Military-Relevant Traumatic Brain Injuries: A Pressing Research Challenge

Ibolja Cernak

Blast-induced neurotrauma, i.e., traumatic brain injuries caused by a complex environment generated by an explosion and diverse effects of the resulting blast, currently represents one of the highest research priorities in military medicine. Both clinical experience and experimental results suggest specific blast–body–brain interactions causing complex, interconnected physiological and molecular alterations that can lead to long-term neurological deficits. The Biomedicine Business Area at APL has developed a capability to reproduce all major types of military-relevant traumatic brain injuries (primary blast-induced, penetrating, and blunt head injures) comparable to those caused by explosions in theater by using well-defined experimental mouse models. The overarching research approach aims at obtaining a full understanding of blast-induced neurotrauma; developing reliable, fieldable, and user-friendly diagnostic methods; and designing novel treatments and preventive measures.

INTRODUCTION

The DoD Personnel & Procurement Statistics data[1] show that more than 73% of all U.S. military casualties in Operation Enduring Freedom and Operation Iraqi Freedom were caused by explosive weaponry (e.g., rocket-propelled grenades, improvised explosive devices, and land mines). The incidence of primary blast injury increased from 2003 to 2006, and return-to-duty rates in patients injured in explosions decreased by half.[2] Although the mortality caused by explosion has remained low and unchanged, the incidence of long-term consequences, including those related to blast-induced traumatic brain injuries (TBIs), shows an increasing trend. The Defense and Veterans Brain Injury Center (DVBIC) estimated, on the basis of medical records and actual medical diagnoses, that more than 202,000 service members were diagnosed with TBI between 2001 and 2010, with the overwhelming distribution of mild TBI caused by blast. Nevertheless, it has been hypothesized that this number could be even higher given the fact that many warfighters with

potential TBI are undiagnosed or have delayed diagnosis. Among the symptoms the warfighters experience are irritability; memory and speech problems such as reduced verbal fluency, working memory, and executive functioning;[3] headache; dizziness and balance problems; and psychological impairments including depression, posttraumatic stress disorder, substance abuse, and suicide.[4,5] It is important to note that nearly half of the time these problems developed or were noted after the acute phase.[4] Nevertheless, blast-induced neurotrauma (BINT) is not only a military problem: a high proportion of diffuse brain injury due to blasts and relative to all other types of injuries has been observed among civilians both during wartime[6] and during peacetime.[7]

Accumulating experimental data and clinical evidence show that blast waves can cause brain injury with or without blunt impact or penetrating head wounds. This is caused by a series of effects such as primary blast, or the shock wave itself; secondary blast, where fragments of debris are propelled by the explosion; tertiary blast, which is the acceleration/deceleration of the whole body or part of the body by a blast wind (Fig. 1); and quaternary blast, where flash burns are a consequence of the transient but intense heat of the explosion.[8,9]

There are numerous controversial opinions about the cause of neurological deficits that develop after exposure to blast. These opinions range from the belief that blast-induced mild TBI is a mere concussion, and as such the nature of its symptoms is similar to those of a temporary postconcussive syndrome,[10] to the belief that blast-induced neurological deficits are caused by unique interactions of systemic, local, biomechanical, and cerebral responses to blast.[11–13] Despite concentrated efforts to clarify the molecular mechanisms underlying BINT, definitive diagnosis and specific evidence-based therapy have yet to be developed for preventing and/or reducing the detrimental effects of blast exposure on the brain.

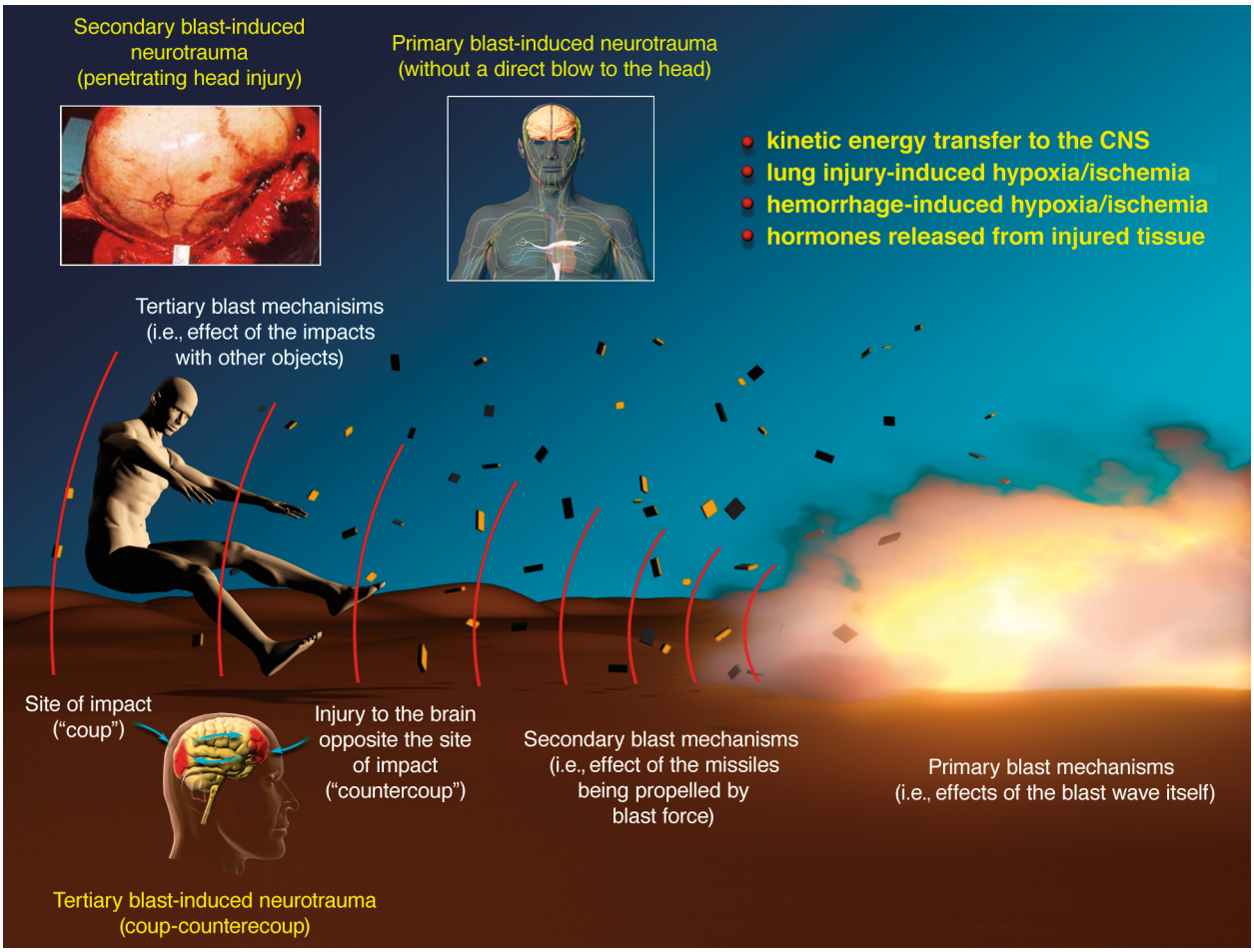

Figure 1. The complex injurious environment generated by explosion: primary blast effects of the blast wave itself causing primary blast injury; secondary blast effects due to fragments generated and propelled by blast-force causing secondary blast injury (blunt or penetrating); and tertiary blast effects caused by acceleration and deceleration of the body thrown by the kinetic energy of the blast and colliding with other objects (similar to "coup-countercoup" injuries). CNS, central nervous system. (Reproduced with permission from Ref. 13.)

RESEARCH APPROACH

Experimental models of blast injuries used to study BINT differ widely from one another, making the comparison of the experimental results extremely difficult. Often, instead of mimicking real-life conditions by generating blast signatures with multiple shocks and expansion fronts as seen in theater, blast injury models replicate the ideal blast wave from an open-field explosion. This significantly reduces their clinical and military relevance. The APL team designed and developed a modular, multichamber shock tube capable of tailoring pressure wave signatures and reproducing complex shock wave signatures seen in the military operational environment. Moreover, we developed and standardized a mouse model able to replicate the main pathophysiological consequences of graded blast injuries and primary BINT, including the long-term neurological effects seen in warfighters. Additionally, aiming to compare the mechanisms of a blunt head injury caused by a direct impact to the skull with the primary blast-induced TBI, we also developed a weight-drop blunt head injury mouse model. Finally, in collaboration with the Karolinska Institutet, we built a penetrating head injury device to study mechanisms and consequences of penetrating brain injury in mice.

RESULTS

Physiological parameters, functional (motor, cognitive, and behavioral) outcomes, and underlying molecular mechanisms involved in brain inflammation measured in the brain over the 30-day postblast period showed that this model is capable of reproducing major neurological changes caused by clinical BINT. We have established a causal relationship between the intensity of the mechanical force (i.e., overpressure of the blast) and long-term functional (i.e., motor performance, cognitive performance, and behavior) outcomes; demonstrated a graded decline in functional performance; and showed an intensity-dependent difference between mild and moderate injury groups. Similar to the main systemic and neural impairments seen in patients[4, 5, 14, 15] our model reproduced weight loss, unstable heart and respiratory rates, motor deficits, memory decline, depression, and loss of interest toward environment after blast exposure. In animals with moderate blast injuries, the alterations in vital functions suggested impaired autonomous nervous system control, memory decline, and behavioral impairments that remained permanent after 1 month, which is a substantial period in the life of a mouse.

Also, we confirmed the importance of body position in determining blast injury outcome and the severity of blast-induced neurological deficits. Namely, placing the animals in the supine position, i.e., a body position facing the shock wave front, resulted in higher lethality and more severe organ damage than when the animals were positioned so that their backs were turned toward the shock wave front. This has been previously confirmed in both animals and people[16–19] and can be explained, at least in part, by the interaction between the shock wave's kinetic energy and the elastic abdominal wall.[13, 20, 21]

It is important to note that we confirmed the vital importance of the systemic response to blast exposure in the pathobiology of blast-induced TBI. In an attempt to link systemic and cerebral inflammation as one of the potential mechanisms underlying long-term neurological deficits caused by blast, we performed real-time, in vivo imaging of myeloperoxidase (MPO) activity of activated polymorphonuclear leukocytes in mice exposed to mild-intensity blast during a 1-month postinjury period. Namely, migration and accumulation of polymorphonuclear leukocytes are among the major hallmarks of the host response to injuries.[22–24] Briefly, mice were anesthetized, mounted in supine position to the animal holder secured inside the driven section of the helium-driven shock tube, and exposed to mild-intensity shock wave [measured rupture pressure: 183 ± 14 kPa (26.5 ± 2.1 psig); measured total pressure: 103 kPa (14.9 psig), causing 5% mortality].[20] Subsets of animals (n = 5) were exposed to whole-body blast; blast with torso (chest and abdomen) protection using a custom-made rigid plexiglass "body armor" with the head exposed; or blast with head protection using a custom-made plexiglass "helmet" covering the skull, face, and the neck of the animal with the torso exposed. Ten minutes before imaging, mice were injected with a XenoLight RediJect Inflammation Probe (Caliper Life Sciences, Hopkinton, Massachusetts) at 200 mg/kg (150 μl per mouse) intraperitoneally. The XenoLight RediJect Inflammation Probe is a chemiluminescent reagent in a ready-to-use format that allows for longitudinal tracking of MPO level and inflammation status, in vivo, in a variety of disease models. Bioluminescence imaging was performed using the IVIS Imaging System 3-D Series (Caliper Life Sciences). During the imaging, the animals were anesthetized with a gas mixture (isoflurane:nitrous oxide:oxygen at 1:66:33 proportions, respectively) using the integrated system for gas anesthesia. The duration of imaging was 5 min. Sham control animals did show only low-intensity localized bioluminescence at the injection site (results not shown). Figure 2 shows the distribution of increased bioluminescence in mice with whole-body blast exposure 1 month after blast exposure. Significant inflammation was observed in the gastrointestinal tract and the diaphragmatic and costal parts of the lungs, as well as in the brain. Experiments using rigid body or head protection in animals subjected to blast showed that head protection failed to prevent inflammation in the brain or reduce neurological deficits, whereas body protection was successful in alleviating the blast-induced functional and morphological impair-

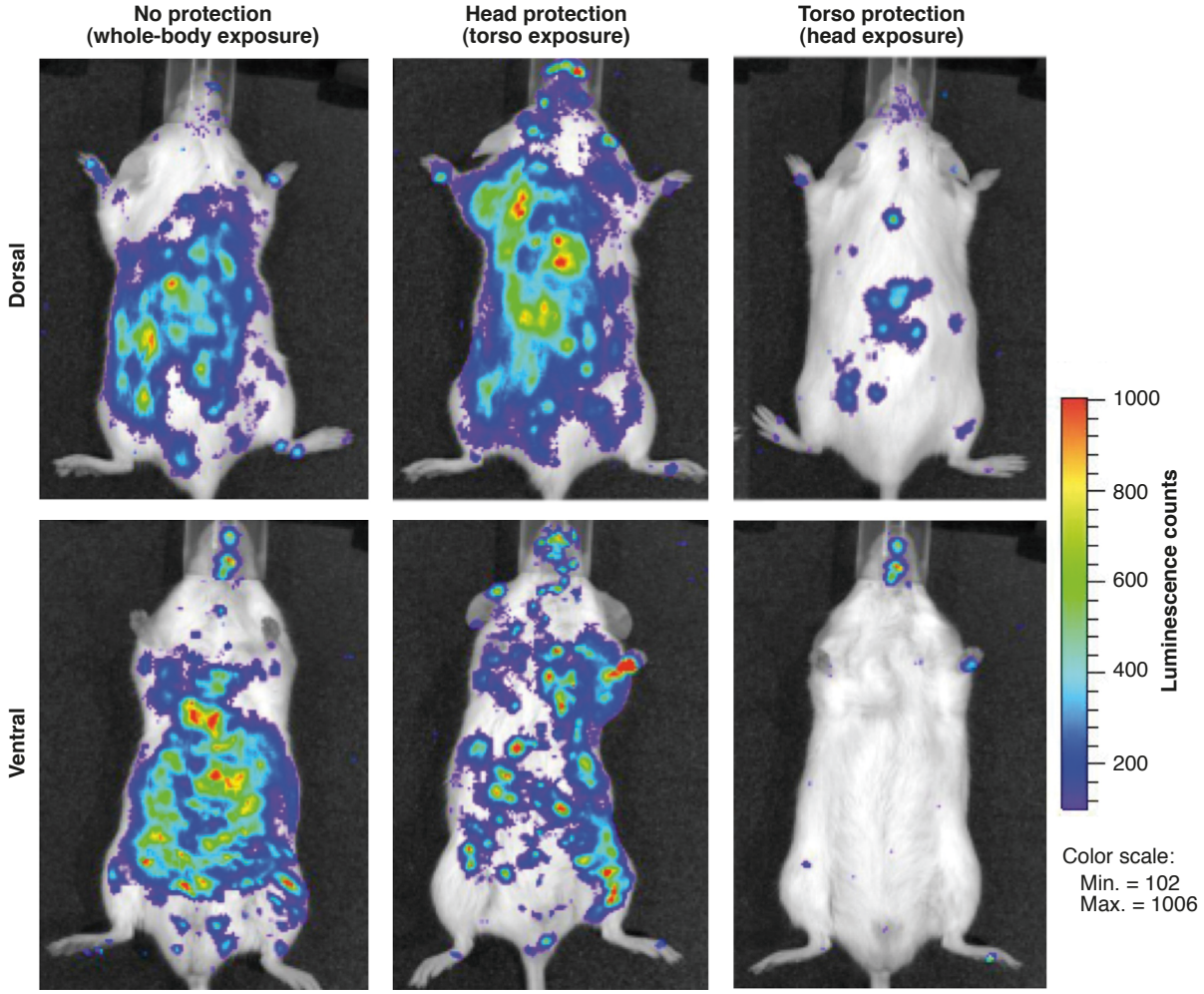

Figure 2. Distribution of increased bioluminescence showing MPO activity of activated polymorphonuclear leukocytes in mice subjected to whole-body blast exposure, imaged 1 month after mild blast exposure. The photographs show the same representative animals imaged in both dorsal and ventral positions. The intensity of bioluminescence was scaled based on the photon counts. The nose cone that can be discerned in the photos is part of the anesthesia device that is the integral part of the IVIS Imaging System 3-D Series and does not represent a head cover. (Reprinted in part with permission from Ref. 25.)

ments in the brain. Indeed, the increase in MPO activity observed in the mice with head protection was similar to corresponding changes found in the brains of animals exposed to whole-body blast, suggesting that inflammatory cells of systemic origin play an important role in the pathobiology of blast-induced inflammatory processes in the brain.[25] These results clearly suggested the importance of the indirect, i.e., blast–body, interaction as well as the decisive role of autonomous nervous–neuroendocrine–immune systems interaction in the pathogenesis of BINT.

Finally, aiming to identify the similarities and differences between BINT and blunt-impact TBI, we used well-standardized corresponding mouse models to analyze physiological (arterial blood oxygen saturation, heart rate, respiratory rate, and pulse distention), functional (motor performance, exploratory activity), and molecular (glial fibrillary acidic protein) alterations in the brainstem and hippocampus that occurred more than 30 days after injury. Our results demonstrate that the generalizable consequences of a brain insult, such as a decrease in motor performance and exploratory activity as well as the stimulation of astrocytes, have differing temporal profiles, suggesting injury specificity that should be taken into account when developing diagnostic and differential diagnostic methods.

CONCLUSION

Understanding of the mechanisms of blast injuries and BINT and having a reliable model, and thus a powerful research tool, is crucial in developing reliable diagnostic tools, treatments, and preventive measures for our warfighters. The Biomedicine Business Area has developed a unique research environment that supports military-relevant, state-of-the-art research focusing on psycho-

logical and physical impairments of the central nervous system. Fostering multidisciplinary research, our efforts are focused on improving the quality of our warfighters' lives.

ACKNOWLEDGMENT: This work was supported by APL internal research and development funds.

REFERENCES

[1]*Global War on Terrorism By Reason: October 7, 2011 Through October 31, 2011*, DOD Personnel and Procurement Statistics: Personnel and Procurement Reports and Data Files, http://siadapp.dmdc.osd.mil/personnel/CASUALTY/gwot_reason.pdf (accessed 8 Nov 2011).

[2]Ritenour, A. E., Blackbourne, L. H., Kelly, J. F., McLaughlin, D. F., Pearse, L. A., et al., "Incidence of Primary Blast Injury in US Military Overseas Contingency Operations: A Retrospective Study," *Ann. Surg.* **251**(6), 1140–1144 (2010).

[3]Nelson, L. A., Yoash-Gantz, R. E., Pickett, T. C., and Campbell, T. A., "Relationship Between Processing Speed and Executive Functioning Performance Among OEF/OIF Veterans: Implications for Postdeployment Rehabilitation," *J. Head Trauma Rehabil.* **24**(1), 32–40 (2009).

[4]Terrio, H., Brenner, L. A., Ivins, B. J., Cho, J. M., Helmick, K., et al., "Traumatic Brain Injury Screening: Preliminary Findings in a US Army Brigade Combat Team," *J. Head Trauma Rehabil.* **24**(1), 14–23 (2009).

[5]Peskind, E. R., Petrie, E. C., Cross, D. J., Pagulayan, K., McCraw, K., et al., "Cerebrocerebellar Hypometabolism Associated with Repetitive Blast Exposure Mild Traumatic Brain Injury in 12 Iraq War Veterans with Persistent Post-Concussive Symptoms," *Neuroimage* **54**(Suppl 1), S76–S82 (2010).

[6]Levi, L., Borovich, B., Guilburd, J. N., Grushkiewicz, I., Lemberger, A., et al., "Wartime Neurosurgical Experience in Lebanon, 1982-85. II: Closed Craniocerebral Injuries," *Isr. J. Med. Sci.* **26**(10), 555–558 (1990).

[7]Bochicchio, G. V., Lumpkins, K., O'Connor, J., Simard, M., Schaub, S., et al., "Blast Injury in a Civilian Trauma Setting Is Associated with a Delay in Diagnosis of Traumatic Brain Injury," *Am. Surg.* **74**(3), 267–270 (2008).

[8]Mellor, S. G., "The Pathogenesis of Blast Injury and Its Management," *Br. J. Hosp. Med.* **39**(6), 536–539 (1988).

[9]Owen-Smith, M. S., "Explosive Blast Injury," *Med. Bull. US Army Eur.* **38**(7/8), 36–43 (1981).

[10]Hoge, C. W., Goldberg, H. M., and Castro, C. A., "Care of War Veterans with Mild Traumatic Brain Injury—Flawed Perspectives," *N. Engl. J. Med.* **360**(16), 1588–1591 (2009).

[11]Cernak, I., Savic, J., Malicevic, Z., Zunic, G., Radosevic, P., et al., "Involvement of the Central Nervous System in the General Response to Pulmonary Blast Injury," *J. Trauma* **40**(3 Suppl), S100–S104 (1996).

[12]Cernak, I., Savic, J., Zunic, G., Pejnovic, N., Jovanikic, O., and Stepic, V., "Recognizing, Scoring, and Predicting Blast Injuries," *World J. Surg.* **23**(1), 44–53 (1999).

[13]Cernak, I., and Noble-Haeusslein, L. J., "Traumatic Brain Injury: An Overview of Pathobiology with Emphasis on Military Populations," *J. Cereb. Blood Flow Metab.* **30**(2), 255–266 (2010).

[14]Brenner, L. A., Ladley-O'Brien, S. E., Harwood, J. E., Filley, C. M., Kelly, J. P., et al., "An Exploratory Study of Neuroimaging, Neurologic, and Neuropsychological Findings in Veterans with Traumatic Brain Injury and/or Posttraumatic Stress Disorder," *Mil. Med.* **174**(4), 347–352 (2009).

[15]Warden, D. L., French, L. M., Shupenko, L., Fargus, J., Riedy, G., et al., "Case Report of a Soldier with Primary Blast Brain Injury," *Neuroimage* **47**(Suppl 2), T152–T153 (2009).

[16]Bowen, I. G., Fletcher, E. R., and Richmond, D. R., "Estimate of Man's Tolerance to the Direct Effects of Air Blast," Technical Report DA-49-146-XZ-372, Defense Atomic Support Agency, Washington, DC (1968).

[17]Chiffelle, T. L., "Pathology of Direct Air-Blast Injury," in Technical Progress Report DA-49-146-XY-055, Defense Atomic Support Agency, Department of Defense, Washington, DC (1966).

[18]Richmond, D. R., Damon, E. G., Bowen, I. G., Fletcher, E. R., and White, C. S., "Air-Blast Studies with Eight Species of Mammals," Technical Progress Report DASA 1854, Fission Product Inhalation Project, Lovelace Foundation for Medical Education and Research, Albuquerque, NM, pp. 1–44 (1967).

[19]Richmond, D. R., Damon, E. G., Fletcher, E. R., Bowen, I. G., and White, C. S., "The Relationship Between Selected Blast-wave Parameters and the Response of Mammals Exposed to Air Blast," *Ann. N Y Acad. Sci.* **152**(1), 103–121 (1968).

[20]Cernak, I., Merkle, A. C., Koliatsos, V. E., Bilik, J. M., Luong, Q. T., et al., "The Pathobiology of Blast Injuries and Blast-Induced Neurotrauma as Identified Using a New Experimental Model of Injury in Mice," *Neurobiol. Dis.* **41**(2), 538–551 (2011).

[21]Cernak, I., Wang, Z., Jiang, J., Bian, X., and Savic, J., "Ultrastructural and Functional Characteristics of Blast Injury-Induced Neurotrauma," *J. Trauma* **50**(4), 695–706 (2001).

[22]Menezes, G. B., Rezende, R. M., Pereira-Silva, P. E., Klein, A., Cara, D. C., and Francischi, J. N., "Differential Involvement of Cyclooxygenase Isoforms in Neutrophil Migration in Vivo and in Vitro," *Eur. J. Pharmacol.* **598**(1–3), 118–122 (2008).

[23]Maslinska, D., and Gajewski, M., "Some Aspects of the Inflammatory Process," *Folia Neuropathol.* **36**(4), 199–204 (1998).

[24]Toft, P., Andersen, S. K., and Tonnesen, E. K., "The Systematic Inflammatory Response after Major Trauma," *Ugeskr. Laeger* **165**(7), 669–672 (2003).

[25]Cernak, I., "The Importance of Systemic Response in the Pathobiology of Blast-Induced Neurotrauma," *Front. Neur.* **1**(Dec 10), 1–9 (2010).

The Author

Ibolja Cernak led the biomedical research concerning psychological and physical impairments of the central nervous system due to injuries. She was a Principal Professional Staff member and Medical Director in APL's former National Security Technology Department's Biomedicine Business Area and provided overall direction of the experimental, theoretical, and clinical aspects of the military-relevant neurological and psychological deficits. Dr. Cernak has more than 20 years of experience in both clinical and experimental blast injury research. Her current research is focused on blast injuries, including BINT; ballistic injuries; and stress response and resilience to military-relevant stressors (extreme environmental conditions and repeated exposures to nonlethal, nonimpact injurious factors, among others). She is currently the Canadian Military and Veterans' Chair in Clinical Rehabilitation at the University of Alberta. Her e-mail address is cernak@ualberta.ca.

The *Johns Hopkins APL Technical Digest* can be accessed electronically at **www.jhuapl.edu/techdigest**.