Neurosteroids in PTSD and Co-occurring Conditions
Biomarkers to Therapeutics

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Duke University Medical Center;
Staff Psychiatrist
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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

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 Repository:** Mira Brancu  
  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 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)
Peripheral Serum Pregnenolone Levels are Correlated with Central Hippocampal Pregnenolone Levels

$r = 0.95$
$p < 0.0001$

PREG in Serum (ng/mL)

PREG in Hippocampus (ng/g)
CSF *Pregnenolone* vs. Temporal Cortex Preg. (HUMAN)

![Graph showing correlation between CSF Pregnenolone levels and Temporal Cortex Pregnenolone levels]

- **CSF Pregnenolone (pg/mL)**
- **Temporal Cortex Pregnenolone (pg/g)**

- **Correlation**: $r = 0.57$, $p < 0.0001$

Pregnenolone Levels in CSF are Correlated with Pregnenolone Levels in Temporal Cortex
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?)
Pregnenolone Treatment Results in 5-Fold Increases in Serum Allopregnanolone
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 (Rasmussen 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)


- 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:**
  

  *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)
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)

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

**Graph:**
- **Title:** ALLO in PARIETAL CORTEX is Correlated in CSF ALLO
- **Equation:** $r = 0.52$, $p < 0.0001$

**Axes:**
- **Y-axis:** ALLO PARIETAL CX (pg/mL)
- **X-axis:** ALLO CSF (pg/mL)
Allopregnanolone level associated with left cortical thickness (n=115)

- Allopregnanolone level associated with left cortical thickness (n=115)
- p < .05; FDR corrected
- 1. Inferior Occipital Gyrus
- 2. Inferior Occipital Gyrus
- 15. Middle frontal gyrus
- 14. Triangular part of the inferior frontal gyrus
- 54. Superior Frontal Sulcus
- 29. Precentral Gyrus
- 67. Postcentral sulcus
- 27. Superior Parietal Lobule
- 26. Supramarginal Gyrus
- 25. Angular gyrus
- 56. Intraparietal Sulcus
- 19. Middle occipital gyrus
- 58. Superior occipital sulcus
- 22. Lingual gyrus
- 16. Superior frontal gyrus
- 8. Middle-posterior of the cingulate gyrus
- 42. Occipital Pole
- 44. Calcarine Sulcus
- 11. Cuneus
- 65. Parieto-occipital sulcus
- 69. Superior part of the precentral sulcus
- 57. Superior Temporal Sulcus
- 73. Superior Temporal 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 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

<table>
<thead>
<tr>
<th>Davidson Trauma Scale (DTS)</th>
<th>N</th>
<th>%</th>
<th>DHEAS LS Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (&lt;10)</td>
<td>291</td>
<td>44.2</td>
<td>1877.7</td>
<td>63.7</td>
</tr>
<tr>
<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>
</tr>
</tbody>
</table>
PTSD Symptoms Assessed by DTS
LS Means for DHEAS Levels

p=0.033

DHEAS LS mean

Low (<10) | Medium (10-39) | High (≥40)
Depression Symptoms Assessed by BDI-II LS Means for DHEAS Levels

<table>
<thead>
<tr>
<th>Beck Depression Inventory-II</th>
<th>N</th>
<th>%</th>
<th>DHEAS LS MEAN</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<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>
</tr>
</tbody>
</table>
Depression Symptoms Assessed by BDI-II
LS Means for DHEAS Levels

- Low (<10)
- Medium (10-19)
- High (≥20)

p < 0.026
Pearson Partial Correlation Coefficients
(n=621; adjusting for age, smoking)

<table>
<thead>
<tr>
<th></th>
<th>DHEA</th>
<th>DHEAS</th>
<th>Ratio: DHEA/DHEAS</th>
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<tbody>
<tr>
<td><strong>RESILIENCE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONNOR-DAVIDSON</td>
<td>0.00132</td>
<td>0.14989</td>
<td>-0.07845</td>
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<tr>
<td>RESILIENCE SCALE</td>
<td>0.9738</td>
<td>*0.0002</td>
<td>0.0511</td>
</tr>
<tr>
<td>(CD-RISC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>SCL-90 (Anxiety)</strong></td>
<td>-0.01797</td>
<td>-0.13071</td>
<td>0.11174</td>
</tr>
<tr>
<td></td>
<td>0.6554</td>
<td>*0.0011</td>
<td>*0.0054</td>
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<tr>
<td><strong>SCL-90 (Depression)</strong></td>
<td>0.00208</td>
<td>-0.12992</td>
<td>0.10690</td>
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<tr>
<td></td>
<td>0.9589</td>
<td>*0.0012</td>
<td>*0.0078</td>
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<tr>
<td><strong>SCL-90 (GSI)</strong></td>
<td>-0.01643</td>
<td>-0.13806</td>
<td>0.10999</td>
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<tr>
<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 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
<table>
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<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Q1</th>
<th>Median</th>
<th>Q3</th>
<th>Max</th>
<th>p.value</th>
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<tr>
<td><strong>Pregnenolone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>103</td>
<td>544.7</td>
<td>278.2</td>
<td>77.7</td>
<td>345.5</td>
<td>542.4</td>
<td>684.4</td>
<td>1752.1</td>
<td>0.041 *</td>
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<tr>
<td>PTSD</td>
<td>109</td>
<td>506.8</td>
<td>411.9</td>
<td>69.9</td>
<td>273.1</td>
<td>417.7</td>
<td>645.1</td>
<td>3598.2</td>
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<tr>
<td>Overall</td>
<td>212</td>
<td>525.3</td>
<td>353.0</td>
<td>69.9</td>
<td>306.5</td>
<td>470</td>
<td>669.0</td>
<td>3598.2</td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td>&lt;0.001 *</td>
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<td>32.2</td>
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<td>42.8</td>
<td>54.9</td>
<td>75.1</td>
<td>200.8</td>
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<tr>
<td>PTSD</td>
<td>107</td>
<td>47.9</td>
<td>33.2</td>
<td>5.8</td>
<td>25.2</td>
<td>40.1</td>
<td>58.1</td>
<td>203.4</td>
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<td>Overall</td>
<td>210</td>
<td>55.7</td>
<td>33.6</td>
<td>5.8</td>
<td>33.1</td>
<td>47.4</td>
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<td>104.23</td>
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<td>0.008 *</td>
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<td>97.45</td>
<td>127.2</td>
<td>173.075</td>
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**SUMMARY STATISTICS (by diagnosis group); PTSD, irrespective of TBI**
## 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| <0.0000  |
| 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

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>p.value</th>
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<td><strong>Pregnenolone</strong></td>
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<tr>
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<td>103</td>
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<td>TBI</td>
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<td>103</td>
<td>63.7</td>
<td>32.2</td>
<td>54.9</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>TBI</td>
<td>129</td>
<td>46.7</td>
<td>28.6</td>
<td>40.1</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>232</td>
<td>55.7</td>
<td>46.1</td>
<td>46.1</td>
<td></td>
</tr>
<tr>
<td><strong>Pregnanolone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>103</td>
<td>172.6</td>
<td>90.6</td>
<td>150.8</td>
<td></td>
</tr>
<tr>
<td>TBI</td>
<td>129</td>
<td>177.9</td>
<td>113.1</td>
<td>149.8</td>
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<tr>
<td>Overall</td>
<td>232</td>
<td>174.0</td>
<td>103.5</td>
<td>150.5</td>
<td>0.85</td>
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<tr>
<td><strong>Androsterone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>103</td>
<td>151.4</td>
<td>63.9</td>
<td>135.1</td>
<td></td>
</tr>
<tr>
<td>TBI</td>
<td>129</td>
<td>138.5</td>
<td>92.0</td>
<td>119.6</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>232</td>
<td>144.2</td>
<td>80.8</td>
<td>125.9</td>
<td>0.02 *</td>
</tr>
</tbody>
</table>
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

Pregnenolone 2pg
m/z=298.2
S/N=15.6:1

Androsterone 2pg
m/z=486.2
S/N=54.7:1
Pregnanolone is significantly reduced in OEF/OIF Veterans with blast-related TBI compared to deployed control veterans.
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)

ANDROSTERONE in PARIETAL CX is Correlated with CSF Androstereone

$\text{ANDROS PARIELTAL CX (pg/mL)}$

- $r = 0.73$
- $p < 0.0001$
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
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)

Decreases in CAPS Cluster D Sxs

- Placebo
- PREG

-7.5 -5.0 -2.5 0.0
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)
- Builds upon recent data showing amygdala and DLPFCC 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
- **Primary Behavioral Endpoint**: Cluster D Symptoms
CAPS: Cluster D Symptoms
(Follow-up Study – NEED TO UPDATE)
Neurosteroids and Mild TBI: Elevations Following Pregnenolone (Follow-up Study)
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.
In collaboration with Raj Morey, MD MS
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<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>Demographic</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>
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</table>

*Age: Mean = 37*
Muscle Soreness, Chest Pain, and Headache are Associated with Reduced Serum Levels of ALLO\textsuperscript{*} and Androsterone\textsuperscript{*} 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>0.003</td>
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<tr>
<td>Androsterone</td>
<td>-0.0025</td>
<td>-0.0007</td>
<td>11.67</td>
<td>0.001</td>
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<td>Pregnanolone</td>
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<td>Pregnenolone</td>
<td>-0.0004</td>
<td>0.0002</td>
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</table>

<table>
<thead>
<tr>
<th>Chest Pain</th>
<th>Neurosteroid</th>
<th>95% Confidence Limits</th>
<th>Chi-Square</th>
<th>P value</th>
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<tr>
<td>Allopregnanolone</td>
<td>-0.0080</td>
<td>-0.0004</td>
<td>4.62</td>
<td>0.032</td>
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<tr>
<td>Androsterone</td>
<td>-0.0028</td>
<td>-0.0001</td>
<td>6.42</td>
<td>0.031</td>
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<td>Pregnanolone</td>
<td>-0.0014</td>
<td>0.0007</td>
<td>0.38</td>
<td>0.536</td>
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<td>Pregnenolone</td>
<td>-0.0007</td>
<td>0.0001</td>
<td>2.28</td>
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<table>
<thead>
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<th>Headache Pain</th>
<th>Neurosteroid</th>
<th>95% Confidence Limits</th>
<th>Chi-Square</th>
<th>P value</th>
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<td>Allopregnanolone</td>
<td>-0.0042</td>
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<td>Androsterone</td>
<td>-0.0019</td>
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<td>-0.0005</td>
<td>0.0001</td>
<td>2.08</td>
<td>0.149</td>
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</tbody>
</table>
Unadjusted Raw ALLOPREGNANOLONE Levels in Serum Samples

- **No/Little Pain**
  - Chest Pain: n=431
  - Headache: n=347
  - Muscle Soreness: n=339

- **Moderate/Severe Pain**
  - Chest Pain: n=54
  - Headache: n=138
  - Muscle Soreness: n=146
Unadjusted Raw Androsterone Levels in Serum Samples

- **Chest Pain**
  - No/Little Pain: n=431, pg/mL = 260
  - Moderate/Severe Pain: n=54, pg/mL = 240

- **Headache**
  - No/Little Pain: n=347, pg/mL = 250
  - Moderate/Severe Pain: n=138, pg/mL = 230

- **Muscle Soreness**
  - No/Little Pain: n=339, pg/mL = 260
  - Moderate/Severe Pain: n=146, pg/mL = 240
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

Graph showing the correlation between Allopregnanolone levels (pg/mL) and C-Reactive Protein (mg/dL) with a Pearson correlation coefficient of r = -0.23 and p = 0.047.
Neurosteroids and Possible Anti-inflammatory Actions: 

**CRP - Androsterone, Pregnenolone**

C-Reactive Protein and Neurosteroid Levels:

**ANDROSTERONE:**
- $R = -0.22$
- $P < 0.0001$
- $N = 480$

**PREGNENOLONE:**
- $R = -0.33$
- $P < 0.0001$
- $N = 479$
# Neurosteroids and Possible Anti-inflammatory Actions: Interleukin-6 (IL-6) and Allopregnanolone, Androsterone, Pregnenolone

<table>
<thead>
<tr>
<th>Neurosteroid Levels:</th>
<th>IL-6 and Neurosteroid Levels:</th>
<th>IL-6 and Neurosteroid Levels:</th>
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<tbody>
<tr>
<td><strong>ALLOPREGNANOLONE:</strong></td>
<td><strong>ANDROSTERONE:</strong></td>
<td><strong>PREGNENOLONE:</strong></td>
</tr>
<tr>
<td>R= -0.22</td>
<td>R= -0.19</td>
<td>R= -0.25</td>
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<tr>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>N=480</td>
<td>N=480</td>
<td>N=479</td>
</tr>
</tbody>
</table>
Neurosteroids and Possible Anti-inflammatory Actions:  
*TNF-α and Androsterone, Pregnenolone*

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

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

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