Three Experimental Models for Evaluation of Three Different Mechanisms in Blast TBI

Mårten Risling, MD, PhD
Experimental Traumatology
Department of Neuroscience, Karolinska institutet
Retzius väg 8
SE-171 77 Stockholm
SWEDEN

Marten.Risling@ki.se

Ulf P. Arborelius, PhD
Experimental Traumatology
Department of Neuroscience,
Karolinska institutet
Retzius väg 8
SE-171 77 Stockholm
SWEDEN

Ulf.P.Arborelius@ki.se

Johan Davidsson, PhD
Injury Prevention Group,
Division of Vehicle Safety
Applied Mechanics
Chalmers University of Technology
SE-412 96 Göteborg
SWEDEN

johan.davidsson@chalmers.se

ABSTRACT

Traumatic Brain Injuries (TBI) induced by blast waves from detonations provides huge diagnostic problems. It may be assumed that several mechanisms contribute to the injury. Thus, the primary blast overpressure, acceleration movements, focal impacts as well as heating could contribute to the injury. In a number of experiments we have evaluated the injuries induced by these mechanisms. Our experimental models include a blast tube in which an anesthetized rat can be exposed to controlled detonations of PETN explosives that result in a pressure wave with a magnitude between 130 and 600 kPa. In this model, the animal is fixed with a metal net to avoid head acceleration forces. The pressure wave is of simple Friedländer type, with a duration of less than 0.5 ms. Animals that are exposed side on suffer from lethal bleedings from the lungs if the peak pressure exceeds 300 kPa. In recent experiments we have mounted animals in a rigid metallic body protection that covers all parts of the body except for the head, which rests on a bar that prevents from acceleration movements. With this protection the animals survive 600 kPa. The second model is a controlled penetration of a 2 mm thick needle, which is assumed to represent the focal impact of fragments. In the third model the animal is subjected to a high-speed sagittal rotation angular acceleration. This model is assumed to be relevant for rapid acceleration movements that can occur after explosions. Immunohistochemical labeling for amyloid precursor protein revealed signs of diffuse axonal injury (DAI) in the penetration and rotation models. Signs of punctuate inflammation were observed after focal and rotation injury. Exposure in the blast tube did not induce DAI or detectable cell death, but functional changes. Affymetrix Gene arrays showed changes in the expression in a large number of gene families including cell death, inflammation and neurotransmittors in the hippocampus after both acceleration and penetration injuries. Exposure to primary blast wave induced limited shifts in gene expression in the hippocampus. The most interesting findings were a down regulation of genes involved in neurogenesis and synaptic transmission. These experiments indicate that rotational acceleration may be a critical factor for DAI and other acute changes after blast TBI. The further exploration of the mechanisms of blast TBI will have to include a search for long-term effects. Detailed studies on the anxiety related pathways from the brainstem represent an important part these continued studies.

1.0 INTRODUCTION

1.1 The Different Types and Mechanisms for Traumatic Brain Injury (TBI)

TBI is a leading cause for death and disability in both civilian life and at the battlefield. The signature TBI has evidently changed from penetrating TBI to blast induced TBI in recent military conflicts. All types of TBI may occur in both the civilian and military setting. However, there are some important considerations for military TBI, mostly relating to the extreme energy transfer. TBI can be graded from mild to severe. TBI can be classified as diffuse or focal, and very often a complex mixture of both.

1.1.1 Mild TBI

A TBI is often classified as mild (concussion or commotio) if loss of consciousness or confusion is shorter than 30 minutes. MRI (magnetic resonance imaging) and CT (computer tomography) scans are usually normal but the patient may have headache, cognitive problems (memory problems, mood disturbance, attention deficits) and the effect on the patient can be devastating. The majority of blast induced TBI fall into this category, although the patophysiology is largely unknown. Cerebral concussion is often associated with other types of brain injury.

1.1.2 Moderate and Severe TBI

These injuries can be divided into closed head injuries and penetrating injuries. Closed head injuries may be both diffuse and focal [1]. The penetrating TBI will always induce a focal injury and often diffuse secondary injuries.

1.1.3 Diffuse TBI

The most common diffuse injury is the diffuse axonal injury (DAI), which is defined as the presence of diffuse damage to axons in the cerebral subcortical parasagittal white matter, corpus callosum, brain stem and cerebellum. This is usually the result of an acceleration/deceleration trauma. Different parts of the brain move at different speeds because of their relative density. If this is a rotational trauma, the positions of the axis of rotation will be an important factor in the injury mechanism and areas at a greater distance from this axis will sustain larger forces. This can lead to shearing injury and DAI. Beta-amyloid precursor protein (APP) has been proven to be an excellent marker for axonal injury in histology. Modern imaging techniques, such as MRI with DTI (diffusion tensor imaging), have provided improved possibilities to detect DAI.

1.1.4 Secondary Traumatic Brain Damage

Secondary traumatic brain damage occurs as a complication of the different types of TBI and includes ischemic and hypoxic damage, swelling, raised intracranial pressure and infection. The secondary TBI is potentially reversible with adequate treatment. Axonal damage in both DAI and focal injuries interferes with axoplasmic transport. Severe traumatic injury results in primary axonal disruption or transection termed primary axotomy or sets in train a series of ill understood events culminating in secondary axonal degeneration or secondary axotomy. Thus, adequate treatment could probably limit the axonal damage.

1.2 TBI in a Military Setting

The frequent use of Improvised Explosive Devices (IED) in contemporary asymmetric warfare has changed the scene and spectrum of TBI at the battlefield. At the same time, new equipment for body protection has increased the survival rate after TBI at the battlefield. It may be assumed that several mechanisms contribute to the injury. The US government has initiated the largest coordinated research

programs ever in neurotrauma. The specific focus is to get insight in mechanisms and to provide better treatment for blast induced traumatic brain injuries. The background should probably be well known for anyone working in the field of traumatic brain injuries (TBI). The asymmetric warfare in the recent military conflicts in Iraq and Afghanistan has resulted in numerous attacks with Improvised Explosive Devices (IED) and large losses in terms of deaths and wounded soldiers. TBI has been identified as major health problem in military personnel returning from service. The injuries range from severe multitrauma to a number of mild TBI that still has to be settled. Propagation of blast waves is very complex. It could involve both direct propagation through the skull and indirect propagation via blood vessels and it is obvious that a systemic response comes along with the blast TBI [2]. It is difficult to identify a reliable borderline between mild blast TBI and posttraumatic stress syndrome (PTSD). Many of the symptoms are similar and many patients might suffer from both TBI and PTSD [3, 4].

The energy transfer during an explosion is extremely complex and it is difficult to predict how energy is propagated into the body and absorbed. Exposure data from clinical situations at the battlefield are usually lacking. One way to generate a better understanding of the mechanisms of TBI after a blast exposure is to perform controlled experiments in animals . In this paper we will describe 3 models (Fig. 1) that are intended to simulate specific mechanisms in TBI and how they will be employed for further studies on basic mechanisms in blast induced neurotrauma.

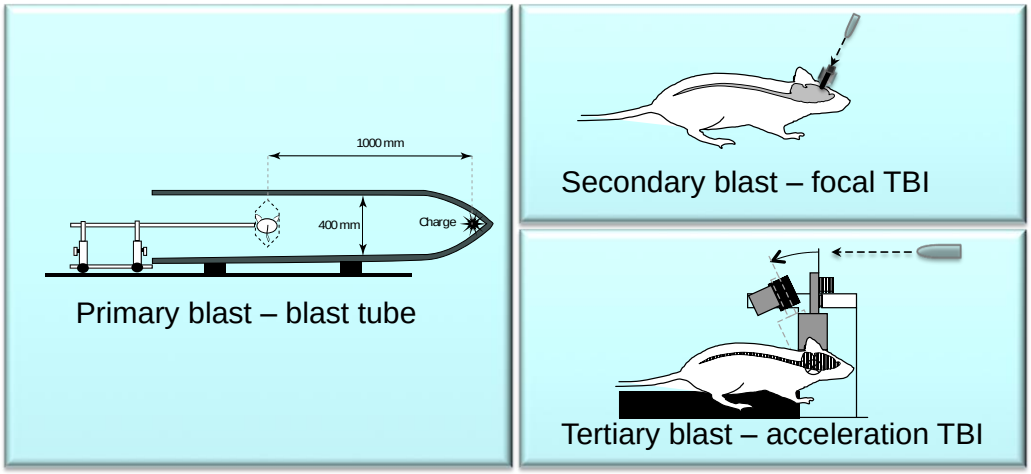

Figure 1: Schematic illustration of 3 models for primary, secondary and tertiary blast. Acceleration movements have been limited in the blast tube. The focal injuries from impact have been represented by a model for focal penetration with a relatively high velocity. Effects of acceleration are studied by the use of a model for rotational injury. The models generate TBI with different distribution and outcome.

2.0 THREE MODELS FOR EXPERIMENTAL TBI

2.1 A Blast Tube for Studies on Primary Blast

A tradition of in research on biological effects of blast started in the 1940´s. Carl-Johan Clemedson published a thesis titled ”*An experimental study on air blast injuries”* in 1949 at the university in Uppsala [5]. He continued his research at FOA (Swedish Defence Research Establishment) using compressed air [6] and finally a newly constructed blast tube [7] in which a charge of plastic explosive (pentaerythritol tetranitrate PETN) was used. The system was composed of a cylindrical 400mm wide and 565 mm long cast iron tube, with a cone shaped tip where the charge is placed. The wall thickness at the tip is 100mm. At the other end the iron tube is elongated to a total length by an extension tube of steel (wall thickness

10mm). The open end of the extension tube was by a steel disc connected to a pendulum in Clemedsons initial experiments. At the conical end of the tube there is a threaded screw plug for the insertion of the charge that is mounted on the detonator. The charge is fixed at a distance of 100mm from the inner tip. Clemedson and his coworkers published a number of studies on vascular and respiratory effects of blast [8, 9]. After some time, this work was extended to include the central nervous system [10] and the cerebral vasculature [11]. The blast tube (without the pendulum door) was modified for work with rodents by Anders Suneson in the 1990´s and Annette Säljö used this system in her thesis work [12, 13]. The ignition system and system for animal montage was later been improved. The current setting employs the NONEL non-electric ignition from Nobel. The work with the blast tube has been continued in the Unit for Experimental Traumatology at the Swedish Defence Research Agency (FOI), which succeeded FOA in 2001. The trauma unit was transferred from FOI to Karolinska institutet (KI) in 2008 after an agreement between the Supreme Commander of the Armed Forces and the President of KI. The experimental traumatology unit does also represent Sweden in NATO RTO HFM-189, -193 and -98.

The anesthetized rat is mounted in the blast tube at a distance of 1m from the charge. The PETN generates a pulse with rapid raise time and very short duration (<0.3 msec) that corresponds to a position to the far left side of the Bowen curve. An increase in the charge has a direct and proportial effect on the peak pressure, but only a small effect on the duration. Secondary reflections are limited. By moving the object to the orifice of the tube (1.5 m from the charge) the peak pressure drops to about half, whereas the duration is increased somewhat. All rat experiments have been performed at the 1 m distance from the charge, which has been varied from 0.5g to 5g PETN (Spherical charges of Swedish army plastic explosive m/46, containing 86% pentaerythritol tetranitrate PETN and mineral oil, where used. In the following text the weights of the plastic explosive charges are given in g). We have previously reported that the peak pressure during detonation of 2.5g PETN would be 260kPa and nearly 600kPa with a 5g charge. To get representative recordings of the peak pressure during a detonation of explosives is connected to substantial methodological difficulties due to the extreme requirement for good dynamics. Recent recordings with a set of open silicon piezoresistive sub-miniature pressure transducers (Entran Sensors & Electronics) indicate a peak pressure exceeding 10 bar during detonation of 5g PETN, as recorded in front of a rat dummy at the 1m position in the tube (Fig 2).

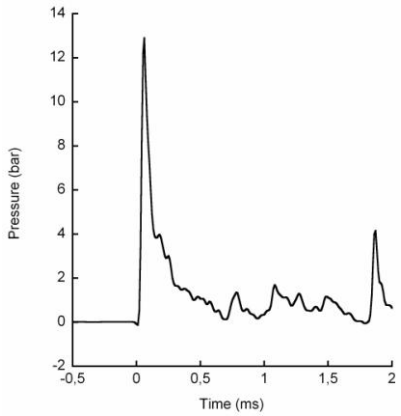

Figure 2: A pressure curve recorded at 1m from the charge during detonation of 5g PETN in the blast tube. Note the rapid rise time and very short duration of the primary peak.

A charge of 2.5g in an unprotected animal (side-on at 1m from the charge) results in about 50% lethality and at 3g the majority of the exposed animals die from pulmonary bleedings (Fig 3). On the other hand, the lethality is less than 10% at 2g PETN. In recent experiments we have used a full protection (rigid steel tube) of the body, except for the head and had no lethality with 5g charges.

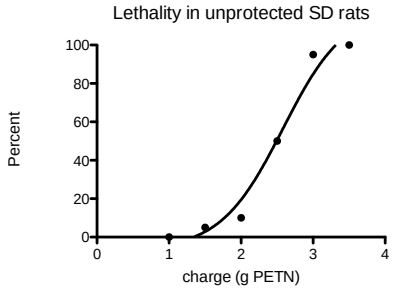

Figure 3: A dose-response lethality curve. Unprotected adult anesthetized Sprague Dawley rats exposed to side-on detonation in the blast tube, at a distance of 1000mm. The charge is indicated on the x-axis. Body protection (steel tubing) of the torso improves survival rate to nearly 100% with a 5g charge.

In spite of the substantial lethality with this system (in unprotected animals) structural changes in the brain seem very limited or absent. The cell death in the brain that was reported by Säljö and coworkers [13], employing a similar protocol, has not been possible to verify. However, there are a number of functional changes such as amplitude and frequency changes in the electro encephalogram (EEG) signals (non-digital system) and a transient change in beam walk behavior [14]. There were also significant cell death [15] and gene expression changes [16] in the inner ear. The effects on the brain in unprotected rats after exposure to a blast from a 2g charge has been evaluated with MRI (magnetic resonance imaging) using a 4.7T scanner and protocols for T2, diffusion-weighted imaging (DWI) and ADC (apparent diffusion coefficient) mapping [17]. The T2 and DWI did not reveal any lesions, whereas the ADC indicated a variation of values in the cortex and white matter that indicates a need for further exploration that will be performed with a new 9.4T system.

The hippocampus of the brain of rats exposed to a 2g charge has been subjected to examination of the gene expression by use of Affymetrix Rat Gene Arrays. In such experiments the expression of all known genes can be evaluated at the same time. It was found that about 100 genes had a significant expression change 24 h survival after the blast. The majority (76 genes) had a decrease in their expression whereas an increased expression was found in 39 genes [18]. Affected systems include synaptic transmission and neurogenesis (Fig 4).

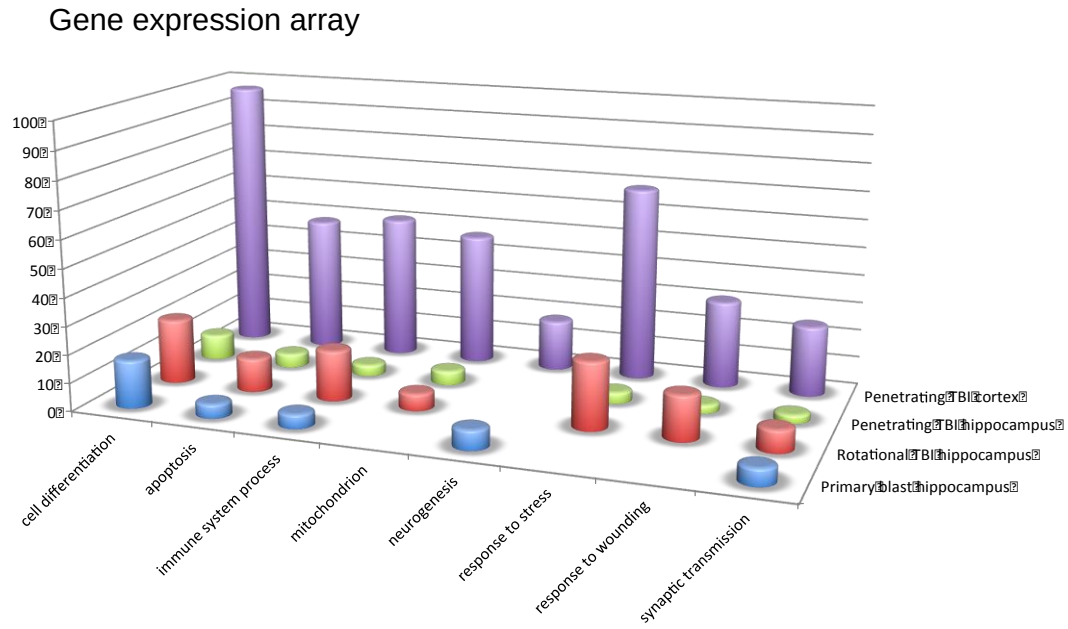

Figure 4: A diagram illustrating the number of significant genes in selected categories after primary blast, in the hippocampus after rotational TBI, in the hippocampus after penetrating TBI and in the border zone after penetrating TBI. The reader should concentrate on the differences in the profiles between the different materials and not on absolute numbers since the energy and severity of the lesion is different for the different injury mechanisms.

In collaboration with Dr Jia Lu and Kian Chye Ng from the DSO National Laboratories in Singapore a number of behavioral tests have been performed. Both unprotected rats exposed to detonation of 2g PETN and rats with the torso protected by a rigid steel tube during exposure to a 5g blast have been examined. The tests have included the radial maze for reference and working memory evaluation, motor activity tests, decision-making tests and anxiety tests. Neither memory nor anxiety was significantly affected in these tests. The brains from these animals will be evaluated at DSO for inflammatory reactions. Anxiety and stress reactions as well as gene expression in brain stem nuclei that are important for such reactions will be examined in collaboration with prof Tomas Hökfelt from KI and Chorin Kawa from the Imperial College in London.

Taken together, the lack of structural damage combined with some functional changes (EEG and gene expression) indicates that the primary blast in this model generates a mild TBI. Future work with the blast tube will include repeated exposures and variations in body position. The blast tube in the current setting creates a very simple and short pulse that may imitate the situation at short distance with an open field charge. This pulse form is probably not at all similar to the situation in a protected vehicle that is hit by a road bomb. It should therefore be of interest to modify the length of the blast tube and introduce reflecting objects that could create a more complex pulse form. One aspect with this type of blast tube is the heat and gas emission that is a result of the use of explosives instead of compressed air. Thus, quaternary blast is added to the primary blast. Acceleration movements are however limited, by the montage of the animal, and impacts of flying objects do not occur. Secondary and tertiary blast is instead imitated and better controlled in other models.

2.2 A Penetrating TBI Device for Evaluation of Secondary Blast

This device was presented at the INTS international neurotrauma meeting in Adelaide 2004 [19]. The anesthetized rats are placed in a stereotactic frame and a 3 mm craniotomy is performed with a center

about 3 mm posterior and 3 mm lateral to the bregma. A lead bullet is accelerated by air pressure in a specially designed rifle and impacts a secondary projectile. The second projectile consisted of a metal cylinder with an attached carbon fiber pin. The pin of the secondary projectile, guided by a narrow tube, penetrates the surface of the brain with a speed of 100 m/s. The shape of the narrow tube provides good control of the path of the projectile. The base of the projectile is surrounded by compressible ring that provides control of the penetration depth into the brain, which usually is set to 5 mm. This model is specifically aimed to evaluate effects of the rapid impact that can be the result of shrapnel and other flying objects and during an explosion. Speed and shape of the penetrating secondary projectile can be changed to get a variation of physical properties. The model has recently been modified for work in mice, in collaboration with dr. Ibolja Cernak at Applied Biophysics Laboratory at Johns Hopkins University. Combined primary blast and secondary blast exposures, using this model and a blast tube, is one possibility for controlled evaluation of more complex situations.

The studies on rats show that the penetrating TBI results in a central cavity corresponding to the actual penetration. A zone of reactive tissue, which contains a mixture presence of dying cells, reactive cells and invading inflammatory cells, surrounds this cavity. The size of this zone is related to both the speed and shape of the projectile. Cell death in this zone is both apoptotic and necrotic and most intense 1-3 days after the injury. Oedema and blood brain barrier defect are transient features in the reactive zone. After 3 days an invasion of ED1-positive macrophages is apparent both in the lesion cavity and in the surrounding borderzone and there are signs of angiogenesis. Reactive glial cells show an upregulation of GFAP and nestin. However, confocal microscopy has shown a reduced covering of capillaries by astrocytic end-feet. Thus, the astrocyte reaction in the penumbra involved both retraction and proliferation. Electron microscopy has demonstrated mainly extracellular perivascular edema [18].

Gene expression arrays have shown an upregulation in many gene families both in the hippocampus and the reactive zone that surrounds the cortical penetration. About 2000 genes were found to be affected at both locations. In the cortex an upregulation was the dominating response, whereas in the hippocampus the majority of affected genes had a decrease in their expression. Affected systems include transmission, cell death and metabolism (see Fig 4).

Behavioral evaluation performed together with the group from DSO show a moderate and partly reversible decrease in memory functions and motor functions, as well as a shift to a more exploratory behavior in anxiety tests.

Taken together, this model of focal injury results in a moderate TBI, which can be varied in size and location.

2.3 A Device for Acceleration/Deceleration TBI for Evaluation of Tertiary Blast

This model was described in detail at the IRCOBI conference 2009 [20]. It is designed to produce diffuse brain injuries by in sagittal plane rearward rotational acceleration. The skull of an anesthetized adult rat is tightly secured to a rotating bar. The bar is impacted by a striker that causes the bar and the animal head to rotate rearward; the acceleration phase last 0.4 ms and is followed by a rotation at constant speed and a gentle deceleration when the bar makes contact with a padded stop. The total head angle change is less than 30 . By adjusting the air pressure in the rifle used to accelerate the striker, rotational acceleration between 0.3 and 2.1 Mrad/s2 can be produced [21].

The signature injury with this model is diffuse axonal injuries in the corpus callosum, subcortical white matter and the brain stem. The lesions can be demonstrated with antibodies for APP, which is accumulated in affected axons within a couple of hours. After a few days positive labeling with silver stain for degenerating axons occurs in the same regions. There is also an increase in the injury biomarker S100B in

serum, starting at the same threshold for acceleration as the diffuse axonal injuries can be detected. Vascular lesions and focal impacts such as contrecoup contusions may occur at acclerations high above the threshold for the axonal injuries.

The gene expression arrays showed an increased expression in the hippocampus for many gene families. Changes were observed in more than 1000 genes [18] and affected systems include stress and transmission (see Fig 4).

Behavioral tests with the DSO group indicate very limited shifts in memory and motor functions. Thus, this model results in a rather mild TBI. Future work with the model will include changes in the duration the pulse and a shift of the axis of rotation.

2.4 Ongoing Studies and Future Work the Models

The described TBI models have been employed for work to identify relevant serum biomarkers, since each of the models generate a different signature in terms of structural and functional changes. This work includes evaluation of axonal biomarkers, since the timetable for axonal injuries is different in the models for penetrating and rotational TBI. This work is conducted in collaboration with dr Denes Agoston at Uniformed Services University USU in Bethesda, USA and takes use of Reverse Phase Protein Microarray technique. In other studies the Golgi technique combined with software for Extended Depth of Focus is employed for studies on dendrite morphology in identified types of neurons. The aim is to reveal changes is to analyze if retraction of dendrites or changes in postsynaptic elements such as dendritic spines (Fig 5) correlate to changes in behavior or gene expression. The 3 models will also be further analyzed with imaging techniques including positron emission tomography (PET) in collaboration with dr Balázs Gulyás at KI in Stockholm.

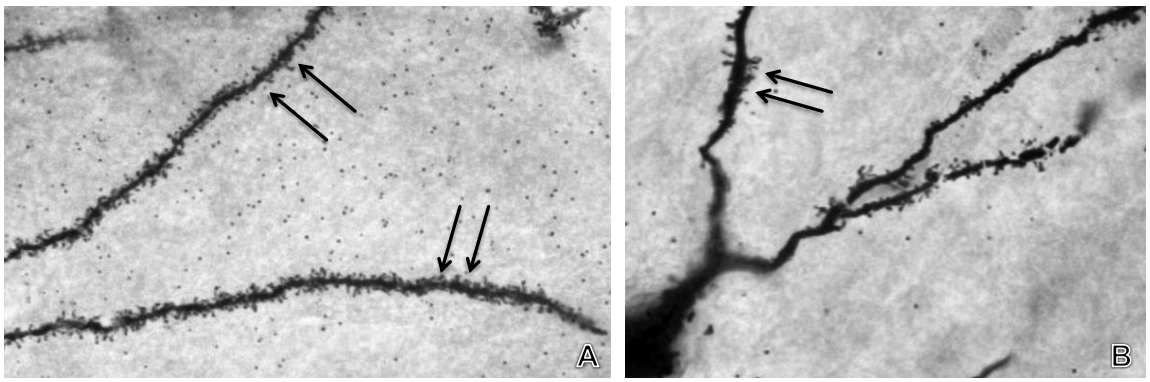

Figure 5: Examples of dendrites in the hippocampus in one normal rat (A) and one rat exposed to a 1.8 Mrad/s2 sagittal plane rearward rotational acceleration (B). Golgi stain and software for Extended Depth of Focus was used. Each image is composed of about 150 images captured at different focal planes. The arrows indicate dendritic spines, which represent a component in the synaptic transmission.

3.0 CONCLUSIONS AND COMMENTS

The situation and the physics that generates blast-induced neurotrauma are extremely complex and many scientific and practical problems must be solved to provide a full picture of the actual injury mechanisms [22]. Exposure data including peak pressure, direction of forces and pulse forms are generally lacking from the clinical cases at the battlefield. In addition, basic knowledge about propagation of pulses in the human body and how this can be modified body protection are missing. It may be assumed that many

cases of blast TBI are the result of a combination of primary blast and other mechanisms such as acceleration injuries. The work with defined experimental TBI models, such the models described in this paper, can presumably help to dissect the contributing mechanisms in blast TBI. However, it is a demanding task to connect experimental data with the clinical situation. The step between experimental work in rodents and obtaining a good picture of critical loads and thresholds in humans is huge. Analysis of advanced computerized mathematical models of the head may provide better understanding of propagation of forces and pressure in TBI [23]. That type of work should be essential for scaling between species and could enhance the possibilities to compensate for differences in head dimensions, differences in the meningeal anatomy, subarachnoid space and brain size, etc. The development of models using blood vessels and fiber orientation [24] will without any doubt contribute to generate more precise and realistic information. However, such models must be based on injury criteria for which detailed biological data are necessary. It will therefore be important to generate detailed models of the rat head that can be used to correlate with data from experimental work, such as the models we have described in this paper. Such data for many basal injury mechanisms are not available at the moment and thoroughly planned work with controlled and validated experimental for each mechanism is needed.

One other way to provide links between data from experimental work in animals and clinical cases, is to use comparable methods for the evaluation of the injury. Data from imaging such as MRI and PET, serum biomarkers, EEG recordings, microdialysis and monitoring of intracranial pressure could therefore be a valuable in the strategy for evaluation of new animal models. It would, however, also be very appreciated if precise exposure data including peak pressure and acceleration forces together with eg results from biomarker analysis could be provided from clinical blast cases. That type of background material seems essential for the design of realistic and useful animal experiments and the information must be conveyed in both directions.

Thus, experimental research in animals may provide insights in injury mechanisms that can facilitate diagnosis and prevention of injuries. Specific biomarkers for trauma can be identified using experimental models. Improved knowledge about injury mechanisms and thresholds are important for development of better body protection. In addition, specific pharmacological treatment for TBI is largely lacking. Any preclinical testing of drug candidates would require realistic, validated and reproducible animal models for TBI.

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