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PERSONNEL AND
READINESS

JUN 25 2015

The Honorable Thad Cochran
Chairman
Subcommittee on Defense
Committee on Appropriations
United States Senate
Washington, DC 20510

Dear Mr. Chairman:

The enclosed report is in response to Senate Report 113-211, page 255, to accompany H.R. 4870, the Department of Defense Appropriations Bill, 2015, which requests the Assistant Secretary of Defense for Health Affairs to provide a report outlining strategies for overcoming roadblocks to post-mortem brain donation in the military, including consent issues that are preventing access to needed resources.

Traumatic Brain Injury has been called the "signature injury" of the recent conflicts in Iraq and Afghanistan. In order to ideally prevent, or diagnose and treat, this potentially devastating injury, systematic and critical research of the human brain is necessary, requiring brain specimens from U.S. Service members. The Brain Tissue Repository within the Center for Neuroscience and Regenerative Medicine at the Uniformed Services University of the Health Sciences was established to conduct research addressing this very issue. Despite the significant complexity and sensitivity of obtaining postmortem specimens, considerable progress is now being made to obtain specimens with proposed collaborations with national Organ Procurement Organizations and the National Disease Research Interchange.

Thank you for your interest in the health and well-being of our Service members, veterans, and their families.

Sincerely,

Brad Carson
Acting

Enclosure:
As stated

cc:
The Honorable Richard J. Durbin
Vice Chairman

Department of Defense Report to Congressional Defense Committees
Senate Report 113-211, page 255, Accompanying H.R. 4870
Department of Defense Appropriations Bill, 2015

Overcoming the Challenges of Obtaining Postmortem Brain Specimens from U.S. Service Members

The estimated cost of this report for the Department of Defense is approximately $ 8,900 for the 2015 Fiscal Year. This includes $0 expenses and $ 8,900 labor.

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Executive Summary

Since the September 11, 2001, attacks on the United States, approximately 2.5 million U.S. Service members have deployed overseas to defend our Nation. The weapon of choice used by enemy forces has been high explosives, especially improvised explosive devices (IED). Traumatic brain injury (TBI) from blast exposure has been called a "signature injury" of the conflicts in Iraq and Afghanistan. Blast causes damage through a variety of mechanisms; when the blast wave reaches the victim, its pressure and energy can be transmitted through the skull to the brain. Milliseconds later, the blast wind, can propel bomb fragments, other objects and debris at high speed, or hurl a Service member's body against another object. The complex interplay of factors is quite different from the impact and deceleration head injuries commonly seen in the civilian world. Currently, we know little about the effect of blast waves as they pass through the human brain. Furthermore, even less is known about the effect of the blast wave in rapid succession with injuries from the blast wind.

Another challenge in managing blast-related TBIs is the phenomenon of repetitive mild traumatic brain injuries, also referred to as "concussions." During prolonged and repeated deployments, Service members face a higher risk of exposure to multiple blast-related episodes. We know from observational studies that such events increase the risk of progression to chronic symptoms and complicate our understanding of the underlying pathology. To better care for Service members who still face these weapons, future Service members who serve, and veterans who are struggling with persistent behavioral, psychiatric, and/or neurologic sequelae from past exposure to IED blasts, researchers need to compare exposure histories and clinical findings with detailed examinations of brain tissue donated after death. There is no other practical way to get answers to these questions.

Although science has made remarkable progress with various approaches to brain imaging, microscopic examination of brain tissue is still the only way to detect and define the acute and long-term pathology of TBIs caused by blast exposure. The pathologic findings can then be used to validate and improve neuroimaging approaches for future clinical use. Furthermore, access to specially prepared postmortem brain tissues is required for additional techniques, such as the extremely powerful tools of modern molecular neurobiology that can be used to provide further valuable information on the nature of disease processes present in the human brain after blast exposure. Importantly, studies must characterize the initial injuries through the long-term effects to examine a range of neurodegenerative and psychological health concerns.

To address these needs, the Department of Defense (DoD) has established a specialized brain tissue repository (BTR) to determine the pathologic consequences of TBI in the context of the military experience. This BTR is a resource of the Uniformed Services University of the Health Sciences (USUHS) within the Center for Neuroscience and Regenerative Medicine (CNRM) (hereafter referred to as the USUHS-CNRM BTR). The USUHS-CNRM BTR is well positioned to support this research effort based on the stable infrastructure combined with the clinical and research expertise associated with USUHS and collaborating federal partnerships with the Joint Pathology Center (JPC), Walter Reed National Military Medical Center (WRNMMC), and the National Institutes of Health (NIH).

Many challenges have been successfully addressed as the USUHS-CNRM BTR has worked through regulatory, organizational, and logistical issues related to respectfully collecting the necessary brain donations and handling them in an appropriate manner. One of the most important challenges for the USUHS-CNRM BTR has been how to properly collect brain donations in a

manner that is ethical and respectful of those who have died and sensitive to the loss experienced by the loved ones of the deceased. The efforts to understand TBI in military context can be further supported by refining and optimizing policies that help those interested in brain donation to advance our understanding of blast-related brain injury connect with the USUHS-CNRM BTR. Additional efforts to support brain donations from consenting Service members or their representatives have included working with the Armed Forces Medical Examiner System (AFMES), JPC, and Organ Procurement Organizations (OPOs). Efforts are now being focused on the JPC and OPOs as primary resources, along with outreach efforts to make the community aware of the opportunity to donate to the USUHS-CNRM BTR to advance research in military TBI.

OPOs represent a preferred pathway for broadly reaching out to those interested in brain donation, a pathway in which the USUHS-CNRM BTR has focused since 2012. Several OPOs are interested in assisting the USUHS-CNRM BTR in collecting brain specimens from deceased Service members to support military TBI research. The OPOs have the infrastructure and experience needed to obtain consent for donation from the legally authorized representative (LAR) and collect brain specimens. In 2013, the Office of the Assistant Secretary of Defense for Health Affairs (OASD(HA)) wrote to the directors of all 58 OPOs in the United States asking if they would be interested in partnering with the USUHS-CNRM BTR to assist in identifying deceased active duty and former Service members who would be willing to consent to brain donation to support military TBI research. This letter stimulated five positive responses from OPOs expressing an interest in working on this project. To initiate this approach, the USUHS-CNRM BTR is currently completing arrangements to partner with the OPO Virginia Lifenet to obtain such specimens. Based on Lifenet's past experience with obtaining donations of other kinds of tissue for research and their frequent interactions with military families, they predict that they can provide at least 2 brain specimen donations per month within a feasible cost structure. We expect to complete the Memorandum of Understanding (MOU) and receive Institutional Review Board (IRB) approval for this initiative by June 2015.

In addition, discussions are underway with the National Disease Research Interchange (NDRI) about working with OPOs to obtain additional brain donations. NDRI is a non-profit organization that has been in operation for over 30 years and serves to collect a wide variety of tissue specimens for use in biomedical research. They collect these specimens from hospitals and pathologists working closely with the OPO network. The NDRI, through their extensive outreach operations, has agreed to partner with the USUHS-CNRM BTR to obtain brain specimens. This organization has extensive experience in obtaining properly consented specimens, including brains, for use in research. Based on funding recently obtained through the OASD(HA), an MOU is close to completion with NDRI to expect approximately 20 brains per year. Importantly, NDRI has the infrastructure to collect frozen brain samples with relatively short postmortem intervals, which are both important considerations to maximize the utility of the tissues for specific research techniques.

In summary, the proper collection, storage and examination of appropriate brain specimens will provide critical information to guide diagnostic, preventive and therapeutic measures related to the problem of the acute and long-term effects of military TBI, particularly related to blast exposure. Although it has been challenging to navigate the ethical and respectful ways in which those specimens can be properly collected, a number of means have been outlined by which those challenges can be addressed. The availability of the necessary brain specimens and expertise at the USUHS-CNRM BTR can enable advances in research on this critically important problem for the sake of current, former and future Service members, the DoD, and our Nation.

Overcoming the Challenges of Obtaining Postmortem Brain Specimens from US Service Members

The Nature of TBI in the Military

Since September 11, 2001, following the attacks on the United States, approximately 2.5 million U.S. Service members have deployed in defense of our Nation. Following the early months of Operations ENDURING FREEDOM (OEF) and IRAQI FREEDOM (OIF), the weapon of choice used by our adversaries has been high explosives, especially IEDs. Simple and inexpensive to construct and easily placed in buildings, vehicles, on roadways and even worn as suicide vests, these devices enabled otherwise outmatched adversaries to inflict substantial casualties among allied forces. At the height of the war, U.S. troops were attacked almost daily with high explosives, which accounted for over 60 percent of combat casualties (1-4). High explosive blast exposure was the leading cause of death among combatants in both OIF and OEF, and remained a high risk during Operation NEW DAWN. Because of modern military armor and dramatic advances in combat casualty care, many troops survived injuries that would have been fatal in previous conflicts. The challenge now is to provide appropriate treatment for those who survived, many of whom have TBIs related to these blast exposures.

Because TBI following blast exposure is so common, it has rightfully been called a "signature injury" of the recent conflicts. Most of the time, Service members have a mild form of TBI, often a concussion or "mild TBI" (mTBI). Unlike external wounds or broken bones, which we can easily diagnose with medical examination and X-ray, it is more difficult to confidently diagnose mTBI. Currently there are no validated blood tests or neuroimaging methods, such as computed tomography or magnetic resonance imaging (MRI), to non-invasively diagnose mTBI. Functional tests are commonly used, but they are imprecise as well. To make matters even more challenging, no effective treatment is available, other than the recommendation to rest and avoid activity that would risk another mild TBI. Fortunately, the majority of those who experience an mTBI recover fully (5). However, some Service members develop persistent and disabling symptoms, such as persistent headaches, sleep disorders, trouble concentrating and difficulty in remembering even simple things (4,6). For an unfortunate few, the symptoms are far worse and may include disturbing behavioral and psychiatric problems including abrupt mood swings, feelings of depression and despair, outbursts of anger, inability to concentrate, impulsive behaviors, and substance abuse – symptoms that clearly overlap with those of posttraumatic stress disorder (PTSD). In extreme cases, suicide may follow (7,8). Therefore, a diagnosis of a TBI as "mild" refers to the initial evaluation shortly after the injury, but a subset of TBIs, particularly when the individual sustains multiple injuries of this sort, can result in chronic symptoms that have a significant impact on quality of life. Determining the factors that increase likelihood of progression to chronic symptoms is one of the important challenges facing TBI researchers.

Blast-related TBIs are the result of a mix of different forces. At the moment of detonation, a high-pressure blast wave is formed that moves outward faster than the speed of sound and strikes any nearby personnel before they can react to take protective actions. When the blast wave reaches the victim, its pressure and energy can be transmitted through even a well-designed helmet to reach the skull and the brain (10,11). The precise effects of this blast wave, as it passes through the human brain, remains largely unknown. It is likely, however, that the damage it causes to vital brain structures most likely underlies some of the persistent neurologic/behavioral symptoms that are subsequently observed. Milliseconds after the blast wave comes the blast wind, which can forcefully propel bomb fragments, objects and debris or physically hurl a Service member's body

against the interior of the vehicle or another object (2,12,13). We understand more of what happens to the brain from penetrating and blunt impacts because of numerous pathology studies in civilian victims of assault and victims of impact-deceleration head injuries, such as motorcycle and car crashes. However, currently we know little about the effect of blast waves, as they pass through the human brain. Furthermore, even less is known about the effect of the blast wave in rapid succession with penetrating or blunt impact injuries.

Blast modeling, experimental animal model studies, preclinical testing, and symptom reporting from Service members, each suggest that high explosives can produce significant disruption in the brain. This damage may underlie many of the persistent symptoms noted in exposed Service members. Several recent studies have shown that there is a dramatically increased likelihood of Service members developing PTSD symptoms after being exposed to blast (9,14-16). Moving beyond reasonable speculation to actual knowledge of how symptoms associated with blast exposure relate to damage of the brain by the blast wave requires examination of brain specimens from deceased Service members.

In summary, although neuroscientists have learned a great deal about blast-related injuries in animal models, we still do not completely understand what happens in the brain when someone has mTBI related to single or repetitive blast exposure, either immediately or over the long term. This critical gap in knowledge needs to be addressed for present Service members who still face these weapons, for veterans who are struggling to reintegrate into the community with persistent behavioral/psychiatric/neurologic sequelae following high explosive exposure, and for future military personnel who will serve our nation. The study of appropriate brain specimens will provide critical information to guide diagnostic, preventive and therapeutic measures.

The Need for Neuropathological Examination of Brain Tissues to Understand the Effects of Blast Exposure

Over the past 40 years, there has been a severe downward trend in the number of autopsies conducted in medical facilities. Although in the 1960's it was common for major civilian academic medical centers to perform autopsies on over 60 percent of deaths, today fewer than 5 percent of deaths undergo postmortem examination. One cause of this trend has been a mistaken impression, at least for diseases of the brain, that modern neuroimaging techniques provide information that is comparable to what might be learned from an autopsy. Unfortunately, this is not true in most types of brain disease, especially mTBI. MRI provides the most precise imaging capabilities that are available for study of the brain in a living person. Current state-of-the-art MRI instruments are able to detect structures that are approximately 0.5 to 1 mm in diameter (that is, about the size this circle - o -). However, because of inherent limitations of the technology, an MRI cannot provide information on the cellular level. Cells are typically 500 to 1000 times smaller than what the best MRI can detect. In contrast, the pathologist using a standard light microscope can see individual cells and even subcellular features. Furthermore, in the current practice of brain pathology (neuropathology), brain specimens can be prepared to identify pathologic changes present in individual cells, characterize the abnormalities present within those cells and define their nature, extent and distribution of their involvement. With this in mind, one cannot rely on findings obtained from neuroimaging approaches to determine the presence of many brain diseases or use this approach to characterize the nature of a poorly defined condition of the brain. Brain tissue specimens must be studied under the microscope to detect and define the pathology of TBI caused by blast exposure. The pathology can then be used to validate neuroimaging approaches that detect the relevant changes when present in a sufficient number of cells to non-invasively identify that pathology in patients. Access to specially prepared postmortem brain tissues is required to apply advanced techniques of modern molecular neurobiology. These approaches can then be used to

provide further valuable information on the nature of disease processes present in the human brain after blast exposure.

The current challenge with mTBI in the military is that few pathology studies have been performed to examine human brain tissues under the microscope for the purpose of identifying damage to brain tissues and correlating it with symptoms. These methods of pathology are trusted tools in medicine. For example, in 1907 Dr. Alois Alzheimer, a psychiatrist and pathologist working in Germany, was taking care of a patient who suffered from rapidly progressing dementia and other disturbing behavioral symptoms. After the patient died, Dr. Alzheimer examined the patient's brain under the microscope. Based on his studies, the basic brain lesions which characterize the disease that bears his name – Alzheimer's disease – were first identified (17). This case was the first time that a disease characterized clinically by diverse psychiatric symptoms had been defined based on cellular abnormalities in the brain that could only be seen under the microscope. Even today, the definitive diagnosis of Alzheimer's disease is made at autopsy based on a person's symptoms and a microscopic examination of the brain. These pathological findings continue to lead efforts to better diagnose the disease, for example, through targeting neuroimaging of specific molecular and cellular abnormalities, and inform strategies to develop treatments. The same classic approach is required to evaluate the effects of mTBI, with a focus on exposure to blast in warfare. Neuropathological studies are now far more sophisticated than in the past. Use of these advanced techniques can provide important insights about pathology and the progression of blast-related TBIs.

Currently, there are no non-invasive techniques that can be used to reveal the damage in the brain that is associated with a clinical diagnosis of mTBI. Therefore, while recommending a neuropathological approach to study mTBI in the military, postmortem studies should also take full advantage of neuroimaging technologies with the potential to detect relevant pathological features in brain specimens as the first step toward translating findings into effective approaches for early detection in patients. Because medical imaging alone is not adequate to detect the changes associated with mTBI, new cutting-edge imaging methods are being developed. Neuroimaging scientists need information about what to look for, that is, information about the underlying pathology, to succeed in designing improved techniques to detect the appropriate changes. Indeed, development of new imaging technology will both be informed by pathology and will subsequently depend on the support of pathology to validate the results. More importantly, neuroimaging scientists need information about what to look for, that is, information about the underlying pathology, to succeed in designing improved techniques to detect the appropriate pathology. The same can be said for development of other diagnostic tools for mTBI, such as biochemical markers in the blood or other fluids or tissues.

Innovative and progressive ideas for evaluation and treatment of TBI are dependent on a functioning brain bank (BTR) in the DoD. The only way to examine human brain tissues under the microscope is to take tissue samples from the brain of a Service member or veteran who has recently died. After death the brain tends to deteriorate more rapidly than other organs and consequently the brain specimen needs to either be frozen or put into preservative fluids (a process referred to as fixation) relatively quickly. After fixation, the whole brain can then be examined and samples can be taken for further tissue analysis. One of the most important challenges has been properly collecting brain donations in a manner that is ethical and respectful of those who have died and sensitive to the loss experienced by the loved ones of the deceased. Furthermore, the BTR has to have the appropriate collection of brains to support identification of generalizable findings, which must take into consideration the heterogeneous pathology of impact TBI along with the additional concerns about blast and stress among deployed Service members.

Accomplishments of Specialized Brain Banks

Beginning in the 1980's, brain banks were developed to support modern neurobiologic research on a variety of nervous system diseases. These brain banks have mostly specialized in the collection of brain specimens related to the study of specific neurologic disorders, such as Alzheimer's disease, amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease), schizophrenia, Parkinson's disease, etc. There are many reasons for such specializations, including funding sources, availability of specific cohorts of clinically-followed patients suffering from such conditions, local clinical and research interest and expertise, and requirements for specialized dissection approaches to study specific aspects of the particular disorder. Another important issue is that for each disease entity to be studied, the brain bank must identify and collect specimens without that disorder that can be compared to the diseased brains. The most useful controls must be matched for specific characteristics relevant to the diseased population. For example, controls may need to be matched for age or a life experience such as pharmacologic treatment, participation in contact sports, or military deployment. In essence, the process of research inevitably involves a comparison of observations, whether they be morphologic, molecular, biochemical, etc., made between what is present in diseased brains and appropriate controls. By using this approach, there has been a rapid expansion of knowledge about each of the diseases under study that has led to a greater understanding of their nature and the development of new modalities for diagnosis and treatment.

Congruent with these research efforts, brain specimen collections have expanded and, to some extent, can now be used for further analysis by sharing cases to increase the number of specimens in an analysis, access control cases, and facilitate comparisons between diseases. As a means to enhance coordination across a set of six brain banks, the NIH has recently developed the NeuroBioBank. This online resource (https://neurobiobank.nih.gov/) supports requests for specific brain regions from investigators with appropriate regulatory approvals. The NeuroBioBank also helps facilitate requests from those interested in brain donation. The NeuroBioBank is an important step to help optimize access and utilization of brain tissues for research, especially by investigators who otherwise would not have access to human specimens. However, this resource complements the specialized brain banks, which typically require much more extensive clinical detail such as symptoms and medical history and often require examination of the whole brain or specialized cutting of the specimen to expose brain regions in a specific orientation or combination that connects to other regions. Importantly, the specialized brain bank approach is best to lead research when pathological analysis must be coordinated with clinical diagnosis to characterize unknown disease entities, as related above for identification of the pathology in Alzheimer's disease and as is likewise needed for military TBI.

A recent example of how the availability of a specialized brain bank can provide important scientific breakthroughs for human disease is the history of the identification of chronic traumatic encephalopathy (CTE). CTE is a disorder associated with athletes who receive repeated head trauma through their participation in contact sports. CTE was first described as a clinical entity in retired boxers by Martland in 1928 (18). It was not until 1973 that the disease was defined on the cellular level based on the neuropathologic descriptions of Corsellis and colleagues (19). At the time, CTE was thought to be an extremely rare and poorly understood condition that was seen almost exclusively among former boxers. Following a single case report of CTE in a former National Football League player (20), a group at the Boston University School of Medicine developed a brain bank to collect and study the brains of former athletes who engaged in a wide variety of contact sports (e.g., football, rugby, soccer, ice hockey, etc.). They quickly found that a very high percentage of the brain specimens they received from deceased contact sport athletes showed microscopic evidence of the same disease that had been described in former boxers

(namely, CTE) (21,22). Affected individuals may show prominent neurologic and behavioral symptoms, including mood disturbance, headaches, inability to concentrate, and memory loss that can lead to profound dementia.

The pathology of CTE involves the widespread accumulation of a protein called *tau* in two major cell types, neurons and astrocytes, according to specific patterns in the brain (21,22). It is important to recognize that the *tau* deposits that characterize this disorder are not visible by standard non-invasive neuroimaging techniques. Currently, the only way to diagnose the disorder is through examining the brain tissue under the microscope at autopsy. Indeed, cases of CTE with profound symptomatology and extensive microscopic abnormalities can look entirely normal using state-of-the-art neuroimaging techniques (22). Through the development of this brain bank specialized for concussion in contact sports, the concept of what CTE is and who might suffer from it has completely changed. This has led to widespread discussion in the media, among the sports world and among a concerned lay public. There are many important unanswered questions about CTE. Answering those questions has been difficult, mostly related to the lack of appropriate control brains, i.e. brains from athletes with and without a history of concussions but free of symptoms, against which the affected athlete cases can be compared.

The Complexity of Neuropathology Studies of Military TBI

A similar course of biomedical discovery can be achieved through research focused on military TBI, particularly following blast exposures. However, it should be recognized that the situation for TBI among Service members is particularly complex. Service members who experience blast TBI frequently also suffer from blunt impact TBI related to the effects of the blast wind. In addition, with prolonged and repeated deployments, Service members face a higher risk of repeated blast-related episodes (4). The combined effects of such serial injuries remain unclear. Furthermore, frequently Service members have also suffered multiple non-combat related TBIs related to falls, vehicular or training accidents, participation in sports associated with repeated head trauma, e.g. football, boxing or mixed martial arts. The combined effects of these experiences remain unclear. It is not clear how prior TBI episodes contribute to long-term effects among military Service members exposed to additional physical and emotional trauma, or the prolonged stresses of deployment. To address the questions most relevant to understanding TBI among military populations, an appropriate brain bank would need donations from Service members who have experienced TBI, including blast and repetitive head injuries. However, for comparison purposes, the brain bank would also need donations from military personnel who served in deployed settings but did not experience a blast TBI.

The complexity of these issues can be readily seen among the few available studies in which evidence of brain pathology was sought in brain specimens obtained from blast-exposed veteran populations. Findings of CTE were reported in five cases among three recent studies (8,21,23). A separate study of six veterans failed to show evidence of CTE, and instead described prominent pathology of blood vessels and neuronal pathways (axons) (24). Among the cases with CTE findings, three of these five cases had known histories of non-blast mTBIs from activities common in military and civilian life, such as motor vehicle accidents and contact sport participation (7,19,21). McKee *et al.* also published a study of 68 cases with CTE, including 21 veterans; however, with the exception of the three veterans included in the above cited studies, blast history was not reported in the other 18 cases, and most of the 21 veterans had also been contact sport athletes (21). In contrast, a study led by investigators at Johns Hopkins University failed to show abnormal *tau* immunostaining in the brains of six veterans (24). This more recent study not only failed to confirm the presence of CTE but emphasized the importance of damage found in the white matter of the brain. However, these six cases were also noted to suffer from significant drug abuse.

It is clear from the conflicting results that the acute and long-term effects of blast on the structure and function of the brain are complex and remain largely unknown. Additional detailed studies of many more military cases, particularly those with more complete histories and more extensive pathologic study, will be needed to sort out these complex issues. Importantly, identifying and collecting proper control brains from which to compare will be critical for valid comparisons to be made.

Filling the Need for Research Specialized for Military TBI - Development of the USUHS-CNRM BTR

The DoD has established a BTR dedicated to the study of TBI experienced by military Service members. This BTR is a resource of the USUHS-CNRM BTR.

In 2008, the CNRM was established. The CNRM has rapidly grown into a strong and productive research program composed of core resources and investigator-initiated studies that address the current needs of the medical community to better diagnose and develop strategies to intervene in the prevention or treatment of the long-term consequences resulting from TBI, especially TBI due to blast exposure. The CNRM involves over a hundred federal intramural investigators in the National Capital Area from within the DoD and the NIH. The CNRM truly acts as a research "center" which integrates the expertise of clinicians and scientists across numerous disciplines to catalyze innovative approaches to TBI research. The CNRM research programs have an emphasis on aspects of high relevance to the military populations, particularly Service members cared for at the WRNMMC and those exposed to blast events. USUHS is responsible for the overall operational and fiscal management of the CNRM, on behalf of the DoD.

In the earliest days of the CNRM it became clear that there was little neuropathological information available on the effects, both immediate and long term, of TBI as a consequence of exposure to high explosives. Furthermore, as explained above, Service members commonly experienced persistent neurologic impairment as well as behavioral and psychiatric disturbances following episodes of blast-related TBI. This lack of understanding of the nature of these clinical manifestations represented a major stumbling block for clinical diagnosis, prevention and treatment of Service members.

The consequences of blast-related TBI remain an extremely important medical issue for the military and will continue to be so for the lifetimes of those who have already been exposed to high explosives and for those who will fight in future conflicts. The CNRM has been charged with addressing these critical issues. The following sections will address the establishment and function of the USUHS-CNRM BTR. To do so, the process of obtaining consent for brain donation for research must first be addressed. The limitations of initial efforts to collaborate for neuropathological studies of military TBI will be explained as relevant to the development of the USUHS-CNRM BTR to fill the critical need of performing research on brains donated from military Service members.

Consent is Required for Brain Donation for Research Purposes

To utilize a brain specimen for research in a brain tissue repository (brain bank), one must receive consent to do so either prospectively from the person who wishes to bequeath his/her brain to science after death (when permitted, as determined by the state statutes) or from an appropriate representative of the deceased, either the LAR or next-of-kin. This consent explains that the brain will be obtained postmortem and that the specimen will be stored and used to support research. Consent for this purpose does not involve the more detailed process that is referred to as "informed

consent," which addresses the balance between the possible risks vs. anticipated benefits to the person giving consent for a surgical procedure, to enter a clinical trial, etc. If a bequest is not made and recorded prior to death, next-of-kin consent for brain donation must be obtained relatively quickly since the brain specimen must be procured within 24-36 hours of death to be useful in most research procedures. Ideally, specimens should be collected less than 12 hours after death to take full advantage of most of the techniques used in modern research studies.

Clearly, obtaining consent for brain donation soon after the death of a loved one requires great sensitivity and tact. The death of a loved one is always tragic and a family's grief must be respected. To assure that this process is managed in a caring and professional manner, brain tissue repositories generally have trained individuals assigned to sensitively approach families and request consent. It requires special skill to deal compassionately with individuals undergoing the immediate trauma of just having learned of the death of a family member. Nevertheless, experience shows that when interactions with the family members are properly handled, donations of this type are often welcomed by the family. Knowing that a timely donation after death may produce scientific advances that will help other warfighters and their loved ones brings many families a sense of comfort and closure in their time of loss.

Development of the USUHS-CNRM BTR

To enable the necessary research capabilities, the CNRM established a state-of-the-art research facility for human neuropathology research, including the development of the USUHS-CNRM BTR dedicated to the study of military TBI. This led to the recruitment of Dr. Daniel Perl to the faculty of the USUHS in September of 2010 to lead the CNRM supported Neuropathology Core. Dr. Perl is a widely-recognized expert on brain banking. Over the course of his career, he has consulted with the NIH, numerous universities and has lectured throughout the world on aspects of running an effective brain bank repository and maximizing the utility of the human tissues collected for a wide range of modern neurobiologic research studies. Furthermore, Dr. Perl had been one of the few neuropathologists in the world who had previously conducted research on CTE and was an early consultant to the Boston University group who identified and characterized this disease among former athletes.

The CNRM Neuropathology Core provides infrastructure, equipment, and expertise as a stable foundation for the USUHS-CNRM BTR. The infrastructure includes leased space, program management, and a faculty steering committee to oversee policies and approve requests for access to tissue specimens. Core equipment is available for an optimized work flow of high quality tissue sectioning and staining, as needed to characterize each brain donation. The U.S. Army Medical Research and Materiel Command provided a multi-year grant to advance the research capabilities beyond the level of CNRM support of the Core infrastructure. Additional equipment and supplies are also obtained through specific funding as needed to advance the capabilities of the USUHS-CNRM BTR to address specific research needs. The Core functions and budget are reviewed annually by CNRM leadership and the Programmatic Oversight Committee of the CNRM.

The USUHS-CNRM BTR is designed as a specialized brain bank focused on military TBI, especially blast. This focus enables the research to take advantage of the collection of appropriate cases that are relevant to the injuries and stresses experienced by Service members. Importantly, this emphasis extends to the collection and analysis of control cases that will be matched to the TBI and blast cases, including deployment status. Clinical data, symptom progression, and patient histories will be collected in association with specimens to the fullest extent possible since this information is extremely useful for interpreting the research findings and making appropriate comparisons with the control cases. This in-depth characterization of each case in a collection of

military TBI cases is critical for sorting out significant pathological features associated with blast, repetitive concussion, and stress in relationship to the short and long term progression of symptoms. The CNRM Neuropathology Core capability facilitates characterization of the cases into appropriate cohorts. The Core has a steering committee that works through an approved process to enable sharing of tissues with qualified researchers.

The USUHS-CNRM BTR has the additional advantages of the larger CNRM research program. These interactions greatly facilitate insights from the neuroimaging studies ongoing at NIH, WRNMMC, and USUHS that involve a diverse population of TBI patients as well as animal models of TBI and blast exposure. Similarly, CNRM investigators have ongoing work in biochemical markers of TBI, PTSD, and blast that provides additional insights and supports potential translational of diagnostic tools. Importantly, the CNRM includes ongoing studies with neurological and psychiatric components that bring expertise to the interpretation of the known symptoms among the cases of brain specimens in comparison with a larger cohort of military and civilian patients.

CNRM Interactions with the AFMES

The AFMES may conduct a forensic pathology investigation of active duty Service members, to include an autopsy, under the circumstances found at 10 USC 1471. Soon after coming to USUHS, Dr. Perl was asked to serve as a consultant in neuropathology at the AFMES. Among the cases Dr. Perl saw in consultation were a small number of brains derived from Service members who had experienced TBI. However, because these were AFMES autopsied cases and had not received appropriate consent from the LAR, they could not be used for research.

The primary mission of the AFMES is to determine the "cause and manner" of death of the cases they examine. Accordingly, their activities do not include participation in research. AFMES pathologists have limited contact with the LAR of their cases, posing a challenge with obtaining consent for donation of the brain for use in research. Thus, few brain donations have resulted among the AFMES cases coming to autopsy.

Toward the end of 2013, with considerably less battlefield conflict, the number of autopsies performed by the AFMES had decreased and examples of cases showing the acute effects of IED exposure became quite rare. On the other hand, those Service members suffering with the long-term effects of blast exposure are numerous. Furthermore, many active duty and, in particular, former Service members suffering from the long-term effects do not come to autopsy by the AFMES in Dover. Service members that die long after active duty are often examined by local medical examiners or coroners.

Requirements Specific to Military Service Members for Obtaining Consent for Donation of Brain Specimens for Research

If brain donation is not facilitated by another organization on behalf of the USUHS-CNRM BTR, the consent issue is further complicated for brains from deceased Service members. The USUHS IRB communicated that the USUHS-CNRM BTR staff should not directly approach the LAR of deceased Service members to request donation of the brain to the USUHS-CNRM BTR. The IRB expressed concern that there might be either an actual or perceived conflict of interest having the researchers directly approach potential LAR donors in order to obtain necessary specimens for the research. The families of deceased Service members rarely know of the existence of the opportunity to participate in the donation program. Furthermore, the family would have to know how to reach the USUHS-CNRM BTR in a timely manner if the donation were to be

successfully completed. The only permissible ways to provide information on brain donation are either through a party separate from the BTR personnel or USUHS-CNRM-BTR outreach efforts through the approved website and materials distributed through community activities.

If consent and donation of the brain for research occurs, the studies that will be done with the tissues also have very specific requirements. For the USUHS-CNRM BTR studies, an approved human subjects research protocol process is used in order for the USUHS IRB to serve in the role of a Privacy Board to ensure health information is collected and maintained appropriately from the point of brain donation through use of tissues for de-identified research purposes. It should be noted that recently the USUHS IRB has been able to provide approval of USUHS-CNRM BTR protocols on an expedited basis.

USUHS-CNRM BTR Pathways to Obtain Brain Donations from Military Service Members

The USUHS-CNRM BTR has pursued several pathways to enable brain donations, while following the approved guidelines for interactions of the BTR personnel. Avenues considered in the discussion of potential pathways include community outreach, Service Casualty Office interactions, CNRM support at AFMES, and utilizing OPOs. Each avenue will be discussed to communicate both the options that have and have not been explored, and the relevant experiences in the decisions regarding each.

a. *Outreach efforts*

CNRM has a variety of materials approved by the USUHS IRB for distribution within the community along with information that is available on a website (http://www.researchbraininjury.org/) designed for public access and education about brain donation procedures. Links from the widely used DVBIC website (http://dvbic.dcoe.mil/audience/service-members-veterans) have also been helpful.

b. *Consideration of interactions with the Service Casualty Office*

The potential for members of the various Service Casualty Corps to mention brain donation as part of their check-list of items discussed with the LAR following notification of the death of a Service member was discussed with the leadership of the Casualty Corps. The Casualty Corps leadership was concerned that the casualty officer would not be knowledgeable of the details of the BTR function, would be unable to answer any questions the family might have about it, and would be unfamiliar with the consent process. Since there are numerous casualty officers doing this job, it would be very difficult to adequately train them on how to handle these critical details. Participation of the Casualty Officers would have to be approved by the Casualty Assistance Board and likely each of the individual branches of the Armed Services. Based on all these concerns, this option was not pursued.

c. *Considerations of placing support personnel at AFMES*

Representatives of the AFMES emphasize that their primary mission is to determine the "cause and manner of death" for the cases they examine and that their duties do not inherently include a research mission. AFMES representatives indicated that as long as their primary mission could be effectively and efficiently carried out, they were not opposed to properly consented research being conducted by others, including the USUHS-CNRM BTR. The AFMES representatives stated that AFMES personnel could not be directly involved in the consent process. Concern was also expressed that obtaining donated specimens might interfere with AFMES personnel carrying out their duties, especially given the logistics of the relatively short window of time in which cases were in the Dover facility for their forensic examinations. One possibility raised was embedding USUHS-CNRM BTR personnel at AFMES to interact

with the LAR. The AFMES personnel would have to determine whether there was an interest in discussing brain donation based on prior indications by the deceased, or from interactions with family members or the LAR. A USUHS-CNRM BTR coordinator could then be immediately available to meet with anyone who might be interested in participating and explain the brain donation program. The AFMES representatives agreed that this might assist in obtaining consents. AFMES representatives also agreed to put the USUHS-CNRM BTR in contact with other medical examiners outside of the AFMES who also conduct autopsies on deceased Service members. However, in each case, similar concerns about timing and consent issues would need to be resolved.

On reflection, the concept of placing trained personnel at the Dover AFMES facility to be available to discuss potential brain donation was not considered feasible for the USUHS-CNRM BTR. With active battlefield conflicts winding down, there has been a considerable decrease in AFMES cases in which there is an opportunity to obtain a brain specimen from an acute death with blast-related head trauma. Realizing this, the USUHS-CNRM BTR leadership felt that hiring and maintaining an individual in Dover who would serve to coordinate brain collection would not be a viable strategy to support sufficient brain donations for the research needed. CNRM remains interested in exploring options for partnering with the AFMES since the acute injury cases are of high interest and may provide unique insights.

d. Utilizing Organ Procurement Organizations

OPOs represent a pathway for broadly reaching out to those interested in brain donation. Many Service members and veterans declare their interest in serving as an organ and tissue donor by indicating such on their driver's license. Each state maintains a list of names of potential organ and tissue donors that were obtained mostly from drivers' license inquiries and these names and identifiers are maintained on statewide databases. According to Donate Life America, as of July 12, 2013, a total of 117,108,378 Americans have registered as organ/tissue donors (a total of 48 percent of all individuals with over age 18 years) (25). In all 50 states and the District of Columbia, such databases are readily accessible and used by OPOs to identify potential organ and tissue donors. OPOs represent a nationwide network of 58 facilities that are responsible for two main functions: 1) increasing the number of registered donors, and 2) coordinating the donation process when actual donors become available. In order to facilitate this, the OPOs receive hourly updates of all deaths within their catchment area and use the organ/tissue donor lists when contacting next-of-kin asking for consent to donate.

Most of this activity is related to the Universal Anatomic Gift Act (UAGA), written under a federal program designed to stimulate the availability of organs and tissues for use in therapeutic transplantation. In this context, the term "organ" connotes a structure in the body that must be removed immediately at death for it to be used for transplantation (examples include heart, liver, kidney), whereas the term "tissue" signifies any part of the body that may be removed hours following the death of the donor yet still be useful therapeutically (examples include cornea, skin, bone, tendons, etc.). Under this distinction, the brain represents a tissue, although it is not used therapeutically. The means by which organs and tissues are obtained in different jurisdictions is declared on a state-by-state basis under their individual laws. However, the UAGA was proposed for wide adoption and each state, with minor variations, has used its language for adoption by their respective legislatures.

Importantly, for this discussion, the UAGA defined anatomic gifts as follows: "a donation of all or part of a human body to take effect after a donor's death for the purposes of transplantation, therapy, ***research*** or education." [emphasis added] Virtually every state includes donation for research in the basic language of their UAGA legislation. This statutory

language has led several OPOs to use their donor registry databases to also request consent for donation of tissue specimens for research purposes.

Of note, the DoD-issued Common Access Card (CAC) card that is carried by all Service members provides an additional opportunity for all DoD personnel to declare their desire to be an organ/tissue donor. A significant percentage of Service members have declared their willingness to serve as organ/tissue donors on their CAC card. However, it is somewhat unclear how, or if, this information is used. The CAC card donor declaration is not placed in the Record of Emergency Data database, nor is the information available to OPOs for placement in their potential donor databases. DoD is now reviewing potential new procedures to facilitate organ and tissue donations by military members. It is anticipated that as this program matures, the patient will have additional mechanisms of updating their donor election status.

The possibility of approaching the OPOs as a means of facilitating the brain donation process became a primary focus of the USUHS-CNRM BTR since 2012. This led to discussions of this concept among the USUHS-CNRM BTR, the office of the Deputy Assistant Secretary of Defense for Clinical and Program Policy (DASD(C&PP)) and the Clinical Support Division of the Defense Health Agency. Based on these discussions, and following a full review by DoD administrative and legal staff, on November 23, 2013, the DASD(C&PP) wrote to the directors of all 58 OPOs in the United States asking if they would be interested in partnering with the USUHS-CNRM BTR to assist in identifying deceased active duty and former Service members who would be willing to consent to brain donation to support military TBI research. This letter stimulated five positive responses from OPOs expressing an interest in working on this project.

The USUHS-BTR evaluated the logistics and costs of pursuing brain donations through the interested OPOs. Discussions were initiated with Lifenet Virginia, an OPO that expressed enthusiastic interest in working with the USUHS-CNRM BTR and was physically close enough to facilitate interactions. Importantly, this OPO was already actively involved in collecting other tissues for use in research. In addition, this OPO serves a location, particularly in the Portsmouth, Virginia area, in which a large number of active duty Service members and recently retired veterans reside. The USUHS-CNRM BTR is close to signing an MOU with Virginia Lifenet in which they will obtain consented brain donations through their donor network. Under this agreement, the OPO personnel upon contacting the next-of-kin will ask if the deceased had been a member of the Armed Forces. If the answer is yes, and if a few other conditions are met, they will then introduce the concept of the CNRM BTR and ask for consent to donate the brain donation for military TBI research. Based on Lifenet's past experience with obtaining donation of other kinds of tissue for research and their frequent dealings with military families, they predict that they can provide at least 2 brain specimen donations per month within a feasible cost structure. We expect to complete the MOU and receive IRB approval for this initiative by June 2015.

Continuing along similar lines, the USUHS-CNRM BTR has also had in-depth discussions with the NDRI about helping us work with OPOs to obtain additional brain donations. NDRI is a non-profit organization that has been in operation for over 30 years and serves to collect a wide variety of tissue specimens for use in biomedical research. They collect these specimens from hospitals and pathologists working closely with the OPO network. Over the years, the NDRI has served a number of federally-funded research projects including the GTEx study, a very large NIH-funded project investigating gene expression in donated surgery and autopsy-derived tissue specimens. The NDRI, through their extensive outreach operations, has agreed to partner with the USUHS-CNRM BTR to obtain brain specimens. This organization has

extensive experience in obtaining properly consented specimens, including brains, for use in research. Based on funding recently obtained through the office of the ASD(HA), an MOU is close to completion with NDRI to obtain at least 20 brains per year. Importantly, NDRI has the infrastructure to collect frozen brain samples with relatively short postmortem intervals, which are both important considerations to maximize the utility of the tissues for specific research techniques.

Moving Forward to Address the Needs in Military TBI Research

In summary, the USUHS-CNRM BTR is the specialized brain bank established by the DoD to determine the pathologic consequences of TBI in the context of the military experience, particularly with regard to exposure to high-explosive blasts. Addressing the neuropathology of blast TBI is a critical issue in the care of Service members. Blast TBI, along with repetitive head injury and stress, may include neurodegenerative and psychiatric effects that may progress with time after the initial injuries. The USUHS-CNRM BTR is well positioned to support this research effort based on the stable infrastructure combined with the clinical and research expertise associated with USUHS and collaborating federal partnerships with the JPC, WRNMMC, and NIH. Many challenges have been addressed as the USUHS-CNRM BTR has worked through regulatory, organizational, and logistical issues related to collecting the necessary brain donations in the appropriate manner. The efforts to understand TBI in military context could be further supported by refining and optimizing policies to help those interested in brain donation to advance our understanding of blast-related brain injury connect with the USUHS-CNRM BTR. The current plans utilizing outreach efforts and OPOs are expected to be successful for obtaining brain donations from Service members. Once specific findings are identified from integrated analysis of clinical data, symptoms, and pathology among a cohort of cases and appropriately matched controls, partnership with the JPC should support development of potential diagnostic indicators within a more generalizable population of Service members. The availability of the necessary brain specimens and expertise will enable the USUHS-CNRM BTR to advance research on this critically important problem for current, former and future Service members, the DoD, and our Nation.

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