Neuroscience

Military blast-induced synaptic changes with distinct vulnerability may explain behavioral alterations in the absence of obvious brain damage

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Sadly many military veterans, who left home to serve their country honorably, return from service with permanent life-changing injuries. It is easy to remember our debt to those who have incurred such visible injuries, and all too easy to forget the invisible wounds that afflict so many of our military servicemen and women. Brain injuries can be invisible during initial medical evaluations and are often caused by military explosives that create blast shockwaves of varying intensity. One of the most common types of traumatic brain injury (TBI) linked to military service is blast-induced neurotrauma. To better understand this type of injury, a recently published study subjected rat brain slice cultures to detonations of RDX military explosives, resulting in reduced levels of specific synaptic markers. Such alterations have in fact been linked to depressive behavior, anxiety, and cognitive rigidity, and the blast-induced synaptic modifications may underlie the behavioral changes in those TBI sufferers who do not exhibit measurable brain damage. This research has the potential to improve diagnoses by identifying indicators of synapse integrity for the assessment of subtle synaptopathogenesis linked to blast-induced neurotrauma.

Traumatic Brain Injury (TBI) | neurotrauma | blast-induced shockwaves | military explosives

Many veterans return from military service with life-changing injuries. Some have lost limbs; some are confined to wheel chairs, and some have other permanent disfigurements to their bodies. All too often they also come home with invisible injuries such as a type of traumatic brain injury (TBI) known as blast-induced neurotrauma, an injury caused by shockwaves emitted from explosives. While victims of blast-induced neurotrauma experience cognitive and behavioral changes, they fail to exhibit the neuropathology expected to accompany a TBI, thus making this type of TBI difficult to detect. As a result, a number of veterans with these injuries have not been properly diagnosed and therefore have been denied certain benefits. Last year a member of the United States Congress, Representative Louise Slaughter, urged the Pentagon on more than one occasion to study blast exposure and its effects. As she points out, because of the advances in the types of weapons used and the increase in their prevalence, blast-related injuries occur more frequently today than they did in past military engagements (1). In the Iraq and Afghanistan wars, improvised explosive devices (IEDs) became the common weapon used by insurgents to attack the coalition forces. As described by Hamilton (2), tens of thousands of military personnel were exposed to bomb blasts and many of them subsequently experienced memory loss and confusion. Hamilton also points out the important work of elite doctors who challenged the historical dogma that head injuries were not serious unless "obvious and bloody."

Statistics show that military veterans in the United States returning from Iraq and Afghanistan are affected by TBI at a significantly higher rate than the general population. While almost 2% of the U.S. population lives with a TBI-associated disability, 10-20% of veterans returning from Iraq suffer the effects of a traumatic brain disorder (3). Although the statistics are startling, they should not be surprising when one considers the levels of blast exposures to which these men and women are subjected. Postdeployment surveys from members of the U.S. Army and Marine Corps are particularly striking. They show that 86-92% of those deployed in Iraq experienced artillery, rocket, or mortar fire (4). In addition to receiving incoming adversarial blasts, other combat and non-combat experiences can subject military personnel to blast events of various intensities (see Table 1). In both combat missions and training exercises, different size shockwaves occur when delivering artillery, rocket, or mortar fire, discharging large caliber munitions, or when firing recoilless weapons that emit powerful bursts of gases from the back of the firearm. A wide range of blast levels is also associated with obstacle and urban breaching for troop maneuvers and related training. With the large number of people exposed to explosive blasts, studies of blast-induced neurotrauma are important to better understand these injuries so that they may be prevented, properly diagnosed, and treated.

Primary blast-induced neurotrauma can be caused by a variety of explosive devices and weapons. To determine the neuronal effects after survivable blasts, recent studies took advantage of a new experimental protocol that generates distinct blast waves from detonated cyclotrimethylene trinitramine (RDX) military explosives directed at cultured cells and brain slices (5-9). The *in vitro* model was specifically developed using real explosives for investigating blast-induced injuries, especially to understand the vulnerability of brain tissue. In Smith et al. (6), long-term cultures of rat hippocampal slices were used since the hippocampus is particularly susceptible to traumatic and excitotoxic injuries and since this brain region plays a key role in higher order behavioral functions including learning and memory. The organotypic slice cultures maintain the distinct neuronal subfields of the hippocampus as well as the unique synaptic connectivity that underlies memory encoding. When detonations of 1.7-g assemblies of RDX explosives were directed at the cultured brain slices, indicators of synaptic integrity were lost in a progressive manner across the number of consecutive blasts applied to the tissue.

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Table 1. Wide range of blast intensities in combat and training experiences that involve blast events.

Blast Events	Blast Intensity
receiving artillery, rocket, or mortar fire	high levels
delivering artillery, rocket, or mortar fire	low levels
being in close proximity to IED detonation	high levels
participating in obstacle breaching	low to high levels
firing large-caliber weapons	low levels

For assessing the direct blast effects on brain tissue in the Smith et al. study, hippocampal slice cultures were positioned inside a specialized chamber, and then reproducible constructs of RDX explosives were detonated outside the chamber in order to generate the type of shockwave experienced by blast-induced TBI victims. Pressure sensors positioned just above the brain tissue detected the progress of the shockwave as it penetrated the wall and traveled through the chamber. The explosive blasts caused a subsequent, time-dependent loss of specific proteins of synapses (6, 9), the neuronal connections of the hippocampal circuitry. The presynaptic marker synaptophysin and the excitatory postsynaptic protein GluR1 exhibited 50 to 70% reductions after consecutive RDX detonations as compared to their measures from mock-treated control slice samples.

Whereas these two synaptic proteins declined in dendritic zones of the hippocampal slices, the cytoskeletal protein actin remained unchanged across multiple blast exposures. Moreover, another ubiquitous cytoskeletal component found in the brain, spectrin, exhibited no proteolytic deterioration after RDX blasts, further indicating that the pre- and postsynaptic markers are distinctly vulnerable to military blast-induced pathology. Indeed, this subtle synaptic pathology was detected in the absence of any overt tissue or cellular damage in the brain slice model. Cell death in the hippocampal slice cultures was assessed with the sensitive Fluoro-Jade B staining method after a triple RDX blast insult, and the lack of such staining indicates that the blasts lead to the compromise of synaptic integrity in the absence of neuronal deterioration. Further analysis found that blast-mediated synaptic alterations were associated with the disruption of a signaling pathway for facilitating functional plasticity in neuronal connections (6). Such subtle but distinct changes to neurons may explain blast-induced alterations to mechanisms underlying cognition and behavior. Altered central synapses evident in the blast model also warrants the Defense Advanced Research Projects Agency (DARPA) research on brain stimulation therapy that is being pursued to help memory problems caused by blast-induced TBIs (10), particularly for troops returning from Iraq and Afghanistan. In addition to the direct brain stimulation, concussion-reducing technology may one day be used to reduce injuries resulting from both contact sports and military service (11). Note that veterans who experienced a TBI have also been found to exhibit evidence of chronic traumatic encephalopathy (CTE), a unique pathological condition also found in athletes.

Perhaps the most important finding of the Smith et al. study is that the level of military explosives used in the *in vitro* brain tissue model produces a unique type of synaptic pathology. The blast-induced changes to two vital proteins of hippocampal synapses – GluR1 and synaptophysin – have a distinct vulnerability, occurring well before overt damage becomes apparent in the brain slices. Interestingly, these specific synaptic alterations have been linked to depressive behavior, aggression, anxiety, and cognitive stress in different types of experimental models (12-15) and have served as the basis of potential therapeutic avenues (see refs. 16-18). The selective pathogenic changes targeting synapses may explain the altered behavior evident in some blast-induced TBI sufferers that have no detectable neuropathology, and it may shed light on how exposure to military blasts may influence the risk to dementia. Further research is imperative in order to understand the effects of repetitive exposure to military-related blasts as well as the long-term effects of mild TBI, the signature injury in many that have served in recent wars.

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