Neurosteroids in PTSD and Co-occurring Conditions *Biomarkers to Therapeutics*

Chris Marx, MD MA

Professor and Vice Chair for Faculty
Division Director, Translational Neuroscience
Duke University Medical Center;

Staff Psychiatrist
Director, Interventions & Metabolomics Core
VA Mid-Atlantic MIRECC and Durham VA Medical Center

Disclosures

FDA IND numbers and discussion of off-label use

- **#71,768 (pregnenolone/schizophrenia)**
- **#73,099 (pregnenolone/PTSD)**
- **#78,101 (omega-3 fatty acids/PTSD)**
- **#78,270 (pregnenolone/mild TBI)**
- **#114,799 (pregnenolone/pain)**
- **#129,623 (DHEA/PTSD)**

Co-applicant, pending patent applications

(NO PATENTS ISSUED, NO LICENSING IN PLACE; VA 208 waiver in place)

- **Neurosteroids and Derivatives for CNS Disorders**

Study Drug and Matching Placebo – Marinus Pharmaceuticals (Ganaxolone in PTSD)

Support

- **Department of Veterans Affairs**
  VA Mid-Atlantic Mental Illness, Research, Education and Clinical Center (MIRECC),VA ARCD, VA REAP, VA CDTA, VA Merit Review
- **Department of Defense (INTRuST, Concept Award)**
- **NIMH/NIH**
- **NARSAD**
- **Bryan Alzheimer's Disease Research Center (ADRC), Duke University School of Medicine**

VA Mid-Atlantic MIRECC

(Mental Illness, Research, Education and Clinical Center)

Director: John Fairbank
Deputy Director: Mira Brancu

Funded in 2005
Durham VA, Salisbury VA, Richmond VA, Hampton VA, other collaborating VAs

Components:

Research:	**Chris Marx**
	Jean Beckham
Education:	**Robin Hurley**
	Katherine Taber
Clinical:	**Keith Shaw**

Laboratories:

Interventions & Metabolomics	**Chris Marx**
Neuroimaging:	**Raj Morey**
PDMH and:	**Mira Brancu**
Repository	**Jen Runnels**
Health Services:	**Pat Calhoun**
Neurocognition:	**Larry Tupler**
Genetics:	**Jean Beckham, Mike Hauser, Alison Ashley-Koch**
Neuroscience:	**Scott Moore**

Statistical Expertise
Ryan Wagner
Robert Hamer

INTRuST Consortium
Injury and Traumatic Stress Center

PI: Murray Stein
Co-PI: Ariel Lang

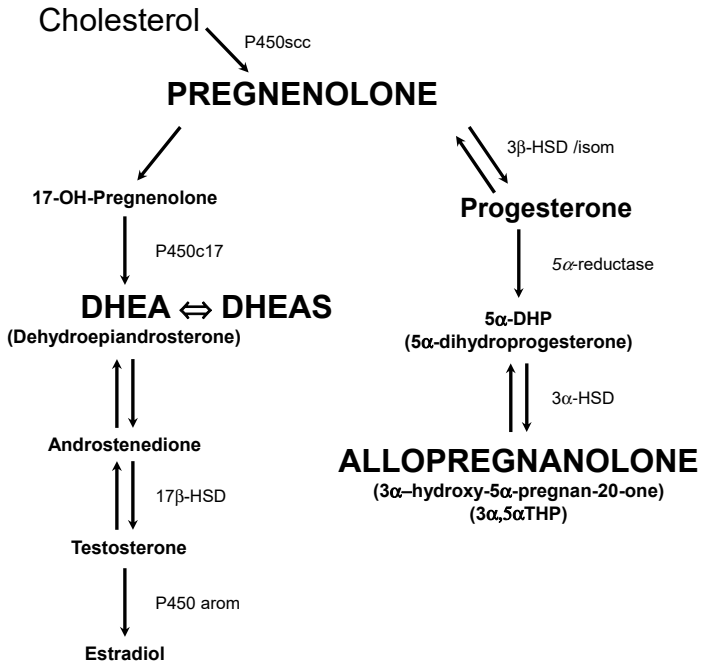
Funded 2008 – 2017 (NCE)
Department of Defense

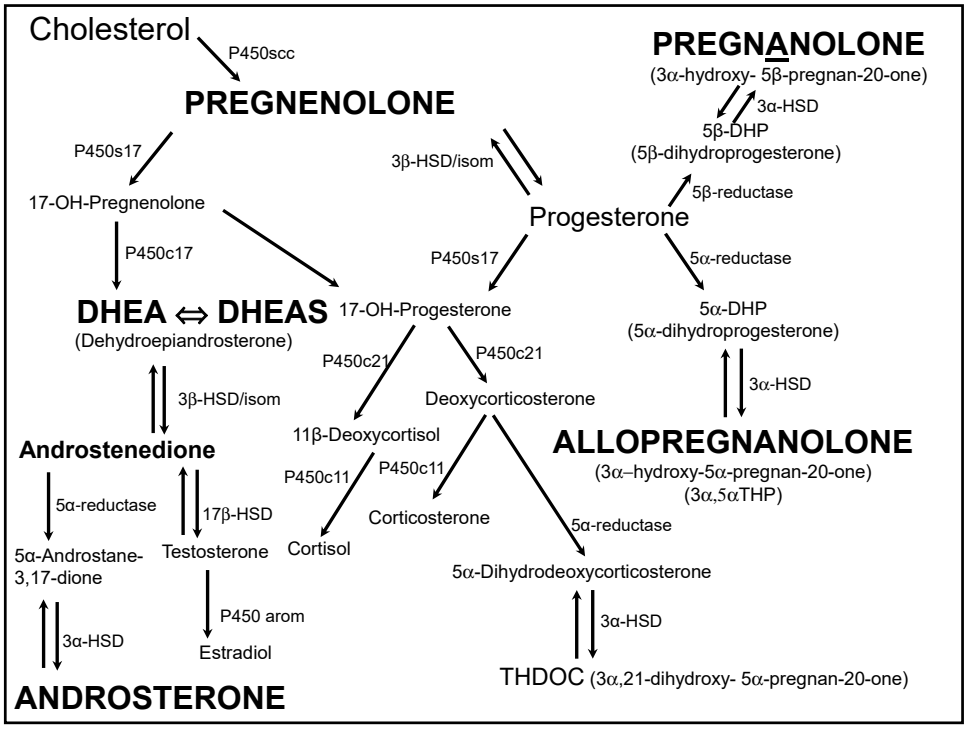
BIOREPOSITORY
Contributing Sites
6 institutions participated in the Biorepository effort:
Dartmouth, Duke and Durham VAMC, South Carolina, Spaulding-Harvard, University of California San Diego, University of Cincinnati

Biorepository PIs: Gerry Grant
Chris Marx
Mike Hauser

Neuroimaging PI: Marty Shenton

Datacore: Sonia Jain
Feng He





Strategy

- **Biomarker investigations:**
  To identify potential risk / resilience factors for TBI (and frequently co-occurring disorders such as PTSD, depression, pain disorders and other CNS conditions) in serum samples from the VA Mid-Atlantic MIRECC Post-Deployment Mental Health (PDMH) study by characterizing neurosteroid "signatures"

- **New therapeutic investigations:**
  Conduction of proof-of-concept randomized controlled trials that are biomarker-informed, supported by preclinical/clinical data, and demonstrate potential for prediction of therapeutic response (neurosteroids as interventions)

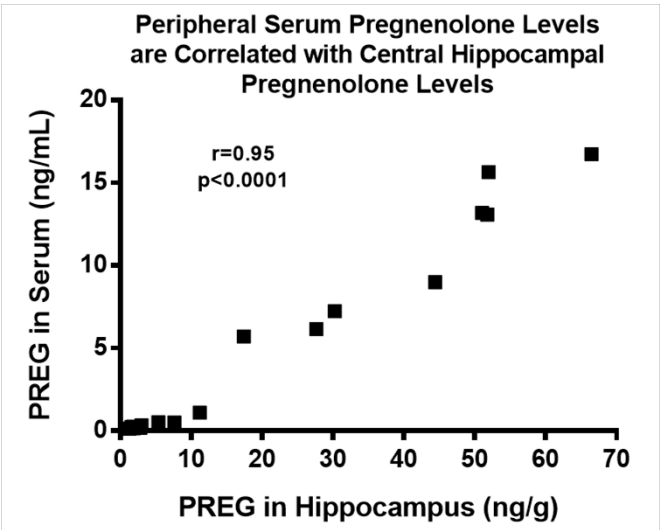
Neurosteroids as Promising Pharmacological Interventions: *Pregnenolone*

- Enriched in brain, also synthesized in the adrenal, other tissues
- Precursor to many neurosteroids, glucocorticoids, other steroids
- Classified as a "dietary supplement" by the FDA (Dietary Supplement Health and Education Act 1994)
- Paucity of clinical trials; 1940s, early 1950s
- Additional neurosteroid candidates (DHEA, derivatives)
- ***Biomarker alterations → New therapeutics***

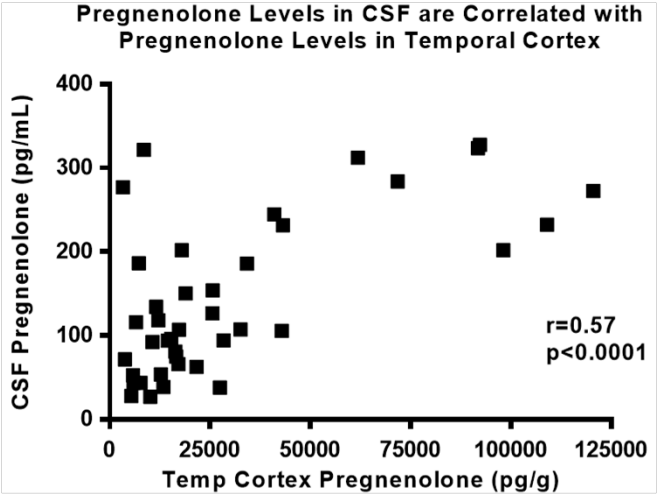
Pregnenolone

- Enhances myelination (Zhu and Glaser 2008, Koenig et al 1995), improves locomotor behavior in myelin mutant rats (Bloom et al 2002); increases neuritic outgrowth (Fontaine-Lenoir et al 2006)
- Stabilizes microtubules (Hsu et al 2006); binds to MAP2 and enhances microtubule polymerization (Fontaine-Lenoir et al 2006, Murakami et al 2000; Hsu et al 2006), enhances microtubule growth and cell migration (Weng et al 2103)
- Neuroprotective actions - Protects against glutamate & amyloid β-protein toxicity (Gursoy et al 2001) and dose-dependently protects vs. amyloid β-peptide toxicity in PC-12 cells (Akan et al 2009)
- ↓ apoptosis (Leskiewicz et al 2008), impacts synaptic plasticity (Bu and Zu 2013)
- Enhances learning and memory in rodent models (Flood et al 1992)
- Reductions in CSF associated with depressive sxs (George et al 1994)
- Reduces depression symptoms - bipolar depression (Brown et al 2014)

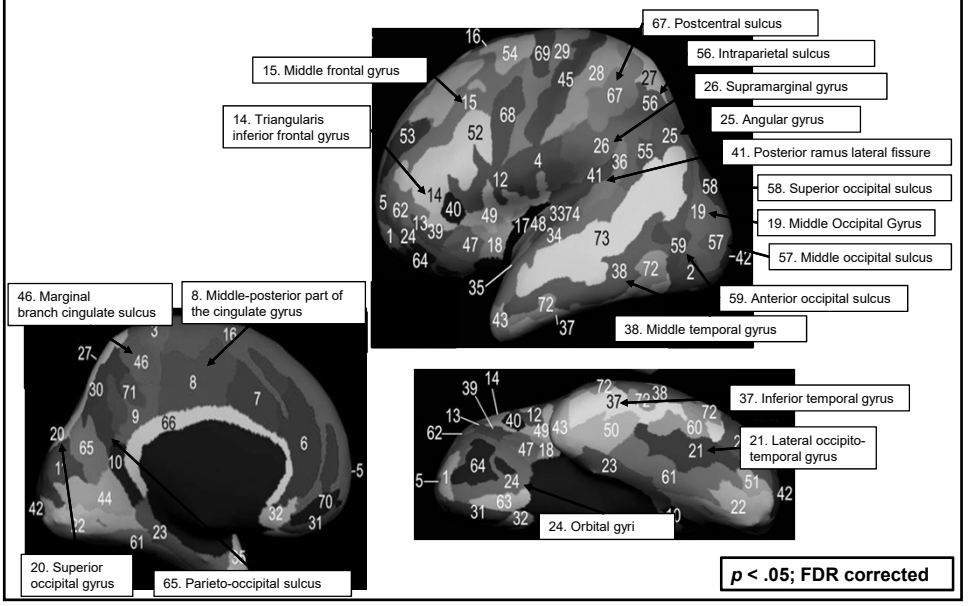
Brain vs. Serum *Pregnenolone* (RAT)



CSF *Pregnenolone* vs. Temporal Cortex Preg. (HUMAN)

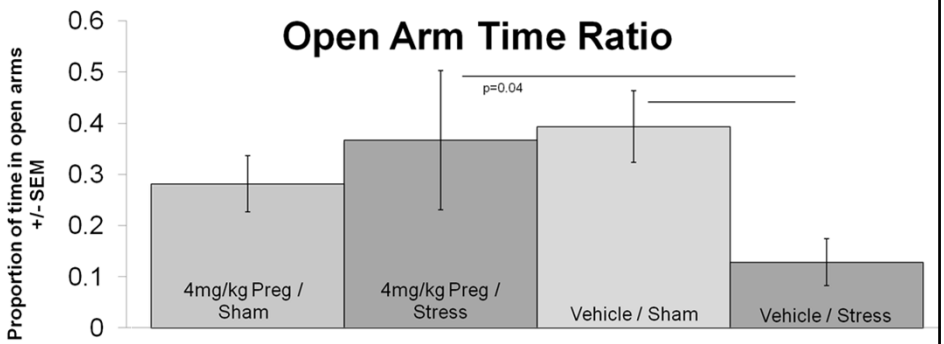


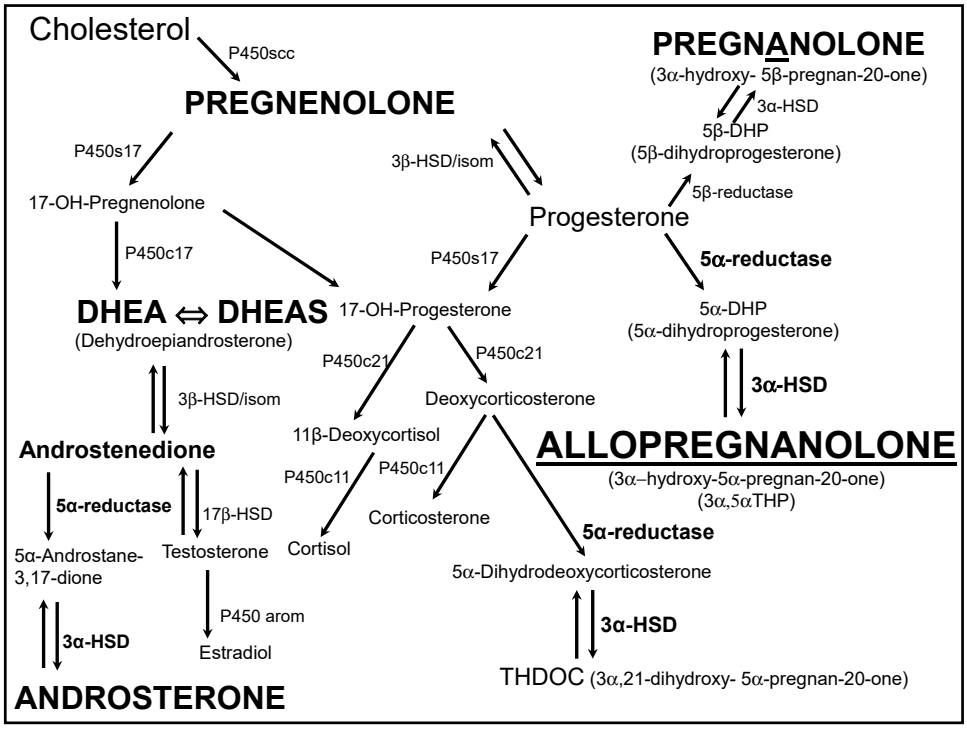
Pregnenolone levels (serum) are associated with left cortical thickness (n=115)

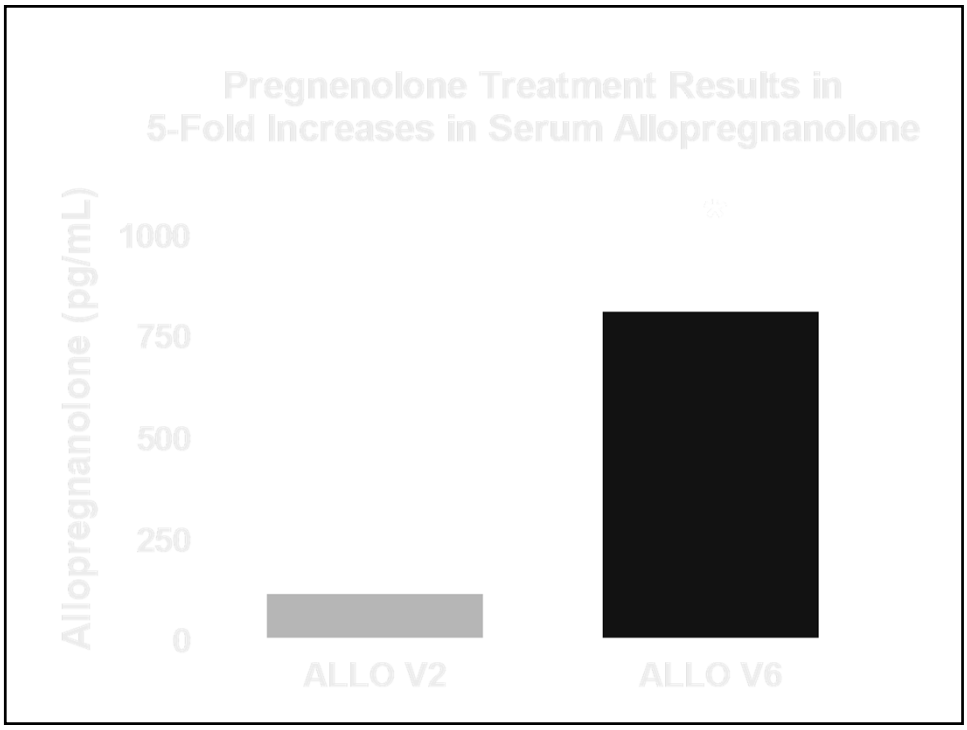


Pre-Treatment with Pregnenolone Decreases Predator Stress-Induced Anxiety Behaviors:

(Potential for Secondary Prevention?)




Cholesterol
P450scc
PREGNENOLONE
P450s17
17-OH-Pregnenolone
P450c17
DHEA ⇔ DHEAS
(Dehydroepiandrosterone)
3β-HSD/isom
Androstenedione
5α-reductase
17β-HSD
5α-Androstane-3,17-dione
Testosterone
3α-HSD
ANDROSTERONE
P450 arom
Estradiol
3β-HSD/isom
Progesterone
P450s17
17-OH-Progesterone
P450c21
P450c21
11β-Deoxycortisol
Deoxycorticosterone
P450c11
P450c11
Cortisol
Corticosterone
5α-reductase
5α-Dihydrodeoxycorticosterone
3α-HSD
THDOC (3α,21-dihydroxy- 5α-pregnan-20-one)
PREGNANOLONE
(3α-hydroxy- 5β-pregnan-20-one)
3α-HSD
5β-DHP
(5β-dihydroprogesterone)
5β-reductase
5α-reductase
5α-DHP
(5α-dihydroprogesterone)
3α-HSD
ALLOPREGNANOLONE
(3α–hydroxy-5α-pregnan-20-one)
(3α,5αTHP)


Pregnenolone Treatment Results in
5-Fold Increases in Serum Allopregnanolone
Allopregnanolone (pg/mL)
1000
750
500
250
0
ALLO V2
ALLO V6

Neurosteroids and PTSD

Allopregnanolone:

- Relevance to fear conditioning:
  Decreased allopregnanolone levels during social isolation enhances contextual fear (Pibiri et al 2008)
- Cerebrospinal fluid levels decreased in females with PTSD compared to control subjects (Rasmusson et al 2006)

DHEA/DHEAS:

- Possible resilience factors against stress (Morgan et al 2004; 2009)

Allopregnanolone

- Positively modulates $GABA_A$ receptors at physiologically relevant nanomolar [ ], potentiating $GABA_A$ receptor responses 20-fold more potently than benzodiazepines/200-fold more potently than barbiturates (Majewska et al 1986; Morrow et al 1987, 1990)
- Anxiolytic-like actions (Wieland et al 1991, Modol et al 2011; Engin, Treit 2007; Finn et al 2003)
- Antidepressant-like actions in rodent behavioral models (Khisti et al 2000 Rodriguez-Landa et al 2007, 2009; Shirayama et al 2011)
- Recent positive Phase II RCT in severe post-partum depression (Sage)
- Anticonvulsant effects in rodent models (Belelli et al 1989, Devaud et al 1995)
- Anticonvulsant actions in humans
  - Super-refractory status epilepticus (SRSE); SAGE-547 (positive Phase II data); 73% of patients successfully weaned from anesthetic agent (concentration 200nM)

Allopregnanolone

- HPA axis effects:
  Modulates the stress response →
  decreases CRF, ACTH, corticosterone release in rodents (Patchev et al 1994, 1996; Calogero et al 1988; Guo et al 1995); inhibits CRH gene promoter activity, (Budziszewska et al 2010).
  Endogenous autoregulatory mechanism?
- Enhances myelination and increases MBP expression (Chen et al. 2011; Brinton 2013; Ahmad et al 2005; Ghoumari et al 2003; Schumacher et al 2003)
- Anti-inflammatory actions (Djebaili et al 2005, 2004: He et al 2004), reduces microglia activation (Ahmad et al 2005), anti-apoptotic actions (Charalampapoulos et al 2004, 2006)
- Enhances neurogenesis; increases proliferation in rodent and human neural progenitor cells (Wang et al 2005, Brinton et al 2006)

Allopregnanolone

Neuroprotective actions:

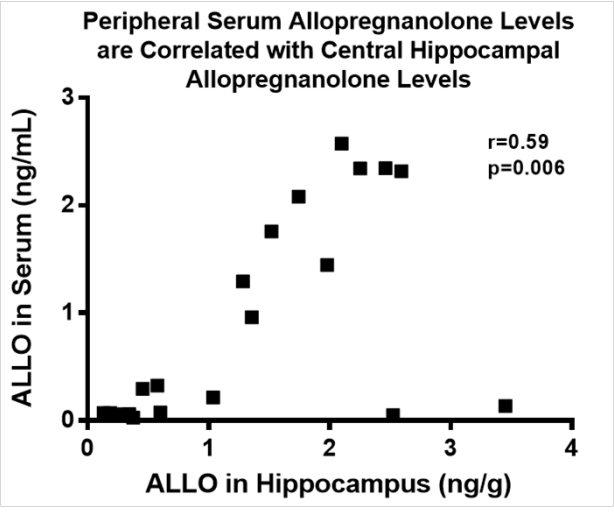
- One-time administration doubles lifespan in Niemann-Pick type C mice and delays neurological symptom onset (Griffin et al 2004)
- Neuronal toxicity induced by tributyltin (Ishihara et al 2013)
- Protects against ischemia (Knight et al 2012; Kelley et al 2008), ischemia-induced learning and memory impairment (Morali et al 2011)
- Reduces infarct size and decreases blood-brain barrier breakdown following traumatic brain injury [TBI] (Ishrat et al 2010), reduces infarct volume in a rodent stroke model (Sayeed et al 2006)

Allopregnanolone

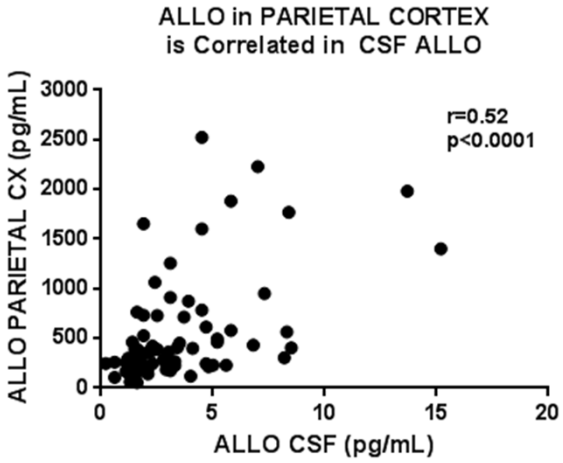
Neuroprotective actions (cont'd):

- Decreases cell death, neuronal loss, and gliosis, and enhances cognitive performance and recovery following TBI (Djebaili et al 2004 and 2005; He, Hoffman, Stein 2004)
- Protects against oxygen-glucose deprivation (Radley et al 2012, Ardeshiri et al 2006) and kainic acid excitotoxicity (Ciriza et al 2004)
- Protects against hypoxia-induced astrogliosis (Kruse et al 2009)
- Decreases NMDA-induced toxicity and decreases neuronal apoptosis (Charalampopoulos et al 2004 and 2006;).

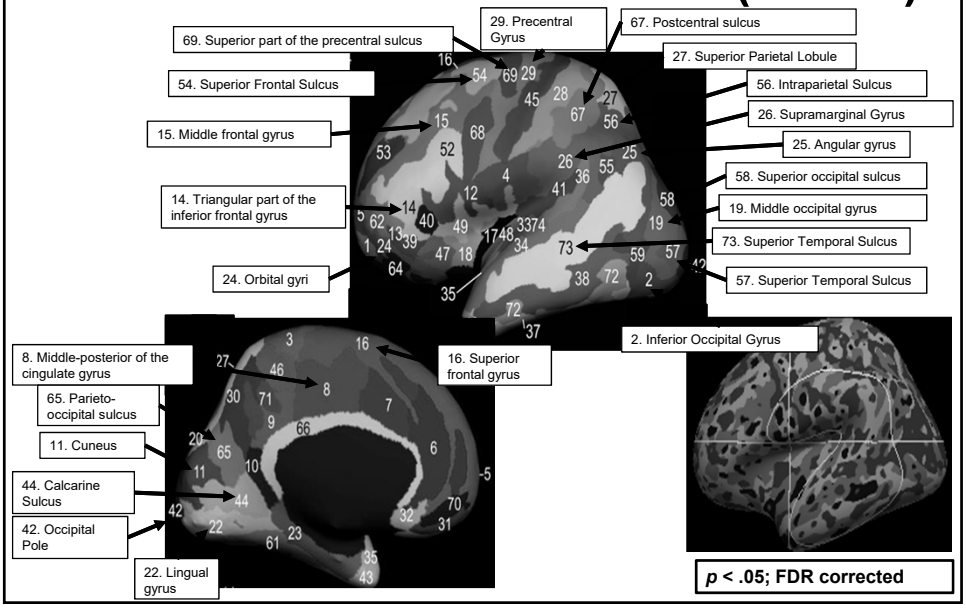
Brain vs. Serum *Allopregnanolone* (RAT)



CSF *Allopregnanolone* vs. Parietal Cortex Allo. (HUMAN)



Allopregnanolone level associated with left cortical thickness (n=115)



PRIORITY COMMUNICATION

Allopregnanolone Elevations Following Pregnenolone Administration Are Associated with Enhanced Activation of Emotion Regulation Neurocircuits

Rebecca K. Sripada, Christine E. Marx, Anthony P. King, Jessica C. Rampton, S. Shaun Ho, and Israel Liberzon

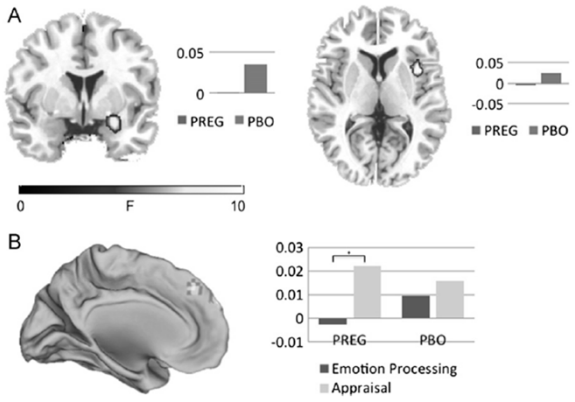


Figure 2. (A) Compared with placebo, pregnenolone administration decreased activation in right amygdala (y = 2) and right insula (z = −6) across conditions and face types. **(B)** Compared with placebo, pregnenolone administration increased dorsal medial prefrontal cortex activation during appraisal (x = 0). Percent signal change is displayed next to each figure. PBO, placebo; PREG, pregnenolone administration group.

Biological Psychiatry
PRIORITY COMMUNICATION
2013. 73(11):1045-1053

Neurosteroids and Traumatic Brain Injury (TBI)

VA Mid-Atlantic MIRECC Registry

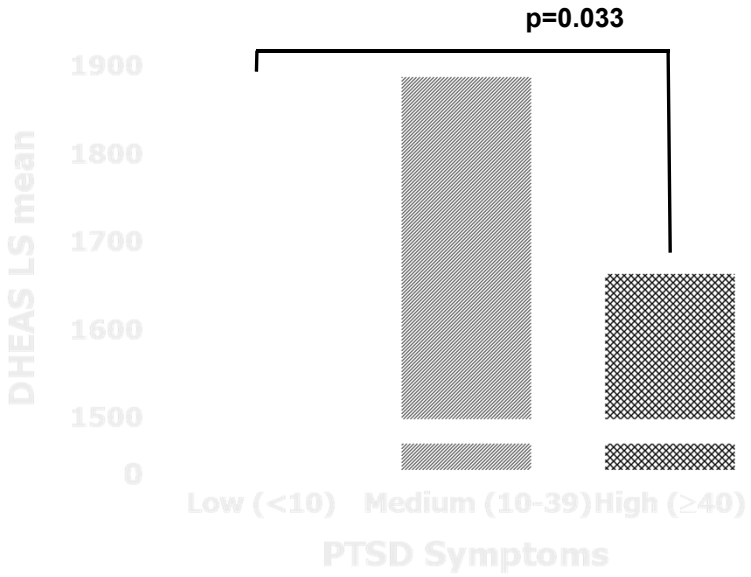
Neurosteroid Investigations in the VA Mid-Atlantic MIRECC Registry Cohort

- DHEA and DHEAS Levels in Serum Samples
- Male OEF/OIF/OND Era Veterans (n=662); RIA
- Blood draw between 10:30AM - 2:30PM
- Enrolled at Durham VA Medical Center

PTSD Symptoms Assessed by DTS LS Means for DHEAS Levels

Davidson Trauma Scale (DTS)	N	%	DHEAS LS MEAN	SEM
Low (<10)	291	44.2	1877.7	63.7
Medium (10-39)	154	23.4	1889.8	86.2
High (≥40)	213	32.4	1666.6	74.3

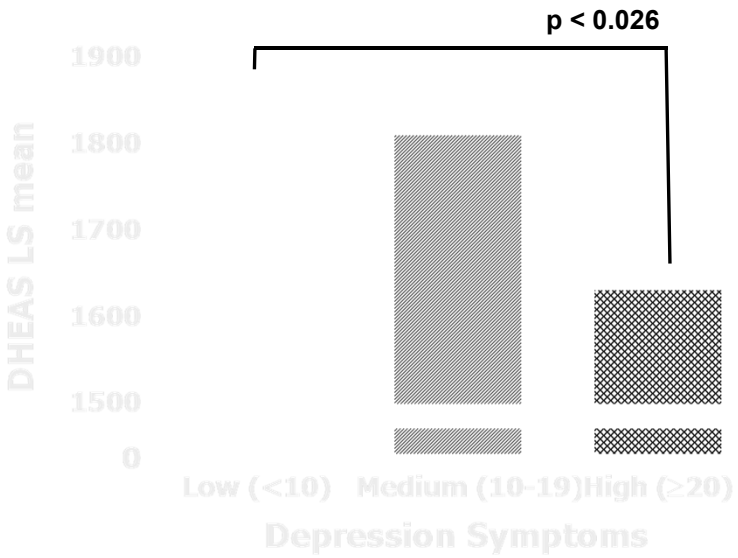
PTSD Symptoms Assessed by DTS
LS Means for DHEAS Levels



Depression Symptoms Assessed by BDI-II
LS Means for DHEAS Levels

Beck Depression Inventory-II	N	%	DHEAS LS MEAN	SEM
Low (<10)	359	53.7	1867.7	56.7
Medium (10-19)	160	24.0	1812.0	84.9
High (≥ 20)	149	22.3	1632.4	88.2

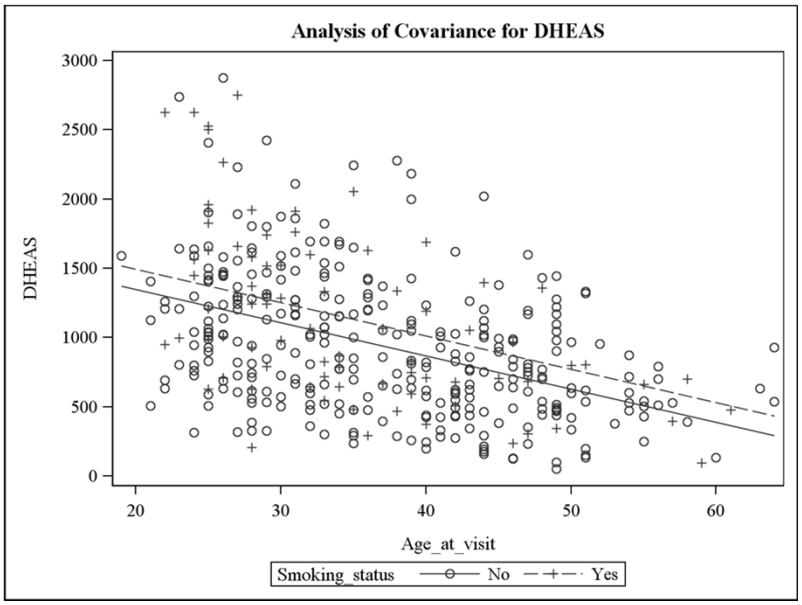
Depression Symptoms Assessed by BDI-II LS Means for DHEAS Levels



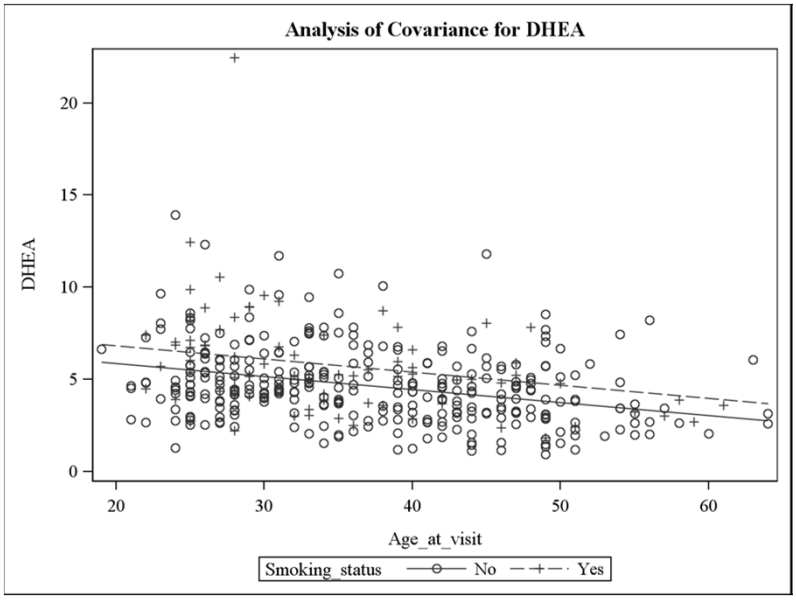
Pearson Partial Correlation Coefficients (n=621; adjusting for age, smoking)

	DHEA	DHEAS	Ratio: DHEA/DHEAS
RESILIENCE CONNOR-DAVIDSON RESILIENCE SCALE (CD-RISC)	0.00132 0.9738	**0.14989** ***0.0002**	-0.07845 0.0511
SCL-90 (Anxiety)	-0.01797 0.6554	**-0.13071** ***0.0011**	**0.11174** ***0.0054**
SCL-90 (Depression)	0.00208 0.9589	**-0.12992** ***0.0012**	**0 .10690** ***0.0078**
SCL-90 (GSI)	-0.01643 0.6832	**-0.13806** ***0.0006**	**0.10999** ***0.0062**

DHEAS Decreases with Age
DHEAS is Elevated in Smokers



DHEA Decreases with Age
DHEA is Elevated in Smokers



Neurosteroid Investigations in the VA Mid-Atlantic MIRECC Registry Cohort

- *DHEA and DHEAS in Male OEF/OIF/OND Era Veterans (n=662)*
  - DHEAS decreased in PTSD
  - DHEAS decreased in depression
  - DHEAS inversely correlated with SCL-90R *anxiety* and *depression* subscales
  - DHEAS positively correlated with resilience (Connor-Davidson Resilience Scale)
  - Both DHEA and DHEAS increased with smoking
  - Both DHEA and DHEAS decreased with age

INTRuST Biorepository: *Neurosteroids and PTSD*

Methods

- **Summary statistics:**
  - --Summary table with N, mean, standard deviation, min, Q1, median, Q3, and max)
  - --Statistical tests conducted to compare the difference in each variable between the groups using Wilcoxon Rank Sum test.
- **Regression analysis:**
  - --Outcome ~group + age + current smoking (predetermined co-variates)
  - --Outcome is neurosteroid and inflammatory markers
  - --Neurosteroid variables: allopregnanolone, pregnenolone, androsterone, pregnanolone
  - --Inflammatory markers: c-reactive protein, IL-6, IL1β, TNF-α, IL-8, others
  - --For group variable, control is the reference group
  - --Current smoking has two categories: Not smoking at all (reference group) vs. now smoking every day or smoking some days
  - --The following two types regression are performed:
    - * Linear regression without transformation
    - * Box-Cox transformed regression model

SUMMARY STATISTICS (by diagnosis group); PTSD, irrespective of TBI

	N	Mean	SD	Min	Q1	Median	Q3	Max	p.value
Pregnenolone									
Control	103	544.7	278.2	77.7	345.5	542.4	684.4	1752.1	
PTSD	109	506.8	411.9	69.9	273.1	417.7	645.1	3598.2	
Overall	212	525.3	353.0	69.9	306.5	470	669.0	3598.2	**0.041 ***
Allopregnanolone									
Control	103	63.7	32.2	14.5	42.8	54.9	75.1	200.8	
PTSD	107	47.9	33.2	5.8	25.2	40.1	58.1	203.4	
Overall	210	55.7	33.6	5.8	33.1	47.4	69.7	203.4	**<0.001 ***
Pregnanolone									
Control	103	172.6	90.6	48.2	114.4	150.8	219.5	457.9	
PTSD	107	177.9	116.3	16.9	96.7	149	238.0	784.1	
Overall	210	175.3	104.23	16.9	105.0	150.5	223.9	784.1	**0.915**
Androsterone									
Control	103	151.4	63.9	46.3	106.3	135.1	191	355.4	
PTSD	107	128.1	60.3	32.7	92.15	120	153.7	396.8	
Overall	210	139.5	63.0	32.7	97.45	127.2	173.075	396.8	**0.008 ***

Allopregnanolone

Regression Without Transformation

	Estimate	Std. Error	t value	Pr(>|t|)
GroupPTSD	-11.02	4.82	-2:28	**0.02343**
Age	-0.820	0.20	-4.13	0.00005
Smoking (every day or some days)	0.917	4.97	0.14	0.88947

Box-Cox Transformed Regression

	Estimate	Std. Error	t value	Pr(>|t|)
GroupPTSD	-0.288	0.083	-3.44	**0.0007**
Age	-0.017	0.0034	-4.82	0.0000
Smoking (every day or some days)	-0.013	0.086	-0.15	0.8789

Androsterone

Regression Without Transformation

	Estimate	Std. Error	t value	Pr(>|t|)
GroupPTSD	-15.73297	8.14785	-1.93094	**0.0549**
Age	-2.45073	0.33487	-7.31855	<0.00001
Smoking	24.98435	8.39488	2.97614	0.00328

Box-Cox Transformed Regression

	Estimate	Std. Error	t value	Pr(>|t|)
GroupPTSD	-0.10645	0.05529	-1.92554	**-0.0556**
Age	-0.02101	0.00227	-9:24462	-0:00000
Smoking	0.16340	0.05696	2.86868	0.00456

Continuous Outcomes

Neurosteroids and PTSD (PCL)
Neurosteroids and Depression (PHQ9)

PTSD Symptom Checklist (PCL)
Allopregnanolone

Box-Cox Transformed Regression Model

	Estimate	Std. Error	Tvalue	Pr(>|t|)
Allopregnanolone	-0.00054	0.00021	-2.61709	**0.00944**
Age	0.00182	0.00059	3.05500	0.00251
Smoking	0.05989	0.01414	4.23583	0.00003

PHQ9 (Patient Health Questionnaire); Depression Allopregnanolone

Box-Cox Transformed Regression Model

	Estimate	Std. Error	Tvalue	Pr(>|t|)
Allopregnanolone	-0.01721	0.00540	-3.18701	**0.00163**
Age	0.04961	0.01506	3.29458	0.00114
Smoking	1.38617	0.36921	3.75437	0.00022

INTRuST Biorepository: *Neurosteroids and TBI*

SUMMARY STATISTICS (by diagnosis group);
TBI, irrespective of PTSD

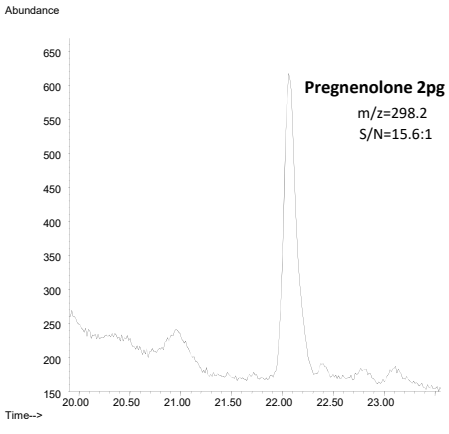
	N	Mean	SD	Median	p.value
Pregnenolone					
Control	103	544.7	278.2	542.4	
TBI	130	504.7	39259	421.3	
Overall	233	522.4	346.6	462.3	**0.049 ***
Allopregnanolone					
Control	103	63.7	32.2	54.9	
TBI	129	46.7	28.6	40.1	
Overall	232	55.7	46.1	46.1	**<0.001 ***
Pregnanolone					
Control	103	172.6	90.6	150.8	
TBI	129	177.9	113.1	149.8	
Overall	232	174.0	103.5	150.5	**0.85**
Androsterone					
Control	103	151.4	63.9	135.1	
TBI	129	138.5	92.0	119.6	
Overall	232	144.2	80.8	125.9	**0.02 ***

Neurosteroids and Traumatic Brain Injury (TBI)

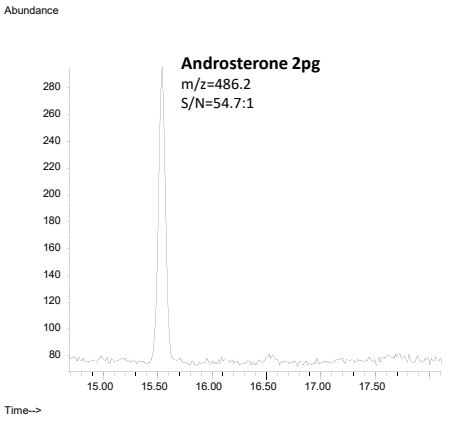
Pilot Neurosteroid Investigation: Blast-Related TBI vs. Deployed Control OEF/OIF Era Veterans

- VA Mid-Atlantic MIRECC Registry Investigation
- Blast-Related TBI (either with or without LOC)
  vs.
  Deployed OEF/OIF Veterans with no history of blast-related TBI (n=55/group)
- GC/MS preceded by HPLC
- Matched for:
  - Time of blood draw
  - Age
  - Smoking Status (smoker/non-smoker)
  - All males

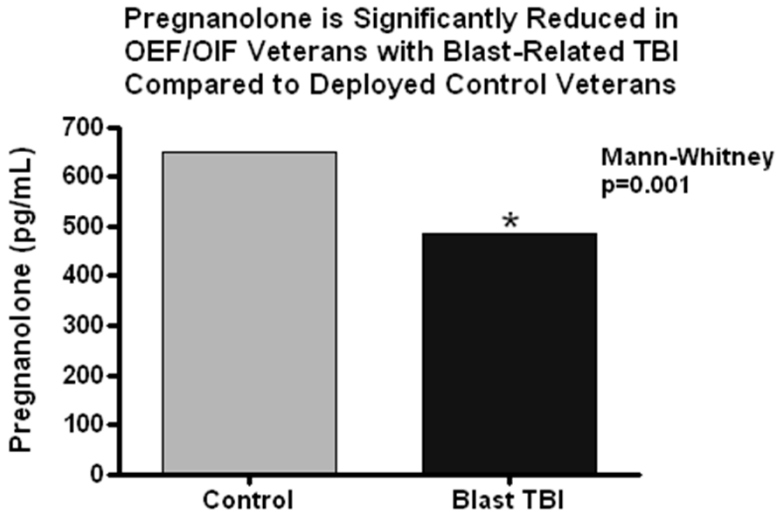
Pregnenolone



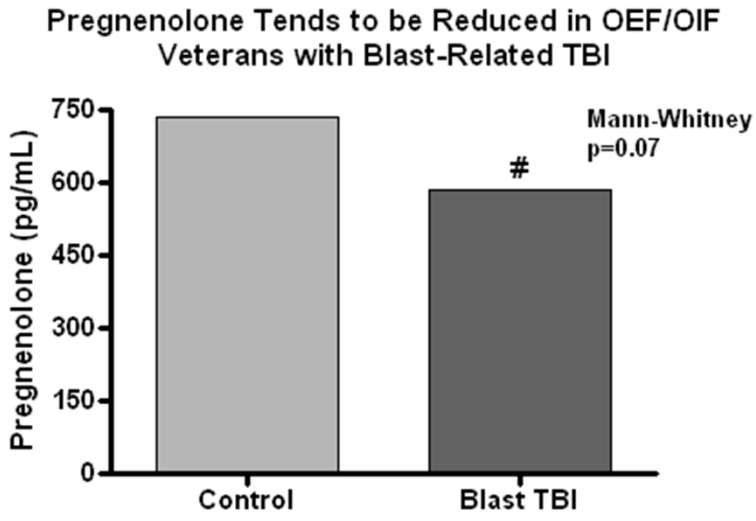
Androsterone



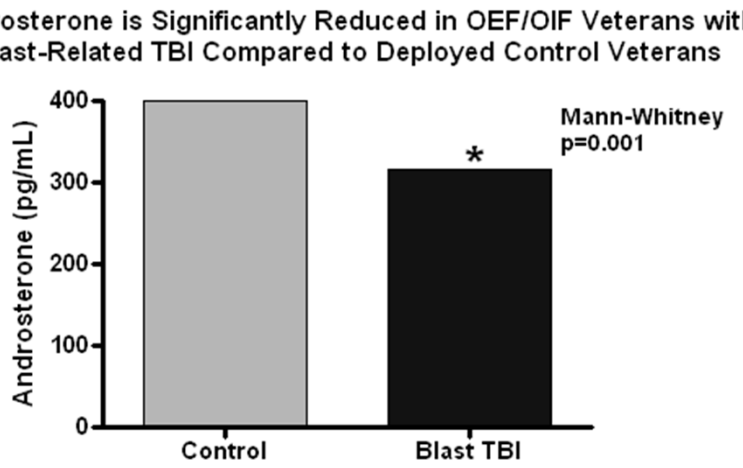
Pregnanolone is Significantly Reduced in OEF/OIF Veterans with Blast-Related TBI



Pregnenolone Tends to be Reduced in OEF/OIF Veterans with Blast-Related TBI



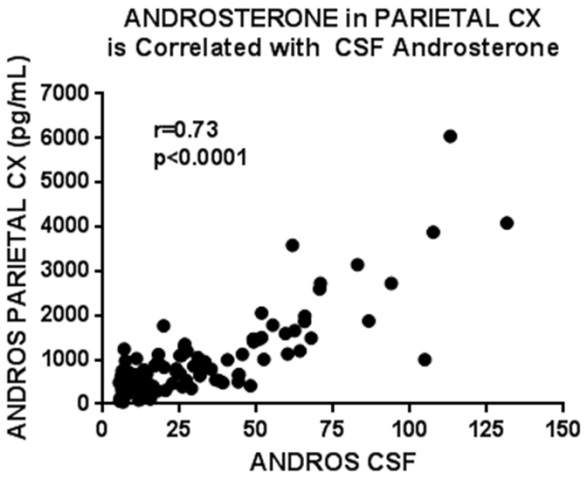
Androsterone is Significantly Reduced in OEF/OIF Veterans with Blast-Related TBI



Androsterone

- $GABA_A$ receptor modulator (Peters et al 1988; Park-Chung et al 1999)
- Anticonvulsant (Zolkowska et al 2014; Kaminski et al 2005)
- Neuroprotective actions vs. pilocarpine-induced seizure (Cho et al 2014)
- Anxiolytic-like actions (Zajda et al 2012)

CSF *Androsterone* vs. Parietal Cortex Andros (HUMAN)



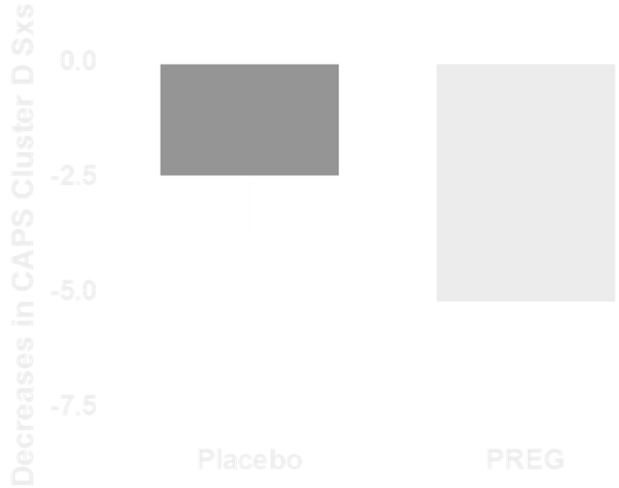
Pilot RCT in Mild TBI in Iraq and Afghanistan Era Veterans

- **Randomized, placebo-controlled, double-blind**
- **FDA IND #78,270**
- **Single-blind placebo lead-in period all pts (2 wks)**
  Randomization to pregnenolone or placebo (8 wks):
  - **50 BID x 2 weeks, followed by**
  - **150 BID x 2 weeks, followed by**
  - **250 BID x 4 weeks**
- **Psychiatric medications (if any) stable:**
  no change in dosing ≥ 4 weeks prior to enrollment;
  no change in psychiatric medication throughout study
- **22 reached 4 wks post-randomization / 73% of 30 randomized**

Pilot RCT in Mild TBI in Iraq and Afghanistan Era Veterans

- **Inclusion Criteria:**
  - **18-55 years of age, any ethnic group, either sex**
  - **History of mild TBI since September 2001**
  - **Definition of mild TBI: World Health Organization Task Force (Holm et al 2005), with the exception of the Glasgow Coma Scale Score criteria (generally not available for these participants)**
  - **Ability to participate fully in the informed consent process.**

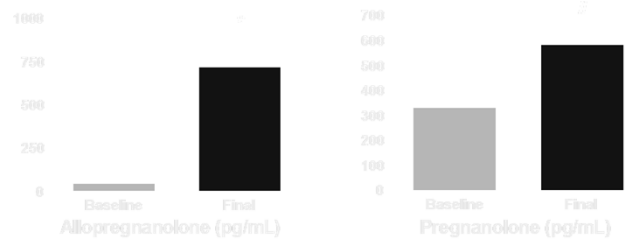
CAPS: Cluster D Symptoms (Pilot Study)

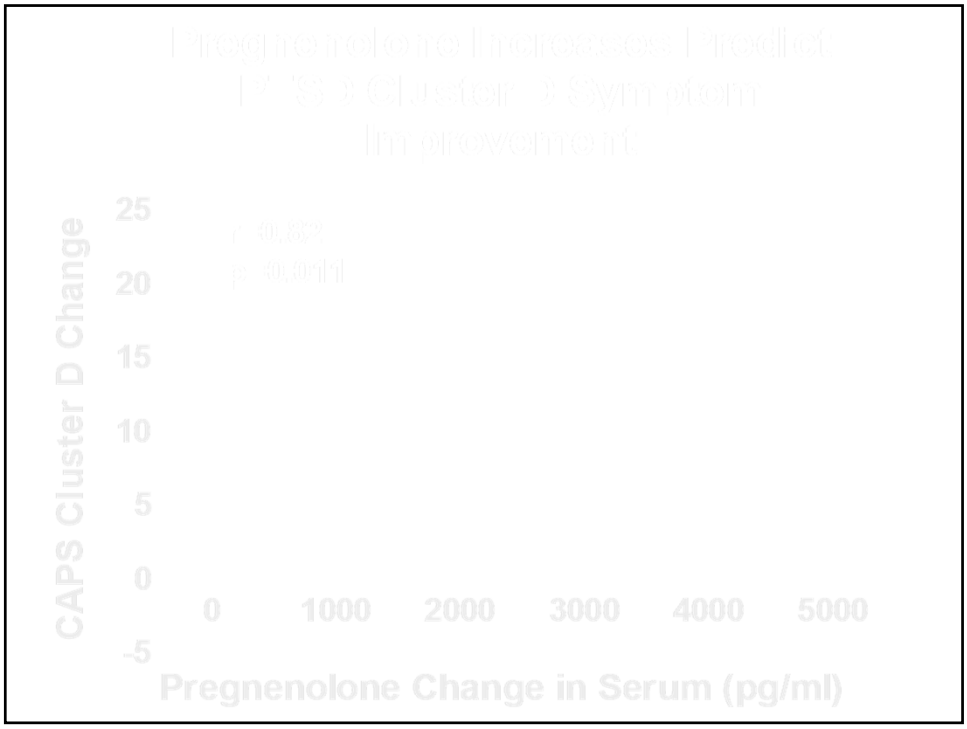


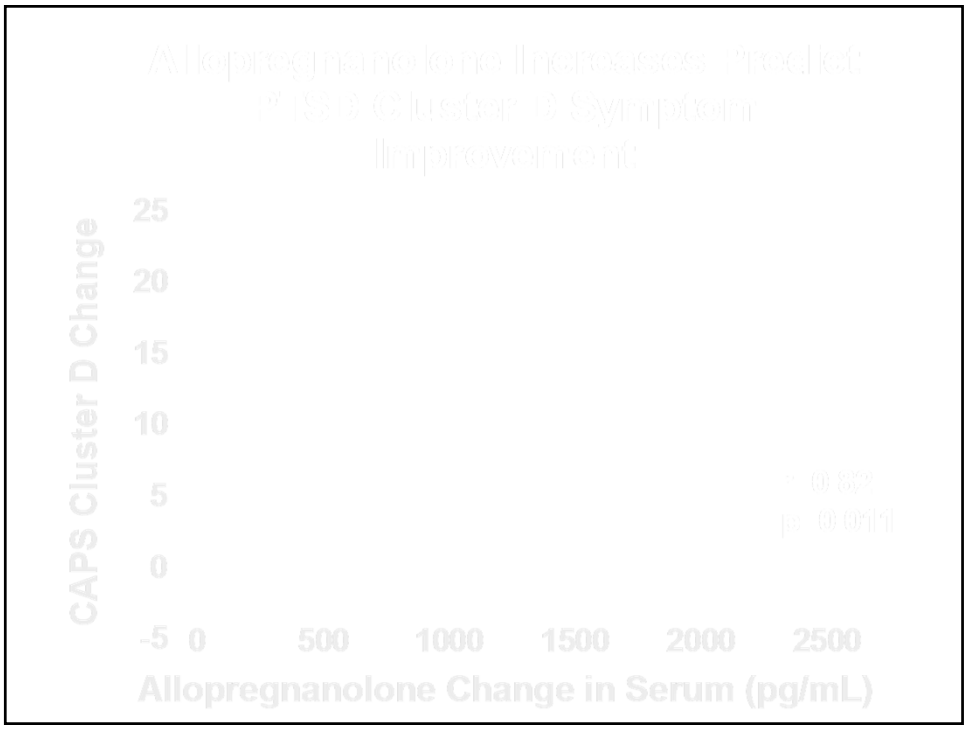
CAPS Cluster D Symptoms

- **Cluster D: Hyperarousal:**
  - **Sleep difficulty**
  - **Irritability or outbursts of anger**
  - **Concentration difficulty**
  - **Hypervigilance**
  - **Exaggerated startle response**

Neurosteroids and Mild TBI: Elevations Following Pregnenolone
(Pilot Study)




Pregnenolone Increases Predict PTSS Cluster D Symptom Improvement
r = 0.82
p = 0.001
CAPS Cluster D Change
25
20
15
10
5
0
-5
0
1000
2000
3000
4000
5000
Pregnenolone Change in Serum (pg/ml)


Allopregnanolone Increases Predict PTSS Cluster D Symptom Improvement
r = 0.82
p = 0.001
CAPS Cluster D Change
25
20
15
10
5
0
-5
0
500
1000
1500
2000
2500
Allopregnanolone Change in Serum (pg/mL)

Proof-of-Concept RCT with Pregnenolone in Mild TBI

(Follow-up Investigation)

- **Larger randomized controlled trial (same design; VA Merit); last patient visit March 2016 (n=53 randomized; 44 to Week 4 post-randomization)**
- **Neurosteroids as potential biomarkers of therapeutic response**
- **Participants with relative deficits in baseline neurosteroids more likely to respond to a neurosteroid intervention?**
  (i.e. that potentially restores neurosteroid levels to physiologically optimal concentrations)
- **Neuroimaging component in subset of participants pre/post neuroimaging (DTI)**
- **Builds upon recent data showing amygdala and DLPFC changes on fMRI following one-time neurosteroid administration**

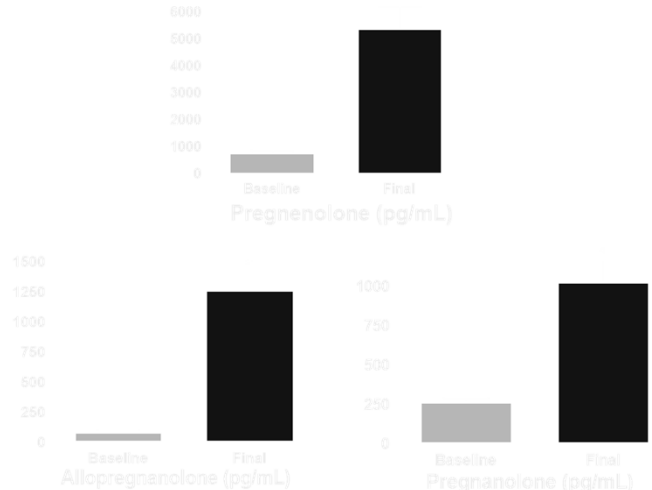
Proof-of-Concept RCT in Mild TBI in Iraq and Afghanistan Era Veterans

(Follow-up Investigation)

- **Psychiatric medications (if any) stable:**
  no change in dosing ≥ 4 weeks prior to enrollment;
  no change in psychiatric medication throughout study
- **FDA IND #78,270**
- **Randomized, placebo-controlled, double-blind (45 reached 4 weeks post-randomization / 88% of 51 randomized)**
- **Single-blind placebo lead-in period all pts (2 wks)**
  Randomization to pregnenolone or placebo (8 wks):
  50 BID x 2 weeks, followed by
  150 BID x 2 weeks, followed by
  250 BID x 4 weeks
- **Total Duration 10 weeks**
- **<u>Primary Behavioral Endpoint</u>: <u>Cluster D Symptoms</u>**

CAPS: Cluster D Symptoms
(Follow-up Study – NEED TO UPDATE)

Neurosteroids and Mild TBI: Elevations Following Pregnenolone
(Follow-up Study)



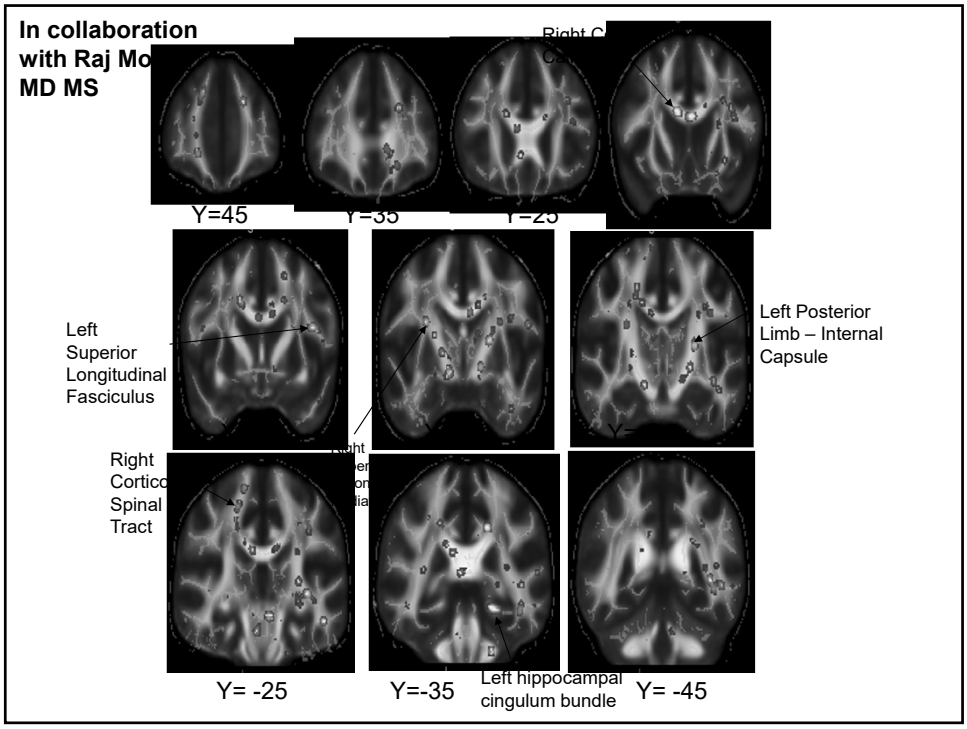
NEUROIMAGING CORRELATES
Randomized Control Trial
(with Raj Morey, MD)

Sample size

- 13 pre/post assessments in pregnenolone group
- 7 pre/post assessments in placebo group

DTI at baseline/randomization visit and post-treatment x 8 weeks
(pregnenolone n=13 vs. placebo n=7)

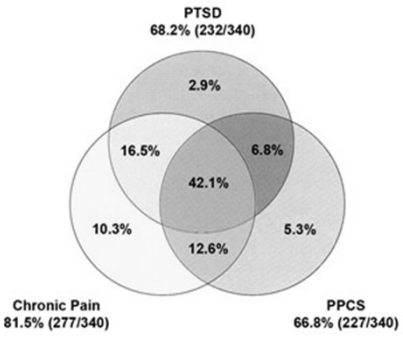
- DTI data analyzed with Tract-Based Spatial Statistics (TBSS) approach.
- DTI results show interaction time X treatment.
  - In other words, highlighted voxels show greater posttreatment vs. pretreatment changes in the pregnenolone group compared to the placebo group ($p < .05$; two tailed, uncorrected).
- The clustering of significant voxels (uncorrected) suggest effects that are unlikely to be noise, but do not meet the corrected threshold for significance
- Conduction of a spatially independent analysis of time X treatment in progress



Neurosteroids and Pain
(Iraq/Afghanistan Era Veterans)

Pain and Co-Occurring Conditions

- **Chronic pain disorders are challenging to treat in OEF/OIF Veterans** (Taylor et al., 2012; Helmer et al., 2009; Gironda et al., 2009; Lew et al., 2009, Cohen et al., 2009).
- **Mental health diagnosis increases likelihood of receiving opiates and increases risk of adverse clinical outcomes** (Seal et al., 2012).
- **Need for effective, safe, and non-habit forming pharmacological treatments**



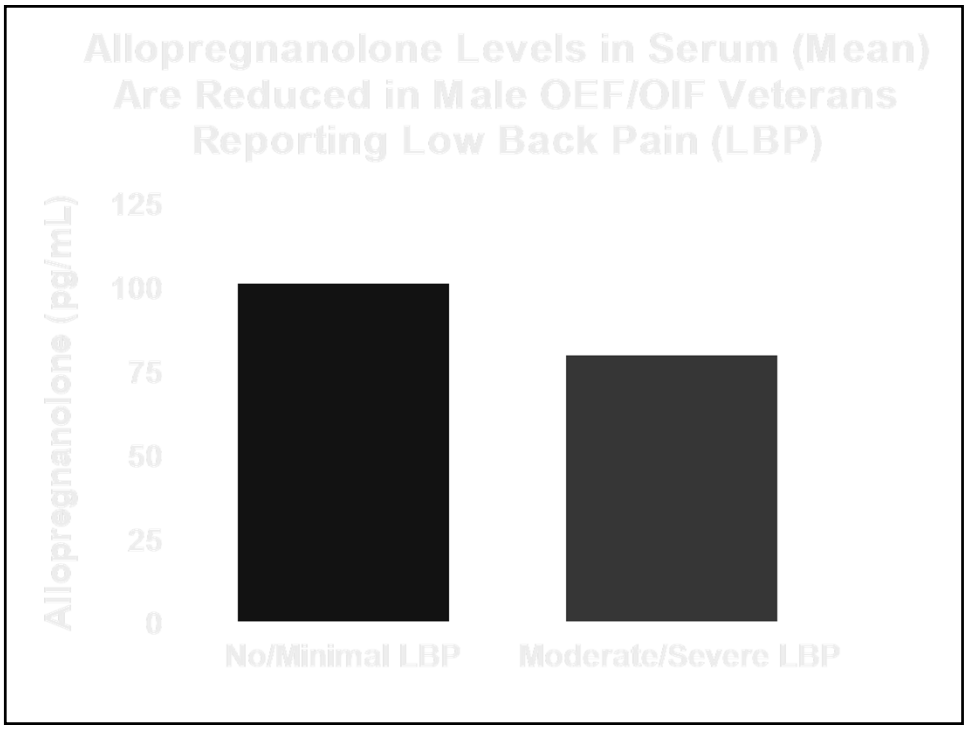
Polytrauma Clinical Triad.
Adapted from: Lew et al., 2009

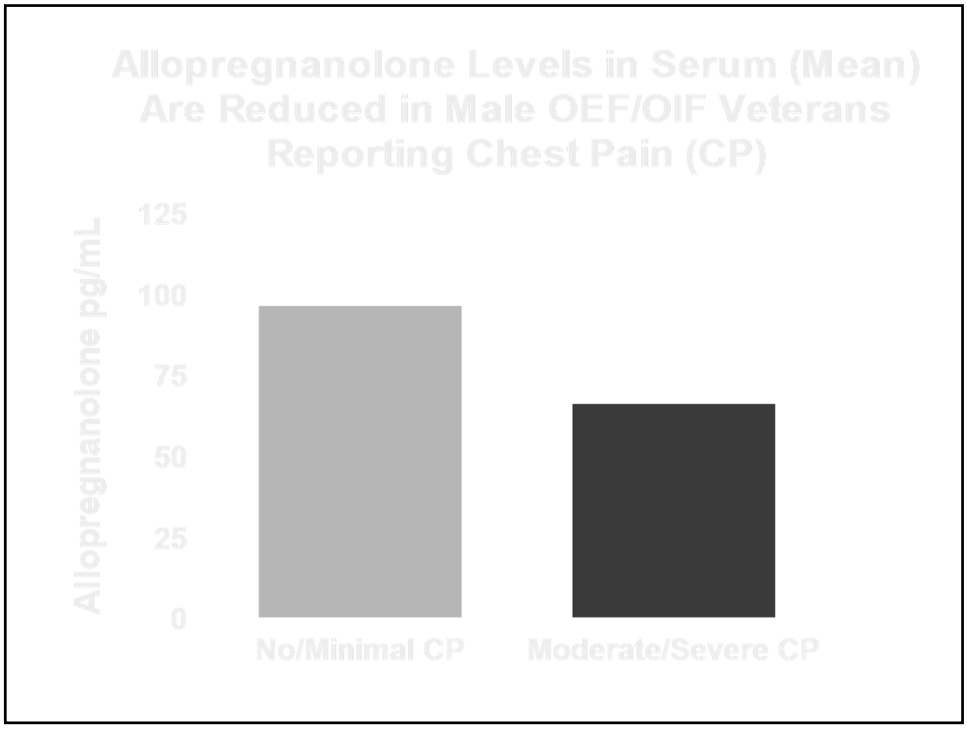
Neurosteroids as Biomarker Candidates and Potential New Therapeutics for Pain

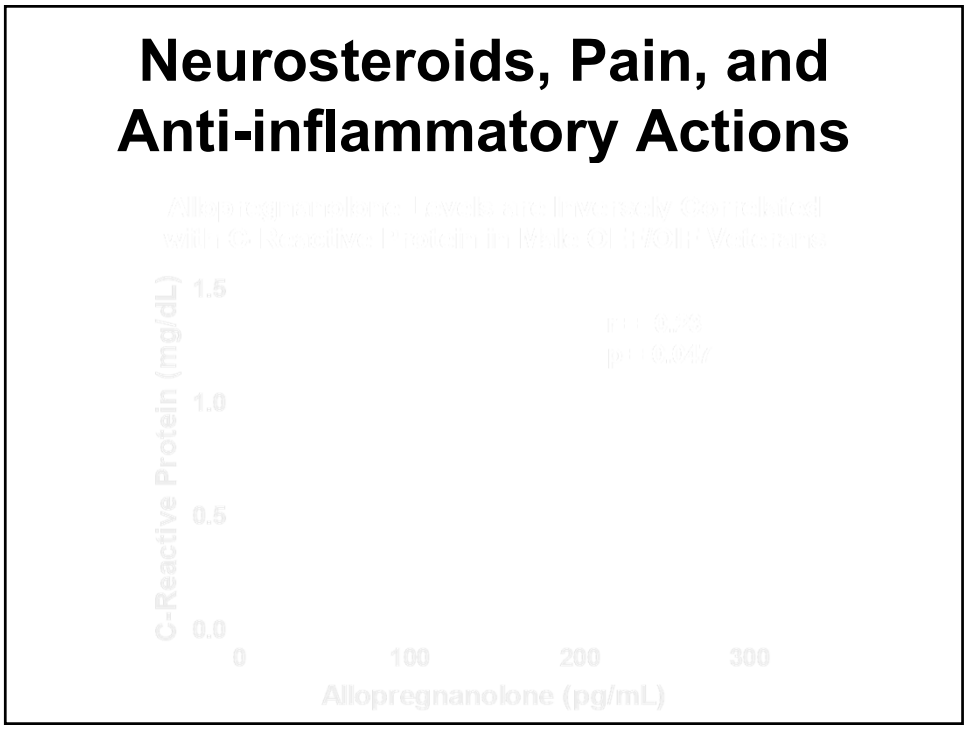
- Allopregnanolone positively modulates inhibitory $GABA_A$ receptors (Majewska et al., 1986; Morrow et al., 1987).
- Neurosteroids that positively modulate $GABA_A$ receptors demonstrate the following actions:
  - anxiolytic (Crawley et al., 1986; Wieland et al., 1991; Bitran et al., 2000; Jain et al., 2005),
  - anticonvulsant (Landgren et al., 1987; Belelli et al., 1989; Kokate et al., 1994; Devaud et al., 1995; Kokate et al., 1996)
  - anti-aggression (Kavaliers, 1988; Pinna et al., 2003)
- Additional evidence of analgesic actions of neurosteroids, particularly ALLO and other GABAergic neurosteroids.

Allopregnanolone and Analgesic Properties

- **PRECLINICAL EVIDENCE:**
  - **ALLO increases response latencies to thermal stimuli in both rats** (Kavaliers et al., 1987) **and invertebrates** (Kavaliers et al., 2000).
  - **ALLO increases response latencies to tailflick in rats** (Frye & Duncan, 1994).
  - **ALLO and alphaxalone (a synthetic neurosteroid derivative) reverse thermal and mechanical hyperalgesia in rodent model** (Svensson et al., 2013).
  - **ALLO protects against noxious mechanical visceral stimuli in rats** (Winfree et al., 1992).
  - **ALLO implicated in neuropathic pain analgesia** (Afrazi et al., 2014, Patte-Mensah et al., 2010; Aouad et al., 2014; Xu et al., 2014; Kawano et al., 2011)
  - **ALLO** (Meyer et al., 2011) **and 3-alpha androstanediol** (Meyer et al., 2013) **prevent and suppress chemotherapy-induced neuropathies in rats.**




Allopregnanolone Levels in Serum (Mean) Are Reduced in Male OEF/OIF Veterans Reporting Chest Pain (CP)
Allopregnanolone pg/mL
125
100
75
50
25
0
No/Minimal CP
Moderate/Severe CP


Neurosteroids, Pain, and Anti-inflammatory Actions
Allopregnanolone Levels are Inversely Correlated with C-Reactive Protein in Male OEF/OIF Veterans
C-Reactive Protein (mg/dL)
1.5
1.0
0.5
0.0
0
100
200
300
Allopregnanolone (pg/mL)

Replication in 485 Male Veterans from the VA Mid-Atlantic MIRECC Registry

- **Independent cohort of 485 male participants from VA Mid-Atlantic MIRECC Registry**
  (blood draw between 10:30AM and 2:30PM)
- **Outcome Measures:**
  - **Symptom Checklist-90 (SCL-90, low back pain, chest pain, muscle soreness, and headache)**
  - **Analyses:**
    - **Poisson Regression**
      - **Predictor Variable: Neurosteroid**
      - **Response Variable: Pain rating**
  - **NS levels quantified by gas chromatography/mass spectrometry, preceded by high performance liquid chromatography purification (sensitivity 1 picogram)**

Demographics

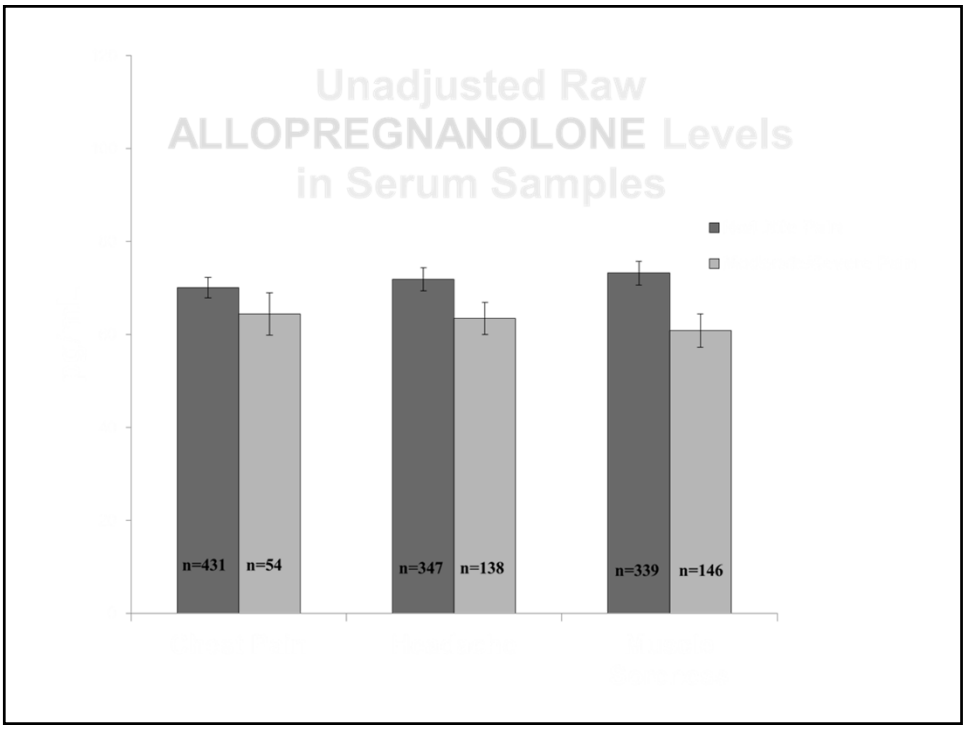
African American	48%
Caucasian	40%
Native American	5%
Hispanic	7%
Age	Mean=37

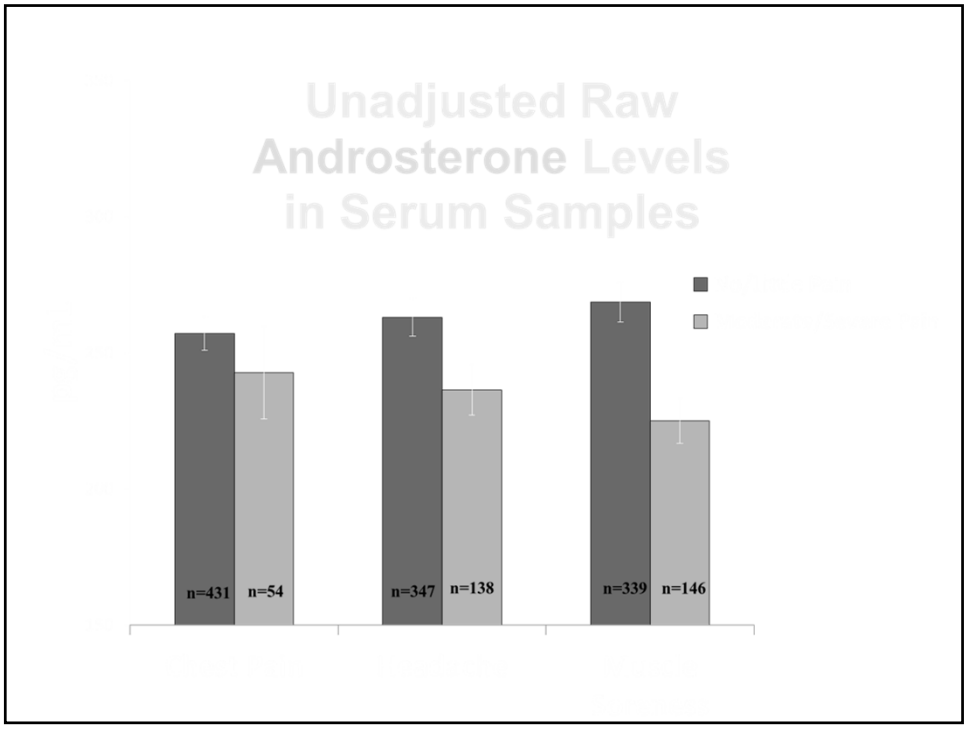
Muscle Soreness, Chest Pain, and Headache are Associated with Reduced Serum Levels of ALLO* and Androsterone* in Male Veterans

Muscle Soreness				
Neurosteroid	95% Confidence Limits		Chi-Square	P value
Allopregnanolone	-0.0064	-0.0013	8.96	**0.003**
Androsterone	-0.0025	-0.0007	11.67	**0.001**
Pregnanolone	-0.0005	0.0008	0.12	0.734
Pregnenolone	-0.0004	0.0002	0.71	0.401

Chest Pain				
Neurosteroid	95% Confidence Limits		Chi-Square	P value
Allopregnanolone	-0.0080	-0.0004	4.62	**0.032**
Androsterone	-0.0028	-0.0001	4.64	**0.031**
Pregnanolone	-0.0014	0.0007	0.38	0.536
Pregnenolone	-0.0007	0.0001	2.28	0.131

Headache Pain				
Neurosteroid	95% Confidence Limits		Chi-Square	P value
Allopregnanolone	-0.0042	0.0002	3.07	**0.080**
Androsterone	-0.0019	-0.0002	6.42	**0.011**
Pregnanolone	-0.0008	0.0005	0.16	0.689
Pregnenolone	-0.0005	0.0001	2.08	0.149





Neurosteroids as Biomarker Candidates

Summary:

- **ALLO:**
  - **Significant inverse association between serum ALLO and *muscle soreness***
  - **Significant inverse association between serum ALLO and *chest pain***
  - **Marginally significant inverse association between ALLO and *headache***

  Replicates, in large part, prior ALLO findings in 82 OEF/OIF Veterans in a larger independent cohort of 485 OEF/OIF/OND Veterans

- **Androsterone:**
  - **Significant inverse association between androsterone levels and *chest pain***
  - **Significant inverse association between androsterone levels and *headache***
  - **Significant inverse association between androsterone levels and *muscle soreness***

Neurosteroids and Inflammation

Neurosteroids and Possible Anti-inflammatory Actions: *Allopregnanolone and C-Reactive Protein (CRP)*

C-Reactive Protein and Allopregnanolone Levels:

R=-0.26
P<0.0001

Discovery Cohort:
N=82

Replication Cohort:
N=480

Neurosteroids and Possible Anti-inflammatory Actions: *CRP - Androsterone, Pregnenolone*

C-Reactive Protein and Neurosteroid Levels:

ANDROSTERONE:

R= -0.22
P<0.0001

N=480

C-Reactive Protein and Neurosteroid Levels:

PREGNENOLONE:

R= -0.33
P<0.0001

N=479

Neurosteroids and Possible Anti-inflammatory Actions: *Interleukin-6 (IL-6) and Allopregnanolone, Androsterone, Pregnenolone*

IL-6 and Neurosteroid Levels:

ALLOPREGNAN-OLONE:

R= -0.22
P<0.0001
N=480

IL-6 and Neurosteroid Levels:

ANDROSTERONE:

R= -0.19
P<0.0001
N=480

IL-6 and Neurosteroid Levels:

PREGNENOLONE:

R= -0.25
P<0.0001
N=479

Neurosteroids and Possible Anti-inflammatory Actions: *TNF-α and Androsterone, Pregnenolone*

Tumor Necrosis Factor-α (TNF- α) and Neurosteroid Levels:	Tumor Necrosis Factor-α (TNF- α) and Neurosteroid Levels:
ANDROSTERONE:	**PREGNENOLONE:**
R= -0.13 P<0.0043	R= -0.18 P<0.0001
N=480	N=479

Neurosteroids, Pain, and Possible Anti-inflammatory Actions

- **C-Reactive Protein and Neurosteroids**
  - Allopregnanolone levels are *inversely correlated* with c-reactive protein (r = -0.26; p<0.0001; n=480); replication
  - Also *inversely correlated* to C-reactive protein: androsterone (r = -0.22; p<0.0001; n=480) & pregnenolone (r= -0.33; p<0.0001; n=479)

Neurosteroids, Pain, and Possible Anti-inflammatory Actions

- **IL-6 (pro-inflammatory cytokine)**

  Allopregnanolone levels are inversely correlated with IL-6 levels

  ($r = -0.22$; $p<0.0001$; $n=480$), as are androsterone ($r = -0.20$; $p<0.0001$; $n=480$) & pregnenolone ($r= -0.25$; $p<0.0001$; $n=479$)

Lab shout-outs!! *with gratitude*

Larry Shampine – since Nov. 2002 (!)
Gillian Parke
Jennifer Naylor
Jason Kilts
Trina Allen
Karen Smith
Susan O'Loughlin
Brian Cuffe
Steven Szabo

Thank you!