

**Project Title:** Membrane Biophysics of TBI

**Principal Investigator:** Sergey M. Bezrukov, Ph.D.

## **ABSTRACT**

While most investigations focus on the long term patho-physiological processes that follow the primary injury, our first goal is to determine the biophysical consequences during the first milliseconds following the blast shock wave that passes through the brain and the subsequent cellular responses. Our second goal is to develop a theory to quantitatively understand the physical mechanisms of the injury by examining shockwave interactions with the membrane. It is well known that high hydrostatic pressure up to hundreds of atmospheres does not rupture membranes. However, for a medium devoid of air cavities, like the brain, the blast wave can induce a transient with an absolute negative pressure.

We have examined how negative pressure and the dynamics of a rapidly changing pressure can lead to membrane injury. We have introduced a new theoretical injury model where cavitation happens within cell membranes. Available simulations suggest the existence of additional shear forces between different parts of the brain (i.e., white and gray matter, CFS etc.). To investigate the possible interactions between the blast shockwave and cell membranes giant unilamellar vesicles (GUV) of various lipid compositions were used. To model the shock wave we explored several approaches, including high frequency ultrasound and laser-pulse-induced cavitation. We have found that the conditions that best duplicate blast are achieved when we use a short (5 – 100 msec) pulse of high intensity, low-frequency (40kHz) ultrasound. GUVs were exposed to a blast wave and, in parallel to real time observations; the statistics of GUV numbers and sizes before and after the pulse were collected.

In order to directly model TBI in human tissue, we developed a new system of tissue culture based on adult and fetal human brain. Briefly, we developed three different systems: 1) Adult organotypic human brain, 2) adult dissociated human glioblastoma multiforme (GBM) culture, and 3) fetal dissociated human brain culture. All of these systems were optimized for long-term time-lapse recordings, and calcium imaging. The systems develop internal connectivity, show high viability and preservation of cell type, and respond to blasts.

In order to emulate the pressure profile of a blast, while maintaining the ability to observe cells in real time, we developed a new pneumatic device based on an air gun. To our knowledge, no other in vitro or in vivo model has had the spatial-temporal resolution to observe the effect of a primary blast on single and small networks of cultured human cells. By imaging cells in real time as they react to the transient pressure wave, cellular response can be followed on a per-cell basis, and associations between early cellular response and long term behavior can be made. This system is amenable to evaluating pharmacological treatments of TBI; these experiments have been initiated. We developed three different primary human cell cultures that can be maintained for continuous microscopic observation for one week or longer.

In summary, for the cellular studies, we have shown that the physical properties of a blast can be mimicked and applied to cells maintained in culture over the microscope. We find that the wounding response is not the result of network electrical activity, that the wounding response in this system is mediated by purinergic signaling, that the wounding response can be pharmacologically manipulated using P2 receptor antagonists and apyrase, and that our new in vitro system of adult human neurons and glial captures the potentiating effects observed in vivo following secondary insult.

**Project Title:** Pathological and Pathophysiological Alterations in Rat Hippocampus in mTBI

**Principal Investigator:** Maria F.M. Braga, D.D.S., Ph.D.

## **ABSTRACT**

TBI is one of the most important predisposing factors for acquired epilepsy. In 68% of patients suffering from posttraumatic epilepsy, the epileptic focus resides in temporal lobe structures such as the hippocampus and the amygdala. Yet, the current knowledge of the morphological and functional alterations that occur in these brain regions after TBI leading to epileptogenesis is very limited. Here, we utilized the controlled cortical impact (CCI), a widely used experimental form of closed head injury, to investigate pathophysiological alterations in the amygdala and hippocampus that underlie the development of posttraumatic epilepsy. Whole-cell recordings from CA1 and BLA pyramidal neurons showed a significant reduction in the frequency and amplitude of GABA<sub>A</sub>-mediated spontaneous inhibitory postsynaptic currents (IPSCs), seven days after CCI. This reduction was accompanied by a decrease in the levels of the  $\alpha 1$ ,  $\beta 2/3$  and  $\gamma 2$  subunits of the GABA<sub>A</sub> receptor expressed in the membrane, as determined by biotinylation assay and Western blots. We have also observed loss of inhibitory neurons in the BLA whereas we have not detected loss of the total number of neurons. Activation of  $\alpha 7$  containing nicotinic receptors by fast application of acetylcholine or choline showed that the function of  $\alpha 7$  nicotinic receptors in the CA1 region was unaltered. Accordingly, there is no change in the level of  $\alpha 7$  containing nicotinic receptors in the hippocampus one week after CCI. However, in the BLA, the function of  $\alpha 7$  nicotinic receptors was significantly increased seven days after CCI, suggesting that compensatory mechanisms involving the cholinergic system may take place after CCI. Our results show that CCI exposure causes a significant reduction in the inhibitory tonus of the CA1 and BLA regions seven days after CCI. This reduction may significantly underlie the behavioral abnormalities that follow TBI. Genome-wide expression patterns for mRNA, and the microarray (miRNA) which epigenetically influences mRNA activity, can reveal long-term effects of brain injury. An Illumina microarray comprising a comprehensive set of rat expressed genes was interrogated with hippocampal tissue from the ipsilateral side of sham-operated or cortically injured male rats at 1, 7 and 30 days post-injury. The chemokine ligands Ccl2 and Ccl7, the blood-brain-barrier regulator Cldn1, the GABA type b2 receptor (Gabbr2), and the Pou4f1, Klf4 and Klf2 transcription factors were identified as early (Ccl2, Ccl7, Cldn1), intermediate (Gabbr2) and late (Klf2, Klf4 and Pou4f1) markers for the hippocampal response to CCI. Illumina Solexa sequencing revealed 31 significantly up- or down-regulated miRNAs. A series of bioinformatic tools identified miRNAs and putative target mRNAs whose expression was altered inversely after CCI, indicating potential miRNA-dependent repression or de-repression of mRNA after brain injury. Several-fold up-regulation of Ccl2 and Ccl7 24 hours after CCI, subsiding by one week after injury, suggests short-term inflammatory responses which resolve quickly and are not under miRNA control. Altered expression of Gabrb2, changes in GABA receptor trafficking after CCI and Pou4f1/Klf4 suppression might all contribute to excitatory/inhibitory neuronal imbalance in hippocampus with long-term behavioral consequences.

**Project Title:** Neuroprotective and Neurotrophic Effects of Mood Stabilizing Drugs in TBI

**Principal Investigator:** De-Maw Chuang, Ph.D.

## **ABSTRACT**

In developed countries, TBI is a leading cause of morbidity and death in young adults, and the United States alone averages an estimated 1.7 million TBI cases every year. Approximately 200,000 military service members were diagnosed with TBI since 2000. TBI causes primary injury (mechanical damage to neurons, glia, and vascular structures) followed by secondary injury including excitotoxicity, oxidative stress, neuroinflammation, mitochondrial dysfunction, and axonal degeneration, and the latter one is often associated with cognitive and behavioral dysfunction. Despite extensive studies aimed at finding successful pharmaceutical therapies, however, the FDA has not yet approved a drug for the treatment of TBI. The complex pathology of TBI suggests that treating it will require combined therapy or an agent capable of interfering with multiple pathways for cell survival or death.

Lithium and valproic acid (VPA) have long been used to treat bipolar mood disorder. Both mood stabilizers are effective in the treatment and prophylaxis of acute mania and, to a lesser extent, the depression episode associated with the disorder. Emerging evidence supports the notion that both lithium and VPA exhibit neuroprotective and neurotrophic properties. They suppress neuronal apoptosis and neuroinflammation in cellular and animal models of neurodegenerative diseases including cerebral ischemia, Huntington's disease, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, spinal cord injury and retinal degeneration, among others. A number of clinical trials using lithium or VPA for brain disorders are now underway. These drugs have a limited number of direct targets, and it is generally believed that inhibition of glycogen synthase kinase-3 (GSK-3) by lithium and inhibition of HDACs by VPA contribute to their neuroprotective/neurotrophic effects. We recently demonstrated that combined treatment with lithium and VPA induces synergistic or additive neuroprotective effects in cultured neurons against glutamate induced excitotoxicity and in a transgenic mouse model of amyotrophic lateral sclerosis. The synergistic mechanisms involve, at least in part, enhanced inhibition of GSK-3. These observations suggest that combined treatment with lithium and VPA may enhance the beneficial effects elicited by either drug alone.

The present project was to investigate neuroprotective and neurotrophic actions of lithium and VPA in an experimental mouse TBI model using the method of controlled cortical impact (CCI). Two specific aims are proposed:

- (1) To study short and long-term effects of post-insult treatment with lithium and/or VPA on CCI-induced brain damage, glial activation, blood-brain barrier breakdown, motor imbalance and memory deficits.
- (2) To identify the targets and mechanisms underlying the beneficial effects of mood stabilizers including the role of GSK-3 and brain-derived neurotrophic factor (BDNF) in mediating these actions.

**Project Title:** Neurobehavioral Phenotyping of Male and Female Rats to BOP, HIFU, and Stress

**Principal Investigator:** Neil E. Grunberg, Ph.D.

## **ABSTRACT**

The wars in Iraq and Afghanistan are taking physical and psychological tolls on American service members. Fifteen to thirty percent of returning Warriors have been diagnosed with TBI and 40% returning Troops have been diagnosed with mental health problems (including anxiety, depression, and substance abuse). Moderate to severe stress is comorbid with TBI. The purpose of this research project is to evaluate effects of mTBI and stress on behavioral and neurochemical responses at two time points after injury in male and female rats. The specific aims of this project are to: (1) characterize the behavioral phenotype of mTBI in rats; (2) compare responses of male and female rats; (3) compare responses with and without exposure to stress; and (4) compare behavioral phenotypes with biological responses. This research project is investigating effects of injury; stress; sex; and time after injury (1-4 or 8-11 days). Injury is manipulated by blast overpressure (BOP) to model effects of blast waves from improvised explosive devices (IED) without shrapnel or penetrating injury. Stress is manipulated using the Warrior Stress Paradigm (WSP) that combines predator stress (exposure to synthetic fox urine) and unpredictable environmental stimuli (e.g., noise, lights). WSP is designed to model the Warriors' stress exposure to unpredictable threats of death and disruptive environmental stimuli. Subjects are assessed behaviorally pre- and post-injury and blood and brain samples are collected post-mortem for analyses. The behavioral dependent variables measure movement, sensory and motor reflexes, balance, attention, memory, nociception, and anxiety- and depression-related behaviors. Blood is assayed for corticosterone, adrenocorticotrophic hormone (ACTH), prolactin, cytokines and chemokines. Brain samples are micropunched from: orbital frontal cortex, prefrontal cortex, insula cortex, hippocampus, amygdala, nucleus accumbens, and ventral segmental area and assayed for total protein, catecholamines and their metabolites (DA, DOPAC, HVA, NE) and serotonin and its metabolites (5HT, 5HIAA). Electrophysiological recording from the hippocampus and amygdala provide additional information about neurobiological activity in these brain regions relevant to memory and emotion.

**Project Title:** Effects of Boosting Brain Endocannabinoid Levels on Fear Extinction Behavior

**Principal Investigator:** Andrew Holmes, Ph.D.

## **ABSTRACT**

Extinction of aversive memories is a form of learning in which the expression of a conditioned fear response is reduced after repeated experience of a conditioned stimulus in the absence of an unconditioned aversive stimulus. Impaired fear extinction is a major symptom of anxiety disorders including PTSD. Fear extinction is readily quantifiable in laboratory mice, providing important behavioral models for translational studies of anxiety disorders. The endocannabinoid (ECB) system has been strongly implicated in rodent fear extinction. For example, endocannabinoid levels increase in the amygdala during extinction and mutant mice deficient in the ECB CB1 receptor (CB1R) exhibit impaired fear extinction. These data suggest that increasing ECB activity via the CB1R could facilitate rodent fear extinction and, to the extent this behavior provides a model of persistent traumatic memory in PTSD, open an avenue for development of novel pharmacotherapeutics for PTSD.

CB1Rs mediate manifold functions in the central nervous system and periphery. Therefore, indiscriminate facilitation of CB1R function with, for example, a CB1R-selective agonist could have effects that interfere with the facilitation of fear extinction and/or produce unwanted side effects in patients. However, an important characteristic of the ECB system is that ECB function is increased 'on-demand.' Thus, selectively boosting ECB levels that are being endogenously released on-demand could augment ECB functions at CB1Rs (for example on fear extinction) without producing the broad profile of unwanted effects produced by application of an exogenous CB1R agonist. One effective approach to boosting ECB levels is to inhibit the activity of fatty acid amide hydrolase (FAAH), the enzyme responsible for degradation of the ECB anandamide. The objective of our project was to determine the effects of FAAH inhibition on fear extinction, using a novel FAAH inhibiting drug (AM3506). To this end, we address 5 specific aims:

Aim 1 was to determine the magnitude and duration of FAAH inhibition after systemic treatment with AM3506. We planned to measure *ex vivo* levels of FAAH activity in the prefrontal cortex at various time points after intraperitoneal injection of AM3506.

Aim 2 tests the effects of AM3506 on fear extinction. AM3506 will be systemically administered prior to extinction training and we quantified the long-term reduction in fear during a retrieval test 10 days later. In order to verify that effects of AM3506 were CB1R-mediated, a subgroup of mice will be co-administered the CB1R antagonist SR141716A.

Aim 3 extended our behavioral experiments to test how predicted extinction-facilitating effects of AM3506 were manifest as changes in patterns of prefrontal and amygdala activation during extinction. Mice were drug-treated and behaviorally tested as in Aim 2, and 2 hours following extinction training sacrificed for immunohistochemical analysis of the immediate-early genes, *c-Fos*, *Zif268* and *pCreb*.

Aim 4 provided measures of neuronal activity in the amygdala and prefrontal cortex, *in vivo*, via multi-electrode electrophysiological recordings of these regions while mice undergo extinction testing under the influence of AM3506 (or vehicle).

Aim 5 was designed to further test the clinical relevance of AM3506 by examining the drug's ability to block the effects of exposure to stress on extinction. Stress is a known risk factor for PTSD and impairs extinction in mice (6). We will test whether AM3506 reverses these stress effects.

**Project Title:** Blast-Induced Injury of Blood-Brain Barrier and Neuroimmune Sequelae

**Principal Investigator:** Chantal Moratz, Ph.D.

## **ABSTRACT**

Our long-term goal is to understand how mild-to-moderate exposure to blast injury results in the associated long-term physiological, cognitive and behavioral symptoms. The specific goal of this proposal is to begin to determine the biological mechanisms underlying mild to moderate blast-induced TBI, by addressing the hypothesis that transient interruption of the blood brain barrier (BBB) mediate alterations in normal neuroimmunological functions. The rationale for examining the BBB integrity and function are that the type of changes in BBB function will determine the type of neuropathology which occurs and the resulting alterations in neuroimmune function. Severe disruption of the BBB, such as shearing, would illicit an immediate neuropathology due to hemorrhage and ischemic consequences. Disruption of the tight junctions and alterations of the activation states of BBB cells (endothelium, astrocytes, microglia, pericytes, neurons), may result in neuroimmune dysfunction resulting in a chronic neurodegenerative condition. This proposal will employ two models of blast-induced TBI and will evaluate blast effects in rats and mice. The two models of blast used in the proposal are, the blast overpressure (BOP) shock tube and a potentially useful new approach that employs high intensity focused ultrasound (HIFU). However, since this is a rat model of blast injury, the valuable resource to identify key biological pathways using genetically-modified urine strains is not available.

In addition, manipulation of the focus and intensity of the blast is limited due to current technical limitations of the blast tube. The advantages of the HIFU model are: 1. the HIFU wave form can be methodically manipulated and mimic critical impulse energy and rise times of real blasts, 2. mice can be used in this model, which allows the use of genetically-altered strains to evaluate key biological pathways, and 3. physiological monitors implanted in the abdominal cavity (for heart rate, blood pressure, body temperature and respiration) are not destroyed because the blast is limited to the head. Initial results indicate an early neuro-inflammatory response with the blood brain barrier vasculature and a selective alteration of BBB integrity allowing small molecular weight molecules to cross within the first 24 hours after exposure in both models. The correlation of the established rat work to a mouse model is imperative to translation of existing blast data to the mouse system. Taken together, this project will determine how blast wave exposure disrupts the BBB and alters normal neuroimmunological functions, and what implications these alterations have for long term physiologic, cognitive and behavioral functions.

**Project Title:** Genetics and neural factors determining vulnerability and resilience to trauma

**Principal Investigator:** Andrew Holmes, Ph.D.

## **ABSTRACT**

Exposure to stress is a major risk factor for various neuropsychiatric diseases, including Post Traumatic Stress Disorder (PTSD) and other anxiety disorders. Susceptibility to stress, however, varies considerably between individuals, in part due to the modulating influence of genes. Reflecting this, recent reports estimate that while significant proportion of soldiers serving in Operations Iraqi Freedom/Enduring Freedom exhibit symptoms of PTSD, the majority do not. This implies that some individuals are susceptible to the deleterious impact of stress, while others are resilient. Despite being the subject of enormous research efforts, the genetic and neurobiological factors underlying stress susceptibility and resilience remain poorly understood. Studying these factors is extremely difficult in human populations given the myriad genetic, lifestyle, and environmental differences between individuals. Animal models, and mouse models in particular, are an extremely powerful tool for parsing the relative influence of genes and stress on risk for PTSD. In keeping with the utility of mouse models, we have recently demonstrated that different genetic strains of mice differ in their anxiety-related responses to chronic (restraint) stress, with one commonly used inbred strain, C57BL/6J, exhibiting an active-coping response with another commonly used strain, DBA/2J, exhibiting a passive coping response. Using microarray analysis of brain tissue, our preliminary studies also found that these different coping responses were associated with divergent expression of genes regulating excitatory neurotransmission in the prefrontal cortex and amygdala. These findings are very intriguing because dysfunction of these brain regions has been strongly implicated in the pathophysiology of PTSD and other mood and anxiety disorders associated with trauma exposure. The main overall goals of this project are to identify patterns of neural activation associated with active-coping versus passive-coping responses to stress, and then to target those specific brain regions identified with glutamatergic drugs in order to normalize stress responses. These findings would provide a foundation for further studies to uncover the genetics of susceptibility and resiliency, which could lead to new screening tools for at-risk soldiers and improved drug treatments for soldiers suffering from the devastating effects of traumatic combat.

**Project Title:** Gender-specific differences in regional cerebral blood and glucose metabolism after moderate lateral fluid percussion injury (LFPI)

**Principal Investigator:** Marguerite Littleton-Kearney PhD, RN FAAN

## **ABSTRACT**

The complex effects of TBI that trigger brain hemodynamic dysfunction and lead to injury progression remain poorly understood. Both the severity of the primary injury, combined with secondary injuries associated with persistent cerebral ischemia can impact outcomes. Therefore, initiation of early diagnostic testing and intervention, as well as the administration of appropriate pharmacologic agents is vital in minimizing brain damage due to both primary and secondary injury. Currently, it is inconclusive whether gender has a positive, negative or neutral effect on outcomes after TBI. Conclusions based on data from existing published reports remain equivocal. In order to advance our understanding of TBI pathophysiology, gender-specific differences that may affect treatment and recovery need to be established. Longitudinal studies are required to determine if gender affects amount of brain damage, regional cerebral blood flow, tissue metabolism and ultimately injury progression. We propose to use clinically useful imaging techniques to evaluate real-time changes in brain tissue metabolism (FDG-PET) and regional cerebral blood flow (CBF) recovery (perfusion MRI) using arterial spin labeling (ASL) to map these changes in a rat model of TBI (LFPI). Imaging techniques such as positron emission tomography (PET) or magnetic resonance imaging (MRI) enable early detection of subtle changes in brain tissue metabolism and CBF recovery after TBI. Additional studies that clarify the correlation among hemodynamic alterations in local cerebral blood flow (CBF), histological changes in brain and the gender-specific responses following TBI are warranted.

Perfusion MRI and FDG-PET scanning techniques are practical diagnostic tools because they are less invasive and there is minimal radiation exposure. These features are important for repetitive use in clinical settings. Further, perfusion MRI combined with FDG-PET will permit examination of the relationships between regional CBF and glucose metabolism after TBI. Once we have completed these baseline studies we propose to ascertain if one potential pharmacologic neuroprotective intervention (administration of a KOR agonist), which is known reduce the size of brain tissue injury after focal stroke in males, can reduce changes in regional CBF and glucose metabolism after LFPI-induced brain injury. Therefore, our specific aims for this pilot study are.

1. To ascertain if alterations in CBF and glucose metabolism after moderate TBI are gender-specific. We will examine the effects of moderate LFPI-induced brain damage on regional CBF and brain glucose metabolism and determine if there are differences between males and females. We will subject the animals to [18F] fluoro-2-deoxy-D-glucose (FDG)-PET and MRI imaging procedures at 4 hr, 1 day, 2 days, and 7 days post injury. The brains of the animals will be harvested and subjected to triphenyltetrazolium chloride (TTC) staining for histologic analysis of ischemic injury volume.

2. To determine if stimulation of KOR's alter the brain tissue recovery and CBF after moderate TBI in male and female rats. We will evaluate the effects of LFPI-induced CBF changes and brain glucose metabolism in male and female rats treated with a specific KOR agonist. All rats will undergo FDG-PET and MRI procedures for evaluation of CBF at 4 hr, 1 day, 2 days, and 7 days post injury. The brains of the animals will be harvested and subjected to TTC staining for histologic analysis of ischemic injury volume.

**Project Title:** Brain Protein SUMOylation for Neuroprotection

**Principal Investigator:** Joseph McCabe, Ph.D.

## **ABSTRACT**

The goal of this research project is to perform experiments that will determine whether or not a process in cells called SUMOylation reduces the effects of traumatic brain injury (TBI). Briefly, under certain circumstances cells can attach a “SUMO molecule” to proteins through a series of biochemical steps. Previous research indicates protein SUMOylation can protect cells from destruction when they are stressed, as occurs during TBI and strokes. To test whether or not SUMOylation reduces the effects of TBI, we will use genetically modified mice that carry extra gene copies of an enzyme. By having additional copies of the enzyme, mice express higher cellular levels of SUMOylated proteins. We hypothesize that elevation in the amount of SUMOylated proteins will “protect” cells from destruction, reducing the severity of TBI, and result in these mice exhibiting better performance on behavioral tests compared to mice that express normal levels of protein SUMOylation. At the present time there are no known drugs that stimulate protein SUMOylation, but if protein SUMOylation is beneficial, we will explore mechanisms that might increase protein SUMOylation as a means of protecting the brain after TBI.