

# Associations between PTSD-Related extinction retention deficits in women and plasma steroids that modulate brain GABA<sub>A</sub> and NMDA receptor activity

Suzanne L. Pineles<sup>a,b,\*</sup>, Yael I. Nillni<sup>a,b</sup>, Graziano Pinna<sup>c</sup>, Andrea Webb<sup>d</sup>,  
Kimberly A. Arditte Hall<sup>e</sup>, Jennifer R. Fonda<sup>f,g</sup>, John Irvine<sup>h</sup>, Matthew W. King<sup>a,b</sup>,  
Richard L. Hauger<sup>i,j</sup>, Patricia A. Resick<sup>k</sup>, Scott P. Orr<sup>l</sup>, Ann M. Rasmusson<sup>a,b</sup>

<sup>a</sup> National Center for PTSD Women's Health Sciences Division at VA Boston Healthcare System, Boston, MA, 02130, USA

<sup>b</sup> Department of Psychiatry, Boston University School of Medicine, Boston, MA, 02118, USA

<sup>c</sup> The Psychiatric Institute, Department of Psychiatry, University of Illinois at Chicago, Chicago, IL, 60612, USA

<sup>d</sup> The Charles Stark Draper Laboratory, Inc., Cambridge, MA, 02139, USA

<sup>e</sup> Framingham State University, Framingham, MA, 01701, USA

<sup>f</sup> Translational Research Center for TBI and Stress Disorders (TRACTS) and Geriatric Research, Education and Clinical Center (GRECC), VA Boston Healthcare System Boston, MA, 02130, USA

<sup>g</sup> Department of Epidemiology, Boston University School of Public Health, Boston, MA, 02118, USA

<sup>h</sup> The MITRE Corporation, Bedford, MA, 01730, USA

<sup>i</sup> Center of Excellence for Stress and Mental Health, San Diego VA Healthcare System, San Diego, CA, 9216, USA

<sup>j</sup> Department of Psychiatry, School of Medicine, University of California, San Diego, La Jolla, CA, 92093, USA

<sup>k</sup> Department of Psychiatry and Behavioral Sciences, Duke University, Durham, NC, 27701, USA

<sup>l</sup> Psychiatry Department, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA, 02129, USA

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## ABSTRACT

Several studies have demonstrated poor retention of extinction learning among individuals with posttraumatic stress disorder (PTSD). Gonadal hormone signaling in brain appears to influence the retention of extinction learning differently in women with and without PTSD. Women with PTSD, compared to trauma-exposed women without PTSD, show relative deficits in extinction retention during the mid-luteal phase (mLP) of the menstrual cycle, compared to the early follicular phase (eFP). A PTSD-related reduction in conversion of progesterone to its GABAergic metabolites allopregnanolone (Allo) and pregnanolone (PA) may contribute to these findings. The current study in trauma-exposed women with (n = 9) and without (n = 9) PTSD investigated associations between extinction retention and plasma Allo + PA levels, as well as the ratio of Allo + PA to 5 $\alpha$ -dihydroprogesterone (5 $\alpha$ -DHP), the immediate steroid precursor for Allo. The study also investigated the relationship between extinction retention and the ratio of Allo + PA to dehydroepiandrosterone (DHEA), an adrenally-derived GABA<sub>A</sub> receptor antagonist. Study participants completed differential fear-conditioning during both the eFP and mLP of the menstrual cycle. Analyses revealed a strong positive relationship between resting plasma Allo + PA levels and extinction retention during the mLP in the women with, but not without, PTSD (e.g., diagnosis X Allo + PA interaction controlling for early extinction:  $\beta = -.0008$ ,  $p = .003$ ). A similar pattern emerged for the Allo + PA to 5 $\alpha$ -DHP ratio ( $\beta = -.165$ ,  $p = .071$ ), consistent with a PTSD-related block in production of Allo and PA at the enzyme 3 $\alpha$ -hydroxysteroid dehydrogenase. The ratio of Allo + PA to DHEA appeared to influence extinction retention only during the eFP when Allo + PA and DHEA levels are comparable and thus may compete for effects on GABA<sub>A</sub> receptor function. This study aligns with male rodent PTSD models linking experimental reductions in brain Allo levels to deficits in extinction retention and suggests that targeting PTSD-related deficits in GABAergic neurosteroid synthesis may be therapeutic.

## 1. Introduction

Posttraumatic stress disorder (PTSD) has been conceptualized as a

failure to recover from normative acute trauma-related symptoms (Rothbaum et al., 1992). As such, there has been growing attention to laboratory studies measuring extinction of conditioned fear and

\* Corresponding author. VA Boston Healthcare System (116B-3), Boston, MA, 02130, USA.

E-mail address: [Suzanne.Pineles@va.gov](mailto:Suzanne.Pineles@va.gov) (S.L. Pineles).

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retention of extinction learning. Several studies have shown that PTSD and normal comparison groups often, but not always, show comparable acquisition and extinction of conditioned fear, while individuals with PTSD demonstrate impaired extinction retention (Milad et al., 2008, 2009).

Given significant sex differences in the development and maintenance of PTSD (Breslau et al., 1998), it is possible that specific biological factors place women at greater risk for developing this disorder (Pineles et al., 2017). For example, hormone signaling in the central nervous system (CNS) has been shown to influence the retention of extinction learning (Lebron-Milad and Milad, 2012), and the associations appear to differ for women with and without PTSD (Pineles et al., 2016). Healthy women with relatively high levels of estradiol and/or progesterone during extinction learning better retain the learning when subsequently tested (Pineles et al., 2016; Milad et al., 2010). In contrast, women with PTSD show poorer retention of extinction learning during the mid-luteal phase (mLP) of the menstrual cycle when both estradiol and progesterone levels are high, compared to the early follicular phase (eFP) when both estradiol and progesterone are low and stable (Pineles et al., 2016). As these effects could not be attributed to PTSD-related deficits in either estradiol or progesterone, we considered whether the PTSD-related deficits in extinction learning during the mLP might be due to reduced conversion of progesterone into the stereoisomers allopregnanolone (Allo) and pregnanolone (PA), neurosteroids that equipotently and positively modulate the effects of gamma-aminobutyric-acid (GABA) at GABA<sub>A</sub> receptors (Rasmusson et al., 2006; Pineles et al., 2018).

Indeed, a marked decrease in the level of Allo + PA (measured together by gas chromatography-mass spectrometry: GC-MS) was observed in the cerebrospinal fluid (CSF) of women with PTSD in association with increased PTSD reexperiencing and depressive symptoms (Rasmusson et al., 2006). A PTSD-related decrease in the ratio of Allo + PA to 5 $\alpha$ -dihydroprogesterone (5 $\alpha$ -DHP, the immediate steroid precursor for Allo) also was found (Rasmusson et al., 2006) and was replicated in plasma (Pineles et al., 2018), suggesting the presence of a PTSD-related deficiency in the expression or activity of the enzyme 3 $\alpha$ -hydroxysteroid dehydrogenase (3 $\alpha$ -HSD). In plasma, PTSD-related decreases in this ratio were evident both at rest and after exposure to a stressful fear conditioning procedure (Pineles et al., 2018), suggesting that measurement of this ratio in a resting plasma sample might feasibly serve as a quantitative trait marker for 3 $\alpha$ -HSD deficiency in both the CNS and periphery of women with PTSD.

In turn, preclinical work has demonstrated the importance of Allo in extinction learning and retention. Allo-deficient male mice, compared to mice with normal Allo levels, are slow to extinguish conditioned contextual fear responses and exhibit greater spontaneous recovery of conditioned fear after completion of extinction learning (Pinna and Rasmusson, 2014; Pibiri et al., 2008; Locci and Pinna, 2019). Furthermore, a single systemic injection of either a synthetic Allo-derivative (ganaxolone) (Pinna and Rasmusson, 2014) or N-palmitoyl ethanolamine (PEA: a peroxisome proliferator-activated receptor-alpha agonist that induces Allo synthesis) (Locci and Pinna, 2019) to Allo-deficient mice after one brief exposure to a fear-conditioned context markedly reduced freezing during subsequent extinction training sessions and prevented the spontaneous recovery of contextual fear after completion of extinction learning. These findings suggest that Allo-based compounds might be used to block fear memory reconsolidation, correct deficits in extinction learning, and/or enhance extinction learning retention related to Allo deficiency (Pinna and Rasmusson, 2014; Locci and Pinna, 2019; Rasmusson and Pineles, 2018).

Based on these clinical and preclinical findings, we evaluated the relationship between extinction retention and deficient production of Allo and PA in women with PTSD. We are reporting results from secondary analyses of data collected during our previously published study of fear conditioning across the menstrual cycle in trauma-exposed women with and without PTSD (Pineles et al., 2016, 2018).

Specifically, we assessed whether extinction retention deficits that were most pronounced in the mLP were associated with either lower resting plasma Allo + PA levels or the ratio of (Allo + PA) to 5 $\alpha$ -DHP. We also assessed whether extinction retention deficits were related to the Allo + PA to dehydroepiandrosterone (DHEA) ratio. DHEA is an adrenally-derived neuroactive steroid that may compete with Allo and PA effects by allosteric antagonism of GABA<sub>A</sub> receptors. DHEA also facilitates the function of N-methyl-D-aspartate (NMDA) receptors (Chalbot and Morfin, 2006). Previous studies in men and follicular phase women demonstrated somewhat stronger correlations between PTSD symptoms and the CSF (Allo + PA)/DHEA ratio than CSF Allo + PA levels alone (Rasmusson et al., 2006, 2019).

As described elsewhere (Pineles et al., 2018), each study participant was tested during both the eFP and mLP. Based on our previous findings, we hypothesized that an association between Allo + PA levels or the (Allo + PA)/5 $\alpha$ -DHP ratio and extinction retention would be strongest for the women with PTSD during the mLP when PTSD-related extinction retention deficits are most pronounced (Pineles et al., 2016). We also noted that resting plasma Allo + PA and DHEA levels in our subjects were comparable during the eFP, whereas during the mLP, Allo + PA levels were about 6 times higher than DHEA levels, which do not change across the menstrual cycle (see Results section below: Table 1). This suggested that DHEA might not be able to compete well with the effects of Allo and PA on GABA<sub>A</sub> receptor function during the mLP. Therefore, we hypothesized that there would be a stronger relationship between the (Allo + PA)/DHEA ratio and extinction retention during the eFP than mLP.

## 2. Methods and materials

### 2.1. Participants

Participants included 18 trauma-exposed women ( $M_{Age} = 28.61$  years,  $SD = 8.33$ ) with PTSD ( $n = 9$ ) and without PTSD (trauma-exposed controls, TC;  $n = 9$ ), who were a subset of participants from a larger investigation examining psychophysiological responses to provocative stimuli across the menstrual cycle (Pineles et al., 2016, 2018). Participants in the current study met the inclusion and exclusion criteria for the larger study and: a) had a complete set of blood biomarkers, b) had valid skin conductance (SC) measures available for both phases of the menstrual cycle, and c) were SC responders across the 4 laboratory sessions. The reduced sample size in this secondary analysis, compared to previous papers, reflects the large number of participants who were either SC non-responders or for whom we were

**Table 1**  
Neurosteroid means and SDs by menstrual cycle phase (pg/mL).

	Early Follicular Phase		Mid-luteal Phase		P-value
	Mean	SD	Mean	SD	
<b>Overall Sample</b>					
Allo + PA	260.11	217.13	1091.61	498.69	< .001
5 $\alpha$ -DHP	854.65	675.65	932.58	610.55	.231
DHEA	190.74	126.09	170.34	102.32	.865
<b>PTSD Group</b>					
Allo + PA	179.33	139.94	1360.89	391.80	.004
5 $\alpha$ -DHP	1082.59	854.79	1208.42	716.49	.109
DHEA	164.03	154.38	145.81	95.30	.820
<b>Trauma Control Group</b>					
Allo + PA	340.89	256.74	822.33	460.23	.055
5 $\alpha$ -DHP	626.71	350.01	656.73	333.94	.945
DHEA	217.46	91.34	194.88	108.68	.820

*Note.* Allo: allopregnanolone; PA: pregnanolone; 5 $\alpha$ -DHP: 5 $\alpha$ -dihydroprogesterone; DHEA: dehydroepiandrosterone. Two participants were missing the mid-luteal phase 5 $\alpha$ -DHP measurement (1 missing in the PTSD group; 1 missing in the trauma control group). P values were determined using the Wilcoxon signed rank test.

unable to start an IV for blood draws at one laboratory session or another in the parent study (Pineles et al., 2016, 2018). Women in the PTSD group met criteria for a PTSD diagnosis based on DSM-IV criteria, as assessed with the Clinician Administered PTSD Scale (CAPS) (Blake et al., 1995), and had a total CAPS score  $\geq 40$ . Women in the TC group experienced at least one DSM-IV PTSD Criterion A traumatic event, but did not meet criteria for: a) current, partial, or lifetime PTSD, b) any other current Axis I psychological disorder, with the exception of specific phobia or public speaking phobia, or c) more than a single episode of past major depression.

Participants were required to have a normal physical exam and standard screening medical laboratory test results. Exclusion criteria included: infectious illness, organic brain disorder, major neurological problems, endocrine disorders, psychotic disorders, bipolar I disorder, active substance or alcohol abuse or dependence within the past 6 months, irregular menstrual cycle, use of hormonal contraceptives, current pregnancy, and current participation in trauma-focused psychotherapy. Psychotropic medications, as well as other medications that could affect psychophysiological responding or neuroactive steroid levels, were not allowed during the month before the experimental procedures (or longer if an antidepressant with a long half-life).

## 2.2. Fear conditioning procedures

Participants completed the fear conditioning procedures during both the eFP of the menstrual cycle, 1–5 days from the start of menses, and the mLp, 6–10 days after the luteinizing hormone (LH) surge. The menstrual cycle phase during which participants first completed the conditioning procedures was determined by random assignment.

The following procedures were used to avoid potential confounding influences on neurosteroid hormone levels: a) laboratory sessions were scheduled between 1:00 p.m. and 3:00 p.m., b) participants were asked to abstain from alcohol for 1 week, and other illicit substances for 2 weeks, prior to their visits, c) nicotine users ( $n = 2$ ) were asked to abstain from nicotine for 1 h prior to arrival at the laboratory, and d) participants were asked to abstain from caffeine and food for 4 h and 2–3 h, respectively, prior to arriving at the laboratory. Abstinence was verified at each study visit by alcohol breathalyzer and urine drug tests. Participants also took a urine pregnancy test at each study visit.

Fear acquisition (conditioning), extinction learning, and extinction retention procedures occurred on 2 consecutive days and followed a standard laboratory paradigm (Pineles et al., 2016; Orr et al., 2000). The unconditioned stimulus (UCS) was a 500-ms electric pulse generated by a Coulbourn Finger Stimulator set at a level determined by the participant to be “highly annoying but not painful” during a previous screening session and delivered through electrodes attached to the second and third fingers of the left hand. The conditioned stimuli (CSs) were represented by 2 different colored shapes (e.g., blue vs. white circles). During the acquisition phase, one colored shape was paired with the electric pulse (CS+), while the second colored shape was not (CS-). A different set of colors and shapes was used during the conditioning session that occurred in the alternate menstrual cycle phase. Participants were seated 4 feet from a monitor that displayed the 16-cm colored stimuli.

### 2.2.1. Day 1

The electrodes used to measure SC and administer the UCS were attached and participants received instructions for the study procedures. They were told that there were 3 phases during which they would see 2 different colored shapes. Although they were told that they would receive the electrical stimulus during the second phase of the task (i.e., the fear acquisition phase), they were not informed about the possible presence or absence of the electrical stimulus during the third phase (i.e., the extinction learning phase).

Next, the participant underwent a 5-min baseline recording period followed by completion of the 3 task phases: a) habituation, b)

acquisition, and c) extinction learning. During the habituation phase, participants were shown 5 presentations each of the to-be CS+ and CS- (i.e., presentation of the 2 different colored shapes without the electrical stimulus). During the acquisition phase, they were shown 5 presentations each of the CS+ and CS- with the 500-ms electrical stimulus occurring immediately after each CS + offset. During the extinction learning phase, they were shown 10 presentations of the CS+ and CS- without administration of the electrical stimulus. Each CS was presented in pseudorandom order for 8 s (inter-trial interval =  $20 \pm 5$  s).

### 2.2.2. Day 2

Following attachment of the SC recording electrodes and finger stimulator, participants were told, “Two different colored shapes will be presented on the monitor. There may also be electrical stimulus administration.” Following a 5-min baseline recording, participants viewed 10 presentations of the CS+ and CS- with a shock administered following the fifth CS + only. Because the shock was administered in the interval after the fifth CS + trial, only responses to the first 5 CS+ and CS- presentations were used to assess extinction retention.

## 2.3. Psychophysiology measures

A Coulbourn Modular Instrument System (Allentown, PA) was used to administer the conditioning protocol. SC level was measured by an Isolated Skin Conductance coupler using a 0.5 V constant direct current through 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).

### 2.3.1. Response scores

Prior to calculating SC response (SCR) scores, data were screened for recording artifacts and outliers. Next, participants were screened to ensure that they were SC responders (i.e., the average unconditioned response to the 5 electrical stimulus presentations during acquisition was  $> 0.1 \mu\text{S}$ ). All participants in this study completed the conditioning task during both menstrual cycle phases and were SC responders across the 4 laboratory sessions included in SCR analyses (Pineles et al., 2016).

SCR scores for each CS presentation were calculated by subtracting the mean SC level during the 2-s interval immediately prior to CS onset from the highest SC level recorded during the 8-s CS interval. SCR values were then square root transformed. The percentage of missing SCR scores for all participants with SC data ranged from 0 to 16% ( $M = 4.3\%$ ,  $SD = 5.0\%$ ) (Pineles et al., 2016). Missing scores were replaced using multivariate imputation by chained equations based on random forest recursive partitioning (Shah et al., 2014), and analyses reflect pooled outcomes from 5 imputed datasets.

### 2.4. Menstrual cycle phase monitoring and verification

For eFP testing, participants notified study staff when they began menses and were scheduled for their visit 1–5 days later. For mLp testing, participants tested for their LH surge using a home ovulation kit. They were scheduled for their mLp visit 6–10 days later. Menstrual cycle phase was confirmed by assay of progesterone and  $17\beta$ -estradiol levels in plasma collected prior to psychophysiological testing. For the eFP, the mean  $17\beta$ -estradiol level was 25.7 pg/mL ( $SD = 11.6$ ) and the mean progesterone level was 0.43 ng/mL ( $SD = 0.23$ ). For the mLp, the mean  $17\beta$ -estradiol level was 73.6 pg/mL ( $SD = 20.8$ ) and the mean progesterone level was 12.3 ng/mL ( $SD = 6.9$ ). Plasma concentrations of progesterone and  $17\beta$ -estradiol levels were measured by a solid-phase radioimmunoassay (RIA) (Siemens Healthcare Diagnostics, Deerfield, Ill). The progesterone assay had an intra-assay coefficient of variation (CV) of  $\sim 4\%$  and an inter-assay CV of  $\sim 6\%$ . The standard range was from 0.1 to 40 ng/mL, with an assay sensitivity of  $\sim 0.03$  ng/mL. The estradiol assay had a sensitivity of 8 pg/mL and a working range of 12–3900 pg/mL. At the 50% intercept, the intra- and inter-

assay CVs for this assay were 4% and 7%, respectively.

## 2.5. Measurement of neurosteroids

For each menstrual cycle phase, we investigated plasma Allo, PA, and 5 $\alpha$ -DHP, as well as salivary DHEA levels at a resting baseline, 45 min after placement of an intravenous line and electrodes, and before the fear conditioning and extinction task on day 1 of the 2-day laboratory paradigm. Resting day 1 eFP and mLP plasma and saliva steroid levels were used to assess associations with day 2 eFP and mLP indices of extinction retention, respectively.

Samples collected by blood draw or collection of saliva into a tube (without cotton insert) were immediately placed on wet ice and centrifuged at 3000 rpm for 20 min. Plasma (500  $\mu$ L) and saliva (1000  $\mu$ L) then were aliquotted into cryotubes tubes for storage at  $-80^{\circ}$ C. As previously described (Rasmusson et al., 2006; Pineles et al., 2018; Pinna et al., 2000), the plasma samples were extracted in ethylacetate and lyophilized, after which Allo, PA, and 5 $\alpha$ -DHP were separated using HPLC and measured by GC-MS. Saliva DHEA concentrations were measured by an enzyme-linked immunoassay (Salimetrics LLC, Carlsbad, CA) with a standard curve range of 10.2 pg/mL - 1000 pg/mL; the linear range was from 20 to 500 pg/mL. The functional sensitivity of the assay was 8.32 pg/mL DHEA. The assay's average intra-assay CV was 5.6%, while the average inter-assay CV was 8.2%. The DHEA antibody exhibits only 0.063% cross-reactivity with DHEA-sulfate.

## 2.6. Data analytic plan

The primary aim of this study was to test whether deficits in production of Allo and PA might underlie extinction retention deficits observed in women with PTSD, compared to trauma-exposed women without psychopathology.

To calculate extinction retention (Milad et al., 2010), we averaged the first 5 CS + trials and first 5 CS- trials during day 2 extinction retention testing and subtracted the average CS- SCR from the average CS + SCR. Similarly, CS + minus CS- SCR difference scores were calculated for early extinction learning and late extinction learning on day 1 (Milad et al., 2010). Extinction learning was calculated separately for early and late extinction because we'd observed increased (although not statistically significant) differential SCR during late vs. early extinction (Milad et al., 2010). We then used these variables to calculate two separate extinction retention scores for the eFP and mLP (i.e., extinction retention subtracting early extinction; extinction retention subtracting late extinction). Larger (i.e., more positive) difference scores indicate poorer extinction retention.

We calculated the mean and standard deviation for resting state biomarkers Allo + PA, 5 $\alpha$ -DHP, and DHEA, for the eFP and mLP. These measurements violated the normality assumption, as determined by the Shapiro-Wilk test and Q-Q plots. Thus, we used the Wilcoxon signed rank test to determine if there were significant differences between eFP and mLP for each biomarker. These analyses were computed in the overall sample, and within each diagnostic group (PTSD and TC).

We used a linear regression modeling to test the relative predictive effects of diagnostic group status (PTSD vs. TC), resting state biomarkers of interest, i.e., Allo + PA, (Allo + PA)/5 $\alpha$ -DHP, and (Allo + PA)/DHEA, and their interaction on extinction retention. Separate models were used to examine these relationships during the eFP and mLP. Spearman's rank-order correlations were performed between the two indices of extinction retention and the plasma biomarkers of interest for each phase of the menstrual cycle and for the PTSD and TC groups separately. These correlation coefficients were computed to assist in interpreting significant interactions. Because of the small sample size, these correlation coefficients also were used in an exploratory manner as estimates of effect size in order to inform the design of possible future studies in larger samples. Spearman's correlations were used in lieu of Pearson's correlations due to the small

**Table 2a**

Extinction retention deficits as predicted by diagnostic group, Allo + PA, and the interaction between diagnostic group and Allo + PA.

Fixed Effect	Coefficient	SE	T-ratio	Unadjusted p-value
<b>eFP</b>				
<b>Early Extinction</b>				
Intercept	$\gamma_{00}$ .335	.185	1.805	.093
Dx (0,1)	$\gamma_{01}$ -.312	.259	-1.203	.249
Allo + PA	$\gamma_{02}$ -.000369	.000443	-.832	.419
(Allo + PA) x Dx	$\gamma_{03}$ .000637	.000927	.688	.503
<b>Late Extinction</b>				
Intercept	$\gamma_{00}$ .295	.216	1.371	.192
Dx (0,1)	$\gamma_{01}$ -.133	.302	-.441	.666
Allo + PA	$\gamma_{02}$ -.000420	.000516	-.815	.429
(Allo + PA) x Dx	$\gamma_{03}$ -.000083	.001077	-.077	.940
<b>mLP</b>				
<b>Early Extinction</b>				
Intercept	$\gamma_{00}$ -.056	.127	-.442	.665
Dx (0,1)	$\gamma_{01}$ 1.147	.259	4.429	.001
Allo + PA	$\gamma_{02}$ .000082	.00014	.599	.559
(Allo + PA) x Dx	$\gamma_{03}$ -.000756	.000210	-3.594	.003
<b>Late Extinction</b>				
Intercept	$\gamma_{00}$ -.148	.168	-.882	.392
Dx (0,1)	$\gamma_{01}$ 1.135	.342	3.318	.005
Allo + PA	$\gamma_{02}$ .000199	.000180	1.103	.289
(Allo + PA) x Dx	$\gamma_{03}$ -.000866	.000278	-3.114	.008

Note. The dependent variable is the skin conductance value for extinction retention (see Methods). Diagnostic group (Dx) is coded 0 for trauma control (TC: n = 9) and 1 for posttraumatic stress disorder (PTSD: n = 9). eFP: early follicular phase; mLP: mid-luteal phase; Allo: allopregnanolone; PA: pregnanolone.

sample size (n = 9 in each diagnosis group) and the violation of the normality assumption for several of the extinction retention and plasma biomarker measures. For all statistical analyses described above, a p-value of  $< .05$  was considered statistically significant.

## 3. Results

Descriptive statistics for Allo + PA, 5 $\alpha$ -DHP, and DHEA and are presented in Table 1.

The linear regression model examining the effects of Allo + PA on extinction retention calculated in comparison to early extinction during the mLP revealed a main effect of diagnosis such that individuals with PTSD showed poorer extinction retention ( $\beta = 1.147$ ,  $p = .001$ ; Table 2a), a finding consistent with that previously reported for the larger sample (Milad et al., 2010). There was also a significant diagnosis by Allo + PA interaction ( $\beta = -.0008$ ,  $p = .003$ ) indicating a strong positive relationship between resting plasma Allo + PA levels and better extinction retention for the PTSD, but not TC group (Table 2a). Parallel results were found for the mLP model examining extinction retention calculated in comparison to late extinction (diagnosis main effect:  $\beta = 1.135$ ,  $p = .005$ ; diagnosis X Allo + PA interaction:  $\beta = -.0009$ ,  $p = .008$ ). The correlations between mLP Allo + PA levels and extinction retention based on early and late extinction are depicted for the PTSD and TC groups in Table 3 and Fig. 1a and b.

As predicted, the plasma resting (Allo + PA)/5 $\alpha$ -DHP ratio performed like Allo + PA during the mLP in predicting extinction retention (Table 2b), although the effects of this ratio appeared to be somewhat weaker (diagnosis main effect controlling for early extinction:  $\beta = .403$ ,  $p = .026$ ; diagnosis main effect controlling for late extinction:  $\beta = .348$ ,  $p = .101$ ). There was a trend for an interaction between diagnosis and the (Allo + PA)/5 $\alpha$ -DHP ratio with extinction retention (calculated in comparison to early extinction) ( $\beta = -.165$ ,  $p = .071$ ), such that a higher ratio was associated with better extinction retention in the PTSD, but not TC, group. Parallel results were found for



**Table 2b**  
Extinction retention deficits as predicted by diagnostic group, (Allo + PA)/5α-DHP, and the interaction between diagnostic group and (Allo + PA)/5α-DHP.

Fixed Effect		Coefficient	SE	T-ratio	Unadjusted p-value
<b>eFP</b>					
<i>Early Extinction</i>					
Intercept	γ00	.038	.234	.164	.872
Dx (0,1)	γ01	.034	.272	.124	.903
Ratio	γ02	.290	.354	.820	.426
Ratio x Dx	γ03	-.293	.416	-.705	.493
<i>Late Extinction</i>					
Intercept	γ00	-.049	.272	-.182	.858
Dx (0,1)	γ01	.161	.316	.510	.618
Ratio	γ02	.344	.412	.835	.418
Ratio x Dx	γ03	-.443	.483	-.918	.374
<b>mLP</b>					
<i>Early Extinction</i>					
Intercept	γ00	.020	.115	.175	.863
Dx (0,1)	γ01	.403	.162	2.495	.026
Ratio	γ02	-.006	.059	-.100	.921
Ratio x Dx	γ03	-.165	.084	-1.954	.071
<i>Late Extinction</i>					
Intercept	γ00	-.001	.141	-.007	.994
Dx (0,1)	γ01	.348	.198	1.758	.101
Ratio	γ02	.011	.073	.149	.884
Ratio x Dx	γ03	-.193	.103	-1.869	.083

*Note.* The dependent variable is the skin conductance value for extinction retention (see Methods). Diagnostic group (Dx) is coded 0 for trauma control (TC: n = 9) and 1 for traumatic stress disorder (PTSD: n = 9). eFP: early follicular phase; mLP: mid-luteal phase; Allo: allopregnanolone; PA: pregnanolone; 5α-DHP: 5α-dihydroprogesterone.

**Table 2c**  
Extinction retention deficits as predicted by diagnostic group, (Allo + PA)/DHEA, and the interaction between diagnostic group and (Allo + PA)/DHEA.

Fixed Effect		Coefficient	SE	T-ratio	Unadjusted p-value
<b>eFP</b>					
<i>Early Extinction</i>					
Intercept	γ00	.184	.214	.862	.403
Dx (0,1)	γ01	-.120	.270	-.443	.664
Ratio	γ02	.015	.110	.133	.896
Ratio x Dx	γ03	-.011	.127	-.090	.929
<i>Late Extinction</i>					
Intercept	γ00	.109	.245	.443	.664
Dx (0,1)	γ01	.065	.309	.209	.837
Ratio	γ02	.026	.127	.207	.839
Ratio x Dx	γ03	-.078	.146	-.539	.598
<b>mLP</b>					
<i>Early Extinction</i>					
Intercept	γ00	-.028	.147	-.191	.852
Dx (0,1)	γ01	.171	.241	.711	.489
Ratio	γ02	.007	.020	.334	.743
Ratio x Dx	γ03	-.004	.024	-.174	.865
<i>Late Extinction</i>					
Intercept	γ00	-.096	.166	-.577	.573
Dx (0,1)	γ01	.036	.271	.134	.895
Ratio	γ02	.019	.022	.842	.414
Ratio x Dx	γ03	-.007	.027	-.277	.786

*Note.* The dependent variable is the skin conductance value for extinction retention (see Methods). Diagnostic group (Dx) is coded 0 for trauma control (TC: n = 9) and 1 for traumatic stress disorder (PTSD: n = 9). eFP: early follicular phase; mLP: mid-luteal phase; Allo: allopregnanolone; PA: pregnanolone; DHEA: dehydroepiandrosterone.

extinction retention based on late extinction (diagnosis main effect:  $\beta = .348, p = .101$ ; diagnosis X Allo + PA interaction:  $\beta = -.193, p = .083$ ). **Table 3** depicts correlations between the mLP (Allo + PA)/5α-DHP ratio and indices of extinction retention.

In contrast to the above findings for the mLP, the eFP models yielded no significant main effects of diagnostic status, Allo + PA levels, or their interaction for indices of extinction retention (**Table 2a**).

**Table 3**  
Spearman correlations between the GABAergic neurosteroid indices of interest and indices of extinction retention.

	Spearman Correlation (rho)	Coefficient	P-value
<b>PTSD</b>			
<b>eFP</b>			
<i>Early Extinction</i>			
Allo + PA	.167 (-.559, .748)		.668
Allo + PA to 5α-DHP Ratio	.233 (-.510, .777)		.546
Allo + PA to DHEA Ratio	-.117 (-.725, .593)		.765
<i>Late Extinction</i>			
Allo + PA	-.433 (-.852, .324)		.244
Allo + PA to 5α-DHP Ratio	-.400 (-.841, .360)		.286
Allo + PA to DHEA Ratio	-.650 (-.918, .025)		.058
<b>mLP</b>			
<i>Early Extinction</i>			
Allo + PA	-.867 (-.972, -.477)		.003
Allo + PA to 5α-DHP Ratio	-.571 (-.910, .223)		.139
Allo + PA to DHEA Ratio	.083 (-.615, .708)		.831
<i>Late Extinction</i>			
Allo + PA	-.533 (-.884, .203)		.139
Allo + PA to 5α-DHP Ratio	-.382 (-.856, .443)		.352
Allo + PA to DHEA Ratio	.217 (-.523, .770)		.576
<b>Trauma Control</b>			
<b>eFP</b>			
<i>Early Extinction</i>			
Allo + PA	-.200 (-.763, .536)		.606
Allo + PA to 5α-DHP Ratio	.150 (-.571, .740)		.700
Allo + PA to DHEA Ratio	-.167 (-.748, .559)		.668
<i>Late Extinction</i>			
Allo + PA	-.433 (-.852, .324)		.244
Allo + PA to 5α-DHP Ratio	-.017 (-.673, .655)		.966
Allo + PA to DHEA Ratio	-.317 (-.810, .440)		.406
<b>mLP</b>			
<i>Early Extinction</i>			
Allo + PA	.250 (-.497, .784)		.517
Allo + PA to 5α-DHP Ratio	-.381 (-.856, .443)		.352
Allo + PA to DHEA Ratio	.233 (-.510, .777)		.546
<i>Late Extinction</i>			
Allo + PA	.333 (-.425, .817)		.381
Allo + PA to 5α-DHP Ratio	.190 (-.594, .789)		.651
Allo + PA to DHEA Ratio	.233 (-.510, .777)		.546

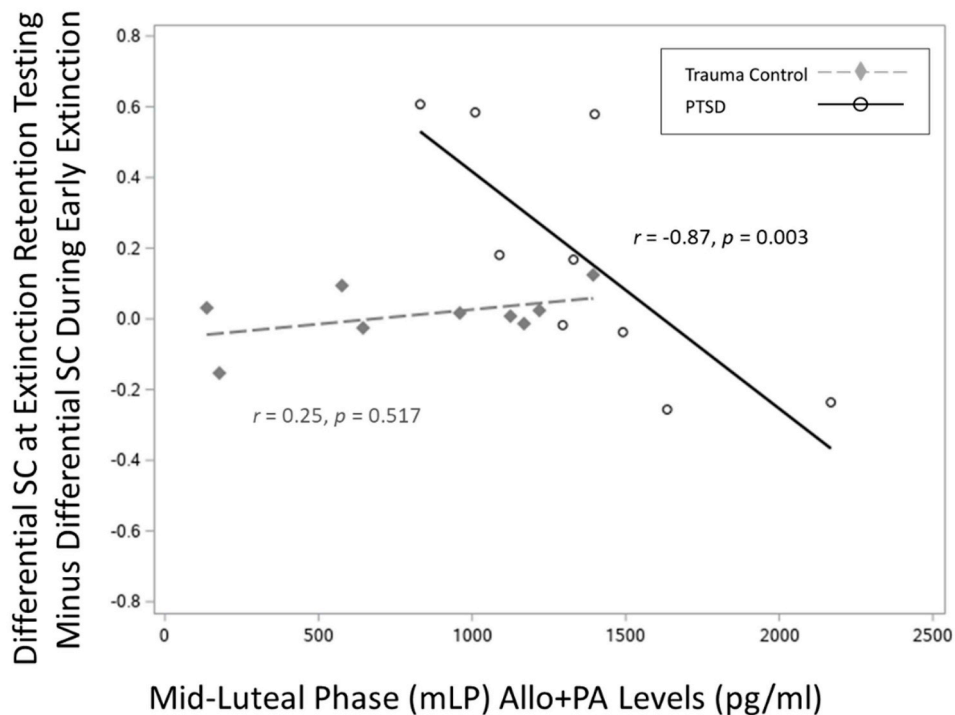
*Note.* CI = confidence interval; eFP: early follicular phase; mLP: mid-luteal phase; Allo: allopregnanolone; PA: pregnanolone; 5α-DHP: 5α-dihydroprogesterone; DHEA: dehydroepiandrosterone.

Nor were there significant main effects of diagnostic status, the (Allo + PA)/5α-DHP ratio, or their interaction on extinction retention (**Table 2b**). Also, as noted in **Table 2c**, there were no significant main effects of diagnostic status, the plasma resting (Allo + PA)/DHEA ratio, or their interaction on extinction retention. Nevertheless, exploratory correlation analyses for the PTSD group during the eFP revealed a trend for a correlation between resting plasma (Allo + PA)/DHEA ratio and extinction retention ( $r = -.65, p = .058$ ) based on late but not early extinction (**Table 3**). As can be seen in **Fig. 2a**, the difference between the average differential SCRs for extinction retention testing vs. *early* extinction was similar across individuals with PTSD regardless of their (Allo + PA)/DHEA ratio. In contrast (**Fig. 2b**), the difference between the average differential SCRs for extinction retention testing vs. *late* extinction decreased for individuals with higher (Allo + PA)/DHEA ratios to levels consistent with achievement of good extinction retention, but increased for individuals with lower ratios.

#### 4. Discussion

The current study suggests that low resting plasma Allo + PA levels in women with PTSD, apparently related to deficient 3α-HSD activity,

a.



b.

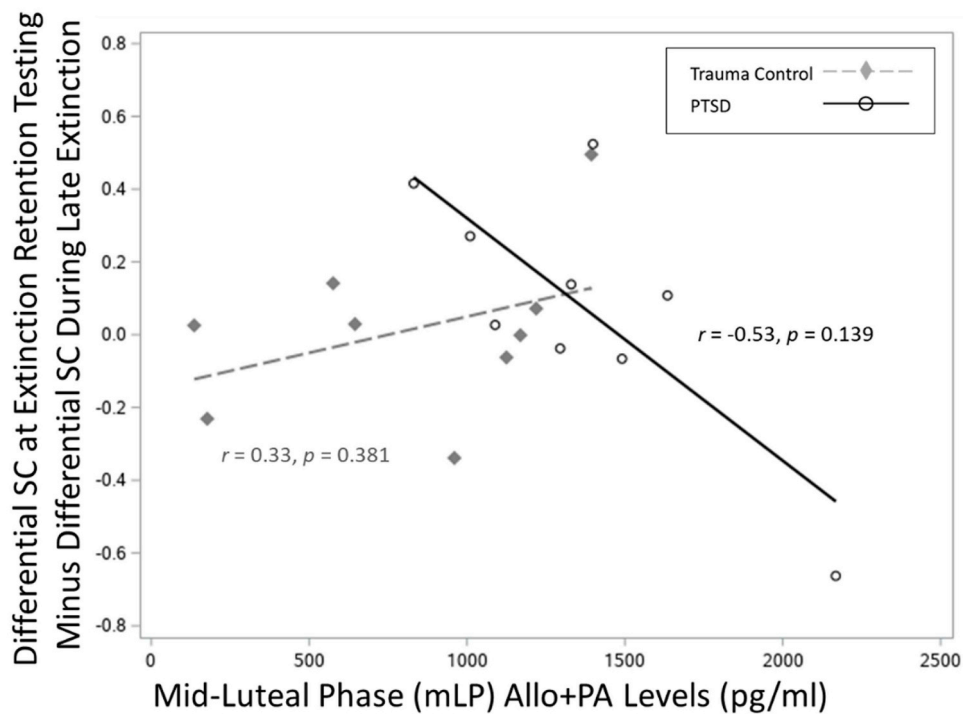
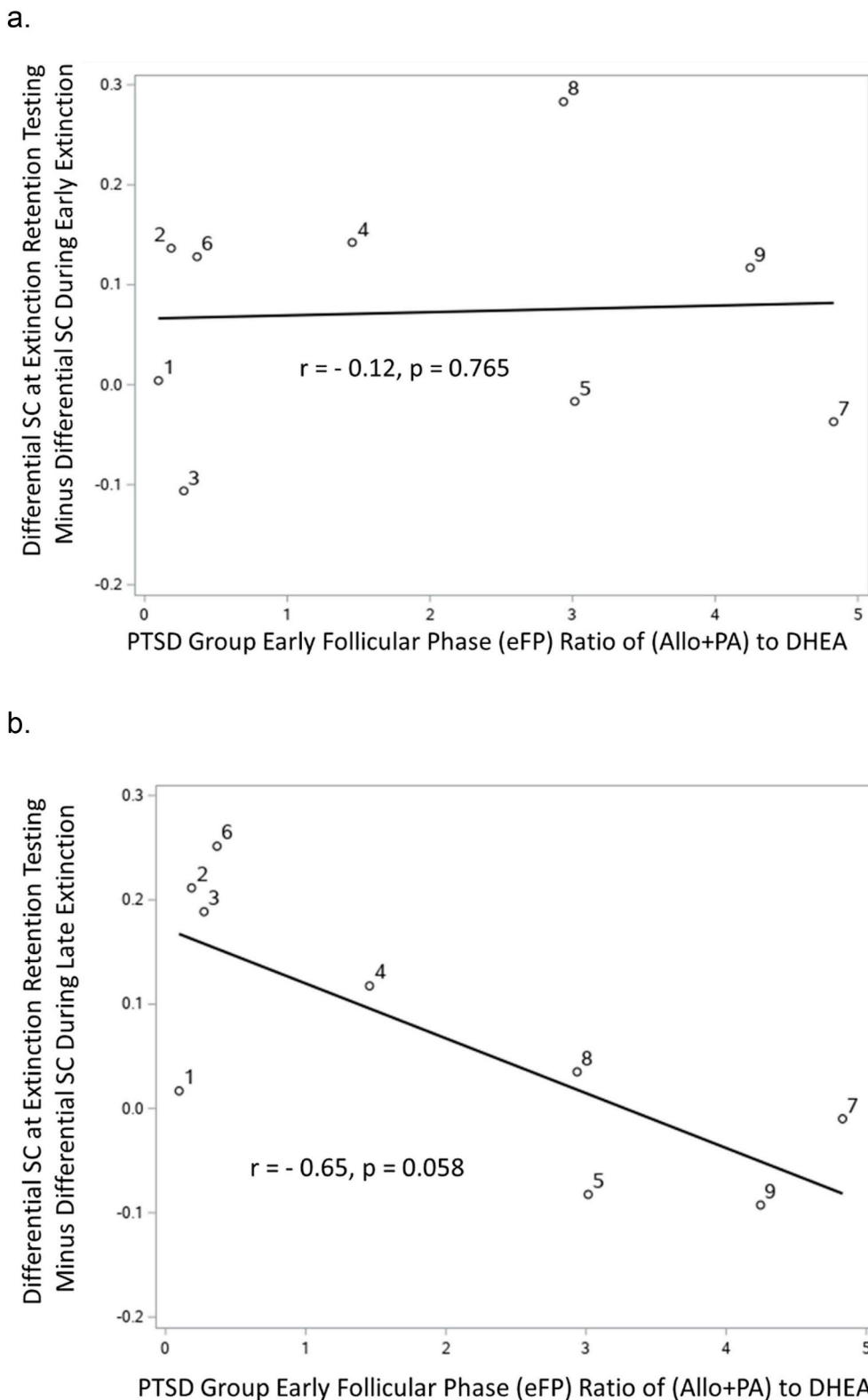


Fig. 1. The associations between Allo + PA and extinction retention, adjusting for (a) early extinction or (b) late extinction for individuals with and without PTSD [Note that higher values on the y-axis (i.e., indicating differences between differential SC during extinction retention and early extinction) indicate poorer extinction retention.].



**Fig. 2.** Correlations between the (Allo + PA)/DHEA ratio and extinction retention controlling for (a) early extinction or (b) late extinction [Note that higher values on the y-axis (i.e., indicating differences between differential SC during extinction retention and early extinction) indicate poorer extinction retention. Participant numbers are provided to allow comparisons between their extinction retention performance compared to late vs. early extinction.].

are associated with extinction retention deficits during the mLP of the menstrual cycle when  $17\beta$ -estradiol and progesterone levels are both high and stable (Pineles et al., 2016). Studies in rodents (Zhang et al., 2014) and in women exposed to extreme stress (Rasmussen et al., 2006) suggest that peripheral deficits in the capacity for Allo and PA synthesis

are mirrored in the CNS. A limited capacity for intraneuronal Allo + PA synthesis has been shown to forestall development of long-term depression (LTD) and/or emergence of long-term potentiation (LTP) interference (Izumi et al., 2013). LTD and LTP are critical to extinction (Maren, 2015). In turn, studies suggest that the emergence of LTP

interference across learning (e.g., extinction learning) may protect the recently strengthened or weakened synapses from further modulation (i.e., from being “overwritten”) during memory consolidation (Rasmusson and Pineles, 2018; Stefan et al., 2005; Cantarero et al., 2013). Thus, it follows that adequate neuronal synthesis of the GABAergic neurosteroids, Allo and PA, may support both extinction learning and the retention of extinction learning, as suggested by this study.

In contrast, during the eFP, when progesterone and estradiol levels are low, Allo + PA levels alone were not a significant predictor of extinction retention. However, the ratio of Allo + PA to DHEA was positively associated with extinction retention based on late but not early extinction. As levels of Allo + PA (which facilitate GABA<sub>A</sub> receptor function and antagonize NMDA receptor function) and DHEA (which antagonizes GABA<sub>A</sub> receptor function and facilitates NMDA receptor function) are comparable during the eFP (Table 1), it is possible that their effects compete. Thus, a low ratio of inhibitory vs. excitatory neurosteroids may impact net neuronal excitability with effects on a) neuronal LTD or LTP interference, as well as b) frontal lobe and hippocampal circuits that inhibit amygdala reactivity during extinction learning or extinction recall. Consistent with our hypothesis regarding potential menstrual phase-specific effects of DHEA, we saw a trend for a relationship between the (Allo + PA)/DHEA ratio and extinction retention during the eFP when Allo + PA and DHEA levels are comparable. We did not see a relationship between the (Allo + PA)/DHEA ratio and extinction retention during the mLP when plasma Allo + PA levels are an order of magnitude higher than plasma DHEA levels.

Our previously published work based on a larger subsample ( $n = 32$ ) of the parent study (Pineles et al., 2016) examined only the effects of diagnostic status and menstrual phase on extinction retention. Consistent with earlier work by other investigators (Milad et al., 2010), that study showed healthy trauma-exposed women to have better extinction retention during the mLP when both estradiol and progesterone levels are high, compared to the eFP when levels of both are at their lowest. In contrast, women with PTSD performed worse during the mLP than the eFP (when their performance was comparable to that of healthy women). 17 $\beta$ -estradiol has been shown to upregulate 3 $\alpha$ -HSD expression in the brain of female rodents (Mitev et al., 2003). It is possible that effects of 17 $\beta$ -estradiol on 3 $\alpha$ -HSD expression are compromised during the mLP in a subpopulation of women with PTSD. A variety of factors may be responsible (Rasmusson et al., 2017). For example, possession of a loss-of-function polymorphism in the estrogen sensitive pituitary adenylate cyclase-activating polypeptide (PACAP) receptor (PAC1) has been associated with increased risk for PTSD in women, as well as with poor safety signal learning during fear acquisition (Ressler et al., 2011). As previously discussed (Rasmusson and Pineles, 2018), this polymorphism would be expected to reduce on-demand neurosteroidogenesis. Reduced glucocorticoid receptor signaling due to deficits in cortisol synthesis, as seen in individuals with various forms of congenital adrenal hyperplasia (CAH) or genetically impaired glucocorticoid receptor function, also may reduce 3 $\alpha$ -HSD expression and Allo + PA synthesis under stress (Hou et al., 1998). For example, common variants of the *FKBP5* gene are associated with higher *FKBP5* protein expression, which results in impaired glucocorticoid receptor function. These variants also have been associated with increased risk for PTSD, both directly and interactively with early life stress (Binder, 2009; Hawn et al., 2019; Watkins et al., 2016). In addition, deficits in the reduced form of nicotinamide adenine dinucleotide phosphate (i.e., NADPH), a necessary cofactor for the reductive function of 3 $\alpha$ -HSD, or possession of a loss-of-function polymorphism in the 3 $\alpha$ -HSD gene itself, including in the steroid response element that binds estradiol, might contribute to Allo + PA deficits or a reduced ratio of Allo + PA to DHEA (Penning et al., 2004). Finally, well described but relatively rare, deficiencies in 3 $\beta$ -HSD function that result in elevated DHEA synthesis (Al Alawi et al., 2019) would be expected to limit synthesis of Allo and PA and, thereby, may contribute to increased

PTSD risk, severity or chronicity. Indeed, homozygosity for 3 $\beta$ -HSD deficiency has been associated with increased rates of depression, alcohol misuse and suicidality (Al Alawi et al., 2019), which are common comorbidities of PTSD. Thus, heterozygosity for 3 $\beta$ -HSD deficiency, which may present with hyperandrogenism, also should be investigated for its possible relationship to PTSD (Al Alawi et al., 2019; Nayak et al., 1998).

Examination of the impact of neurosteroids on extinction retention is a relatively new area of research in humans. As discussed above, the current study has a small sample size due to the number of SC non-responders and individuals for whom we were unable to obtain blood samples. Given this limited sample size, the present findings must be viewed with caution and in need of replication. However, the findings agree with previous preclinical work in male rodents demonstrating an association between experimentally induced reductions in brain Allo levels and impaired extinction retention in similarly sized cohorts (Pinna and Rasmusson, 2014; Pibiri et al., 2008; Schüle et al., 2014; Nagaya et al., 2015). As noted in the introduction, these preclinical studies also suggest that it might be possible to improve extinction retention in women with deficient synthesis of Allo and PA through interventions aimed at increasing levels of these GABAergic neurosteroids. However, the timing and dose of such GABAergic neurosteroid based treatments may be critical and bear close investigation in humans for their impact on PTSD symptom severity, as well as learning and memory processes inherent to PTSD recovery.

There is a long tradition of using laboratory extinction models to inform mechanisms underlying exposure therapy efficacy in humans (Hofmann, 2008). Although exposure therapies are first line treatments for PTSD and often produce significant symptom reductions (Powers et al., 2010), they have a high dropout rate (Hembree et al., 2003) and most clients are not asymptomatic at the end of treatment (Barlow et al., 2002; Schnurr et al., 2007). Consequently, research is increasingly being targeted toward development of adjunctive pharmacological interventions that might enhance exposure therapy outcomes. Several clinical trials have tested D-cycloserine (i.e., a partial NMDA agonist) and have shown variable results (McGuire et al., 2014; Singewald et al., 2015). Another recent study showed promising effects for propranolol (a noradrenergic beta-receptor blocker) in enhancing the efficacy of written exposure treatment for PTSD (Brunet et al., 2018). Findings from the current study provide preliminary support for Allo as another promising pharmacological adjunctive treatment to enhance exposure therapy outcomes for PTSD. Allo + PA levels also have been shown to increase in response to non-pharmacological interventions such as brief, intense exercise (Scioli-Salter et al., 2016), which was recently shown in a pilot study to improve outcomes in women with PTSD treated with Prolonged Exposure therapy (Powers et al., 2015). Pharmacological Allo-related therapies are also currently being used or investigated for treatment of disorders other than PTSD, such as major depression, postpartum depression, and premenstrual dysphoric disorder (ClinicalTrials.gov identi, 2016). These disorders have been associated with decreases in CSF or plasma Allo levels in some studies, and all have substantially increased co-prevalence with PTSD among women. These observations as well as the results of the current study point to the potential importance of developing clinically feasible tests for deficient GABAergic steroid synthesis that may be useful in the development and prescription of transdiagnostic Allo-based treatments.

## 5. Conclusion

The findings of this study suggest that reduced Allo + PA levels may contribute to extinction retention deficits observed during the mLP in women with PTSD. During the eFP, it appears that a low resting plasma (Allo + PA)/DHEA ratio might contribute to extinction retention deficits in women with PTSD. Together with repeatedly observed associations between GABAergic neurosteroid synthesis deficits and PTSD



severity in men and women, our findings suggest a mechanism that may link early post-trauma PTSD severity with risk for chronic PTSD (Shalev et al., 2019). The present work also supports the importance of investigating interventions that may increase Allo + PA levels in order to reduce PTSD risk and enhance PTSD recovery.

### Financial disclosures

Dr. Rasmusson reports receiving in-kind travel compensation during the past 3 years as a scientific consultant to Cohen Veterans Bioscience, a non-profit organization. Dr. Pinna has two pending patent applications; one on PEA and PPAR- $\alpha$  agonists (US, 2018/0369171) and one on allopregnanolone's analogs in the treatment of neuropsychiatric disorders (WO, 2018/237282). Dr. Pinna also received funding from Marinus Pharmaceuticals, Inc. for studies on ganaxolone. Other authors report no conflict of interests related to this study.

### CRedit authorship contribution statement

**Suzanne L. Pineles:** Conceptualization, Methodology, Investigation, Writing - original draft, Project administration, Funding acquisition. **Yael I. Nillni:** Conceptualization, Writing - original draft, Writing - review & editing. **Graziano Pinna:** Conceptualization, Investigation, Writing - review & editing. **Andrea Webb:** Conceptualization, Methodology, Formal analysis, Writing - review & editing. **Kimberly A. Arditte Hall:** Writing - review & editing. **Jennifer R. Fonda:** Methodology, Formal analysis, Visualization, Writing - review & editing. **John Irvine:** Conceptualization, Writing - review & editing. **Matthew W. King:** Methodology, Formal analysis, Writing - review & editing. **Richard L. Hauger:** Investigation, Writing - review & editing. **Patricia A. Resick:** Conceptualization, Methodology, Resources, Writing - review & editing, Supervision. **Scott P. Orr:** Conceptualization, Methodology, Writing - review & editing, Supervision. **Ann M. Rasmusson:** Conceptualization, Methodology, Writing - review & editing, Supervision.

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