Neurosteroids in PTSD and Co-occurring Conditions

Biomarkers to Therapeutics

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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

Cholesterol

\[ \text{Pregnrenolone} \]

17-OH-Pregnrenolone

\[ \text{DHEA} \leftrightarrow \text{DHEAS} \]

(Dehydroepiandrosterone)

Androstenedione

Testosterone

Estradiol

\[ \text{Progesterone} \]

\[ \text{Allopregnolone} \]

(3α,5α-hydroxy-5α-pregn-20-one)

(3α,5αTHP)

\[ \text{Cholesterol} \]

\[ \text{P450scc} \]

17α-HSD

3β-HSD/iso

P450c17

5α-reductase

5α-DHP

(5α-dihydroprogesterone)

3α-HSD
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**

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**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 sx (George et al 1994)
- **Reduces depression symptoms** - bipolar depression (Brown et al 2014)
**Brain vs. Serum**

*Pregnenolone (RAT)*

![Graph showing correlation between Peripheral Serum Pregnenolone Levels and Central Hippocampal Pregnenolone Levels.](image)

- $r=0.95$
- $p<0.0001$

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

![Graph showing correlation between Pregnenolone Levels in CSF and Temporal Cortex Pregnenolone Levels.](image)

- $r=0.57$
- $p<0.0001$
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?)

Open Arm Time Ratio

Proportion of time in open arms +/- SEM

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

p < .05; FDR corrected
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)
- 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)

- **Anti-inflammatory actions** (Djebaili et al 2005, 2004; He et al 2004); reduces microglia activation (Ahmad et al 2005), anti-apoptotic actions (Charalampopoulos 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 (Djebieli 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 (Cariza et al 2004)

- Protects against hypoxia-induced astrogliosis (Kruse et al 2008)

- Decreases NMDA-induced toxicity and decreases neuronal apoptosis (Charalampopoulos et al 2004 and 2006).

Brain vs. Serum

Allopregnanolone (RAT)

![Graph showing the correlation between peripheral serum allopregnanolone levels and central hippocampal allopregnanolone levels. The correlation coefficient (r) is 0.59, and the p-value is 0.006.](image)
Neurosteroids in PTSD and Co-occurring Conditions
Christine Marx, MD
Saturday, September 16, 2017
General Session

CSF Allopregnanolone vs. Parietal Cortex Allo. (HUMAN)

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

$p < .05; FDR corrected$
Neurosteroids in PTSD and Co-occurring Conditions
Christine Marx, MD

Saturday, September 16, 2017
General Session

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>
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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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<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>
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</table>
PTSD Symptoms Assessed by DTS
LS Means for DHEAS Levels

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>
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</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>
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</table>
Depression Symptoms Assessed by BDI-II
LS Means for DHEAS Levels

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>RESILIENCE</td>
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<tr>
<td>CONNOR-DAVIDSON RESILIENCE SCALE (CD-RISC)</td>
<td>0.00132</td>
<td>0.14989</td>
<td>-0.07845 0.0511</td>
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<td></td>
<td>0.9738</td>
<td>*0.0002</td>
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<tr>
<td>SCL-90 (Anxiety)</td>
<td>-0.01797</td>
<td>-0.13071</td>
<td>0.11174 *0.0054</td>
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<td></td>
<td>0.6554</td>
<td>*0.0011</td>
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<tr>
<td>SCL-90 (Depression)</td>
<td>0.00208</td>
<td>-0.12992</td>
<td>0.10690 *0.0078</td>
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<td></td>
<td>0.9589</td>
<td>*0.0012</td>
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<tr>
<td>SCL-90 (GSI)</td>
<td>-0.01643</td>
<td>-0.13806</td>
<td>0.10999 *0.0062</td>
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<td></td>
<td>0.6832</td>
<td>*0.0006</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>
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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>
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<td>PTSD</td>
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<td>411.9</td>
<td>69.9</td>
<td>273.1</td>
<td>417.7</td>
<td>645.1</td>
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<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>
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<tr>
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<td>PTSD</td>
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<td>47.9</td>
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<td>25.2</td>
<td>40.1</td>
<td>58.1</td>
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<td>210</td>
<td>55.7</td>
<td>33.6</td>
<td>5.8</td>
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<td>47.4</td>
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<tr>
<td>Control</td>
<td>103</td>
<td>172.6</td>
<td>90.6</td>
<td>48.2</td>
<td>114.4</td>
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<td>PTSD</td>
<td>107</td>
<td>177.9</td>
<td>116.3</td>
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<td>Overall</td>
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<td>175.3</td>
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<td>97.45</td>
<td>127.2</td>
<td>173.075</td>
<td>396.8</td>
<td></td>
</tr>
</tbody>
</table>
## 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
### Neurosteroids and Traumatic Brain Injury (TBI)

<table>
<thead>
<tr>
<th>Neurosteroid</th>
<th>Control (N=103)</th>
<th>TBI (N=130)</th>
<th>Overall (N=233)</th>
<th>p.value</th>
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</thead>
<tbody>
<tr>
<td><strong>Pregnenolone</strong></td>
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<td></td>
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<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
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<tr>
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<td>544.7 (278.2)</td>
<td>504.7 (392.59)</td>
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<td>Median (p.value)</td>
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<td>Mean (SD)</td>
<td>Mean (SD)</td>
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<tr>
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<td>63.7 (32.2)</td>
<td>46.7 (28.6)</td>
<td>55.7 (46.1)</td>
<td>&lt;0.001 *</td>
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<td>Median (p.value)</td>
<td>54.9</td>
<td>40.1</td>
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<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
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<tr>
<td></td>
<td>172.6 (90.6)</td>
<td>177.9 (113.1)</td>
<td>174.0 (103.5)</td>
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</tr>
<tr>
<td><strong>Androsterone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>151.4 (63.9)</td>
<td>138.5 (92.0)</td>
<td>144.2 (80.8)</td>
<td>0.02 *</td>
</tr>
<tr>
<td></td>
<td>Median (p.value)</td>
<td>135.1</td>
<td>119.6</td>
<td></td>
</tr>
</tbody>
</table>
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

- m/z=298.2
- S/N=15.6:1

### Androsterone

- m/z=486.2
- S/N=54.7:1
Neurosteroids in PTSD and Co-occurring Conditions
Christine Marx, MD
Saturday, September 16, 2017
General Session

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

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

<table>
<thead>
<tr>
<th></th>
<th>Mann-Whitney p=0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>700</td>
</tr>
<tr>
<td>Blast TBI</td>
<td>500</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th></th>
<th>Mann-Whitney p=0.07</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>750</td>
</tr>
<tr>
<td>Blast TBI</td>
<td>600</td>
</tr>
</tbody>
</table>
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

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)
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)
### 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
- **Primary Behavioral Endpoint:** Cluster D Symptoms
Neurosteroids in PTSD and Co-occurring Conditions
Christine Marx, MD

**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)
Neurosteroids in PTSD and Co-occurring Conditions

Christine Marx, MD

Saturday, September 16, 2017
General Session

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

Neurosteroids as Biomarker Candidates and Potential New Therapeutics for Pain

- Allopregnanolone positively modulates inhibitory GABA\(\alpha\) receptors (Majewska et al., 1986; Morrow et al., 1987).

- Neurosteroids that positively modulate GABA\(\alpha\) 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 androstenediol (Meyer et al., 2013) prevent and suppress chemotherapy-induced neuropathies in rats.
Neurosteroids in PTSD and Co-occurring Conditions
Christine Marx, MD

Saturday, September 16, 2017
General Session

**Neurosteroids, Pain, and Anti-inflammatory Actions**

- Allopregnanolone levels are reduced in male OEF/OIF veterans reporting chest pain (CP).
- Allopregnanolone levels are correlated with C-reactive protein.

\[ Allopregnanolone \text{ (pg/mL)} \times \text{C-reactive protein (mg/dL)} = \text{Allopregnanolone-CRP product} \]

\[ p = 0.05 \]

\[ p = 0.002 \]
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>0.003</td>
</tr>
<tr>
<td>Androsterone</td>
<td>-0.0025</td>
<td>-0.0007</td>
<td>11.67</td>
<td>0.001</td>
</tr>
<tr>
<td>Pregnanolone</td>
<td>-0.0005</td>
<td>0.0006</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>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>0.032</td>
</tr>
<tr>
<td>Androsterone</td>
<td>-0.0028</td>
<td>-0.0001</td>
<td>4.64</td>
<td>0.031</td>
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<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>2.78</td>
<td>0.131</td>
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</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>0.080</td>
</tr>
<tr>
<td>Androsterone</td>
<td>-0.0019</td>
<td>-0.0002</td>
<td>6.42</td>
<td>0.011</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>

Unadjusted Raw ALLOPREGNANOLONE Levels in Serum Samples
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)*

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>C-Reactive Protein and Neurosteroid Levels:</th>
<th>C-Reactive Protein and Neurosteroid Levels:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ANDROSTERONE:</strong></td>
<td><strong>PREGNENOLONE:</strong></td>
</tr>
<tr>
<td>R= -0.22</td>
<td>R= -0.33</td>
</tr>
<tr>
<td>P&lt;0.0001</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>N=480</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>IL-6 and Neurosteroid Levels:</th>
<th>IL-6 and Neurosteroid Levels:</th>
<th>IL-6 and Neurosteroid Levels:</th>
</tr>
</thead>
<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>
</tr>
<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**

<table>
<thead>
<tr>
<th>Neurosteroid</th>
<th>Tumor Necrosis Factor-α (TNF-α) Levels:</th>
<th>Neurosteroid</th>
<th>Tumor Necrosis Factor-α (TNF-α) Levels:</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANDROSTERONE:</td>
<td>R = -0.13</td>
<td>PREGNENOLONE:</td>
<td>R = -0.18</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.0043</td>
<td></td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>N = 480</td>
<td></td>
<td>N = 479</td>
</tr>
</tbody>
</table>

• **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
  \[ r = -0.22; \text{p}<0.0001; n=480 \], as are
  androsterone \[ r = -0.20; \text{p}<0.0001; n=480 \] &
  pregnenolone \[ r= -0.25; \text{p}<0.0001; n=479 \]

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!