Facilitation of Extinction Retention and Reconsolidation Blockade by IV Allopregnanolone in PTSD

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1 LIST OF ABBREVIATIONS

Abbreviation	Abbreviation Definition
3α-HSD	3α-Hydroxysteroid Dehydrogenase
5α-DHP	5α-Dihydroprogesterone
Aβ	Amyloid-β
ACTH	Adrenocorticotropin Hormone
AD	Alzheimer's Disease
AE	Adverse Event
Allo	Allopregnanolone
Allo-S	Allopregnanolone Sulfated
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AST	Aspartate Aminotransferase
BAT-L	Boston Assessment of TBI-Lifetime
BDNF	Brain Derived Neurotrophic Factor
BMC	Boston Medical Center
BPI	Brief Pain Inventory
BUD	Beyond-Use-Date
BUN	Blood Urea Nitrogen
BUMC	Boston University Medical Campus

CADSS	Clinician-Administered Dissociative States Scale
CAPS-5	Clinician-Administered PTSD Scale for DSM-5
CBC	Complete Blood Count
CDC	Center for Disease Control
CNS	Central Nervous System
Co-I	Co-Investigator
CPT	Cognitive Processing Therapy
CREB	Cyclic AMP Response Element Binding Protein
CRP	C Reactive Protein
CRSC	Clinical Research Services Center
CS	Conditioned Stimulus
CS+	Fear Conditioned Stimulus
CS-	Neutral Conditioned Stimulus
CSF	Cerebrospinal Fluid
CSSRS	Columbia Suicide Severity Rating Scale
CRSC	Clinical Research Service Center
CTOB	Clinical Trials Operations Branch
DHEA	Dehydroepiandrosterone
DAMPs	Damage-associated molecular patterns
DSMB	Data Safety Monitoring Board
DSMP	Data Safety Monitoring Plan
EDA	Electrodermal Activity
eFol	Early Follicular
eFP	Early Follicular Phase
eGFR	Estimated Glomerular Filtration Rate
EKG	Electrocardiogram
ERK	Extracellular Signal-Regulated Kinase
FDA	Federal Drug Administration
fMRI	Functional Magnetic Resonance Imaging
FPS	Fear-Potentiated Startle
GABA	Gamma-Aminobutyric Acid
GC-MS	Gas Chromatography-Mass Spectrometry
GCRU	General Clinical Research Unit
GH	Group-Housed
GMP	Good Manufacturing Practices
GNX	Ganaxolone
GGT	Gamma-Glutamyl Transferase
HbA1c	Hemoglobin A1c
HCG	Human Chorionic Gonadotropin
HDL	High-density Lipoproteins
HIPAA	Health Insurance Portability and Accountability Act
HPA	Hypothalamic- Pituitary-Adrenal
ICF	Informed Consent Form
IF	Inflammation
IL	Interleukin

IFN-γ	Interferon-gamma
IMM	Independent Medical Monitor
IPS	Investigational Pharmacy Service
IRB	Institutional Review Board
ISI	Insomnia Severity Index
ITT	Intent-to-Treat
IUD	Intrauterine Device
IV	Intravenous
LDL	Low-density Lipoproteins
LEC	Life Events Checklist
LH	Luteinizing Hormone
LOC	Loss of Consciousness
LTD	Long-Term Depression
LTP	Long-Term Potentiation
MAPK	Mitogen-Activated Protein Kinase
MCAT	MethCathinone
MCI	Mild Cognitive Impairment
MCP	Monocyte Chemoattractant Protein
MDMA	Methylenedioxymethampetamine
MDPV	Methylenedioxypyrovalerone
mLut	Mid-Luteal
mLP	Mid-Luteal Phase
MMP	Matrix Metalloproteinase
NA	Noise Alone
NBFs	Neurobiological Factors
NE	Norepinephrine
NFAT	Nuclear Factor of Activated T-cells
NF-kb	Nuclear Factor kappa-light-chain-enhancer of activated B cells
NIA	National Institute of Aging
NLRP	Nucleotide-binding oligomerization domain, Leucine rich Repeat and Pyrin domain containing
NMDA	N-Methyl-D-Aspartate
NS	Neurosteroid
O_2	Oxygen
OCR	Office of Clinical Research
PA	Pregnanolone
PANAS	Positive and Negative Affect Schedule
PAS	Pregnanolone Sulfated
PBMC	Peripheral Blood Mononuclear Cell
PCL-5	PTSD Checklist for DSM-5
PDAC	Psychopharmacologic Drugs Advisory Committee
PdG	Pregnanediol Glucuronide
PE	Prolonged Exposure
PEA	Palmitoylethanolamine
PFC	Prefrontal Cortex

PHQ	Patient Health Questionnaire
PI	Principal Investigator
PK	Pharmacokinetic
PKA	Protein Kinase A
PPAR	Peroxisome Proliferator-Activated Receptor
PPD	Postpartum Depression
PPX	Propoxyphene
PT	Prothrombin Time
PTSD	Post-Traumatic Stress Disorder
PTT	Partial Thromboplastin Time
RAVLT	Rey Auditory Verbal Learning Test
REMS	Risk Evaluation and Mitigation Strategy
ROI	Release of Information
ROS	Review of Systems
SAE	Serious Adverse Event
Sat	Saturation
SCID-5	Structured Clinical Interview for DSM-5
SF	Short Form
SI	Social Isolation
SCR	Skin Conductance Response
spTMS	Single Pulse Transcranial Magnetic Stimulation
STAXI	State-Trait Anger Expression Inventory
T4	Thyroxine
TBI	Traumatic Brain Injury
THC	Marijuana
TLR	Toll-Like Receptor
TNF	Tumor Necrosis Factor
TSH	Thyroid Stimulating Hormone
UAE	Unanticipated Adverse Event
UC	University of California
UM	University of Michigan
US	Unconditioned Stimulus
VAN	Ventral Attention Network
VAS	Visual Analogue Scale
VEH	Vehicle
WCCVT	Waggoner Computerized Color Vision Test
WSU	Wayne State University

2 PROTOCOL SUMMARY

Title:	"Facilitation of Extinction Retention and Reconsolidation Blockade by Intravenous (IV) Allopregnanolone (Allo) in Post-Traumatic Stress Disorder (PTSD)"
Population:	Males and females with chronic PTSD who are aged 18-55 (at time of enrollment), generally healthy and free from prohibited medications will be recruited to participate in the study at either Boston University School of Medicine in Boston, Massachusetts or Wayne State School of Medicine in Detroit, Michigan. We aim to have a total of 256 study completers in the main parts of the study: n=128 for Expt. 1, "Effects of IV Allo vs. Placebo on Extinction Retention" and n=128 for Expt. 2, "Effects of IV Allo vs. Placebo on Reconsolidation Blockade". A conservative attrition rate of 20% is assumed, so that we anticipate recruiting up to 308 participants (154 participants for Expt. 1 and 154 participants for Expt. 2) to achieve the expected number of *study completers*. We anticipate that about half of the study completers will participate at the Boston site and about half at the Detroit site. Six to 12 other individuals with PTSD (and otherwise meeting the same eligibility criteria as participants in the main study) will participate at either the Boston site or Detroit site in each of the preliminary Expt. 1 and Expt. 2 PK studies. PLEASE NOTE: Participants can take part in only one of the study paradigms: **either** in the PK study for Expt. 1 **or** Expt. 1 **or** the PK study for Expt. 2 **or** Expt. 2. Therefore, a participant can receive study drug (IV Allo or IV placebo) just once.
Intervention:	**I. Study Drugs: IV Allopregnanolone (Allo) and Placebo** The Good Manufacturing Practice (GMP) IV Allo and placebo to be used in the study is manufactured by UC Davis and will be administered in accordance with IND 152138 Notice to Proceed granted to Dr. Ann M. Rasmusson, MD, 7/29/2020. The IV Allo is a USP equivalent of brexanolone, which was approved by the FDA in 2019 for the treatment of PPD (Zulresso™, Sage Therapeutics). The only difference is their concentrations. The UC Davis IV Allo product will be a 2 mg/mL Allo concentrate with 24% sulfobutyl ether-β-cyclodextrin sodium salt [USP: Dexolve; CycloLab, Budapest, Hungary) in 0.9% saline for injection. Before use, it is diluted 4-fold with 0.9% saline to give a final Allo concentration of 0.5 mg/mL with a final cyclodextrin concentration of 6%. This compares to brexanolone (Sage Therapeutics), which is supplied as a 5 mg/mL concentrate with sulfobutyl ether-β-cyclodextrin (USP: Captisol) and diluted before use to 1 mg/mL. The FDA considers the excipients Dexolve and Captisol

to be USP equivalents. The UC Davis product was initially licensed to Sage Therapeutics; the excipient was from changed to Dexolve to Captisol for patenting purposes. Both products have been used safely in humans.

II. Expt. 1: Effects of IV Allo on Extinction Retention

A. IV Allo Pharmacokinetic (PK) Study

Day 1: To confirm that the planned IV Allo dose raises plasma Allo + pregnanolone (PA) to the targeted level: A 1.7 mcg/kg IV bolus of Allo will be administered over 5 minutes to raise plasma Allo levels by ~1500 pg/ml and continue at 2.6 mcg/kg/hr over 5-hours to reach and maintain resting plasma Allo+PA levels associated with optimum extinction retention in a previous study in trauma-exposed individuals with and without PTSD (Pineles et al., 2020). Blood for neurosteroids (NS) and safety measures will be collected at a resting baseline before the IV Allo bolus, and at +0', +15', +30', +1hr, +2hr, +3hr, +4hr, and +5hr after the bolus.

Day 2: A follow-up resting blood draw will be drawn for NS and safety measures.

B. 3-Day Fear-Conditioning, Extinction, & Extinction Retention Test Paradigm

Dependent variables:

a) Skin conductance response (SCR) to fear conditioned stimulus (CS+) and neutral "safety signal" (CS-).
b) Fear-potentiated startle (FPS) to auditory startle probe.

Day 1: Fear acquisition will be induced by exposing subjects to a brief, noxious, but not painful airblast to the larynx (unconditioned stimulus; US) paired with one of two colored geometric shapes (i.e., the conditioned stimulus; CS) displayed on a computer monitor. There will be 12 repetitions of each CS+ and CS- type. A brief auditory stimulus will serve as a startle probe. Blood will be collected after a 1-hour rest prior to fear acquisition for assay of NS and other neurobiological factors (NBFs).

Day 2: Extinction training (16 presentations of the CS+ and CS- *without airblast*) will be followed by a 5' IV Allo vs. placebo bolus and a subsequent continuous 5-hour infusion (dosing per PK study). Blood will be collected for assay of NS, other NBFs, and safety measures after a 1-hour rest just before extinction training. Blood will be collected for NS and other NBFs post extinction training, and at +0', +30', +1hr, +3hr, and +5hr after the IV Allo vs. placebo bolus.

Day 3: Extinction retention will be tested during exposure to 4 CS+ and CS- stimuli without airblast. The capacity for fear reinstatement will be assessed by re-exposure to 1 airblast immediately after the

initial set of 4 CS+ and CS- stimuli, and will be followed by exposure to 4 more CS+ and CS- stimuli without airblast. Blood will be collected for NS, other NBFs, and safety measures after a 1-hour rest before extinction retention testing, and for NS and other NBFs 10' after testing for fear reinstatement.

C. Episodic Memory Testing

Effects of IV Allo vs. placebo on visual episodic memory for neutral stimuli (i.e., non-aversive learning) will be tested using a source memory paradigm. Encoding and immediate recall testing will occur on Day 1 of PK testing (if paradigm is ready) and Day 2 of the 3-day conditioning paradigm ~30-60 minutes before the IV Allo infusion (PK study) or IV Allo vs. placebo infusion (Expt. 1 fear conditioning paradigm). Delayed recall and source memory will be tested about 24 hours later at rest on Day 2 of PK testing (if paradigm is ready) and Day 3 of the fear conditioning paradigm.

III. Expt. 2: Effects of IV Allo on Reconsolidation Blockade

A. IV Allo PK Study

Day 1: To confirm that the planned IV Allo dose raises plasma Allo+PA levels to levels observed at 28-40 weeks of pregnancy, a 28 mcg/kg dose of IV Allo will be infused over 30 minutes, after which *IV fluid only* will be administered over the next 5 hours. Blood will be collected for NS and safety measures at a resting baseline before the bolus and at +0' +15' +30', +1hr, +2hr, +3hr, +4hr, +5hr after the 30' IV Allo infusion.

Day 2: Follow-up resting blood draw for NS and safety measures.

B. 3-Day Fear-Conditioning, Fear Reconsolidation Blockade, & Fear Memory Testing

Dependent variables:
a) Skin conductance response (SCR) to CS+ and CS-.
b) Fear-potentiated startle (FPS) to auditory startle probe.

Day 1: Fear acquisition will be performed as outlined for Expt. 1.

Day 2: The fear memory will be reactivated with *one presentation* of the CS+ (including a startle probe); no airblast will be administered. Afterwards, IV Allo vs. placebo will be infused over 30 minutes (dosing per PK study), after which *IV fluid only* will be administered over the next 5 hours. Blood will be collected for NS, other NBFs, and safety measures at a resting baseline before fear reactivation, after fear activation just prior to initiation of the IV Allo vs. placebo infusion, 15' into the infusion, and at +0', +1hr, +3hr, and +5hr after the 30' IV Allo vs. placebo infusion.

	Day 3: Fear memory and the capacity for reinstatement will be tested and blood will be collected as outlined for Expt. 1. **C. Episodic Memory Testing** Effects of IV Allo vs. placebo on visual episodic memory for neutral stimuli (i.e., non-aversive learning) will be tested using a source memory paradigm as outlined in Expt. 1.
Objectives:	**A.** The objective of the PK studies is to verify that the planned IV Allo doses raise endogenous Allo+PA levels to target levels: Expt. 1: an optimum resting level (per Pineles et al., 2020) sustained over 5 hours. Expt. 2: observed at 28-40 weeks of pregnancy by the end of the 30-minute IV Allo infusion, after which only normal saline will be continued for 5 hours during which endogenous Allo+PA levels are expected to return to the resting baseline. **B.** The objectives of the main studies are to determine whether IV Allo vs. placebo: Expt. 1: administered after extinction training and continued over the 5-hour window of memory consolidation to maintain optimum resting plasma levels enhances fear extinction retention in individuals with PTSD. Expt. 2: administered at a supra-physiological dose (i.e., for non-pregnant individuals) immediately after fear reactivation blocks reconsolidation of the conditioned fear memory in chronic PTSD. **C.** The objectives of the exploratory non-aversive episodic memory studies are to determine whether IV Allo vs. placebo also enhances non-aversive memory consolidation when given ~30-60' minutes after memory encoding at doses that either sustain optimum *resting* endogenous Allo+PA levels (Expt. 1) or reach supra-physiological Allo+PA levels that return to resting levels across the subsequent 5-hour window of memory consolidation (Expt. 2).
Total Study Duration:	August 1, 2021, through April 30, 2025

Subject Participation Duration:	The pre-screening questionnaire will take about 20 minutes to complete. The telephone/zoom or in-person psychological screening evaluation will take about 3½ hours to complete. The in-person medical screening evaluation will take about 2½ hours to complete. In-person screening evaluations will take place at the General Clinical Research Unit (GCRU) at the Boston University Medical Campus (BUMC) or Clinical Research Service Center (CRSC) at WSU. For the Expt. 1 PK study conducted at the GCRU and/or Boston Medical Center (BMC) or CRSC at WSU, the Day 1 and 2 visits, respectively, will take about 8½ and 3 hours to complete. For the Expt. 2 PK study conducted at the GCRU and/or BMC or CRSC at WSU, the Day 1 and 2 visits, respectively, will take about 9 and 3 hours to complete. For the Expt. 1 and Expt. 2 main studies, the Day 1, 2 and 3 visits at BMC or the WSU CRSC, respectively, will take 3½, 9 and 3½ hours to complete. The telephone/zoom check-in one week later will take about 45 minutes to complete.

Please note that this study will be conducted in compliance with the protocol, applicable regulatory requirements, and BMC/BU Medical Campus Human Research Protection policies and procedures, which align with GCP procedures.

3 BACKGROUND/RATIONALE & PURPOSE

3.1 BACKGROUND

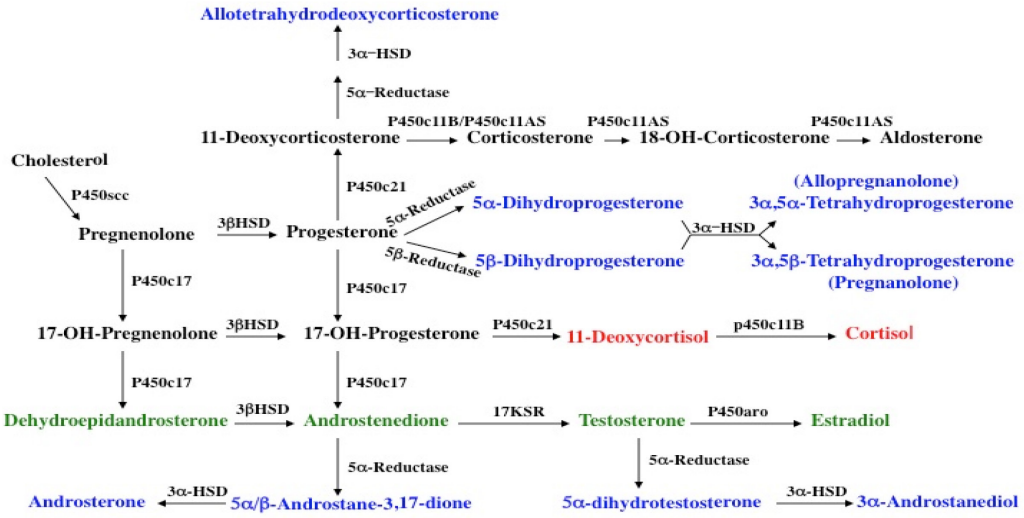
A. Variable Treatment Response in PTSD. Posttraumatic stress disorder (PTSD) is a major public health concern, affecting 6.4% of the general U.S. population, 8.6% of women and 4.1% of men, with more than double these rates among individuals exposed to particularly high-risk types of trauma such as combat, sexual assault and compound community trauma. Trauma-focused psychotherapies show general efficacy in the treatment of PTSD, but outcomes vary substantially among individuals with PTSD and across PTSD subpopulations and many clients do not achieve clinically meaningful reductions in symptoms by the end of treatment (Schottenbauer et al., 2008; Cusack et al., 2016; Amundson et al., 2019). For example, among published studies of Cognitive Processing Therapy (CPT) for PTSD, initial PTSD symptom severity is similar across studies, but the percent reduction in symptoms from pre- to post-treatment ranges from 15% to 86%, with a general pattern reflecting substantially less improvement in veterans and active duty military personnel (both men and women) compared to civilian non-veterans (Resick et al., 2002; Chard et al., 2005; Monson et al., 2006; Galovski et al., Resick et al., 2008; 2012; Forbes et al., 2012; Kaysen et al., 2013; Suris et al., 2013; Morland et al., 2014; Resick et al., 2015; Maieritsch et al., 2016; Galovski et al., 2016).

B. Allo and PTSD. Several factors may contribute to poor responses to these often highly effective PTSD psychotherapies, including *genetic and environmental or stress effects on neurobiological factors* involved in learning and memory processes (Rothbaum and Davis, 2003) critical to: a) reprocessing of trauma memories and related thoughts about the trauma (e.g., Nijdam et al., 2013; Scott et al., 2015; Etkin et al., 2019), b) extinction of threat-conditioned behavioral and neurophysiological defense responses (e.g., Orr et al., 2000; Norrholm et al., 2011; Glover et al., 2012; Jovanovic et al., 2012; Zuj et al., 2016), and c) consolidation of resulting reconfigured brain circuits (e.g., Milad et al., 2008; Pineles et

al., 2016). Recent work by Etkin et al. (2019) demonstrated that *both (not either)* poor delayed recall on a verbal memory word-list learning task *and* aberrant functional connectivity within the ventral attention network (VAN) during functional magnetic resonance imaging (fMRI) defined a subgroup of PTSD patients who responded poorly to Prolonged Exposure (PE) therapy. Further, reduced VAN connectivity was associated with prolonged below-baseline alpha-range desynchronization in response to single pulse transcranial magnetic stimulation (spTMS) — a phenomenon thought to be related to aberrant gamma-amino-butyric acid (GABA) system function. This work thus suggests that resistance to the beneficial effects of trauma-focused psychotherapy may be associated with dysfunction in more than one relevant neural domain (i.e., coordinated neuronal connectivity patterns and learning and memory)—or in a neurobiological factor or factors with impact across multiple neural domains critical to PTSD recovery.

Allopregnanolone (Allo) and pregnanolone (PA) are neurosteroids produced from progesterone in the brain, adrenal gland, ovaries and testes (Fig. 1) that equipotently facilitate the effects of GABA at $GABA_A$ receptors by increasing Cl^- influx 7-10 times. **As recently reviewed (Rasmusson and Pineles, 2018), preclinical and clinical research from this investigative team's laboratories implicate deficits in the synthesis of these GABAergic neurosteroids in the pathophysiology and recovery from PTSD.** For example, our group was the first to demonstrate markedly reduced cerebrospinal fluid (CSF) levels of Allo+PA (measured together by gas chromatography-mass spectrometry or GC-MS) in follicular phase women with PTSD (Rasmusson et al., 2006). These women with PTSD also exhibited a decrease in the

Fig. 1 Neurosteroid Synthesis Pathways



ratio of Allo+PA to the Allo precursor 5α-dihydroprogesterone, suggesting the presence of a block in Allo synthesis at the enzyme 3α-hydroxysteroid dehydrogenase (3α-HSD) (Fig. 1). A PTSD-related block in the synthesis of Allo from its steroid precursors was later confirmed by our group in the CSF of men with PTSD, although the block was at the enzyme 5α-reductase instead of 3α-HSD (Rasmusson et al., 2018; Fig. 1), consistent with previous findings of a PTSD risk polymorphism in the 5α-reductase II gene in men, but not women (Gillespie et al., 2011). Moreover, in both men and women with PTSD, CSF Allo+PA levels correlated negatively and strongly with PTSD reexperiencing and depressive symptoms, accounting for about 50% of the variance—and the ratio of (Allo+PA) to dehydroepiandrosterone (DHEA), an adrenally-produced neuroactive steroid that allosterically *antagonizes* $GABA_A$ receptors and facilitates N-Methyl-

D-Aspartate (NMDA) receptor function, correlated even more strongly, accounting for over 60% of the variance. This suggests that an imbalance in inhibitory to excitatory neurotransmission in the central nervous system (CNS) contributes to PTSD severity. Consistent with these studies, Cruz et al. (2019) recently reported reduced Allo in post-mortem medial orbitofrontal cortex of males with PTSD; androsterone (a GABAergic neurosteroid produced from androstenedione; see Fig. 1) was reduced when controlling for age, post-mortem interval, and smoking.

Recently, Pineles et al. (2018) confirmed a PTSD-related reduction in the (Allo+PA) to 5α-DHP ratio in *plasma* of women with PTSD tested in both the early follicular (eFol) and mid-luteal (mLut) phases of the menstrual cycle. The women with PTSD also failed to increase this ratio in response to a moderately stressful laboratory fear-conditioning task, while healthy trauma-exposed women responded to the stress of this task with a robust increase in this ratio. This study also revealed a strong positive correlation between resting state Allo+PA levels and extinction retention in the mLut phase women with PTSD (See Fig. 2a, Preliminary Studies & Pineles et al., 2020). Among the women with PTSD tested in the eFol phase, the resting state Allo to DHEA ratio correlated strongly with extinction retention (Fig. 2b). As previously reported (Pineles et al., 2016), women with PTSD perform like healthy trauma-exposed women on tests of extinction retention during the eFol phase. However, healthy women perform best in the mLut phase when estradiol, progesterone and Allo levels are all high; in contrast, women with PTSD perform poorly in the mLut phase. Therefore, it is important to note that estradiol upregulates expression of 3α-HSD in females, a mechanism by which estradiol may contribute to the positive association between estradiol levels and extinction retention in healthy women (Milad et al., 2010; Glover et. al, 2005). In women with PTSD, however, estradiol may be inadequate in the mLut phase or at least unable to overcome the impact of other causes of 3α-HSD dysfunction.

Finally, it should be noted that a role for Allo in the pathophysiology of PTSD in humans aligns with male rodent models of PTSD developed in the laboratory of Dr. Graziano Pinna (who will be measuring the neurosteroids for the current study) and confirmed in other laboratories. In these studies, experimentally induced brain and blood Allo deficits are associated with behavioral manifestations of anxiety, depression, and aggression, as well as enhanced contextual fear conditioning, slow extinction, and poor extinction retention. In turn, administration of the Allo analogue, ganaxolone (Kaminski et al. 2004; Reddy et al. 2004), or drugs that enhance Allo synthesis reverse these PTSD-like behaviors (Pibiri et al., 2008 Pinna and Rasmusson, 2014, Zhang et al., 2014; Locci and Pinna, 2019). (See Fig. 3 under the Preliminary Studies section below depicting published data from the Pinna laboratory demonstrating the effects of ganaxolone and other drugs on extinction, extinction retention, and/or reconsolidation blockade).

Recent work in very large samples by Shalev and collaborators from the International Consortium to Predict PTSD (2019) demonstrated a remarkably accurate predictive relationship between *PTSD symptom severity* rated within a month of trauma exposure and development of chronic PTSD 4-15 months later. Thus, PTSD severity appears closely linked to inability to recover from traumatic stress and PTSD. Further research will be necessary to determine if this important observation is related to the presence of deficits in Allo or Allo synthesis capacity, which have been associated in multiple studies now with PTSD or PTSD severity—as well as with deficient consolidation (i.e., retention) of extinction learning.

C. Fear conditioning paradigms with relevance to PTSD recovery. During trauma-focused therapies, activation of a threat-related memory renders the memory "labile" and engages two competing processes: **extinction** and **reconsolidation** (Mamiya et al., 2009; Monfils et al., 2009). Extinction involves both: a) prefrontal cortical (PFC) inhibition of amygdala-mediated sympathetic nervous system, cardiovascular responses, hypothalamic-pituitary-adrenal (HPA) axis, and behavioral defense responses,

and b) acquisition and consolidation of new learning (e.g., the conditioned threat stimulus or CS^+ no longer signals threat, at least in the new time-space context) (Pitman et al., 2012; Dunsmoor et al., 2019). At the molecular level, extinction involves both synaptic long-term potentiation (LTP) and long-term depression (LTD) (Marens, 2015). Extinction thus improves function, but is not permanent, as amygdala-mediated defense responses may be 'renewed' in a new context, 'reinstated' upon re-exposure to the unconditioned threat stimulus (US), or 'spontaneously recover' with the passage of time (Monfils et al., 2009). As previously noted, PTSD has been associated with deficits in both extinction learning (Orr et al., 2000; Norrholm et al., 2011; Glover et al., 2012; Jovanovic et al., 2012; Zuj et al., 2016) and extinction retention (Milad et al., 2008; Pineles et al., 2016). Reconsolidation blockade also may contribute to PTSD recovery (reviewed in Elsey et al., 2018). -blockers (e.g., propranolol: Debiec and LeDoux, 2004; Hu et al., 2007), protein kinase A (PKA) inhibitors (Tronson et al., 2006), and protein synthesis inhibitors (not feasible in humans) (Nader et al., 2000) block reconsolidation (if given within an hour of *brief* threat memory reactivation) by blocking phosphorylation of serine 845 residues on Glu-R1 α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, thus limiting their synaptic incorporation—a prerequisite for memory reconsolidation (Oh et al., 2006; Monfils et al., 2009). Some but not all human studies have demonstrated propranolol-induced blockade of episodic and aversive memory reconsolidation (Elsey et al., 2018), as well as PTSD symptom improvement in paradigms combining reconsolidation blockade and extinction (e.g., Brunet et al., 2018).

In a series of human laboratory studies using propranolol, Soeter and Kindt (2010, 2011, 2012) showed advantages of reconsolidation blockade in that after the intervention fear responses could not be reinstated or renewed; in addition, reacquisition of fear was no faster than during the initial fear conditioning experience, suggesting that there was no "savings" of the CS^+-US association. However, it is important to point out that fear potentiated startle, but not skin conductance responses to the CS+ were reduced, and that emotional distress but not declarative memory for the conditioning procedure was blocked—suggesting that the sites of molecular disruption by reconsolidation blockade were in hippocampus and amygdala, consistent with preclinical work by Mamiya et al., 2009. Clinical treatment investigations modeled on these laboratory designs have been mixed but generally promising. A study by Wood et al. (2015) using mifepristone or D-cycloserine was negative but has been criticized for design flaws (Elsey et al., 2018). Preliminary studies by Brunet et al. (2008; 2011) using trauma-related scripts were promising but were conducted using only the active propranolol intervention (without a placebo control). The most recent placebo-controlled study (Brunet et al., 2018) administered propranolol immediately before re-exposure of the participant to the participant's trauma script—with the aim of reaching therapeutic propranolol levels just after script re-exposure—over a series of up to six sessions. While this design may be confounded by uncertainty about propranolol levels achieved relative to trauma memory reactivation and by trauma script re-exposure over multiple treatment sessions (thereby invoking possible effects of propranolol on extinction), the effect sizes for the advantage of propranolol over placebo were promising: intention-to-treat vs. per protocol pre- to post-treatment effect sizes (Cohen's d), respectively, were 1.76 and 1.64 in the propranolol group and 1.25 and 0.72 in the placebo group based on Clinician-Administered PTSD Scale (CAPS) scores; CAPS scores improved 38% vs. 36% in the propranolol group and 24% vs. 13% in the placebo group. At 6-months follow-up, the mean CAPS scores were 52.0 (n=9) and 69.2 (n=5) for propranolol vs. placebo; PCL-S scores were 38.4 vs. 69.0 for propranolol vs. placebo. Thus, while there appeared to be a clear propranolol advantage, overall symptom change based on the CAPS was more limited than often achieved for in trauma-focused therapies such as CPT and PE. This suggests that there is room for improvement in the application of reconsolidation blockade approaches to treating PTSD.

D. Role of Allo in extinction retention and reconsolidation blockade.

Extinction retention: As noted above, individuals with PTSD are at high risk for Allo deficiency, and deficits in Allo production have been associated with *poor extinction retention* in male rodents and women with PTSD. The *de novo* neuronal synthesis of Allo and its equipotent stereoisomer pregnanolone (PA) is initiated and facilitated, respectively, by NMDA and G-protein coupled receptor activation (reviewed in Rasmusson and Pineles, 2018). The synthesis of these GABAergic neurosteroids, which peaks 30-60 minutes after synthesis initiation (Purdy et al., 1991: rodent swim stress; Izumi et al., 2013: activation of hippocampal neurons; Scioli et al., 2016: maximum load exercise in humans), is essential to the production of LTD and/or LTP interference (Izumi et al., 2013). While LTD contributes directly to extinction (Marens, 2015), LTP interference likely contributes to extinction retention, as it is thought to protect recently modulated synapses from further modification during the 1-6 hour period of memory consolidation (e.g., Stefan et al., 2005). Two phenomena may contribute: 1) rising sulfated metabolites of Allo and PA (i.e., Allo-S and PAS) antagonize NMDA receptor activation (Park-Chung et al., 1997; Gibbs et al., 2006), and 2) rising Allo+PA facilitates $GABA_A$ receptor inhibition of p38MAPK/ERK pathway production of PKA (Tyagi et al., 2009; Kim et al., 2012), thus disrupting Glu-R1 serine 845 phosphorylation. Enhancement of extrasynaptic $GABA_A$ receptor function by Allo and PA in amygdala also likely restrains activation of brainstem monoamine inputs to prefrontal cortex (PFC) that, if too high, disrupt working memory and PFC inhibition of the amygdala (Pitman et al., 2012). Reconsolidation blockade: In contrast to the potential beneficial effects of Allo administered *after extinction training* on extinction retention, Allo given *after a single CS^+ re-exposure* is expected to block CS^+-US reconsolidation—possibly more effectively than propranolol or PKA inhibitors. Resulting increases in Allo-S may directly antagonize NMDA receptors; facilitation of $GABA_A$ receptor function by Allo and PA would be expected to disrupt Glu-R1 serine 845 phosphorylation by inhibiting the PKA/cyclic AMP response element binding protein (CREB) signaling pathway directly and indirectly via inhibition of amygdala activation of the locus coeruleus, thus reducing norepinephrine (NE) release. In contrast, while propranolol may block NE activation of the PKA/CREB signaling pathway, it could also reduce activation of neurosteroidogenesis (Rasmusson and Pineles, 2018; Liang and Rasmusson, 2019) and thus diminish potential inhibitory effects on reconsolidation of Allo and PA and their sulfated metabolites. Thus we hypothesize that dosing Allo immediately after a single CS+ exposure will be more effective than propranolol in blocking reconsolidation.

Episodic Memory: Given the potential effects of rising Allo levels in response to NMDA receptor activation on LTD (LTP not yet investigated) and LTP interference (which theoretically should also protect synapses newly modulated by LTP from further modification during the subsequent 1-6 hour memory consolidation window, which is dependent on protein synthesis), we have elected to also explore the effects of low vs. high dose Allo administration *on consolidation of episodic memory for neutral material* as an exploratory aim. While we hypothesize that high as well as low dose IV Allo will facilitate consolidation of episodic memory when learning occurs in the hour before Allo administration, it is also possible that high dose Allo (as in Expt. 2 testing effects of Allo on reconsolidation blockade) will interfere with PKA/CREB pathway dependent protein synthesis, despite potential advantageous effects on LTP interference. Execution of this exploratory aim will therefore allow us to gather preliminary data regarding potential effects of IV Allo administration on non-aversive episodic memory consolidation. This may be pertinent to the use of IV Allo in the context of trauma-focused psychotherapies that include important non-aversive 'teaching' of therapeutic techniques, tools, and ideas, in addition to implicit induction of extinction of conditioned defensive responses.

E. Role of Inflammation in Stress Exposure and Fear Conditioning.

Stress may contribute to fear learning and memory by propagating and regulating inflammatory responses via activation of the toll-like receptor (TLR-4)/ nuclear factor (NF)--kappa-light-chain-enhancer of activated B cells (kB) inflammatory cascade and release of pro-inflammatory cytokines and inflammasomes. Exposure to a stressor may also enhance inflammation via activation of the NLRP3 inflammasome. In a rat model of PTSD, exposure to footshock increased interleukin (IL)-1β mRNA in the dorsal hippocampus at 6, 24, 48, and 72 hours (Jones et al., 2015). Dong et al. (2020) demonstrated an increase in cleaved caspase-1 and IL-1β in the hippocampus of mice 3 hours after footshock, consistent with activation of the Nucleotide-binding oligomerization domain, Leucine rich Repeat and Pyrin domain containing (NLRP)-3 inflammasome. Transcriptional upregulation of IL-1β and tumor necrosis factor (TNF)-α was also detected in isolated microglia 3 hours after footshock exposure. Furthermore, hippocampal neuroinflammation (evidenced by an increase in the number of activated microglia) and loss of post-synaptic density proteins (PSD) (important for synaptic stabilization during LTP) was seen 72 hours after footshock exposure. Finally, NLRP3 inflammasome inhibition or knockout and IL-1β inhibition enhanced the extinction of contextual fear memory and attenuated anxiety-like behaviors, while IL-1β administration inhibited both the acquisition and extinction of the contextual fear memory (Dong et al., 2020) suggesting that activation of these inflammatory factors impedes learning generally during stress—and of specific relevance to this proposal—may be associated with extinction retention deficits.

Inflammation pathways also appear to be involved in tipping the balance between reconsolidation of conditioned fear and fear extinction. In experiments conducted by Scholz et al. (2016), rat hippocampal tissue was collected 2 hours after 'no reactivation of the conditioned fear memory', 'reactivation of fear without extinction', or 'fear reactivation with extinction'. Increased NF-kB, IL-1β, and IL-6 gene expression was associated with fear reactivation alone, while increased TNF gene expression was associated with extinction (Scholz et al., 2016). Furthermore, longer fear reactivation (10 minutes vs. 2 minutes) was associated with reductions in NF-kB gene expression and extinction. De la Fuente et al. (2011) also found that NF-kB activation was crucial for contextual fear memory reconsolidation and impaired extinction. In contrast, calcineurin phosphatase blockade of NF-kB activation allowed extinction to occur. Calcineurin phosphatase also induced 'nuclear factor of activated T-cells' (i.e., NFAT) activation and translocation, which is required for expression of IL-2, IL-4, TNF, and other cytokine genes (Abbas et al., 2014; de la Fuente et al., 2011). These findings thus suggest that NF-kB gene regulation determines whether gene transcription supports fear reconsolidation or extinction of conditioned fear.

Finally, Young et al. (2018) found that fear acquisition increased plasma levels of interferon (IFN)-γ in mice, while extinction training produced an increase in plasma IL-6 manifest at 0 and 10 minutes, but not 60 minutes. Reactivation of the CS without extinction training also produced a rapid elevation in plasma IL-6. Inhibition of IL-6 reduced freezing behavior only when conditioned fear reactivation was followed by extinction training, suggesting that actions of IL-6 in fear memory learning are important for extinction but not reconsolidation (Young et al., 2018). Therefore, increased plasma IL-6 levels in individuals with PTSD may play a role in poor extinction learning or extinction retention.

F. Role of Allo in Inflammation.

Allo has also been observed to be low in PTSD and many PTSD-comorbid conditions associated with inflammation including chronic pain, depression, psychosis, panic attacks and Alzheimer's Disease (Cai et al., 2018; Marx et al., 2006; Naylor et al., 2016; Romeo et al., 1998; Ströhle et al., 2003). Allo or agents that increase the synthesis of GABAergic neurosteroids (Pinna & Rasmusson, 2012) may reduce inflammation. Interestingly, the efficacy of certain SSRIs and antipsychotics currently used to treat PTSD may be related to their ability to increase Allo (Romeo et al., 1998; Uzunova et al., 1998; Marx et al.,

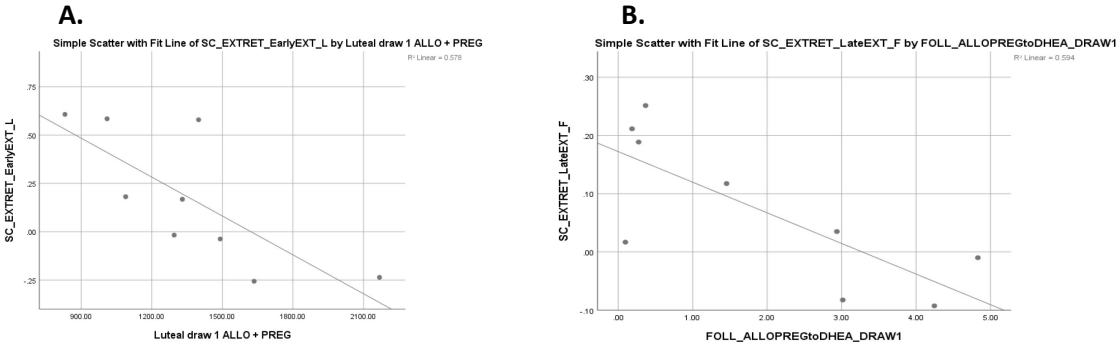
2003) and reduce inflammation, in addition to having multiple other salutary effects (Rasmusson et al., 2017). For instance, in rodent traumatic brain injury (TBI) models, Allo reduced IL-1β and TNF-α protein levels 4 hours post-injury (He et al., 2004). Allo also reduced IL-6 and matrix metalloproteinase (MMP)-9 while reducing blood-brain barrier disruption and the extent of brain injury 72 hours after blunt neurotrauma in rodents (Ishrat et al., 2010). Allo mediated inhibition of TLR-4 signaling in monocyte/macrophage cells and reduced monocyte chemoattractant protein (MCP)-1 in alcohol-preferring rats (Balan et al., 2019) and reduced oxidative stress by enhancing activity of the antioxidant enzyme superoxide dismutase (Qian et al., 2015). Finally, Chen et al. (2011) showed that Allo reduced microglial activation and amyloid-β (Aβ) generation in cortex, hippocampus, and amygdala in a rodent model of Alzheimer's disease. No studies have been conducted in humans to assess the effect of IV Allo on inflammation. Therefore, an exploratory aim of this study is to investigate whether IV Allo vs. placebo administered on Day 2 decreases inflammation over the course of Day 2 and on Day 3 in individuals with PTSD. Furthermore, the potential differential effects of low vs. high IV Allo on inflammation will be examined. Given the heightened inflammatory response observed in PTSD, IV Allo may be used as a potential treatment to mitigate inflammation and therapeutically tip the balance between conditioned fear consolidation vs. reconsolidation blockade or extinction of conditioned fear.

3.2 INNOVATION

a) First test of IV Allo on extinction retention and reconsolidation blockade in humans
b) There has been a call to evaluate clinical mechanisms in men and women simultaneously rather than developing a standard in men and then testing women. Even if these studies are underpowered to detect sex differences, they can be used to determine if there is a sufficient signal to conduct larger studies powered to detect sex differences or designed separately by sex or menstrual status (Shansky, 2019).
c) The growing evidence for biological heterogeneity underlying the PTSD phenotype (Pitman et al., 2012; Rasmusson and Pineles, 2018) has provoked a call for medications that address individually variable PTSD- pathophysiological processes (Friedman and Bernardy, 2016). Our exploratory examination of the relationship between resting Allo levels (prior to extinction training or reconsolidation blockade procedures) will allow an examination of variation in Allo synthesis capacity on extinction retention in the placebo group, and on potential benefits of IV Allo administration on extinction retention.
d) The experimental paradigms, as designed, allow testing of potential effects of IV Allo administration on both aversive memory (main aims) and episodic memory (preliminary aim).
e) First examination of the balance between neurosteroids that positively vs. negatively modulate effects of GABA at GABAA receptor or positively vs. negatively modulate NMDA receptor function on fear learning, extinction, extinction retention, and reconsolidation.
f) First study of the effects of IV Allo on inflammatory factors that also appear to play important roles in learning under conditions of threat or stress.

3.3 PRELIMINARY STUDIES

Fig. 2 Relationships of Resting Plasma GABAergic Neurosteroids and Extinction Retention in Women

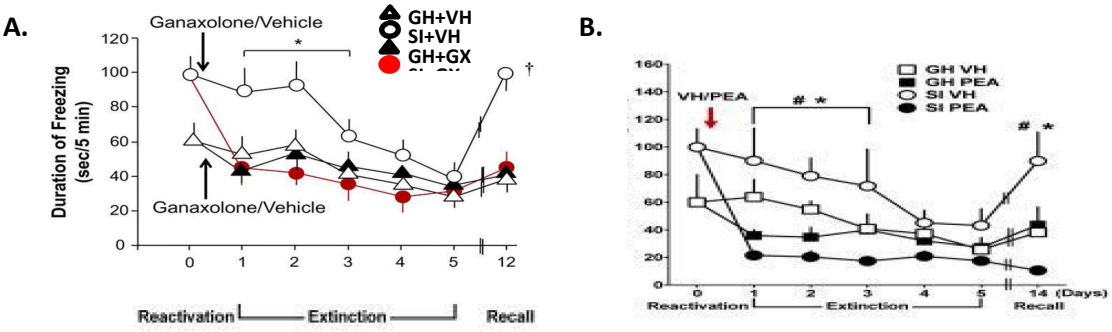


A. The preliminary data above (**Fig. 2**) (Pineles et al., 2020) show the strong positive relationships between resting plasma Allo+PA and extinction retention mLut phase of women with PTSD ($r^2 = 0.58$) (A-left) and between the resting plasma (Allo+PA)/DHEA ratio and extinction retention in eFol phase women ($r^2 = 0.59$) (B-right), as tested using the differential fear conditioning paradigm previously published in Pineles et al. (2016, 2018). Note that the measure of extinction retention depicted along the Y-axis is: the differential skin conductance (SC) measure of arousal on the day of extinction retention testing minus the differential SC measure of arousal at the end of extinction training on the previous day. Thus, a higher value along the Y-axis indicates worse extinction retention. It should also be noted that in the newly proposed study, a similar differential fear conditioning paradigm will be used but will be improved upon as we plan to execute the paradigm over 3 days instead of 2 (with fear acquisition on Day 1, extinction training on Day 2 and extinction retention testing on Day 3). We do, however, expect similar results given that in the previous work by Pineles et al. (depicted) all participants were brought to full extinction of their conditioned fear response on Day 1 (after fear acquisition)—thus the time gap between extinction training and extinction testing is the same as in the proposed study.

B. The studies below (**Fig. 3**) were conducted in the laboratory of Co-I Graziano Pinna, Ph.D., which has led the field in development of rodent models of Allo deficiency and treatments that rectify resulting PTSD-like behavioral disturbances. This laboratory first described the capacity of serotonin-selective reuptake inhibitors to raise brain Allo levels at doses <1/10 of those that block serotonin reuptake, doses that restore normal brain Allo levels and normal behavior in Allo-deficient rodents (Pinna et al., 2009). The study below (Pinna and Rasmusson, 2014; Fig. 3, left) shows the capacity of a single dose of ganaxolone (GNX: a synthetic 3□eta-analogue of Allo with similar effects at $GABA_A$ receptors; Kaminski et al. 2004; Reddy et al. 2004) to induce what appears to be *reconsolidation blockade* when administered to male mice with social isolation (SI)-induced brain Allo deficiency. Notably, the treatment had no effect on group-housed (GH) mice with normal brain Allo levels. As depicted, brief reactivation of contextual fear by re-exposure of the mice to the chamber in which the mice were previously exposed to footshock was immediately followed by subcutaneous administration of GNX (10 mg/kg) or vehicle (VEH). Contextual freezing was markedly reduced on re-exposure to the context on the following day in the GNX-treated SI mice (red line). Freezing remained low and followed the course of freezing in the non-Allo deficient mice over the subsequent extinction training trials—and showed no spontaneous recovery a week after extinction training was completed. In contrast, the SI mice treated with VEH extinguished very slowly (as seen in other studies; Pibiri et al., 2008; Locci and Pinna, 2019) and showed spontaneous recovery of fear one week after extinction training ended. Notably, GNX had no apparent effect in mice with normal brain Allo levels. In the second study using the same

conditioning and single injection treatment paradigm (Locci and Pinna, 2019; Fig. 3, right), the endocannabinoid congener N-palmitoylethanolamine (PEA: 5 mg/kg i.p.) had similar effects. PEA activates the peroxisome proliferator-activated receptor (PPAR)-alpha, which induces biosynthesis of Allo; the effects of PEA in the SI-induced Allo deficient rodents were blocked by the prior administration of finasteride, which blocks activity of 5α-reductase and synthesis of Allo. Possible effects of PEA on GH rodents with normal Allo also were seen.

Fig. 3 Effects of Drugs that Augment Allo in Mouse Models of Reconsolidation Blockade



C. Previous Clinical Studies Using the IV Allo

Formulation to be Administered in the Current Study

As previously noted, the University of California (UC) Davis GMP IV Allo formulation to be utilized in the proposed study is the United States Pharmacopeia (USP) equivalent of brexanolone (Zulresso™), which has been approved for treatment of postpartum depression (PPD) using the Federal Drug Administration (FDA) required Zulresso™ Risk Evaluation and Mitigation Strategy (REMS). Both the UC Davis GMP IV Allo formulation and brexanolone have been administered to many patients. Patients on active drug experienced adverse events (AEs) at the same incidence as those receiving placebo, except for transitory dizziness, sedation, somnolence, and mild nausea, which were seen more frequently with Allo, consistent with the activity of Allo at $GABA_A$ receptors. While PTSD patients in the proposed study will be treated with substantially lower doses of IV Allo than administered in previous studies, suggesting that they will be at even less risk for these AEs, we will have staff and even more conservative REMS procedures in place to manage such AEs if they occur. According to Dr. Rogawski who manufactures the IV Allo at UC Davis, Allo levels are based on body mass index and *Allo infusion kinetics are linear.* In a pharmacokinetic (PK) study by Timby et al. (2006), three IV injections of Allo were given over a few minutes each at 30-min intervals, using doses of 15, 30, and 45 mcg/kg, for a cumulative dose of 90 ug/kg. Based on Timby et al., Allo levels in the currently proposed study are expected to fall by half over the hour after the 30-min 28 mcg/kg bolus and continue to fall to resting levels by 4-5 hours after the bolus.

D. Summary of Preliminary Data

In sum, our clinical studies have shown strong relationships between CSF Allo+PA levels or plasma indices of deficient Allo+PA synthesis and PTSD severity in both men and women —suggesting that Allo deficits may contribute to PTSD risk and/or poor recovery from traumatic stress. Further, we have demonstrated links between low resting plasma Allo+PA levels in women or low brain Allo levels in male mice and poor extinction retention. Together these studies support a role for Allo in moderating symptom severity as well as in learning and memory processes critical to PTSD recovery. Based on these findings and the results of our promising rodent treatment studies, we plan to investigate the use of

acute IV Allo administration to facilitate extinction retention and reconsolidation blockade in men and women with PTSD. We propose to use a 3-day differential fear-conditioning paradigm to test effects of acute IV Allo vs. placebo administration on extinction retention (Expt. 1) and reconsolidation blockade (Expt. 2) in men and women with chronic PTSD. Since Allo infusion kinetics are linear we are confident that we do not have to run a dose-response study. However, we are performing PK studies prior to initiation of Expt. 1 and Expt. 2 to confirm the IV Allo doses proposed (and as prepared) increase endogenous Allo+PA levels to those desired as outlined above in the study objective.

4 OBJECTIVES

4.1 STUDY OBJECTIVES

We aim to evaluate the effects of acute IV administration of Allo vs. placebo on extinction retention (Expt. 1) and reconsolidation blockade (Expt. 2).

Aim 1: Compare effects of IV Allo vs. placebo administered after extinction training on *extinction retention* (Expt. 1) in chronic PTSD.

- *Hyp-1*: IV Allo versus placebo given at completion of extinction training on Day 2 (to raise plasma Allo to resting levels previously associated with optimal extinction retention) will enhance extinction retention on Day 3.
- *Hyp-2:* IV Allo versus placebo treatment will reduce conditioned fear reinstatement after US re-exposure on Day 3.
- *Hyp-3 (exploratory):* Resting Allo + PA levels before or 3 hours after extinction training will correlate positively with extinction retention. The ratio of Allo + PA to: a) their 3β-isomers, or b) DHEA, neurosteroids that allosterically antagonize $GABA_A$ receptors, will more strongly predict extinction retention.

Aim 2: Compare IV Allo vs. placebo effects on reactivated fear memory *reconsolidation* in chronic PTSD (Expt. 2).

- *Hyp-1:* A high dose IV bolus of Allo versus placebo given immediately after re-exposure to a single CS+ (no US) on Day 2 will significantly decrease conditioned fear on Day 3.
- *Hyp-2:* A high dose IV bolus of Allo versus placebo given immediately after re-exposure to a single CS+ (no US) on Day 2 will prevent its reinstatement on Day 3.

Exploratory Aims:

A) Evaluate effects of low vs. high dose IV Allo vs. placebo on *non-aversive episodic memory consolidation* in chronic PTSD**.**

- *Hyp:* Delayed recall, recognition and source memory of pictorial stimuli encoded on Day 2, 60 minutes before low (Expt. 1) or high (Expt. 2) dose IV Allo vs. placebo will be better in Allo-treated participants tested on Day 3.

B) Evaluate effects of low vs. high doses of IV Allo vs. placebo on *inflammation.*

- *Hyp-1* IV Allo vs. placebo on Day 2 in both Expt. 1 and Expt. 2 will significantly reduce inflammation across time on Day 2.
- *Hyp-1*: IV Allo vs. placebo on Day 2 in both Expt. 1 and Expt. 2 will significantly reduce inflammation on Day 3.
- *Hyp-2*: High dose IV Allo (Expt. 2) will be more effective in reducing inflammation on Day 3 compared to low dose IV Allo (Expt. 1).

4.2 STUDY OUTCOME MEASURES

4.2.1. Primary Outcome Measures: The effects of IV Allo vs. placebo (given on Day 2) on the primary outcome efficacy measures, extinction retention (Expt. 1) and reconsolidation blockade (Expt. 2) on Day 3 will be assessed as follows:

A. Extinction Retention

1. **Skin Conductance Response (SCR):** To determine extinction retention, the difference between the average SCR to the first 4 fear conditioned stimuli (CS+) trials and the average SCR to first 4 neutral conditioned stimuli (CS-) trials on Day 3 will be calculated. Extinction retention for SCR will be defined as this differential SCR minus differential SCR for Day 2 (calculated as the difference between the average SCR to the last 4 CS+ trials and average SCR for last 4 CS- trials– the index of Day 2 extinction).
2. **Fear-Potentiated Startle (FPS):** The degree of FPS to the CS+ during the first 4 CS+ trials on Day 3 will be examined and compared to the FPS for the last 4 CS+ trials during extinction. Lower scores indicate better extinction retention.

B. Reconsolidation blockade

1. **SCR:** Reconsolidation blockade will be assessed by comparing the difference between the average SCR to the first 4 CS+ trials minus the average SCR to the first 4 CS- trials. Lower scores indicate better reconsolidation blockade.
2. **FPS:** Reconsolidation blockade will be calculated as the average FPS to the first 4 CS+ trials

4.2.2 Secondary Outcome Measures

A. Fear acquisition on Day 1 will involve the pairing of a brief, noxious/aversive but not painful airblast to the larynx (the unconditioned stimulus or US) to a conditioned stimulus (CS) (Expts. 1 and 2). The CS will be different colored shapes appearing on a computer monitor. An auditory stimulus will serve as the startle probe. Fear acquisition will be defined as the difference between the average SCR to the last 4 CS+ and average SCR to the last 4 CS- trials during the acquisition phase (i.e., differential SCR). For FPS, the last 4 trials of acquisition for each CS will be examined. Since FPS is calculated as the difference between startle to the CSs compared to Noise Alone (NA) trials, the standard is to use FPS to CS+ trials as the dependent variable rather than the difference between FPS to CS+ versus CS- as is typically done for SCR. Higher scores indicate greater conditioned fear acquisition.

B. Reinstatement of conditioned fear on Day 3 will be assessed in Expt. 1 and Expt. 2. Reinstatement of conditioned fear will be defined as the average SCR to the last 4 CS+ trials minus the average SCR to the last 4 CS- trials. For FPS, the last 4 CS+ trials will be examined. Higher scores indicate greater reinstatement of conditioned fear.

4.2.3 Exploratory Outcome Measures

A. Non-Aversive Episodic Memory: On Day 3 in Expt. 1 and Expt. 2, *delayed recall* will be calculated to assess *recognition* and *source memory*. Participants will be presented with 15-30 objects from set 1 and 15-30 objects from set 2 seen and recalled on Day 2, as well as 15-30 objects not previously seen, all shown against a uniform white background. Participants will be asked to indicate whether an object was presented on the previous day (assessing recognition memory), and if yes, whether it was presented in the first set or the second set, thus assessing source memory.

B. Relationship between Neurosteroid (NS) and Psychophysiology Measures: Relationship between resting and post activation blood NS levels and measures of fear acquisition, extinction, and extinction retention (Expt. 1) or between the ratios of the GABAergic/GABA receptor-antagonizing or NMDA receptor facilitating NS and psychophysiology measures: acquisition, extinction, and extinction retention or reconsolidation blockade (Expt. 1 and Expt. 2).

C. Relationships of resting and stress activated NS to PTSD severity [CAPS and PTSD Checklist (PCL)]: assessed separately in men, early follicular phase (eFP) women and mid-luteal phase (mLP) women.

D. Inflammation: The effect of IV Allo vs. placebo on inflammation will be assessed by change in blood levels of C-Reactive Protein (CRP), IFN-γ, IL-1β, IL-6, TNF-α, MCP-1, and MMP-9 from Day 2 to Day 3 in Expt. 1 and Expt. 2.

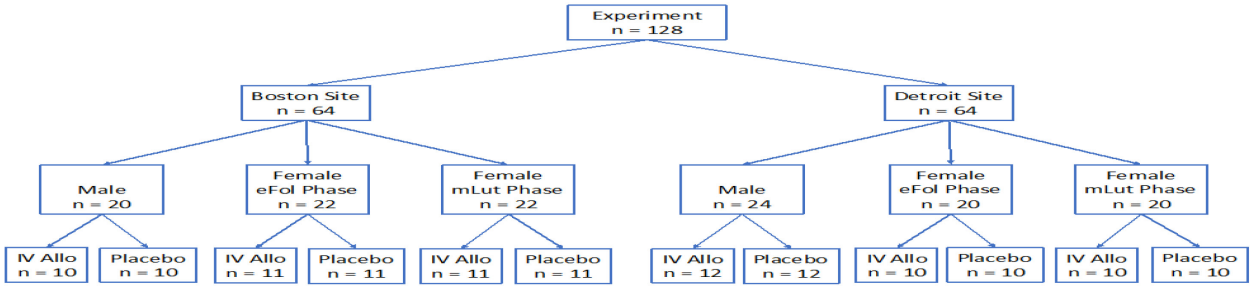
5 STUDY DESIGN

5.1 GENERAL STUDY DESIGN CHARACTERISTICS

This is a Phase II randomized, proof-of-concept, placebo-controlled study of the effects of IV Allo vs. placebo on extinction retention (Expt. 1) and reconsolidation of reactivated conditioned fear memories (Expt. 2) in generally healthy participants not on prohibited medications and aged 18-55 (male, eFol female, mLut female) with chronic PTSD.

5.2 RANDOMIZATION

For both Expt. 1 and Expt. 2 (n=128 for each experiment; n=256 total sample), we will employ stratified randomization of IV Allo vs. placebo by site and the following subgroups: men (n=44; 22 per treatment group), women in the eFol phase (n=42, 21 per treatment group), and women in the mLut phase (n=42, 21 per treatment group). Dr. Fonda will create and oversee randomization schedules for the Boston and Detroit sites and will create separate randomization schedules for each site and subgroup (3 randomization schedules per site) using block randomization. Dr. Fonda will expand the randomization schedule to account for a potential 20% attrition within any subgroup to ensure that the minimum number assigned to the treatment groups within each subgroup is attained (see Fig. 4 for a flow diagram of the final number randomized and retained in the study for each site and subgroup). Investigators and participants will be blinded to treatment assignment; only the biostatistician, pharmacist dispensing the study drug, and Data Safety Monitoring Board (DSMB) will have access to the randomization schedule. Dr. Fonda will provide the SAS statistical program that creates the randomization schedule, including the block size used in the block randomization for each site and subgroup, to the DSMB.



5.3 RATIONALE FOR STUDY DESIGN

5.3.1 General: This study does not aim to test the effects of IV Allo vs. placebo on PTSD or other psychiatric symptoms per se. Rather the main aims of this Phase II clinical trial are to test the capacity of IV Allo vs. placebo to affect underlying specific time-dependent neural processes thought to be critical to recovery from PTSD: extinction retention (Expt. 1) vs. reconsolidation of reactivated fear memories (Expt. 2). Previous work suggests that de novo synthesis of the GABAergic neurosteroids Allo and PA is initiated by NMDA receptor activation in brain and adrenocorticotropin hormone (ACTH) stimulation in the adrenals, and that Allo and PA levels rise slowly in brain and the blood to peak 30-60 minutes later. Early low levels favor fear memory reconsolidation of the labile reactivated synapses; later higher levels contribute to LTD, as well as LTP interference, which is thought to protect newly modulated synapses from further modification during the subsequent several-hour window of memory consolidation. The timing of IV Allo augmentation for the treatment of PTSD thus may be critical: a) High Allo levels immediately *before* fear memory reactivation (which is essential to extinction) may prevent fear memory reactivation, b) raising Allo to high levels immediately *after* fear memory activation is expected to prevent fear memory reconsolidation and thus prevent fear reinstatement, and c) raising Allo at the end of extinction to high optimum resting levels (see Fig. 2 above) is expected to enhance retention of fear extinction. Use of IV Allo to promote extinction or block reconsolidation may be a useful strategy for the treatment of chronic PTSD—the former for individuals with low endogenous Allo and PA synthesis; reconsolidation blockade may be a useful treatment strategy for individuals with normal Allo+PA synthesis but dysregulation of other neurobiological systems that contribute to chronic PTSD. Data from this study thus may support future development of IV Allo for the prevention or treatment of chronic PTSD.

5.3.2 Accounting for Sex Differences: Study of women and men separately, as well as women in both the eFol and mLut phases of the menstrual cycle is important given documented differences in neurosteroid levels and performance on extinction retention between women across these menstrual phases.

5.3.3 Preliminary PK Testing: We decided that PK testing was essential due to possible PTSD-related stress-induced increases in p450 CYP3A4 metabolism of Allo. A previous study of ganaxolone in the treatment of PTSD dosed ganaxolone in accordance with PK studies previously performed in non-PTSD populations. Trough ganaxolone levels in the PTSD study were substantially lower than expected and presumably subtherapeutic.

5.3.4 Valid, Innovative Psychophysiology & Neurosteroid Assay Methods Across Sites:
Psychophysiological systems used to conduct Expt. 1 and Expt. 2 are uniform across sites; expert investigators from Detroit will assist in person with set-up and alignment of the system in Boston.
Neurosteroids of interest in the study will be measured under treatment-blind conditions in expert laboratories that use state-of the art methodologies for plasma neuroactive steroid measurements (see section 9.4.14 Blood Biomarkers).

6 POTENTIAL RISKS AND BENEFITS

6.1 RISKS AND PROTECTIONS AGAINST RISKS

6.1.1 Psychological Risks

I. Emotional Discomfort: Participants may feel embarrassment or stress when talking about their exposure to traumatic or other stressful life events, psychological/psychiatric/emotional health or medical experiences.

Protections: To minimize discomfort, all telephone/Zoom or in-person visits will take place in a private, comfortable setting. If participants experience distress during the interview, the person conducting the interview will work with the participant to provide support or connect the participant to a mental health provider as needed. Additionally, the participants are informed that if they feel upset or uncomfortable with any particular question, they can refuse to answer or stop as needed until the distress lessens. They are also informed that if symptoms emerge or increase to a distressing level at home, they should contact the study team so that additional clinical support or a safety evaluation and management can be provided if needed. Participants are also informed that if they are at risk of hurting themselves or others, they may be referred (against their wills if necessary) for an emergency evaluation at a local emergency room.

II. Interview Burden: Participants may get tired or anxious from participation in potentially long interviews.

Protections: Participants are informed that if they become too tired or stressed during potentially long interviews (often longer if a participant has experienced more traumatic events), they can take a break or stop the visit early and continue the interview by phone or in-person, as appropriate, at a later date.

III. Risk of Harm to Self/Others: It is possible that participants in the study screening or after might already pose a risk to self or others, or it is possible that participation in the study could surface memories of traumatic or other experiences that increase distress or trigger thoughts leading to risk to self or others. In our many years of experience engaged in similar studies in participants with PTSD or depression or other disorders (perhaps present but disqualifying during screening), such occurrences are rare.

Protections: A Columbia Suicide Severity Rating Scale (CSSRS; Oquendo et al., 2003) to assess history and immediate risk for self/other harm will be completed at the remote (IRB-approved and COVID-compliant or in-person when allowed) eligibility screening evaluation, as well as at each of the 2-day PK study visits, each of the 3-day psychophysiology testing visits and the 1 week follow-up remote visit for both Expt. 1 and 2. The PHQ-9 (Spitzer et al., 1999) also will be administered at the first screening evaluation for study eligibility and at the Day 1 and follow-up visits for both Expts. 1 and 2; one PHQ item queries suicidal ideation: "Thoughts that you would be better off dead or of hurting yourself in some way." Participant answers to the questions on these documents will be concurrently reviewed by a trained masters level coordinator who will have immediate access to a credentialed doctoral level psychologist or psychiatrist. The latter will evaluate the individual further. Participants with active risk to self/others will be referred immediately for further clinical evaluation and treatment as appropriate (including engagement of 911 services if needed and against the participant's will if necessary), as outlined during the informed consent process. Study participants with active current risk to self/others or with a history of a suicide attempt or harm to others within the past year will not be eligible for entry

into the study. Participants who are not eligible for the study will be offered information about clinical care options and a referral if desired. Participants also will be advised that they are welcome to contact the investigators if their PTSD or depressive symptoms, general distress, or thoughts of harm to self or others occur or increase in response to the screening evaluation or at any time while in the study. As described during the informed consent process, contact with the potential participant's clinician is a prerequisite to study participation (to ensure appropriateness of participant enrollment from a safety point of view as well as to confirm use of any medications), and will require a signed release of information (ROI) form by the participant at the time of consent.

IV. Scheduling Inconvenience: While we will aim to schedule participants' study appointments as soon as possible and at their convenience, unexpected delays could occur, for example, due to the COVID-19 pandemic. Participants will receive a rapid COVID-19 test at the beginning of each in-person study visit. If a participant tests positive on a rapid COVID-19 test at a screening evaluation or Day 1 study visit, they will be rescheduled for that study visit in accordance with the Center of Disease Control (CDC) guidelines. However, if a participant tests positive for COVID-19 at a Day 2 or Day 3 visit, they will be discontinued from further participation in the study. In addition, scheduling of eligible females during the desired menstrual cycle phase can sometimes be a challenge and may have to be delayed until the next month.

Protections: We will work with participants to schedule their appointments for the study as soon as possible and at their convenience. However, we will also inform them that unexpected delays could occur, for example, due to the COVID-19 pandemic. In addition, scheduling of eligible females during the desired menstrual cycle phase can sometimes be a challenge and may have to be delayed until the next month. Participants will be informed of these potential inconveniences during the informed consent process.

V. Menstrual Cycle Monitoring (Females): This involves dipping a test strip into the first morning urine and urine once in the afternoon or evening and informing the investigators when the LH surge test strip become 'positive'. Women using hormonal IUDs or other devices (e.g., Nuvaring) will also be asked to check their urine for a rise in their pregnanediol glucuronide (PdG) levels subsequent to a positive LH surge test.

Protections: This presents a minor inconvenience. We provide the kits directly to the participants at the in-person evaluation or mail them if needed. The female participants are informed of this process at the time of consent. We routinely use these procedures in many of our studies and subjects have never complained. They are typically interested and eager to do this—based on their observations that their mood or symptoms is often influenced by their menstrual cycle.

VI. Potential Delay in Starting Clinical Treatment: Participants are advised in the ICF that they will be allowed to participate in certain treatments that don't directly target PTSD symptoms and may stop participating at any time if a delay in getting clinical care is too long or puts the individual at risk.

Protections: We expect there to be minimal delays to starting any desired clinical treatments for PTSD due to being in this study—though for the reasons described above, eligible females may experience greater delays. Participants can decide to leave the study (without penalty except for payment for sessions in which they have not yet participated) if they prefer to start a clinical treatment before completion of this study.

VII. Financial Costs: Participants are advised during informed consent that they will be compensated for travel and paid for study sessions to help offset time away from work or other costs, such as childcare.

However, if a visit is ended by the investigators and the participant is disqualified because they tested positive for nicotine, drugs, or alcohol at that session, the participant will not be paid for that study visit, although travel will be paid. Participants will be paid for the study visit if they are positive on a COVID-19 rapid test performed at the beginning of that study visit.

Protections: To minimize misunderstandings about payments, participants are advised in the ICF that: a) They will be compensated for travel and paid for study sessions to help offset time away from work or other costs, such as childcare; b) If they decide to withdraw from the study, they will be paid for sessions in which they already participated; c) *They will not be paid if a visit is ended by the investigators because the participant tested positive for nicotine, drugs or alcohol (which excludes a participant from further participation);* d) They will be paid if a visit is ended by the investigators because the participant tested positive for COVID-19 on the rapid test. Participants will be discontinued from further participation in the study if they cannot be rescheduled (i.e., if the positive COVID-19 test is on a Day 2 or Day 3 visit or if they no longer meet study criteria or cannot arrange to be rescheduled for participation). However, as we do not want participants with limited resources to incur a potentially unaffordable transportation cost or be stranded without transportation, we will pay costs of travel for that session. Note, that we have adopted the practice of not paying participants who present with positive urine toxicology or saliva alcohol test strip results and do not agree to abstain from cannabinoids and nicotine for one month prior to the experimental procedures, have a follow-up negative screening test, and test negative for these substances on the morning of experimental procedures because a substantial number of individuals have used research opportunities with us to make money (often to buy drugs) without a real prospect of qualifying for the study. We thus make these payment contingencies very clear in both the pre-screening and first telephone/Zoom or in-person screening evaluation. Once we instituted this practice in our previous studies, we saw a substantial decline in persons presenting for in-person screenings (or subsequent experimental visits) with positive urine toxicology screens (Japuntich SJ, Hall A, Kimberly A, Joos CM, Rasmusson AM, Pineles SL. Methods to reduce false reporting of substance abstinence in clinical research. *International Journal of Methods in Psychiatric Research* 2018e1603, doi: 10.1002/mpr.1603). Excluding individuals using drugs or alcohol ensures safety, preserves limited study resources, and protects the integrity of study results.

VIII. Coercion: Participants can sometimes feel pressured into participating in studies that may not be in their best interest due to undue pressure by investigators or out of financial need or because the compensation is inflated.

Protections: Participants who do not wish to participate in the study or who wish to drop out of the study after enrollment will not experience any negative consequences (apart from not receiving payment for sessions not yet attended) and will be referred to community-based treatment for PTSD or other conditions diagnosed if desired. We believe the rate of compensation for the current study is fair and matched to time needed to complete procedures and additional inconveniences that are part of some procedures. Travel is compensated to address a potential disincentive to study participation. Participants will be compensated for most study sessions based on the time required—as payment may be needed to offset babysitting costs or time away from work. We plan to pay at rates commensurate with time spent (~$15-20 an hour), apart from the sessions, for which we pay more to encourage retention. Of note, the last session is critical to achieving the study aims and present little risk to participants and require less time than the prior day's session.

6.1.2 Medical Risks

I. Electrocardiogram (EKG): Sticky EKG pads are placed after using alcohol pads to remove skin oil and shaving of hair from the chest if it interferes with secure adhesion and transmission of the EKG signals from the heart. This can cause some redness, swelling or itching where the pads were placed.

Protections: Participants are informed that the EKG adhesive pads could cause some redness, swelling or itching. If the participant has a history of allergy or skin irritation to adhesives or EKG pads, they can decide not to participate in the study. Otherwise, if this occurs, the area can be cleansed with soap and water. The participant can, if desired, obtain an over-the-counter cream to diminish itching and speed resolution, or if needed be referred to a medical provider for treatment.

II. Attachment of Wireless Electrodes for Startle Testing: Mild irritation/allergic reaction to application of the wireless electrodes (for psychophysiology testing), which involve mild abrasion and the use of alcohol to cleanse the surface of the participants' skin.

Protections: Staff applying the electrodes are carefully trained so as to minimize irritating the participants' skin. The participant can, if desired, obtain an over-the-counter cream to diminish itching and speed resolution, or if needed be referred to a medical provider for treatment.

III. Airblasts to the Larynx: Participants may find the airblasts distressing, as they are intended to elicit a startle response.

Protections: While experienced as aversive, the airblasts are not painful. In addition, the emotional reactions to them are typically brief. Participants are advised during the informed consent process that brief airblasts to the neck will occur and may be startling or distressing but are not otherwise harmful.

IV. Blood Draw or Intravenous (IV) Placement: IV placement and blood draws by needle stick may cause discomfort or pain, bruising at the site, lightheadedness, fainting, or infection (rare). Sometimes a needle stick or IV placement may need to be attempted more than once. The amount of blood collected over the course of the study is up to 120 cc for the PK studies (about 8 tablespoons or about 1/4th of a standard blood donation) and up to 410 cc and 404 cc for Expts. 1 and 2, respectively (about 27 tablespoons or about 4/5ths of a standard blood donation). This could increase the severity of anemia in an otherwise anemic individual.

Protections: To reduce risks associated with IV placement and blood drawing, a trained phlebotomist or research nurse will use standard techniques and infection precautions when performing these procedures. To increase the ease of blood drawing, we will ensure that participants are well hydrated. We also inform participants that sometimes a needle stick or IV placement may need to be attempted more than once, but that they can decide to end their participation if that is not okay. The researchers also may end their participation if it doesn't make scientific sense to continue (e.g., if the entire IV Allo vs. placebo infusion can't be completed). In either case, the participant would be paid for that session. We also require that anemia detected at the medical screening evaluation must be treated before the participant can continue in the study.

V. Side Effects or Other Reactions to the IV Allo or Placebo Infusions:

A. Reported Side Effects of IV Allo

The following side effects were reported in a study of the effects of IV Allo vs. placebo on episodic memory (Timby et al., 2006) 10 healthy women in the follicular phase of the menstrual cycle. Three increasing IV Allo boluses of 15, 30 and 45 ug/kg were administered at 3-minutes intervals for a total dose of 90 ug/kg (3x the IV Allo dose planned for Expt. 2) to. AEs included **mild feelings of alcohol-like intoxication** (3 participants), **mild nausea** (3 participants), **flushing** (1 participant), and an **anxiety attack 24 hours after the infusion** (1 participant).

The UC Davis formulation (co-investigator M. Rogawski, personal communication) was administered in a recently completed National Institute of Aging (NIA)-funded study of men and women aged ≥ 55 years of age, "Allopregnanolone Regenerative Therapeutic for mild cognitive impairment (MCI)/ Alzheimer's disease (AD): Dose Finding Phase 1" (ClinicalTrials.gov; NCT02221622). The active drug vs. placebo was infused over 30 minutes once per week x 12 weeks at escalating doses based on the safety of the previous dose: 2 mg, 4 mg, and 6-18 mg. For a 70 kg individual, these doses translate to 28.6 mcg/kg, 57.1 mcg/kg and ≥ 85.7 mcg/kg. The lowest 28.6 mcg/kg dose is equivalent to the highest dose planned for Expt. 2 in the current study (28 mcg/kg infused once over 30 minutes). Adverse events in the MCI/AD study (Hernendez et al., 2020) are reported to be consistent with results publicly reported in various other trials using IV Allo including the FXTAS trial, which used the UC Davis formulation (Wang et al., 2017) and the brexanolone (Zulresso™) trials in PPD. In all of these studies, subjects receiving active drug experienced adverse effects at the same rate as those receiving placebo, except for **transitory dizziness, sedation, somnolence and mild nausea** seen more frequently with active drug, consistent with the activity of Allo at $GABA_A$ receptors.

As reported in the SAGE Therapeutics package insert for Zulresso™, the most common adverse reactions, characterized as an incidence ≥ 5% and at least twice the placebo rate, were **sedation/somnolence, dry mouth, loss of consciousness (LOC), and flushing/hot flash**. One participant in the SAGE trials developed suicidal ideation once home after the 60-hour study infusion; this AE was not thought to be related to Zulresso. The most serious adverse events (AEs) were reported in an on-line 11/2/18 FDA Briefing Document that included patient blood Allo levels proximal to the AEs, as detailed below.

1. Sudden LOC "as if in deep sleep" associated with *infusion pump malfunction* 14 hours after starting the 90 ug/kg/hr infusion. The patient felt well 10 minutes after the infusion was stopped.
   Allo level: 79.3 ng/ml = 79,300 pg/ml (2.5 hrs before episode).
2. Sudden onset of snoring associated with *infusion pump malfunction* 90 minutes into 30 ug/kg/hr continuous infusion; the patient fully recovered 14 minutes after the infusion was stopped.
   Allo level: 29.5 ng/ml = 29,500 pg/ml (2.25 hrs after event).
3. Somnolent and unaware of surroundings 30 hours into a 60 ug/kg/hr infusion. The patient improved 15 minutes after the infusion was stopped and recovered 45 minutes it stopped.
   Allo level: 103 ng/ml = 103,000 pg/ml (1.2 hrs after event).
4. Serious adverse event (SAE): Sudden LOC while eating 5 hours into a 60 ug/kg/hr infusion. The patient opened his eyes to verbal stimuli in 10 minutes, but otherwise was non-responsive for 60 minutes. Sent to emergency room. No further treatment required. No memory for event.
   Allo level: 51.6 ng/ml = 51,600 pg/ml (30 min. before event).
5. Presyncope/vertigo 13 hours after starting a 90 µg/kg/hr infusion. The patient sat down and the infusion continued. The presyncope resolved in 10 minutes, and the vertigo in 2 hrs.
   Allo level: 138 ng/ml = 138,000 pg/ml (2.75 hrs after event).
6. A healthy, non-obese 55-year-old man participating in a SAGE Phase I cardiac repolarization study experienced severe somnolence and <1 minute of apnea while receiving two times the maximum recommended dosage of ZULRESSO™ for treatment of PPD (i.e., 180 ug/kg/hour).
   Allo level: 150 ng/ml = 150,000 pg/ml.
7. Vasovagal syncope during blood drawing in participant with fear of needles.
   Allo level: 64.6 ng/ml = 64,600 pg/ml.

It should be noted that in the current study, we are using much lower doses of IV Allo than the studies in which the above AEs were reported. For **Expt. 1**, we are administering an IV bolus of 1.7 mcg/kg followed by an infusion of 2.6 mcg/kg/hr for 4-5 hours. This is expected to raise plasma Allo levels to a high normal resting level. For **Expt. 2**, we are administering a bolus of 28 mcg/kg over 30 minutes followed by IV saline only for the next 4-5 hours. This is equivalent to 1/3 of the dose in the Timby et al. study in which minimal sedation (manifest as a decrease in saccadic eye movements; no somnolence was observed) and is also much lower than delivered in the Sage trials. The above AE-related Allo levels in the Sage PPD trials were 16-73 times higher than the highest Allo+PA blood levels targeted *in men by IV Allo infusions in the current study* (approximate range expected in men = 1549-1900 pg/mg). The Sage AE-related blood Allo levels were 15-70 higher than the highest Allo+PA blood levels *targeted in eFP women* (approximate range expected in eFP women= 1,510-1,967 pg/ml). They were 8-38 times higher than the highest Allo+PA blood levels targeted *in mLP women* (approximate range expected in mLP women = 2,232 to 3,600 pg/ml).

However, although the extreme sedation or loss of consciousness reported in the Sage PPD studies were at IV Allo doses that were substantially higher than in the current study, these AEs could potentially be seen at lower IV Allo doses or due to unexpected events such as IV infusion pump malfunction.

Protections:

1. General: The proposed study infusions will be conducted in accordance with the FDA Risk Evaluation and Mitigation Strategy (REMS) protocol stipulated for Zulresso (generic: IV Allo or brexanolone) at a certified healthcare facility (i.e., BMC) or a free-standing outpatient research facility (WSU CRSC, which has at least a decade of experience conducting FDA regulated clinical trials) with a) access to emergency medical services, b) monitoring by a qualified BUMC GCRU nurse detailed to BMC or a WSU CRSC nurse, respectively, and a study MD or NP, and c) continuous pulse oximetry with an alarm. The researchers will ask participants to stay awake and to let the researchers know if they feel sleepy during the 4-5 afternoon hours after the IV Allo vs. placebo infusions. PTSD patients have difficulty sleeping at night may become sleepy while sitting at rest for 4-5 hours during either the placebo infusion or the Expt. 1 low dose (1.7 mcg bolus over 2 minutes followed by 2.6 mcg/kg/hour) or Expt. 2 higher dose (28 mcg/kg over 30 minutes followed by normal saline) IV Allo infusion. Nevertheless, based on the studies reviewed above and a newly published PK study by Hernandez et al. (2020) in which no sedation was observed when GMP IV Allo (provided by UC Davis) was infused over 30 minutes at doses of 2 mg (~28 mcg/kg), 4 mg (~57 mcg/kg), and 6 mg (~86 mcg/kg) to individuals with Alzheimer's disease, we expect that such fatigued participants should be readily awakened to an alert, oriented, and coherent state. **If not—see the following safety plan.**

2. In case of hypoxia: The IV Allo vs. placebo infusion will be immediately stopped and will not be resumed if pulse oximetry reveals **hypoxia.** Gries et al. (1996) reviewed of all-night pulse oximetry data (with care to exclude periods of motion artifact) in 350 apparently healthy individuals without previously diagnosed sleep apnea. In these healthy individuals, the mean ± SD low Sat was 90.4% ± 3.1%. The mean Sat 10 (i.e., O2 sat below which the patients spent 10% of the time was 94.7% ± 1.6%. The mean Sat 50 was 96.5% ± 1.5%. There was no relationship between any of the O_2 Sat measures and sex, race, or obesity as measured by body mass index. However, older subjects (>60 years of age) had lower Sat 10 (92.8% ± 2.3%) and Sat 50 (95.1% ± 2.0%) than did younger subjects. Therefore, based on Gries et al. (1996), hypoxia will be defined for the current study as an O_2 saturation < 96% while the participant is awake or < 90% if the participant falls asleep, as long as not due to artifact. Specifically:

a) To ensure the baseline health of participants, we will exclude anyone with an O_2 sat <96% at the medical screening evaluation or baseline O_2 sat measurement on the day of the study infusion (PK Studies Day 1; Expts. 1 and 2, Day 2).
b) If the O_2 sat in an awake participant falls below 96% during the IV Allo or placebo infusion, the study will be stopped.
c) If during sleep, the O_2 sat falls below 90% and does not appear to be due to artifact (i.e., persists >30 seconds and has good signal quality), the study will be stopped.
d) If during sleep, the O_2 sat falls to 90-93% for > 10 minutes continuously or for > 5 minutes continuously on 2 occasions, the study will be stopped.*

* Criterion "d" recognizes (per Gries et al., 2006) that it is normal to spend some sleep time below a 94.7% O_2 sat, while it cautiously errs on the side of stopping the infusion if O_2 sats are starting to look shaky. Criterion "d" thus would allow the infusion to continue if a participant's O_2 sat was mostly at >94% during sleep, but just occasionally dropped down to low 90s before popping back up. An O_2 sat of 90-93% does not itself harm a person; the issue is whether it is a warning sign of a potentially dangerous drop to below 90%.

It should be pointed out, however, that hypoxemia during an IV Allo infusion would be expected only in the context of ***excessive* sedation**, which is not expected at the planned IV Allo doses, but could occur unexpectedly (e.g., due to IV pump malfunction or if a participant violated study procedures and was on a concomitant sedating drug). See below for protections.

3. In case of excessive sedation:
a) For **Expt. 1,** the (low dose) IV Allo vs. placebo infusion will be stopped immediately if the participant develops signs or symptoms of *excessive sedation or a change in consciousness* (i.e., the participant cannot be fully awakened/alerted to a sustained, fully oriented and coherent state).

If a participant who falls to sleep, awakens readily to a fully oriented and coherent state in response to staff, and it is determined that the IV pump did not malfunction, the IV Allo vs. placebo infusion will be resumed at 50% of the initial planned dose as a matter of caution. As can be seen in section **8.2.3 Dosing Protocols**, dosing at this level will decrease blood Allo levels to the *lower end* of previously observed normal resting Allo levels associated with optimum extinction retention. However, to be cautious, if the participant falls asleep *again* at this lower IV Allo vs. placebo dose (even if still readily awakened to a fully oriented and coherent state), the infusion will be substituted with a slow (keep vein open or 'KVO' normal saline infusion) to preserve IV access for safety purposes and to allow further blood sampling. Blood sampling then will continue under close monitoring until the end of the 4-5 hour window of memory consolidation—after which the individual will be examined/cleared for discharge home if appropriate.

If a pump malfunction is discovered, we will not be able to tell how much IV Allo was received. In that case, the IV Allo or placebo infusion will be stopped and substituted with a slow 'KVO' normal saline infusion to preserve IV access for safety purposes and allow further blood sampling. Blood sampling will continue under close monitoring until the end of the 4-5 hour window of memory consolidation (as long as the individual can maintain an awake, oriented and coherent state—after which the individual will be examined/cleared for discharge home if appropriate.

If at any point, the individual cannot be fully awakened or maintain an oriented and coherent state, the infusion will be stopped and substituted with normal saline. A code will be called if appropriate (e.g., if hypoxia or confused/agitated behavior occurs) and/or the participant will be transferred as appropriate to a higher level of care. Blood sampling will be recommended (if not clinically

contraindicated) in order to later shed light on the possible cause of such an event (e.g., pump malfunction or possible undetected use of a sedating agent—see the Drug Interactions section below).

b) For **Expt. 2,** the higher dose IV Allo vs. placebo infusion takes place over 30 minutes, after which only normal saline is infused over the next 4-5 hours. If excessive sedation occurs (as defined above) during the 30-minute infusion, the IV Allo vs. placebo infusion will be stopped, normal saline will be substituted at a slow KVO rate to preserve IV access for safety purposes and to allow further blood sampling. Blood sampling will continue under close monitoring until the end of the 4-5 hour window of memory consolidation—after which the individual will be examined/cleared for discharge home if appropriate. If at any point (during or after the 30 minutes infusion) the individual cannot be fully awakened to an alert, oriented and coherent state, a code will be called if needed (e.g., if hypoxia or confused/agitated behavior) and/or the participant will be transferred as appropriate to a higher level of care. Blood sampling will be recommended (if not clinically contraindicated) in order to later shed light on the possible cause of such an event.

4. If a participant experiences an alcohol intoxication-like reaction to the IV Allo, we will offer support the participant as needed (e.g., phone calls, encourage attendance at an AA meeting, trouble shooting) to avoid relapse to alcohol or drugs (e.g., benzodiazepine) if that has been a problem for the participant in the past.

5. Before discharge, an MD or other qualified healthcare provider will evaluate the participant to ensure that it is safe for the participant to leave. Participants also will be cautioned against engaging in potentially dangerous activities that require mental alertness, and someone else must drive the participant home (friend, family member, commercial ride service paid by the study). In addition, we will remind the participants to avoid use of alcohol or drugs or medications not approved by the study team the evening after the infusion, and we recommend that the participant have a trusted person available.

B. Potential Side Effects of the IV Allo or Placebo Infusion (Not Previously Reported)

Allergic reaction: This is not likely to occur in reaction to the Allo, as this is a naturally occurring neurosteroid. However, participants could have an allergic reaction to the excipient in which the Allo is dissolved/stored for IV administration; the excipient is identical for the placebo formulation.

Protections: In the informed consent form (ICF), we advise participants that they could have an allergic reaction to the substance in which the Allo or placebo substance is dissolved (sulfobutyl ether beta-cyclodextrin). If the participant has an allergic reaction, the IV Allo or placebo treatment will be stopped and appropriate medication or other treatment will be provided in accordance with the BUMC GCRU or WSU CRSC Emergency Response and Medication Administration protocols (see the attached document in INSPIR). Participants are also informed that there could be costs associated with referral for such treatment.

Drug Interactions: IV Allo could interact negatively with another drug or illicit substance taken by the participant to increase risk of sedation, nausea, or other side effects.

Protections: Concurrent use sedating drugs or other substances (e.g., alcohol) may increase risk of sedation or hypoxemia due to respiratory depression in the context of IV Allo infusions. Use of alcohol, illicit drugs and nicotine also would confound interpretation of the study data (e.g., by altering neuroactive steroid levels and/or the capacity to respond to psychophysiology testing), which would influence the study risk/benefit ratio. Therefore, use of prescribed medications that may increase risk of sedation or confound interpretation of the study results is prohibited during this study. Diagnosis of moderate or severe substance use disorders within three months of screening is also an exclusion

criterion. Participants are also asked to abstain from alcohol for 2 weeks and illicit drugs, marijuana, and nicotine for at least 4 weeks prior to participation in the PK Study Day 1-2 procedures and the Psychophysiology Study Day 1-3 procedures.

In addition, to deter attempted study enrollment by misusers of alcohol, illicit drugs, marijuana, or nicotine, individuals are informed during the pre-screening and telephone/in-person psychological screening examination that they will not be paid for their in-person visits if they test positive for these substances. In addition, urine toxicology and saliva alcohol testing will be conducted at every in-person visit (i.e., the in-person medical screening evaluation, PK Study Days 1-2, and Psychophysiology Study Days 1-3) to determine whether the individual is free from alcohol, illicit drugs, marijuana, and tobacco. On the day of the IV Allo vs. placebo infusion, we will test the participants' urine for illicit drugs and saliva for alcohol before IV placement. Please see section **9.4.17 Urine Toxicology for Illicit Drugs and Cotinine** for the list of urine toxicology tests to be conducted. We also perform a stat urine test for fentanyl in the hospital chemistry laboratory before administration of study drug (active or placebo). Note that carfentanil cannot currently be adequately detected by available laboratory tests, highlighting the importance of having naloxone available per BMC protocols to reverse potential dangerous sedative effects of this opiate and negative effects on respiratory drive.

Harm to a Developing Fetus or Breastfed Infant: There is limited information on the reproductive safety of IV Allo. The *general class of medications* to which IV Allo belongs (i.e., drugs that facilitate $GABA_A$ receptor function) may cause degeneration of the developing brain. Therefore, this research should not be done during pregnancy or by women who are breastfeeding.

Protections: In accordance with FDA regulations, we will test eligible females for pregnancy at each in-person study session (the medical screening evaluation, both days of PK testing, and each of the 3 days of psychophysiology testing (i.e., startle testing as described in the ICFs). In the event of pregnancy, the individual will be referred to clinical care if desired. In addition, eligible females of childbearing potential must use two highly effective forms of contraception for a week before receiving IV Allo vs. placebo and for 28 days afterwards in accordance with recommendations by the FDA. Systemic hormonal contraceptives are not allowed because they will interfere with the results of the study [e.g., many suppress allopregnanolone synthesis—as demonstrated in a recent preliminary study by the PI—and previous researchers have found that healthy women without psychiatric disorders on oral contraceptives had deficits in extinction retention (Graham and Milad, 2013)]. Some hormonal intrauterine devices (IUDs) (e.g., Mirena, Kyleena, Liletta, and Skyla) or other contraceptive devices (e.g., Nuvaring) will be allowed if we can ascertain that the participant using the hormonal IUD or other device is still menstruating and ovulating (see Study Procedures). While these IUDs and other devices are intended to have local effects in the uterus that prevent pregnancy, in many women, the hormones released from these devices raise blood levels sufficiently to directly suppress ovulation. Other effective contraceptive methods the participant or participant's partner can use include a diaphragm or cervical cap with spermicide, condoms with spermicide, vasectomy, and the copper IUD. Females of childbearing potential will be instructed to immediately contact the study MD if pregnancy is suspected while they are in the study.

Risk to individuals with end stage renal disease: Zulresso is not recommended for PPD treatment in patients with *end stage kidney disease* (estimated Glomerular Filtration Rate 15-29 mL/minute/1.73 m^2) due to renal accumulation of the solubilizing agent betadex sulfobutylether sodium, which can cause further renal impairment. Sulfobutyl ether beta-cyclodextrin is the equivalent excipient for the IV

allopregnanolone solution to be delivered to research subjects in this project and thus presents a risk of renal toxicity with high exposures to individuals with chronic renal failure.

Protections: In the current study, IV Allo is given at a much lower dose over a much shorter period of time than in the Zulresso™ studies and as currently prescribed treatment for PPD. We also test renal function at the in-person medical screening evaluation to ensure that participants' renal function is normal; we will exclude individuals with an estimated glomerular filtration rate (eGFR) of <30 ml/min. To be especially cautious, we will test renal function again just before and the day after the IV Allo treatment. If any abnormalities are found, we will refer the participant for further evaluation.

The PI (Dr. Rasmusson) and Dr. Rogawski (Co-I who provided the reference Investigational New Drug [IND] for the UC Davis, GMP product to be used in the study) do not believe there is a substantial risk of renal deterioration in response to the doses of IV Allo or placebo (and excipient) to be delivered to participants in this study because of: a) the study's stringent medical inclusion/exclusion criteria, and b) the low sulfobutyl ether beta-cyclodextrin exposure. In support of this contention, we have prepared the following comparisons between a) the total dose of IV sulfobutyl ether beta-cyclodextrin to be delivered to participants using the proposed dosing regimens for Expt. 1 and Expt. 2, and b) the dose of sulfobutylether-beta-cyclodextrin delivered by an FDA-approved product (Voriconazole for Injection) when dosed according to recommendations in the label. Please note that:

1. The maximum *sulfobutylether-beta-cyclodextrin* load that a 110 kg experimental subject will receive is about 370 mg (0.37 g). This compares with 21 g that a patient would receive when treated with IV voriconazole dosed according to the recommendations in the label.
2. *Sulfobutylether-beta-cyclodextrin in Voriconazole for Injection (FDA-approved)*
   Composition of voriconazole for injection: 200 mg voriconazole and 3,200 mg sulfobutylether-beta-cyclodextrin sodium (SBECD); Dose of voriconazole: 6 mg/kg IV q 12 h.
   a) For 70 kg subject: 840 mg voriconazole; 13.4 g sulfobutylether-beta-cyclodextrin
   b) For 110 kg subject: 1,320 mg voriconazole; 21.1 g sulfobutylether-beta-cyclodextrin
3. *Sulfobutylether-beta-cyclodextrin in UC Davis IV allopregnanolone (i.e., experimental drug)*
   The UC Davis GMP product consists of 0.5 mg/cc allopregnanolone in 6% (6g/100 cc or 60 mg/1cc) sulfobutyl ether beta-cyclodextrin (GMP) in 0.9% sodium chloride injection, USP.

   Expt. 1: Target plasma concentration: 1.5 ng/ml*;* dosing regimen: 1.7 mcg/kg bolus over 5 min, followed by 5-hour drip infusion at 2.6 mcg/kg/hr

   IV) *For 70 kg subject:*

   Bolus: 0.119 mg allopregnanolone; Infusion: 0.91 mg allopregnanolone over 5 hours
   TOTAL ALLOPREGNANOLONE: 1.029 mg
   Bolus: 14.28 mg sulfobutylether-beta-cyclodextrin; Infusion: 109.2 mg sulfobutylether-beta-cyclodextrin
   TOTAL SULFOBUTYLETHER-BETA-CYCLODEXTRIN: **123.5 mg**

   IV) *For 110 kg subject:*

   Bolus: 0.187 mg allopregnanolone; Infusion: 1.43 mg over 5 hours
   TOTAL ALLOPREGNANOLONE: 1.62 mg
   Bolus: 22.44 mg sulfobutylether-beta-cyclodextrin; Infusion: 171.6 mg sulfobutylether-beta-cyclodextrin

TOTAL SULFOBUTYLETHER-BETA-CYCLODEXTRIN: **194 mg**

Expt. 2: Target plasma concentration: 25 ng/ml; dosing regimen: 28 mcg/kg over 30 min

IV) *For 70 kg subject:*

Bolus: 1.96 mg allopregnanolone

TOTAL ALLOPREGNANOLONE: 1.96 mg

Bolus: 235.2 mg sulfobutylether-beta-cyclodextrin

TOTAL SULFOBUTYLETHER-BETA-CYCLODEXTRIN: **235 mg**

IV) *For 110 kg subject*

Bolus: 3.08 mg allopregnanolone

TOTAL ALLOPREGNANOLONE: 3.08 mg

Bolus: 369.6 mg sulfobutylether-beta-cyclodextrin

TOTAL SULFOBUTYLETHER-BETA-CYCLODEXTRIN: **370 mg**

6.1.3 Other Risks

There may be additional **unknown or unanticipated reactions** to the IV Allo or placebo infusion or to other procedures associated with the study.

6.2 ADEQUACY OF PROTECTION FROM RISK

Given the means by which the range of study risks will be mitigated and given that the FDA has provided an IND and Notice to Proceed based on the proposed means of mitigating the specific risks of IV Allopregnanolone and placebo administration, we believe that the participants will be adequately protected.

6.3 POTENTIAL BENEFITS

There are no anticipated direct benefits to participants in this study. It is possible that some participants might benefit from diagnosis of mental (or medical) health disorders of which they were unaware or uncertain, or referral for appropriate treatment outside of the study if desired.

6.4 ANALYSIS OF RISKS IN RELATION TO BENEFITS

We believe that procedures described above sufficiently mitigate risks to participants such that the ratio potential risks to potential benefits to the participants and society is acceptable.

Findings from this study may advance development of more efficacious treatments for PTSD and/or increase our understanding of neurobiological factors (NBFs) that contribute to less than optimum outcomes for these treatments. The findings may also advance development of precision medicine for PTSD by identification of individually variable factors that possibly can be addressed to facilitate natural and treatment-facilitated recovery from PTSD. Findings also may directly inform the development of pharmaceutical agents that could target individually variable neurobiological deficits that may impede PTSD recovery. We thus believe that the risks to participants are reasonable given the importance of knowledge expected to result from this study.

7 SUBJECT SELECTION

7.1 SUBJECT INCLUSION CRITERIA

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

- Between the ages 18 and 55 (at time of enrollment) and reproductively mature.
- Meet criteria for chronic PTSD (i.e., CAPS-5 determined PTSD duration more than 3 months).
- Generally healthy and not on any prohibited medications (that could affect study outcomes).
- Willing to abstain from alcohol for 2 weeks and from nicotine, marijuana or illicit drugs for 4 weeks before experimental procedures and throughout the study.
- For biological females:
    - Natural menstrual cycle.
    - If of childbearing potential, female and partner must use 2 types of effective birth control (except for hormonal contraceptives, unless IUD or a device like Nuvaring) for a week before the IV Allo or placebo infusion, and for one month after.

7.2 SUBJECT EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

- Present an imminent risk to self or others or require clinical intervention to maintain safety
- Diagnosis of moderate or severe substance use disorder within three months of screening per administration of the SCID substance abuse evaluation. Diagnosis of a *mild* substance use disorder within three months of screening will be allowed if the participant agrees to abstain from illicit drugs for one month and/or alcohol for 2 weeks prior to the experimental procedures, has a negative screening or follow-up urine toxicology and/or saliva alcohol test (if the screening test is positive), and tests negative for these substances on the morning of the experimental procedures.
- Bipolar I disorder, schizophreniform disorders, or clinically significant psychotic symptoms apart from the presence of trauma-related sensory hallucinations or negative beliefs.
- History of a suicide attempt within 1 year of enrolling.
- A history of *severe* TBI is exclusionary for the PK-1 and PK-2 studies. A history of *moderate or severe* TBI is exclusionary for the main studies (i.e., Expt. 1 and Expt. 2).
- Diagnosis of sleep apnea
- Awake resting O_2 saturation <96%
- Severe renal failure with an eGFR <30 ml/min
- Use of medications or substances (by report or toxicology testing) will be exclusionary under the following conditions:

    1. <u>During screening for eligibility</u>:

    IV) Use of illicit substances, as well as prescribed opiates or benzodiazepines (either reported or detected on urine toxicology testing), will be exclusionary.

    IV) Reported *non-dependent* use of cannabinoids or nicotine (with indication of such by a positive urine toxicology or cotinine test at screening) <u>will not</u> be exclusionary if the individual agrees to abstain from cannabinoids and nicotine for one month prior to the experimental procedures, has a follow-up negative screening test, and tests negative for these substances on the mornings of experimental procedures.

    IV) A positive saliva alcohol test at screening (which indicates uncontrolled alcohol use and likely moderate to severe alcohol dependence) will be exclusionary.

    IV) A high serum gamma-glutamyl-transferase (GGT) test at screening (indicative of more

remote recent drinking but not necessarily moderate to severe alcohol use or dependence) will not be exclusionary if the individual does not meet criteria for a moderate or severe alcohol use disorder within three months of screening, agrees to abstain from drinking for 2 weeks prior to the experimental procedures, and has a normal follow-up salivary alcohol and serum GGT test.

e) Use of non-illicit over-the-counter or prescribed medications that may increase the risk of IV Allo side effects or adversely affect the experimental results is exclusionary. Participants may agree to stop non-psychotropic medications used on a prn basis, such as acetaminophen, ibuprofen, or loratadine (a CYP-3A inhibitor) for 5 half-lives of the parent drug or active metabolite (whichever is longer) before the experimental procedures. Regular psychotropic medications (including those used to treat non-psychiatric conditions, such as alpha1-antagonists prescribed for hypertension or urinary hesitancy), may be discontinued under the management of the potential participant's non-study prescriber for 3 months before evaluation for eligibility and participation in subsequent experimental procedures.

2. <u>On the mornings of the PK-1, PK-2, Expt. 1 or Expt. 2 experimental procedures</u>:

a) Use of any medications or substances that may increase the risk of IV Allo side effects or adversely affect the experimental results (indicated by report, urine toxicology or cotinine testing, or saliva alcohol testing) will be exclusionary.
b) Systemic hormone therapy or contraception will be exclusionary [Exception: hormonal IUDs (e.g., Mirena, Kyleena, Liletta, and Skyla) or other contraceptive devices (e.g., Nuvaring)] will be allowed if the participant still has normal menstrual periods and is found to ovulate using commercial urine test kits provided by study).

- Pregnancy (urine pregnancy tests given at each in-person session).
- Breast-feeding.
- Unable to tolerate IV placement or blood drawing by needle stick.
- Wear hearing aid(s) (For Expt. 1 and 2, <u>not</u> PK studies).
- Fail hearing test (For Expt. 1 and 2, <u>not</u> PK studies).

8 STUDY INTERVENTION

The investigation focuses on the effects of single infusions of IV Allo vs. placebo on three neural processes thought to underlie PTSD recovery: a) conditioned fear extinction retention (Expt. 1), b) conditioned fear reconsolidation blockade (Expt. 2), and c) non-aversive episodic memory (Expt. 1 and Expt. 2).

8.1 BEHAVIORAL INTERVENTION

Participants will be individuals with chronic PTSD recruited through Boston University Medical Campus (BUMC)/Boston Medical Center (BMC) (Site 1), and Wayne State University (WSU) in Detroit (Site 2). At BUMC, eligibility evaluations will take place at the GCRU. The 3-day series of psychophysiology tests of extinction retention (Expt. 1) and reconsolidation blockade (Expt. 2), as well as non-aversive episodic memory (Expt. 1 and 2) will take place at BMC (to afford adequate safety/access to emergency services during the IV Allo vs. placebo infusions; in addition, all 3 days of the fear conditioning psychophysiology paradigm must be conducted in the same context. The infusions for the PK studies will take place at BMC; the Day 2 follow-up visit will take place in the same context in which the non-aversive episodic

memory testing occurred on Day 1. See details below under section 9 “Study Procedures”. At WSU, the eligibility screening evaluations, 2-day PK studies, and the 3-day fear condition psychophysiology paradigms will take place at the CRSC.

8.2 STUDY DRUG: IV ALLOPREGNANOLONE AND PLACEBO INTERVENTION

8.2.1 Manufacture, Shipping, Storage & Dispensing

As noted under section 2.0 Protocol Summary: IV Allo and matching IV Placebo will be provided by the UC Davis GMP Laboratory via arrangements made with Co-Investigator, Michael Rogawski, MD, PhD (UC Davis site-PI). The UC Davis product is a 2 mg/mL Allo concentrate with 24% sulfobutyl ether-β-cyclodextrin sodium salt, USP (Dexolve; CycloLab, Budapest, Hungary) in 0.9% saline for injection. Prior to shipping to the BMC and WSU CRSC Investigational Pharmacy Services (IPS), the Allo or matching placebo will be diluted 4-fold with 0.9% saline at the UC Davis GMP laboratory to give a final Allo concentration of 0.5 mg/mL with a final 6% Dexolve concentration for active drug or final 6% Dexolve concentration for matching placebo.

Both investigational drug products (IV Allo and placebo) manufactured at the UC Davis GMP Laboratory will be packaged in a flexible, plastic single-use IV container (bag). When stored in the bags, the finished drug products are certified for 5-month (150-day) sterility and stability when stored at 2°- 8°C. The products will be marked with a beyond-use date (BUD) 150 days following the manufacture (i.e., compounding). The investigational drug products will be delivered in temperature-controlled shipping containers by overnight courier to the BMC and WSU CRSC IPS. Product solutions will be stored at 2°-8°C at the BMC and WSU CRSC IPS until used. Note that the investigational drugs will not be prepared or altered in any way after shipment by the supplier. They will simply be stored by the BMC or WSU CRSC IPS and dispensed per the randomization schedule for IV administration according to the dosing protocols for Expt. 1 and Expt. 2 (below).

Boston Site: Nursing staff responsible for destroying any investigational drug remaining after administration to the participant will be blind as to whether the dispensed drug was the schedule IV active Allo product or placebo. Therefore, nursing staff will destroy any investigational product remaining after administration to the participant per nursing policy for the waste of controlled substances. Nursing will document the waste of IV allopregnanolone or placebo solution in the Record of Destruction of Clinical Product Log.

Following dispensing, if the IV allopregnanolone or placebo infusion bag is not administered to the subject, it will be returned to IPS for destruction. Therefore, any waste will be destroyed in accordance with DEA regulations and nursing policy/procedures for C-IV controlled substances. Any unused or expired IV allopregnanolone bags still stored in the IPS will be destroyed on-site in accordance with the DEA regulations per the IPS destruction SOP. Any unused or expired matching placebo still stored in the IPS will be destroyed via the IPS vendor Clean Harbors per the IPS SOP.

Detroit site: CRSC nursing staff will document hand-off of any remaining dispensed study drug (IV Allopregnanolone or Placebo) to the WSU CRSC research pharmacist for destruction on the study visit session flowsheets. The WSU CRSC research pharmacist then will destroy the study drug in accordance with DEA regulations for C-IV controlled substances and document destruction on the Record of Destruction of Clinical Product Log.

Any unused or expired IV allopregnanolone bags still stored in the WSU CRSC Research Pharmacy will be destroyed using a reverse distributor licensed with the DEA per Standard Operating Procedures (SOPs). Unused or expired matching placebo still stored in the WSU CRSC Research Pharmacy will be destroyed per site SOPs.

8.2.2 Investigational Drug Product Label

Investigational product shipped from UC Davis to IPS will have the following label with a tear-off part indicating whether the product is active allopregnanolone or placebo. The tear-off part of the label for the active IV Allo product will indicate that the product is a DEA schedule C-IV drug. IPS will remove the tear-off labels when the infusion bags are dispensed by IPS staff for administration to study participants.

I.V. Bag #:_____ Subject #: _____

Allopregnanolone 0.5mg/mL or Placebo C-IV
in 0.9% sodium chloride injection, USP

Total Volume: 50 mL

Beyond-Use Date (BUD): _____/____/_____

Protocol Name: FACILITATION OF EXTINCTION RETENTION AND RECONSOLIDATION BLOCKADE BY IV ALLOPREGNANOLONE IN PTSD
Reference: IND 152,138
Store at 2–8°C; dispense at room temperature.
For intravenous use only.
Caution: New Drug – Limited by Federal Law to Investigational Use
Manufactured by University of California, Davis

8.2.3 Dosing Protocols

I. Expt. 1. Effects of IV Allo vs. Placebo on Extinction Retention: The dose of IV Allo to be administered targets normal luteal phase *resting* levels of Allo + PA (the natural equipotent stereoisomer of Allo) previously determined by gas chromatography, mass spectrometry in a published study by our group (Pineles et al., 2020). The anticipated (target) plasma Allo levels to be reached for each of the study groups (men, eFP women, mLP women) in response to the initial 5-minute bolus and sustained over the following 4-5 hour period of memory consolidation follow:

A. Target Plasma Allo+ PA Levels in Participants Randomized to IV Allo

Early Follicular Phase (eFP) Females. Endogenous resting (Allo+PA) levels in eFol phase women ranged from ~10 pg/ml to 467 pg/ml. If we raise endogenous (Allo+PA) levels of this group by 1500 pg/ml after extinction training, post-training resting levels are expected to range from ~ 1510 to 1967 pg/ml.

Mid-Luteal Phase (mLP) Females. In our previous studies (Pineles et al., 2018; 2020), optimum extinction retention was observed in 4 of 9 mLut phase participants with natural resting (Allo + PA) levels above 1500 pg/ml. The lowest resting value was 732 pg/ml and the highest was 2100 pg/ml. We therefore plan to raise endogenous resting (Allo+PA) levels after extinction training by 1500 pg/ml Allo to 'optimized' resting Allo ranges of ~2232 pg/ml (lowest) to ~3600 pg/ml (highest) during the post-extinction 1-5 hour window of memory consolidation.

Men. In our recently completed study of CSF and plasma (Allo+PA) levels in trauma-exposed men with and without PTSD (Rasmusson et al., 2019), endogenous plasma (Allo+PA) levels in men with PTSD after

60 minutes of rest ranged from 40 pg/ml to 905 pg/ml. If we raise plasma levels of (Allo+PA) in men with PTSD by 1500 pg/ml after extinction training, post-training resting levels would range from ~1549 pg/ml to 1900 pg/ml—a range similar to that expected in eFol phase women and associated in mLut phase women with optimum extinction retention. Further evidence for optimization of brain function at this level of Allo is provided by a study of *young healthy men* by Sripada et al. (2013). In that study, pregnenolone (a precursor for Allo and PA) was administered 60-min before an emotion regulation task performed during fMRI. This dose of pregnenolone raised serum (Allo+ PA) levels from an average of 575 pg/ml to 1320 pg/ml (range: 545 pg/ml to ~2060 pg/ml) in association with improvement in mean emotion regulation performance during exposure to aversive stimuli.

B. Calculation of Allo dosing based on PK data (provided by Co-I M. Rogawski):

The following yields the size **bolus** needed to raise plasma Allo levels by 1500 pg/ml or 1.5 ng/ml: Bolus dose=C0*Vd=(1.5 ng/ml)*(1,110 ml/kg)= 1665 ng/kg = 1.7 mcg/kg. The following yields the **infusion rate** needed to maintain the increased resting plasma levels at > 1500 pg/ml or 1.5 ng/ml. According to Dr. Rogawski, Allo infusion kinetics are linear. As 86 mcg/kg/hr results in a plasma level of 50 ng/ml, a plasma level of 1.5 ng/ml will result from an infusion of 2.6 mcg/kg/hr [i.e., 86 ug/kg/hr divided by 50 ng/ml multiplied by 1.5 ng/ml = 2.6 mcg/kg/hr. Note: C0=concentration; Vd=volume of distribution.

II. Expt 2. Effects of IV Allo vs. Placebo on Reconsolidation Blockade: The substantially higher level of IV Allo to be administered over 30 minutes immediately after reactivation of the fear memory (and then stopped) is intended to disrupt reconsolidation during the 1-hour reconsolidation window wherein this has previously been shown to be possible) via positive modulatory effects of Allo at $GABA_A$ receptors and negative modulatory effects of Allo-S at neuronal NMDA receptors—while minimizing risks to the participant typically associated with higher doses of IV Allo given in the postpartum depression studies.

A. Target Plasma Allo+ PA Levels in Participants Randomized to IV Allo:

We thus chose to target a plasma level of 25 ng/ml (or 25,000 pg/ml), which approximates Allo+PA levels measured by GC-MS between 28 and 40 weeks of pregnancy (Parizek et al., 2005, Hill et al., 2007, Gilbert Evans et al., 2005). This plasma level is about 10 times higher than average Allo+PA levels in the mid-luteal phase of the menstrual cycle. Based on the findings of Timby et al. (2006), plasma Allo+PA levels are expected to fall by half over the hour after the 30-min bolus and continue to fall to reach resting baseline levels 4-5 hours after the bolus.

B. Calculation of Allo dosing based on PK data (provided by Co-I M. Rogawski):
The following yields the size **bolus** needed to raise plasma Allo levels to ~25 ng/ml: Bolus dose=C0*Vd= (25 ng/ml)*(1,110 ml/kg) = 27,750 ng/kg = 28 mcg/kg. Note: C0=concentration; Vd=volume of distribution.

9 STUDY PROCEDURES

9.1 SCHEDULE OF EVENTS

Please the Appendix.

9.2 STUDY TIMELINE

	Year 1				Year 2				Year 3				Year 4				Year 5			
Quarter	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Start of Quarter	May 1	Aug 1	Nov 1	Feb 1	May 1	Aug 1	Nov 1	JFeb1	May 1	Aug 1	Nov 1	Jfeb1	May 1	Aug 1	Nov 1	Jfeb1	May 1	Aug 1	Nov 1	Feb 1
Study Prep																				
Recruitment																				
Expt. 1																				
Expt. 2																				
Non-aversive memory study																				
Data analysis																				
Dissemination of Results																				

a) Expt. 1 PK testing: 2/2022 (at BU site) and 6/2022 (at WSU site)

b) Expt. 1 full protocol (both sites): 4/2022 (at WSU site) or 5/2022 (at BU site: depending on completion of the BMC clinical space renovation)

c) Expt. 2 PK testing (both sites): 3/2023 or 3/2022 (depending on completion of the BMC clinical space renovation)

d) Expt. 2 full protocol (both sites): 5/2023

9.3 SERIES OF GENERAL STUDY PROCEDURES

1. Self or other referral of potential participants who may be a good fit for the study (specific methods to be described in a future amendment).
2. IRB-approved in-person or telephone pre-screening of potential participants who have indicated interest in participation in the study. The general rationale and procedures involved in the study will be explained in addition to general inclusion and exclusion criteria, so that the participant can decide whether he/she/they are interested in participating in the more detailed remote (or in-person when allowed) informed consent process.

3. Screening Evaluation following remote or in-person informed consent depending on whether remote or in-person psychological screening or in-person medical evaluation is done first.
   (a) remote or in-person psychological screening evaluation for eligibility (see below for details).
   (b) in-person medical screening evaluation with medical history, physical exam, EKG, and laboratory testing for eligibility, as well as neurocognitive testing.
4. Prior to beginning Expt. 1 and Expt. 2, perform PK study to confirm IV Allo dosing is accurate. Participants in PK testing will not be allowed to participate in Expt. 1 or Expt. 2 procedures.
5. If eligible, schedule for Day 1, 2, and 3 Expt. 1 or Expt. 2 procedures. These days are contiguous. Females are assigned to perform procedures in either the eFP or mLP of the menstrual cycle (procedures below). If these procedures are not performed within 3 months of the screening medical examination, the screening medical examination and laboratory testing must be repeated. Under any circumstances, a medical review of systems (ROS) is repeated on each day of the research procedures to ensure that the individual remains well (and thus eligible for continued participation) and to monitor for any potential side-effects from the IV Allo vs. placebo infusion.
6. As needed visits to the BUMC GCRU or WSU CRSC: An in-person medical/neurological exam, EKG, and laboratory tests or psychological evaluation (remote or in-person) will be repeated, as indicated, for purposes of establishing safety and continued eligibility should the participant exhibit or report a change in health status.
7. One-week follow-up telephone/zoom call and discharge from the study as long as no further safety monitoring is indicated.

9.4 STUDY PROCEDURE DETAILS

IV.4.7 Recruitment

BUMC/BMC:
In general, participants will be recruited by IRB-approved posted advertisements or pamphlets placed on bulletin boards, in print media, at universities or community colleges, on the internet, on transportation routes and vehicles) or by scripts prepared for radio or social media. The Boston University affiliated research participant recruitment service www.researchmatch.org may be utilized to distribute IRB-approved flyers and text advertisements to potentially eligible participants. Since the clinical trial is taking place at the GCRU and BMC, recruitment efforts will target individuals who have utilized or requested services through BMC (including, but not limited to) Departments of Psychiatry, Obstetrics and Gynecology, and Primary Care affiliated units and centers including Behavioral Health Outpatient Services, the Boston Emergency Services Team, the Boston Center for Refugee Health and Human Rights, the Center for Multicultural Mental Health, the Psychiatry Consult and Liaison Service, Psychiatric Emergency Services, Psychiatric Neuropsychology, Psychiatric Psychology, Family Medicine and Internal Medicine. Recruitment efforts may also target individuals who utilize services at St. Elizabeth's Medical Center, which is a Boston University affiliated teaching hospital. To recruit participants from BMC and St. Elizabeth's Medical Center, study personnel may post IRB-approved flyers and text advertisements at these sites with the permission of unit supervisors and throughout the hospital as permitted. Study personnel may also set up information booths at recruitment BMC sites periodically and in BMC and BUMC lobbies (if COVID-19 measures allow) to provide information to interested patients and answer questions regarding the clinical trial. Potentially interested participants will be instructed in the advertisements to call study personnel for more information or directed to scan a QR code or click on a

URL link that will take them to a REDCap contact management database where they can enter their contact information for contact by study personnel at a later time. Study personnel will then arrange a time for the Pre-Screening via text sent through Microsoft Teams, telephone, or email.

A specialized participant recruitment service, Trialfacts, also may be utilized to distribute IRB-approved advertisements to potential participants. Trialfacts combines extensive marketing and advertising expertise with clinical trial experience to create effective marketing solutions for participant recruitment while adhering to GCP and IRB requirements. Potential participants can choose to click on a social media, search, or display advertisement that takes them to the Trialfacts landing page for this study. Potential participants can then choose to enter their contact details and complete an online pre-screening questionnaire (included in content) to determine whether they are eligible to continue to a phone screening. If eligible, potential participants then can book an appointment time for phone screening with research staff. If ineligible, potential participants can contact Trialfacts for further information on the study. TrialFacts will provide the contact information and screening responses for those who pass the online screening questionnaire. The research team will not retain the online screening questionnaire responses for anyone, regardless of whether they end up qualifying for the study after the pre-screening.

A specialized participant recruitment service, BuildClinical, also may be utilized to distribute IRB-approved advertisements to potential participants. BuildClinical is a data-driven platform that helps academic researchers recruit participants for research studies more efficiently using social media, software, and machine learning. BuildClinical adheres to all the appropriate GCP and IRB guidelines and procedures. BuildClinical utilizes study-specific advertisements to engage potential participants on digital platforms such as Facebook, Google, WebMD, etc. and redirect them to a study-specific landing page should the individual click on it. On the landing page, the person can complete an online pre-screening questionnaire that gets routed into BuildClinical's platform. BuildClinical's Secure Socket Layer (SSL) software encrypts all inputted information and keeps information private and HIPAA compliant. The backend servers are stored in the U.S. at some of the most secure data centers in the world. BuildClinical will provide the contact information and screening responses for those who pass the online screening questionnaire to the study staff. Potentially eligible participants then will be contacted for phone pre-screening with research staff.

Study personnel may also use the "Epic" electronic medical record system to generate a list of potential participants between 18 to 55 years of age who receive medical care at BMC and have a diagnosis of acute stress disorder or PTSD, are not on any prohibited medications, and do not have any exclusionary conditions per the medical chart. Study personnel will reach out to discuss the clinical trial with physicians and clinicians who have potential participants generated on the EPIC list. A letter will be provided to the physicians and clinicians that briefly describes the study and broad eligibility criteria. We will provide physicians and clinicians with recruitment materials to give to interested individuals who may then contact study personnel for more information about the clinical trial. The list of upcoming appointments for potential participants will be regularly updated. This list will be kept in a secure private, password-protected BU/BMC BOX, which is HIPAA compliant and will only be accessed by study personnel. Study personnel will contact the potential participant's provider about any upcoming appointments, determine whether the provider thinks the individual may be a good fit for the study, and see if the provider is willing to introduce the study to the potential participant at the end of the appointment.

Study personnel may post the clinical trial information on the BMC StudyFinder website and will post on clinicaltrials.gov. The research team may also describe this initiative in the monthly Department of Psychiatry newsletter.

Study personnel will reach out to Massachusetts Veteran Service Officers caring for veterans and their families to discuss the clinical trial. A letter will be provided to the Veteran Service Officers that briefly describes the study and broad eligibility criteria. Veteran Service Officers will be provided with IRB-approved advertisements to distribute to potential participants.

The Psychiatry Research Opportunities for Volunteer Enrollment (PROVEn) registry (H-35897) may also be utilized. PROVEN is a registry of individuals with diverse psychiatric conditions who have expressed interest in taking part in research activities at BUMC. Individuals who consented to enter the PROVEn registry and who meet broad eligibility requirements will be contacted by the study team via telephone or secure email with opportunities to participate in this clinical trial.

Recruitment Procedures During COVID-19 Pandemic while In-Person Research is Limited:
While in-person research and recruitment are limited at BUMC and BMC, recruitment may occur via the posting of advertisements in areas where permitted and by the virtual recruitment approaches described above. At the beginning of recruitment, study staff may send letters to patients who have acute stress disorder or PTSD, have received care at BMC since January 1, 2011, and are potentially eligible (based on the inclusion criteria stated above). Subsequently, each week study staff may send letters to all new potentially eligible patients who have not already received a recruitment letter. Letters will describe the study, broad eligibility criteria, provide study contact information if patients want to participate, and state that a follow-up phone call will occur 1-2 weeks later, unless the potential participant lets us know by phone or mail that they are not interested in participating in the study. Study personnel may call individuals who do not contact us to inquire about their interest in the study and then arrange a time for the Pre-Screening if the individual is interested.

WSU:
In general, participants will be recruited by IRB-approved posted advertisements or pamphlets placed on bulletin boards, in print media, at universities or community colleges, on the internet, on transportation routes and vehicles (near hospital) or by scripts prepared for radio or social media. The Boston University affiliated research participant recruitment service www.researchmatch.org also may be utilized to distribute IRB-approved flyers and text advertisements to potentially eligible participants. Study personnel will post the clinical trial information on clinicaltrials.gov.

Recruitment efforts out of the Beaumont system may take place in the following locations (including but not limited to): Royal Oak, Troy, Wayne, Dearborn, Farmington Hills, Grosse Point, and Canton.

Participants may be recruited by flyers provided to patients via clinicians and posted inside the hospital on the appropriate floors and elevators. Flyers may also be posted in the University of Michigan Hospital in Ann Arbor, Henry Ford Hospital in Detroit, and the Ann Arbor VA.

WSU has also partnered with University of Michigan (UM) to post flyers on their UM Health Research portal, which currently has over 60,000 people registered on it. Therefore, UM Health Research portal may be utilized for recruitment purposes. Their site will build the study into the portal and all contacts will be forwarded to the WSU study team. UM IRB has already approved the recruitment plan and procedures.

Study personnel may also post the clinical trial information on WSU's Academica site and the Department of Psychiatry at the WSU School of Medicine.

A specialized participant recruitment service, BuildClinical, also may be utilized to distribute IRB-approved advertisements to potential participants. BuildClinical is a data-driven platform that helps academic researchers recruit participants for research studies more efficiently using social media, software, and machine learning. BuildClinical adheres to all the appropriate GCP and IRB guidelines and procedures. BuildClinical utilizes study-specific advertisements to engage potential participants on digital platforms such as Facebook, Google, WebMD, etc. and redirect them to a study-specific landing page should the individual click on it. On the landing page, the person can complete an online pre-screening questionnaire that gets routed into BuildClinical's platform. BuildClinical's Secure Socket Layer (SSL) software encrypts all inputted information and keeps information private and HIPAA compliant. The backend servers are stored in the U.S. at some of the most secure data centers in the world. BuildClinical will provide the contact information and screening responses for those who pass the online screening questionnaire to the study staff. Potentially eligible participants then will be contacted for phone pre-screening with research staff.

Potentially interested participants will be instructed in the advertisements to call study personnel for more information or to scan a QR code or click on a URL link that will take them to a Qualtrics contact management database where they can enter their contact information for contact by study personnel at a later time. Study personnel will then arrange a time for the Pre-Screening via text sent through Microsoft Teams, telephone, or email. Information from the Qualtrics contact management database may also be stored in Ripple, which is a HIPAA compliant research management software.

9.4.2 In-Person or Telephone Pre-Screening

The IRB-approved in-person or telephone pre-screening will take approximately 15 to 20 minutes and will be used to broadly evaluate the potential participants' eligibility. Potential participants will be a) asked some specific questions about their psychiatric and medical health history, b) read broad eligibility criteria, and c) asked whether or not they think they might be eligible. If conducted in-person, potential participants will be given the option to discuss the pre-screening in a private office or private clinical laboratory space or complete the questionnaire, which contains sensitive information, in a semi-public space. If conducted in-person in a semi-public space, individuals will be asked to read and fill in their answers to the pre-screening questions on a piece of paper. The individual will be told that if they have questions, they might be reluctant to ask in the semi-public space, they can arrange then to meet instead with the study staff in a private office or clinical laboratory space or complete the questionnaire by phone. If they do appear to meet the broad requirements, they will be scheduled for either the telephone/zoom or in-person informed consent process and subsequent psychological screening or the in-person informed consent process and subsequent medical screening, depending on the individual's schedule or transportation availability and the availability of research personnel. To reduce risks to confidentiality associated with the pre-screening, answers to the prescreening questionnaire will be shredded or destroyed if the individual is not eligible or ultimately chooses not to participate in the study. If an individual ultimately qualifies for participation in the study, the prescreening questionnaire will become part of the participant's research record and will only be identifiable by the participant's study ID number.

9.4.3 In-Person or Telephone/Zoom Informed Consent Process

Prior to completing any study procedures (except IRB-approved pre-screening following verbal assent), the participant will undergo the informed consent process. Individuals conducting the informed consent

process (research coordinator, co-investigator, MD, DO, NP, PA) will be trained by the PI and monitored over the course of the first several sessions to ensure adequacy of training. If non-licensed independent practitioners are involved in conducting the informed consent process, they will introduce the study, review the informed consent form, and give participants time to think about participating in the study. A licensed independent practitioner then will be brought in to review and discuss the purpose, risks, benefits, and alternatives of the study, answer any questions, and sign the informed consent form. If consenting is done remotely (e.g., prior to the remote/in-person psychological screening evaluation if that is done first), study staff will text or email the participant either a password-protected DocuSign link or a REDCAP link to the consent form which contains a written explanation of the study and the associated risks and benefits for the participant to review. DocuSign will be used initially because it is FDA Part 11 Compliant. BUMC REDCAP will only be used once it is validated as FDA Part 11 Compliant. IRB-approved personnel administering consent will be available for spontaneous questions. Then, the member of the study team will verbally explain the purpose of the study, general procedures and timeline, and the risks and benefits associated with participation again. Participants will be given an opportunity and will be encouraged to ask questions; they will be referred to the PI as appropriate to answer questions about procedures or medical/psychological safety risks. The participant will be asked questions to ensure inclusion and comprehension of the study procedures along with risks and benefits. Participants also will be advised that they can stop participating at any time if they become too uncomfortable and that withdrawal from the study will not affect their healthcare. They will be advised that the study does not provide treatment per se for PTSD, and that if they desire, they can be informed of clinical treatments available for PTSD or any conditions discovered and for which they may not yet be in treatment. In addition, participants will be informed that if they are found to be at imminent risk to self or others, they will be referred for further psychiatric evaluation and intervention to an emergency or urgent care center as appropriate (against their will if necessary). If unsure of interest, they will be advised to query family or friends or others before signing forms indicating comprehension of the study and interest in participating. If interested, individuals will electronically sign the consent form via REDCAP or DocuSign (see consent procedures); the form will be co-signed by the person obtaining consent. Participants will receive a copy of the signed consent form and Health Insurance Portability and Accountability Act (HIPAA) authorization/revocation form for their records. The participant also will be given explicit instructions about how to contact researchers should the participant decide against allowing use of already collected but not yet analyzed genetic samples. Participants will receive a copy of the signed consent form for their records by email, mail or in person, as desired.

9.4.4 Telephone/Zoom or In-person Psychological Screening Evaluation

If the psychological screening evaluation is done first, it will be conducted either immediately after informed consent (during the same telephone/Zoom or in-person session), or if preferred, during a subsequent remote or in-person session. If in-person, participants will be reminded of the evaluation scheduled at the BUMC GCRU or WSU CRSC a few days ahead and again the day before the evaluation to minimize no-shows. Results of the evaluations will be used to ascertain demographic information and diagnoses bearing on inclusion and exclusion. This visit may be broken into two sessions if needed given the administration time of these assessments can vary significantly depending on the complexity of the psychiatric history of an individual. The neuropsychiatric assessments administered over the telephone or Zoom or in-person will be audio recorded for inter-rater reliability. Altogether the assessments will take approximately 3½ hours to complete. The clinician-administered assessments include the CAPS-5 for DSM-5 (Weathers et al., 2013a), the Structured Clinical Interview for DSM-5 Axis I Disorders (SCID-5; First et al., 2014), and the CSSRS (Oquendo et al., 2003). PTSD will be diagnosed with the CAPS-5, which is the current gold standard interview for PTSD assessment. The CAPS-5 takes approximately 45 to 60

minutes to administer. This 30-item structured interview yields a dichotomous diagnosis of PTSD and a continuous score of severity for each symptom. The CAPS-5 is currently being validated, but the previous CAPS-IV version demonstrated excellent sensitivity (.81) and specificity (.95). Raters will be trained to criterion on the CAPS-5 by a certified expert CAPS-5 trainer, who will also ensure continued inter-rater reliability across both experiments in the study. PTSD-comorbid diagnoses and exclusionary diagnoses will be ascertained with the SCID-5 research version. The SCID-5 takes approximately 45 to 90 minutes to complete depending on the complexity of the individual's psychiatric history. Since we are using the CAPS-5 for diagnosis, the SCID-5 PTSD section will be excluded. Additionally, most individuals with exclusionary diagnoses, such as schizophrenia, bipolar I disorder, and/or alcohol or substance use disorders should already have been excluded based on information obtained via by our recruitment efforts and pre-screening questionnaire. For these reasons, the SCID-5 is likely to only take 45 to 60 minutes to complete. The CSSRS assesses past and current risk to self/others and is currently recommended for use in clinical psychiatry treatment trials. Assessment time can vary depending on an individual's history or current situation with regard to such risks, but typically takes about 5 minutes to complete.

Participants also will complete the following online REDCap self-assessments: Life Events Checklist (LEC-5) for DSM-5 (Weathers et al., 2013b), Clinician Administered Dissociative States Scale (CADSS) (Bremner et al., 1998), 12 item Short Form Health Survey (Ware et al., 1996), Boston Assessment of TBI-Lifetime (BAT-L; Fortier et al., 2014), Patient Health Questionnaire (PHQ)-9 (Spitzer et al., 1999), PTSD Checklist for DSM-5 (PCL-5; Blevins et al., 2015; Bovin et al., 2015; Wortman et al., 2016), Insomnia Severity Index (ISI; Morin et al., 2011), and the Positive and Negative Affect Schedule (PANAS) (Watson and Clark, 1988). The State-Trait Anger Expression Inventory-2 (STAXI-2) (Lievaart et al., 2016) will be completed via PARiConnect. If individuals do not have access to a computer or smartphone, these assessments will be administered over the telephone or Zoom or in-person by the research team. The LEC-5 takes approximately 10 to 20 minutes to complete and will be used to identify the DSM-5 Criterion A traumatic event for the CAPS-5 interview. The Clinician Administered Dissociative States Scale (CADSS) will be used to measure level of dissociation (Bremner et al., 1998) and takes approximately 10 minutes to complete. The BAT-L survey will assess lifetime blunt/blast head trauma. Time to administer the BAT-L varies depending on the number of TBIs, but we expect the survey to take 5 to 10 minutes. Again, most potential participants with a history of moderate to severe TBI should have been excluded prior to the telephone/zoom or in-person psychological screening evaluation, thus reducing the duration of the BAT-L. The Short Form Health Survey, PHQ-9, PCL-5, ISI, PANAS, and STAXI-2 will take approximately 40 minutes total (Short Form Health Survey: 5 min; PHQ-9: 5 min; PCL-5: 10 min; ISI: 5 min; PANAS: 5 min; STAXI-2: 10 min) and will be used to assess general health, depression severity, PTSD severity, functional sleep disturbance, affect, and trait anger, respectively. If participants continue to meet eligibility criteria after the neuropsychiatric assessments, an in-person screening evaluation at the BUMC GCRU or WSU CRSC and 3-day experimental psychophysiology and memory testing sessions will be scheduled as appropriate.

9.4.5 In-Person Screening Evaluation: Medical Examination and Cognitive Assessment

Medical Exam: Participants will be reminded of the scheduled in-person medical screening evaluation at the BUMC GCRU or WSU CRSC a few days ahead and again the day before the evaluation to minimize no-shows. If done before the psychological screening evaluation, informed consent will be conducted first (as described above). The medical evaluation will take about 2½ hours to complete and will determine whether the participant has any medical conditions that would: a) make it potentially unsafe to participate (e.g., end stage renal disease), or b) interfere with interpretation of the research neurosteroid or psychophysiological measurements. A review of medical history, review of systems, and

a physical/neurological examination will be conducted by a study MD or NP and take ~45 minutes to complete.

A certified BUMC GCRU or WSU CRSC nurse will obtain an EKG and perform pulse oximetry. Certified BUMC GCRU or WSU CRSC nurses or other qualified phlebotomists will draw blood for medical testing. Certified nurses or other qualified personnel will also collect saliva for an alcohol test and urine for a) clinical urinalysis, b) pregnancy testing in females, and c) urine toxicology (see section **9.4.17 Urine Toxicology for Illicit Drugs and Cotinine** below for specific tests to be performed). Blood drawing, saliva collection, urine collection, and the EKG with pulse oximetry will take about 30-45 minutes to complete.

Any women positive for unsuspected pregnancy will be referred, if desired, for follow-up by the individual's medical provider. The saliva alcohol test strip and blood GGT test results will be used to ascertain acute and somewhat more remote alcohol use, respectively. If a participant is acutely intoxicated, the individual will be evaluated for safety to leave according to current state law. Individuals with potential substance use problems will be referred for substance use disorder treatment if desired. Medical blood tests will include but are not limited to (additional tests may be necessary to clarify eligibility): a complete blood count and differential (with other pertinent follow-up tests such as those for ferritin, vitamin B12 or folate if indicated), diabetes (glucose and hemoglobin A1c [HbA1c]), inflammatory (CRP), and coagulation measures (prothrombin time [PT] and partial thromboplastin time [PTT]), cholesterol (total), lipids (low-density lipoproteins [LDL], high-density lipoproteins [HDL], triglycerides), and thyroid (thyroxine [T4], thyroid stimulating hormone [TSH]), kidney (electrolytes [potassium, sodium, chloride, and carbon dioxide], blood urea nitrogen [BUN], creatinine), and liver function tests (aspartate aminotransferase [AST], alanine amino transferase [ALT], GGT). Participants will be advised that some tests may need to be repeated to a) ensure accuracy, or b) if abnormal, to confirm or reevaluate if reflective of a transient condition (e.g., urinary tract infection). If so, subjects will be asked to return to the BUMC GCRU or WSU CRSC for the repeat test and will be paid for that visit; see Payment section below). Please note that at the BUMC/BMC site, the urine fentanyl and medical blood test results <u>will</u> be put in participant's medical chart because these tests are conducted at the BMC clinical laboratory. At the WSU site, participants will place their initials on the ICF indicating their decision as to whether or not the results of their medical blood tests can be put in their medical chart. However, at the WSU site, urine fentanyl test results will <u>not</u> be put in the participant's medical chart. The results of the saliva alcohol test, urinalysis, and urine toxicology (except fentanyl), cotinine and pregnancy tests will <u>not</u> be put in the participant's medical chart at either the BUMC/BMC or WSU site.

Color Vision and Hearing Tests: A color vision test and hearing test will be conducted by the research coordinator and/or research assistant. The Waggoner Computerized Color Vision Test (WCCVT) will be completed on a study staff computer via the downloaded WCCVT software. Non-aversive memory test data from color deficient participants will not be included in analyses. The participant must be able to hear 1) 1000 Hz and 2000 Hz and 2) either 500 Hz or 4000 Hz in both ears at less than or equal to 35 decibels on the hearing test to participate in Expt. 1 or 2. Individuals participating in the PK studies will not be given the hearing test as these individuals are not participating in the psychophysiology procedures.

Cognitive Assessment: The following cognitive tests from the NIH Toolbox will be administered by computer: Flanker task, Picture Sequence Memory, List Sorting Working Memory, Dimensional Card Sort, Pattern Comparison Processing Speed. This will take ~30 minutes to complete. The Rey Auditory Verbal Learning test (RAVLT) also will be administered given recent evidence that delayed verbal memory may help define a phenotype with poor response to prolonged exposure treatment (Etkin et al., 2019). The RAVLT takes ~ 10 to 15 minutes to complete with a 30-minute delay between administration of immediate and delayed recall.

Participants who continue to meet eligibility criteria after the in-person medical screening evaluation will be scheduled for the telephone/Zoom or in-person psychological screening evaluation (if not already completed) or the 3-day experimental psychophysiology and memory tests, as appropriate.

9.4.6 Randomization to IV Allo or Placebo

Participants eligible for the 3 days of startle testing will be randomized to receive either IV Allo or placebo on the second day of testing. Neither the participant nor the researchers will know which study drug (IV Allo or placebo) the participant receives. If there is a need to know at any point for safety reasons, the research pharmacy will provide the information. See Section 5.2 for the methods.

9.4.7 Scheduling Eligible Participants for the 3-Day Psychophysiology and Memory Experiments

IV. General

Eligible participants will be scheduled for standardized tests of aversive and non-aversive learning and memory testing 3 days in a row in the same context at BMC or WSU to evaluate effects of IV Allo vs. placebo administration on extinction retention (Expt. 1) (Pineles et al., 2016) and reconsolidation blockade (Expt. 2) (Elsey et al., 2018). Participants must be scheduled for Day 1 of the 3-day paradigm ≤ 90 days of both the remote/in-person psychological screening evaluation and in-person medical/cognitive screening evaluation; out-of-window screening evaluations must be repeated prior to the Day 1 procedures to ensure continued eligibility.

Due to known menstrual phase effects on Allo levels, extinction retention, and the relationship between Allo and extinction retention, potential effects of IV Allo in women with PTSD will be tested in separate groups in the eFol and mLut phases of the menstrual cycle. Prior to each Expt., a PK study will be conducted to confirm the accuracy of dosing.

II. Number of subjects

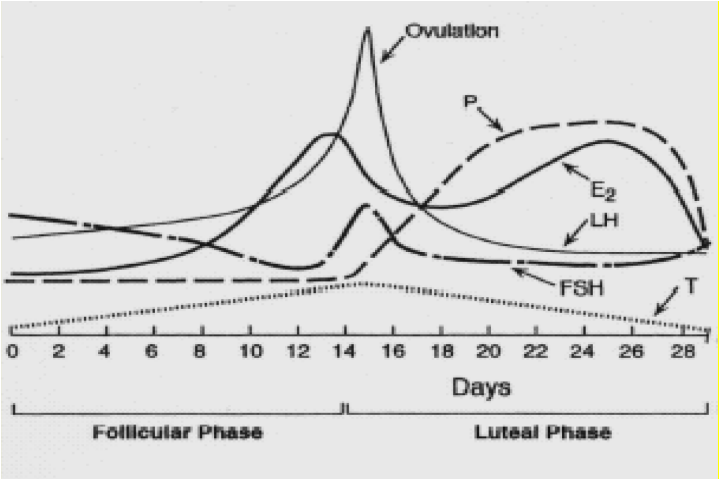
For each experiment (1 and 2), 128 participants will complete the 3-day assessments—about half (n=64) in Boston and half (n=64) in Detroit, for a total of 256 participants. At each site, for each experiment (1 and 2), ~22 participants will be men, ~21 will be women in the eFol phase and ~21 in the mLut phase.

IV. Special Scheduling Procedures for Eligible Females

Females will be scheduled *either* during the eFP of the menstrual cycle (about 2-6 days after the menstrual period begins, though this can vary depending on the length of the menstrual cycle) or during the mLP (about 6-10 days after the luteinizing hormone or 'LH' surge, though this may vary depending on the length of their menstrual cycle). The LH surge occurs just before ovulation, about 2 weeks after the start of menstruation. Participants scheduled during the mLP will be asked to test their urine for the LH surge every morning and every afternoon or evening with a dipstick beginning 6 days after onset of menses and continuing until the dipstick is positive (usually 7-10 days later). Individuals on hormonal IUDs or a device like Nuvaring will be asked to test their urine after the LH surge with a different dipstick for pregnanediol glucuronide (PdG–the urine metabolite of progesterone), which rises in association with ovulation. This will ascertain whether the individual still ovulates and qualifies to continue in the study. Ovulation will be confirmed in all participants at the end of the study by the rise in plasma progesterone and estradiol into the normal luteal phase range at the time of testing. Data from subjects without phase-appropriate eFP and mLP progesterone levels will be excluded from analyses.

"Scheduling Exceptions for Females with Menstrual Cycles Longer than 28 Days: Please see the figure from Carr 1998 (see below) for a depiction of a standard menstrual cycle upon which the protocol

rationale for scheduling females is based. For eFP participants, the intent is study females when their estrogen levels are at their lowest before the substantial rise in estrogen preceding the LH surge. For mLP participants, the intent is to study females during the timeframe when PROG (and levels of ALLO and PA) are highest—and before the precipitous drop in these steroids at the end of the luteal phase that triggers the onset of menses and transition back to the eFP. Individuals with menstrual cycles longer than the average 28-day cycle typically have extended eFPs and/or mLPs; thus, scheduling exceptions for these participants will be allowed to maximize eligibility if scientifically reasonable. For example, the follicular and luteal phases for a female with a 32-day menstrual cycle may each last 16 days instead of 14 days. Therefore, it would be scientifically reasonable to schedule such a female in the eFP on days 2 to 8 after the start of her menses since estrogen levels would be similar to those of a female with a standard/typical 28-day menstrual cycle on days 2 to 6 after menses onset. It would also be scientifically reasonable to study this female during the mLP on days 6 to 12 after her LH surge since her PROG, ALLO, and PA levels would be similar to those of a female with a typical/standard 28-day menstrual cycle on days 6 to 10 after the LH surge. Such scheduling exceptions must be reviewed and approved by the BUMC/BMC Lead PI or designated study team member trained by that PI.



From: Carr, Bruce R. Disorders of the Ovaries and Female Reproductive Tract, Chapter 15, "Reproduction" in Williams Textbook of Endocrinology, 9th Edition, 1998, Jean Wilson, Daniel Foster, Henry Kronenberg, P. Reed Larsen (eds). W.B. Saunders Co., Philadelphia, PA, p. 769.

IV. Protection Against Pregnancy for Eligible Females

As noted under **Section 6** (Risks and Adequacy of Protection from Risks), eligible females of childbearing potential must use two highly effective birth control methods for one week before the IV Allo vs. placebo infusion and for 28 days after. Systemic hormonal contraceptives will not be allowed because they will interfere with the results of the study. As previously noted, some hormonal intrauterine devices (IUDs) (e.g., Mirena, Kyleena, Liletta, and Skyla) or other contraceptive devices (e.g., Nuvaring) will be allowed if the participant is still menstruating and ovulating, which indicates that very little of the hormones are getting into the blood. To see if the participant is ovulating, we will ask the participant to do urine dipstick testing at home for both the LH surge (as above) and the rise in PdG that follows if the individual does ovulate. Other effective contraceptive methods that the female participant or partner can use include a vasectomy, diaphragm or cervical cap with spermicide, condoms with spermicide, and the copper IUD. Females are advised that if they suspect they have become pregnant while participating in the study, they should immediately contact the study physician.

9.4.8 General Pharmacokinetic (PK) and Psychophysiology Study Procedures for Expt. 1 and Expt. 2

Prior to the initiation of Expt. 1 and Expt. 2 a PK study will be conducted to verify the PK of IV Allo doses proposed for Expt. 1 and Expt. 2. PK characterization will be performed in 2-4 participants from each of the 3 study groups (2-4 men, 2-4 eFol females, 2-4 mLut females). Participants will undergo the same pre-screening and screening evaluation procedures and must meet the same inclusion and exclusion criteria. They will not participate in psychophysiology testing but may take part in non-aversive episodic source memory testing.

I. Expt. 1: Effects of IV Allo vs. Placebo on Extinction Retention and Non-Aversive Memory Consolidation

A. General Procedures for 2-Day PK Study

1) Day 1 (about 8 ½ hours): Participants will be asked to arrive at the BUMC GCRU or WSU CRSC **by about 9:30 a.m**. Vital signs will be taken and repeated several times later during this visit. The CSSRS will be administered. Participants will be weighed, eat breakfast (protein bars, 7 kcal/kg with water) and provide a urine sample for nicotine, drug and (if eligible female) pregnancy testing. They also will provide a saliva sample for an alcohol test. If any of these tests are positive, the participant will not be able to continue in the study (please see potential effects on payment in the **"Payment"** section below). A pulse oximeter will be placed on a finger to monitor blood oxygen levels. If the participant's blood oxygenation saturation is below 96%, the participant will not be able to continue in the study. If still eligible bases on these tests, the participant will complete the first portion of the non-aversive memory test (if paradigm is ready; details below). An IV then will be placed and kept open by a slow stream of IV fluid and the participant will be accompanied to BMC. At BMC, the participant will sit at rest for about 1 hour while completing psychiatric and medical symptom ratings (see 15.1.1. Schedule of Events for PK Studies in the Appendix). The participant will complete the Monk Visual Analogue Scale (VAS) for sleepiness (Monk, 1989) and the nurse, physician or NP will complete a qualitative sedation rating (Joint Commission on Accreditation of Healthcare Organizations in the United States, 2004). Then blood will be drawn through the IV, and IV Allo will be given in a bolus at 1.7 mcg/kg over 5 minutes followed by a 2.6 mcg/kg/hr drip over the next 4-5 hours. During this time, small amounts of blood (5cc) will be drawn 8 more times through the IV. After the IV Allo infusion, a neurological exam will be performed to ensure that the participant can safely leave. As sedation is a possible side effect of IV Allo, the participant must arrange for a ride home by a friend or family member or use a transportation service (paid by the study). Participants will be reminded that they should avoid using alcohol or drugs or medications not approved by the study team the evening after the infusion.

2) Day 2 (about 3 hours): Participants will be asked to arrive at the BUMC GCRU or WSU CRSC **by about 9:30 a.m**. They will eat breakfast (protein bars, 7 kcal/kg with water), have their vital signs taken, provide a urine sample for nicotine, drug and pregnancy (if eligible female) testing, and provide a saliva sample for an alcohol test. If any of these tests are positive, the participant will not be able to continue in the study (please see potential effects on payment in the **"Payment"** section below). If the tests are negative, the participant will sit at rest for about 1 hour, participate in the second part of the non-aversive memory test (if the paradigm is ready), do psychiatric and medical symptom ratings (see 15.1.1. Schedule of Events for PK Studies in the Appendix) and have a blood draw by needle stick.

B. General Procedures for 3-Day Fear Conditioning, Extinction, & Extinction Retention Study with 1-Week Follow-Up

1) Day 1 (about 3 ½ hours): Participants will be asked to arrive at our laboratory at BMC or WSU CRSC **by about 9:30 a.m**. Vital signs (temperature, respiratory rate, heart rate and blood pressure) will be taken and may be repeated later during this visit. Participants will eat breakfast (protein bars, 7 kcal/kg with water) and provide a urine sample for nicotine, drug and pregnancy (if eligible female) testing. Participants also will provide a saliva sample for an alcohol test. If any of these tests are positive, they will not be able to continue in the study (please see potential effects on payment in the **"Payment"** section below). If these tests are negative, the participant will be prepared for *startle testing* by cleaning the skin for sensor placement below the eye, behind the ear, and on the palm, chest, stomach and/or wrist. We will then use sticky tape to attach 8 small sensors (tiny metal discs with wires): 2 below the eye, 1 behind the ear, 2 to the palm, 2 to the chest, and 1 to the stomach or wrist. The participant then will sit at rest for 1 hour and fill out ratings of psychiatric and medical symptoms (see 15.1.2. Schedule of Events for Expt. 1 and Expt. 2 in the Appendix). Severity and pain interference questions from the Brief Pain Inventory (BPI) and the sickness questionnaire (which have been shown to be associated with inflammation) will be assessed (Cleeland and Ryan, 1994; Andreasson et al., 2018). At the end of the hour, the participant's blood will be drawn, and psychophysiology testing (conditioned fear acquisition) will be carried out over 15-30 minutes (details below).

2) Day 2 (about 9 hours): Participants will be asked to arrive at our laboratory at BMC or WSU CRSC **by about 9:30 a.m**. Vital signs will be taken and repeated several times later during this visit. Participants will eat breakfast (protein bars, 7 kcal/kg with water) and provide a urine sample for nicotine, drug and pregnancy (if eligible female) testing. Participants also will provide a saliva sample for an alcohol test and a pulse oximeter will be placed. If any of these tests are positive or if the blood oxygen saturation is below 96%, the participant will not be able to continue in the study. If these tests are negative, 1) an IV will be placed and kept open by a slow stream of IV fluid, 2) the participant will be prepared for psychophysiology testing (as described above), and 3) the participant will sit for about 1 hour while doing a brief memory test and ratings of psychiatric and medical symptoms (see 15.1.2. Schedule of Events for Expt. 1 and Expt. 2 in the Appendix). Then the participant's blood will be drawn through the IV and psychophysiology testing (i.e., extinction training) will be carried out over 15-30 minutes (details below). Afterwards, blood will be drawn again through the IV, study drug (Allo or placebo) will be given through the IV over 5 minutes, and a lower dose will be continued over the next 4-5 hours. During this time, blood will be drawn several more times more through the IV (See section **9.4.12 Blood Collection Schedule)**. At the end of the infusion, a neurological exam will be performed to ensure that it is safe for the participant to leave. As sedation is a possible side effect of IV Allo, the participant must arrange for a ride home by a friend or family member or use a transportation service (paid by the study). Participants will be reminded that they should avoid using alcohol or drugs or medications not approved by the study team the evening after the infusion.

3) Day 3 (about 3 ½ hours): Participants will be asked to arrive at our laboratory at BMC or WSU CRSC **by about 9:30 a.m**. Vital signs will be taken and may be repeated later during this visit. The participant will eat breakfast (protein bars, 7 kcal/kg with water) and provide a urine sample for nicotine, drug and (if eligible female) pregnancy testing. The participant will also provide a saliva sample for an alcohol test. If any of these tests are positive, the participant will not be able to continue in the study. If the tests are negative, the participant will be prepared for psychophysiology testing and sit for 1 hour while doing a brief memory test and ratings of psychiatric and medical symptoms (see 15.1.2. Schedule of Events for Expt. 1 and Expt. 2 in the Appendix). At the end of the hour psychophysiology testing (extinction

retention and reinstatement) will be carried out over 15-30 minutes (details below). The participant's blood will be drawn by needle stick once before and once after the testing.

4) Follow-up Phone/Zoom Call (about 45 minutes): About one-week after the experimental testing is complete, a follow-up telephone/Zoom call will include interviews and questionnaires about the participant's psychiatric and medical symptoms or adverse events (see 15.1.2. Schedule of Events for Expt. 1 and Expt. 2 in the Appendix). If preferred, some of the questionnaires can be filled out online instead. The call will be audio recorded. If no other safety measures are necessary, the participant will be discharged from the study after this call.

II. Expt. 2: Effects of IV Allo vs. Placebo on Reconsolidation Blockade and Non-Aversive Memory Consolidation

A. General Procedures for 2-Day PK Study

1) Day 1 (about 9 hours): Procedures will be the same as above except that the IV Allo (28 mcg/kg) will be given over 30 minutes and stopped. Only IV fluids will be continued at a slow drip for the next 4-5 hours.

2) Day 2 (about 3 hours): Procedures are the same as for Day 2 of Expt. 1.

B. General Procedures for 3-Day Fear Conditioning, Fear Reactivation, & Reconsolidation Blockade Study with 1-Week Follow-Up

1) Day 1 (about 3 ½ hours): Participants will be asked to arrive at our laboratory at BMC or WSU CRSC **by about 9:30 a.m**. Vital signs (temperature, respiratory rate, heart rate and blood pressure) will be taken and may be repeated later during this visit. Participants will eat breakfast (protein bars, 7 kcal/kg with water) and provide a urine sample for nicotine, drug and pregnancy (if eligible female) testing. Participants also will provide a saliva sample for an alcohol test. If any of these tests are positive, they will not be able to continue in the study (please see potential effects on payment in the **"Payment"** section below). If these tests are negative, the participant will be prepared for *startle testing* by cleaning the skin for sensor placement below the eye, behind the ear, and on the palm, chest, stomach and/or wrist. We will then use sticky tape to attach 8 small sensors (tiny metal discs with wires): 2 below the eye, 1 behind the ear, 2 to the palm, 2 to the chest, and 1 to the stomach or wrist. The participant then will sit at rest for 1 hour and fill out ratings of psychiatric and medical symptoms (see 15.1.2. Schedule of Events for Expt. 1 and Expt. 2 in the Appendix). At the end of the hour, the participant's blood will be drawn, and psychophysiology testing (conditioned fear acquisition) will be carried out over 15-30 minutes (details below).

2) Day 2 (about 9 hours): Participants will be asked to arrive at our laboratory at BMC or WSU CRSC **by about 9:30 a.m**. Vital signs will be taken and repeated several times later during this visit. Participants will eat breakfast (protein bars, 7 kcal/kg with water) and provide a urine sample for nicotine, drug and pregnancy (if eligible female) testing. Participants also will provide a saliva sample for an alcohol test and a pulse oximeter will be placed. If any of these tests are positive or blood oxygen saturation is below 96%, the participant will not be able to continue in the study. If these tests are negative, 1) an IV will be placed and kept open by a slow stream of IV fluid, 2) the participant will be prepared for psychophysiology testing (as described above), and 3) the participant will sit for about 1 hour while doing a brief memory test and ratings of psychiatric and medical symptoms (see 15.1.2. Schedule of Events for Expt. 1 and Expt. 2 in the Appendix). Then a blood sample will be drawn through the IV and the subject will participate in brief psychophysiology testing (fear reactivation) (details below).

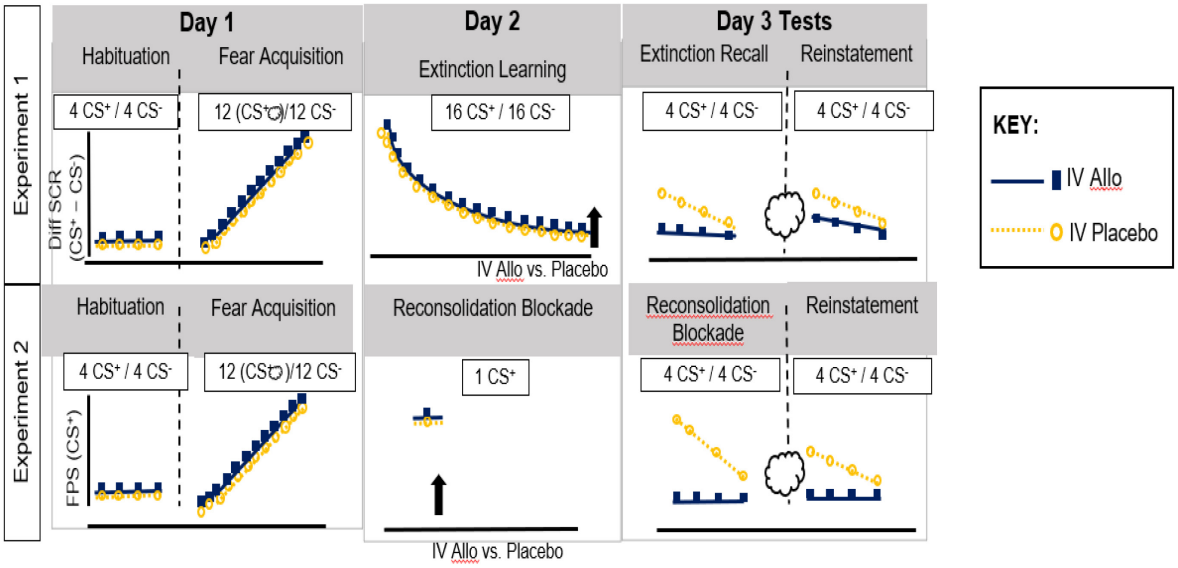
Afterwards, blood will be drawn again through the IV, study drug (IV Allo or placebo) will be given over 30 minutes, and IV fluids only will be continued for the next 4-5 hours. During this time, blood will be drawn several more times more through the IV (See section **9.4.12 Blood Collection Schedule**). A neurological exam then will be performed to ensure that it is safe for the participant to leave. As sedation is a possible side effect of IV Allo, the participant must arrange for a ride home by a friend/family member or use a transportation service (paid by the study). Participants will be reminded that they should avoid using alcohol or drugs or medications not approved by the study team the evening after the infusion.

3) Day 3 (about 3 ½ hours): Participants will be asked to arrive at our laboratory at BMC or WSU CRSC **by about 9:30 a.m**. Vital signs will be taken and may be repeated later during this visit. The participant will eat breakfast (protein bars, 7 kcal/kg with water) and provide a urine sample for nicotine, drug and pregnancy (if eligible female) testing. The participant will also provide a saliva sample for an alcohol test. If any of these tests are positive, the participant will not be able to continue in the study. If the tests are negative, the participant will be prepared for psychophysiology testing and sit for 1 hour while doing a brief memory test and ratings of psychiatric and medical symptoms (see 15.1.2. Schedule of Events for Expt. 1 and Expt. 2 in the Appendix). At the end of the hour psychophysiology testing (for reconsolidation blockade and reinstatement) will be carried out over 15-30 minutes (details below). The participant's blood will be drawn by needle stick once before and once after the testing.

4) Follow-up Phone/Zoom Call (about 45 minutes): About one week after the experimental testing is complete, a follow-up telephone/Zoom call will include interviews and questionnaires about the participant's psychiatric and medical symptoms or adverse events (see 15.1.2. Schedule of Events for Expt. 1 and Expt. 2 in the Appendix). If preferred, some of the questionnaires can be filled out online instead. The call will be audio recorded. If no other safety measures are necessary, the participant will be discharged from the study after this call.

9.4.9 Detailed Psychophysiology Procedures

Fig. 4 Psychophysiology Paradigms with Expected Outcomes for Expt. 1 (top) and Expt. 2 (bottom)



I. Psychophysiological Testing on Days 1,2, and 3 (Procedural Details)

A. Stimuli

The fear conditioning paradigm will include fear acquisition (Day 1), fear extinction (Day 2), and extinction recall (Day 3). Dependent variables will be skin conductance response (SCR) and fear-potentiated startle (FPS). The unconditioned stimulus (US) will be a 250 ms airblast of 120 p.s.i. directed to the larynx, which is aversive but not painful. The conditioned stimuli (CS's) will be different colored shapes appearing on a computer monitor in front of the participant for 6.5 sec (CS+) or 6 sec (CS-). Six seconds after CS onset, an auditory stimulus (106-dB SPL, 40ms burst of broadband noise with near instantaneous rise time, delivered binaurally through headphones) will be presented to serve as the startle probe. At CS+ offset, the US will be presented. A CS- will never be paired with the US and will represent a safety signal (Norrholm et al., 2011a; Glover et al., 2012). In addition, startle probes will be presented outside the context of the CS (i.e., noise alone (NA) trials).

B. Dependent Measures

1) Data Collection: Skin Conductance (SC) data will be collected via 8-mm Ag/AgCl surface electrodes filled with isotonic paste and placed on the hypothenar surface of the non-dominant hand (Fowles et al., 1981). The eyeblink component of the acoustic startle response will be measured by electromyography (EMG) recordings of the right *orbicularis oculi* muscle with two 5-mm Ag/AgCl electrodes filled with electrolyte gel (Norrholm et al., 2011a; Glover et al., 2012). The startle response data will be acquired at a sampling rate of 1000 Hz using the EMG module and SC will be acquired using the electrodermal activity (EDA) module of the Biopac MP160 for Windows (Biopac Systems, Inc.) as described in our previous studies (Norrholm et al., 2011a; Glover et al., 2012). Heart rate variability also will be collected using the EKG module of Biopac system as described in previous studies (Jovanovic et al., 2011; Kamkwalala et al., 2012). The acquired data will be filtered, rectified, and smoothed using MindWare Technologies software.

2) Primary Outcomes: Although SCR and FPS will be used as outcome measures for both experiments, SCR will be used as the primary outcome measure for the extinction retention study (Expt. 1); FPS will be used as the primary outcome measure for the reconsolidation blockade study (Expt. 2) SCR was chosen as the primary outcome measure for Expt. 1 based on its extensive use in past fear conditioning work (Orr et al., 2000) and our Preliminary Data. FPS was chosen as the primary outcome for Expt. 2 based on its extensive and reliable use in past tests of reconsolidation blockade (Elsey et al., 2018).

3) Data Cleaning and Response Scoring: SC and FPS responses will be scored after artifacts and outliers are removed; a) A SCR score for each CS presentation will be calculated by subtracting the mean SC level during the 2s interval immediately prior to CS onset from the highest SC level recorded during the first 6 sec of the CS interval. b) A startle response score will be calculated for each startle probe and defined as the peak amplitude of the eye-blink muscle contraction 20-200 msec after presentation of the startle probe. c) FPS will be calculated by subtracting average startle magnitude on the noise alone (NA) trials from the CS trials. Inclusion of startle will allow evaluation of conditioning in all participants, including SC non-responders.

C. Participant Experience

1) Day 1: Conditioned Fear Acquisition (Expts. 1 & 2): The participant will be told that there will be two phases during which two different colored shapes will appear on the screen and that during the second phase, airblasts will be administered. Following the instructions and a 1-minute baseline recording of psychophysiological measures, the participant will complete the two phases of the task: (i) habituation, and (ii) acquisition. Three startle probes will be administered during habituation to reduce initial startle

reactivity. The acquisition session will consist of 12 trials of each type: NA, CS+, and CS-. Note that 100% of CS+ trials will be reinforced.

2) Day 2: For Expt. 1 and Expt. 2, participants will be told that they will see the colored shapes seen the previous day but the presence/absence of the airblast is not mentioned. After a 1-min baseline recording period during which 3 startle probes will be administered for the purpose of startle habituation, either (Expt. 1) extinction training will begin and consist of 16 presentations of the CS+ and CS- with no airblast administered. Each CS will be presented for 6 s (inter-trial interval of 9-22 s) in pseudorandom order. Startle probes will be administered during each CS presentation; an additional 16 startle probes will be administered alone, or (Expt. 2) standard procedures for inducing reconsolidation blockade will be used and include a 1-minute baseline recording period and 3 trials of startle probes administered for the purposes of startle habituation. The fear memory will be reactivated with one presentation of the CS+ (including a startle probe); no shocks will be administered.

3) Day 3: For both Expt. 1 and Expt. 2, testing of responses to each CS+ and CS- in the absence of airblast over 4 trials of each type will be followed by a test of capacity for reinstatement of conditioned fear. After the electrodes to measure SC and FPS are attached, participants will be told, "Two different colored shapes will be presented on the monitor. There may also be airblast administration." Following the instructions and a 1-minute collection of baseline psychophysiological data followed by 3 startle probes alone, the participant will experience 4 CS+ and 4 CS- presentations. Startle probes will be administered during each CS presentation and an additional 5 startle probes will be administered alone. The first set of 4 CS+/CS- presentations without airblast will gauge extinction retention (Expt. 1). In Expt. 2, exposure to the 4 CS+/CS- presentations will ensure adequate extinction before reinstatement is potentially induced by administering 3 unpaired airblasts. Five NA trials will be presented after the unpaired airblasts to reduce sensitization. Participants then will be exposed to 4 additional CS+/CS- presentations without airblast, as well as 4 additional NA trials. This latter set of 4 CS+/CS- presentations will be used to assess capacity for reinstatement of conditioned fear. As noted in the Aims, we expect all participants in Expt. 1 to show reinstatement, but the IV Allo group to show less reinstatement than the IV placebo group. In Expt. 2, we expect the IV placebo group but not the IV Allo group to show reinstatement.

9.4.10 Scoring of Psychophysiology Experiment Outcomes by Day

I. Day 1

For both Expts. 1 and 2, the degree of conditioned fear acquisition will be defined as the difference between the average SCR to the last 4 CS+ and the average SCR to the last 4 CS- trials during the acquisition phase (i.e., the differential SCR). For FPS we will examine the last 4 trials of acquisition for each CS (Norrholm et al., 2011a). (Because FPS is calculated as the difference between startle to the CSs compared to the NA trials, the standard is to use FPS to CS+ trials as the DV rather than the difference between FPS to CS+ vs. CS- as is typically done for SCR). Higher scores indicate greater conditioned fear acquisition. The literature and our previous work indicate that the reinforcement schedule used will ensure conditioned fear acquisition in all participants.

II. Day 2

For Expt. 1, the degree of extinction learning on Day 2 will be defined as the difference between average SCR to the last 4 CS+ trials and the average SCR to the last 4 CS- trials. The literature and our previous work show that exposure to 16 CS+/CS- extinction trials will ensure full extinction in most participants. For FPS we will examine early, mid and late extinction to the CS+ (Norrholm et al 2015), as well as difference between early and late extinction.

III. Day 3

A. *Extinction Retention (Expt. 1):*
We will first calculate the difference between the average SCR to the first 4 CS+ trials and the average SCR to the first 4 CS- trials on Day 3. This differential SCR will be subtracted from the differential SCR for Day 2 (calculated as the difference between the average SCR to the last 4 CS+ trials and the average SCR for the last 4 CS- trials—the index of Day 2 extinction). For FPS, we will examine the degree of FPS to the CS+ during the first 4 CS+ trials as compared to the FPS for the last 4 CS+ trials during extinction. Higher scores indicate less extinction retention.

B. Reconsolidation Blockade (Expt. 2)
Reconsolidation blockade will be calculated by examining the average FPS to the first 4 CS+ trials and comparing the difference between the average SCR to the first 4 CS+ trials minus the average SCR to the first 4 CS- trials.

C. *Conditioned Fear Reinstatement (Expt. 1 and Expt. 2)* Reinstatement of conditioned fear will be defined as the average SCR to the last 4 CS+ trials minus the average SCR to the last 4 CS- trials. For FPS, we will examine the last 4 CS+ trials (Norrholm et al., 2011b). Higher scores indicate greater reinstatement of conditioned fear.

9.4.11 Non-Aversive Memory Testing
For both Expt. 1 and Expt. 2, effects of IV Allo vs. placebo on episodic memory for neutral stimuli (i.e., non-aversive learning) will be tested in the context of a source memory paradigm. If participants fail the color vision test, they will still complete the source memory paradigm, but their data will not be included in the episodic memory analyses. During encoding (Day 1 for PK studies [if the paradigm is ready] and Day 2 for the psychophysiology studies), participants will see pictures of objects, presented one at the time for 3 seconds each. The first set of 15-30 objects will be presented on a computer monitor. Following a 5 min distractor task, the second set of 15-30 objects will be presented. Thus, a distinct temporal context will be created for the two stimulus sets. Immediately following encoding of the two sets, participants will be asked to recall as many objects as they can. Delayed recall will be tested about 24 hours later (Day 2 for PK studies [if paradigm is ready] and Day 3 for the psychophysiology studies), followed by a test of source memory. Here, participants will be presented with the 15-30 objects from set 1, the 15-30 objects from set 2, and 15-30 objects not previously seen, all shown against a uniform white background. Participants will be asked to indicate whether an object was presented on the previous day (assessing recognition memory), and if yes, whether it was presented in the first set or in the second set, thus assessing source memory. Assignments of pictures to sets (set 1, set 2, unstudied), and of studied sets will be counterbalanced across subjects. Selection of stimulus set size and presentation parameters are based studies from the laboratory of Co-Investigator Mieke Verfaellie.

9.4.12 Blood Collection Schedule

Note: **IF:** inflammation; **NS:** neurosteroids; **NE:** neuroendocrine

I. Expt. 1 PK-1 Study

A. Total Blood Volume: up to **120 cc** (about 8 tablespoons or ~ 1/4th blood donation)
1) Screening Evaluation Clinical Laboratory Assessments: **30 – 50 cc**
2) 2-Day PK-Study: **70 cc**

B. Blood Draws Across Experimental Days

1) Day 1: PK Testing (9 draws): resting baseline, post 5-min infusion: +0', 15', 30', 1hr, 2hr, +3hr, 4hr, 5hr
-Resting baseline*: 6 cc purple (NS), 5 cc green lithium heparin separator (Renal: creatinine) = **11 cc**
-Next 8 blood draws: 8 x 6 cc purple tops (NS) = **48 cc**
*Resting baseline buffy coat will be saved for DNA sequencing, methylation/acetylation
Total Blood Volume for Day 1: **59 cc**

2) Day 2: Follow-Up (1 draw)
-Resting baseline: 6 cc purple top (NS), 5 cc green lithium heparin separator (Renal: creatinine)
*Resting baseline buffy coat saved for methylation/acetylation
Total Blood for Day 2: **11 cc**

Total Blood for Expt. 1 2-Day PK-1 Study: ***70 cc***

II. Expt. 1 Extinction Retention Study

A. Total Blood Volume*:* up to **410 cc** (about 27 tablespoons or ~4/5ths of a standard blood donation)
1) Screening Evaluation: **30 – 50 cc**
2) 3-Day Psychophysiology Study: **360 cc**

B. Blood Draws Across Experimental Days

1) Day 1: Acquisition (1 draw)

-Pre-Acquisition 10 cc purple, 6 cc purple (IF); 2 x 10 cc purple* (NS, NE)
*****Buffy coat saved for DNA sequencing, methylation/acetylation
Total Blood for Day 1: **36 cc**

2) Day 2: Extinction (13 draws): resting baseline/pre-extinction training, post extinction training, post 5-min infusion: +0', 30', 1hr, 1.5 hr, 2 hr, 2.5 hr, 3hr, 3.5 hr, 4 hr, 4.5 hr, 5hr post infusion

-Resting Baseline Pre-Ext Training: 10 cc purple, 6 cc purple (IF); 20 cc purple* (NS, NE), 5 cc green lithium heparin separator (Renal: creatinine) = **41 cc**
-Post-Extinction Training/Pre-Drug: 10 cc purple, 6 cc purple (IF); 20 cc purple* (NS, NE) = **36 cc**
-Post 5-min bolus infusion: +0': 1 x 6 cc purple (NS, NE) = **6 cc**
-Post-start of continuous drug infusion:
a) 10 cc purple, 6 cc purple (IF): +30', 1hr, 3 hr, 5 hr [(4 x 10 cc) + (4 x 6 cc)] = **64 cc**
b) 10 cc purple* (NS/NE): +30', 1hr, 1.5hr, 2hr, 2.5hr, 3hr, 3.5hr, 4hr, 4.5hr, 5hr = 10 x 10 cc = **100 cc**
*Buffy coat saved for DNA methylation/acetylation
Total Blood for Day 2: **247 cc**

3) Day 3: Extinction Retention Testing (2 draws): resting pre-test, 10' post-test

-Resting Pre-Test: 10 cc purple, 6 cc purple (IF); 2 x 10 cc purple* (NS, NE), 5 cc green lithium heparin separator (Renal: creatinine) = **41 cc**
-10' Post-Test: 10 cc purple, 6 cc purple (IF); 2 x 10 cc purple* (NS, NE) = **36 cc**
*Buffy coat saved for DNA methylation/acetylation
Total Blood for Day 3: **77 cc**

Total Blood for Expt. 1. 3-Day Extinction Retention Study: ***360 cc***

III. Reconsolidation Blockade PK-2 Study

A. Total Blood Volume: up to **120 cc** (about 8 tablespoons or ~1/4th blood donation)
1) Screening Evaluation: **30 – 50 cc**
2) 2-Day PK-Study: **70 cc**

B. Blood Draws Across Experimental Days

1) Day 1: PK Testing (9 draws): resting baseline, post 30-min infusion: +0', 15', 30', 1hr, 2hr, 3hr, 4hr, 5hr

-Resting Pre-Infusion: 6 cc purple (NS), 5 cc green lithium heparin separator (Renal: creatinine) = **11 cc**
-Next 8 blood draws: 8 x 6cc purple tops (NS) = **48 cc**
Total Blood for Day 1: **59 cc**

2) Day 2: Follow-Up (1 draw):

-Resting 6 cc purple top* (NS), 5 cc green lithium heparin separator (Renal: creatinine)
*Resting buffy coats saved for methylation/acetylation
Total Blood for Day 2: **11 cc**

Total Blood for Expt. 2 2-Day PK-2 Study: ***70 cc***

IV. Expt. 2 Reconsolidation Blockade Study

A. Total Blood Volume: up to **404 cc**, about 27 tablespoons or ~ 4/5ths of a standard blood donation
1) Screening Evaluation: **30 – 50 cc**
2) 3-Day Psychophysiology Study: **354 cc**

B. Blood Draws Across Experimental Days
1) Day 1: Acquisition (1 blood draw)

-Pre-Acquisition 10 cc purple, 6 cc purple (IF); 2 x 10 cc purple* (NS, NE)
*****Buffy coat saved for DNA sequencing, methylation/acetylation
Total Blood for Day 1: **36 cc**

2) Day 2: 1 CS re-exposure (14 draws): Resting baseline/Pre-stress reactivation, post-CS reactivation/pre-drug, 15' into infusion, post 30-min infusion: +0', 30', 1hr, 1.5 hr, 2 hr, 2.5 hr, 3hr, 3.5 hr, 4 hr, 4.5 hr, 5hr

-Resting Baseline Pre-Stress Reactivation: 10 cc purple, 6 cc purple (IF); 20 cc purple* (NS, NE), 5 cc green lithium heparin separator (Renal: creatinine) = **41 cc**
-Post-Stress Reactivation/Pre-Drug: 10 cc purple, 6 cc purple (IF)= **16 cc**
-15' into infusion: 10 cc purple* for NS and ACTH to be drawn 15' into infusion = **10cc**
-Post 30-min infusion:
a) 10 cc purple, 6 cc purple (IF): +0', 1hr, 3 hr, 5 hr [(4 x 10cc) + (4 x 6cc)] = **64cc**
b) 10 cc purple* (NS, NE): + 0', 30', 1hr, 1.5hr, 2hr, 2.5hr, 3hr, 3.5hr, 4hr, 4.5hr, 5hr = 11 x 10cc=**110 cc**
*Buffy coat saved for DNA methylation/acetylation
Total Blood for Day 2: **241 cc**

3) Day 3: Reconsolidation Blockade Testing (2 draws)

-Resting Pre-Test: 10 cc purple, 6 cc purple (IF); 2 x 10 cc purple* (NS & NE), 5 cc green lithium heparin separator (Renal: creatinine) = **41 cc**
-10' Post-Test: 10 cc purple, 6 cc purple (IF); 2 x 10 cc purple* (NS & NE) = **36 cc**
*Buffy coat saved for DNA methylation/acetylation

Total Blood for Day 3: **77 cc**

Total Blood for Expt. 2. 3-Day Reconsolidation Blockade Study: ***354 cc***

9.4.13 Blood Processing and Storage in the Department of Laboratory Medicine at BMC, BUMC GCRU and WSU CRSC

Blood for clinical laboratory testing conducted at the department of laboratory medicine at BMC for safety and eligibility purposes will be collected in lavender top EDTA, plain red top (for serum), gold top, green top lithium heparin, and light blue top sodium citrate tubes and kept at room temperature. These tubes will be transported to the department of laboratory medicine at BMC as soon as possible but within 2 hours of collection for processing.

At WSU, clinical laboratory blood testing for safety and eligibility purposes will be conducted at LabCorp. Samples will be collected from participants in lavender top EDTA, plain red top (for serum), gold top, green top lithium heparin, and light blue top sodium citrate tubes and kept at room temperature. These tubes will be collected from the WSU CRSC within 6 hours maximum and sent to LabCorp for testing.

Blood for research purposes will be collected in lavender top EDTA tubes and placed immediately on wet ice. These tubes will be immediately transported to the laboratory in the BUMC GCRU or WSU CRSC where the blood in the EDTA tubes will be spun in a refrigerated centrifuge at 3000 rpm x 15 minutes to obtain plasma, which will be pipetted into 500 ul vials; buffy coat from the EDTA tubes will be collected for peripheral blood monocytes (PBMCs) from which DNA and RNA can be extracted. The samples will be immediately stored at -80^{o}C in the BUMC GCRU or WSU 3rd floor wet lab until shipping on dry ice to appropriate laboratories external to BUMC/BMC or WSU for measurement of the neurobiological factors of interest.

9.4.14 Blood Biomarkers

The neuroactive steroids of interest will be measured in selected expert laboratories that use state-of-the-art methodologies for plasma neuroactive steroid measurements. The methodologies below may be tested and confirmed for accuracy across laboratories before use in the main Expt. 1 and 2 studies:
a) In the laboratory of our expert Co-I, Graziano, Ph.D., University of Illinois at Chicago (with whom the study team has a long history of successful collaboration), plasma concentrations of estradiol, progesterone, 5α-DHP, Allo, AlloS, PA, PAS, DHEA, and the 3β-hydroxylated isomers of Allo (isoallopregnanolone) and PA (epipregnanolone) will be determined using high performance liquid chromatography (HPLC) and state of the art GC-MS techniques, which allow the simultaneous measurement of several steroid from a single sample with high sensitivity femtomolar and unsurpassed structural specificity). Extraction, derivatization, and quantification of Allo, its un-conjugated isomer pregnanolone, and its precursors and their sulfated forms will be corrected for procedural losses by adding deuterium-labeled neuroactive steroid(s) to serve as internal standards (Pinna et al., 2000; Locci and Pinna, 2019). Steroids will be extracted, purified, and separated by HPLC. The aqueous phase (2 mL) containing the sulfated neurosteroids AlloS and PAS will undergo a solvolysis prior to extraction. After derivatization, GC-MS analysis in the standard electron impact mode will be performed.
b) Other state-of-the-art methodologies may include liquid chromatographay/mass spectrometry/mass spectrometry (LC/MS/MS), as used by Jimmy Wu in the UC Davis laboratory of Michael Rogawski. While this methodology can be somewhat less sensitive than GC/MS, it has been previously used successfully to measure higher post-GMP IV Allo infusion concentrations of allopregnanolone (e.g., as in Hernandez et al., 2020, a study that infused approximately the same dose of GMP IV Allo as proposed for Expt. 2).
c) Other state-of-the-art methodologies established in other expert laboratories yet to be determined.

Inflammatory and other neurobiological markers of interest (except ACTH) are not currently funded. We plan to apply for additional funding from university, foundation, or federal sources so as to measure inflammatory factors such as CRP, IFN-γ, IL-1β, IL-6, TNF-alpha, MCP-1, and MMP-9 and other factors of possible relevance to the neurobiology of the neural processes under consideration such as norepinephrine (NE), neuropeptide Y, dipeptidyl peptidase (DPP-4), cortisol, GABA, glutamate, pituitary adenylate-cyclase-activating polypeptide (PACAP), etc.

9.4.15 Urinalysis by Dipstick

A clinical urinalysis will be conducted at the BUMC GCRU or WSU CRSC screening visit to screen for potential exclusionary diagnoses (e.g., diabetes, kidney disease, urinary tract infection, cancer).

9.4.16 Pregnancy Testing

The premenopausal women in the study must have normal menstrual cycles. Due to unknown risks of IV Allo to the fetus, females with childbearing potential must use two highly effective forms of contraception (other than hormonal contraceptives except for IUDs or a device like Nuvaring) for a week before the IV Allo or placebo infusion and for 28 days after. Generally, systemic hormonal contraceptives are not allowed because they would interfere with the results of the study. However, hormonal IUDs (e.g., Mirena, Kyleena, Liletta, and Skyla) or other contraceptive devices (e.g., Nuvaring) will be allowed if testing for the luteinizing hormone (LH) surge and follow-up PdG testing confirms ovulation. Other effective contraceptive methods include use of a diaphragm or cervical cap with spermicide, condoms in combination with spermicide, vasectomy, or a copper IUD.

Urine beta-human chorionic gonadotropin (HCG) testing for pregnancy will be conducted for women at every in-person visit (i.e., in-person medical screening, PK Study Days 1-2, Psychophysiology Study Days 1-3) to ensure that the individual is not pregnant; pregnancy is exclusionary.

9.4.17 Urine Toxicology for Illicit Drugs and Cotinine

The specific urine toxicology tests to be used in the study are below. If necessary (e.g., due to unanticipated product unavailability or concerns regarding cost), functionally equivalent test products may be substituted.

a) Abbott/Alere Toxicology NicQuick urine cotinine test (+/-; cut-off 200 ng/ml) will be used to detect possible nicotine use. Cotinine is a major nicotine metabolite with a 10-40 hour half-life).

b) Abbot Labs 'ICUP 13' will be used to test urine for the following drugs (sensitivity in parentheses):
-benzodiazepines (>300 ng/ml)*
-barbiturates (>300ng/ml)*,
-opiates (morphine, codeine, heroin; >2000 ng/ml)**
-buprenorphine (>10 ng/ml)*
-methadone (>300 ng/ml)*
-oxycodone (>100 ng/ml)*
-propoxyphene (PPX; e.g., Darvocet; 300 ng/ml)*
-cocaine (300 ng/ml)**
-amphetamine (1000 ng/ml)**
-methamphetamine (>1000 ng/ml)*
-phencyclidine (PCP: >25 ng/ml)**
-marijuana (THC: > 50ng/ml)**
-tricyclic antidepressants (TCA: 1000 ng/ml)*

*At present, the Substance Abuse and Mental Health Services Administration (SAMHSA) does not have a recommended screening cut-off for positive specimens.
**This is the suggested screening cut-off for positive specimens set by the Substance Abuse and Mental Health Services Administration (SAMHSA, USA).

c) **A NarcoCheck** urine test will be used to test for methylenedioxymethamphetamine (MDMA: 500 ng/ml sensitivity)
*At present, the Substance Abuse and Mental Health Services Administration (SAMHSA) does not have a recommended screening cut-off for positive specimens.

d) A **NarcoCheck® multi-NPS** test will be used to detect use of new illicit synthetic drugs:
-K2 / Spice: first generation synthetic cannabinoid that appeared in mid-2000.
-K3 / AB-Pinaca: second generation synthetic cannabinoids that appeared in 2014-15.
-K4 / UR-144: third type of synthetic cannabinoid first detected in 2013-2015.
-MCAT: MethCathinones derived from cathinone, the main active substance in Khat (or Chat), a sub-Saharan plant cultivated and consumed for strong psychotropic effects close to amphetamines and ecstasy. This test detects the dozens of methcathinone sub-molecules (mephedrone, 3-MMC, 4-MEC, butylone, methylone etc.
-MDPV: MethyleneDioxyPyroValerone, another powerful synthetic cathinone popularized and sold under innocuous names as "bath salts" or "fertilizers".

e) Urine will be sent to the BMC/Ford Biomedical (WSU) for fentanyl testing (with 2 ng/ml sensitivity), which is 5 times more sensitive than commercial test strips currently available. Turn-around time is regularly 4 hours, but if rushed is 60 minutes (BMC) or 2 hours (Ford Biomedical), allowing use of this method on the day of the IV Allo vs. placebo infusions as well as for the initial screening evaluation. The methodology also detects carfentenyl at 4 ng/ml. However, carfentanyl is 100 times more potent than fentanyl; thus, it is unlikely to effectively test for this drug in participants, highlighting the need for closing monitoring, the potential use of BUMC GCRU or WSU CRSC Emergency Response and Medication Administration protocols, and the potential use of naloxone per BMC or WSU CRSC protocols.

9.4.18 Saliva Alcohol Test Strips

A saliva alcohol test strip will be used at every in-person visit (in-person medical screening, PK Study Days 1-2, Psychophysiology Study Days 1-3, 1-week follow-up visit) to assess recent acute alcohol use which would increase risk of sedation by IV Allo vs. placebo as well as confound the physiological and neuropsychological responses to the psychophysiological procedures or follow-up measures of neurosteroids or other neurobiological factors. The saliva alcohol test strip measures the level of alcohol in human saliva with aligned indices of relative Blood Alcohol Concentration (BAC) at 0.0%, 0.02%, 0.04%, 0.08%., and 0.30%. The test strip turns blue when alcohol is present. A very small amount of saliva will be collected, just enough to allow us to coat the tip of the test strip. Saliva collection should take place at least 15 minutes after the last sip of a beverage. The saliva sample should be brought to room temperature for 15 minutes before testing. It should be noted that participants are excluded if they have been diagnosed with an alcohol use disorder in the 3 months prior to enrollment in the study and are asked to abstain from alcohol for at least two weeks prior to psychophysiological test procedures.

9.4.19 Payment

Participants will be paid $50 for the psychological screening evaluation, $50 for the medical screening evaluation, $135 for Day 1 of the PK testing, and $45 for the Day 2 PK follow-up blood, saliva, and urine collection. Therefore, they can receive up to $280 for taking part in all PK study visits. For Expts. 1 and 2, participants will receive $50 for the psychological screening evaluation, $50 for the medical screening evaluation, $60 for the first day of startle testing, $175 for the second day of startle testing, $100 for the third day, and $15 for the follow-up telephone/Zoom call. Therefore, they can receive up to $450 for taking part in all study visits. If participants return to the BUMC GCRU or WSU CRSC to repeat a lab test,

they will be paid $20. Participants will receive these payments on a Clin Card. Individuals will be paid for participation in study sessions unless they are disqualified at that session based on a positive saliva alcohol test or positive urine drug or nicotine test. If a visit is ended by the investigators because the participant tested positive for COVID-19 on the rapid test, they will be paid for that study visit.

10 ASSESSMENT OF SAFETY AND DATA SAFETY MONITORING PLAN (DSMP)

10.1 SAFETY DEFINITIONS

As the BMC/BU Medical Campus PI is also the FDA sponsor (that is, holds the IND), FDA definitions are merged with OHRP definitions which may overlap. Specific provisions for pregnancy in female subjects have been previously described.

Adverse Event (AE) is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research. The FDA definition for AE is narrower: "*Adverse Event (AE)* is any untoward medical occurrence associated with use of a drug in a human, whether or not considered drug related" and is encompassed in this broader definition.

Suspected adverse reaction means any AE for which there is a reasonable possibility that the drug caused the AE. "Reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the AE. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a drug.

An AE or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. 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 subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or substance use disorder.

An AE or serious suspected adverse reaction is considered *"life-threatening"* if, in the view of the investigator or sponsor its occurrence places the subject at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

An AE or unexpected suspected adverse reaction or suspected adverse reaction is considered "*unexpected*" if it is not listed in the Zulresso (brexanolone) package insert (as it is a pharmacological equivalent of the UC Davis GMP IV Allopregnanolone used in the current study) or is not listed at the specificity or severity that has been observed; or, if it is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. "Unexpected," as used in this definition, also refers to AEs or SAEs that are mentioned in the package insert and risk information as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation.

Unanticipated Problem is defined as an event, experience or outcome that meets **all three** of the following criteria:

- is unexpected; AND
- is related or possibly related to participation in the research; AND
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

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.

Regarding this specific protocol, abnormal results of diagnostic procedures (except in the case of preexisting condition—see below) are considered to be AEs if the abnormality:

1. results in study withdrawal.
2. is associated with a serious AE.
3. is associated with clinical signs or symptoms.
4. leads to additional treatment or further diagnostic tests.
5. is considered by the investigator to be of clinical significance.

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an AE if the frequency, intensity, or the character of the condition worsens during the study period.

A clinical laboratory abnormality should be documented as an AE if any one of the following conditions is met:

- the known or foreseeable risk of AEs associated with the procedures involved in the research that are described in (a) the protocol–related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document, and (b) other relevant sources of information, such as product labeling and package inserts;
- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g., more frequent follow-up assessments, further diagnostic investigation, etc.

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as a SAE unless specifically instructed otherwise in this protocol.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an AE in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition.
- Surgery should ***not*** be reported as an outcome of an AE if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.

10.2 SAFETY REVIEW

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an AE must also be recorded and documented as an AE.

At each contact with the subject, the investigator must seek information on AEs by specific questioning and, as appropriate, by examination. Information on all AEs should be recorded immediately in the source document All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis.

All AEs occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study infusion or participation is not the cause. SAEs that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any SAE that occurs within 30 days after the study period and is considered to be possibly related to the study infusion or study participation should be recorded and reported immediately to the IRB, Data Safety Monitoring Board (DSMB) and FDA.

Minimum information required for each AE includes type of event, duration (start and end dates), severity, seriousness, causality to study drug, action taken, and outcome.

All unresolved AEs should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the AE is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The IRBs, DSMB, and FDA should also be notified if the investigator should become aware of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study or if there is a suspicion of drug exposure in utero.

Evaluating Adverse Events

Assessment should include the intensity (severity) of the event and the relationship to Study Agent(s)/Intervention(s). Severity of AEs will be graded by the Investigator using the following criteria as guidelines. Note, a severe AE doesn't necessarily mean that it meets criteria for a Serious Adverse Event (SAE) (defined above in Section 10.1):

1. Mild: Nuisance, barely noticeable.
2. Moderate: Uncomfortable, troublesome symptoms not significantly interfering with activities or sleep.
3. Severe: Symptoms significantly interfere with daily activities or sleep.
4. Life-Threatening: Symptoms place the participant at imminent risk of death or urgent intervention indicated.
5. Death

The relationship of the AE to the study drug should be specified by the Investigator, using the following definitions:

1. Not Related: Concomitant illness, accident or event with no reasonable association with study drug.
2. Unlikely: The reaction has little or no temporal sequence from administration of the study drug, and/or a more likely alternative etiology exists.
3. Possibly Related: The reaction follows a reasonably temporal sequence from administration of the drug and follows a known response pattern to the suspected drug; the reaction could have been produced by the study drug or could have been produced by the subject's clinical state or by other modes of therapy administered to the subject.
4. Probably Related: The reaction follows a reasonable temporal sequence from administration of study drug; is confirmed by discontinuation of the study drug or by rechallenge; and cannot be reasonably explained by the known characteristics of the subject's clinical state.

5. Definitely Related: The reaction follows a reasonable temporal sequence from administration of study medication; that follows a known or expected response pattern to the study medication; and that is confirmed by improvement on stopping or reducing the dosage of the study medication, and reappearance of the reaction on repeated exposure.

Reporting Adverse Events

Site Principal Investigators (PIs): The WSU site PI and BMC/BUMC study personnel will report all unexpected and expected AEs or suspected AEs to the BMC/BUMC lead site or Sponsor-PI (Dr. Rasmusson), who will in turn forward the reports to the Independent Medical Monitor (IMM) for review. Any SAEs will be reported to the IMM within 24 hours of the lead site/Sponsor PI learning of the event to the IMM; a summary of non-serious AEs will be provided to the IMM quarterly. The lead site /Sponsor PI will report non-serious AEs to the IMM in-between quarterly reports (if expected or unexpected) if of a nature, frequency or pattern that raises potential questions about safety, a new potential relationship to study drug, or relationship to other study procedures that may benefit from amendment. Dr. Rasmusson also will interact with all appropriate entities (e.g., the BMC/BUMC central IRB of record, NIMH DSMB, and FDA) to ensure rigorous compliance with the regulatory aspects of the trial's design and conduct. In addition to the DSMB, the lead BMC/BUMC lead site and Sponsor-PI, the WSU site PI, and the IMM will regularly consider whether any AE (or group of related AEs) or major protocol deviation/violation affects the Risk/Benefit ratio of the study and determine whether modifications to the protocol procedures, risks section, or consent form (risks and inconveniences sections) will be needed. Such modifications will be discussed and thereafter submitted by Dr. Rasmusson to the BMC/BUMC IRB (IRB of record).

Independent Medical Monitor (IMM): The IMM, George O'Connor, MD, MS, who is not associated with the study, will be required to review all unanticipated problems (UPs), SAEs, major protocol deviations/violations, unblinding, and volunteer deaths associated with the protocol and provide an unbiased written report of the event to the lead site PI for reporting to the DSMB. At a minimum the Medical Monitor should comment on the outcomes of the event, major protocol deviation/violation, or problem, and in the case of a serious adverse event, unblinding, or death, comment on the relationship to participation in the study. The IMM should also indicate whether he/she concurs with the details of the report provided by the lead site PI. The IMM will provide a summary report on such events back to the lead site PI as soon as possible, but ideally within 3 to 4 calendar days, to enable reporting by the lead site PI to the central BMC/BUMC IRB or record, DSMB, and FDA in accordance with their required reporting timeframes.

The IMM will review non-serious AEs on a quarterly basis and provide a report back quarterly as to whether or not there are any safety issues. He will also assimilate the AEs and analyze them for patterns, and report back to the Sponsor-PI. If any safety concerns, these will be discussed in a meeting including the Medical Monitor, Sponsor-PI and WSU PI.

DSMB: Per guidelines, the NIMH will be providing a NIMH-constituted DSMB for monitoring of data integrity and participant safety. The DSMB will be composed of three to eight individuals. Please see the NIMH Charter for further details regarding NIMH-constituted DSMBs. The NIMH DSMBs are managed under the auspices of the Office of Clinical Research (OCR) in the NIMH Office of the Director. Each NIMH DSMB has a Scientific Administrator assigned from the Human Research Protections Branch from the NIMH OCR who serves in an Executive Secretary capacity and is responsible for the coordination of all NIMH DSMB activities. The Clinical Trials Operations Branch (CTOB) DSMB will ensure that monitoring

systems are in place for the study. The study team will provide two interim data reports and one annual data report to the DSMB for review. The site IRBs, PIs, and NIMH staff will be informed of directives made by the NIMH DSMB. In addition, the DSMB will weigh in after preparation of the interim statistical analysis for Expt. 2 (only), "The effects of IV Allopregnanolone on Reconsolidation Blockade", regarding the possibility of early stopping for efficacy. The DSMB also can make directives for stopping for safety.

Unblinding Procedures

If a clinical situation arises so that the PI or WSU site PI feels it is necessary to break the blind to make a therapeutic decision for a particular subject, he/she should consult with the other site PI and IMM prior to breaking the blind, whenever possible. The blind is to be broken by the study biostatistician or dispensing pharmacist for the relevant site only in the event of an emergency or SAE when the identification of the medication is critical for treatment decisions. If the blind is broken, the PI must immediately notify the DSMB and provide a full explanation as to the reason for doing so and provide an unbiased report from the IMM. This information must be documented in the study file.

10.3 REPORTING PLANS

Investigator Obligations

Investigators must conform to the AE reporting timelines, formats, and requirements of the various entities to which they are responsible, but at a minimum those events that must be promptly reported to the lead site Sponsor-PI are those that are:

- Serious Adverse Events, whether related or unrelated to study participation or study drug and whether expected or unexpected in relation to the study or study drug.
- Any other unanticipated problems (UPs) involving risks to subjects or others.

The study period during which AEs must be reported will be defined as the period from the initiation of any study procedures to 30 days after the participant is discharged from the study (which will also encompass the time period during which female participants are required to use two effective means of contraception in order to protect against pregnancy (1 week prior to the IV Allo or placebo infusion and for 28 days after receiving the IV Allo infusion).

Notifying the Local IRB

The overall study lead and Sponsor-PI (Dr. Ann Rasmusson) will be responsible for reporting AEs and major protocol deviations/violations from both the Boston and WSU sites to the central BMC/BUMC IRB of record via the electronic IRB system. The WSU site PI (Dr. Tanja Jovanovic) will be responsible for safety reporting to the WSU IRB only if required by the WSU IRB, which will otherwise be under a Reliance Agreement with the BMC/BUMC Central IRB. Copies of each report and documentation of IRB notification and receipt will be kept in each site Pis study file.

The Sponsor-PI (Dr. Rasmusson) will report to the BMC/BU Medical Campus Central IRB all Unanticipated Problems, major protocol deviations/violations, safety monitors' reports, and AEs in accordance with that Central IRB's policies:

- Unanticipated Problems and major protocol deviations/violations occurring at the Boston or WSU site will be reported to the central IRB of record within 7 days of the Sponsor-PI learning of the event.
- Reports from safety monitors with recommended changes also will be reported to the IRB within 7 days of the Sponsor-PI receiving the report.

● Reports from the study Independent Medical Monitor and the NIMH Data Safety Monitoring Board (DSMB) with no recommended changes will be reported to the IRB of record and WSU IRB at the time of continuing review.

Notifying the Lead investigator

Unanticipated problems posing risks to subjects or others, major protocol deviations/violations, and serious adverse events (SAEs) associated with the research will be forwarded to the Sponsor-PI (Dr. Rasmusson) within 24-hours. Non-serious AEs assimilated in the study database will be forwarded to the Sponsor-PI quarterly.

Notifying the Independent Medical Monitor

Unanticipated problems posing risks to subjects or others, and serious adverse events (SAEs) associated with the research will be forwarded to the IMM) by the Sponsor-PI within 24-hours of learning of the event. Major protocol deviations/violations will be forwarded by the Sponsor-PI to the IMM within 7 days of learning of the event unless the PI determines that reporting should occur earlier based on the need to consult with the IMM to evaluate or resolve any immediate issues that may impact study safety or scientific integrity or to develop the most effective corrective and preventive action plan. The IMM for the study will provide an unbiased evaluative written report back to the SponsorPI of all life-threatening AEs, major protocol deviations/violations, and other unanticipated problems and SAEs as soon as possible, but ideally within 1 to 4 calendar days for review and submission to the WSU PI and appropriate regulators (e.g., central IRB of record, DSMB, FDA).

Notifying the NIMH DSMB

Unanticipated problems posing risks to subjects or others and serious, unexpected AEs associated with the research will be forwarded to the DSMB as soon as possible, no later than 10 business days after the study PI becomes aware of the event, accompanied by the evaluation of the event conducted by the study PI, WSU site PI and Medical Monitor. Deaths will be reported to the DSMB within 5 business days after the study PI becomes aware of the death. These reports should indicate that the monitoring entities (i.e., the IRBs) and appropriate regulatory entities (e.g., FDA) have been notified in accordance with the approved monitoring plan and federal regulations. For all protocol deviations/violations and AEs and SAEs deemed expected/unexpected and/or related/unrelated to the study, a summary will be submitted three times a year (two interim data reports and one full data report).

Notifying the FDA

The BUMC/BMC PI must notify the FDA and all participating Co-investigators (i.e., all investigators to whom the BUMC/BMC PI is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the BUMC/BMC PI determines that the information qualifies for expedited reporting. Information that qualifies for reporting based on above criteria includes serious and unexpected suspected adverse reaction, findings from other studies, findings from animal or in vitro testing, and increased rate of occurrence of serious suspected adverse reactions. In each IND safety report, the BUMC/BMC PI must identify all IND safety reports previously submitted to FDA concerning a similar suspected adverse reaction and must analyze the significance of the suspected adverse reaction in light of previous, similar reports, or any other relevant information.

The BUMC/BMC PI must report any suspected adverse reaction that is both serious and unexpected. The PI must report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:

(a) A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome);
(b) One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture);
(c) An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

The BUMC/BMC PI must report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies, whether or not conducted under an IND, and whether or not conducted by the PI, that suggest a significant risk in humans exposed to the drug. Ordinarily, such a finding would result in a safety-related change in the protocol, informed consent, risk information (excluding routine updates of these documents), or other aspects of the overall conduct of the clinical investigation.
The BUMC/BMC PI must report any findings from animal or in vitro testing, whether or not conducted by the PI, that suggest a significant risk in humans exposed to the drug, such as reports of mutagenicity, teratogenicity, or carcinogenicity, or reports of significant organ toxicity at or near the expected human exposure. Ordinarily, any such findings would result in a safety-related change in the protocol, informed consent, risk information (excluding routine updates of these documents), or other aspects of the overall conduct of the clinical investigation.

The BUMC/BMC PI must report any clinically important increase in the rate or severity of a serious suspected adverse reaction over that listed in the protocol or Zulresso (brexanolone) package insert.

The BUMC/BMC PI must submit each IND safety report in a narrative format or on FDA Form 3500A or in an electronic format that FDA can process, review, and archive. FDA will periodically issue guidance on how to provide the electronic submission (e.g., method of transmission, media, file formats, preparation and organization of files). Reports of overall findings or pooled analyses from published and unpublished in vitro, animal, epidemiological, or clinical studies must be submitted in a narrative format. Each notification to FDA must bear prominent identification of its contents, i.e., "IND Safety Report," and must be transmitted to the review division in the Center for Drug Evaluation and Research or in the Center for Biologics Evaluation and Research that has responsibility for review of the IND. Upon request from FDA, the sponsor must submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

The BUMC/BMC PI must also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information.

The BUMC/BMC PI must promptly investigate all safety information it receives in response to an IND safety report. Relevant follow-up information to an IND safety report must be submitted as soon as the information is available and must be identified as such, i.e., "Follow-up IND Safety Report."

If the results of a PI’s investigation show that an AE not initially determined to be reportable is so reportable, the PI must report such suspected adverse reaction in an IND safety report as soon as possible, but in no case later than 15 calendar days after the determination is made.

10.4 STOPPING RULES

The NIMH DSMB may recommend stopping the study for safety at any point. One interim efficacy analysis for early stopping is planned for Expt. 2 only, and the DSMB then may recommend stopping for early efficacy. In order to maintain the overall two-sided type I error rate of 0.05, the Lan-DeMets (O'Brien-Fleming) α-spending function will be used. The interim analysis for efficacy will be done when 64 evaluable subjects have completed the study while the final analysis will be done when all 128 evaluable subjects have completed the study. The table below shows the critical values and other design characteristics used in this analysis. Subject to the DSMB approval, a z-statistic greater than the critical value of 2.963 will provide evidence of early stopping for efficacy.

Critical values and design characteristics

	Information Rate	Critical Value	Nominal P-Value	Sample Size
Interim	50%	2.96259	0.00306	64
Final	100%	1.96860	0.04900	128

11 DATA HANDLING AND RECORD KEEPING

11.1 CONFIDENTIALITY

11.1.1 Subject Confidentiality

All of the data for this project will be collected specifically for research purposes. Hard copy files will be kept in locked file cabinets within locked offices. If a psychological screening is conducted remotely, hard copy files that may be generated will be placed in a locked storage box for transport to locked file cabinets within designated locked study research offices. Electronic data (e.g., digital audio-files of diagnostic interviews used for consensus diagnostic purposes) will be kept in a secure folder on a secured, password protected server, with access restricted to staff for this specific research study. Participant numbers without personal identifiers assigned to each participant will be the only means by which collected information is labeled. The master code is the only list that will link the names of the participants with their participant numbers will be kept in a secure, password- protected computer account on a separate drive from research coded data and will be accessible only to IRB- approved members of the research team. Research team members associated with the outside laboratory at University of Illinois at Chicago where samples will be processed for neurosteroid measures will not have access to the link and only see samples with a coded research number.

11.1.2 Data Accuracy

The research team will assure the accuracy and integrity of the data by checking completeness of assessments prior to the ends of research visits, and also check participant safety assessments (e.g., results of CSSRS, ECG results) before the participant leaves the research session to facilitate opportunities for further evaluation and treatment as needed. Data will be entered within a day of collection and verified within a week. These strategies will reduce likelihood of missing data.

11.1.3 Data Management

Data entered into the Redcap and NIMH Data Archive databases will be verified by the study Biostatistician or trained and supervised designate. Standard quality assurance procedures for data management also will be followed. The study biostatistician (Dr. Fonda) developed a quality assurance

guideline that addresses common errors in data collection and scoring and provides standardized methods to minimize these errors. The guidelines are updated as needed to correct for any new quality assurance issues. Dr. Fonda will train the research coordinator on the quality assurance guidelines. The research coordinator with the supervision of Dr. Fonda will develop database programs to read in the data files and check for inconsistent or incomplete fields and outliers. These programs also automate mathematical computation of subscale, total, and standardized scores to minimize computational error. The biostatistician will ensure that the data monitoring and qualitative assurance procedures are followed at the Boston and Detroit sites and will oversee data transfer from Detroit to Boston.

11.1.4 Data Sharing

Data from this study will be submitted to the National Institute of Mental Health Data Archive (NDA) at the National Institutes of Health (NIH). NDA is a large database where deidentified study data from many NIMH studies is stored and managed.

11.1.5 Clinicaltrials.gov

The study has already been registered on clinicaltrials.gov. The reporting of summary results information (including adverse events) will occur no later than 1 year after the completion date; grant and progress report forms also will include a certification that all required submissions have been made to ClinicalTrials.gov.

11.1.6 Document and Record Inspections

The study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

11.2 SOURCE DOCUMENTS

11.2.1 Procedures will ensure that source data meet the “ALCOA” standards: Attributable, Legible, Contemporaneous, Original, and Accurate.

11.2.2 Sources of Data

A) Recruitment and Screening

(1) the initial pre-screen,
(2) telephone/zoom or in-person psychological screening evaluation comprised of questionnaires to obtain demographic information, self-rated symptom ratings, and a psychiatric diagnostic interview
(3) in-person medical screening evaluation that includes a a) review of medical history and systems, b) cognitive assessment, c) physical examination to assess current physical health with vital signs, EKG, urine toxicology and saliva alcohol test strip results, urine pregnancy results (women), and clinical urine and blood laboratory results, d) hearing and color vision test results, e) biological female scheduling results, f) RAVLT results
(4) Source data and documents to obtain medical history for inclusionary and exclusionary purposes may also include hospital records, clinical and office charts, clinical laboratory results, pharmacy dispensing records.

B) PK Studies
(1) Day 1 and 2 pre-experimental a) vital signs, b) urine toxicology and saliva alcohol test strip results, c) urine pregnancy results and menstrual phase information (women)
(2) Day 1 a) pharmacy drug storage, dispensing and destruction records, b) drug administration records, c) vital signs and continuous pulse oximetry results immediately before and after drug dispensing until discharge, d) focused physical and neurology examination prior to discharge, e) blood renal (creatinine) safety results, f) research blood biomarker data, g) results of non-aversive episodic memory testing (if the paradigm is ready)
(3) Day 2 a) symptom reports, b) blood biomarker data, c) blood renal (creatinine) safety results, d) results of non-aversive episodic memory testing
(4) Adverse event reports

C) Expt. 1 and Expt. 2
(1) Day 1, 2, and 3 pre-experimental a) vital signs, b) urine toxicology and saliva alcohol test strip results, and c) urine pregnancy results and menstrual phase information (women)
(2) Day 1 a) blood biomarker data
(3) Day 2 a) pharmacy drug storage, dispensing and destruction records, b) drug administration records, c) vital signs and continuous pulse oximetry results immediately before and after drug dispensing until discharge, d) focused physical and neurology examination prior to discharge, e) blood renal (creatinine) safety results, f) results from psychophysiology tests of fear conditioning, extinction or reconsolidation blockade, extinction retention or confirmation of reconsolidation blockade and conditioned fear reinstatement, g) blood biomarker data, h) results of non-aversive episodic memory testing
(4) Day 3 a) symptom reports, b) blood biomarker data, c) blood renal (creatinine) safety results, d) results of non-aversive episodic memory testing
(5) One-week follow-up symptom reports
(6) Adverse event reports

Clinical laboratory tests (*not urine toxicology or saliva alcohol test strip results*) generated by the methods described in the protocol will be recorded in the subjects' medical records (for WSU: if indicated “yes” on ICFs) and/or study progress notes. Data may be transcribed legibly on CRFs supplied for each subject or directly inputted into an electronic system or any combination thereof.

11.3 CASE REPORT FORMS

The study case report form (CRF) will be the primary data collection instrument for the study. All data requested on the CRF will be recorded. All missing data will be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, “N/D” will be written. If the item is not applicable to the individual case, “N/A” will be written. All entries will be printed legibly in black ink. If any entry error has been made, to correct such an error, a single straight line will be drawn through the incorrect entry and the correct data will be entered above it. All such changes will be initialed and dated. There will be no erasures or white-out on CRFs. For clarification of illegible or uncertain entries, the clarification will be printed above the item, then initialed and dated. The following source data will be recorded directly on the CRFs: biological females scheduling results, demographics, neuropsychiatric assessments, physical and neurological examination, hearing test results, color vision test results, EKG, laboratory results, pharmacy drug dispensing form, session flow sheets, adverse event reports.

11.4 STUDY RECORDS RETENTION

Study records, including documentation of informed consent of subjects will be retained for at 7 years after completion of the study in accordance with BMC/BUMC requirements. Such records will be preserved in hardcopy, electronic or other media form and will be accessible for inspection and copying by authorized individuals.

Drug/Biologics: In accordance with FDA requirements for IND research, the sponsors and investigators will retain FDA required records and reports for 2 years after a marketing application is approved for the drug; or if an application is not approved for drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA so notified

12 STATISTICAL PLAN

12.1 STUDY HYPOTHESES (not null)

Aim 1: Compare IV Allo vs. placebo effects on extinction retention in chronic PTSD (Expt. 1).

- *Hyp-1:* IV Allo vs. placebo given at completion of extinction training on Day 2 (to raise plasma Allo to resting levels previously associated with optimal extinction retention) will enhance extinction retention on Day 3.
- *Hyp-2:* IV Allo vs. placebo infusion given at completion of extinction training on Day 2 will reduce conditioned fear reinstatement after US re-exposure on Day 3.
- *Exploratory Hypotheses:* 1) Resting Allo+PA levels before or 3 hours after extinction training will correlate positively with extinction retention. 2) The ratio of Allo+PA to: a) the sum of their 3β-isomers, or b) DHEA, NSs that allosterically antagonize $GABA_A$ receptors, will be positively associated with extinction retention.

Aim 2: Compare IV Allo vs. placebo effects on reactivated fear memory *reconsolidation* in chronic PTSD (Expt. 2).

- *Hyp-1:* A high dose IV bolus of Allo vs. placebo given immediately after re-exposure to a single CS+ (no US) on Day 2 will significantly decrease conditioned fear on Day 3.
- *Hyp-2:* A high dose IV bolus of Allo vs. placebo given immediately after re-exposure to a single CS+ (no US) on Day 2 will prevent conditioned fear reinstatement on Day 3.

Exploratory Aims: Evaluate effects of low vs. high dose IV Allo vs. placebo on *non-aversive episodic memory consolidation* and *inflammation* in chronic PTSD**.**

A) Non-aversive source memory
Hyp: Delayed recall, recognition and source memory of pictorial stimuli encoded on Day 2, 60' before low (Expt. 1) or high (Expt. 2) dose IV Allo or placebo will be better in the Allo- vs. placebo-treated participants tested on Day 3.

B) Inflammation
Hyp-1: IV Allo vs. placebo given on Day 2 will reduce inflammation across time on Day 2.
Hyp-2: IV Allo vs. placebo given on Day 2 will reduce inflammation on Day 3.
Hyp-3: High dose IV Allo (Expt. 2) will be more effective in reducing inflammation on Day 3 compared to low dose IV Allo (Expt. 1).

12.2 SAMPLE SIZE DETERMINATION

Power calculations were conducted for primary Aims 1 and 2 so as to observe an equal variance t-test effect of Cohen's d=0.5 or larger with a two-tailed $p < 0.05$ and ≥ 80% power. For both Expt. 1 and Expt. 2, the primary analyses will compare the effects of IV Allo to placebo collapsing across the male, eFol phase female and mLut phase female subgroups. Although we anticipate that the IV Allo infusion will perform better than placebo, we selected a two-tailed p-value to be conservative. Additionally, we selected a conservative medium effect size using a Cohen's d=0.50, even though results from Brunet et al. (2018), the rodent research depicted in the Preliminary Studies section, and our preliminary results in women for the association between resting Allo levels or the Allo/DHEA ratio and extinction retention suggest that we might observe larger effect sizes. The univariate t-tests comparing the IV Allo infusion to placebo for each of the primary outcome scores (Aim 1 for Expt. 1 and Aim 2 for Expt. 2) require a total sample size of 128 (n=64 for each treatment arm). Applying a conservative attrition rate of 20%, we will enroll and randomize up to 154 participants to ensure a final total sample of 128 participants who complete each of the 3-day experiments. In order to ensure adequate recruitment, the study is being conducted at 2 sites. Expt. 1 and Expt. 2 will each recruit and run subjects over 2 years. Thus, each site will be expected to complete 128 subjects/2 years/12 months = 5 subjects per month or ~1-2 subjects per week. To accomplish this, multiple streams of recruitment will be used at both sites.

12.3 STATISTICAL METHODS

The primary analysis for each aim will use an intent-to-treat (ITT) approach (i.e., all randomized subjects will be used in the analysis as long as the outcome measure of extinction retention or reconsolidation blockade on Day 3 has been acquired). ITT is optimal since it provides the least biased estimate of the treatment effect—and the randomization balances IV Allo and placebo groups on known and unknown baseline confounders. We will implement quality assurance procedures to minimize protocol deviations and ensure correct treatment assignment according to the randomization. We do not anticipate non-compliance to treatment due to blinding and single administration of the assigned treatment; thus, we do not anticipate that a per-protocol analysis will be required. We will address missing data, under the assumption of missing at random, using multiple imputation. If age, race/ethnicity, baseline Allo+PA levels, and history of mild TBI, are associated with both the treatment group (see Section on Baseline Comparability) and outcome measures, the final statistical models will be adjusted for these factors.

For each primary aim we will first conduct t-tests comparing the IV Allo and placebo groups on extinction retention (Aim 1, Hyp-1 and 2) and reconsolidation blockade (Aim 2) outcomes at the end of Expt. 1 and Expt. 2, respectively. While we do not anticipate a significant interaction by site, we will evaluate the 2-way interaction for the treatment group and site using mixed models (PROC MIXED in SAS). If there are no differences by site and the interaction term is not significant, we will use ANOVA/ANCOVA for the remaining analyses. If there are any imbalances by age, race/ethnicity, baseline Allo+PA, or history of mild TBI, we will consider the factor as a confounder in the ANCOVA model. We will assess the assumptions of linearity, normality, and homogeneity of variance. If there are any violations, we will apply a transformation of the outcome measure or use a non-parametric approach as necessary. As an exploratory sub-analysis, we will repeat the analyses in Aims 1 and 2 within each subgroup (men, eFol women, eLut women). These analyses will provide estimates of effect size that can be used, if needed, to power future RCTs evaluating the efficacy of IV Allo vs. placebo in these subgroups.

In Aim 1, exploratory Hyp-3, we will compute correlation between extinction retention and the following NS: (1) resting Allo+PA; and (2) ratios of Allo+PA to a) their 3β-isomers, or b) DHEA. We will check for normality and use Pearson or Spearman correlation based on whether or not that assumption is met. For the reconsolidation experiment (Aim 2), one interim analysis (as described above under **Section 10 "STOPPING RULES"**) and one final analysis for efficacy are planned.

For continuous variables, the mean, median, standard deviation, minimum, maximum, interquartile range, and sample size for each intervention group will be reported for each treatment group. For categorical variables, the frequency distribution will be reported for each treatment group.
We do not anticipate any substantial differences between the IV Allo vs. placebo intervention groups given the eligibility restrictions on clinically important confounders and use of randomization. We will use t-tests and chi-square tests to determine if the IV Allo and placebo groups are balanced on age, race/ethnicity, Allo+PA, and history of mild TBI at the baseline assessment and adjust for these confounders as appropriate.

For the Exploratory Aim A, we will first conduct t-tests to compare the effects of low dose IV Allo vs. placebo (Expt. 1) and high dose IV Allo vs. placebo (Expt. 2) on delayed recall, recognition (hits-false alarm scores), and source memory outcomes. We do not expect differences in immediate recall as a function of treatment, but if such differences were to be observed, we will consider it as a confounder in an ANCOVA model. Additionally, we will conduct a 2-way ANOVA evaluating the effect of low vs. high dose IV Allo across the gender subgroups. Similar methods will be used to evaluate Exploratory Aim B addressing potential effects of IV Allo vs. placebo on the inflammatory factors of interest.

For the Exploratory Aims (A and B), we will use analysis methods as described for Expt. 1.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

This study is to be conducted according to applicable US federal regulations and institutional policies (which are based in federal regulations, guidance, and ICH Good Clinical Practice guidelines).
This protocol and any amendments will be submitted to the BMC/BUMC IRB, which is the single IRB of record for this 2-site study, for formal approval of the study conduct. WSU will set up a Reliance Agreement with the BMC/BUMC IRB. The decision of the IRB concerning the conduct of the study will be made in writing to the principal investigator at the Boston site, who will share the decision with the WSU PI. A copy of the initial IRB approval letter will be provided to the sponsor (NIMH) and the FDA before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The consent form will be submitted with the protocol for review and approval by the IRB. The consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. Consent will be documented as required by the IRB.

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15 APPENDIX

15.1 SCHEDULE OF EVENTS

15.1.1. Schedule of Events for PK Studies

Schedule of Events	Recruitment and Screening				Experiment 1 PK Study		Experiment 2 PK Study	
	Phone Pre-Screen	Psych Screen	Medical Screen	Before Day 1	Day 1	Day 2	Day 1	Day 2
Pre-Screening Questionnaire	X							
Informed Consent		X[1]	X[1]					
ISI: sleep		X						
PHQ-9: depression		X						
CSSRS: risk self/others harm		X	X		X	X	X	X
LEC-5: trauma inventory		X						
BAT-L: TBI screen		X						
PCL-5: self-rated PTSD		X						
CAPS-5: clinician-rated PTSD (Dx & severity)		X						
SCID-5 (DSM-5 Dx)		X						
PANAS: affect		X						
CADSS: dissociation		X						
SF-12: Health Survey		X						
STAXI-2: trait anger		X						
Rapid COVID-19 Test		X	X		X	X	X	X
Cognitive Battery			X					

Schedule of Events	Recruitment and Screening				Experiment 1 PK Study		Experiment 2 PK Study	
	Phone Pre-Screen	Psych Screen	Medical Screen	Before Day 1	Day 1	Day 2	Day 1	Day 2
Rey Auditory Verbal Learning Test			X					
Color Vision Test			X					
Urinalysis			X					
Urine Cotinine Test			X		X	X	X	X
Urine Toxicology			X		X	X	X	X
Urine Pregnancy Test			X		X	X	X	X
Saliva Alcohol Test			X		X	X	X	X
Pulse Oximetry: Baseline Measurement			X		X		X	
Blood Collection: Clinical			X All		X Renal	X Renal	X Renal	X Renal
EKG			X					
Review of Medical Systems			X		X	X	X	X
Physical & Neurological Examination			X					
Urine LH Surge & PdG Testing for Scheduling & Eligibility of Women[2]				X				
Qualitative Sedation Rating					X	X	X	X
Monk VAS: sleepiness scale					X	X	X	X
IV placement					X		X	

Schedule of Events	Recruitment and Screening				Experiment 1 PK Study		Experiment 2 PK Study	
	Phone Pre-Screen	Psych Screen	Medical Screen	Before Day 1	Day 1	Day 2	Day 1	Day 2
Research Blood Collect Neurosteroids/Other Neurobiological Factors					X	X	X	X
By IV line					X		X	
By needle stick						X		X
IV Allopregnanolone Infusion					X		X	
Continuous Pulse Oximetry with an Alarm & Close Observation by MD/NP/Nurse					X		X	
Record AEs		X	X		X	X	X	X
Episodic Memory Test (if the paradigm is ready)					X	X	X	X

[1] If phone/in-person psychological screening evaluation or in-person medical screening evaluation is the first study visit, obtain informed consent.

[2] Women are scheduled for the PK Expt. 1 or Expt. 2 Days 1 and 2 study procedures either during the early follicular phase (eFP) or mid-luteal (mLP) of the menstrual cycle. Our group has extensive experience and routine success with such scheduling using the following procedures: eFP testing will occur on Days 2-6 after onset of menses when plasma progesterone and estrogen levels are both low. The mLP occurs 6-10 days after the luteinizing hormone (LH) surge (Days 19-23 of a standard cycle). The LH surge, which signals impending ovulation, is detected via a commercial urinary test kit. Women test their first morning urine and urine once in the afternoon or evening with the kit dipsticks starting 6 days after onset of menses and continuing until the LH surge occurs, usually day 13-15 of a 28-day cycle. Women using hormonal IUDs or a device like Nuvaring also will be asked to check their urine for pregnanediol glucuronide (PdG) levels for 4 consecutive days starting 7 days after a positive LH surge test to confirm ovulation. Menstrual phase will be confirmed at the end of the study by measuring progesterone and estradiol levels. Data from subjects without phase-appropriate eFP and mLP steroid levels will be excluded from data analyses.

15.1.2. Schedule of Events for Expt. 1 and Expt. 2.

Schedule of Events	Recruitment and Screening				Experiment 1 ~Jan. 2021-Dec. 2022			Experiment 2 ~Jan 2023-Dec. 2025			1-Wk Follow Up
	Pre-Screen	Psych Screen	Medical Screen	Before Day 1	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	~Day 10
Pre-Screening Questionnaire	X										
Informed Consent		X[1]	X[1]								
ISI: sleep		X			X			X			X
PHQ-9: depression		X			X			X			X
CSSRS: risk self/other harm		X	X		X	X	X	X	X	X	X
LEC-5: trauma inventory		X									
BAT-L: TBI screen		X									
PCL-5: self-rated PTSD		X			X			X			X
CAPS-5: clinician-rated PTSD (Dx & severity)		X									
SCID-5 (DSM-5 Dx)		X									
PANAS: affect		X									X
CADSS: dissociation		X									
SF-12: Health Survey		X									
STAXI-2: anger		X									
Rapid COVID-19 Test		X	X		X	X	X	X	X	X	
Color Vision Test			X								
Cognitive Battery			X								
Rey Auditory Verbal Learning Test			X								

Schedule of Events	Recruitment and Screening				Experiment 1 ~Jan. 2021-Dec. 2022			Experiment 2 ~Jan 2023-Dec. 2025			1-Wk Follow Up
	Pre-Screen	Psych Screen	Medical Screen	Before Day 1	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	~Day 10
Hearing Test			X								
Saliva Alcohol Test			X		X	X	X	X	X	X	
Clinical Urinalysis			X								
Urine Toxicology			X		X	X	X	X	X	X	
Urine Cotinine Test			X		X	X	X	X	X	X	
Urine Pregnancy Test			X		X	X	X	X	X	X	
Pulse Oximetry Baseline Measurement			X			X			X		
Blood Collection: Clinical (Needle Stick or IV)			X All			X Renal	X Renal		X Renal	X Renal	
ECG			X								
Review of Medical Systems			X		X	X	X	X	X	X	
Physical & Neurological Examination			X								
Urine LH Surge & PdG Testing for Eligibility & Scheduling of Women[2]				X							
Qualitative Sedation Rating					X	X	X	X	X	X	
Monk VAS: sleepiness scale					X	X	X	X	X	X	
Sickness Questionnaire					X			X			X
BPI: pain inventory					X			X			X
IV placement						X			X		
Collect Research Blood					X	X	X	X	X	X	

Schedule of Events	Recruitment and Screening				Experiment 1 ~Jan. 2021-Dec. 2022			Experiment 2 ~Jan 2023-Dec. 2025			1-Wk Follow Up
	Pre-Screen	Psych Screen	Medical Screen	Before Day 1	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	~Day 10
By IV line						X			X		
By needle stick					X		X	X		X	
IV Allo vs. Placebo						X			X		
Continuous Pulse Oximetry with an Alarm & Close Observation by MD/NP/Nurse						X			X		
Record AEs		X	X		X	X	X	X	X	X	
Episodic Memory Test						X	X		X	X	
Fear Acquisition					X			X			
Fear Extinction						X					
Test for Extinction Retention							X				
Reconsolidation Blockade (Single CS+ without US)									X		
Test for Reconsolidation Blockade							X			X	
Test for Fear Reinstatement							X			X	

[1] If phone/in-person psychological screening evaluation or in-person medical screening evaluation is the first study visit, obtain informed consent.

[2] Women are scheduled for the Expt. 1 or Expt. 2 Days 1, 2 and 3 psychophysiology procedures either during the early follicular phase (eFP) or mid-luteal (mLP) of the menstrual cycle. Our group has extensive experience and routine success with such scheduling using the following procedures: eFP testing will occur on Days 2-6 after onset of menses when plasma progesterone and estrogen levels are both low. The mLP occurs 6-10 days after the luteinizing hormone (LH) surge (Days 19-23 of a standard cycle). The LH surge, which signals impending ovulation, is detected via a commercial urinary test kit. Women test their first morning urine and urine once in the afternoon or evening with the kit dipsticks starting 6 days after onset of menses and continuing until the LH surge

occurs, usually day 13-15 of a 28-day cycle. Women using hormonal IUDs or a device like Nuvaring also will be asked to check their urine for pregnanediol glucuronide (PdG) levels for 4 consecutive days starting 7 days after a positive LH surge test to confirm ovulation. Menstrual phase will be confirmed at the end of the study by measuring progesterone and estradiol levels. Data from subjects without phase-appropriate eFP and mLP steroid levels will be excluded from data analyses.

15.2 NIMH DSMB CHARTER

15.2.1. Charter for the National Institute of Mental Health Constituted Data and Safety Monitoring Boards (DSMBs) December 2016

The National Institutes of Health (NIH) requires that each of its Institutes and Centers (IC) have a system in place for the appropriate oversight and monitoring of the conduct of clinical trials to ensure the safety of participants and the validity and integrity of the data for NIH- supported and conducted clinical trials[1]. Data and safety monitoring of a clinical trial is to be commensurate with the risks posed to study participants and with the nature, size and complexity of the study. In 1999, NIH issued a policy announcement[2] requiring all multi-site trials with data safety monitoring boards (DSMB) to forward summary reports to each Institutional Review Board (IRB) involved in the study. In addition, this policy announcement noted that NIH-funded Project Directors/Principal Investigators (PD/PI(s)) are to identify the DSMB to the IRB and ensure reports of assessments of adverse events are transmitted from the DSMB to each IRB. NIH also issued Further Guidance on Data and Safety Monitoring for Phase I and Phase II Trials in which investigators are required to submit a data and safety monitoring plan for phase I and II clinical trials to the funding IC before the trial begins[3].

The establishment of a DSMB is required for multi-site clinical trials involving interventions that entail potential risk to the participants. The data and safety monitoring functions and oversight of such activities are distinct from the requirement for study review and approval by an IRB. An IC may elect to either conduct the monitoring of ongoing studies or delegate such functions to the research funding recipient. While the IC may delegate the monitoring functions, it cannot delegate oversight of the monitoring activities.

National Institute of Mental Health (NIMH) funded clinical trials (including extramural trials funded via grants/cooperative agreements/contracts, intramural trials, and intramural-extramural collaborations), may be assigned to an NIMH-constituted Data and Safety Monitoring Board (NIMH DSMB) for monitoring of data integrity and participant safety. The NIMH DSMB will be responsible for ensuring that appropriate monitoring systems are in place, that the quality of the monitoring activity is adequate, and that the IRB, PDs/PIs, and NIMH staff are informed of recommendations emanating from monitoring activities overseen by the NIMH DSMB. Trials that involve interventions of significantly increased risk, include vulnerable subjects, treatment delays, medication washouts and/or are of large and/or complex nature may also be assigned for review by a NIMH DSMB. The NIMH Director may assign any study to the NIMH DSMB at his/her discretion.

The NIMH DSMBs are managed under the auspices of the Office of Clinical Research (OCR) in the NIMH Office of the Director (OD). Each NIMH DSMB has a Scientific Administrator (SA) assigned from the Human Research Protections Branch (HRPB) from the NIMH OCR who serves in an Executive Secretary capacity and is responsible for the coordination of all NIMH DSMB activities. The responsibilities of the NIMH DSMB outlined herein are in addition to (and not in lieu of) IRB, Office for Human Research Protections (OHRP) and Food and Drug Administration (FDA) requirements, and any additional applicable NIH policies guidelines.

Scope of Responsibilities
The NIMH DSMB provides monitoring of NIMH-sponsored clinical trials via independent, non-conflicted review of protocols, consent documents, adverse events, recruitment, retention, plans for and results of interim analyses, and safety/outcome data. The DSMB will determine if any study procedures should be altered or stopped based on indications of clinical benefit or harm to trial participants that are attributable to the interventions under evaluation. The NIMH DSMB has decisional authority over studies under review.

Examples of trials that may be considered for assignment to an NIMH DSMB include, but are not limited to, the following:

- Studies that involve high risk interventions, include vulnerable subjects, and/or are of large and/or complex nature
- Phase III trials
- Phase I or II trials that involve multiple clinical sites, are blinded, and/or employ particularly high-risk interventions or vulnerable populations
- Trials that have potential concerns regarding the financial interests of study investigators that may impact subject safety or data integrity

Membership
Membership on the NIMH DSMBs reflects the scientific disciplines and medical specialties necessary to interpret the safety of enrolled subjects and the integrity of accumulating data from the trials being monitored. All NIMH DSMB members and Chair(s) are appointed by the NIMH Director or designee. Appointed NIMH DSMB members serve 3-year terms of service which can be renewed. The typical NIMH DSMB committee roster is comprised of between three and eight members with one of those members serving as the Chair. *Ad hoc* advisors may serve as voting members for the review of specific studies to expand expertise as appropriate for the trial. To hold an official NIMH DSMB meeting a quorum consisting of at least half of the voting members on the roster must participate.

Conflict of Interest (COI): No member of the NIMH DSMB will participate in the oversight of a study in which they have direct involvement. Furthermore, members are required to declare financial, proprietary, professional, or other interests that may affect his/her impartial, independent decision-making. DSMB members may be affiliated with the investigator's institution or other participating sites, but cannot be a scientific collaborator or co-author, supervisor, mentor/mentee, subordinate of the investigators, or a member of the investigator's institutional department within the last three years. Members may recuse themselves in the case of such conflicts or appearance of conflicts. Some examples of conflicts of interest that may be noted for a NIMH DSMB member include the following:

- Involvement in the research project under consideration and/or planned co-authorship on a paper reporting the results of the trial;
- Having an employment position that may affect the study in other ways than through the NIMH DSMB work; and/or
- Serving as a lead investigator on a different but similar trial.

Confidentiality: At time of their appointment to the NIMH DSMB, each member will affirm to uphold confidentiality of the clinical trials data under review by the NIMH DSMB. Each NIMH DSMB member will agree not to discuss any data, deliberations, or decisions of the NIMH DSMB. Confidential interim data

will be distributed on a need to know basis. A proviso to uphold confidentiality requirements is announced at the beginning of each NIMH DSMB meeting and is noted in the NIMH DSMB meeting report summary.

Management/Meeting Planning
The SA oversees membership, meeting planning, and agenda development, as well as distribution of materials to be reviewed at the meeting. Following the meeting, the SA prepares and distributes the meeting summary report to NIMH staff and PD/PIs. The SA also serves as the primary point of contact for inquiries regarding the NIMH DSMB.

Each NIMH DSMB will meet at least two to four times per year (depending upon the nature of the study under review). NIMH DSMB meeting are typically held via teleconference or a web-based format, with the NIMH aiming to convene at least one in-person meeting per year in North Bethesda, Maryland. The meetings dates are determined on an annual basis. Ad hoc meetings may be called outside the normal time frame for matters that warrant the NIMH DSMB's immediate discussion. Such meeting logistics will be determined based upon clinical urgency and the availability of NIMH DSMB members.

Meeting Format
Typically, there are three types of meeting formats that may occur: Open Session, Closed Session, and Executive Session.

Open session: This session is open to observers, including investigators, other study staff, NIMH staff and program staff (and potentially staff from other ICs). Discussions during the open session include the general conduct and progress of the study such as participant accrual, participant demographics and other baseline characteristics, data quality control, adherence to the protocol, retention, and follow-up. Outcome results should not be discussed during open session- only non-confidential information may be discussed during this session. If unexpected sensitive issues should arise, the chair may call for the session to be closed or table the discussion until the closed session.

Closed session: Participation in this session is limited to NIMH DSMB members, designated ad-hoc reviewers, the assigned NIMH Program Official (PO), the assigned Clinical Trial Operations and Biostatistics Branch (CTOBB) study liaison, and the DSMB Scientific Administrator (including scientific administrator backup). NIMH senior staff may be invited by the DSMB to attend a closed session to provide their specific expertise on an issue under discussion, but Investigators, NIH scientific collaborators, and general NIMH staff are not invited to attend. Discussions during the closed session include the review of confidential material such as study progress and safety data used in formulating NIMH DSMB decisions regarding the continuation of a study.

Executive Session: This session is called for by NIMH DSMB members and includes only DSMB members. The SA will attend unless recused by the DSMB chair. In the instance that the SA does not attend this session, it is the responsibility of the DSMB Chair to summarize the discussion in the meeting summary report. This session may be used to discuss unblinded and comparative interim data.
In some circumstances, identification of treatment groups may be necessary for meaningful discussion and interpretation of results. In situations when a NIMH DSMB requests that data be unblinded, the treatment codes will be provided but will only be revealed to the voting members of the NIMH DSMB in attendance, and the SA. The DSMB has a right to view unblinded data at its discretion. In the event that the DSMB is unblinded, the CTOBB study liaison will ensure that the DSMB deliberations do not unblind the investigators.

Deliberation Procedures

During the NIMH DSMB meeting, the Chair will call for a review of the issues and, as necessary, put each study to a vote. While consensus is desirable, a majority vote (with 50% or more of the voting members participating as a minimum quorum) will constitute a decision. If one or more members disagree with the majority decision, that concern(s) will be documented as a minority opinion statement in the NIMH DSMB meeting summary report.

With the vote on new clinical trials, NIMH DSMB members review, then approve, disapprove, defer, or require modifications or revisions to NIMH-sponsored clinical trial protocols, consent documents, and other study-related documents.

In the monitoring of ongoing trials, a NIMH DSMB may vote for continuation, to defer for further information, to suspend, or to terminate all or parts of a study under NIMH DSMB review. When a termination decision has been made by a NIMH DSMB, this decision will be conveyed expeditiously to the NIMH Director and relevant NIMH staff. In such circumstances, the report must explicitly state the reason(s) for discontinuation of all or part of a study. The NIMH Director has the opportunity to request further information from the NIMH DSMB regarding the vote, to ask the NIMH DSMB to consider further data/information, and/or to request another vote following the consideration of further data/information. If, after responding to the NIMH Director's request, the NIMH DSMB's conclusion remains to discontinue the study, then the NIMH Director, CTOBB study liaison, and NIMH Program Officer are notified of this decision by the Scientific Administrator.

Meeting Reports

Meeting reports will be prepared by the NIMH SA, signed off by the DSMB chair, and provided to the NIMH Director and Deputy Director within four weeks following the corresponding meeting. The Director or Deputy Director will not receive the report for a given study if they have a conflict of interest. The study- specific meeting summaries will also be sent to the assigned NIMH Program Officer and CTOBB study liaison.

In fulfillment of the requirement for NIMH DSMBs to report their evaluations to the Institutional Review Board, the NIMH Scientific Administrator will also distribute a statement documenting the occurrence of the meeting reflecting any concerns about the continuation of the study and/or any emerging safety concerns to CTOBB and/or the program officials responsible for programmatic monitoring of the NIMH-funded study. CTOBB staff will distribute this document to the PD/PI(s) and the PD/PI(s) is required to forward the document to his/her IRB of Record.

This charter should be reviewed/updated every 3 years.

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[1] National Institutes of Health. "NIH Policy for Data and Safety Monitoring (NOT-98-084)," last modified June 10, 1998, http://grants.nih.gov/grants/guide/notice-files/not98-084.html

[2] National Institutes of Health. "Guidance on Reporting Adverse Events to Institutional Review Boards for NIH- Supported Multicenter Clinical Trials (NOT-99-107)," last modified June 11, 1999, http://grants.nih.gov/grants/guide/notice-files/not99-107.html

[3] National Institutes of Health. "Further Guidance on a Data and Safety Monitoring for Phase I and Phase II Trials (NOT-00-038)," last modified June 5, 2000, http://grants.nih.gov/grants/guide/notice-files/NOT-OD-00-038.html

15.3 SUICIDE AND SELF HARM RISK MANAGEMENT PLAN

Risk to self or others could be spontaneously reported by a participant or may be indicated by the participant's answer to questions on either of two of the neuropsychiatric assessments (see below) administered by the research coordinator or other qualified research team member. Both assessments are administered at the in-person/telephone/Zoom psychological screening evaluation, Day 1 visit, and 1-week follow-up telephone/Zoom visit. The Columbia Suicide Severity Rating Scale (CSSRS) is also administered at each in-person study session.

The Patient Health Questionnaire (PHQ-9) is self-reported and asks:

"Over the last 2 weeks, how often have you been bothered by…
#9 Thoughts that you would be better off dead, or of hurting yourself:
____ 0- not at all ____ 1- several days ____ 2 more than half the days ____ 3- everyday"

The CSSRS is a structured interview that assesses past and current risk to self/others and is currently recommended for use in clinical psychiatry treatment trials.

The research coordinator or other qualified research team member will review the filled-out measures before the participant ends the telephone/Zoom visit or leaves the Boston University Medical Campus (BUMC) General Clinical Research Unit (GCRU) or Boston Medical Center (BMC)/Wayne State University (WSU) Clinical Research Service Center (CRSC) to ensure that potential risk to self/others is immediately evaluated (see below).

If a participant a) spontaneously reports potential risk to self/others, b) endorses any answer other than "0-not at all" for the PHQ-9, or c) endorses risk to self/others within the past year on the CSSRS at screening (or change in that status after screening, indicating recent/current risk), the procedures below will be followed:

1. If the participant is in the BUMC GCRU or at BMC or the WSU CRSC, the research coordinator will contact one of the clinically qualified investigators (e.g., psychiatrist/psychologist/social worker) to evaluate the participant.
2. For phone/Zoom calls, the assessor (e.g., coordinator) will: a) have ascertained ahead of time that a clinically qualified investigator is available by phone or in-person for emergency consultation if needed, and b) obtain the physical location of the participant at the onset of the phone/Zoom call. If the assessor is told of possible risk to self/others by the participant, they will strive to keep the person on the phone/Zoom while contacting the clinically qualified investigator (or if available, psychiatric social worker on the unit), to help evaluate the patient. If in the interim, the patient ends the call, the social worker or clinically qualified investigator would assist in making a determination about calling 911.

Possible Evaluation Outcomes:

1. If the participant is determined not to be at imminent risk of self/other harm, the participant will be referred for mental health services as appropriate and told how to get emergent help in the future, if needed: i.e., a) call 911 or the National Suicide hotline at 988, or b) go to the BMC emergency room or an emergency room closer to where the participant lives. If the individual no longer qualifies for the study based on risk to self/others, appropriate steps to end participation in the study will be taken.
2. If a participant is thought to present an imminent risk to self/others (per procedures outlined above) the individual will be accompanied or transported to the emergency room (against their will if necessary) for further evaluation; in addition, their provided will be contacted.

Note: The participant is informed in the ICF that reporting potentially imminent risk to self/others will eventuate in the procedures above, which may lead to loss of confidentiality with regard to participation in the study and risk status.