Bridging Bench and Bedside: Translational Neurosteroid Investigations - from Rodent Models to FDA INDs

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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 (Duke/VA)

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

Consultant - Zogenix
Support

- **Department of Veterans Affairs**
  - VA Merit Reviews
  - VA Mid-Atlantic Mental Illness, Research, Education and Clinical Center (MIRECC)
  - VA CDTA, VA ARCD, VA REAP

- **Department of Defense**
  - DoD INTRuST Consortium
  - DoD INTRuST Biorepository
  - DoD Concept Award

- **NIMH/NIH; NIAAA/NIH**
  - K23 Career Development Award
  - R01 (subcontracts)

- **Singapore Institute of Mental Health**
  - TCR Flagship Programme

- **NARSAD Young Investigator Awards**

- **Bryan Alzheimer’s Disease Research Center, Duke SOM**
VA Mid-Atlantic MIRECC
(Mental Illness, Research, Education and Clinical Center)

**Director:** John Fairbank  
**Deputy Director:** Mira Brancu

*Begun in 2005*
Durham VA, Salisbury VA, 
Richmond VA, Hampton VA, 
other collaborating VAs

**Components:**

**Research:**  
Jean Beckham  
Chris Marx

**Education:**  
Robin Hurley  
Katherine Taber

**Clinical:**  
Keith Shaw  
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Nathan Kimbrel

**Laboratories:**

- Interventions & Metabolomics  
  Chris Marx  
  Raj Morey  
  Mira Brancu  
  Jen Runnels

- Neuroimaging:  
  Raj Morey  
  Mira Brancu  
  Jen Runnels

- PDMH and Repository  
  Pat Calhoun  
  Larry Tupler

- Health Services:  
  Pat Calhoun  
  Larry Tupler

- Neurocognition:  
  Scott Moore

- Genetics:  
  Jean Beckham  
  Mike Hauser

**Neuroscience:**

- Statistical Expertise
  - Ryan Wagner
  - Robert Hamer
INTRuST Consortium
Injury and Traumatic Stress Center

**PI:** Murray Stein (UC San Diego)
**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

**Duke SOM/Durham VA Site:**
**Pls:** Gerry Grant
Chris Marx
Thank You

- **Monika Shafi** (Swarthmore)
- **Walter Sokel** (University of Virginia)
- **Michael Wilson and Barth Reller** (Duke 3rd Year Research)
- **Rosa Merino** (Duke 4th Year Sub-I)
- **Leslie Morrow and Jeffrey Lieberman** (Research Fellowship, NIH Extramural at UNC)
- **Richard Weiner/Ranga Krishnan/Dan Blazer/Richard Surwit** (Duke Faculty Recruitment ‘01)
- **Duke Faculty - Bob Stevens** (mass spec), **Bruce Burnett** (FDA)
- **Duke Leadership:** Ranga Krishnan, Marvin Swartz, Holly Lisanby, Richard Weiner, Moira Rynn
- **VA Leadership:** Richard Weiner, Jonathan Leinbach (MHSL); John Fairbank (MIRECC)
- **Duke and VA Collaborators, Colleagues, Administration, and Research Team**
Neurosteroids

• Endogenous molecules synthesized \textit{de novo} in brain from cholesterol

• Enriched in brain, but also synthesized in the adrenal, other tissues

• Many neurosteroids are ‘neuroactive’ - modulating \( \text{GABA}_A \) receptors, NMDA receptors, others (rapidly altering neuronal excitability)

• Precursor to many neurosteroids, glucocorticoids, other steroids
Neurosteroids as Promising Pharmacological Interventions: *Pregnenolone*

- 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*
-Neurological and psychiatric disorders:
  Currently available pharmacological treatment options suboptimal or even non-existent
  There are only 2 FDA-approved drugs for PTSD (both approved > 15 years ago); there are no FDA-approved drugs for TBI; new medication development for PTSD, TBI, schizophrenia, pain, other CNS disorders an urgent unmet clinical need

-Number of mechanistically novel drugs approved by the FDA for CNS disorders in last 50 years extremely low (and many major industry leaders have abandoned efforts in the neurosciences entirely)

-Average time to successful development of a new drug is about 17 years at a cost of 1.8 billion – almost a generation away for drug candidates identified today (Paul et al 2010; *Nature Reviews Drug Discovery*)

-March 10, 2016 – Tufts Center for the Study of Drug Development: ‘Average cost to develop and gain marketing approval for a new drug exceeds 2.5 B’ (*Journal of Health Economics*)

-September 2017: ‘May be as low as 650 million for cancer drugs’ (*JAMA Intern. Med.*)
Strategy

• **Biomarker investigations:**
  To identify potential risk / resilience factors for PTSD, schizophrenia, TBI, depression, pain disorders and other CNS conditions in serum samples from the VA Mid-Atlantic MIRECC Post-Deployment Mental Health (PDMH) study and other cohorts such as INTRuST, others by characterizing neurosteroid signatures (and other small molecule biomarker candidates)

• **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)
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 2013)

- **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)
Neurosteroids and Schizophrenia
Pregnenolone and Clozapine

Clozapine 20 mg/kg Increases Pregnenolone Levels in Hippocampus
Pregnenolone and Clozapine

Clozapine 20 mg/kg Increases
Pregnenolone Levels in Serum

PREG Levels (ng/mL)
Brain vs. Serum Pregnenolone (RAT)

Peripheral Serum Pregnenolone Levels are Correlated with Central Hippocampal Pregnenolone Levels

$r = 0.95$

$p < 0.0001$
CSF Pregnenolone vs. Temporal Cortex Preg. (HUMAN)

Graph showing the correlation between CSF Pregnenolone levels and Temporal Cortex Pregnenolone levels. The correlation coefficient is $r = 0.57$ with $p < 0.0001$.
Pregnenolone in Postmortem Brain Tissue

Neurosteroid Levels in Parietal Cortex

Control
Schizophrenia
Bipolar Disorder
Depression (non-psychotic)
Pregnenolone in Postmortem Brain Tissue

Neurosteroid Levels in Posterior Cingulate

- Control
- Schizophrenia
- Bipolar Disorder
- Depression (non-psychotic)
Pregnenolone levels (serum) are associated with left cortical thickness (n=115)

$\rho < .05; \text{FDR corrected}$
ORIGINAL INVESTIGATION

Proof-of-concept randomized controlled trial of pregnenolone in schizophrenia


*Psychopharmacology* 2014; 231(17):3647-62.

---Participants randomized to pregnenolone (n=56) demonstrated greater improvements in functional capacity (UPSA-B composite changes) compared to placebo (n=55), p=0.03.

---**Pregnenolone was also superior to placebo in the Communication Subscale of the UPSA-B (p<0.001).**

--No significant changes compared to placebo in composite MCCB scores or PANSS scores.
Phase II Randomized Controlled Trial with Pregnenolone

- **Site:** Durham VA Medical Center (single site)
- **Randomization Target:** 88
- **Functional Endpoints:** UPSA Total Score and Communication Subscale
- **Cognitive Endpoints:** MCCB, Subscales; BACS
- **Neurosteroid quantification:** Mass spectrometry (pre/post)
- **Pregnenolone dosing:**

  Placebo Lead-In Phase x 2 weeks (all patients), followed by randomization to adjunctive pregnenolone or placebo:
  - 50 mg BID x 2 weeks, followed by
  - 150 mg BID x 2 weeks, followed by
  - 250 mg BID x 4 weeks (8 wks total tx)
Modified Intention-to-Treat Efficacy (mITT-Efficacy):
The mITT-Efficacy Population includes all randomized subjects who received at least one dose of the planned study medication and who had at least one planned post-baseline efficacy assessment on the primary efficacy endpoints.

A mixed model for repeated measures (MMRM) method specified with an unstructured covariance was used in the Principal Efficacy analyses. *Test of no difference between Placebo and Pregnenolone groups at Week 10 (endpoint, 8 weeks post-randomization) from an MMRM model with treatment, visit and the interaction of treatment and visit as factors and baseline value as covariate.
Inclusion Criteria

- DSM-IV/DSM-IV-TR Schizophrenia or Schizoaffective Disorder
- Age 18-65 years
- Capable of providing informed consent
- Duration of illness $\geq$ one year
- No change in antipsychotic dose $\geq$ 4 weeks
- No change in anticholinergic, benzodiazepine, or mood stabilizer medications for $\geq$ 4 weeks
- Enriched for cognitive symptoms (Composite BACS scores 0-3 SD below the mean)
- No anticipated need to alter medications (antipsychotics, anticholinergics, benzodiazepines, or mood stabilizers) for the 10-week duration of the study
Exclusion Criteria

- Unstable medical or neurological illness (seizures, CVA)
- Alcohol or other substance dependence (other than nicotine) within the last month
- Use of oral contraceptives or other hormonal supplementation such as estrogen [although early studies suggested no effects on menstrual cycle, alterations in downstream metabolites of pregnenolone (such as estradiol) could theoretically impact the efficacy of oral contraceptives and estrogen replacement]
- Other concomitant medications for medical conditions addressed on a case-by-case base to determine if exclusionary
- Active expression of suicidal or homicidal ideation
- Female patients who are pregnant or breast-feeding
- Known allergy to study medication
# Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Pregnenolone</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Participants</strong></td>
<td>46</td>
<td>42</td>
<td>88</td>
</tr>
<tr>
<td><strong>Age (years, SD)</strong></td>
<td>51.6 (8.4)</td>
<td>51.9 (8.5)</td>
<td>51.7 (8.4)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>7 15.2%</td>
<td>6 14.3%</td>
<td>13 14.8%</td>
</tr>
<tr>
<td>Male</td>
<td>39 84.8%</td>
<td>36 85.7%</td>
<td>75 85.2%</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black or African American</td>
<td>36 78.3%</td>
<td>27 64.3%</td>
<td>63 71.6%</td>
</tr>
<tr>
<td>White</td>
<td>9 19.6%</td>
<td>12 28.6%</td>
<td>21 23.9%</td>
</tr>
<tr>
<td>More than one race</td>
<td>1 2.2%</td>
<td>3 7.1%</td>
<td>4 4.5%</td>
</tr>
<tr>
<td>Unknown or Not Reported</td>
<td>0 0.0%</td>
<td>0 0.0%</td>
<td>0 0.0%</td>
</tr>
</tbody>
</table>
Participant Flow: Overall Study

<table>
<thead>
<tr>
<th></th>
<th>TOTAL</th>
<th>PLACEBO</th>
<th>PREGNENOLONE</th>
</tr>
</thead>
<tbody>
<tr>
<td>RANDOMIZED</td>
<td>88</td>
<td>46</td>
<td>42</td>
</tr>
<tr>
<td>COMPLETED</td>
<td>72 (81.8%)</td>
<td>34 (73.9%)</td>
<td>38 (90.5%)</td>
</tr>
<tr>
<td>DROP-OUT</td>
<td>16 (18.2%)</td>
<td>12 (26.1%)</td>
<td>4 (9.5%)</td>
</tr>
</tbody>
</table>
### University of California Performance-based Skills Assessment (UPSA)

<table>
<thead>
<tr>
<th>Participants Analyzed</th>
<th>Placebo</th>
<th>Pregnenolone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>34</td>
<td>38</td>
</tr>
<tr>
<td>UPSA Total score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>76.68 (1.76)</td>
<td>77.49 (1.63)</td>
</tr>
<tr>
<td>Week 4</td>
<td>80.12 (1.63)</td>
<td>77.29 (2.65)</td>
</tr>
<tr>
<td>Week 8</td>
<td>82.03 (2.05)</td>
<td>85.63 (1.31)</td>
</tr>
</tbody>
</table>
## UPSA

<table>
<thead>
<tr>
<th>Outcome</th>
<th>LS Means at week 10 (SE)</th>
<th>Difference between LS means</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo (n=34)</td>
<td>Pregnenolone (n=38)</td>
<td></td>
</tr>
<tr>
<td><strong>UPSA Composite</strong></td>
<td>82.16 (1.74)</td>
<td>84.05 (1.61)</td>
<td>1.89 (2.37)</td>
</tr>
<tr>
<td><strong>UPSA Subscales</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Financial</strong></td>
<td>23.25 (0.50)</td>
<td>23.10 (0.46)</td>
<td>-0.15 (0.68)</td>
</tr>
<tr>
<td><strong>Communication</strong></td>
<td>18.57 (0.72)</td>
<td>20.66 (0.66)</td>
<td>2.09 (0.98)</td>
</tr>
</tbody>
</table>
Increases in Pregnenolone Levels Post-Treatment are Correlated with Improvements in UPSA Total Scores

$r = 0.373$

$p = 0.039$
Increases in Pregnenolone Levels Post-Treatment are Correlated with Improvements in UPSA Total Scores (males only)
Neurosteroid Elevations Post-Treatment with Pregnenolone

![Graphs showing neurosteroid elevations with Pregnenolone treatment](image-url)
## MCCB

<table>
<thead>
<tr>
<th>Outcome</th>
<th>LS Means at week 10 (SE)</th>
<th>Difference between LS means</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PLACEBO (n=34)</td>
<td>PREGNEN. (n=38)</td>
<td></td>
</tr>
<tr>
<td><strong>MCCB composite</strong></td>
<td>33.82 (1.81)</td>
<td>34.81 (1.7)</td>
<td>0.99 (2.49)</td>
</tr>
<tr>
<td><strong>MCCB subscales</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Speed of Processing</td>
<td>38.31 (1.64)</td>
<td>38.38 (1.5)</td>
<td>0.07 (2.22)</td>
</tr>
<tr>
<td>Attention/Vigilance</td>
<td>38.17 (1.81)</td>
<td>37 (1.67)</td>
<td>-1.17 (2.47)</td>
</tr>
<tr>
<td>Working Memory</td>
<td>38.02 (1.63)</td>
<td>38.73 (1.51)</td>
<td>0.7 (2.22)</td>
</tr>
<tr>
<td><strong>Verbal Learning</strong></td>
<td><strong>34.16 (1.3)</strong></td>
<td><strong>38.2 (1.18)</strong></td>
<td><strong>4.04 (1.76)</strong></td>
</tr>
<tr>
<td>Visual Learning</td>
<td>44.13 (2.28)</td>
<td>46.63 (2.09)</td>
<td>2.49 (3.1)</td>
</tr>
<tr>
<td>Reasoning/Problem</td>
<td>41.79 (1.39)</td>
<td>43.65 (1.28)</td>
<td>1.85 (1.89)</td>
</tr>
<tr>
<td>Social</td>
<td>46.47 (2.3)</td>
<td>45.08 (2.12)</td>
<td>-1.38 (3.13)</td>
</tr>
</tbody>
</table>
## Serious Adverse Events

<table>
<thead>
<tr>
<th>Category</th>
<th>PLACEBO</th>
<th>PREGNENOLONE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Serious Adverse Events (SAEs)</strong></td>
<td>4/46 (8.70%)</td>
<td>3/42 (7.14%)</td>
</tr>
<tr>
<td># participants affected / total in group</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Endocrine disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospitalization for Elevated Glucose</td>
<td>0/46 (0.00%)</td>
<td>1/42 (2.38%)</td>
</tr>
<tr>
<td># participants affected / total in group</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gastrointestinal disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical Admission for Observation</td>
<td>1/46 (2.17%)</td>
<td>0/42 (0.00%)</td>
</tr>
<tr>
<td># participants affected / total in group</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Psychiatric disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychiatric Hospitalization</td>
<td>2/46 (4.35%)</td>
<td>2/42 (4.76%)</td>
</tr>
<tr>
<td># participants affected / total in group</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Reproductive system and breast disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast Cancer Diagnosis</td>
<td>1/46 (2.17%)</td>
<td>0/42 (0.00%)</td>
</tr>
<tr>
<td># participants affected / total in group</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Conclusions

- Significant improvement on UPSA Communication Subscale following adjunctive pregnenolone compared to placebo (p=0.0344); replication of Singapore RCT - similar finding of improvement in UPSA Communication Subscale (p<0.001)

- Significant improvement in MCCB Verbal Learning Subscale

- Increases in pregnenolone post-treatment predict improvements in UPSA Total score

- No significant improvements in:
  - MCCB composite (or other subscales), UPSA total
  - BACS composite (or subscales), PANSS, SANS, other assessments
Future Directions

- Possible that higher doses of pregnenolone may be clinically efficacious; dose-finding investigations

- Neurosteroid quantification and biomarker potential for the prediction of therapeutic response

- Pregnenolone decanoate formulation under development; rodent studies underway

- Neuroimaging approaches; preliminary studies
Neurosteroids and PTSD
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)


- **Enhances neurogenesis; increases proliferation in rodent and human neural progenitor cells** (Wang et al. 2005, Brinton et al. 2006)
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)
Cholesterol $\xrightarrow{P450\text{sc}}$ PREGNENOLONE

17-OH-Pregnenolone $\xrightarrow{P450c17}$ DHEA ⇔ DHEAS (Dehydroepiandrosterone)

Androstenedione $\xrightleftharpoons{3\beta\text{-HSD /isom}}$ Testosterone $\xrightarrow{5\alpha\text{-reductase}}$ Progesterone $\xrightarrow{5\alpha\text{-DHP}}$ ALLOPREGNANOLONE (3α−hydroxy-5α-pregnan-20-one) (3α,5αTHP)

Progesterone $\xrightarrow{5\alpha\text{-reductase}}$ P450 arom

17β-HSD

Estradiol $\xrightarrow{P450\text{arom}}$
Allopregnanolone

- Positively modulates GABA$_A$ receptors at physiologically relevant nanomolar concentrations, 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)


- 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)
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;).
Peripheral Serum Allopregnanolone Levels are Correlated with Central Hippocampal Allopregnanolone Levels

Brain vs. Serum Allopregnanolone (RAT)

Peripheral Serum Allopregnanolone Levels are Correlated with Central Hippocampal Allopregnanolone Levels

ALLO in Serum (ng/mL) vs. ALLO in Hippocampus (ng/g)

$r = 0.59$
$p = 0.006$
CSF Allopregnanolone vs. Parietal Cortex Allo. (HUMAN)

ALLO in PARIETAL CORTEX is Correlated in CSF ALLO

$r = 0.52$

$p < 0.0001$
Pregnenolone Treatment Results in Increases in Serum Allopregnanolone
Predator Stress Model of PTSD
Neurosteroid Investigations

Pre-Treatment with Pregnenolone Decreases Predator Stress-Induced Anxiety Behaviors: *(Potential for Secondary Prevention?)*

**Open Arm Time Ratio**

- 4mg/kg Preg / Sham
- 4mg/kg Preg / Stress
- Vehicle / Sham
- Vehicle / Stress

*p = 0.04*
Allopregnanolone level associated with left cortical thickness (n=115)

- Superior Frontal Sulcus
- Inferior Occipital Gyrus
- Middle frontal gyrus
- Triangular part of the inferior frontal gyrus
- Orbital gyri
- Precentral Gyrus
- Postcentral sulcus
- Superior Parietal Lobule
- Intraparietal Sulcus
- Supramarginal Gyrus
- Angular gyrus
- Superior Temporal Sulcus
- Superior occipital sulcus
- Superior part of the precentral sulcus
- Superior frontal gyrus
- Postcentral sulcus
- Superior temporal sulcus
- Superior occipital sulcus

$p < .05; FDR corrected$
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.
Neurosteroids and PTSD

INTRuST Biorepository Investigations
INTRuST Consortium
Injury and Traumatic Stress Center

PI: Murray Stein (UC San Diego)
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

Duke SOM/Durham VA Site:
PIs: Gerry Grant
Chris Marx
Pregnenolone 2pg
m/z=298.2
S/N=15.6:1

Androsterone 2pg
m/z=486.2
S/N=54.7:1
Cholesterol → P450scC → PREGnenolone →

17-OH-Pregnenolone → P450c17 → DHEA ⇔ DHEAS
(Dehydroepiandrosterone)

P450 arom → Estradiol

Androstenedione → 17β-HSD → Testosterone

3β-HSD /isom → Progesterone → 5α-reductase → 5α-DHP
(5α-dihydroprogesterone)

3α-HSD → Allopregnanolone
(3α–hydroxy-5α-pregnan-20-one)
(3α,5αTHP)

5α-DHP → 3α-HSD → Androstenedione → 17β-HSD → Testosterone
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 (ALLO), pregnenolone (PREG)*
    pregnanolone, androsterone
  --Inflammatory markers: *c-reactive protein (CRP), IL-6, IL1β, TNF-α, others*
  --For group variable, control is the reference group
  --Current smoking has 2 categories: *Not smoking at all* (reference group) vs. *now smoking every day/smoking some days*
  --The following two types regression were performed:
    * Linear regression without transformation
    * Box-Cox transformed regression model
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<th></th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Q1</th>
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<td>506.8</td>
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<td>525.3</td>
<td>353.0</td>
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<td>470</td>
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<td></td>
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<td>173.08</td>
<td>396.8</td>
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Allopregnanolone (ALLO)

### Regression Without Transformation

|                  | Estimate | Std. Error | t value | Pr(>|t|) |
|------------------|----------|------------|---------|---------|
| Group PTSD       | -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|) |
|------------------|-----------|------------|---------|---------|
| Group PTSD       | -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|) |
|----------------------|----------|------------|---------|----------|
| Group PTSD           | -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|) |
|----------------------|----------|------------|---------|----------|
| Group PTSD           | -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 (ALLO)

#### 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 (ALLO)**

**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  |
Neurosteroids and PTSD

VA Mid-Atlantic MIRECC Registry Investigations
VA Mid-Atlantic MIRECC
(Mental Illness, Research, Education and Clinical Center)

**Director:** John Fairbank

**Deputy Director:** Mira Brancu

*Began in 2005*
Durham VA, Salisbury VA,
Richmond VA, Hampton VA,
other collaborating VAs

**Components:**

**Research:** Jean Beckham
Chris Marx

**Education:** Robin Hurley
Katherine Taber

**Clinical:** Keith Shaw
Jennifer Naylor
Nathan Kimbrel

**Laboratories:**

- Interventions & Metabolomics
  - Chris Marx

- Neuroimaging:
  - Raj Morey
  - Mira Brancu
  - Raj Morey

- PDMH and:
  - Jen Runnels

- Repository

- Health Services:
  - Pat Calhoun

- Neurocognition:
  - Larry Tupler

- Genetics:
  - Jean Beckham
  - Mike Hauser

- Neuroscience:
  - Scott Moore

**Statistical Expertise**

- Ryan Wagner
- Robert Hamer
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**
PREGNENOLONE

\[ P450c17 \]

17-OH-Pregnenolone

\[ \leftrightarrow \]

DHEA \( \Leftrightarrow \) DHEAS

(Dehydroepiandrosterone)

\[ 17\beta\text{-HSD} \]

Androstenedione

\[ \leftrightarrow \]

Testosterone

\[ 17\beta\text{-HSD} \]

Estradiol

\[ P450\text{ arom} \]

Cholesterol

\[ P450\text{ scc} \]

Progesterone

\[ 5\alpha\text{-reductase} \]

5\(\alpha\)-DHP

(5\(\alpha\)-dihydroprogesterone)

\[ 3\alpha\text{-HSD} \]

5\(\alpha\)-DHP

(3\(\alpha\)–hydroxy-5\(\alpha\)-pregnan-20-one)

(3\(\alpha\),5\(\alpha\)THP)

\[ 3\beta\text{-HSD /isom} \]

ALLOPREGNANOLONE

3\(\alpha\)-HSD
PTSD Symptoms Assessed by DTS
LS Means for DHEAS Levels

p=0.033
### PTSD Symptoms Assessed by DTS LS Means for DHEAS Levels

<table>
<thead>
<tr>
<th>Davidson Trauma Scale (DTS)</th>
<th>N</th>
<th>%</th>
<th>DHEAS LS Mean</th>
<th>SEM</th>
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<tbody>
<tr>
<td>Low (&lt;10)</td>
<td>291</td>
<td>44.2</td>
<td>1877.7</td>
<td>63.7</td>
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<td>Medium (10-39)</td>
<td>154</td>
<td>23.4</td>
<td>1889.8</td>
<td>86.2</td>
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<tr>
<td>High (≥40)</td>
<td>213</td>
<td>32.4</td>
<td>1666.6</td>
<td>74.3</td>
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Depression Symptoms Assessed by BDI-II
LS Means for DHEAS Levels

DHEAS LS mean

<table>
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<th>Depression Symptoms</th>
<th>Low (&lt;10)</th>
<th>Medium (10-19)</th>
<th>High (≥20)</th>
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<tbody>
<tr>
<td>p</td>
<td>p &lt; 0.026</td>
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# Depression Symptoms Assessed by BDI-II LS Means for DHEAS Levels

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<tr>
<th>Beck Depression Inventory-II</th>
<th>N</th>
<th>%</th>
<th>DHEAS LS Mean</th>
<th>SEM</th>
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<td>Low (&lt;10)</td>
<td>359</td>
<td>53.7</td>
<td>1867.7</td>
<td>56.7</td>
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<tr>
<td>Medium (10-19)</td>
<td>160</td>
<td>24.0</td>
<td>1812.0</td>
<td>84.9</td>
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<tr>
<td>High (≥ 20)</td>
<td>149</td>
<td>22.3</td>
<td>1632.4</td>
<td>88.2</td>
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## Pearson Partial Correlation Coefficients
(n=621; adjusting for age, smoking)

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<tr>
<th></th>
<th>DHEA</th>
<th>DHEAS</th>
<th>Ratio: DHEA/DHEAS</th>
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<td><strong>RESILIENCE</strong></td>
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<td>CONNOR-DAVIDSON RESILIENCE SCALE (CD-RISC)</td>
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<td>0.9738</td>
<td>*0.0002</td>
<td>0.0511</td>
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<tr>
<td><strong>SCL-90 (Anxiety)</strong></td>
<td>-0.01797</td>
<td>-0.13071</td>
<td>0.11174</td>
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<td></td>
<td>0.6554</td>
<td>*0.0011</td>
<td>*0.0054</td>
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<td><strong>SCL-90 (Depression)</strong></td>
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<td>0.10690</td>
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<td>0.9589</td>
<td>*0.0012</td>
<td>*0.0078</td>
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<td><strong>SCL-90 (GSI)</strong></td>
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<td>-0.13806</td>
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<td></td>
<td>0.6832</td>
<td>*0.0006</td>
<td>*0.0062</td>
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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 also ↓ in depression
  - DHEAS inversely correlated with SCL-90R anxiety and depression subscales
  - DHEAS positively correlated with resilience (Connor-Davidson Resilience Scale)
  - DHEAS and DHEA ↑ with smoking
  - DHEAS and DHEA ↓ with age
Neurosteroids and Traumatic Brain Injury (TBI)

VA Mid-Atlantic MIRECC Registry Investigations and Randomized Controlled Trials
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
Pregnanolone is Significantly Reduced in OEF/OIF Veterans with Blast-Related TBI Compared to Deployed Control Veterans

Mann-Whitney

p=0.001
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 is Significantly Reduced in OEF/OIF Veterans with Blast-Related TBI Compared to Deployed Control Veterans

Mann-Whitney
p=0.001
Androsterone

- **GABA<sub>A</sub> 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)

ANDROSTERONE in PARIETAL CX is Correlated with CSF Androsterone

$r=0.73$
$p<0.0001$
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
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)

Decreases in CAPS Cluster D Sxs

Placebo

PREG
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 PTSD Cluster D Symptom Improvement

\[ r = 0.82 \]
\[ p = 0.011 \]
Allopregnanolone Increases Predict PTSD Cluster D Symptom Improvement

\[ r = 0.82 \]
\[ p = 0.011 \]
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)
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
- Primary Behavioral Endpoint: Cluster D Symptoms
CAPS: Cluster D Symptoms
(Follow-up Study)
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
White matter integrity may be enhanced with pregnenolone

White matter was assessed with fractional anisotropy from DTI pre-treatment/baseline and post-treatment (pregnenolone n=13 or placebo n=7)
Neurosteroids and Pain (Iraq/Afghanistan Era Veterans)

VA Mid-Atlantic MIRECC Registry Investigations
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<sub>A</sub> receptors (Majewska et al., 1986; Morrow et al., 1987).

• Neurosteroids that positively modulate GABA<sub>A</sub> 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 Low Back Pain (LBP)
Allopregnanolone Levels in Serum (Mean) Are Reduced in Male OEF/OIF Veterans Reporting Chest Pain (CP)
Neurosteroids, Pain, and Anti-inflammatory Actions

Allopregnanolone Levels are Inversely Correlated with C-Reactive Protein in Male OEF/OIF Veterans

$r = -0.23$

$p = 0.047$
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

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>African American</td>
<td>48%</td>
</tr>
<tr>
<td>Caucasian</td>
<td>40%</td>
</tr>
<tr>
<td>Native American</td>
<td>5%</td>
</tr>
<tr>
<td>Hispanic</td>
<td>7%</td>
</tr>
</tbody>
</table>

**Age**

Mean = 37
**Muscle Soreness, Chest Pain, and Headache are Associated with Reduced Serum Levels of ALLO* and Androsterone* in Male Veterans**

<table>
<thead>
<tr>
<th>Muscle Soreness</th>
<th>Neurosteroid</th>
<th>95% Confidence Limits</th>
<th>Chi-Square</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allopregnanolone</td>
<td>-0.0064</td>
<td>-0.0013</td>
<td>8.96</td>
<td><strong>0.003</strong></td>
</tr>
<tr>
<td>Androsterone</td>
<td>-0.0025</td>
<td>-0.0007</td>
<td>11.67</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Pregnanolone</td>
<td>-0.0005</td>
<td>0.0008</td>
<td>0.12</td>
<td>0.734</td>
</tr>
<tr>
<td>Pregnenolone</td>
<td>-0.0004</td>
<td>0.0002</td>
<td>0.71</td>
<td>0.401</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Headache Pain</th>
<th>Neurosteroid</th>
<th>95% Confidence Limits</th>
<th>Chi-Square</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allopregnanolone</td>
<td>-0.0042</td>
<td>0.0002</td>
<td>3.07</td>
<td><strong>0.080</strong></td>
</tr>
<tr>
<td>Androsterone</td>
<td>-0.0019</td>
<td>-0.0002</td>
<td>6.42</td>
<td><strong>0.011</strong></td>
</tr>
<tr>
<td>Pregnanolone</td>
<td>-0.0008</td>
<td>0.0005</td>
<td>0.16</td>
<td>0.689</td>
</tr>
<tr>
<td>Pregnenolone</td>
<td>-0.0005</td>
<td>0.0001</td>
<td>2.08</td>
<td>0.149</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chest Pain</th>
<th>Neurosteroid</th>
<th>95% Confidence Limits</th>
<th>Chi-Square</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allopregnanolone</td>
<td>-0.0080</td>
<td>-0.0004</td>
<td>4.62</td>
<td><strong>0.032</strong></td>
</tr>
<tr>
<td>Androsterone</td>
<td>-0.0028</td>
<td>-0.0001</td>
<td>4.64</td>
<td><strong>0.031</strong></td>
</tr>
<tr>
<td>Pregnanolone</td>
<td>-0.0014</td>
<td>0.0007</td>
<td>0.38</td>
<td>0.536</td>
</tr>
<tr>
<td>Pregnenolone</td>
<td>-0.0007</td>
<td>0.0001</td>
<td>2.28</td>
<td>0.131</td>
</tr>
</tbody>
</table>
Unadjusted Raw ALLOPREGNANOLONE Levels in Serum Samples

- **Chest Pain**: n=431
- **Headache**: n=347, n=138
- **Muscle Soreness**: n=339, n=146

**No/Little Pain**

**Moderate/Severe Pain**
Unadjusted Raw Androsterone Levels in Serum Samples

pg/mL

- Chest Pain: n=431, n=54
- Headache: n=347, n=138
- Muscle Soreness: n=339, n=146

No/Little Pain
Moderate/Severe Pain
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)*

Allopregnanolone Levels are Inversely Correlated with C-Reactive Protein in Male OEF/OIF Veterans

**Discovery Cohort:**
N=82

**C-Reactive Protein and Allopregnanolone Levels:**
R=-0.26
P<0.0001

**Replication Cohort:**
N=480
### Neurosteroids and Possible Anti-inflammatory Actions:

**CRP - Androsterone, Pregnenolone**

<table>
<thead>
<tr>
<th>Neurosteroid</th>
<th>Correlation Coefficient</th>
<th>Significance</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Androsterone</strong></td>
<td>R = -0.22</td>
<td>P &lt; 0.0001</td>
<td>N = 480</td>
</tr>
<tr>
<td><strong>Pregnenolone</strong></td>
<td>R = -0.33</td>
<td>P &lt; 0.0001</td>
<td>N = 479</td>
</tr>
</tbody>
</table>
# Neurosteroids and Possible Anti-inflammatory Actions: Interleukin-6 (IL-6) and Allopregnanolone, Androsterone, Pregnenolone

<table>
<thead>
<tr>
<th>Neurosteroid</th>
<th>Correlation (R)</th>
<th>p-value (P)</th>
<th>Sample Size (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allopregnanolone</td>
<td>-0.22</td>
<td>&lt;0.0001</td>
<td>480</td>
</tr>
<tr>
<td>Androsterone</td>
<td>-0.19</td>
<td>&lt;0.0001</td>
<td>480</td>
</tr>
<tr>
<td>Pregnenolone</td>
<td>-0.25</td>
<td>&lt;0.0001</td>
<td>479</td>
</tr>
</tbody>
</table>
Neurosteroids and Possible Anti-inflammatory Actions: 
*TNFα and Androsterone, Pregnenolone*

**Tumor Necrosis Factor-α (TNF-α) and Neurosteroid Levels:**

**ANDROSTERONE:**
- $R = -0.13$
- $P < 0.0043$
- $N = 480$

**PREGNENOLONE:**
- $R = -0.18$
- $P < 0.0001$
- $N = 479$
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
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 thanks*

Larry Shampine
Gillian Parke
Jennifer Naylor
Jason Kilts
Trina Allen
Karen Smith
Susan O’Loughlin
Brian Cuffe
Steven Szabo
It always seems impossible until it’s done.
Nelson Mandela
3rd Neurosteroid Congress
April 4 – 6, 2018
Washington-Duke Inn
Durham, NC

Frontiers in Neuroscience
Special Issue – Neurosteroids as Biomarkers and Therapeutics
Submissions: Spring 2018 (Eds. Marx, Naylor, Szabo, Kilts)
Thank you
### Other Adverse Events (1 of 4)

<table>
<thead>
<tr>
<th>Disorder Category</th>
<th>PLACEBO</th>
<th>PREGNENOLONE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Other Adverse Events</strong> (not including SAEs)</td>
<td></td>
<td></td>
</tr>
<tr>
<td># participants affected / at risk</td>
<td>38/46 (82.61%)</td>
<td>31/42 (73.81%)</td>
</tr>
<tr>
<td><strong>Blood and lymphatic system disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold Extremities</td>
<td>1/46 (2.17%)</td>
<td>0/42 (0.00%)</td>
</tr>
<tr>
<td><strong>Cardiac disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palpitations</td>
<td>7/46 (15.22%)</td>
<td>4/42 (9.52%)</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>1/46 (2.17%)</td>
<td>0/42 (0.00%)</td>
</tr>
<tr>
<td><strong>Ear and labyrinth disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tinnitus</td>
<td>5/46 (10.87%)</td>
<td>3/42 (7.14%)</td>
</tr>
<tr>
<td><strong>Eye disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blurred Vision</td>
<td>4/46 (8.70%)</td>
<td>1/42 (2.38%)</td>
</tr>
<tr>
<td><strong>Gastrointestinal disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>7/46 (15.22%)</td>
<td>8/42 (19.05%)</td>
</tr>
<tr>
<td>Constipation</td>
<td>12/46 (26.09%)</td>
<td>5/42 (11.90%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>2/46 (4.35%)</td>
<td>6/42 (14.29%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>2/46 (4.35%)</td>
<td>1/42 (2.38%)</td>
</tr>
</tbody>
</table>
Other Adverse Events (2 of 4)

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Pregnenolone</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Restlessness</td>
<td>4/46 (8.70%)</td>
<td>3/42 (7.14%)</td>
</tr>
<tr>
<td>Nasal Congestion</td>
<td>4/46 (8.70%)</td>
<td>1/42 (2.38%)</td>
</tr>
<tr>
<td>Peripheral Edema</td>
<td>1/46 (2.17%)</td>
<td>1/42 (2.38%)</td>
</tr>
<tr>
<td>Malaise</td>
<td>1/46 (2.17%)</td>
<td>1/42 (2.38%)</td>
</tr>
<tr>
<td><strong>Musculoskeletal and connective tissue disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cramps</td>
<td>6/46 (13.04%)</td>
<td>7/42 (16.67%)</td>
</tr>
<tr>
<td>Joint pain/ stiffness</td>
<td>2/46 (4.35%)</td>
<td>5/42 (11.90%)</td>
</tr>
<tr>
<td>Muscle Pain/Stiffness</td>
<td>4/46 (8.70%)</td>
<td>3/42 (7.14%)</td>
</tr>
<tr>
<td>Akathisia</td>
<td>0/46 (0.00%)</td>
<td>1/42 (2.38%)</td>
</tr>
<tr>
<td>Increased Motor Activity</td>
<td>1/46 (2.17%)</td>
<td>0/42 (0.00%)</td>
</tr>
<tr>
<td>Other: &quot;Head/Neck Twitching&quot;</td>
<td>1/46 (2.17%)</td>
<td>0/42 (0.00%)</td>
</tr>
</tbody>
</table>
### Other Adverse Events (3 of 4)

<table>
<thead>
<tr>
<th>Nervous system disorders</th>
<th>Placebo</th>
<th>Pregnenolone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drowsiness</td>
<td>10/46 (21.74%)</td>
<td>11/42 (26.19%)</td>
</tr>
<tr>
<td>Hypersomnia</td>
<td>8/46 (17.39%)</td>
<td>9/42 (21.43%)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>3/46 (6.52%)</td>
<td>7/42 (16.67%)</td>
</tr>
<tr>
<td>Decreased Appetite</td>
<td>6/46 (13.04%)</td>
<td>8/42 (19.05%)</td>
</tr>
<tr>
<td>Increased appetite</td>
<td>9/46 (19.57%)</td>
<td>7/42 (16.67%)</td>
</tr>
<tr>
<td>Dry mouth</td>
<td>8/46 (17.39%)</td>
<td>5/42 (11.90%)</td>
</tr>
<tr>
<td>Headache</td>
<td>9/46 (19.57%)</td>
<td>6/42 (14.29%)</td>
</tr>
<tr>
<td>Tremor</td>
<td>5/46 (10.87%)</td>
<td>5/42 (11.90%)</td>
</tr>
<tr>
<td>Increased Salivation</td>
<td>1/46 (2.17%)</td>
<td>5/42 (11.90%)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>9/46 (19.57%)</td>
<td>4/42 (9.52%)</td>
</tr>
<tr>
<td>Sweating</td>
<td>1/46 (2.17%)</td>
<td>2/42 (4.76%)</td>
</tr>
<tr>
<td>Other: &quot;Lucid Dreams&quot;</td>
<td>0/46 (0.00%)</td>
<td>1/42 (2.38%)</td>
</tr>
<tr>
<td>Decreased Interest in Sex</td>
<td>0/46 (0.00%)</td>
<td>2/42 (4.76%)</td>
</tr>
<tr>
<td>Vertigo</td>
<td>0/46 (0.00%)</td>
<td>1/42 (2.38%)</td>
</tr>
<tr>
<td>Excitement/Agitation</td>
<td>1/46 (2.17%)</td>
<td>1/42 (2.38%)</td>
</tr>
<tr>
<td>Confusion</td>
<td>2/46 (4.35%)</td>
<td>1/42 (2.38%)</td>
</tr>
<tr>
<td>Paresthesia</td>
<td>2/46 (4.35%)</td>
<td>0/42 (0.00%)</td>
</tr>
</tbody>
</table>
### Other Adverse Events (4 of 4)

<table>
<thead>
<tr>
<th>Category</th>
<th>Placebo</th>
<th>Pregnenolone</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Renal and urinary disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nocturnal/Enuresis</td>
<td>1/46 (2.17%)</td>
<td>1/42 (2.38%)</td>
</tr>
<tr>
<td>Other: &quot;Urinary Retention&quot;</td>
<td>1/46 (2.17%)</td>
<td>0/42 (0.00%)</td>
</tr>
<tr>
<td><strong>Reproductive system and breast disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menstrual Disturbance</td>
<td>0/46 (0.00%)</td>
<td>1/42 (2.38%)</td>
</tr>
<tr>
<td><strong>Skin and subcutaneous tissue disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dermatological</td>
<td>4/46 (8.70%)</td>
<td>3/42 (7.14%)</td>
</tr>
</tbody>
</table>