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

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Keystone Conf. 2012

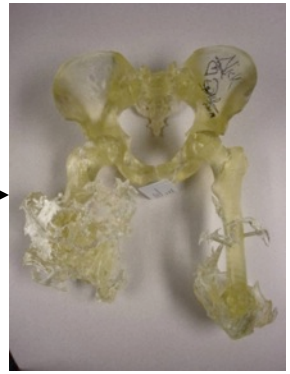
Outline

- 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

Heterotopic Ossification formation



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):

hMSC	1	2	3	4	5	6
Day 9 (Alizarin red stain)						
Day 16						
Condition	HO Wound Effluent 1st Debridement MS161EA (10µL)	HO Wound Effluent Final Debridement MS161EF (10µL)	Non HO Wound Effluent 1st Debridement SC171EA (10µL)	Non HO Wound Effluent Final Debridement SC171EB (10µL)	PBS (10µL)	PBS (10µL)
Media	Bone Media (990µL)	Bone Media (990µL)	Bone Media (990µL)	Bone Media (990µL)	Bone Media (990µL)	Basal Media (990µL)

Wound effluent from HO wounds induces early osteogenic differentiation in culture

Potter BK et al. *J Bone Joint Surg Am*. 2007; 89: 476-86.

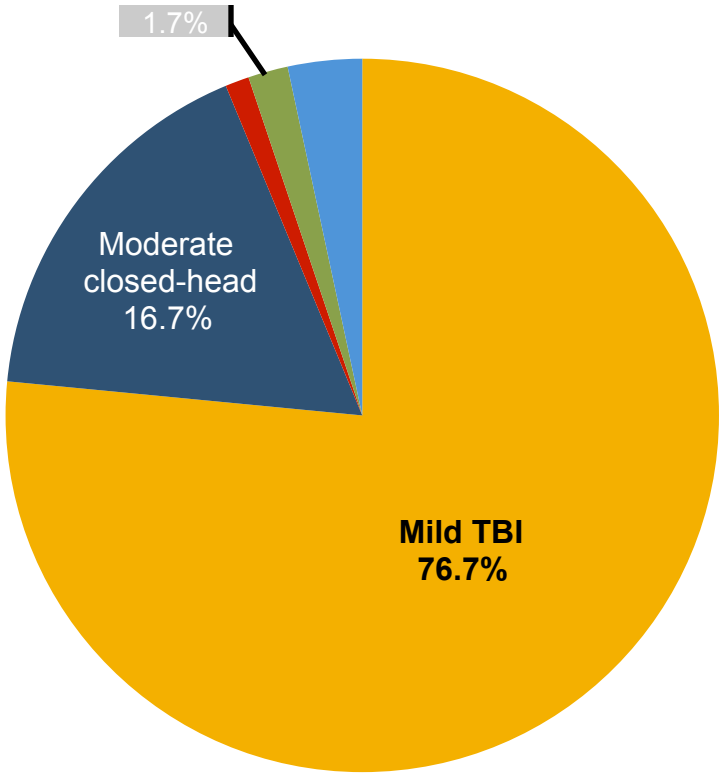
Forsberg JA et al. *J Bone Joint Surg Am* 2009; 91: 1084-1091

Potter, BK et al. *J Bone Joint Surg Am* 2010; 92: 74-89

DoD Traumatic Brain Injury

2000 – 2011 1st quarter

Penetrating	3,573
Severe	2,235
Moderate	35,661
Mild	163,181
Not Classifiable	8,092
TOTAL - All Severity	**212,742**



Source: Military Health System U.S. Dept of Defense
http://www.health.mil/Research/TBI_Numbers/

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**

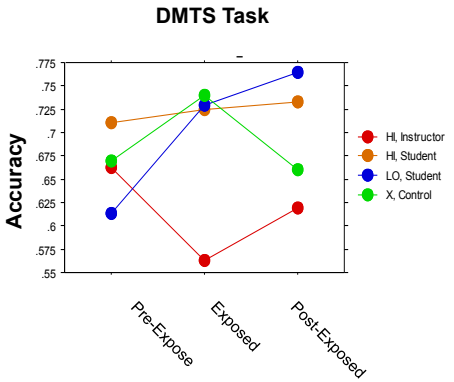
Dynamic entry, Marine Corps Weapon Battalion, Quantico, VA

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

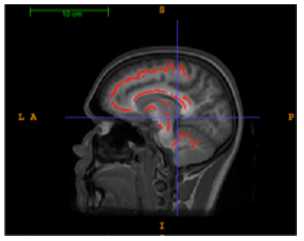


Findings:

- **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**

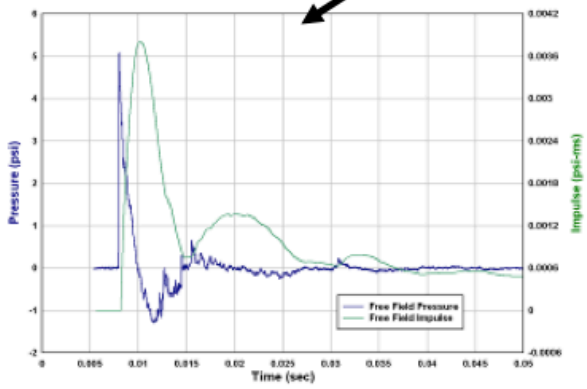


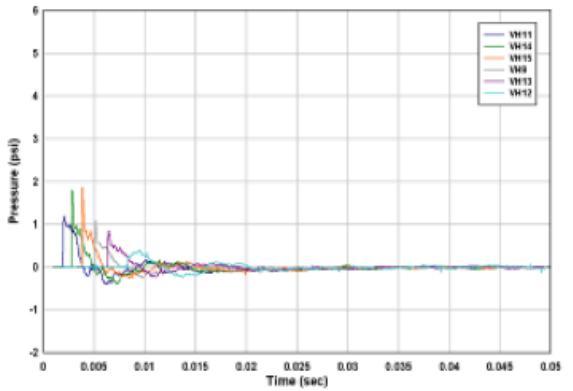
Diffusion Tensor Imaging

Composite Instructor FA

Blast Characterization

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

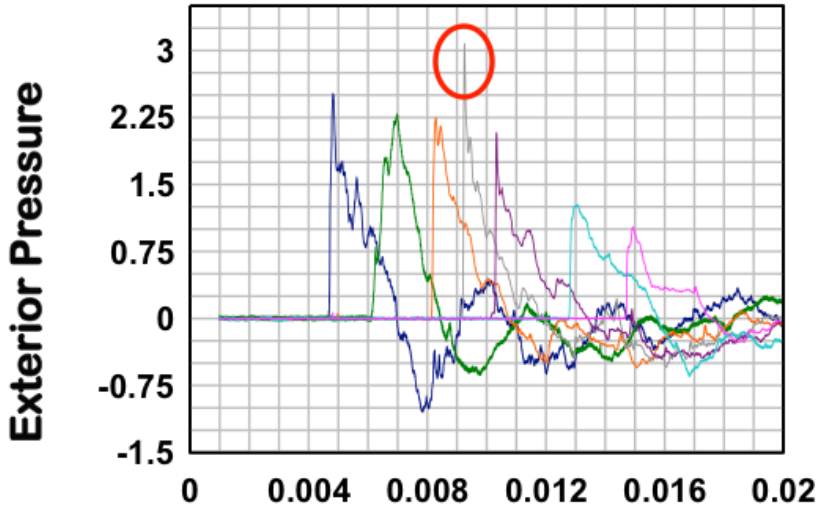


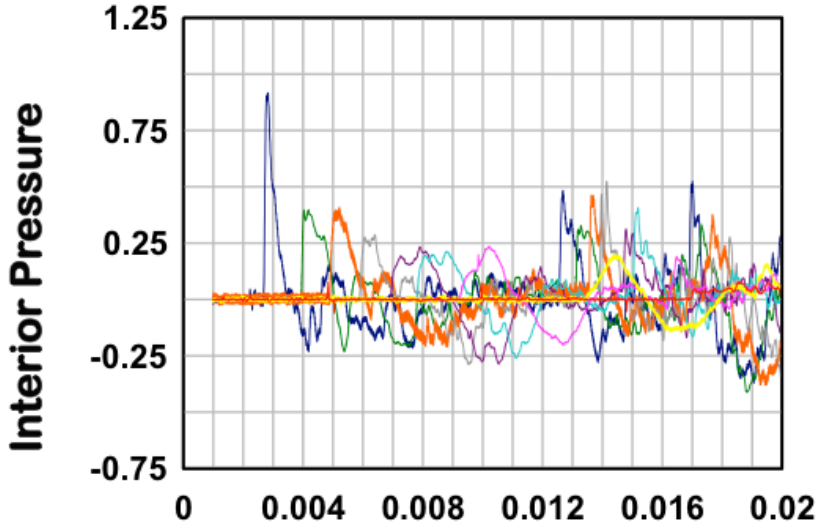


Standard practices reduce the overpressure exposure

Pressure measurements by Applied Research Associates, Inc.

Blast Characterization





Pressure measurements by Applied Research Associates, Inc.

Breacher Blast Forces

Category	Characteristics
1º	Impact of overpressurization wave on body
2º	Due to flying debris, bomb fragments, other projectiles
3º	Due to individuals being thrown by blast winds
4º	explosion-related injury, illness, or disease not due to primary, secondary, or tertiary mechanisms

Associated with higher intensity blast

Breacher Safety

Explosive Breaching Calculator

by Mouretsu

Install $1.00

100-500 downloads, **11** ratings (3.82 average), 2988 kb, Permissions, Contact

Add to list ▼ Like +1 0 Tweet 0 Email QR / more ▼

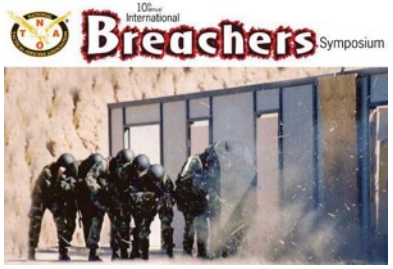
Explosive breaching charge calculator to determine the net weight of explosive charges (primer and main charge) and the PSI effects for interior and exterior shots. This is the same set of calculations used by police SWAT, EOD, Army, Navy, Marine Corp, Air Force, FBI and taught by all of the explosive breaching schools. I have just created an app that allows you to plug in the basic numbers for your explosives and all of the math is done for you and shown on the screen. You can even email the shot information to yourself or someone else so you can have a hard copy of all the data for the shot. There is no internal memory so your shot information will not be saved once the program is closed or you clear out the data to enter another shot. If you want the shot info you must email it to get a hard copy. The app will show you the external PSI values and distance from target for each value. Plug in the values for a room's dimensions and you will be given the internal PSI values for that room. The math has been tested many times during recent training shots and it is reliable. I also included calculations in the screen shots so you can do the math on your own for those weights and see that results are correct. I wanted to keep this app simple but I am willing to add things if certain agencies need anything specific to their breaching program, as long as I have the ability to implement those changes. Any and all feedback is welcomed.

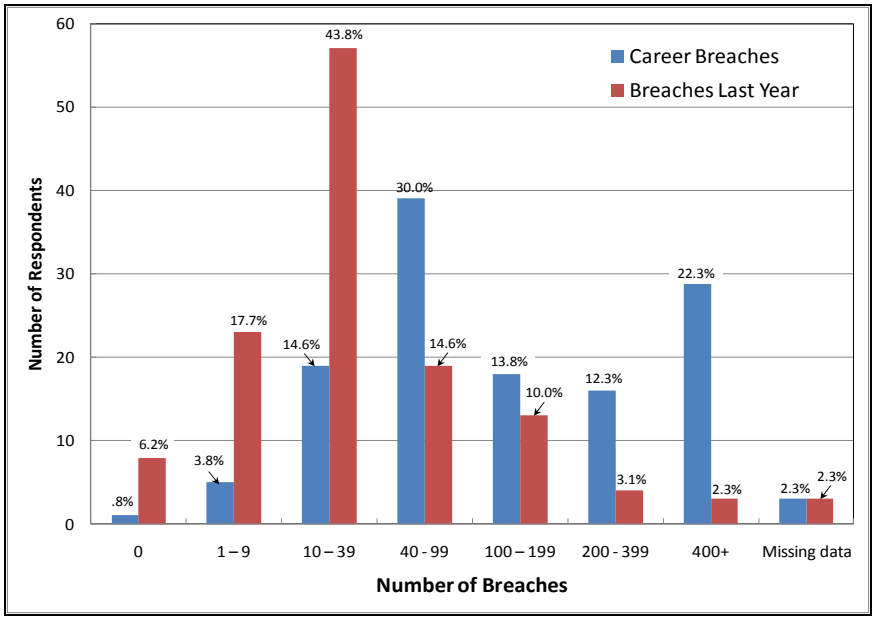
Show full description »

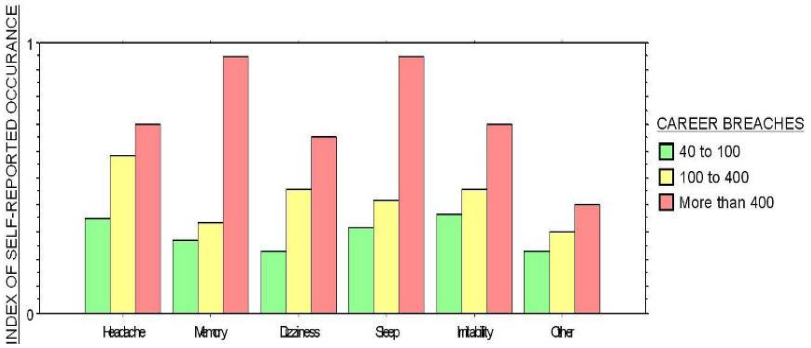
- For acute exposure
- Based upon the Bowen curves for safe standoff distances
- There is no standard for multiple exposures
- Requirement: a “dive table” [e.g., USN93] for multiple blasts?
- Lessons from diving medicine: inflammatory mediators of DCS present a therapeutic target; N_2 bubbles (and bubble 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







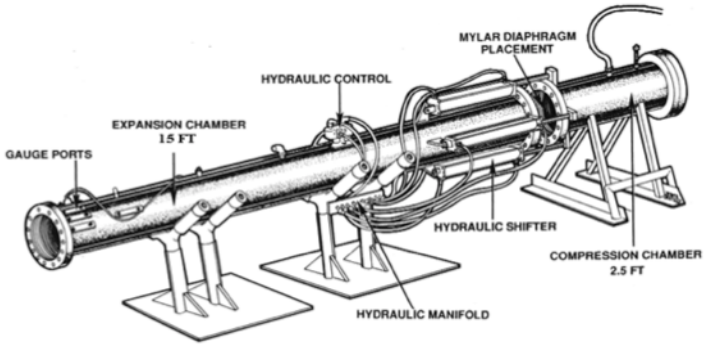
Symptom Set	F-ratio
1 Cognitive/Memory Impacts	2.85**
3 Auditory Impacts	8.53***
4 Diverse	3.65**
5 Neuromuscular Impacts	3.76**
6 PTSD-Specific Impacts	3.90*

Animal Model of Blast (Overpressure)

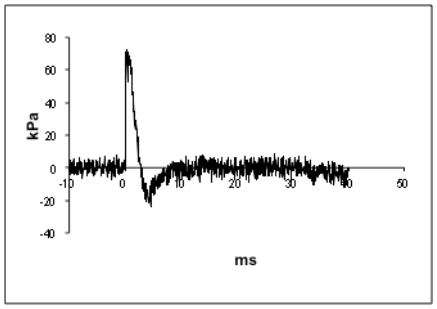
- Goal: Elucidate the *natural history* of *repeated exposure* to blast overpressure (BOP) on brain function and physiology.

- Our focus: Develop an animal model of BOP mTBI

Blast Wave Generated by Explosion



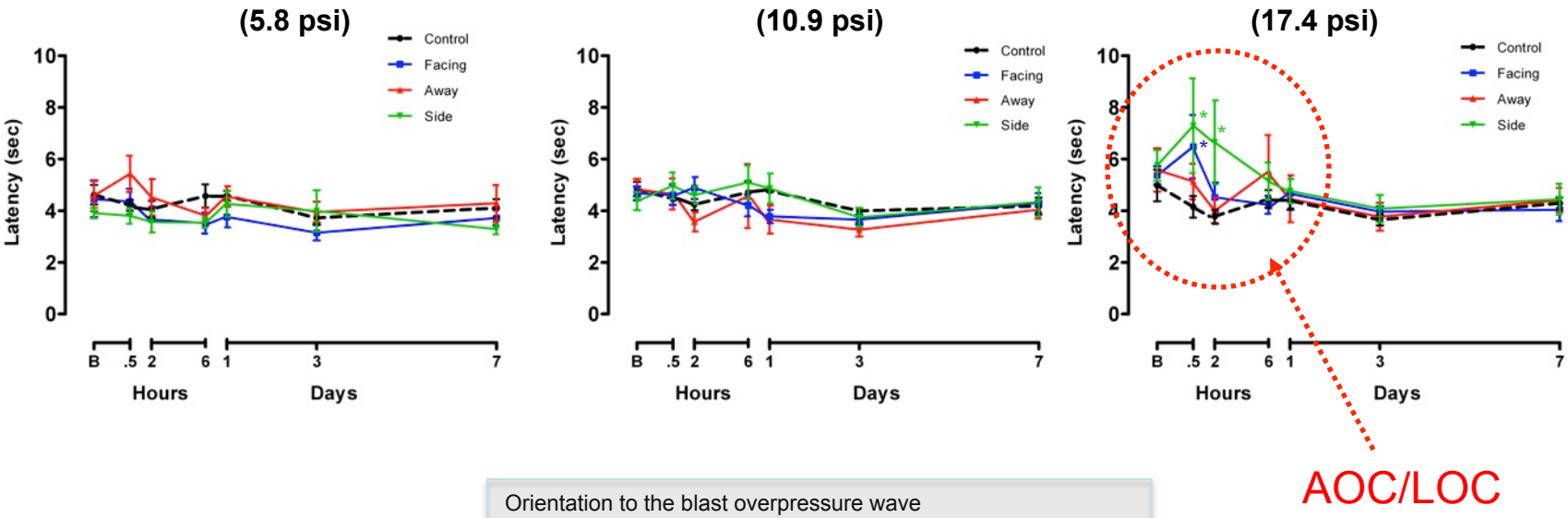
Shock Tube

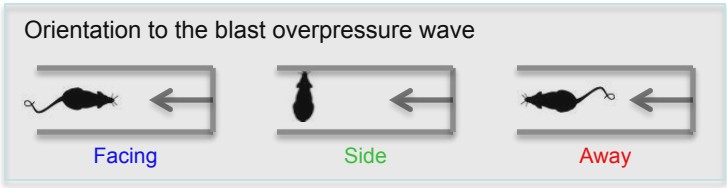


Blast Wave Generated in Shock Tube

Acute Blast Effects

Balance Beam Task (unanesthetized rats)



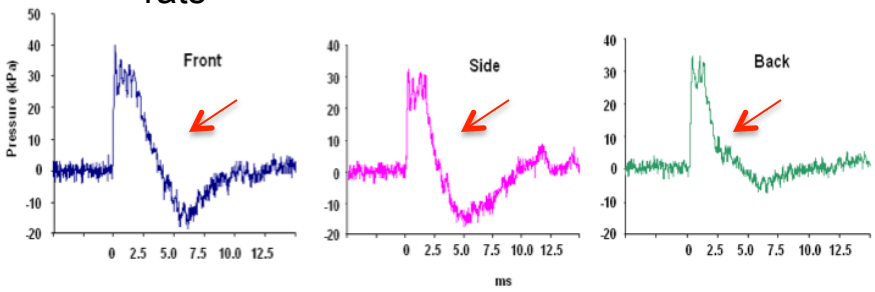


Take away:

- **Threshold for BOP disruption ~11-17 psi**
- **Orientation to the BOP wave matters**

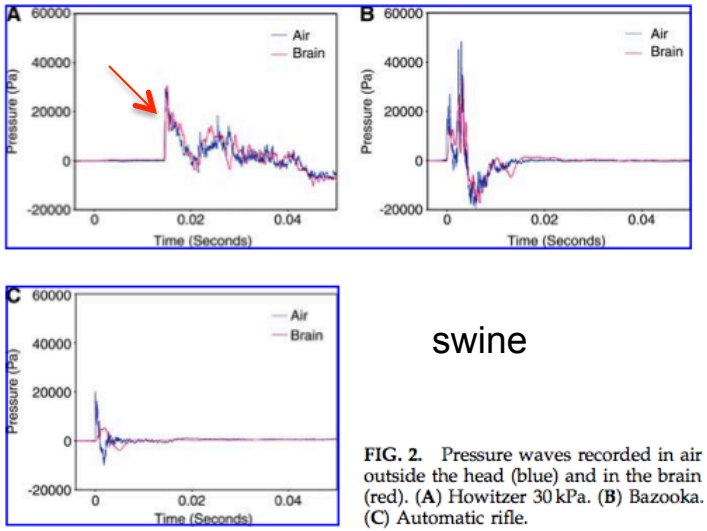
BOP Characterization

rats



Orientation	Peak Overpressure (kPa)	Rise Time (ms)	Overpressure Duration (ms)
Air	36.5 ± 1.6	0.07 ± 0.42	4.1 ± 0.3
Frontal	41.5 ± 1.7*	0.19 ± 0.04*	4.1 ± 0.2
Lateral	33.6 ±3.2	0.26 ± 0.02*	3.3 ± 0.1*
Backward	36.3 ± 2.0	0.31 ± 0.02*	2.9 ± 0.4*

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

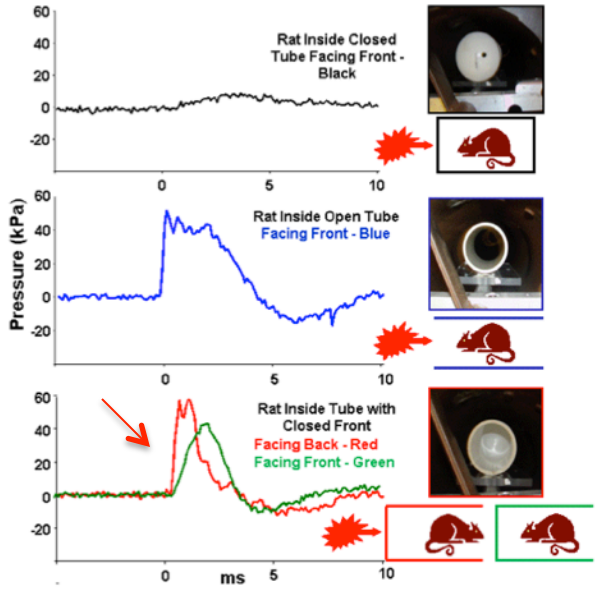


swine

FIG. 2. Pressure waves recorded in air outside the head (blue) and in the brain (red). (A) Howitzer 30 kPa. (B) Bazooka. (C) Automatic rifle.

Saljo, et. al., J. Neurotrauma, 2008

- 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., NATO Conf, 2011

Acute BOP
Functional/Pathologic Outcome

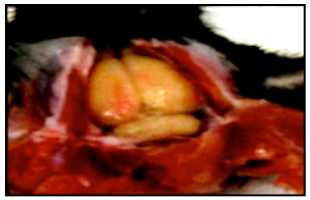
Blast Exposure Levels (psi)

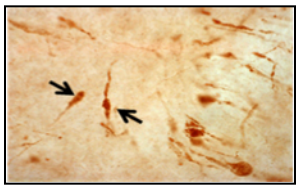
5.8
10.9
17.4

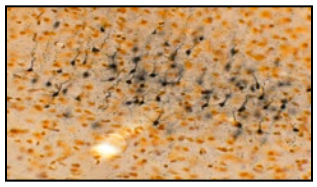
No pathology **Mild TBI (amnesia)**

Overt pathology **Moderate/Severe TBI** **AOC/LOC** **Polytrauma**


Subdural Hemorrhage

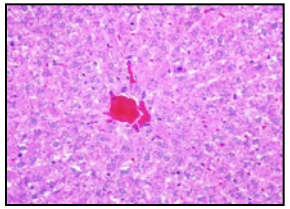

Contusions

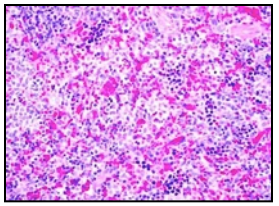

Amyloid Precursor Protein

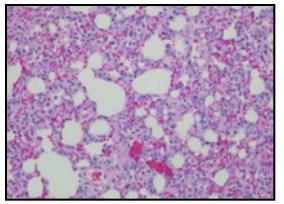

Silver degeneration stain – Amino Cupric Silver

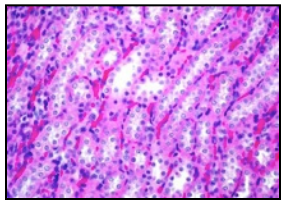
Brain Injury*

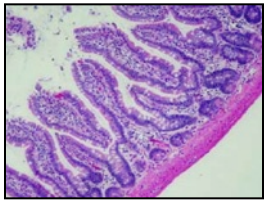
* in ~30% of animals (ED30)

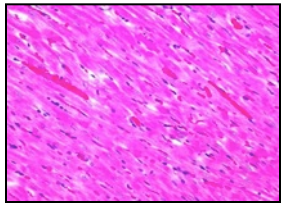

Liver


Spleen


Lung (ED100)


Kidney


Small Intestine

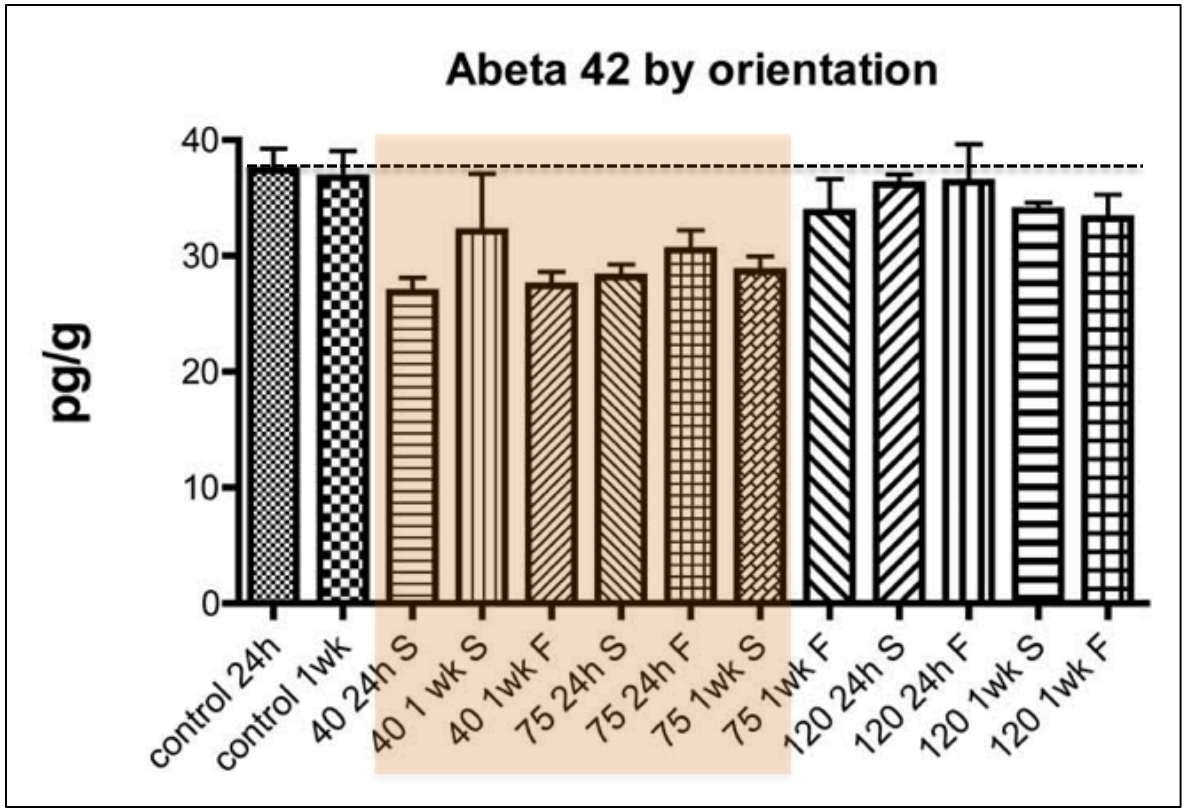

Heart

Unpublished data, courtesy of MAJ J. Dalle Luca, USAISR

Systemic Injury

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

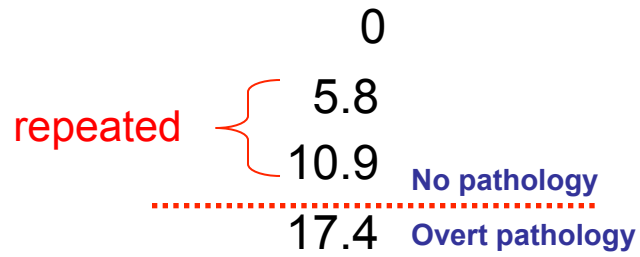
"Relevant" Blast Pressures for Repeated Exposure Studies

NAVY MEDICINE
World Class Care....Anytime, Anywhere

Quantico Breachers

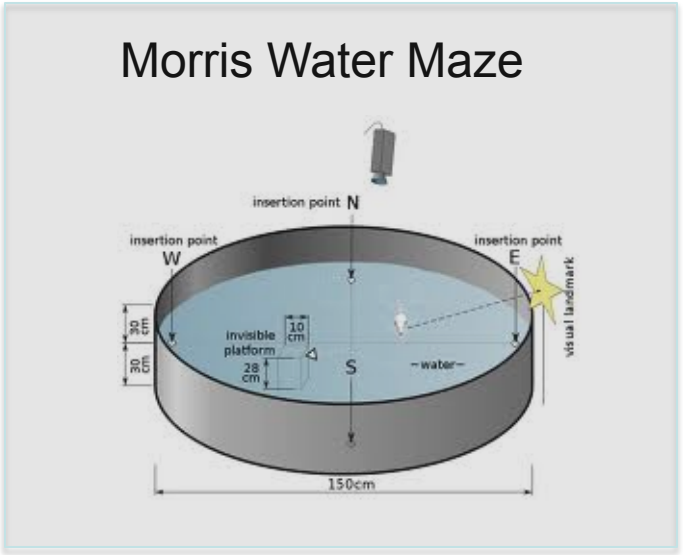
Max Breacher Pressure	12.9
Min Breacher Pressure	0.034
Average Pressure	1.253
0 psi <= Exposures < 1 psi	64%
1 psi <= Exposures < 4 psi	31%
4 psi <= Exposures < 10 psi	4%
Average Charge Weight	0.36
Ave Exterior Charge Weight	0.54
Ave Interior Charge Weight	0.05
No. Exterior Charges	23
No of Interior Charges	14
Total Number of Tests	37

Blast Exposure Levels (psi)

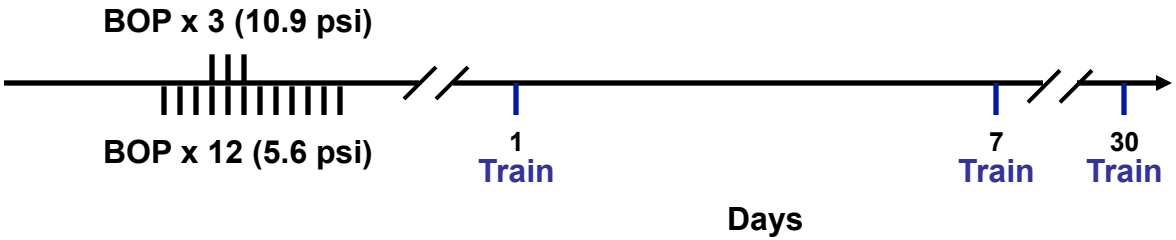


Repeated BOP Exposure

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



Assessment Timeline



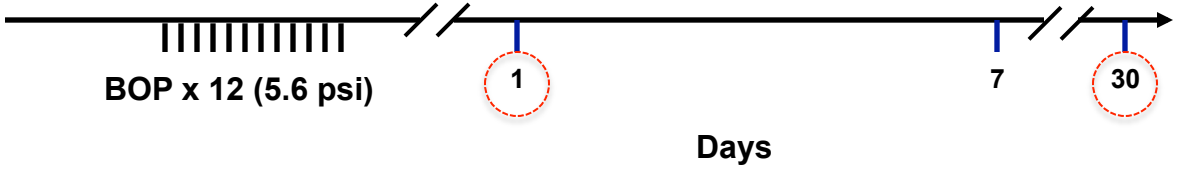
Separate Experiments

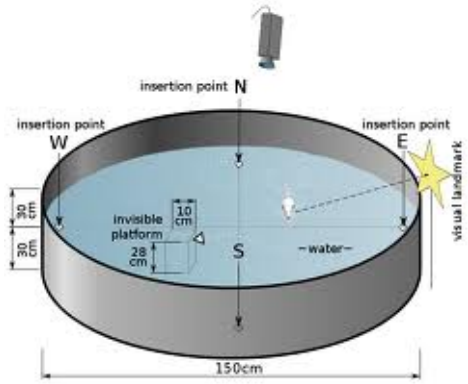
BOP Exposures
- 1 per day (facing or side orientation)
- Isoflurane anesthesia during BOP exposure

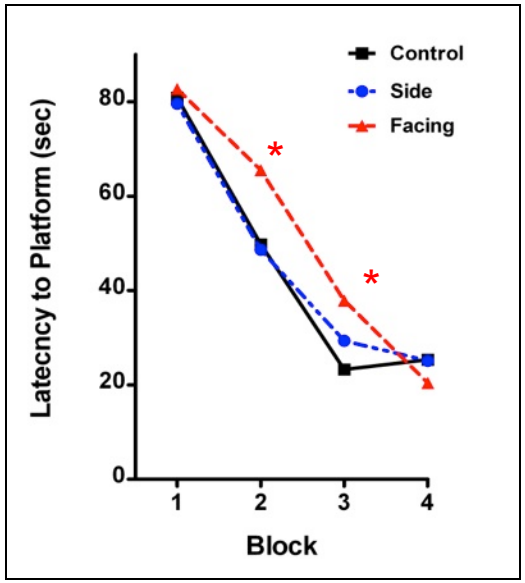
Water Maze Acquisition
- 4 block trials given in a single day
- Each block = four 90 sec trials (N, S, E, W)

Rodent Model of mTBI/PCS

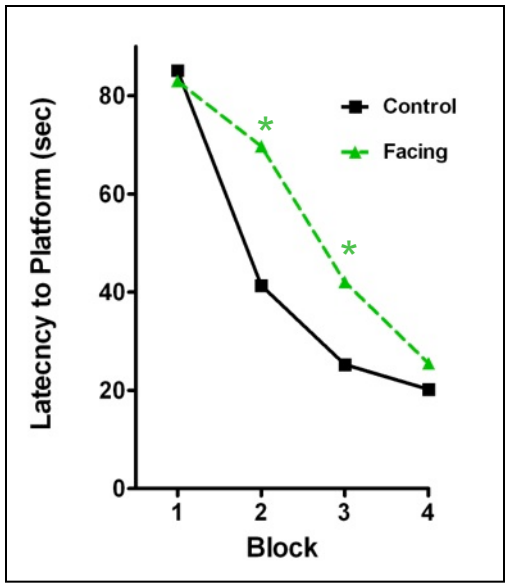
Assessment Timeline







24 Hours post BOP



30 Days post BOP

Rodent Model Pathology

No observable CNS pathology (H&E, APP, GFAP, Silver, etc.)

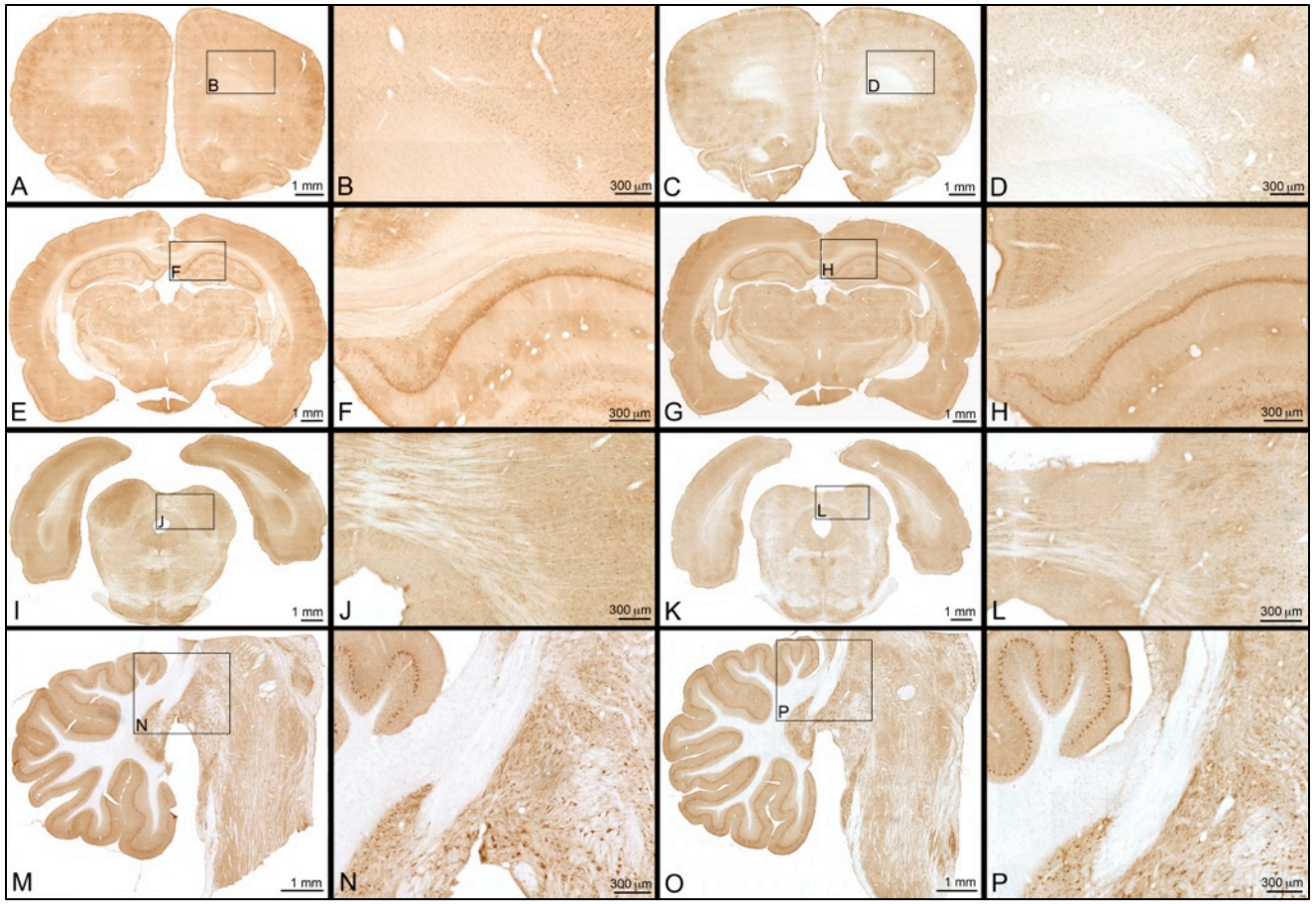


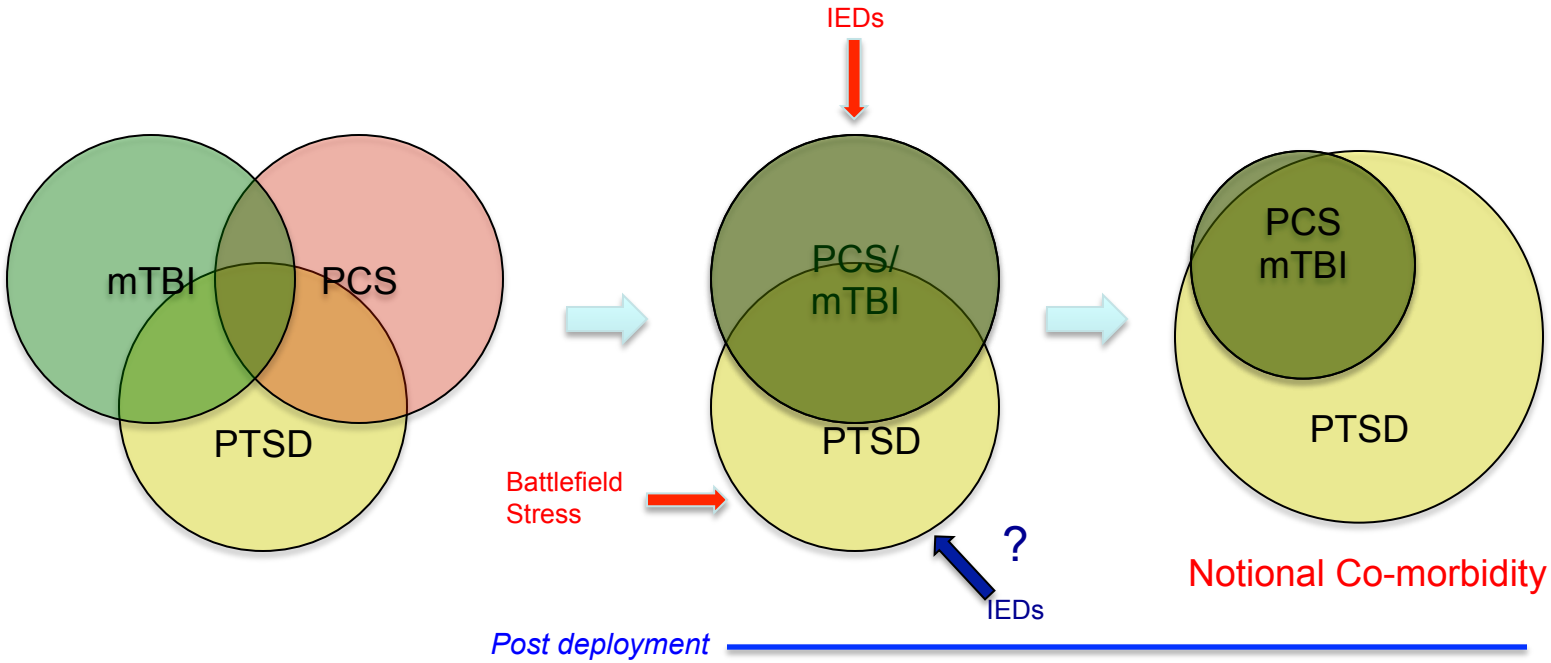
Figure 4: APP immunohistochemistry - frontal overpressure exposure:
APP immunohistochemistry is seen with animals exposed to 12 sessions of 36.6 kPa blast overpressure exposure (A, B, E, F, I, J, M, N) vs. SHAM injured controls (C, D, G, H, K, L, O, P). Photomontages of multiple photomicrographs of frontal (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?

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

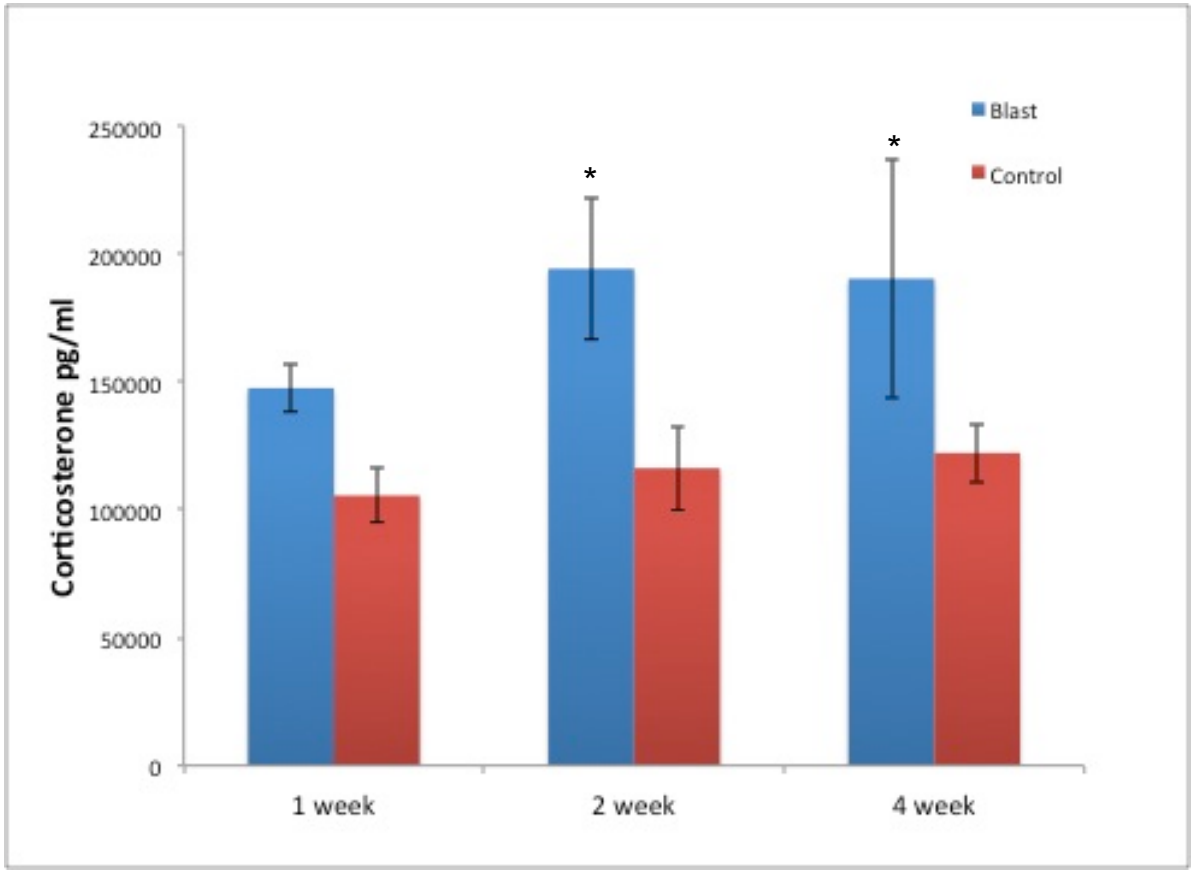
Ahlers et. al., Frontiers Neurotrauma, in press

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

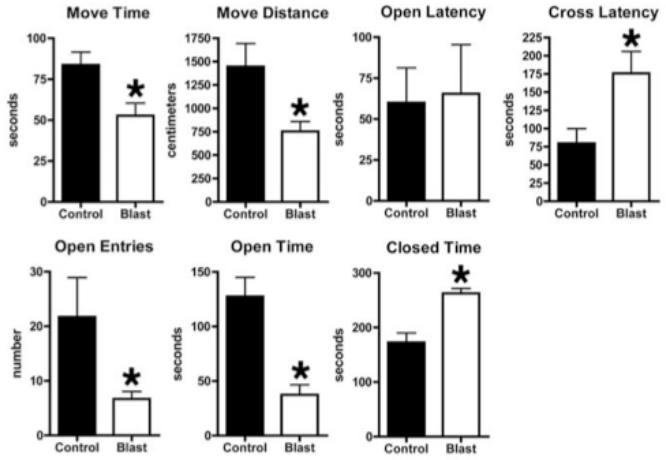


- Rats exposed to 12 x 5.6 psi
- Different groups assessed post BOP

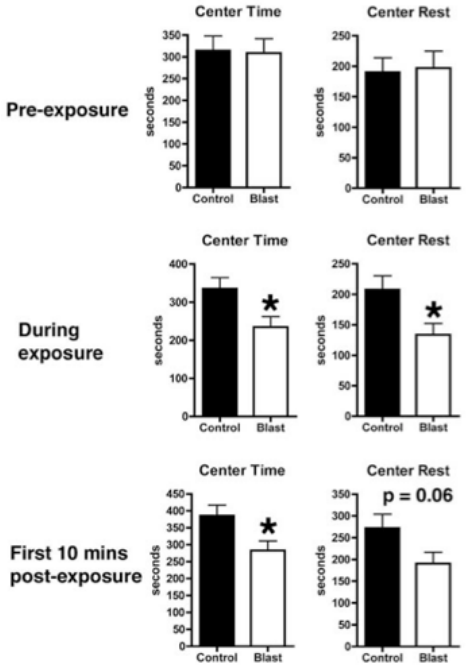
‘Subclinical’ BOP and Anxiety

- Rats exposed to 3 x 10.9 psi
- Tested <u>4.5 months after BOP exposure</u>

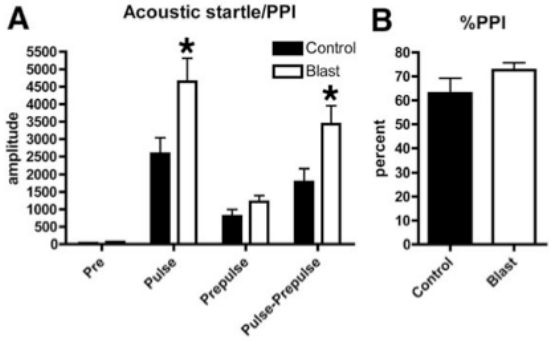
Open Field Test



Predator Scent Assay



Startle Assay



Courtesy of G. Elder, Bronx VA

Pathologic Outcomes of TBI

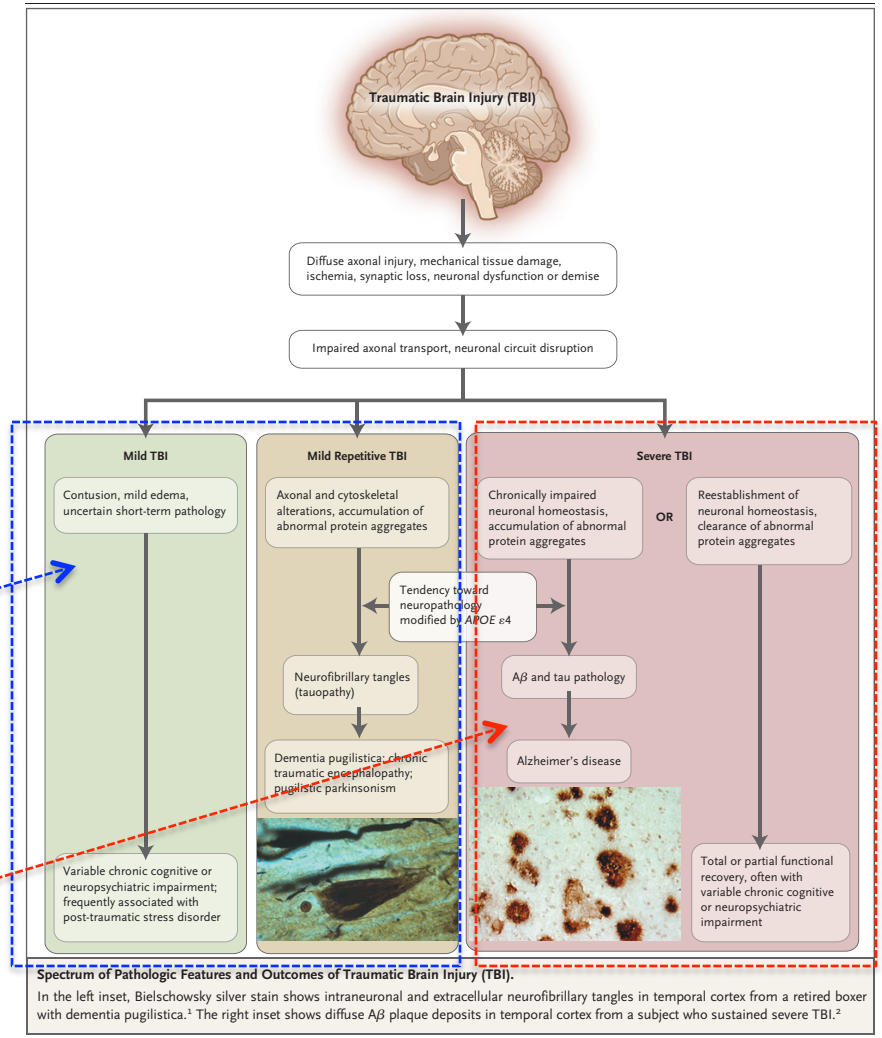
The NEW ENGLAND JOURNAL of MEDICINE

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.

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



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.[1] The right inset shows diffuse Aβ plaque deposits in temporal cortex from a subject who sustained severe TBI.[2]

“Subclinical” BOP and Abeta 42

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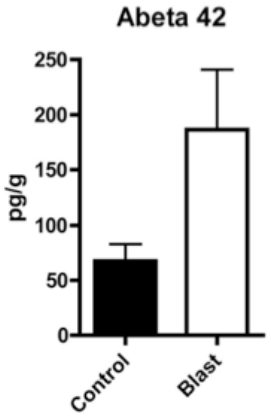

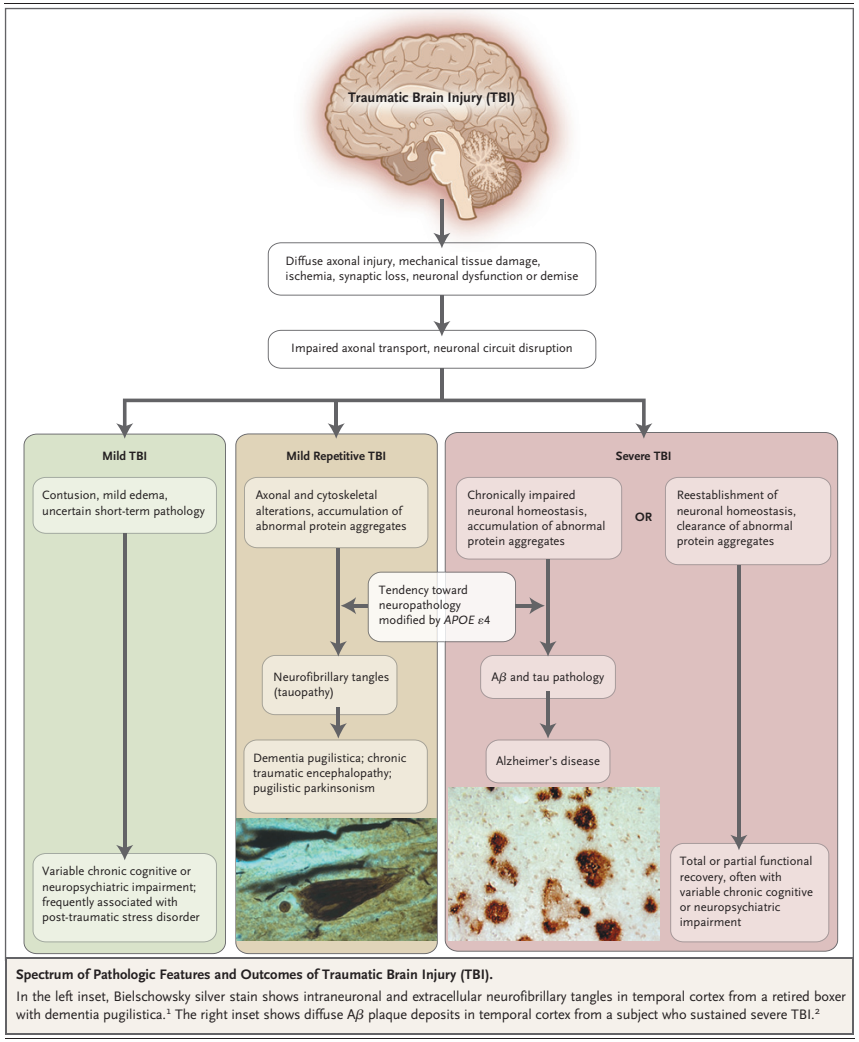

Spectrum of Pathologic Features and Outcomes of Traumatic Brain Injury (TBI).
In the left inset, Bielschowsky silver stain shows intraneuronal and extracellular neurofibrillary tangles in temporal cortex from a retired boxer with dementia pugilistica.[1] The right inset shows diffuse Aβ plaque deposits in temporal cortex from a subject who sustained severe TBI.[2]

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

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

Observed CTE

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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 and 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 antiinflammatory 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 irrita-

neuronal dropout without eosinophilic neuronal necrosis. There was diffuse perineuronal vacuolation, expansion of Virchow Robin spaces and patchy neuropil microspongiosis of both the gray and white matter. There was marked congestion of the arachnoid and pia mater and the penetrating parenchymal vessels. Multifocal sparse perivascular pigment-laden histiocytes were noted in many Virchow Robin spaces.

the brain. 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 tauopathy. 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-

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Chronic traumatic encephalopathy in an Iraqi war veteran with posttraumatic stress disorder who committed suicide

Bennet Omalu, M.D., M.B.A., M.P.H., C.P.E.,[1,2] Jennifer L. Hammers, D.O.,[1,3] Julian Bailes, M.D.,[1,4] Ronald L. Hamilton, M.D.,[5] M. Ilyas Kamboh, Ph.D.,[6] Garrett Webster,[1,2] and Robert P. Fitzsimmons, J.D.[1,7]

[1]Brain Injury Research Institute, Morgantown, West Virginia; [2]Department of Pathology, University of California, Davis, California; [3]Office of the Chief Medical Examiner, Boston, Massachusetts; [4]Department of Neurosurgery, West Virginia University, Morgantown, West Virginia; [5]Department of Pathology, University of Pittsburgh, Pennsylvania; [6]Department of Human Genetics, University of Pittsburgh, Pennsylvania; and [7]Fitzsimmons Law Offices, Wheeling, West Virginia

Observations/ symptoms similar to those observed in breachers

Link to PTSD?

Link to blast?

In 2002 Dr. Bennet Omalu[22] discovered and described CTE in a football player when he performed an autopsy on Mike Webster. Since 2002, Dr. Omalu, the Brain Injury Research Institute, and other researchers have identified and described CTE in numerous football players, wrestlers, boxers, and ice hockey players, which have been reported in the literature.[16,17,19–22,24,25] Following our elucidation of CTE in athletes, we hypothesized that PTSD in war veterans may belong to the CTE spectrum given that active military personnel are high-risk cohorts for repeated subconcussive and concussive traumatic brain injuries; for example, bomb blasts can cause traumatic brain injuries from primary pressure wave and acceleration-deceleration injury mechanisms.[4,28] We expanded our CTE surveillance and brain tissue analyses to include deceased military veterans who were diagnosed with PTSD.

point. He experienced combat and reported exposures to mortar blasts and IED blasts less than 50 m away. During

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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