Considerations of long-term sequelae in an animal model of blast-induced mild traumatic brain injury

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Keystone Conf. 2012
Outline

• Observations from IED War wounds – relevance to TBI
• Challenges of Blast-induced Brain Neurotrauma (BINT)
• Blast Observations:
  – Operational (Breachers)
  – Animal Model
    • Acute exposure
    • Repeated exposure
• Types of Blast Injury
• BINT & PTSD
• BINT & CTE/AD
**IED War Wounds are Different**

Formation of mature lamellar bone in non-osseous tissue

**Risk Factors:**

- 63% of all combat-related amputations\(^1\)
  - Amputation in zone of injury
  - Multiple injuries*/Blast*
  - Injury Severity Scores > 16
- 65% of patients who sustained high energy injuries to the extremity\(^2\)
- 3 times more prevalent than comparable civilian trauma (22%)

**Mechanism(s):**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Day 9 (Alizarin red stain)</th>
<th>Day 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>H0 Wound Effluent Final Debridement MS101/EA (10(\mu)L)</td>
<td><img src="image1.png" alt="Day 9 Image" /></td>
<td><img src="image2.png" alt="Day 16 Image" /></td>
</tr>
<tr>
<td>H0 Wound Effluent Final Debridement MS101/EF (10(\mu)L)</td>
<td><img src="image3.png" alt="Day 9 Image" /></td>
<td><img src="image4.png" alt="Day 16 Image" /></td>
</tr>
<tr>
<td>Non H0 Wound Effluent Final Debridement SC17/EA (10(\mu)L)</td>
<td><img src="image5.png" alt="Day 9 Image" /></td>
<td><img src="image6.png" alt="Day 16 Image" /></td>
</tr>
<tr>
<td>Non H0 Wound Effluent Final Debridement SC17/EB (10(\mu)L)</td>
<td><img src="image7.png" alt="Day 9 Image" /></td>
<td><img src="image8.png" alt="Day 16 Image" /></td>
</tr>
<tr>
<td>PBS (10(\mu)L)</td>
<td><img src="image9.png" alt="Day 9 Image" /></td>
<td><img src="image10.png" alt="Day 16 Image" /></td>
</tr>
<tr>
<td>Basal Media (0(\mu)L)</td>
<td><img src="image11.png" alt="Day 9 Image" /></td>
<td><img src="image12.png" alt="Day 16 Image" /></td>
</tr>
</tbody>
</table>

Wound effluent from HO wounds induces early osteogenic differentiation in culture

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### DoD Traumatic Brain Injury

#### 2000 – 2011 1st quarter

<table>
<thead>
<tr>
<th>Severity Type</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penetrating</td>
<td>3,573</td>
</tr>
<tr>
<td>Severe</td>
<td>2,235</td>
</tr>
<tr>
<td>Moderate</td>
<td>35,661</td>
</tr>
<tr>
<td>Mild</td>
<td>163,181</td>
</tr>
<tr>
<td>Not Classifiable</td>
<td>8,092</td>
</tr>
</tbody>
</table>

| Total - All Severity   | 212,742|

#### Source:
Military Health System, U.S. Dept of Defense

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Improvised Explosive Devices (IEDs)
Blast Induced Brain Injuries – A Grand Challenge in TBI Research

...a few of the specific problems in Blast-induced Neurotrauma (BINT):

- Propagation of blast waves is very complex. It could involve both direct propagation through the skull and indirect propagation via blood vessels.

- Is BINT a specific type injury that will require specific and new types of treatment? ... is the mild TBI from blast exposure more like a classical type of concussion injury?

- Is it possible to identify a reliable borderline between mild BINT and PTSD? Many of the symptoms are similar and many patients might suffer from both TBI and PTSD.

- Is BINT an entirely new problem? The shellshock syndrome that was seen after the enormous artillery battles during World War I had similarities to BINT and post BINT symptoms, but for many years it has been regarded as PTSD rather than physical injuries.

“Well-designed experimental models are required as well as data from acceleration probes and pressure sensors that have been mounted into helmets and body armor will increase the knowledge of the critical mechanisms”

# Mårten Risling, Front. Neurol., 2010
Operational Blast: Breachers

- Assess effects of repeated low-level blast exposures before, during, and after 2 week Breacher training (2008)
- Sponsors: DARPA & ONR

The Breacher Consortium
- Applied Research Associates
- NMRC/WRAMC/USU
- University of Virginia
- US Army Aviation Research Laboratory

Measured:
- Blast exposure parameters
- Neurological function
- Neuroimaging
- Auditory function

Findings:
- No effects in students
- Cognitive impairment in instructors
- Instructors showed neuroimaging changes

Take away:
- # of blast exposures over time is important
- We need to hone subsequent experiments to fully characterize impairment from blast
Blast Characterization

- Measure overpressure in free field and on helmets
- Measure head orientation relative to structure and charge

Standard practices reduce the overpressure exposure

Blast Characterization

Breacher Blast Forces

<table>
<thead>
<tr>
<th>Category</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1°</td>
<td>Impact of overpressurization wave on body</td>
</tr>
<tr>
<td>2°</td>
<td>Due to flying debris, bomb fragments, other projectiles</td>
</tr>
<tr>
<td>3°</td>
<td>Due to individuals being thrown by blast winds</td>
</tr>
<tr>
<td>4°</td>
<td>Explosion-related injury, illness, or disease not due to primary, secondary, or tertiary mechanisms</td>
</tr>
</tbody>
</table>

Associated with higher intensity blast
Breacher Safety

For acute exposure

Based upon the Bowen curves for safe standoff distances

There is no standard for multiple exposures

Requirement: a “dive table” [e.g., USN93] for multiple blasts?

Lessons from diving medicine: inflammatory mediators of DCS present a therapeutic target; N₂ bubbles (and bubble physics) are a major player but not the only one.
Breacher Survey

- Survey (anonymous)
  - 10th & 11th International Breacher Symposiums
  - Breachers (N=130)
  - Military & Law Enforcement

### Number of Respondents vs. Number of Breaches

<table>
<thead>
<tr>
<th>Number of Breaches</th>
<th>Career Breaches</th>
<th>Breaches Last Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8%</td>
<td>6.2%</td>
</tr>
<tr>
<td>1–9</td>
<td>17.7%</td>
<td>14.6%</td>
</tr>
<tr>
<td>10–39</td>
<td>14.6%</td>
<td>30.0%</td>
</tr>
<tr>
<td>40–99</td>
<td>13.8%</td>
<td>10.0%</td>
</tr>
<tr>
<td>100–199</td>
<td>12.3%</td>
<td>12.3%</td>
</tr>
<tr>
<td>200–399</td>
<td>10.0%</td>
<td>14.6%</td>
</tr>
<tr>
<td>400+</td>
<td>22.3%</td>
<td>50.0%</td>
</tr>
<tr>
<td>Missing</td>
<td>2.3%</td>
<td>2.3%</td>
</tr>
</tbody>
</table>

### Symptom Set and F-ratio

<table>
<thead>
<tr>
<th>Symptom Set</th>
<th>F-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Cognitive/Memory Impacts</td>
<td>2.85**</td>
</tr>
<tr>
<td>3 Auditory Impacts</td>
<td>8.53***</td>
</tr>
<tr>
<td>4 Diverse</td>
<td>3.65**</td>
</tr>
<tr>
<td>5 Neuromuscular Impacts</td>
<td>3.76**</td>
</tr>
<tr>
<td>6 PTSD-Specific Impacts</td>
<td>3.90*</td>
</tr>
</tbody>
</table>
Animal Model of Blast (Overpressure)

• **Goal**: Elucidate the *natural history of repeated exposure* to blast overpressure (BOP) on brain function and physiology.

• **Our focus**: Develop an animal model of BOP mTBI
Acute Blast Effects

Balance Beam Task (unanesthetized rats)

Take away:
- Threshold for BOP disruption ~11-17 psi
- Orientation to the BOP wave matters

* Orientation to the blast overpressure wave
  - Facing
  - Side
  - Away

AOC/LOC
BOP Characterization

- Transfer of blast wave into the brain
- Orientation to the wave changes pressure transfer – implications to physiology
- The overpressure wave is “felt” in “protected environment”

**Chavko, et. al., J. Neurosi. Meth, 2011**

**Saljo, et. al., J. Neurotrauma, 2008**
### Acute BOP

#### Functional/Pathologic Outcome

<table>
<thead>
<tr>
<th>Blast Exposure Levels (psi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.8</td>
</tr>
<tr>
<td>10.9</td>
</tr>
<tr>
<td>17.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No pathology</th>
<th>Mild TBI (amnesia)</th>
<th>Overt pathology</th>
<th>Moderate/Severe TBI</th>
<th>AOC/LOC</th>
<th>Polytrauma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Subdural Hemorrhage**
- **Contusions**
- **Liver**
- **Spleen**
- **Kidney**
- **Small Intestine**
- **Lung** *(ED100)*
- **Heart**

- **Amyloid Precursor Protein**
- **Silver degeneration stain – Amino Cupric Silver**

### Brain Injury*

* in ~30% of animals (ED30)

### Systemic Injury

Unpublished data, courtesy of MAJ J. Dalle Luca, USAISR
Abeta After BOP in Brain

In cortex (24 hrs & 1 wk post BOP)

40 kPa = 5.6 psi
75 kPa = 10.9 psi
120 kPa = 17.4 psi
F = frontal orientation
S = side orientation

Courtesy of G. Elder and S. Gandy, Bronx VA
“Relevant” Blast Pressures for Repeated Exposure Studies

Quantico Breachers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max Breacher Pressure</td>
<td>12.9</td>
</tr>
<tr>
<td>Min Breacher Pressure</td>
<td>0.034</td>
</tr>
<tr>
<td>Average Pressure</td>
<td>1.253</td>
</tr>
<tr>
<td>0 psi &lt;= Exposures &lt; 1 psi</td>
<td>64%</td>
</tr>
<tr>
<td>1 psi &lt;= Exposures &lt; 4 psi</td>
<td>31%</td>
</tr>
<tr>
<td>4 psi &lt;= Exposures &lt; 10 psi</td>
<td>4%</td>
</tr>
<tr>
<td>Average Charge Weight</td>
<td>0.36</td>
</tr>
<tr>
<td>Ave Exterior Charge Weight</td>
<td>0.54</td>
</tr>
<tr>
<td>Ave Interior Charge Weight</td>
<td>0.05</td>
</tr>
<tr>
<td>No. Exterior Charges</td>
<td>23</td>
</tr>
<tr>
<td>No of Interior Charges</td>
<td>14</td>
</tr>
<tr>
<td>Total Number of Tests</td>
<td>37</td>
</tr>
</tbody>
</table>

Blast Exposure Levels (psi)

- 0
- 5.8 (repeated)
- 10.9 (No pathology)
- 17.4 (Overt pathology)
Repeated BOP Exposure

Goal: Characterize the effects of repeated exposure to BOP: learning & memory

Assessment Timeline

BOP Exposures
• 1 per day (facing or side orientation)
• Isoflurane anesthesia during BOP exposure

Water Maze Acquisition
• 4 block trials given in a single day
• Each block = four 90 sec trials (N, S, E, W)
Rodent Model of mTBI/PCS

Assessment Timeline

BOP x 12 (5.6 psi)

24 Hours post BOP

30 Days post BOP
No observable CNS pathology (H&E, APP, GFAP, Silver, etc.)

Figure 4: APP immunohistochemistry - frontal overpressure exposure: APP immunohistochemistry is seen with animals exposed to 12 sessions of 36.6 kPa blast overpressure exposure (A, B, E, F, I, J, M, N) vs. SHAM injured controls (C, D, G, H, K, L, O, P). Photomontages of multiple photomicrographs of frontal (A, C), mid (E, G), and posterior (I, K) portions of the cerebrum are shown in coronal section. Sagittal photomicrograph of the brainstem is seen in plates M and O. Magnifications of corresponding boxes within photomontages are seen in plates B, D, F, H, J, L, N, and P. Within all of the plates, no evidence of traumatic injury or difference between experimentally injured and SHAM injured controls is seen. From Ahlers et al., Frontiers Neurotrauma, in press.
Types of blast brain injury?

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Type 1</th>
<th>Type 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blast Frequency</td>
<td>Single</td>
<td>Single or Multiple</td>
</tr>
<tr>
<td>Blast Intensity</td>
<td>17+ psi</td>
<td>≤ 11 psi</td>
</tr>
<tr>
<td>Physical Forces</td>
<td>1° blast wave, 2° penetrating, 3° acceleration/deceleration</td>
<td>1° Blast wave</td>
</tr>
<tr>
<td>Clinical Manifestations</td>
<td>Mild-severe TBI, PCS/PTSD “polytrauma”</td>
<td>Mild TBI/PCS/PTSD “subclinical”</td>
</tr>
<tr>
<td>Clinical Onset</td>
<td>Event-related symptoms</td>
<td>Insidious onset over time</td>
</tr>
<tr>
<td>Radiology/Pathology</td>
<td>CT/MRI hemorrhage, inflammation, vasospasm, edema, white/gray matter damage</td>
<td>No conventional signal DTI, fMRI, MRS (TBD) white matter injury?</td>
</tr>
<tr>
<td>Biomarkers</td>
<td>Inflammatory</td>
<td>GFAP, UCH-L1</td>
</tr>
</tbody>
</table>

Ahlers et. al., Frontiers Neurotrauma, in press
Co-morbidities (mTBI/PCS/PTSD)

- There is significant co-morbidity of mTBI/PCS and PTSD
- PCS observed during the post-deployment period; it may, or may not, be linked to overt mTBI in close temporal proximity to the blast event
- mTBI/PCS results from blast exposure; PTSD results from battlefield stress
- Blast could influence stress physiology (and PTSD)?
- Clinical observations post deployment, post military (VA), suggest that most, if not all, PCS is co-morbid with PTSD, but the reverse is not true
“Subclinical” BOP and Stress

- Rats exposed to 12 x 5.6 psi
- Different groups assessed post BOP
‘Subclinical’ BOP and Anxiety

- Rats exposed to 3 x 10.9 psi
- Tested 4.5 months after BOP exposure

Predator Scent Assay

Open Field Test

- Move Time
- Move Distance
- Open Latency
- Cross Latency

- Open Entries
- Open Time
- Closed Time

Startle Assay

- Acoustic startle/PPI
- %PPI

Courtesy of G. Elder, Bronx VA
Pathologic Outcomes of TBI

Traumatic Brain Injury — Football, Warfare, and Long-Term Effects

Steven T. DeKosky, M.D., Milos D. Ikonomovic, M.D., and Sam Gandy, M.D., Ph.D.

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<td>Single or Multiple</td>
</tr>
<tr>
<td>Blast Intensity</td>
<td>$17 \pm \text{psi}$</td>
<td>$\leq 11 \text{psi}$</td>
</tr>
<tr>
<td>Physical Forces</td>
<td>$1^\text{st} \text{ blast wave, }2^\text{nd} \text{ penetrating, }3^\text{rd} \text{ acceleration/deceleration}$</td>
<td>$1^\text{st} \text{ Blast wave}$</td>
</tr>
<tr>
<td>Clinical Manifestations</td>
<td>Mild-severe TBI, PCS/PTSD “poly-trauma”</td>
<td>Mild TBI/PCS/PTSD “subclinical”</td>
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</table>

Diffuse axonal injury, mechanical tissue damage, ischemia, synaptic loss, neuronal dysfunction or demise

Impaired axonal transport, neuronal circuit disruption

Tendency toward neurophysiopathy modified by APOE 4

Variable chronic cognitive or neuropsychiatric impairment, frequently associated with post-traumatic stress disorder

Neurofibrillary tangles (taylopathy)

Dementia pugilistica, chronic traumatic encephalopathy, frontal lobe parkinsonism

Alzheimer’s disease

Total or partial functional recovery, often with variable chronic cognitive or neuropsychiatric impairment

In the left inset, Bielschowsky silver stain shows intraneuronal and extracellular neurofibrillary tangles in temporal cortex from a retired boxer with dementia pugilistica. The right inset shows diffuse Aβ plaque deposits in temporal cortex from a subject who sustained severe TBI.
“Subclinical” BOP and Abeta 42

Perspective

September 30, 2010

Traumatic Brain Injury — Football, Warfare, and Long-Term Effects

Steven T. DeKosky, M.D., Milos D. Ikonomovic, M.D., and Sam Gandy, M.D., Ph.D.

- Rats exposed to 3 x 10.9 psi
- Tested 4.5 months after BOP exposure

Spectrum of Pathologic Features and Outcomes of Traumatic Brain Injury (TBI).

In the left inset, Bielschowsky silver stain shows intraneuronal and extracellular neurofibrillary tangles in temporal cortex from a retired boxer with dementia pugilistica. The right inset shows diffuse Ab plaque deposits in temporal cortex from a subject who sustained severe TBI.

Courtesy of G. Elder and S. Gandy, Bronx VA
Observed CTE

**Observed CTE**

**Case Report**

Premortem History

This subject was a 27-year-old Caucasian man who committed suicide by hanging approximately 8 months after his honorable discharge from the USMC while

In 2010, he was referred for a neuropsychological screening. His wife reported that he forgot dates, conversations, and trivialities of daily living. He also forgot whether he completed tasks, and sometimes confused his wife’s and sister’s names. He had problems making decisions and therefore avoided them. He believed he snapped at his children too frequently and was increasingly becoming a grumpy person. He admitted to headaches that occurred 3 to 4 times per week, which he described as pressure in his entire head. The headaches were relieved by a nonsteroidal anti-inflammatory agent. He experienced bilateral hearing problems and tinnitus, which he dated back to when he had worked on engines in the military. He reported dizziness when he woke up at night to use the bathroom, slept only 4 hours a night, and had trouble falling asleep. Other reported symptoms included irritability, neuronal dropout without eosinophilic neuronal necrosis. There was diffuse perineuronal vacuolation, expansion of Virchow Robin spaces and pachygyria with necrosis of both the gray and white matter, there was marked congestion of the arachnoid and pia mater and the penetrating parenchymal vessels. Multifocal sparse peri-vascular pigment laden histiocytes were noted in many Virchow Robin spaces.

Chronic traumatic encephalopathy presents clinically after a prolonged latent period as a composite syndrome of mood disorders and neuropsychiatric and cognitive impairment. Direct brain tissue analysis reveals multifocal or diffuse tauopathy, which may be accompanied by low-grade and multifocal white matter rarefaction, microglial activation, and parenchymal histiocytes. Amyloidopathy may be present; however, the primary proteinopathy in CTE is a tauropathy. Some patients with CTE may not exhibit the classic prolonged latency period before clinical symptoms begin.

Posttraumatic stress disorder in war veterans was first designated in 1978 to describe a condition in Viet-

1º blast?
Clinical/Operational/Experimental Findings: Long-term Sequalae

• Low level exposure to blast overpressure is associated with:
  – Long-term cognitive impairment
  – Post-concussive and/or PTSD symptoms
  – Changes in brain that could precipitate long-term pathology

• Unknown:
  – Natural history of BINT
  – Underlying mechanisms
  – Parallel to sports concussion
  – Relevance to CTE/AD
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