REPLICATING BLAST-INDUCED TRAUMATIC BRAIN INJURY IN THE LABORATORY: A COMPREHENSIVE APPROACH

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ABSTRACT

The role of primary blast in blast-induced TBI is controversial. The identification of well documented clinical cases is difficult and rare, while the technical difficulties associated with simulating primary blast in the laboratory are considerable, resulting in an inconsistent literature that is difficult to interpret. This laboratory initiated a multidisciplinary effort in order to understand how primary blast impacts the brain and employed exposure and biological models of increasing complexity. A brain cell aggregate culture model system was developed in order to eliminate the complexity of an organism's whole body response to shock and isolate the damage inflicted at the cellular level. Underwater blast allowed for exposures of the brain cells to free-field underwater shock waves and enabled the assessment of the effect of principal stress without shear or global acceleration. The highly hydrolyzed dialysis tubing used to contain the aggregates in suspension presented no barrier to the shock wave. Subtle biological effects were noted in these samples. An Advanced Blast Simulator (ABS) was then used to expose brain aggregates in suspension and enclosed within a spherical shell, to single pulse air blast. In this more complex and realistic exposure environment, significant differences in the biological endpoints were observed compared to those found in aggregates exposed to the single pulse underwater blast waves. Similar subtle changes were also noted when rats were exposed in head-only fashion to simulated blast using the ABS. In addition, this work showed that exaggerated global acceleration artifacts due to dynamic scaling issues occurred when head movement was not restrained. This resulted in very different changes in brain endpoints compared to when head movement was minimized, and illustrates that a fundamental difference exists between the brain injury caused by primary blast, and that caused by impact-acceleration. Work is ongoing in identifying the specific features of blast and primary blast that are responsible for brain injury.

INTRODUCTION

Explosions cause injuries due to several factors, such as penetrating fragments (secondary blast injury), or the whiplash and impact forces from being thrown (tertiary blast injury). However, a blast event also produces a pressure wave as the gases expand away from the detonation. It is thought that at "far-field" distances from the blast site, outside of where secondary or tertiary injuries are likely to occur, primary blast-induced traumatic brain injury (PbTBI) may be caused by exposure to the blast wave.

Blast experiments are difficult to carry out and are also technically challenging to simulate in the laboratory. This is reflected in the literature, where a variety of conclusions have been made as to the role of primary blast in blast-induced TBI. This is not surprising considering the multitude of technical approaches that have been utilized to produce pressure wave profiles of widely varying shapes, components, overpressures and durations. The pressure waves produced by these efforts are often reported simply as a target peak static overpressure, without dynamic pressure values of the shock wave, anomalies/artifacts within the shock wave form or other characteristics of the wave profile such as rarefaction waves. Superimposed on this is a broad range of exposure conditions used by investigators utilizing traditional blast/shock tubes. Animals are exposed with

or without torso protection, in either whole body or head-only fashion, at varying sites within the shock tube, and with a significant number of laboratories also using endjet testing, where the target is placed immediately at, or just off the end of the tube.

With the broad range of exposure conditions and model platforms utilized to simulate primary blast, a shock or blast exposure having a reported static overpressure in one system, will likely have significantly different total shock wave energy and effect than the same overpressure cited in a different model system. While all of these approaches have the potential to produce brain injury, it is not clear that these disparate methodologies are able to simulate the primary shock wave conditions necessary to produce PbTBI. Similar conclusions have been reached in recent publications that have detailed common sources of confusion in the field (Elder *et al*., 2014; Needham *et al*., 2015). As a result, there is considerable controversy as to whether blast-induced pressure waves are actually damaging to the brain, or whether they play a role in the often delayed mild TBI seen in a large number of returning veterans (Elder *et al*., 2014; Needham *et al*., 2015). In response to this, a research effort was initiated to address PbTBI and its potential role in blast-induced TBI. A multidisciplinary approach has been used that has emphasized that the fidelity and relevance of the exposure conditions are of singular importance.

RESULTS AND DISCUSSION

Development of an Advanced Blast Simulator

In its simplest form, a laboratory shock tube is a straight pipe which is divided into a "driver" section charged with high pressure gas, and separated from the "driven" section by a frangible membrane. Rupture of the membrane results in the suddenly-released high-pressure gas expanding and driving the shock wave into the test section. The wave dynamics developed in this type of system do not intrinsically simulate free-field blast, and in fact introduce very significant artifacts into the exposure (Ritzel *et al*., 2011). To address these concerns, an Advanced Blast Simulator (ABS) was developed in a collaborative effort utilizing expertise from Canadian industry (Dyn-Fx, Inc) and the Suffield Research Center (Ritzel *et al*., 2011). The ABS features a tunable End Wave Eliminator used to prevent rarefaction wave artifacts, a divergent-area driver to assist in shaping the wave form, and controlled pressurization of the driver to eliminate variability in driver pressures at diaphragm breakage (Fig. 1). Using this ABS system, it has been possible to generate highly reproducible single-pulse shock waves (Fig. 2) simulating the static and dynamic pressure conditions of free-field blast exposure, including a planar wave front, secondary shock and negative phase (Ritzel *et al*., 2011; Sawyer *et al*., 2016a). This technology is unique and represents a powerful tool with which to study the effects of primary blast on biological systems.

Figure 1: Advanced Blast Simulator (ABS). The divergent driver is pressurized with helium through a computerized control system. The diaphragm fails at a predetermined driver pressure, sending a shock wave through the transition section and into the test section. The animal restraint device is inserted into a port located 4280 mm downstream from the diaphragm, so that the head is across from the 4280 mm sidewall pressure gauge. A tunable End Wave Eliminator prevents rarefaction waves from re-entering the test section.

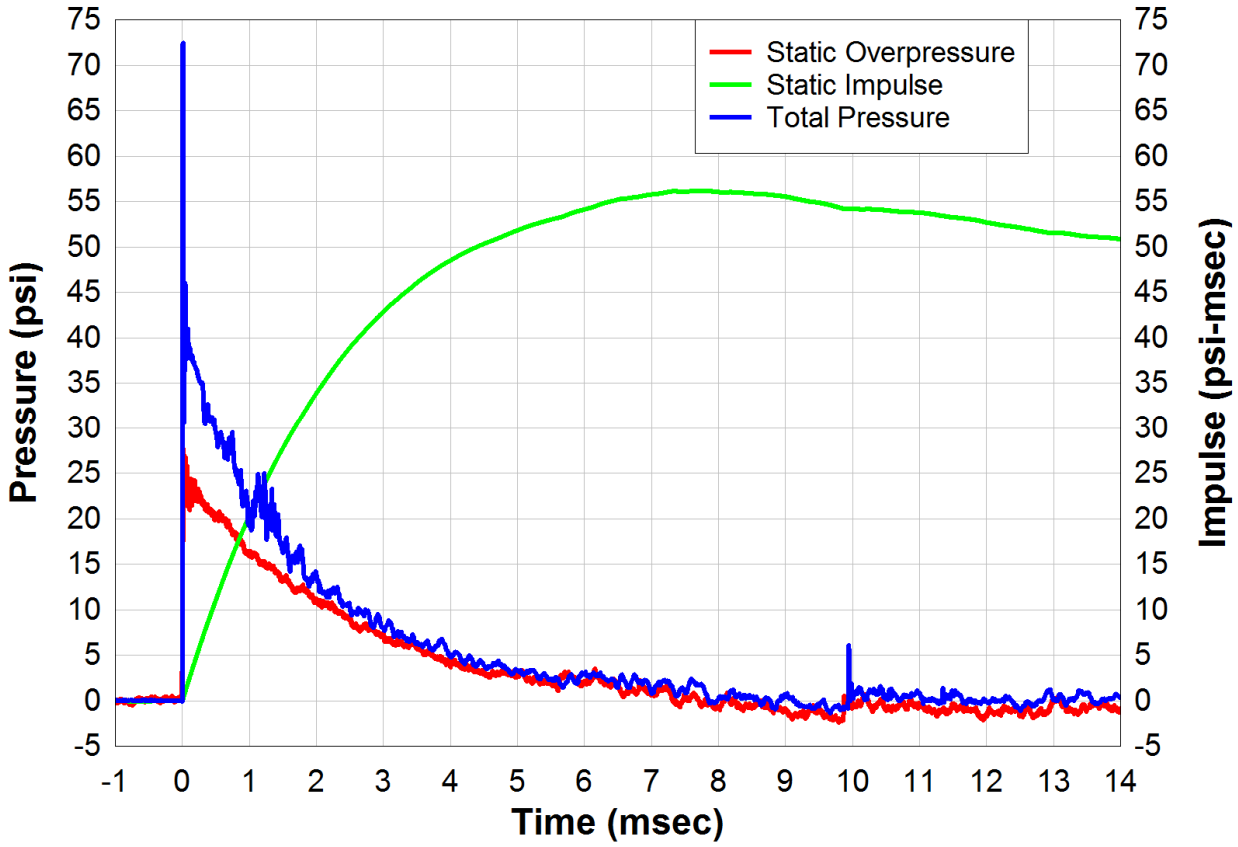

Figure 2: Representative pressure and impulse curves for a 25 psi static overpressure shot. The static overpressures closely follow the Friedlander wave form, with an instantaneous rise-time followed by an exponential decay. The positive phase duration time is approximately 7 msec, followed by a negative phase and a secondary shock wave. The total pressures show an immediate reflective peak, followed by an exponential-like decay.

Primary blast waves cause changes in the brain

Improved body armor and medical treatment have resulted in increased survivability from blast produced by improvised explosive devices (IEDs) during recent conflicts. However, these survivors have the highest frequency of severe traumatic brain injuries (TBIs) since Vietnam, and also often experience delayed symptoms of mild TBIs (mTBIs) that were not recognized prior to discharge. For these reasons, head-only exposures were identified as being an appropriate route of exposure for simulated primary blast. A rat head-only exposure model system was subsequently developed and characterized, and showed that primary blast produced subtle, but reproducible changes in the brain as assessed using a variety of enzymatic, molecular and imaging techniques (Sawyer *et al*., 2016a). Figure 3 shows the effects of primary blast on the neuronal structural protein neurofilament H (NFH). The levels in the cortical brain regions are significantly depressed by seven days, and this depression is persistent out to six weeks.

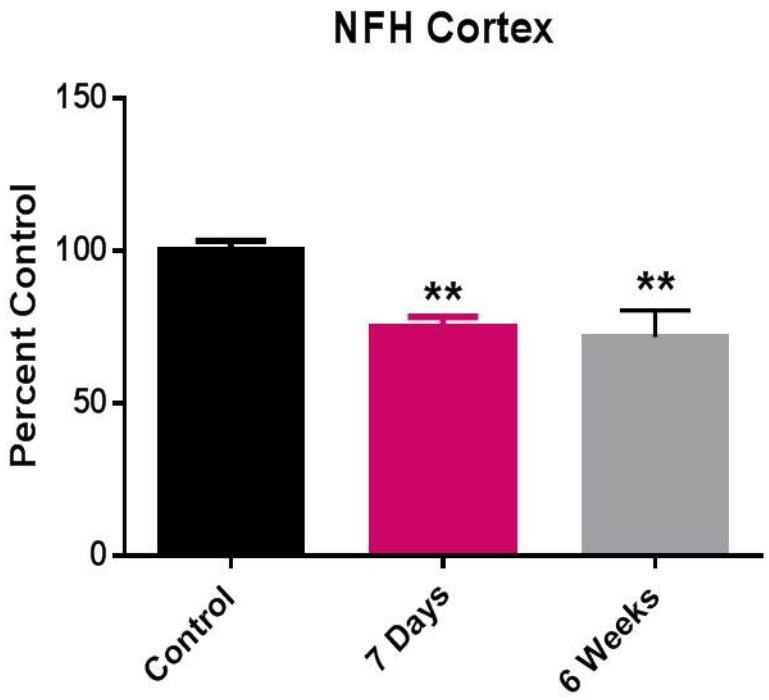

Figure 3: Cortical NFH at one and six weeks. Rats exposed in a head-only fashion to 25 psi overpressure showed significant declines in NFH protein levels in cortical areas of the brain at one and six weeks.

To investigate as to whether primary blast has direct effects on the brain, as opposed to its actions being mediated through whole body systemic effects, a unique brain cell culture system was also developed that has many of the functionalities of whole brain. Rat brain cell aggregate cultures exhibit a multi-cell type composition and can be kept in suspension culture for periods of up to several weeks (Honnegar *et al*. 2003; 2009; Sa Santos *et al*., 2007). They are being used in these laboratories as part of a comprehensive and stepwise research effort to understand the effects of primary blast on the brain. By utilizing highly hydrolyzed dialysis tubing containment during underwater blast exposure, the free floating aggregates were exposed to highly defined shock waves with minimized boundary effects (Sawyer *et al*., 2016b). Subsequent sampling of the exposed cultures showed that the brain tissue responded in a subtle, but reproducible manner to this simple, well defined and measurable shock wave-induced principal stress. The cellular survival proteins Akt and vascular epithelial growth factor (VEGF) were both modified by blast, with the levels of phosphorylated Akt being transiently elevated, while VEGF levels were depressed (Fig. 4). Subsequent experiments increased the complexity (and the realism) of the shock wave exposure by exposing aggregates that were suspended and enclosed within a sphere, to air shock. This work showed that the aggregates are not only sensitive to this pressure insult, but also discriminate it from the much less complex underwater blast with a different profile of biological responses. Notably, the depression of VEGF is much delayed in these latter studies (unpublished observations). Clearly, in these model systems, primary blast causes changes in markers of brain function.

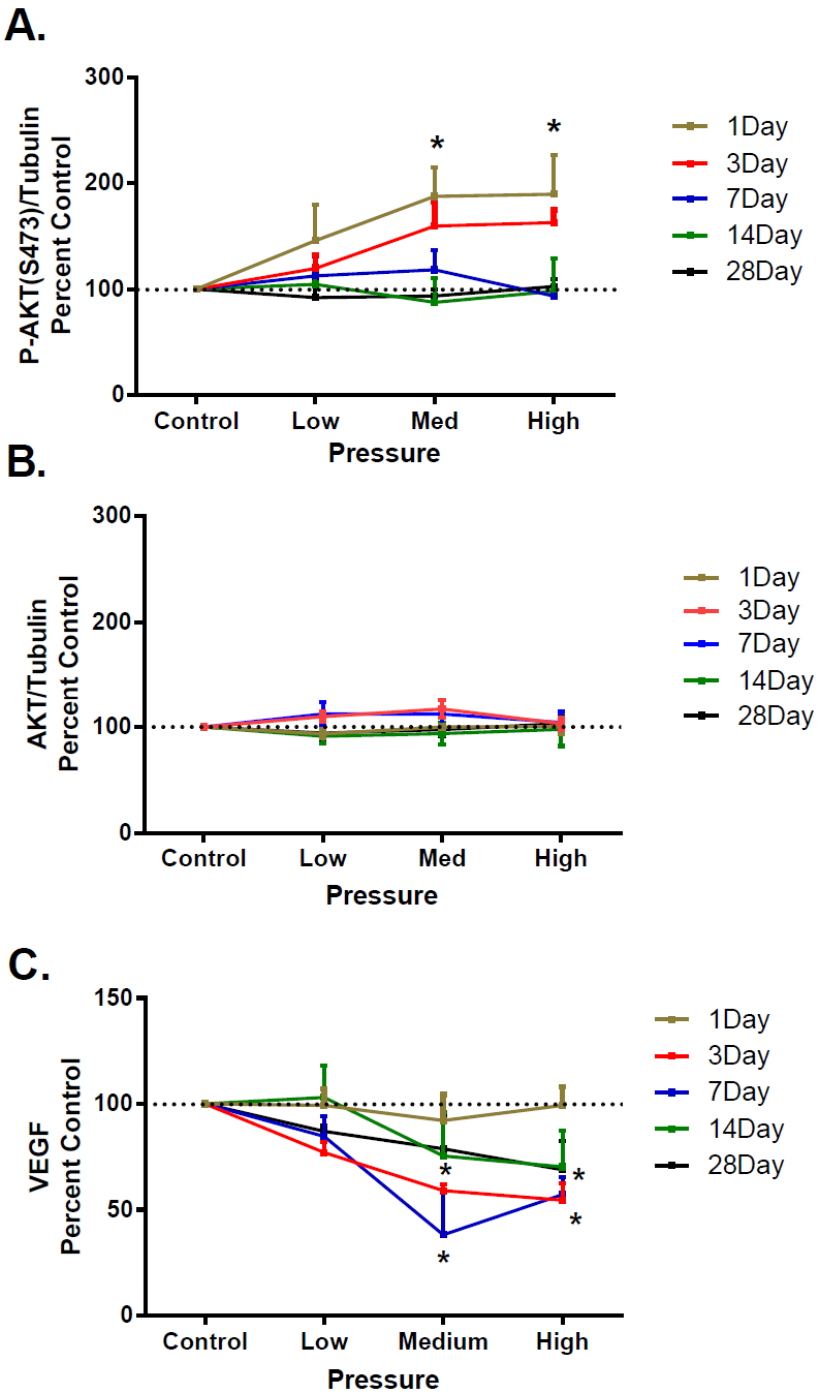

Figure 4: Effect of underwater blast on brain aggregate Akt and on VEGF levels. Aggregate cultures were exposed to low, medium or high levels of underwater blast and then placed back into culture. Significant differences were observed in Akt phosphorylation at medium and high pressures one day after exposure, and in VEGF levels at medium and high pressure exposures on day 3 and 7 (two-way ANOVA and Sidak's multiple comparisons test). Asterisks denote statistical differences from sham control values.

The delayed effects of primary blast waves on the brain

The early effects of primary blast were characterized in both animal and cell culture models and shown to be unique, relatively subtle and not necessarily related to overt cell death or damage Sawyer *et al*., 2016a,b). Efforts were thus directed towards the elucidation of the potential effects of exposure to primary blast at later time points. As noted above, in experiments using cultured brain cell aggregates suspended in, and enclosed in a sphere, air blast caused protein changes that first manifested themselves only two to four weeks after exposure (unpublished observations). At least one of these markers (VEGF) has been implicated in several neurodegenerative disease states. In experiments using rats, degeneration of several brain structural proteins were also first noted four to six weeks after primary blast exposure, most notably in the hippocampal region of the brain. Although the levels of the neuronal NFH, the glial fibrillary acidic protein (GFAP) and the oligodendrocytic protein myelin basic protein were not reduced in this brain structure at one to two weeks, significant decreases in all of these structural proteins were observed in the hippocampal regions of the brains of shock wave-exposed animals four to six weeks post-blast exposure. The decrease in GFAP levels in hippocampus and cortex is depicted in Fig. 5. Uniquely, primary blast causes delayed effects in isolated brain tissue or in primary blast exposed animals.

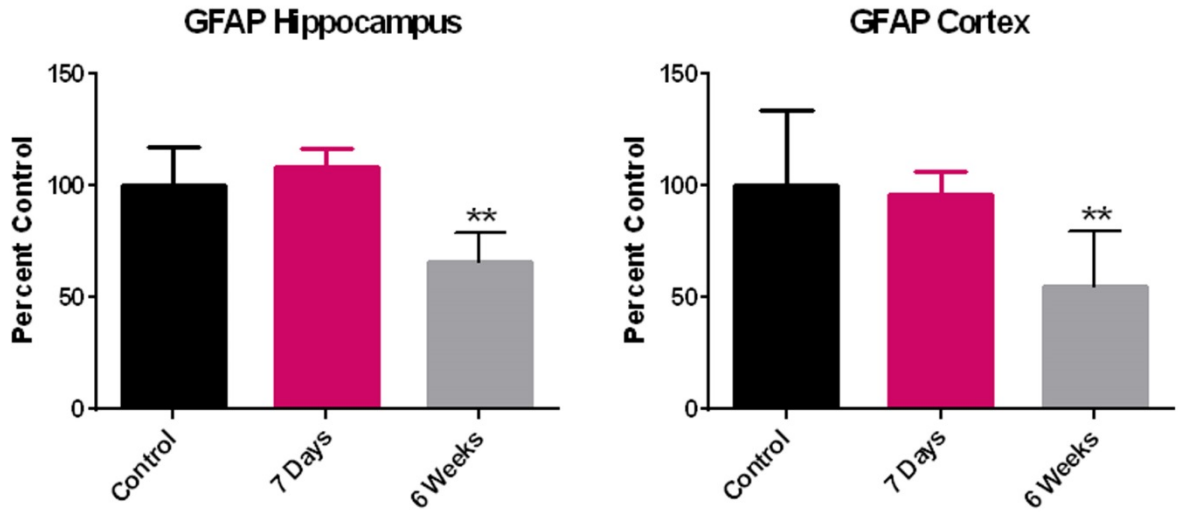

Figure 5: Delayed effects of primary blast on protein levels of glial fibrillary acidic protein (GFAP)in rat hippocampus (left panel) and cortex (right panel). GFAP levels were not changed 7 days after blast but were significantly decreased 6 weeks after blast (two-way ANOVA and Sidak's multiple comparisons test). Asterisks denote statistical differences from sham control values.

Primary blast waves cause brain damage different from that caused by acceleration/impact forces

Early studies in this laboratory examined the effect of head-only exposure of rats to primary blast using high speed photography and showed unexpectedly violent head motion. We thus

investigated the effects of head restraint. A relatively common method used in head-only exposure studies is the provision of a back support for the head contralateral from the blast direction (Garman *et al*., 2011) and we employed this type of head support using nylon netting. In these experiments, the head experienced significant whiplash movement during exposure. This is a scaling artifact due to the small size of the rat's head and similar head movement would not occur in a human exposed to a shock wave of similar properties (15-30 psi overpressure, 6-7 msec duration, 5-22 psi dynamic pressure). In these animals GFAP was very significantly elevated in several areas of the brain by one day post-blast. An increase in this biomarker has been widely utilized and accepted as a diagnostic for the presence of TBI due to concussion and/or whiplash (Diaz-Arrastia *et al*., 2014; Honda *et al*., 2010). In contrast, when head motion was minimized by wrapping it against the back support using additional nylon netting, although several markers of brain damage were observed at one to seven days after exposure, brain levels of GFAP were not elevated (Fig. 6, left panel) and in fact, were depressed in plasma compared to controls (Fig. 6, right panel). We conclude that the GFAP increases observed were due to the artifact of whiplash motion and blast-induced pressure waves do not cause an increase in this biomarker (Sawyer *et al*., 2016a). Clearly, primary blast causes a different profile of brain injury than that caused by acceleration and impact forces in this animal model.

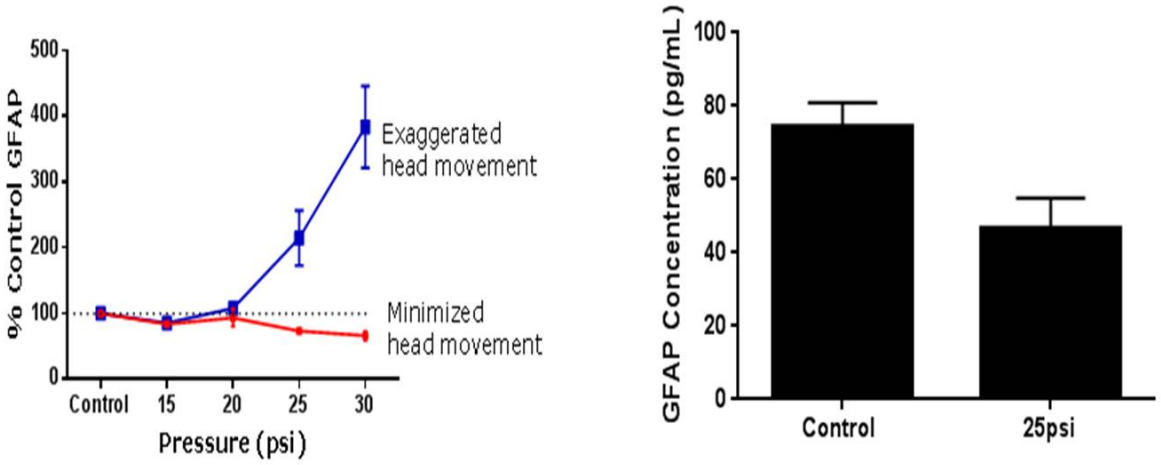

Figure 6: The effect of head motion on brain injury. The cortical brain region levels of the injury biomarker GFAP are not affected by primary blast at one day post-exposure, unless the artifact of exaggerated head movement is allowed (left panel). Animals with minimized head movement (true primary blast simulation) actually exhibit a decrease in plasma GFAP (right panel).

SUMMARY

The recent conflicts in Iraq and Afghanistan have resulted in a high frequency of blast-induced TBIs. While most attention was initially focused on the moderate to severe brain injury caused by penetrating fragments or impact/acceleration, it soon became apparent that the majority of these TBIs were mild, and often not diagnosed at the time of discharge. Although the research and clinical communities have responded to these findings with numerous studies, questions still

remain as to how and even if blast directly affects the nervous system due to blast generated pressure waves. And if so, is the resultant injury different than that encountered in non-blast mTBI caused by whiplash or blunt impact forces? These questions have profound implications for the protection, diagnosis and treatment of individuals exposed to blast.

DRDC research in primary blast has been based on a multidisciplinary approach, and the recognition that the fidelity of the exposure conditions is of singular importance. This has also been recently echoed in the defence TBI community (Elder *et al*., 2014; Needham *et al*., 2015) where it has been acknowledged that increased attention to the details of primary blast exposure are required in the field as a whole. Using the ABS technology, high fidelity simulations of primary blast relevant to survivable IED overpressures can be generated with very high reproducibility (+/- 3%). Recognition of the scaling artifacts that come into play when using small rodents, has led to the development of a rat head-only exposure model that eliminates concussive forces and minimizes whiplash effects. Work using this model system has conclusively shown that pressure waves cause changes to the brains of exposed animals, with resultant functional deficits. In addition to the subtle effects found soon (1 week) after exposure, profound and different changes in brain molecular and imaging endpoints have also been documented up to six weeks post-blast. Importantly, several of these brain changes were very different from those caused by other mechanical means. Several of these primary blast-induced effects have also been duplicated in a unique brain cell culture model, confirming the direct and unique effects of blast-induced pressure waves on brain tissue. Furthermore, these brain cultures were sensitive to the nature of the pressure insult, showing different responses to the highly simplified shock wave produced by underwater blast, versus the complex pressure wave pattern in the spheres due to air shock wave exposure. This illustrates the importance of highly defined exposure parameters in primary blast studies.

The characteristics of PbTBI documented in our studies are strongly reminiscent of those found in the delayed mTBIs that have been diagnosed in many returning veterans and we conclude that primary blast may play a major role in their etiology. The relevance of these model systems is further confirmed by recent reports of the deficits suffered by breachers (Tate *et al*., 2013), as well as MRI studies (Taber *et al*., 2015) that support the contention that low-level blast causes changes in the brains of humans.

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