High Intensity Focused Ultrasound:

A Novel Model of Mild Traumatic Brain Injury

by

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ABSTRACT

Title of Thesis: High Intensity Focused Ultrasound: A Novel Model of Mild Traumatic Brain Injury

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Animal models of traumatic brain injury (TBI) are needed to ethically and experimentally characterize the effects of TBI on Warriors. Numerous techniques have been developed to model TBI, but each animal model of TBI has limitations regarding the induction of the TBI. Non-invasive injuries such as blast overpressure result in diffuse injuries, while invasive injuries such as closed cortical injury result in localized injury. High Intensity Focused Ultrasound (HIFU) has been proposed as a non-invasive model to induce localized, neural-specific mild TBI (mTBI) that could lead to a better understanding of the impact of mTBI on specific brain regions.

Two experiments were conducted to characterize the neurobehavioral effects of HIFU model of mTBI in male and female Sprague-Dawley rats. The first experiment was a 2 (no injury, injury) x 2 (male, female) full factorial mixed design (N=20). The results of this study revealed a between-subjects interaction of sex x injury ($F(1, 16) = 4.539$, $p = .049$) for Vertical Activity, suggesting greater depression-related behavior for HIFU-exposed females compared with the other conditions. The second study was a 3 (control, sham control, injury) x 2 (male, female) full factorial mixed design (N=64). This study sought to replicate and extend the findings of the previous study by

introducing a sham control that would help to distinguish HIFU effects from the HIFU injury preparation and anesthesia. Results show trends towards significant differences for HIFU injury animals compared with both control and sham control for neurobehavioral performance and locomotor activity. These findings suggest HIFU may affect behavior in rats, but the model of TBI is not as robust as other animal models.

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CHAPTER 1: Introduction

Traumatic brain injury (TBI) has been called the signature wound of Operation Enduring Freedom/Operation Iraqi Freedom (90). Given the remarkable advances in protective equipment supplied to U.S. Military personnel and the improvements in medical care provided immediately and over time following injury, OEF/OIF have better combat survival rates than seen in previous conflicts (41). The result of these high survival rates is that many Service Members are facing increasingly difficult recoveries following injury.

Between 2000 and 2012, more than 250,000 U.S. Service Members have experienced a traumatic brain injury (91). Approximately 77% of these TBIs has been classified as mild (39). Traumatic brain injury, though frequently discussed as a condition, is an event in which induced structural injury and/or physiological disruption of brain function is a result of an external force. The disruption can occur as a result of one or more mechanisms of blasts (92). Primary blast injury occurs as a result of the overpressure wave, and it mainly affects air and fluid-filled organs (*e.g.*, lungs). Secondary blast injury occurs as a result of flying debris propelled by the blast wave (*e.g.*, shrapnel) that can penetrate or impact the brain. Tertiary blast injury occurs when an individual is moved by the blast wave and thrown into another object, frequently resulting in blunt trauma.

The TBI is further classified as mild, moderate, severe, or penetrating (25). Mild, moderate, and severe TBIs are differentiated based on a combination of clinical factors including brain imaging results, loss of consciousness, alterations of consciousness, post-traumatic amnesia, and the Glasgow Coma Scale rating (26).

Despite the homogeneity of core symptoms resulting from the traumatic brain injuries, a given individual's recovery from a TBI is not easily predicted, even when the severity of the injury is known. The TBI event sets off numerous, variable down-stream processes in the injured individual. Differences in the TBI event can result in varied post-concussive syndromes, or the pathological condition resulting from a TBI.

Further, there is evidence of gender differences in response to traumatic brain injuries. There are fewer TBIs for women in the adolescent and young adult population (18); however, studies have reported significantly worse outcomes for women with TBIs. Ottochian and colleagues (73) found that women had post-TBI mortality rates of 42% compared with 30% for males. In particular, post-menopausal women had the greatest likelihood of death following severe TBI compared with males and other age groups of women, with an odds ratio of 1.71:1. Given the declines in possibly neuroprotective steroidal hormones post-menopause, there may be hormonal influences contributing to the gender response to and recovery from TBI. Another study reported that women had significantly worse post-concussive symptom (PCS) scores three months after a mild TBI (10).

Commonly experienced symptoms of TBI fall into three primary categories: (1) physical; (2) cognitive; and (3) behavioral/emotional (28). Physical symptoms include headache, nausea, vomiting, dizziness, blurred vision, sleep problems, weakness, paresis/plegia, sensory loss, spasticity, aphasia, dysphagia, dysarthria, apraxia, balance and coordination problems, and possible seizures. Frequently noted cognitive symptoms include changes in attention, concentration, memory, speed of processing, new learning, planning, reasoning, judgment, executive control, self-awareness, language, abstract

thinking. Common behavior and emotional symptoms can include depression, anxiety, agitation, irritability, impulsivity, aggression. Given the heterogeneity of combinations of post-concussive effects resulting from TBI, additional research is needed to refine and expand current scientific understanding of the pathological processes. However, that research must be based on validated models of TBI that accurately reflect the neurological, psychological, and behavioral changes resulting from the injury.

TBI Research

The purest experimental methods of TBI research would involve randomly assigning human subjects to different injury conditions. This controlled, experimental manipulation would provide the ideal data from which to develop predictions about the course of post-concussive symptoms and therapeutic interventions to treat individuals who experience a TBI. However, such an experiment is unethical and cannot be conducted.

Another source of valuable information with which to better understand TBI and post-concussive symptoms is medical records of individuals who have suffered brain injury in naturalistic settings. These records theoretically hold information about the source of injury, affected brain regions, and resulting medical presentation (54). In practice, the records are incomplete, preventing accurate conclusions to be drawn regarding the generalizability of the findings to future injuries (53). Even with the improvements in record keeping, a high degree of experimental control is not possible with this method. Additionally, analyzing records frequently provides correlational results which cannot be used to infer causation (35).

A third option for TBI research is to conduct studies with known TBI patients. This method provides valuable information on current functioning, rehabilitation, and recovery trajectories (35). However, accurate data on pre-morbid functioning can be difficult or impossible to obtain or estimate, injury severity between cases can vary drastically, and matching control subjects for prospective research is less precise than in planned experimental manipulations (42). Fortunately, there are alternatives to human subjects for TBI research.

Animal research

Translational animal research is an alternative to human experimentation that provides many methodological and logistic advantages to study traumatic brain injury. Translational research is defined by the National Institutes of Health (71), in part, as applying basic or pre-clinical research discoveries to the development of research in humans. Translational research using animal models allows for rigorous experimental control of brain injury, randomization to different experimental groups, and a level of environmental control that is virtually impossible to achieve in human studies. Animal research also permits a level of physiological and biological study that is impossible in human research (94). Specifically, animal subjects can be sacrificed at the conclusion of a study to examine morphological and histological changes, both centrally and peripherally, resulting from the experimental manipulation. Controlled behavioral data also can be collected using animal experiments. Numerous behavioral assays provide validated, replicable, and parallel data to distinguish the effects of experimental manipulations. These data provide valuable information that is unavailable or difficult to

obtain in similar human studies, but that can still inform and guide relevant prevention, treatment, and rehabilitation work in humans.

Limitations to animal research must be recognized as well. Shanks et al (78) argue that animal models are not predictive of responses in humans. In particular, they raise concerns regarding the predictive utility of animal models in relation to human outcomes for studies of toxicological and pathophysiological processes. Additional limitations surround the variety of animals that come from different vendors and the different strains of similar animals for establishing gold standard models within research (88).

Despite these concerns, animal research has a long history and has provided valuable insight into the human condition. Darwin's (24) seminal work on the origin of species presented three central tenets: (1) natural selection; (2) spontaneous mutation; and (3) continuity of species. Continuity of species is the basis on which all animal modeling of human conditions is founded. Humans evolved through the same processes and from similar ancestors as other non-human animals; therefore, lessons learned in animal research are relevant to humans.

Subsequent studies utilizing animal models discovered important behavioral principles relevant to the human condition, including classical conditioning (96), operant conditioning (82), as well as psychologically relevant conditions such as depression (74) and anxiety (56). These models allow normal behavior to be studied under controlled conditions and to model the effects of specific stimuli, including TBI, on behavior. As such, animal models are relevant for behavioral and psychological explorations of analogous human conditions.

Of particular interest in conducting animal research is the array of commonly-used animals available for studies. Researchers have utilized mice, rats, rabbits, dogs, pigs, and chimpanzees, among others, for research into the human condition. Entire books have been written covering appropriate animal model selection for different research questions (40; 52). Rats have been bred for laboratory use and are considered a good model of the human condition. Of particular note, the Grunberg Laboratory has been utilizing rats in independent and parallel studies of human conditions for 30 years (43). Relevant examples of the conditions studied with rats include: substance use, eating behaviors, stress, social enrichment, and traumatic brain injury (33; 44-47).

Animal models of brain injury

There are multiple commonly used animal models of traumatic brain injury. Fluid percussion (FP), controlled cortical impact (CCI), weight drop (WD), and blast overpressure (BOP) have well-established procedures; have been used in numerous studies in several different laboratories; and are believed to be relevant to human blast-induced traumatic brain injuries of varying severity.

Fluid percussion

Fluid percussion (FP), initially developed in 1989, is one of the oldest and most commonly used brain injury models in research (31; 80; 99). This model utilizes a direct brain deformation caused by a fluid impulse to the *dura mater* (64). In this model, a craniectomy is performed, a plastic cap is cemented over the hole onto the skull, and sterile saline connected through tubes is used to transmit a controlled pressure to the injury site (3). Following the injury, the scalp is closed and sealed over the skull, but the hole in the skull is not closed, which allows expansion of the brain.

Thompson and colleagues (87) note that fluid percussion has achieved a substantial degree of validity in terms of subject mortality and physiological and neurological changes resulting from the procedure. The impulse delivered in the technique is scalable, resulting in an injury that can model mild to moderate traumatic brain injury. The limitations of the model include the invasive surgical procedure needed to establish the injury site, the time and technical skill needed to perform the procedure, and the significant periods of anesthesia needed to maintain subject immobility during the surgery and injury phases.

Controlled cortical impact

Controlled cortical impact (CCI) was developed to provide greater experimental control over the injury-inducing mechanisms in the model through the use of a pneumatic impactor (57; 59; 98). The pneumatic impactor is highly controlled by an electronic sensor system, providing increased experimental control over injury parameters. CCI improves upon FP by creating a similar range of injury in animals but which results in decreased mortality (27). CCI is most frequently used as an open-head injury; however, it can be used without removing the skull (67). The limitations of this model are similar to those of FP, such as the surgical procedure needed to access the brain in most cases and the deformation of the skull and/or brain tissue resulting from the impact.

Weight drop

Weight drop (WD) is a traumatic brain injury model most similar to controlled cortical impact (CCI). In WD, a free-floating weight is dropped onto a fixture attached to the animal's head (66). The impact can be targeted to multiple areas of the animal's head, and the impact can be made to occur with or without a craniotomy (67). The

impact of the free-floating weight is an acceleration impact from gravitational force, and it arguably models secondary or tertiary blast trauma (21; 79). However, the impact of WD is not as precisely controllable as that of the CCI. Further, the model is difficult to use for focal injury investigations.

Blast overpressure

Blast overpressure (BOP) is the only model that yields primary blast injury through air-blast shockwaves (6; 30; 49). BOP is conducted by securing an animal in a metal restraint in the opening of a blast tube (101). A pressure wave is created by allowing compressed air to rapidly escape through an expansion chamber, which impacts the restrained animal resulting in a primary blast injury. One primary characteristic that sets the model apart from other common injury models is the non-invasive nature of the procedure. There is no direct surgical intervention to the animal's head, unlike the FP or CCI.

Although this model is face valid for the condition of military service members exposed to blasts concussion while deployed, it is a full-body exposure that may result in both central and peripheral damage processes (101). The model's combination of possible injury effect (e.g., damage to peripheral portions of the body as well as air- and fluid-filled organs) is not ideal for isolating neuropathological processes in the study of traumatic brain injury. Further, gross exposure to the blast wave does not permit refined, targeted direction of the injury to different brain regions. As such, behavioral changes resulting from the injury model cannot necessarily be attributed to the brain injury in isolation.

Limitations of Current Animal Models of TBI

Despite the developments made in modeling traumatic brain injury, there is no "gold standard" for studying mild brain injury in isolation (67). The invasive nature of many models allows for targeted injuries, but the requisite surgeries result in injury processes that contribute to impairment beyond the intended mechanism in the model (20). The non-invasive mechanism of blast overpressure prevents the ancillary effects of the surgeries needed in direct impact TBI models (e.g., craniotomy-induced inflammation for fluid percussion). However, the full-body blast exposure in BOP yields peripheral damage (e.g., peripheral inflammation or damage to fluid-filled organs) that may also confound the experimental purity of the brain injury research (101). What is needed is an animal model of brain injury that is both targeted/specific to the brain, yet minimally invasive in the experimental procedure. High Intensity Focused Ultrasound (HIFU) may fill this research need.

High Intensity Focused Ultrasound

Ultrasound has been used in medical and therapeutic settings for over 50 years (29). Ultrasound is defined as any sonic frequency above normal human limits of perception (approximately 20kHz). Ultrasound was first used for medical purposes at the Naval Medical Research Institute in the 1940s (97), and is now commonly used for medical imaging (e.g., fetal sonograms). When ultrasound is focused at high intensities on biological tissue, the resulting acoustic wave can damage the tissue through heating and cavitation. These mechanisms of injury have been used to destroy diseased tissue (e.g., cancers). Recent studies of a specialized ultrasound - high intensity focused ultrasound (HIFU) - have demonstrated that brief administration of the sonic waves yield

varying degrees of tissue damage and may be useful as a model to study traumatic brain injury (67).

High Intensity Focused Ultrasound has recently been utilized to produce brain injury in animals to model mild traumatic brain injuries (mTBI) in humans. Wahab et al (95) reported that HIFU can impair neural axonal functioning. The authors' finding concerning changes in axonal functionality may model the diffuse axonal injury frequently seen in mTBI. Mesiwala et al (68) reported disruptions in the blood brain barrier (BBB) as a result of HIFU. The disruption of the BBB was transient, and in many subjects, the adjacent neural tissue was not noticeably damaged. These findings also may model the subtle disruptions found in mTBI. Further, McCabe and colleagues (2010) explain that the waveform characteristics of the HIFU experimental procedure can be controlled and manipulated to produce similar physical characteristics to those seen in blast shockwaves.

The experimental design of the HIFU model has important benefits to isolate the behavioral effects of the injury. Current HIFU procedures do not require invasive surgical procedures (89). In comparison with other animal models of TBI, the HIFU procedures likely result in decreased mortality of animal subjects because of the localization and non-invasive technique. Another relative benefit of the procedure is the ease with which the procedure can be administered to animal subjects. Because no surgeries are needed, the experimental time is reduced to approximately 2 - 4 minutes per animal, with animals in each of the different stages of the injury manipulation concurrently (i.e., there is not a 3 minute wait between each animal, but closer to a 30 second delay). Additionally, the equipment requirements of BOP are restrictive in many

environments. The size of the blast tube and the specialized protective equipment needed to accommodate the blast procedure require more space and sonic shielding than many laboratories can provide.

Despite the promising advances of the HIFU animal model, there is limited research to support its utility and validity as a model of mild traumatic brain injury. Current research on HIFU suggests it has utility for studying simple neural models in animals such as earthworms (95), but the model has not yet been rigorously applied to complex, mammalian organisms (e.g., rats). While biological or neuropharmacologic changes resulting from the injury are an important foundation to understand the exact action and impact of this new model, translational research emphasizing functional effects of the model on organisms should be the ultimate goal of military-relevant work.

Research is needed to evaluate possible behavioral and psychologically-relevant changes resulting from HIFU-exposure at the level of the organism (e.g., behavioral) to determine its utility as a military-relevant and clinically-relevant model of TBI. The present investigation evaluated High Intensity Focused Ultrasound as a novel model of mTBI by examining alterations in neurobehavioral, nociceptive, and psychologically-relevant locomotor responses (i.e., indices of depression- and anxiety-related behaviors) in male and female rats. The significance of the investigation is that identifying new, military-relevant TBI models may provide an opportunity to develop novel treatment approaches.

CHAPTER 2: Overview and Specific Aims – Experiment 1

The present experiment was designed to examine effects of high intensity focused ultrasound on behavior responses of male and female rats. There were two specific aims: (1) to characterize the behavioral phenotype of high intensity focused ultrasound exposure; and (2) to compare male and female responses to high intensity focused ultrasound exposure.

Hypotheses

There were three hypotheses associated with each specific aim.

Specific Aim 1: To characterize the behavioral phenotype (i.e., behaviors that reflect sensory and motor responses) of high intensity focused ultrasound exposure.

Hypothesis 1a. Rats exposed to HIFU will show impaired neurobehavioral performance compared to controls.

Hypothesis 1b. Rats exposed to HIFU will show different nociceptive response latency compared to controls, either sensitized (decreased latency) or desensitized (increased latency).

Hypothesis 1c. Rats exposed to HIFU will show altered patterns of behavior on psychologically-relevant behavioral measures of locomotor activity compared to controls, including behavioral responses that have been conceptualized as representing anxiety- and depression-related phenomena.

Rationale. Changes in neurological functioning are measurable in humans using neuropsychological assessment, self-report questionnaires, and observational tools. Changes in neurobehavioral functioning is measurable in rodents using the Neurobehavioral Severity Scale – Revised (48). Nociception, or the ability to distinguish

noxious stimuli, is relevant for changes in pain sensitivity and developing chronic pain in humans suffering from TBI (70). Hindpaw lick latency on the hotplate nociception paradigm is a validated animal model of human pain sensitivity (4; 5). Because the goal of this experiment is to characterize the behavioral phenotype of the HIFU injury, there is not sufficient evidence to indicate directionality for nociceptive response at this time. Finally, human studies have reported psychological changes following TBI, including increased depression and anxiety symptoms (13; 55; 60). Locomotor activity, including vertical activity and center time, for rodents is associated with psychologically-relevant conditions in humans (62; 76; 77).

Specific Aim 2: To compare male and female responses to high intensity focused ultrasound exposure.

Hypothesis 2a. HIFU will impair neurobehavioral functioning to a greater extent in females than in males.

Hypothesis 2b. HIFU will alter noceciptive response latency more in females than in males, such that females may either have greater sensitivity or decreased sensitivity compared with males.

Hypothesis 2c. HIFU will show altered patterns of locomotor activity, specifically psychologically-relevant anxiety- and depression-related behaviors, more in females than in males.

Rationale. There is little research on sex differences in traumatic brain injury. Yarnell (100) reported sex differences based on blast overpressure induced TBI in rats. Specifically, female rats exposed to blast overpressure showed deceased center time, vertical activity, and horizontal activity, whereas males did not. Further, stressed and

blast-exposed females showed worsened neurobehavioral performance compared with males. As such, sex differences can be expected in other models of traumatic brain injury. Additionally, Taylor and colleagues (86) have suggested that males and females have different psychological and biological responses to stressors. The authors suggest that females may exhibit a "tend and befriend" response to stress in contrast with the traditional, and characteristically male "fight or flight" response originally proposed by Cannon (19). HIFU exposure may lead to different recovery outcomes on neurobehavioral functioning, nociceptive latency, and locomotor activity. Limited human studies also have reported worsened responses to TBI in females as compared with males (10; 73). However, the sex effects of HIFU are unknown at this time, so directionality of the hypotheses is not predicted for this experiment.

CHAPTER 3: Exploratory Research

Prior to the primary Master's research project, an initial 2 (male, female) x 3 (control, 500 ms, & 1000 ms; 800 mVpp [milliVolts peak to peak]) pilot study (N=16; see Table 1a for subject breakdown) was conducted to become familiar with the HIFU apparatus and procedures and to finalize the experimental timeline. These specific HIFU parameters were selected to be within the range allowed by the equipment and IACUC (range: 0 – 30 sec; 0 – 900 mVpp) and to be greater than parameters that had been tried but which showed no histological damage based on preliminary bioassays (5 – 10 ms; 400 mVpp; J. McCabe, June, 2011, personal communication). Open field activity and neurobehavioral data were gathered before and after exposure to one HIFU exposure, and the preliminary analyses revealed a significant HIFU effect on vertical activity such that rats in both injury conditions displayed significantly decreased vertical activity when controlling for baseline activity ($F(2, 9) = 4.763$, $p < .05$). After we completed this pilot study, we consulted again with Dr. McCabe (who oversees the HIFU laboratory within the USU-NIH CNRM) about our preliminary findings and to schedule the full study. Dr. McCabe then directed us to use parameters of 10 ms and 400 mVpp in order to be comparable with other ongoing CNRM-sponsored biological investigations of the HIFU model. These particular parameters were considered to be the most appropriate settings for a rodent model of mild traumatic brain injury induced by blast overpressure from improvised explosive devices (IEDs). Even though preliminary biological studies had revealed no histological damage in response to these parameters, Dr. McCabe indicated that additional bioassays would be conducted and that our behavioral studies would provide valuable information to determine if and how to proceed with the HIFU model.

CHAPTER 4: Methods – Experiment 1

To address the above hypotheses, this experiment was conducted as a 2 (no HIFU, HIFU) x 2 (male, female) factorial design. This experimental design results in four experimental conditions and allows for analyses of interaction and main effects. There were five subjects in each condition (Table 1b). This sample size (N=20) was used because previous rat experiments in the Grunberg Laboratory have revealed behavioral differences evaluating a variety of independent and dependent variables with similar samples (1; 37; 43; 44). Additionally, this modest sample size was used to accommodate laboratory equipment limitations and as a validation of the recommended HIFU parameters. Animal husbandry conditions, independent variables, dependent variables, experimental timeline, data analytic strategy, and power analyses are explained in detail below.

Animals and Housing

The subjects were 10 male and 10 female Sprague-Dawley (SD) rats from Charles River Laboratories (Wilmington, Massachusetts). Following a parallel TBI study design (7; 100), rats were individually housed in standard polycarbonate shoebox cages (42.5 x 20.5 x 20 cm) with hardwood chip bedding (Pine-Dri). There were no other interactive stimuli in the cage. Social and novel stimuli isolation was used to decrease the possible confounding influence on the experimental variables. Although social exposure (i.e., pair housing) or the inclusion of novel stimuli (e.g., toys) might be relevant for face validity in modeling blast exposure, the introduction of these confounding variables decreases experimental control for identifying the impact of the independent variables (16; 17; 33).

Rats were approximately 55 days old upon arrival. This age, which corresponds to the end of rat adolescence, was used to maintain consistency with previous mTBI research in the Uniformed Services University Center for Neuroscience and Regenerative Medicine and to model approximate age demographics of young adult U.S. Service Members (48; 72; 84). The SD strain was selected because this strain is commonly used for animal research and to be comparable to ongoing blast TBI (bTBI) animal experiments (2; 48; 61; 67). Because sex differences were hypothesized from previous rodent research conducted by the Grunberg Laboratory, male and female rats were used in the present experiment (4; 5; 9; 16; 17; 100). The inclusion of male and female subjects in the experimental design allows for interaction analyses that reveal information about the role of sex on the other experimental variables.

Cages were changed twice a week by Laboratory Animal Medicine (LAM) husbandry staff to maintain ethical and hygienic standards, to maintain uniformity of living conditions, and to decrease environmental stressors (75). Subjects had *ad libitum* access to standard laboratory chow (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. The housing room was maintained at 23°C with 40% relative humidity on a 12 hr reverse light cycle (0600-1800 lights off). A reverse light cycle was used because rats are nocturnal animals, and the behavioral trials were conducted during the animals' active hours.

The rats were handled and experiments conducted under red lighting, which allows experimenters to maneuver in the experimental rooms, but the albino rats are undisturbed by the lighting due to the pigmentation of their eyes (i.e., they only minimally perceive the light source). Prior to data collection with the subjects, each

animal was gentled by trained experiments. The gentling procedure involves handling the rats once per day for approximately 5 minutes per rat to desensitize them to and decrease the effects of transport from cage to the behavioral assays. The experiment was conducted under a protocol approved by USUHS Institutional Animal Care and Use Committee (MPS-09-732; Biobehavioral assessments of traumatic brain injury in rats) and was conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (58).

Independent Variables

There were two independent variables in this study:

(1) injury (no HIFU, HIFU),

(2) and sex (male, female).

There was one within-subject independent variable in this study:

(1) time (Baseline, Post-Injury)

High Intensity Focused Ultrasound (HIFU)

Only rats in the mTBI experimental condition (HIFU) were transported from their housing room to the injury room within the same housing facility on a metal transport cart; rats in the No HIFU condition did not undergo the following procedures. The following procedures are derived from communication with the Center for Neuroscience and Regenerative Medicine HIFU technician (L. Fortin, personal communication, September 29, 2011). The HIFU rats were anesthetized in a sealed, rectangular, Plexiglas box (4.5% isofluorane mixed with oxygen for 3 min) before having the hair removed from their heads with an electric razor and a depilatory cream (Nair, Church & Dwight Co, Princeton, NJ). The shaved portion of the rat's head encompassed an area of

approximately 2.5 cm x 2 cm (Figure 1). Ultrasound transmission gel (Aquasonic, Parker Laboratories, Fairfield, NJ) was placed between the cone membrane and the skin of the animal's head to reduce HIFU signal attenuation. The anesthetized rats were held stable with one hand while the experimenter placed the polyurethane membrane of the cone transducer (64 mm Focused Transducer, Model H-101, Sonic Concepts, Bothell, WA) in contact with the center of the rat's head in the shaved region (Figure 2). The HIFU was initiated by the technician (L. Fortin) starting the software that controlled the injury (details below), and the rat was placed back in its cage at the end of the procedure. The HIFU exposure process was conducted in the following order: (1) anesthesia (approximately 30s); (2) hair removal (approx. 60s); (3) return to anesthesia (approximately 30s); (4) HIFU (approximately 10s, including 10ms of actual HIFU exposure); (5) return to cage with a heating pad for recovery. The entire process from anesthesia to cage-return lasted approximately 2 - 4 minutes per animal, and all HIFU injuries occurred from 1230-1315.

Each animal was examined 10 minutes post-injury to assess for appropriate recovery from anesthesia; all rats recovered normally within that time frame. Normal recovery consisted of awakening from anesthesia and voluntary and controlled locomotion within the home cages. The rats were then returned to the housing room and were allowed 24 hours before beginning behavioral testing the next day. A pea-sized quantity of lubricating ointment (Artificial Tears Ointment, 15% Mineral Oil, 83% White Petrolatum, Webster, Sterling, MA) was administered to cover the shaved portion of each rat's head once per day for the remainder of the experiment to prevent scalp irritation from the hair removal and HIFU injury.

The HIFU system is comprised of the following equipment: 64 mm Focused Transducer, Model H-101, Sonic Concepts, Bothell, WA; Impedence Matching Network, Sonic Concepts, Bothell, WA; RF Power Amplifier A300, Electronics & Innovation, Rochester, NY; AFG 3102 Function Generator, Tektronix, Beaverton, OR; and DPO 3054 Digital Phosphor Oscilloscope, Tektronix, Beaverton, OR (Figure 3). The computer program LabView Signal Express Tektronix Edition (National Instruments, Austin, TX) was used to create a waveform with the following properties: a sine waveform with a frequency of 1.1 MHz (the operating frequency of the transducer) and amplitude of 0.4 Volts peak-to-peak (Vpp). This sine waveform was modulated with an exponential decay waveform with a decay rate of 0.25 and an AM frequency of 100 Hz to produce a decaying blast waveform with a duration of 10 ms. The parameters of 10 ms duration with 0.4 mVpp amplitude were selected to model the exposure time and intensity of an explosive blast wave (22). These parameters were also selected following consultation with the primary researcher responsible for the HIFU apparatus (J. McCabe, Ph.D.). The transducer has a removable cone housing which is filled with degassed water (i.e., water with the gases removed) and capped by a polyurethane membrane. The acoustic waveform produced by the transducer is focused at the plane of the cone's tip, which is held in contact with the rat's shaved head during treatment (L. Fortin, personal communication, September 29, 2011).

Sex

This experiment examined behavioral and biological differences in male and female rats following HIFU exposure. Because of noted human sex differences in response to TBI (10; 73), and in light of recent experimental evidence showing sex

differences in response to blast injury for rodents (7; 9; 100), the present experiment continued to examine sex differences in response to HIFU. Utilizing male and female rats to determine if there are any sex differences in behavior following HIFU follows similar rationale presented in the work of Yarnell (100), specifically: (1) males and females receive traumatic brain injury in numerous, varied settings; (2) information from this study may extend to individuals suffering from TBI induced by other means; and (3) and treatment strategies for TBI may be informed by research findings showing sex differences.

Dependent Variables

The dependent variables were: a measure of neurobehavioral functioning (NSS-R); open-field locomotor activity (OFA); and nociception (hot plate). These measures were included based on previous research and their ability to discriminate behavioral and neurological differences resulting from injury in previous rodent blast TBI (48) and mild TBI work (83; 102). Figure 4 shows the timeline of the experiment. All behavioral assays occurred between the hours of 1300-1700.

Neurobehavioral Severity Scale – Revised (NSS-R)

The Revised Neurobehavioral Severity Scale (48), based on the NSS (51; 65; 81), is a sequence of behavioral tests and observations. This summary of the methods is based on the work of Yarnell (100). The tests assess reflex suppression, general movement, and postural adjustments in response to a challenge. The NSS-R has ten individually-scored components, each with a possible score ranging from zero to two, where zero represents a normal response and one and two indicate increasingly impaired responding (Figure 5). It is noteworthy that the NSS-R uses a three-point scoring scale for each task, whereas

previous scales used a binary (normal, impaired) response. This three point rating increases sensitivity and allows discrimination among different levels of impairment. The NSS-R was scored solely by the lead investigator of the project to increase the reliability of the ratings. The rater's scoring protocol and capability was confirmed on previous experiments using multiple raters of the NSS-R (48; 100). The experimental equipment was cleaned with a 35% alcohol solution between each animal.

As described in Yarnell (100), the testing was conducted using two empty polycarbonate cages (46 cm × 36 cm × 20 cm) with no bedding or lid (Figure 6). The larger cage was used for easier manipulation of the animal and adequate viewing room for the rater. There were two NSS-Rs conducted in this experiment as a within-subject measure: the baseline conducted three days before injury and the post-injury assessment conducted one day after injury (Figure 3). The two assessments were conducted to account for possible individual animal differences. A general description of each measure is listed below based on Grunberg and colleagues (48), and greater detail of each test can be found in Yarnell (100).

General balance test. The rat's ability to walk on a balance beam is assessed first. The rat is gently placed onto a balance beam and observed, and its ability to maneuver is scored.

Landing test. The rat's ability to land appropriately in response to a drop from a height of 29 cm above the test cage floor is observed and evaluated.

Tail raise test. Following the landing test, the rat is gently lifted by the base of the tail above the cage floor, and its reflexes (extension of forelimbs and hindlimbs) are observed

and scored. After the tail raise test, the rat is taken to the second cage to continue the procedure.

Drag test. The rat is carefully dragged across the floor of the cage by the base of its tail at a constant speed with its hindpaws above the floor, resulting in it dragging its forepaws. The rat's behavior during this movement is observed and scored.

Righting reflex. The rat is held still on its back and then released. The rat's reaction to being held upside-down is observed and scored.

Ear reflex. The ear reflex is tested by lightly touching the ear with the cotton-covered tip of a long Q-tip and observing responses.

Eye reflex. The eye reflex is tested by lightly touching the eye with the cotton-covered end of a Q-tip and observing the response.

Sound reflex. This reflex is a movement in response to the noise of a short, sharp clap of the experimenter's gloved hands. The reflex is observed and scored in response to this noise.

Tail reflex. This reflex is tested by applying a brief pinch using the experimenter's thumb and index finger to the middle area of the rat's tail (approximately 2 cm from the base of the tail) and observing its response. The reflex is observed and scored in response to this physical stimulus.

Paw flexion reflex. This reflex is tested by briefly applying a pinch with the experimenter's fingers to the hind paw and observing whether a withdrawal response is elicited.

Open field locomotion

Locomotor activity (OFA) measures naturally occurring behaviors that are exhibited when an animal explores and interacts with its surroundings. OFA provides movement data for each subject, and some of these specific movements are relevant for psychological conditions such as anxious and depressive behaviors (14; 32; 36-38; 44; 69). For the purposes of this experiment, six variables were extracted from each animal's movement within the chambers: total distance moved (OFA – TD), total movement time (OFA – MT), horizontal movement (OFA – HA), vertical movement (OFA – VA), time spent in the center of the chamber (OFA – CT), and time spent in the center as a proportion of the total movement time for the trial (OFA – CT/MT). These variables provide information about general health, gross motor performance, depressive-like behavior, and anxiety-like behaviors.

OFA was measured using Accuscan Superflex Sensor Version 2.2 infrared photocell system in the Accuscan Instruments Standard Animal Cage (measuring 40 x 40 x 30 cm; Accuscan Instruments Incorporated, Columbus, OH) located in a dedicated room designed to minimize acoustic interruptions. The Standard Animal Cage is constructed of Plexiglas with a ventilated, removable Plexiglas lid that prevents the animal's escape during the trial but allows adequate airflow (Figure 7). The animal's locomotion is captured by three, paired 16-photocell Superflex Sensors which transmit the location data to the Accuscan Superflex Node located on the upper-rear of the chamber. The Superflex Node transmits the OFA data to the computer through a universal serial bus connection (USB). The data from the sixteen chambers is processed and aggregated by Accuscan Fusion Software (Version 3.4) on a computer located within

the test room. The open field activity of each rat was measured for 1 hour during its active period. The OFA equipment begins recording data immediately following the rat's entry into the chamber, and the experimenter exited the test room and turned off the light once each rat was placed into its respective chamber. Following the completion of the test, the rat was returned to its home cage and the test chambers were cleaned using a Clidox-S solution. OFA was conducted with the baseline assessment three days before the injury and the post-injury assessment occurring one day after the injury as depicted in the experimental timeline (Figure 4).

Hot Plate

The hot plate is used to test nociceptive response latency in rats (4; 5; 12; 15). The test measures the latency of a rat to lick its hind paw in response to a noxious, thermal stimulus. The hind paw lick is a supraspinally-mediated response (i.e., not a reflex) to the aversive stimulus and involves sensory and motor cortical areas. The hot plate latency (HP) ranges from 0 to 60 seconds, with a longer time indicating decreased sensitivity to the thermal stimulus.

This assay was conducted using an Omnitech Electronics Inc Hotplate (Model HP). The instrument's metal plate (measuring 26.5 x 26.5 cm) is heated to 51ºC, and the plate is enclosed by plexiglass on all sides with an opening in the top through which the animal is placed at the start of the trial and retrieved at its conclusion (Figure 8). This temperature is hot enough to be aversive but will not cause tissue damage to the rat during the trial (4; 5; 12; 15). The trial begins when a single rat is placed on the hot plate, and an observing experimenter simultaneously depresses a foot pedal that begins the digital timer on the hot plate. The rat remains on the hot plate until it licks its hind paw

or for a maximum of 60 seconds. When one of these conditions has been met, the rat is immediately removed from the hot plate and the observing experimenter lifts his or her foot from the foot pedal to stop the digital timer. Each animal was assessed twice using hot plate; the baseline response latency was collected three days prior to the injury, and the post-injury assessment occurred three days after the injury. The hot plate apparatus is cleaned between each trial using a 35% alcohol cleaning solution to ensure no debris or waste interferes with the trial.

Experimental Timeline

On the day that rats arrived, they were singly housed and randomly assigned to an experimental condition. Rats were then handled or "gentled" by the Experimenters for two days to get the animals used to being handled prior to beginning the experiments. The gentling process involves holding and stroking the rats for approximately 5 minutes per rat to desensitize them to the experience of being handled by researchers. On the second day of gentling, the rats were acclimated to the open field chamber for one hour. Baseline OFA, HP, and NSS-R measures were conducted on the third day after arrival. The HIFU injury was conducted about one week after arrival, and no other behavioral measures were collected that day. Post-HIFU behaviors were collected for OFA and NSS-R the day following the HIFU injury, and Post-HIFU HP was collected three days following HIFU. All animals were euthanized on the fourth day following injury in accordance with IACUC-approved standards. Figure 4 presents the experimental timeline.

Data Analytic Strategy

Repeated-measures analyses of variance (ANOVA) were conducted using both independent variables (sex, injury) for both times (BL, PI) of each of the dependent variables (NSS-R, HP, OFA – MT, OFA – HA, OFA – VA, OFA – CT, and OFA – CT/MT) to examine the overall experimental model. Based on apparent baseline differences in the results, analyses of covariance (ANCOVA) were conducted for the overall model including both independent variables (sex, injury) and conducted separately by sex controlling for baseline measurements to look for post-injury differences.

Internal analyses were conducted using ANCOVAs controlling for baseline value that compared injury separated by sex. Additional internal analyses consisted of conducting 2 (baseline, post-injury) x 2 (control, HIFU) repeated-measures ANOVAs independently for males and females. Internal analyses consisted of conducting independent-samples t-tests on the difference score (baseline minus post-injury score) with injury as the independent variable and each change score from the dependent variables. These internal analyses were conducted based on significant findings from previous research (e.g., Yarnell, 2012). OFA scores were separated into five subscales: MT, HA, VA, CT, and CT/MT. All tests were two tailed using alpha = 0.05.

CHAPTER 5: Results – Experiment 1

The significant results and trends towards significance for each dependent variable are presented below. If no significant results were obtained, then the null findings are noted. The primary repeated-measures ANOVA is presented first, followed by the overall model ANCOVA. ANOVAs and ANCOVAs separated by sex are presented next. The independent-samples t-tests are presented last. The results from primary statistical analyses are presented in Appendix B.

Neurological Severity Scale – Revised (NSS-R)

Figure 9 presents the descriptive statistics for male and female rats in NSS-R. No apparent or significant differences were found in the initial repeated-measures ANOVA. There were no main effects and no interactions identified.

After adjusting for baseline NSS-R scores, no main effects for sex or injury were found in the ANCOVA. A trend toward a significant sex*injury interaction was revealed ($F(1, 15) = 4.058$, $p = .062$). The interaction showed impaired performance for female-HIFU rats compared with control and male-HIFU animals. No significant main effects were found in either the female or male ANCOVA.

Hotplate

Figure 10 presents the descriptive statistics for male and female rats in Hotplate. A statistically significant sex difference was found indicating longer latencies for female rats ($F(1, 16) = 5.861$, $p = .028$). This finding is consistent with the literature on nociceptive responses in male and female rats (8; 23).

After adjusting for baseline hotplate scores, neither main effects (sex or injury) nor an interaction of sex*injury were found in the ANCOVA. Internal analyses revealed no further significant findings.

OFA – Movement Time

Figure 11 presents the descriptive statistics for OFA – MT. Visual inspection of descriptive graphs suggested differences in movement time with possible differences for males between baseline and post-injury and possible sex differences with females showing greater overall MT. There was a within-subject effect for Time (Baseline to Post-Injury; $F(1, 16) = 18.625$, $p = .001$) with averages indicating greater movement time post-injury compared with baseline. Additionally, there was a between-subjects effect of sex ($F(1, 16) = 7.045$, $p = .017$), with females moving more than males, and a between-subjects interaction of sex*injury ($F(1, 16) = 4.652$, $p = .047$). This interaction showed increased movement time for the female-HIFU rats compared with the other three groups.

The overall ANCOVA found no main effects for sex or injury and no interaction of sex*injury. Internal analyses revealed a significant main effect for time in the male ANOVA ($F(1, 8) = 21.040$, $p = .002$) with MT increasing post-injury. A trend towards significance was found for time ($F(1, 8) = 3.816$, $p = .087$) with MT increasing post-injury and injury ($F(1, 8) = 4.238$, $p = .074$) with HIFU-exposed rats trending toward greater overall MT in the female ANOVA. No other significant results or trends towards significance were found.

OFA – Horizontal Activity

Figure 12 presents the descriptive statistics for OFA – HA. Apparent differences in the sex and injury conditions were supported by the initial repeated-measures

ANOVA. There was a within-subject effect for Time (Baseline to Post-Injury; $F(1, 16) = 13.377$, $p = .002$) with averages indicating greater horizontal activity post-injury compared with baseline. Additionally, there was a between-subjects effect of sex ($F(1, 16) = 14.720$, $p = .001$), with females showing more horizontal activity than males, and a between-subjects interaction of sex*injury ($F(1, 16) = 6.043$, $p = .026$). This interaction showed a greater increase in HA for male-control rats compared with the other three groups.

After adjusting for baseline HA scores, neither main effects (sex or injury) nor an interaction of sex*injury were found in the ANCOVA. Internal analyses revealed a significant main effect of time ($F(1, 8) = 17.678$, $p = .003$) for the male ANOVA. A main effect for injury ($F(1, 8) = 5.485$, $p = .047$) was found for the female ANOVA, with HIFU-exposed animals showing increased HA compared with control. No other significant results or trends towards significance were found.

OFA – Vertical Activity

Figure 13 presents the descriptive statistics for OFA – VA. There was a within-subject effect for Time (Baseline to Post-Injury; $F(1, 16) = 11.991$, $p = .003$), with averages indicating greater vertical activity post-injury compared with baseline. Additionally, there was a between-subjects effect of sex ($F(1, 16) = 4.543$, $p = .049$), with females showing more vertical activity than males, and a between-subjects interaction of sex*injury ($F(1, 16) = 4.539$, $p = .049$), with control males showing the greatest increase in VA and the HIFU-exposed females demonstrating an attenuated VA increase. After adjusting for baseline VA scores, no main effects for sex or injury and no interaction of sex*injury was found in the ANCOVA.

Internal analyses revealed a trend towards significance for the main effect of injury ($F(1, 8) = 3.971$, $p = .081$) in the female ANOVA. After adjusting for a baseline difference in the male ANCOVA, a main effect of injury ($F(1, 7) = 5.717$, $p = .048$) remained for males, such that HIFU-exposed males showed an attenuated increase in VA compared with control animals.

OFA – Center Time

Figure 14 presents the descriptive statistics for OFA – CT. There was a within-subject effect for Time (Baseline to Post-Injury; $F(1, 16) = 6.059$, $p = .026$), with averages indicating greater center time post-injury compared with baseline. No between-subjects effects or interactions were found for OFA – CT.

The overall ANCOVA found no main effects for sex or injury and no interaction of sex*injury. Internal analyses revealed a significant main effect of time ($F(1, 8) = 5.854$, $p = .042$) for the female ANOVA with both HIFU and control subjects showing increased CT post-injury. A trend towards a significant time*injury interaction ($F(1, 8) = 4.256$, $p = .073$) was found for the male ANOVA, suggesting increased CT for control males compared with slightly decreased CT for HIFU-exposed males post-injury.

OFA – Center Time / Movement Time

Figure 15 presents the descriptive statistics for OFA – CT/MT. A significant between-subjects effect of sex ($F(1, 16) = 7.156$, $p = .017$) was found for CT/MT, indicating males spent a significantly greater proportion of their total movement time in the center compared with females. No other significant results were found.

The overall ANCOVA found no main effects for sex or injury and no interaction of sex*injury. Internal analyses revealed no further significant findings.

CHAPTER 6: Evaluation of Hypotheses – Experiment 1

Specific Aim 1: To characterize the behavioral phenotype of high intensity focused ultrasound exposure.

Hypothesis 1a: The hypothesis that HIFU exposure will result in impaired performance on neurobehavioral measures **was not confirmed**. HIFU and non-HIFU exposed rats had similar NSS-R scores when controlling for baseline differences.

Hypothesis 1b: The hypothesis that HIFU exposure will result in different performance on measures of nociceptive response latency **was not confirmed**. No main effect of HIFU for hotplate was detected in this study.

Hypothesis 1c: The hypothesis that HIFU exposure will result in altered behavior patterns on measures of locomotor activity **was partially supported**. Results for vertical activity showed a significant main effect for injury when controlling for baseline vertical activity. These results are not likely the result of impaired motor functioning, given the absence of an effect of HIFU alone on neurobehavioral performance. No other significant main effects for injury were identified.

Specific Aim 2: To compare male and female responses to high intensity focused ultrasound exposure.

Hypothesis 2a. The hypothesis that HIFU will impair female rats to a greater degree than males, detected using measures of neurobehavioral functioning **was not confirmed**. A trend towards statistical significance was detected ($p = .062$), which may suggest further exploration is needed to detect an interaction effect, but the finding did not meet criteria of alpha = .05 for statistical significance. The trend suggested that HIFU may impair females to a greater extent than males, but further support is needed.

Hypothesis 2b. The hypothesis that HIFU will impair female rats to a greater degree than males, detected using measures of nociceptive response latency **was not confirmed**. No sex by injury interaction was detected in the study.

Hypothesis 2c. The hypothesis that HIFU will result in altered behavior patterns to a greater degree for female rats than males, detected using measures of locomotor activity **was not confirmed**. Significant interactions of sex and injury were identified in the full factorial ANOVA models for movement time, horizontal activity, and vertical activity. However, these interactions were not statistically significant when controlling for baseline activity using an ANCOVA model. Further, these analyses suggested that male rats demonstrated a greater attenuation of VA following HIFU exposure than was seen in female rats, suggesting the effect of HIFU was greater for males on this measure than for females.

CHAPTER 7: Discussion of Experiment 1

The preliminary findings suggest that modest behavioral and psychologically-relevant differences resulting from HIFU injury to male and females rats may be detectable. A notable finding is the significant difference in vertical activity (OFA - VA) between males and females as well as between experimental conditions for the males. Vertical activity in the open field chamber is considered an index of depression-related behavior such that increased VA is associated with decreased depressive-like symptoms (77; 100).

An interaction was found in which female rats exposed to HIFU showed greater VA, indicating decreased depressive-like behavior compared with the other groups. Additionally, male rats in the HIFU condition showed decreased VA (i.e., greater depressive-like behavior) compared with control male rats following the HIFU procedure.

These VA finding have multiple possible interpretations, including: (1) HIFU is affecting balance, yielding decreased VA; (2) HIFU decreases general exploratory behavior; (3) HIFU increases depressive-like behavior. Given that no main effect for injury was found for neurobehavioral performance, HIFU is not likely affecting balance and the first explanation can be ruled out. Because no main effects of injury were found for movement time, horizontal activity, or center time, it is unlikely that HIFU is adversely affecting exploration or general locomotion. Therefore, one possible explanation that fits the results is that HIFU impacts vertical activity as it relates to depressive-like behavior. Further exploration is warranted to better parse out the data and determine if these findings in relation to depression-like behaviors remain significant.

Another interesting trend in the data is the sex*injury interaction for the neurobehavioral task. The trend in the data suggests that females may be more vulnerable to the adverse neurologic effects of HIFU intervention, such that HIFU-injured females may have a higher score on NSS-R (i.e., worse neurobehavioral performance) and that males may not be affected on neurobehavioral measures by the HIFU. A larger sample size will provide additional statistical power to support or refute this interaction.

Overall, the results of Experiment 1 suggest that there may be psychologically- and neurobehavioral-relevant effects of HIFU exposure. The findings from this experiment are promising enough to justify increasing the sample size and continuing to study the effects of high intensity focused ultrasound as an animal model of mild traumatic brain injury. However, the study was not without its limitations, and future iterations of the experiment can address these limitations to improve the procedure and the ability to attribute findings to HIFU exposure.

Limitations of Experiment 1

One limitation of Experiment 1 was the small sample size. The study was an exploration of effects of HIFU as a model of mTBI, and previous studies have yielded measureable results with similarly sized samples. The effects of HIFU are apparently more subtle than other animal models of TBI. In particular, some initial significant differences in repeated-measures ANOVAs were lost when controlling for baseline in the ANCOVAs. The statistical analyses indicated the study was underpowered (see Appendix B for complete listing of power estimates), which limits the ability to identify significant differences between groups and to draw definitive conclusions. Increasing the

number of subjects in each cell of the experiment will increase the likelihood of detecting differences between groups, even when controlling for baseline differences.

Another limitation of the Experiment 1 was the absence of a sham control. Experiment 1 cannot distinguish the impact of the hair removal and anesthesia process on behavioral performance independent of the HIFU intervention. Future iterations of this project should include an additional control group in which the rats are anesthetized, shaved, and exposed to the depilatory cream but not exposed to the HIFU to separate the possible unintended effects of the procedure from the intervention under investigation.

A third consideration to improve the experiment is to evaluate behavioral changes over a longer period of time. Experiment 1 had a single post-HIFU assessment that was conducted one-day following the intervention. Adding a follow-up assessment would allow for an evaluation of possible recovery from HIFU-induced changes over time, or for unidentified changes to become more pronounced over time.

Future Directions and Experiment 2

Experiment 1 provided a proof of concept that high intensity focused ultrasound (HIFU) impacts behavioral measures in male and female rats as well as demonstrated the logistical feasibility of the injury model. Three major changes were made to the experimental protocol to improve the study and address the identified limitations: (1) the sample size was increased; (2) a sham control condition was added; and (3) a follow-up timepoint was included.

CHAPTER 8: Overview and Specific Aims – Experiment 2

The present experiment was designed to examine effects of high intensity focused ultrasound on behavioral responses of male and female rats at two time points. There were two specific aims: (1) to characterize the effects of high intensity focused ultrasound exposure; and (2) to compare male and female responses to HIFU exposure.

Hypotheses

There were multiple hypotheses associated with each specific aim.

Specific Aim 1: To characterize the behavioral phenotype of high intensity focused ultrasound exposure one day and eight days post-exposure.

Hypothesis 1a. HIFU-exposed rats < Sham rats = Control condition rats on measures of neurobehavioral performance one day post-injury.

Hypothesis 1b. HIFU-exposed rats = Sham rats = Control condition rats on measures of neurobehavioral performance eight days post-injury.

Hypothesis 1c. HIFU-exposed rats < Sham rats = Control condition rats on measures of locomotor activity one day post-injury.

Hypothesis 1d. HIFU-exposed rats < Sham rats = Control condition rats on measures of locomotor activity eight days post-injury.

Rationale. As noted for Experiment 1, changes in neurobehavioral functioning and locomotor responses are useful models for human-relevant conditions following TBI. Additionally, the human TBI literature differs on the symptom timecourse following injury. Differing reports have indicated that some self-reported symptoms worsen over time (11; 34), although the Veterans Administration advises potential patients that, in the

majority of cases, symptoms are worse shortly after the injury and decrease in severity over time (93). In either case, it is important to assess for symptom change over time.

Although most rodent studies capture data at only a single time point following injury, recent studies have shown changes in behavior over time following blast exposure (100). Studies have found that psychologically-relevant behavioral measures differed between one and eight days following blast exposure (49; 100). However, the neurobehavioral functioning remained similar between the two times.

Specific Aim 2: To compare male and female responses to HIFU exposure one day and eight days post-exposure.

Hypothesis 2b. HIFU will impair female rats to a greater degree than males one day post-exposure, detected using measures of neurobehavioral performance.

Hypothesis 2b. HIFU will impair female rats to a greater degree than males eight days post-exposure, detected using measures of neurobehavioral performance.

Hypothesis 2c. HIFU will impair male rats to a greater degree than females one day post-exposure, detected using measures of locomotor activity.

Hypothesis 2d. HIFU will impair male rats to a greater degree than females eight days post-exposure, detected using measures of locomotor activity.

Rationale. The limited number of studies including male and female animal subjects suggests there are significant sex differences (4; 5; 7; 17; 32; 46; 100). Yarnell (100) found sex differences between the two time points with female rats showing impaired functioning on psychologically-relevant behavioral variables compared with males following blast exposure. This limited evidence justifies an exploration of the interaction of sex, injury, and time in the experimental design.

CHAPTER 9: Methods – Experiment 2

To address the above hypotheses, this experiment was conducted as a 3 (Control, HIFU, Sham Control) x 2 (male, female) x 2 (one day, eight days post injury) mixed within-between factorial design. This design results in six experimental groups with multiple time points and allows for analysis of interactions and main effects. There were 64 total subjects (Table 30). This sample size (N=64) was based on power estimates to detect significant differences using HIFU and to be comparable to other CNRM-sponsored animal studies of behavioral effects of models of mTBI (48; 100). While the majority of the experimental design was based on the methods of Experiment 1, deviations from the original design are explained in detail below.

Animals and Housing

The subjects were 32 male and 32 female Sprague-Dawley (SD) rats from Charles River Laboratories (Wilmington, MA). We used 10 animals per cell in the four cells replicated from Experiment 1 and 12 per cell in the new sham control cells because this sample size was estimated to show differences between experimental conditions. Additionally, the total sample size (N = 64) was based on the logistical limitations of the animal housing and experimental equipment to be feasible. Housing conditions were maintained identically to those in Experiment 1.

Independent Variables

There were two between-subjects independent variables in this study:

(1) injury (no HIFU, Sham HIFU, HIFU),

(2) and sex (male, female).

There was one within-subject independent variable in this study:

(1) time (Baseline, 1-Day Post-Injury, 8-Days Post-Injury)

High Intensity Focused Ultrasound (HIFU). Rats in the mTBI experimental groups were subjected to identical manipulations as described in Experiment 1 using the same equipment. The same experimenter performed the HIFU intervention to reduce inter-experiment variability.

Sham Control. Rats in the sham control condition were subjected to identical anesthesia and hair removal procedures as described for the HIFU injury; however, they were not subjected to the HIFU and were instead returned to their home cages immediately following hair removal. The sham control process was conducted in the following order: (1) anesthesia (approximately 30s); (2) hair removal (approx. 60s); (3) return to anesthesia (approx. 30s); (4) return to cage with a heating pad for recovery. The exclusion of HIFU from the Sham condition decreased the manipulation time by less than 30s per animal.

Each animal was examined within 5 minutes post-anesthesia to assess for appropriate recovery, and all rats recovered normally within that time frame. The rats were then returned to the housing room and were allowed 24 hours before beginning behavior the next day. A pea-sized quantity of lubricating ointment (Artificial Tears Ointment, 15% Mineral Oil, 83% White Petrolatum, Webster, Sterling, MA) was administered to cover the shaved portion of the rats' heads once per day for the remainder of the experiment to prevent scalp irritation from the hair removal.

Dependent Variables

The dependent variables were a measure of neurobehavioral functioning (NSS-R), open-field locomotor activity (OFA), and a measure of nociception (hot plate). These measures were included based on their ability to discriminate behavioral and neurological differences resulting from injury in previous rodent bTBI work and were conducted in the manner described for Experiment 1 (48). Figure 16 shows the timeline of the experiment, and all behavioral assays were conducted between the hours of 1300-1700.

Data Analytic Strategy

The data analytic strategy was guided by results from Experiment 1. Repeated-measures analyses of covariance (RM-ANCOVA) were conducted using both between-subjects independent variables (i.e., sex, condition), and the two post-injury times (one day, eight days), while controlling for baseline scores on the dependent variables for each of the dependent variables. Based on apparent differences in the results at post-injury time one, analyses of covariance (ANCOVA) were conducted for the dependent variables at time one while still controlling for baseline differences. All tests were two tailed using alpha = 0.05

CHAPTER 10: Results – Experiment 2

The results for each dependent variable are presented in this section. Significant values and values that approach significance are presented in the text; null findings are noted in the absence of significant results. The primary repeated-measures ANCOVA is presented first (controlling for baseline differences), followed by separate ANCOVAs for day one (T1-ANCOVA) and day eight (T2-ANCOVA). Results from primary statistical analyses are presented in Appendix B.

Neurobehavioral Severity Scale – Revised (NSS-R)

Figure 17 presents the descriptive statistics for male and female rats in NSS-R. A repeated-measures analysis of covariance (RMANCOVA) was conducted to identify main effects and interactions of the variables over time. After adjusting for baseline NSS-R scores, a trend towards a significant main effect for sex was found ($F(1, 57) = 3.414$, $p = .070$) with males showing higher scores compared with females, and the interaction of time*condition approached significance as well ($F(2, 57) = 2.852$, $p = .066$). This interaction trend suggests that control and HIFU-injured animal scores decrease by T2, but Sham controls showed increased NSS-R scores at T2. No other significant results were found in the RMANCOVA.

The T1-ANCOVA adjusted for baseline NSS-R scores, but no main effects or interactions were found. The T2-ANCOVA failed to identify any significant differences.

OFA – Movement Time

Figure 18 presents the descriptive statistics for male and female rats in MT. A repeated-measures analysis of covariance (RMANCOVA) was conducted to identify main effects and interactions of the variables over time. The analysis adjusted for

baseline MT scores, and no significant main effects of sex or condition were found, but time was significant ($F(1, 57) = 11.813$, $p = .001$) with increased MT over time. No other significant results were found in the RMANCOVA.

The T1-ANCOVA adjusted for baseline differences, but no main effects or interactions were found. The T2-ANCOVA failed to identify any significant values.

OFA – Horizontal Activity.

Figure 19 presents the descriptive statistics for male and female rats in HA. A repeated-measures analysis of covariance (RMANCOVA) was conducted to identify main effects and interactions of the variables over time. The analysis adjusted for baseline HA scores, and no significant main effects of sex or condition were found, but time was significant ($F(1, 57) = 10.178$, $p = .002$) showing increased HA over time. No other significant results were found in the RMANCOVA.

The T1-ANCOVA adjusted for baseline HA scores, but no main effects or interactions were found. The T2-ANCOVA also adjusted for baseline HA scores, but no main effects or interactions were found.

OFA – Vertical Activity.

Figure 20 presents the descriptive statistics for male and female rats in VA. A repeated-measures analysis of covariance (RMANCOVA) was conducted to identify main effects and interactions of the variables over time. The analysis adjusted for baseline VA scores. Time was found to be significant ($F(1, 57) = 20.569$, $p < .001$) with increased VA demonstrated over time, and the interactions of time*condition ($F(2, 57) = 3.210$, $p = .048$) and time*sex ($F(1, 57) = 4.870$, $p = .031$) were both statistically significant. The time*condition interaction showed a greater increase in VA from T1 to

T2 for Injury and Sham condition animals compared with control, and the time*sex interaction showed a greater increase in VA for males compared to females. No main effects of sex or condition were found in the analysis.

The T1-ANCOVA adjusted for baseline VA scores, and a trend towards significance was found for condition ($F(2, 57) = 2.667$, $p = .078$) with Sham showing greater VA than Injury, and Control showing greater VA than both Injury and Sham. The T2-ANCOVA adjusted for baseline VA scores, and a significant difference was found for sex ($F(1, 57) = 3.893$, $p = .053$) with males showing greater VA than females.

OFA – Center Time.

Figure 21 presents the descriptive statistics for male and female rats in CT. A repeated-measures analysis of covariance (RMANCOVA) was conducted to identify main effects and interactions of the variables over time. The analysis found no baseline difference between groups, and there were no between-subjects main effects or interactions. Time was found to be significant ($F(1, 42) = 7.922$, $p = .007$) with increased CT at T2, but none of the other within-subject contrasts were statistically significant.

The T1-ANCOVA adjusted for baseline VA scores, but no main effects or interactions were found. The T2-ANCOVA failed to identify any significant values.

OFA – Center Time / Movement Time

Figure 22 presents the descriptive statistics for OFA – CT/MT. A repeated-measures analysis of covariance (RMANCOVA) was conducted to identify main effects and interactions of the variables over time. The analysis found no baseline difference between groups, and there were no between-subjects main effects or interactions. Time was found to be significant ($F(1, 42) = 14.257$, $p < .001$) with increased CT/MT at T2.

The T1-ANCOVA adjusted for baseline CT/MT scores, but no main effects or interactions were found. The T2-ANCOVA failed to identify any significant values.

Hot Plate (HP)

Figure 23 presents the descriptive statistics for male and female rats in HP. An ANCOVA was conducted to evaluate the main effects and interactions of the between-subjects variables. The analysis adjusted for baseline HP scores, and an expected significant sex difference was found ($F(1, 57) = 14.057$, $p < .001$) with females showing longer HP latency than males, but no differences were identified for condition or the interaction of the variables.

CHAPTER 11: Evaluation of Hypotheses – Experiment 2

Specific Aim 1: To characterize the behavioral phenotype of high intensity focused ultrasound exposure one day and eight days post-exposure.

Hypothesis 1a. The hypothesis that HIFU-exposed rats < Sham rats = Control condition rats on measures of neurobehavioral performance one day post-injury **was partially supported**. The trend towards a significant interaction of time*condition ($F(2, 57) = 2.852$, $p = .066$) suggests neurobehavioral performance differs based on HIFU exposure.

***Hypothesis 1b*.** The hypothesis that HIFU-exposed rats > Sham rats = Control condition rats on measures of neurobehavioral performance eight days post-injury **was partially supported**. The trend towards a significant interaction of time*condition ($F(2, 57) = 2.852$, $p = .066$) suggests neurobehavioral performance differs based on HIFU exposure.

***Hypothesis 1c*.** The hypothesis that HIFU-exposed rats < Sham rats = Control condition rats on measures of locomotor activity one day post-injury **was partially supported**. The significant time*condition interaction ($F(2, 57) = 3.210$, $p = .048$) as well as the trend towards significance for the main effect of condition at T1 ($F(2, 57) = 2.667$, $p = .078$) support this hypothesis. No other support was found in the locomotor activity.

***Hypothesis 1d*.** The hypothesis that HIFU-exposed rats < Sham rats = Control condition rats on measures of locomotor activity eight days post-injury **was partially supported**. The significant time*condition interaction ($F(2, 57) = 3.210$, $p = .048$) supports this hypothesis. No other support was found in the locomotor activity.

Specific Aim 2: To compare male and female responses to HIFU exposure one day and eight days post-exposure.

Hypothesis 2a. The hypothesis that HIFU will impair female rats to a greater degree than males one day post-exposure, detected using measures of neurobehavioral performance **was not confirmed**. No interaction of sex*condition was found one day post-exposure on neurobehavioral performance.

Hypothesis 2b. The hypothesis that HIFU will impair female rats to a greater degree than males eight days post-exposure, detected using measures of neurobehavioral performance **was not confirmed**. No interaction of sex*condition was found eight days post-exposure on neurobehavioral performance.

Hypothesis 2c. The hypothesis that HIFU will impair male rats to a greater degree than females one day post-exposure, detected using measures of locomotor activity **was not confirmed**. No interaction of sex*condition was found one day post-exposure for locomotor activity.

Hypothesis 2d. The hypothesis that HIFU will impair male rats to a greater degree than females eight days post-exposure, detected using measures of locomotor activity **was not confirmed**. No interaction of sex*condition was found one day post-exposure for locomotor activity.

CHAPTER 12: Discussion of Experiment 2

This experiment used a rat model to determine how high intensity focused ultrasound exposure alters behavior. Three independent variables were manipulated in this experiment: (1) injury (no HIFU, HIFU, sham HIFU); (2) sex (male, female); and time (one day and eight days post injury). The high intensity focused ultrasound (HIFU) model of blast effects on neurological functioning was used to induce traumatic brain injury. The dependent variables were a measure of neurobehavioral functioning (NSS-R) and indices of mental health related behaviors (including anxiety-like and depression-like behavior) using open-field activity. This work included within- and between-subjects statistical comparisons of behavior measured prior to and following injury. The following paragraphs discuss the specific aims, the findings in general, and the limitations of this experiment.

Commentary on Specific Aims

Experiment 2 had two specific aims with four hypotheses associated with each specific aim. Confirmation or failure to find confirmation was presented above. The following section provides discussion of the associated findings for each specific aim.

Specific Aim 1. With regard to Specific Aim 1, the effects of HIFU on behavior one day and eight days after exposure, Experiment 2 found limited statistical support for neurobehavioral changes and locomotor changes.

NSS-R. The overall results for NSS-R present a complicated picture. The repeated MANCOVA showed an interaction of time*condition that approached statistical significance ($F(2, 57) = 2.852$, $p = .066$). These results suggested that, at one day post-injury (T1), Sham = Control < HIFU, and at eight days post-injury (T2), Control < HIFU

< Sham, where higher scores indicate worse neurobehavioral performance. These results suggest that, immediately following injury, HIFU shows worse neurobehavioral performance than Control or Sham, and that both Control and HIFU show improved performance over time. However, Sham appeared to perform worse at T2 which is contrary to expected results.

The time*condition graphs also suggested different patterns based on sex. Data for the males suggested Control < Sham < HIFU at T1, with Control remaining stable; Sham showing slight worsening in performance; and HIFU showing improvement at T2 (Figure 17). Data for the females suggested no difference in performance between Control, HIFU, and Sham at T1, but Control and HIFU both improved while Sham worsened at T2 (Figure 17). A *post hoc* repeated MANCOVA was conducted to split the data by sex to determine if these apparent differences were statistically different. The *post hoc* repeated MANCOVA found no statistical differences for males, but the female results revealed a continued trend towards a significant time*condition interaction ($F(1, 28) = 3.208$, $p = .056$). However, the apparent difference is the result of worsened performance with the Sham condition rather than from effects of the HIFU procedure.

OFA. Vertical Activity (VA) provided additional support for this specific aim. The overall RMANCOVA found a significant time*condition interaction. This interaction showed HIFU < Sham < Control at T1 and found no differences at T2, indicating recovery to similar levels over time. The difference between Control and Sham suggests that the preparation for conducting HIFU (i.e., anesthesia and hair removal) does affect behavior; the difference between Sham and HIFU suggests that there is an additional effect of HIFU above and beyond the preparatory procedure, and

that HIFU adversely impacts VA, a measure of depression-like behavior, immediately following but not eight days post-HIFU exposure. Specifically, HIFU attenuates VA compared with control, and decreased VA approximates increased depressive-like behavior. Further, the absence of a significant difference between conditions for movement time and horizontal activity suggests that the change in VA is a result of the procedure and not a change in general motor behavior.

Specific Aim 2. There were no significant findings associated with Specific Aim 2, the effects of sex and HIFU exposure on behavior one day and eight days after exposure. Experiment 2 failed to identify an interaction of sex*condition or sex*condition*time that would support sex differences based on HIFU exposure.

Limitations - Experiment 2

One unfortunate limitation in this study was the loss of Center Time data for 15 female subjects. There was a technical error with the zone coding in the open field activity system, and the information that allows the calculation of this variable was irretrievably lost. This loss of data prevented a fully-powered analysis of this important mental health relevant (anxiety-like behavior) variable.

Another limitation of Experiment 2 is the findings that the analyses were still underpowered. Despite increasing cell sizes in accordance with estimated power, the effect size of the HIFU intervention may not have been statistically detectable with the present total count. Alternatively, failing to reject the null hypothesis (i.e., there is no effect of the HIFU intervention on the measured behaviors) may be the correct conclusion, and no increase in the subject count would result in statistically significant differences between conditions.

CHAPTER 13: General Discussion

These experiments used a rat model to determine how high intensity focused ultrasound (HIFU) exposure alters behavior. These experiments demonstrated the logistical and methodological advantages of the procedure for behavioral experiments in comparison with other rodent models of brain injury, namely: HIFU is a non-surgically invasive procedure; it is non-resource intensive; it is easily administered to subjects; and it results in decreased subject mortality rates. Additionally, the HIFU intervention showed some modest behavioral effects that are relevant for psychological conditions following brain injury, specifically: there may be an impact on neurobehavioral functioning following HIFU exposure and there may be an increase of depression-related behavior following exposure to HIFU. However, these results were not on par with behavioral findings using the traditional, albeit less logistically favorable, models of brain injury.

These modest to null findings lead to a variety of possible questions regarding future directions of HIFU experimental use. First and foremost, the null findings may indicate that HIFU, as utilized in these studies, has no appreciable effect on psychologically relevant behaviors in rodents. The current studies are limited in that there were no parallel biological or histopathological markers recorded to determine if behavior and biology were correlated. So it is possible that there were biological changes without behavioral changes that were missed in the current study design. One possible future direction would be to incorporate biological assays (e.g., corticosterone, prolactin) into the design and replicate the experiments exactly.

An exact replication is not ideal, as the purpose of the study was to identify and characterize behavioral changes resulting from HIFU exposure, and the current experimental design did not produce the hypothesized results. Given the absence of strong behavioral findings, histopathological and biological findings would not be directly relevant for the primary study question (i.e., does HIFU impact behavior).

An alternative to an exact replication of the study that would preserve the logistical advantages of the HIFU intervention would be to alter the blastwave parameters. Given the possible range of HIFU blast duration from 0 ms to 30 seconds and the amplitude range from 0 mVpp to 900 mVpp, there is a great deal of room for additional exploration. Changes in the parameters would allow for a study to determine if there are ideal HIFU parameters to achieve varying levels of functional physiological impairment in rodents that would be convergent with the behavioral and pathophysiological findings of other animal injury models. The disadvantage to changing the parameters is the loss of blastwave comparability that the HIFU settings currently model.

Wahab and colleagues (95) note that the HIFU parameters can be adjusted to create a pressure amplitude similar to that of a blast tube, but a single pulse has significantly less energy than a true blast. Ensuring mechanical action instead of cavitational or thermal damage is important for HIFU exposure to appropriately model mTBI due to blast, but this experiment may have failed to find statistically significant differences between conditions because the energy of the single HIFU blast could not create a big enough effect

Another disadvantage to simply changing the waveform characteristics of the HIFU is the question of the physics involved with the model. One of the notable concerns regarding the experimental manipulation was the process for administration of the high-intensity focused ultrasound. Currently, there is no manipulation check to ensure uniformity when placing the transducer cone on the rat head. Despite best efforts of laboratory personnel, miniscule variations in pressure or angle of the cone on the head might result in altered HIFU blast exposure.

Additionally, the HIFU was administered through the animals' intact skulls. Although HIFU waves travel well through soft tissue, they do not readily transmit through solid media such as bone (50; 63; 85). The HIFU waves are absorbed, reflected, and refracted when transmitted through bone. There exist complicated phase corrections and magnetic resonance imaging based procedures that correct for the signal aberrations resulting from the skull, but these procedures are expensive and require advanced technical knowledge and equipment (50; 85). The impact of the rats' skulls on the HIFU exposure cannot be accurately accounted for in this experiment. This variable leads to the possibility that different animals received exposure to the HIFU in different relative "doses" in different regions of their brains, depending on individual differences in skull thickness, uniformity, and minor variations in the angle of the transducer cone.

Theoretically, the HIFU injury could be more accurately and more directly applied to subcranial tissue through the use of a craniotomy. Unfortunately, this experimental change would result in the loss of much of the logistical and methodological advantages associated with the procedure as currently practiced. There would be minimal value added to performing this complex procedure that might not

produce comparable injuries to other invasive rodent models of traumatic brain injury (e.g., controlled cortical injury, fluid percussion). There is no obvious advantage and multiple clear disadvantages to changes these procedures. A simpler alternative is to abandon high intensity focused ultrasound as a model of traumatic brain injury at the organismal level and focus on refining and expanding the use of established models such as blast overpressure.

Future Directions

There are a number of possible logistical or methodological changes that could be made to the experimental design to increase the likelihood of significant behavioral findings using high intensity focused ultrasound. Specifically, future experiments could include: (1) multiple HIFU blast exposures; (2) include MRI as a manipulation check to ensure the animals in the intervention conditions are receiving equivalent exposure to and brain injury from the HIFU; or (3) alter the procedure to apply the HIFU directly to subcranial tissue rather than direct it through bone. Despite these possible experimental changes, a more feasible alternative is to abandon HIFU for the purposes of identifying behavioral changes at the level of the organism.

There exist multiple models of traumatic brain injury that yield behavioral changes (e.g., blast overpressure, fluid perussion). Based on these experiments, HIFU does not serve this function well. Given this researcher's training as a scientist practitioner, an ideal future direction would be to focus on possible novel interventions for existing clinical populations suffering from traumatic brain injury and other behavioral and psychological disorders.

CHAPTER 14: Summary

This present work attempted to characterize the behavioral effects of high intensity focused ultrasound (HIFU) on male and female rats at two time points. The current iteration of the experiment provides information on one novel rodent model of military-related, blast-induced traumatic brain injury. These experiments provide some modest support for the behavioral effects of HIFU; however, there are numerous limitations that may have contributed to the dearth of significant results.

Additional research could be conducted to examine: (1) histopathological changes resulting from HIFU; (2) behavioral effects from multiple HIFU blasts; (4) behavioral changes resulting from HIFU blasts using different parameters; or (4) behavioral effects of HIFU administered directly to subcranial tissue. Given the significant methodological changes needed to pursue these alternative experimental designs, it is recommended that traditional models of animal traumatic brain injury, such as blast overpressure, fluid percussion, and controlled cortical impact, be utilized until there is sufficient evidence to support the utility of HIFU for behavioral research.

CHAPTER 15: Conclusions

The present work provided limited support for behavioral changes resulting from high intensity focused ultrasound exposure. However, the findings are not robust enough to support further pursuit of this experimental model of traumatic brain injury.

APPENDIX A – FIGURES

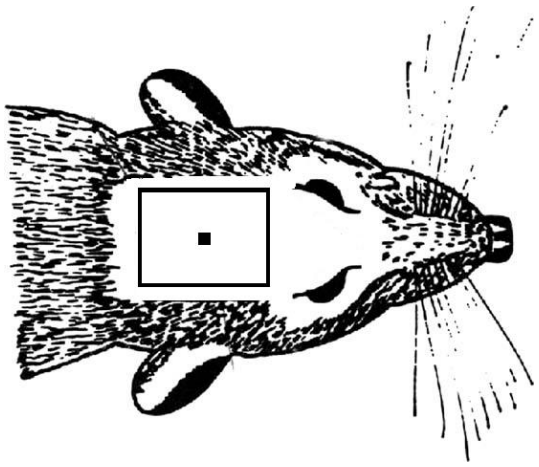

Figure 1. Illustration of the shaved rat head with cone transducer placement indicator.

Figure 2. HIFU injury administration equipment.

***Note*. From left to right: anesthesia chamber, hair removal equipment, and HIFU transducer cone.**

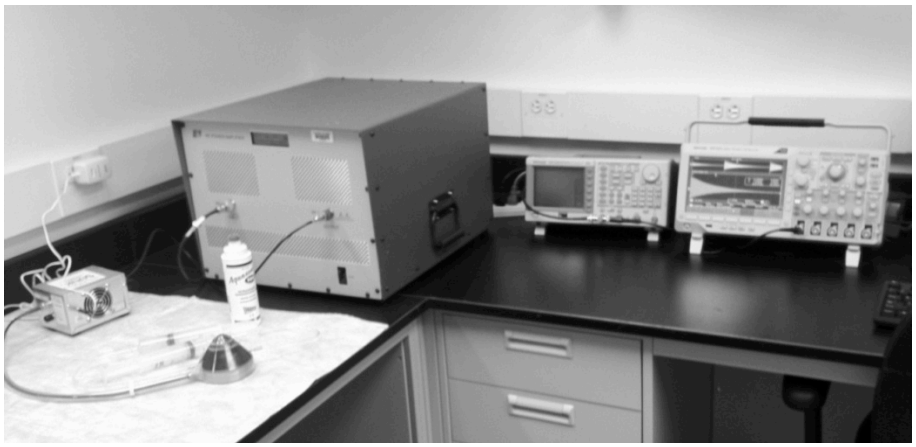

Figure 3. HIFU parameter equipment.

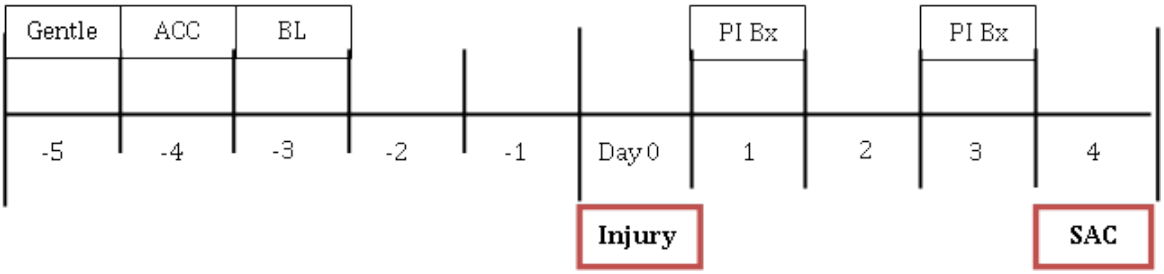

Figure 4. Experiment 1 timeline.

Note. Gentling (Gentle) was conducted prior to behavioral assays on day -5. The acclimation phase (ACC) was conducted on day -4. The baseline measurements (BL) for OFA, HP, and NSS-R were gathered on day -3. The post-injury data (PI Bx) were collected for OFA and NSS-R on day 1, and the post-injury data were collected for HP on day 3. The rats were sacrificed (SAC) on day 4. On the sacrifice day, the rat brains were harvested for study, and trunk blood was collected for additional analyses.

NSS-R

DATE Time _____ Rater _____

*Please circle one of the values per subject

SUBJECT # _____

1. General Balance

0	1	2
balance & walk	balance no walk	no balance/ fall

2. Landing Test

0	1	2
normal landing	partial compromised	no reflex/ falls flat

3. Tail Raise Test

0	1	2
normal reflex	partial/ weak reflex	no reflex/ limp

4. Drag Test

0	1	2
walking motion w/forepaws	partial/ unilateral response	no response

5. Righting Reflex

0	1	2
instant response	delayed/ takes effort	no response

6. Ear Reflex

0	1	2
full response	partial response	no response

7. Eye Reflex

0	1	2
eyeblink	partial response	no response

8. Sound Reflex

0	1	2
flinch then walk	slow mvt &/or no freeze	no response

9. Tail Reflex

0	1	2
turn/bite	turn/no bite	no response

10. Paw Flexion Reflex

0	1	2
turn/bite	turn/no bite	no response

Figure 5. Neurobehavioral Severity Scale – Revised Scoring Sheet.

Figure 6. Neurobehavioral Severity Scale – Revised Equipment.

Photo by A. Yarnell (2012)

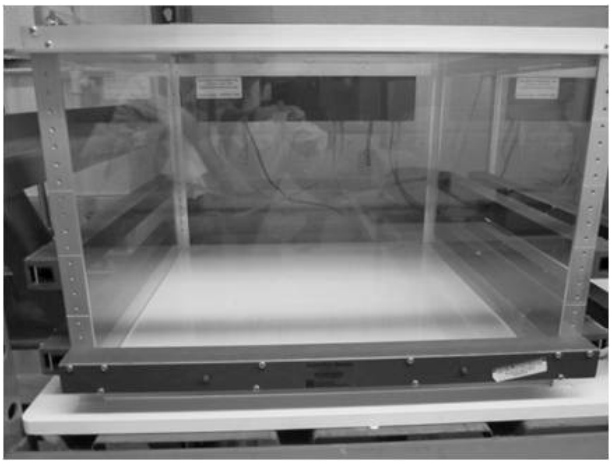

Figure 7. Open Field Activity (OFA) Chamber.

Photo by A. Yarnell (2012)

Figure 8. Hotplate equipment picture.

Photo by B. Finton (2013)

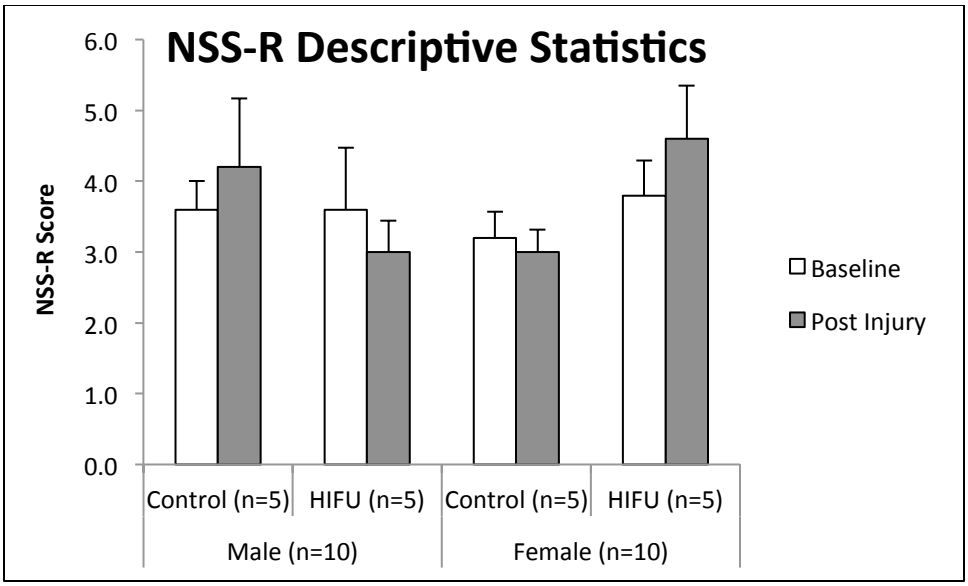

Figure 9. NSS-R descriptives – Experiment 1.

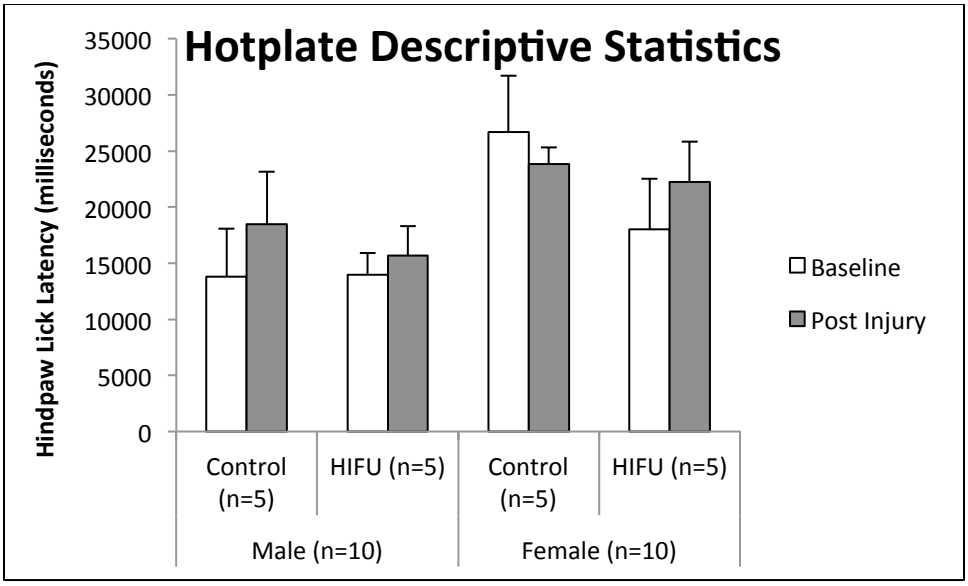

Figure 10. Hotplate descriptives – Experiment 1.

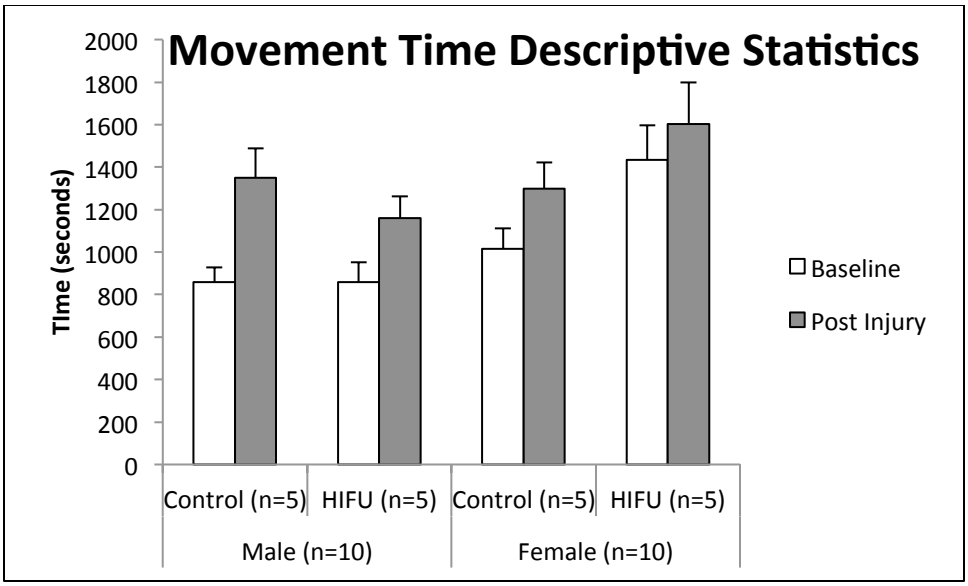

Figure 11. Movement time descriptives – Experiment 1.

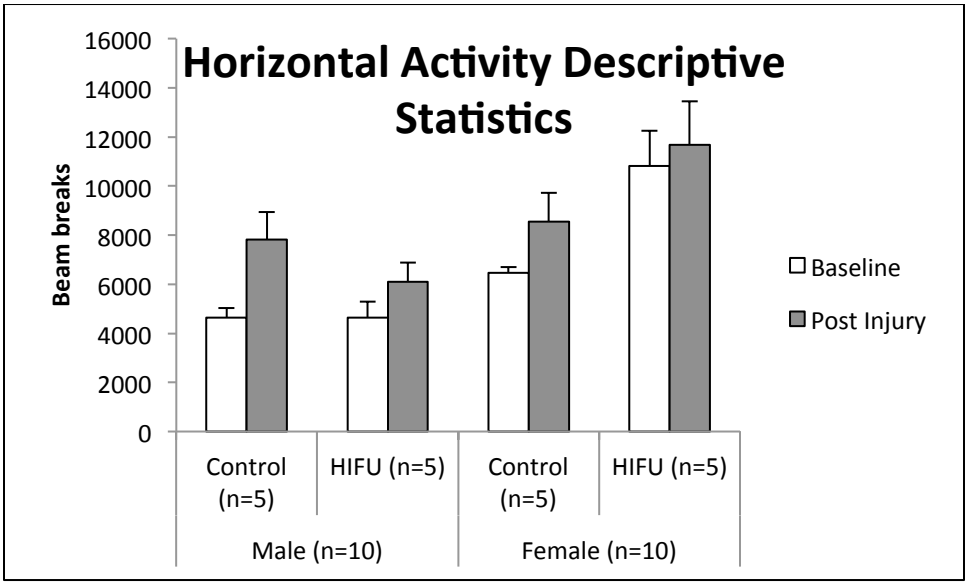

Figure 12. Horizontal activity descriptives – Experiment 1.

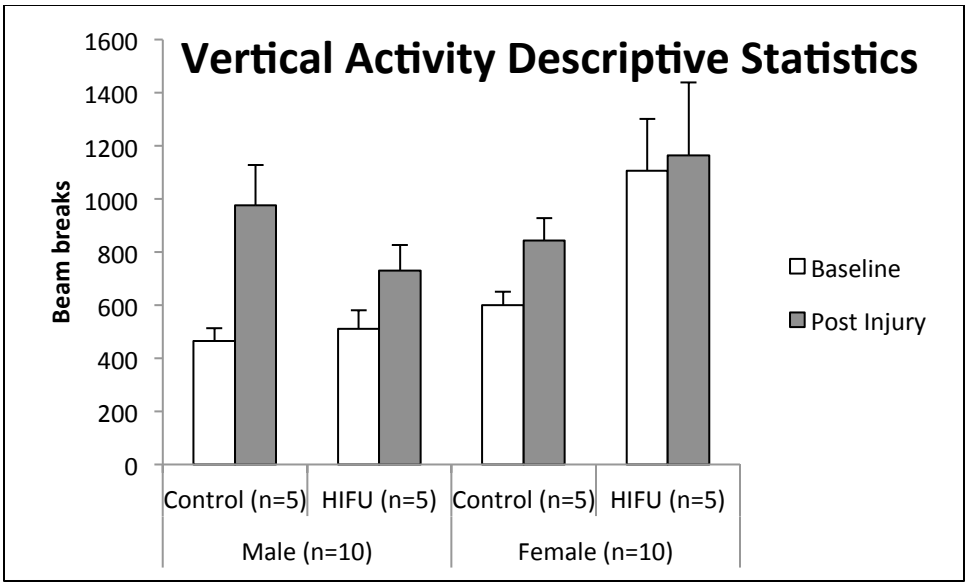

Figure 13. Vertical activity descriptives – Experiment 1.

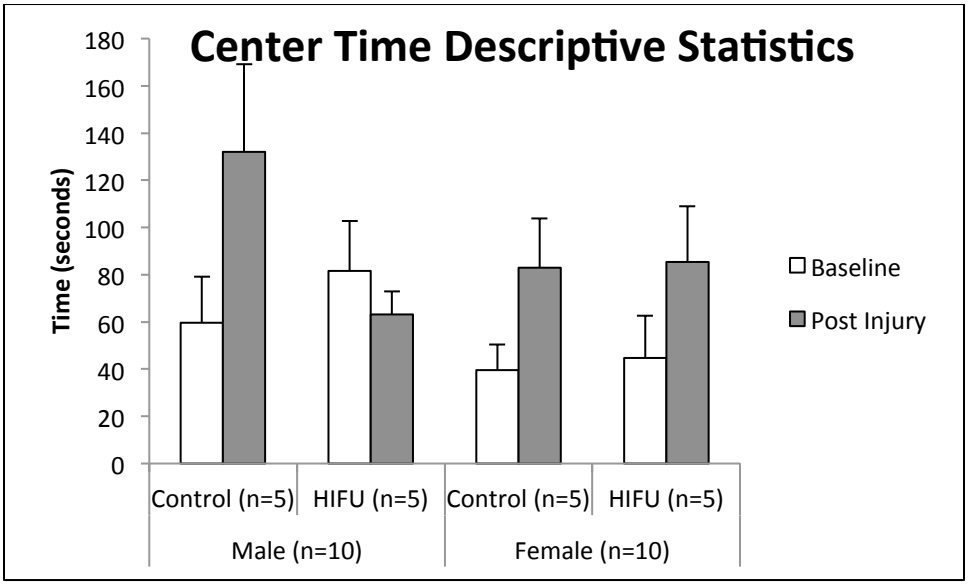

Figure 14. Center time descriptives – Experiment 1.

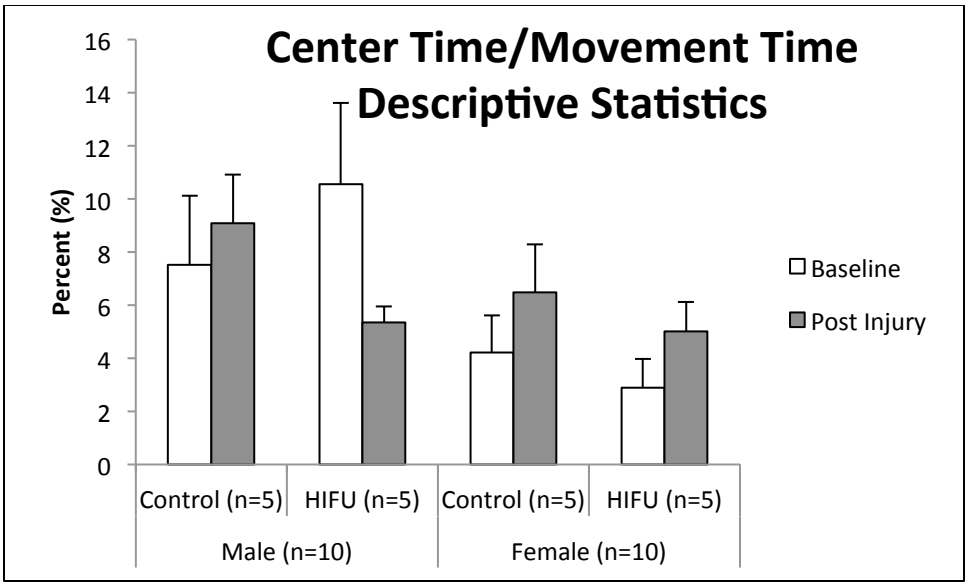

Figure 15. CT/MT descriptives – Experiment 1.

-6	**-5**	**-4**	**-3**	-2	-1	**Day 0**	**1**	2	3	4	5	6	7	**8**	9	**10**
Arrive	**Gentle**	**ACC**	**BL**			***Injury***	**PI1**							**PI2**		**PI2**

Figure 16. Experiment 2 timeline.

Note. Gentling (Gentle) was conducted prior to behavioral assays on day -5. The acclimation phase (ACC) of OFA was conducted on day -4. The baseline measurements (BL) for OFA, HP, and NSS-R were gathered on day -3. The HIFU injury and Sham control procedures were conducted on day 0 (Injury). The post-injury 1 (PI1) data were collected for OFA and NSS-R on day 1, and the post-injury 2 (PI2) data were collected for OFA and NSS-R on day 8. The only post-injury data collection for HP (PI2) was on day 10.

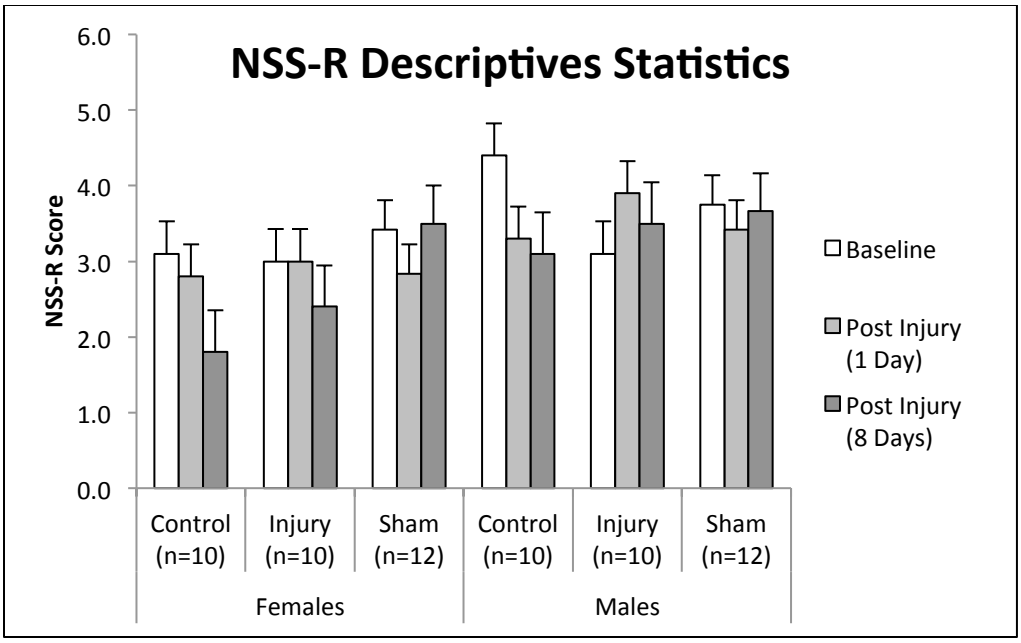

Figure 17. NSS-R descriptives – Experiment 2.

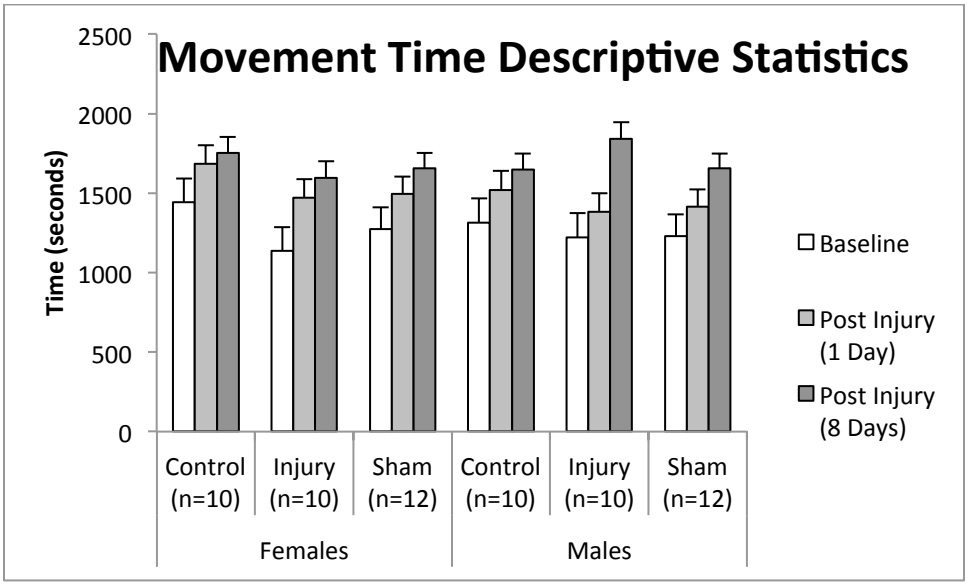

Figure 18. Movement time descriptives – Experiment 2.

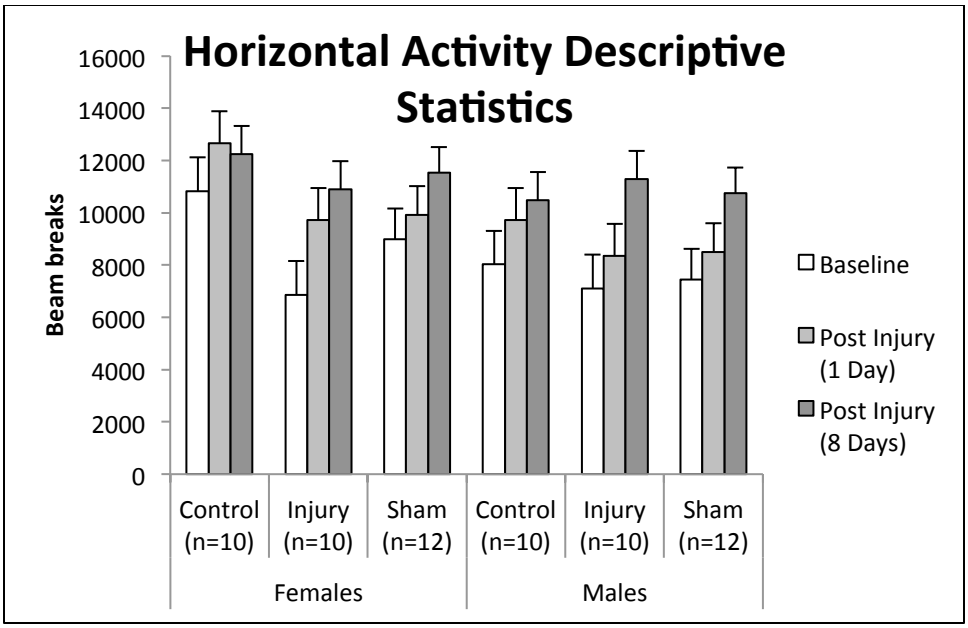

Figure 19. Horizontal activity descriptives – Experiment 2.

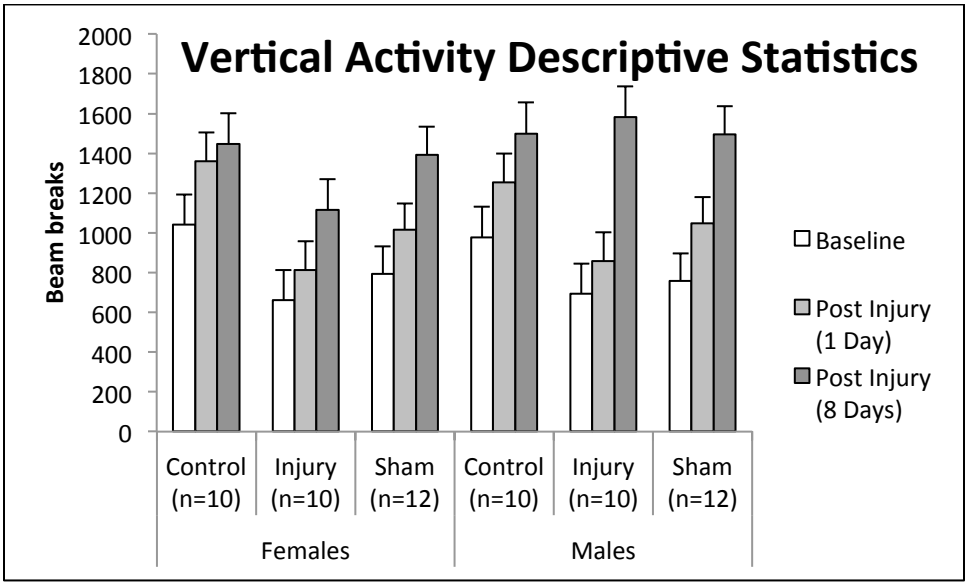

Figure 20. Vertical activity descriptives – Experiment 2.

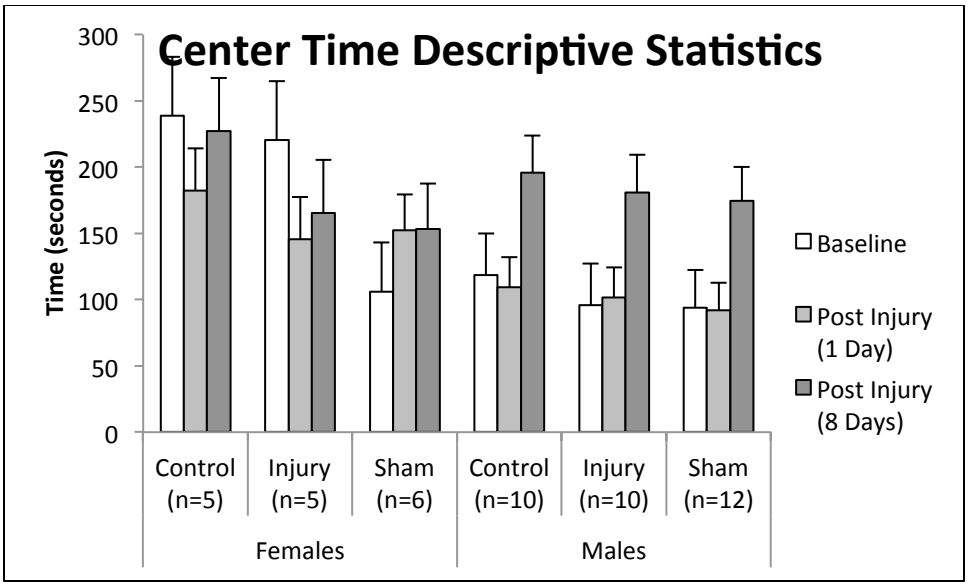

Figure 21. Center time descriptives – Experiment 2.

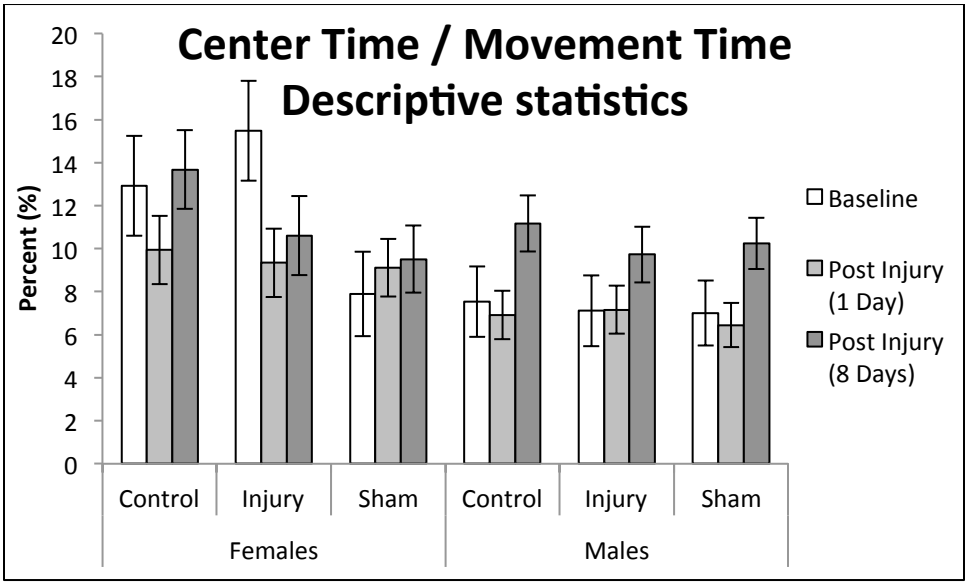

Figure 22. CT/MT descriptives – Experiment 2.

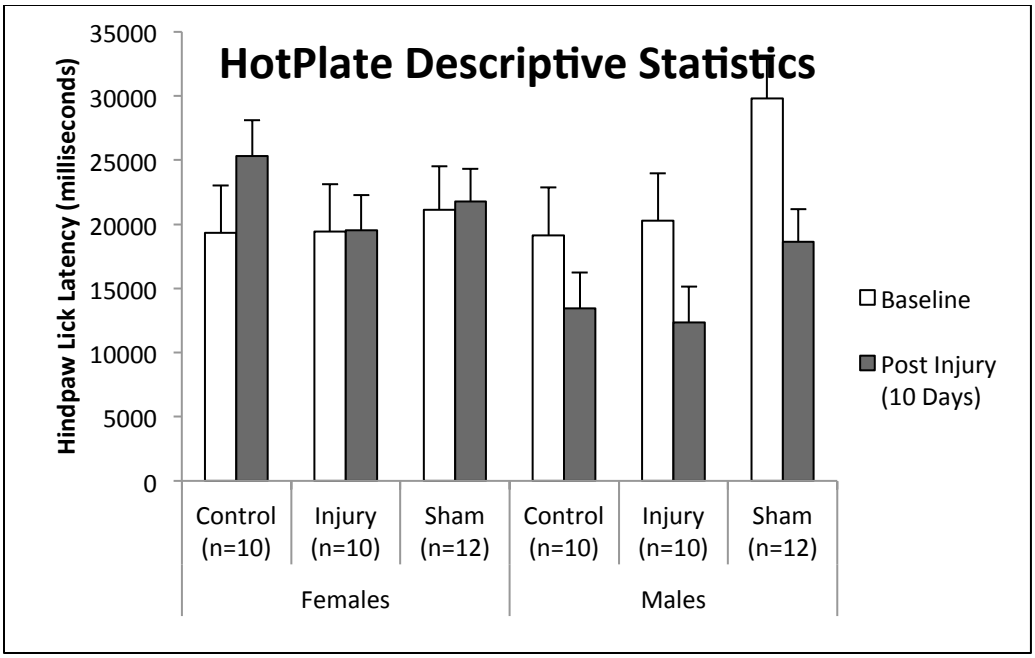

Figure 23. Hotplate descriptives – Experiment 2.

APPENDIX B – TABLES

Table 1. Pilot subject numbers.

Pilot Subject Breakdown (N = 16)		
	Female (n = 8)	Male (n = 8)
Control (n = 10)	2	2
500ms (n = 10)	3	3
1000ms (n = 10)	3	3

Table 1b. Experiment 1 subject numbers.

Experiment 1 Subject Breakdown (N = 20)		
	Female (n = 10)	Male (n = 10)
Control (n = 10)	5	5
HIFU (n = 10)	5	5

Table 2. Neurobehavioral Severity Scale – Revised (NSS-R) Repeated Measures ANOVA

Overall NSS-R Repeated Measures ANOVA: Within-Subject Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Time	.225	1	.225	.225	.642	.014	.073
Time * sex	.225	1	.225	.225	.642	.014	.073
Time * injury	.025	1	.025	.025	.876	.002	.053
Time * sex * injury	3.025	1	3.025	3.025	.101	.159	.373
Error	16.000	16	1.000				

Overall NSS-R Repeated Measures ANOVA: Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	525.625	1	525.625	182.826	.000	.920	1.000
sex	.025	1	.025	.009	.927	.001	.051
injury	.625	1	.625	.217	.647	.013	.072
sex * injury	7.225	1	7.225	2.513	.132	.136	.320
Error	46.000	16	2.875				

a. Computed using alpha = .05

Table 3. Experiment 1 NSS-R ANCOVA

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	18.854[a]	4	4.713	2.585	.080	.408	.581
Intercept	5.103	1	5.103	2.799	.115	.157	.347
NSS_R_BL	8.654	1	8.654	4.747	.046	.240	.531
injury	.004	1	.004	.002	.965	.000	.050
sex	.331	1	.331	.182	.676	.012	.068
injury * sex	7.399	1	7.399	4.058	.062	.213	.470
Error	27.346	15	1.823				
Total	320.000	20					
Corrected Total	46.200	19					

a. R Squared = .408 (Adjusted R Squared = .250)

b. Computed using alpha = .05

Table 4. Experiment 1: Female ANCOVA - NSS-R

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	12.132[a]	2	6.066	5.685	.034	.619	.672
Intercept	.338	1	.338	.317	.591	.043	.078
NSS_R_BL	5.732	1	5.732	5.372	.054	.434	.515
injury	2.602	1	2.602	2.439	.162	.258	.272
Error	7.468	7	1.067				
Total	164.000	10					
Corrected Total	19.600	9					

a. R Squared = .619 (Adjusted R Squared = .510)

b. Computed using alpha = .05

Table 5. Experiment 1: Male ANCOVA - NSS-R

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	7.435[a]	2	3.717	1.372	.314	.282	.207
Intercept	4.759	1	4.759	1.757	.227	.201	.210
NSS_R_BL	3.835	1	3.835	1.415	.273	.168	.178
injury	3.600	1	3.600	1.329	.287	.160	.170
Error	18.965	7	2.709				
Total	156.000	10					
Corrected Total	26.400	9					

a. R Squared = .282 (Adjusted R Squared = .076)

b. Computed using alpha = .05

Table 6. Hotplate Repeated Measures ANOVA

Experiment 1: Overall Hotplate Repeated Measures ANOVA: Within-Subject Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Time	38343514.225	1	38343514.225	.761	.396	.045	.130
Time * sex	15686310.025	1	15686310.025	.311	.585	.019	.082
Time * injury	10527786.025	1	10527786.025	.209	.654	.013	.071
Time * sex * injury	63708284.025	1	63708284.025	1.264	.277	.073	.185
Error	806171877.200	16	50385742.325				

Experiment 1: Overall Hotplate Repeated Measures ANOVA: Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	1.46E+10	1	1.46E+10	164.025	.000	.911	1.000
sex	5.21E+08	1	5.21E+08	5.861	.028	.268	.623
injury	1.04E+08	1	1.04E+08	1.171	.295	.068	.175
sex * injury	3.56E+07	1	3.56E+07	.401	.536	.024	.092
Error	1.42E+09	16	8.88E+07				

a. Computed using alpha = .05

Table 7. Experiment 1: Overall ANCOVA - Hotplate.

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	9.87E+08	4	246767381.677	4.597	.013	.551	.850
Intercept	8.70E+08	1	869659120.378	16.202	.001	.519	.964
HP_BL	8.07E+08	1	807224766.557	15.039	.001	.501	.952
injury	7.12E+06	1	7121132.786	.133	.721	.009	.063
sex	6.44E+07	1	64425168.183	1.200	.291	.074	.177
injury * sex	1.25E+07	1	12546096.671	.234	.636	.015	.074
Error	8.05E+08	15	53674599.190				
Total	1.87E+09	20					
Corrected Total	1.79E+09	19					

a. R Squared = .551 (Adjusted R Squared = .431)

b. Computed using alpha = .05

Table 8. Experiment 1: Female ANCOVA - Hotplate.

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	14330520.302[a]	2	7165260.151	.171	.846	.047	.068
Intercept	6.74E+08	1	674156669.040	16.089	.005	.697	.928
HP_BL	8.23E+06	1	8230910.302	.196	.671	.027	.067
injury	1.13E+06	1	1127575.296	.027	.874	.004	.052
Error	2.93E+08	7	41902429.328				
Total	5.62E+09	10					
Corrected Total	3.08E+08	9					

a. R Squared = .047 (Adjusted R Squared = -.226)

b. Computed using alpha = .05

Table 9. Experiment 1: Male ANCOVA - Hotplate

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	1.30E+08	2	65105398.268	.984	.420	.219	.160
Intercept	1.94E+08	1	193673765.264	2.926	.131	.295	.316
HP_BL	1.10E+08	1	110097884.136	1.663	.238	.192	.201
injury	2.13E+07	1	21253601.689	.321	.589	.044	.078
Error	4.63E+08	7	66195463.409				
Total	3.51E+09	10					
Corrected Total	5.94E+08	9					

a. R Squared = .219 (Adjusted R Squared = -.004)

b. Computed using alpha = .05

Table 10. Movement Time Repeated Measures ANOVA

Experiment 1: Overall Movement Time Repeated Measures ANOVA: Within-Subject Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Time	966124.915	1	966124.915	18.625	.001	.538	.981
Time * sex	73564.071	1	73564.071	1.418	.251	.081	.202
Time * injury	56630.893	1	56630.893	1.092	.312	.064	.166
Time * sex * injury	3713.136	1	3713.136	.072	.792	.004	.057
Error	829949.891	16	51871.868				

Experiment 1: Overall Movement Time Repeated Measures ANOVA: Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	57392658.927	1	57392658.927	511.944	.000	.970	1.000
sex	789832.006	1	789832.006	7.045	.017	.306	.703
injury	180202.434	1	180202.434	1.607	.223	.091	.222
sex * injury	521466.911	1	521466.911	4.652	.047	.225	.527
Error	1793715.039	16	112107.190				

a. Computed using alpha = .05

Table 11. Experiment 1: Overall ANCOVA - Movement Time

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	752300.762[a]	4	188075.191	2.002	.146	.348	.464
Intercept	605908.071	1	605908.071	6.451	.023	.301	.661
OFA_MT_BL	237658.978	1	237658.978	2.530	.133	.144	.319
injury	8025.020	1	8025.020	.085	.774	.006	.059
sex	610.959	1	610.959	.007	.937	.000	.051
injury * sex	85322.867	1	85322.867	.908	.356	.057	.145
Error	1408930.238	15	93928.683				
Total	38786996.381	20					
Corrected Total	2161231.001	19					

a. R Squared = .348 (Adjusted R Squared = .174)

b. Computed using alpha = .05

Table 12. Experiment 1: Female ANCOVA - Movement Time

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	412694.887[a]	2	206347.443	1.632	.262	.318	.239
Intercept	316017.046	1	316017.046	2.500	.158	.263	.278
OFA_MT_BL	177667.590	1	177667.590	1.405	.274	.167	.177
injury	14574.442	1	14574.442	.115	.744	.016	.060
Error	884897.816	7	126413.974				
Total	22348294.553	10					
Corrected Total	1297592.703	9					

a. R Squared = .318 (Adjusted R Squared = .123)

b. Computed using alpha = .05

Table 13. Experiment 1: Male ANCOVA - Movement Time

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	149092.599[a]	2	74546.300	.996	.416	.222	.161
Intercept	252233.821	1	252233.821	3.370	.109	.325	.355
OFA_MT_BL	60129.975	1	60129.975	.803	.400	.103	.122
injury	89144.019	1	89144.019	1.191	.311	.145	.157
Error	523893.834	7	74841.976				
Total	16438701.827	10					
Corrected Total	672986.433	9					

a. R Squared = .222 (Adjusted R Squared = -.001)

b. Computed using alpha = .05

Table 14. Horizontal Activity Repeated Measures ANOVA

Experiment 1: Overall Horizontal Activity Repeated Measures ANOVA: Within-Subject Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Time	36071505.625	1	36071505.625	13.377	.002	.455	.929
Time * sex	1833980.625	1	1833980.625	.680	.422	.041	.121
Time * injury	5275843.225	1	5275843.225	1.957	.181	.109	.260
Time * sex * injury	142444.225	1	142444.225	.053	.821	.003	.055
Error	43144872.800	16	2696554.550				

Experiment 1: Overall Horizontal Activity Repeated Measures ANOVA: Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	2.30E+09	1	2.30E+09	264.298	.000	.943	1.000
sex	1.28E+08	1	1.28E+08	14.720	.001	.479	.949
injury	2.07E+07	1	2.07E+07	2.375	.143	.129	.305
sex * injury	5.27E+07	1	5.27E+07	6.043	.026	.274	.637
Error	1.39E+08	16	8.72E+06				

a. Computed using alpha = .05

Table 15. Experiment 1: Overall ANCOVA - Horizontal Activity

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	1.24E+08	4	31109710.855	5.446	.007	.592	.909
Intercept	7.81E+06	1	7813713.806	1.368	.260	.084	.195
OFA_HA_BL	4.30E+07	1	43034953.271	7.534	.015	.334	.728
injury	5.21E+06	1	5209078.866	.912	.355	.057	.146
sex	3.72E+05	1	371832.629	.065	.802	.004	.057
injury * sex	7.67E+05	1	766765.502	.134	.719	.009	.064
Error	8.57E+07	15	5712121.809				
Total	1.67E+09	20					
Corrected Total	2.10E+08	19					

a. R Squared = .592 (Adjusted R Squared = .483)

b. Computed using alpha = .05

Table 16. Experiment 1: Female ANCOVA - Horizontal Activity

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	54476037.991[a]	2	27238018.996	3.135	.107	.472	.421
Intercept	4.08E+06	1	4077649.665	.469	.515	.063	.092
OFA_HA_BL	3.00E+07	1	30036969.091	3.457	.105	.331	.362
injury	3.65E+05	1	365219.556	.042	.843	.006	.054
Error	6.08E+07	7	8688848.987				
Total	1.14E+09	10					
Corrected Total	1.15E+08	9					

a. R Squared = .472 (Adjusted R Squared = .322)

b. Computed using alpha = .05

Table 17. Experiment 1: Male ANCOVA - Horizontal Activity

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	20612426.055[a]	2	10306213.028	2.946	.118	.457	.399
Intercept	2383757.514	1	2383757.514	.681	.436	.089	.111
OFA_HA_BL	13370416.055	1	13370416.055	3.822	.091	.353	.393
injury	7147655.985	1	7147655.985	2.043	.196	.226	.236
Error	24487452.345	7	3498207.478				
Total	529710778.000	10					
Corrected Total	45099878.400	9					

a. R Squared = .457 (Adjusted R Squared = .302)

b. Computed using alpha = .05

Table 18. Vertical Activity Repeated Measures ANOVA

Experiment 1: Overall Vertical Activity Repeated Measures ANOVA: Within-Subject Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Time	665640.000	1	665640.000	11.991	.003	.428	.901
Time * sex	115347.600	1	115347.600	2.078	.169	.115	.273
Time * injury	140422.500	1	140422.500	2.530	.131	.137	.321
Time * sex * injury	7128.900	1	7128.900	.128	.725	.008	.063
Error	888161.000	16	55510.063				

Experiment 1: Overall Vertical Activity Repeated Measures ANOVA: Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	25587201.600	1	25587201.600	174.891	.000	.916	1.000
sex	664608.400	1	664608.400	4.543	.049	.221	.517
injury	244922.500	1	244922.500	1.674	.214	.095	.230
sex * injury	664092.900	1	664092.900	4.539	.049	.221	.517
Error	2340856.600	16	146303.538				

a. Computed using alpha = .05

Table 19. Experiment 1: Overall ANCOVA - Vertical Activity

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	1074176.505[a]	4	268544.126	2.343	.102	.385	.534
Intercept	342375.025	1	342375.025	2.987	.104	.166	.366
OFA_VA_BL	549438.905	1	549438.905	4.793	.045	.242	.535
injury	103699.885	1	103699.885	.905	.357	.057	.145
sex	46736.214	1	46736.214	.408	.533	.026	.092
injury * sex	47054.523	1	47054.523	.411	.531	.027	.092
Error	1719360.695	15	114624.046				
Total	20046926.000	20					
Corrected Total	2793537.200	19					

a. R Squared = .385 (Adjusted R Squared = .220)

b. Computed using alpha = .05

Table 20. Experiment 1: Female ANCOVA - Vertical Activity

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	570951.259[a]	2	285475.630	1.515	.284	.302	.225
Intercept	230055.514	1	230055.514	1.221	.306	.149	.160
OFA_VA_BL	311096.859	1	311096.859	1.651	.240	.191	.200
injury	146.520	1	146.520	.001	.979	.000	.050
Error	1319112.741	7	188444.677				
Total	11970224.000	10					
Corrected Total	1890064.000	9					

a. R Squared = .302 (Adjusted R Squared = .103)

b. Computed using alpha = .05

Table 21. Experiment 1: Male ANCOVA - Vertical Activity

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	496989.442[a]	2	248494.721	5.929	.031	.629	.691
Intercept	5082.586	1	5082.586	.121	.738	.017	.061
OFA_VA_BL	345207.042	1	345207.042	8.237	.024	.541	.693
injury	239614.193	1	239614.193	5.717	.048	.450	.540
Error	293382.958	7	41911.851				
Total	8076702.000	10					
Corrected Total	790372.400	9					

a. R Squared = .629 (Adjusted R Squared = .523)

b. Computed using alpha = .05

Table 22. Center Time Repeated Measures ANOVA

Experiment 1: Overall Center Time Repeated Measures ANOVA: Within-Subject Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Time	11934.261	1	11934.261	6.059	.026	.275	.638
Time * sex	573.200	1	573.200	.291	.597	.018	.080
Time * injury	5456.429	1	5456.429	2.770	.116	.148	.347
Time * sex * injury	4869.966	1	4869.966	2.472	.135	.134	.315
Error	31516.749	16	1969.797				

Experiment 1: Overall Center Time Repeated Measures ANOVA: Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	216913.984	1	216913.984	81.096	.000	.835	1.000
sex	4433.972	1	4433.972	1.658	.216	.094	.228
injury	960.204	1	960.204	.359	.557	.022	.087
sex * injury	1873.066	1	1873.066	.700	.415	.042	.124
Error	42796.299	16	2674.769				

a. Computed using alpha = .05

Table 23. Experiment 1: Overall ANCOVA - Center Time

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	14051.769[a]	4	3512.942	1.105	.390	.228	.266
Intercept	35069.727	1	35069.727	11.032	.005	.424	.873
OFA_CT_BL	1253.401	1	1253.401	.394	.539	.026	.091
injury	6313.208	1	6313.208	1.986	.179	.117	.262
sex	218.454	1	218.454	.069	.797	.005	.057
injury * sex	6978.530	1	6978.530	2.195	.159	.128	.284
Error	47683.041	15	3178.869				
Total	227038.282	20					
Corrected Total	61734.811	19					

a. R Squared = .228 (Adjusted R Squared = .022)

b. Computed using alpha = .05

Table 24. Experiment 1: Female ANCOVA - Center Time

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	571.942[a]	2	285.971	.105	.902	.029	.061
Intercept	18332.113	1	18332.113	6.699	.036	.489	.605
OFA_CT_BL	555.094	1	555.094	.203	.666	.028	.068
injury	4.259	1	4.259	.002	.970	.000	.050
Error	19155.527	7	2736.504				
Total	90573.358	10					
Corrected Total	19727.469	9					

a. R Squared = .029 (Adjusted R Squared = -.248)

b. Computed using alpha = .05

Table 25. Experiment 1: Male ANCOVA - Center Time

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	12579.974[a]	2	6289.987	1.544	.278	.306	.228
Intercept	16992.095	1	16992.095	4.171	.080	.373	.422
OFA_CT_BL	707.815	1	707.815	.174	.689	.024	.065
injury	12571.954	1	12571.954	3.086	.122	.306	.330
Error	28518.007	7	4074.001				
Total	136464.924	10					
Corrected Total	41097.981	9					

a. R Squared = .306 (Adjusted R Squared = .108)

b. Computed using alpha = .05

Table 26. Center Time / Movement Time (CT/MT) Repeated Measures ANOVA

Experiment 1: Overall Center Time/Movement Time Repeated Measures ANOVA: Within-Subject Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Time	.342	1	.342	.019	.891	.001	.052
Time * sex	39.758	1	39.758	2.251	.153	.123	.292
Time * injury	30.059	1	30.059	1.702	.211	.096	.232
Time * sex * injury	27.432	1	27.432	1.553	.231	.088	.216
Error	282.648	16	17.666				

Experiment 1: Overall Center Time/Movement Time Repeated Measures ANOVA: Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	1631.163	1	1631.163	96.601	.000	.858	1.000
sex	120.834	1	120.834	7.156	.017	.309	.710
injury	7.635	1	7.635	.452	.511	.027	.097
sex * injury	2.692	1	2.692	.159	.695	.010	.066
Error	270.170	16	16.886				

a. Computed using alpha = .05

Table 27. Experiment 1: Overall ANCOVA - Center Time / Movement Time

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	51.550[a]	4	12.887	1.177	.360	.239	.282
Intercept	284.898	1	284.898	26.023	.000	.634	.997
OFA_CTxMT_BL	.100	1	.100	.009	.925	.001	.051
injury	33.320	1	33.320	3.044	.102	.169	.372
sex	8.898	1	8.898	.813	.382	.051	.135
injury * sex	5.729	1	5.729	.523	.481	.034	.104
Error	164.218	15	10.948				
Total	1055.154	20					
Corrected Total	215.768	19					

a. R Squared = .239 (Adjusted R Squared = .036)

b. Computed using alpha = .05

Table 28. Experiment 1: Female ANCOVA - Center Time / Movement Time

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	7.763[a]	2	3.881	.306	.746	.080	.082
Intercept	135.672	1	135.672	10.705	.014	.605	.800
OFA_CTxMT_BL	2.360	1	2.360	.186	.679	.026	.066
injury	6.970	1	6.970	.550	.482	.073	.099
Error	88.718	7	12.674				
Total	425.646	10					
Corrected Total	96.481	9					

a. R Squared = .080 (Adjusted R Squared = -.182)

b. Computed using alpha = .05

Table 29. Experiment 1: Male ANCOVA - Center Time / Movement Time

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	35.167[a]	2	17.583	1.683	.253	.325	.246
Intercept	142.558	1	142.558	13.644	.008	.661	.884
OFA_CTxMT_BL	.104	1	.104	.010	.923	.001	.051
injury	33.709	1	33.709	3.226	.116	.315	.342
Error	73.136	7	10.448				
Total	629.508	10					
Corrected Total	108.303	9					

a. R Squared = .325 (Adjusted R Squared = .132)

b. Computed using alpha = .05

Table 30. Experiment 2 subject numbers

Experiment 2 Subject Breakdown (N = 64)		
	Female (n = 32)	Male (n = 32)
Control (n = 20)	10	10
HIFU (n = 20)	10	10
Sham (n = 24)	12	12

Table 31. NSS-R Repeated Measures ANCOVA

Experiment 2: Overall Repeated ANCOVA of NSS-R - Within-Subject Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
NSSR_time	.258	1	.258	.185	.669	.003	.071
NSSR_time * NSSR_BL	.982	1	.982	.702	.406	.012	.131
NSSR_time * Condition	7.973	2	3.987	2.852	.066	.091	.538
NSSR_time * Sex	.565	1	.565	.404	.528	.007	.096
NSSR_time * Condition * Sex	2.422	2	1.211	.866	.426	.029	.192
Error(NSSR_time)	79.677	57	1.398				

Experiment 2: Overall Repeated ANCOVA of NSS-R - Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	73.563	1	73.563	22.700	.000	.285	.997
NSSR_BL	14.873	1	14.873	4.589	.036	.075	.558
Condition	11.138	2	5.569	1.719	.188	.057	.346
Sex	11.064	1	11.064	3.414	.070	.057	.443
Condition * Sex	2.581	2	1.291	.398	.673	.014	.111
Error	184.719	57	3.241				

a. Computed using alpha = .05

Table 32. Experiment 2: ANCOVA (Time 1) - NSS-R

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	20.924[a]	6	3.487	2.127	.064	.183	.714
Intercept	32.552	1	32.552	19.858	.000	.258	.992
NSSR_BL	11.748	1	11.748	7.167	.010	.112	.749
Sex	3.315	1	3.315	2.022	.160	.034	.287
Condition	4.315	2	2.158	1.316	.276	.044	.273
Sex * Condition	1.550	2	.775	.473	.626	.016	.124
Error	93.435	57	1.639				
Total	771.000	64					
Corrected Total	114.359	63					

a. R Squared = .183 (Adjusted R Squared = .097)

b. Computed using alpha = .05

Table 33. Experiment 2: ANCOVA (Time 2) - NSS-R

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	32.977[a]	6	5.496	1.832	.109	.162	.637
Intercept	41.270	1	41.270	13.760	.000	.194	.954
NSSR_BL	4.106	1	4.106	1.369	.247	.023	.210
Sex	8.314	1	8.314	2.772	.101	.046	.373
Condition	14.796	2	7.398	2.467	.094	.080	.476
Sex * Condition	3.453	2	1.726	.576	.566	.020	.141
Error	170.961	57	2.999				
Total	792.000	64					
Corrected Total	203.938	63					

a. R Squared = .162 (Adjusted R Squared = .073)

b. Computed using alpha = .05

Table 34. Movement Time Repeated Measures ANCOVA

Experiment 2: Overall Repeated ANCOVA of Movement Time - Within-Subject Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
MT_Time	753476.562	1	753476.562	11.813	.001	.172	.922
MT_Time * MT_BL	276300.017	1	276300.017	4.332	.042	.071	.534
MT_Time * Condition	114155.310	2	57077.655	.895	.414	.030	.197
MT_Time * Sex	181997.432	1	181997.432	2.853	.097	.048	.383
MT_Time * Condition * Sex	153085.154	2	76542.577	1.200	.309	.040	.252
Error(MT_Time)	3635747.522	57	63785.044				

Experiment 2: Overall Repeated ANCOVA of Movement Time - Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	24418884.901	1	24418884.901	154.429	.000	.730	1.000
MT_BL	1390276.864	1	1390276.864	8.792	.004	.134	.830
Condition	92259.850	2	46129.925	.292	.748	.010	.094
Sex	20535.511	1	20535.511	.130	.720	.002	.065
Condition * Sex	132320.673	2	66160.336	.418	.660	.014	.115
Error	9013022.023	57	158123.193				

a. Computed using alpha = .05

Table 35. Experiment 2: ANCOVA (T1) - Movement Time

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	2018841.884[a]	6	336473.647	2.910	.015	.234	.861
Intercept	8296768.482	1	8296768.482	71.749	.000	.557	1.000
MT_BL	1453073.501	1	1453073.501	12.566	.001	.181	.936
Sex	162400.835	1	162400.835	1.404	.241	.024	.214
Condition	150266.109	2	75133.054	.650	.526	.022	.154
Sex * Condition	9610.415	2	4805.207	.042	.959	.001	.056
Error	6591281.383	57	115636.515				
Total	151091259.645	64					
Corrected Total	8610123.267	63					

a. R Squared = .234 (Adjusted R Squared = .154)

b. Computed using alpha = .05

Table 36. Experiment 2: ANCOVA (T2) - Movement Time

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	615154.174[a]	6	102525.696	.965	.457	.092	.350
Intercept	16875592.981	1	16875592.981	158.797	.000	.736	1.000
MT_BL	213503.380	1	213503.380	2.009	.162	.034	.286
Sex	40132.109	1	40132.109	.378	.541	.007	.093
Condition	56149.052	2	28074.526	.264	.769	.009	.090
Sex * Condition	275795.412	2	137897.706	1.298	.281	.044	.270
Error	6057488.162	57	106271.722				
Total	189190175.446	64					
Corrected Total	6672642.337	63					

a. R Squared = .092 (Adjusted R Squared = -.003)

b. Computed using alpha = .05

Table 37. Horizontal Activity Repeated Measures ANCOVA

Experiment 2: Overall Repeated ANCOVA of Horizontal Activity - Within-Subject Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
HA_Time	49669854.269	1	49669854.269	10.178	.002	.152	.880
HA_Time * HA_BL	16618349.603	1	16618349.603	3.405	.070	.056	.442
HA_Time * Condition	14871518.627	2	7435759.314	1.524	.227	.051	.311
HA_Time * Sex	6759041.678	1	6759041.678	1.385	.244	.024	.212
HA_Time * Condition * Sex	3116652.299	2	1558326.150	.319	.728	.011	.098
Error(HA_Time)	278164345.972	57	4880076.245				

Experiment 2: Overall Repeated ANCOVA of Horizontal Activity - Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	1.05E+09	1	1.05E+09	74.720	.000	.567	1.000
HA_BL	4.19E+08	1	4.19E+08	29.691	.000	.342	1.000
Condition	7.72E+06	2	3.86E+06	.274	.762	.010	.091
Sex	1.41E+07	1	1.41E+07	.997	.322	.017	.166
Condition * Sex	2.34E+06	2	1.17E+06	.083	.920	.003	.062
Error	8.04E+08	57	1.41E+07				

a. Computed using alpha = .05

Table 38. Experiment 2: ANCOVA (T1) - Horizontal Activity

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	424695859.944[a]	6	70782643.324	7.321	.000	.435	.999
Intercept	3.23E+08	1	323094735.698	33.420	.000	.370	1.000
HA_BL	3.01E+08	1	301183215.807	31.153	.000	.353	1.000
Sex	2.02E+07	1	20164723.176	2.086	.154	.035	.295
Condition	1.85E+07	2	9243954.114	.956	.390	.032	.208
Sex * Condition	2.99E+06	2	1493980.407	.155	.857	.005	.073
Error	5.51E+08	57	9667804.877				
Total	7.09E+09	64					
Corrected Total	9.76E+08	63					

a. R Squared = .435 (Adjusted R Squared = .376)

b. Computed using alpha = .05

Table 39. Experiment 2: ANCOVA (T2) - Horizontal Activity

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	154987598.210[a]	6	25831266.368	2.771	.020	.226	.841
Intercept	7.81E+08	1	780742339.068	83.766	.000	.595	1.000
HA_BL	1.34E+08	1	134316582.210	14.411	.000	.202	.962
Sex	6.62E+05	1	662303.281	.071	.791	.001	.058
Condition	4.11E+06	2	2053440.346	.220	.803	.008	.083
Sex * Condition	2.47E+06	2	1234828.194	.132	.876	.005	.069
Error	5.31E+08	57	9320466.553				
Total	8.71E+09	64					
Corrected Total	6.86E+08	63					

a. R Squared = .226 (Adjusted R Squared = .144)

b. Computed using alpha = .05

Table 40. Vertical Activity Repeated Measures ANCOVA

Experiment 2: Overall Repeated ANCOVA of Vertical Activity - Within-Subject Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
VA_Time	1529559.516	1	1529559.516	20.569	.000	.265	.994
VA_Time * VA_BL	71499.093	1	71499.093	.962	.331	.017	.161
VA_Time * Condition	477398.541	2	238699.270	3.210	.048	.101	.591
VA_Time * Sex	362174.698	1	362174.698	4.870	.031	.079	.583
VA_Time * Condition * Sex	185643.772	2	92821.886	1.248	.295	.042	.261
Error(VA_Time)	4238620.149	57	74361.757				

Experiment 2: Overall Repeated ANCOVA of Vertical Activity - Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	20055598.881	1	20055598.881	78.744	.000	.580	1.000
VA_BL	7361191.009	1	7361191.009	28.902	.000	.336	1.000
Condition	320121.215	2	160060.607	.628	.537	.022	.150
Sex	388558.899	1	388558.899	1.526	.222	.026	.229
Condition * Sex	280678.633	2	140339.317	.551	.579	.019	.137
Error	14517555.732	57	254693.960				

a. Computed using alpha = .05

Table 41. Experiment 2: ANCOVA (T1) - Vertical Activity

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	6760734.925[a]	6	1126789.154	8.269	.000	.465	1.000
Intercept	5253966.365	1	5253966.365	38.557	.000	.403	1.000
VA_BL	4441823.154	1	4441823.154	32.597	.000	.364	1.000
Sex	231.887	1	231.887	.002	.967	.000	.050
Condition	726764.353	2	363382.176	2.667	.078	.086	.509
Sex * Condition	43245.542	2	21622.771	.159	.854	.006	.073
Error	7767182.012	57	136266.351				
Total	85926192.000	64					
Corrected Total	14527916.938	63					

a. R Squared = .465 (Adjusted R Squared = .409)

b. Computed using alpha = .05

Table 42. Experiment 2: ANCOVA (T2) - Vertical Activity

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	4326946.568[a]	6	721157.761	3.741	.003	.283	.942
Intercept	16331192.033	1	16331192.033	84.710	.000	.598	1.000
VA_BL	2990866.948	1	2990866.948	15.514	.000	.214	.972
Sex	750501.710	1	750501.710	3.893	.053	.064	.492
Condition	70755.403	2	35377.701	.184	.833	.006	.077
Sex * Condition	423076.864	2	211538.432	1.097	.341	.037	.233
Error	10988993.869	57	192789.366				
Total	144882938.000	64					
Corrected Total	15315940.438	63					

a. R Squared = .283 (Adjusted R Squared = .207)

b. Computed using alpha = .05

Table 43. Center Time Repeated Measures ANCOVA

Experiment 2: Overall Repeated ANCOVA of Center Time - Within-Subject Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
CT_Time	35216.472	1	35216.472	7.922	.007	.159	.785
CT_Time * CT_BL	3750.958	1	3750.958	.844	.364	.020	.146
CT_Time * Condition	4115.104	2	2057.552	.463	.633	.022	.121
CT_Time * Sex	11119.520	1	11119.520	2.501	.121	.056	.339
CT_Time * Condition * Sex	2848.949	2	1424.475	.320	.728	.015	.098
Error(CT_Time)	186707.536	42	4445.418				

Experiment 2: Overall Repeated ANCOVA of Center Time - Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	498396.297	1	498396.297	58.483	.000	.582	1.000
CT_BL	18845.139	1	18845.139	2.211	.144	.050	.306
Condition	11537.462	2	5768.731	.677	.514	.031	.156
Sex	4670.305	1	4670.305	.548	.463	.013	.112
Condition * Sex	5024.129	2	2512.064	.295	.746	.014	.094
Error	357929.840	42	8522.139				

a. Computed using alpha = .05

Table 44. Experiment 2: ANCOVA (T1) - Center Time

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	63533.763[a]	6	10588.961	2.204	.061	.239	.713
Intercept	134323.331	1	134323.331	27.961	.000	.400	.999
CT_BL	19705.624	1	19705.624	4.102	.049	.089	.508
Sex	15101.267	1	15101.267	3.144	.083	.070	.410
Condition	2134.249	2	1067.125	.222	.802	.010	.082
Sex * Condition	3116.620	2	1558.310	.324	.725	.015	.098
Error	201766.049	42	4803.954				
Total	979872.960	49					
Corrected Total	265299.812	48					

a. R Squared = .239 (Adjusted R Squared = .131)

b. Computed using alpha = .05

Table 45. Experiment 2: ANCOVA (T2) - Center Time

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	22844.193[a]	6	3807.365	.466	.829	.062	.171
Intercept	399289.439	1	399289.439	48.911	.000	.538	1.000
CT_BL	2890.473	1	2890.473	.354	.555	.008	.090
Sex	688.558	1	688.558	.084	.773	.002	.059
Condition	13518.317	2	6759.158	.828	.444	.038	.182
Sex * Condition	4756.458	2	2378.229	.291	.749	.014	.093
Error	342871.328	42	8163.603				
Total	1980303.247	49					
Corrected Total	365715.520	48					

a. R Squared = .062 (Adjusted R Squared = -.071)

b. Computed using alpha = .05

Table 46. CT/MT Repeated Measures ANCOVA

Experiment 2: Overall Repeated ANCOVA of CT/MT - Within-Subject Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
CT_MT_Time	133.863	1	133.863	14.257	.000	.253	.958
CT_MT_Time * CTxMT_BL	41.742	1	41.742	4.446	.041	.096	.540
CT_MT_Time * Sex	.823	1	.823	.088	.769	.002	.060
CT_MT_Time * Condition	26.061	2	13.030	1.388	.261	.062	.282
CT_MT_Time * Sex * Condition	20.446	2	10.223	1.089	.346	.049	.228
Error(CT_MT_Time)	394.343	42	9.389				

Experiment 2: Overall Repeated ANCOVA of CT/MT - Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	1283.931	1	1283.931	68.208	.000	.619	1.000
CTxMT_BL	46.188	1	46.188	2.454	.125	.055	.334
Sex	20.373	1	20.373	1.082	.304	.025	.174
Condition	30.602	2	15.301	.813	.450	.037	.180
Sex * Condition	9.717	2	4.859	.258	.774	.012	.088
Error	790.602	42	18.824				

a. Computed using alpha = .05

Table 47. Experiment 2: ANCOVA (T1) - Center Time / Movement Time

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	168.358[a]	6	28.060	2.576	.032	.269	.790
Intercept	294.324	1	294.324	27.017	.000	.391	.999
CTxMT_BL	87.873	1	87.873	8.066	.007	.161	.792
Sex	14.693	1	14.693	1.349	.252	.031	.206
Condition	2.587	2	1.293	.119	.888	.006	.067
Sex * Condition	11.328	2	5.664	.520	.598	.024	.130
Error	457.552	42	10.894				
Total	3550.446	49					
Corrected Total	625.910	48					

a. R Squared = .269 (Adjusted R Squared = .165)
b. Computed using alpha = .05

Table 48. Experiment 2: ANCOVA (T2) - Center Time / Movement Time

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	68.105[a]	6	11.351	.655	.686	.086	.231
Intercept	1123.470	1	1123.470	64.870	.000	.607	1.000
CTxMT_BL	.056	1	.056	.003	.955	.000	.050
Sex	6.503	1	6.503	.375	.543	.009	.092
Condition	54.076	2	27.038	1.561	.222	.069	.313
Sex * Condition	18.835	2	9.418	.544	.585	.025	.134
Error	727.392	42	17.319				
Total	6311.924	49					
Corrected Total	795.497	48					

a. R Squared = .086 (Adjusted R Squared = -.045)
b. Computed using alpha = .05

Table 49. Experiment 2: ANCOVA (T1) - Hotplate

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Corrected Model	1612639034.598[a]	6	2.69E+08	3.739	.003	.282	.942
Intercept	2.59E+09	1	2.59E+09	36.029	.000	.387	1.000
HP_BL	3.80E+08	1	3.80E+08	5.283	.025	.085	.618
Sex	1.01E+09	1	1.01E+09	14.057	.000	.198	.958
Condition	1.51E+08	2	7.54E+07	1.049	.357	.035	.225
Sex * Condition	1.26E+08	2	6.29E+07	.874	.423	.030	.193
Error	4.10E+09	57	7.19E+07				
Total	2.79E+10	64					
Corrected Total	5.71E+09	63					

a. R Squared = .282 (Adjusted R Squared = .207)
b. Computed using alpha = .05

REFERENCES

1. Acri JB, Grunberg NE, Morse DE. 1991. Effects of nicotine on the acoustic startle reflex amplitude in rats. *Psychopharmacology* 104:244-8
2. Agoston DV, Gyorgy A, Eidelman O, Pollard HB. 2009. Proteomic biomarkers for blast neurotrauma: Targeting cerebral edema, inflammation, and neuronal death cascades. *Journal of Neurotrauma* 26:901-11
3. Alder J, Fujioka W, Lifshitz J, Crockett DP, Thakker-Varia S. 2011. Lateral fluid percussion: Model of traumatic brain injury in mice. *Journal of Visualized Experiments* 54
4. Apatov N. 1998. *The effects of nicotine on antinociception in male and female Sprague Dawley rats with and without stress*. Uniformed Services University of the Health Sciences, Bethesda, MD
5. Apatov N. 1999. *Nicotine-induced antinociception in male and female Sprague Dawley rats*. Uniformed Services University of the Health Sciences, Bethesda, MD
6. Balakathiresan N, Bhomia M, Chandran R, Chavko M, McCarron RM, Maheshwari RK. 2012. MicroRNA let-7i is a promising serum biomarker for blast-induced traumatic brain injury. *Journal of Neurotrauma* 29:1379-87
7. Barry E, Yarnell A, McCarron R, Jacobowitz D, Eidelman O, et al. 2012. Psychological stress alters plasma cytokines and chemokines in male and female rats. In *Center for Neuroscience and Regenerative Medicine Annual Meeting*. Bethesda, MD

8. Bartok R, Craft R. 1997. Sex differences in opioid antinociception. *The Journal of Pharmacology and Experimental Therapeutics* 282:769-78

9. Baumann M, Chwa A, Cravedi K, Barry E, Yarnell A, et al. 2012. Psychological stress and blast overpressure exposure differentially alter central monoamine transmission in male and female rats. In *National Capitol Area TBU Research Symposium*. Bethesda, MD

10. Bazarian J, Blythe B, Mookerjee S, He H, McDermott M. 2010. Sex differences in outcome after mild traumatic brain injury. *Journal of Neurotrauma* 27:527-39

11. Belanger HG, Proctor-Weber Z, Kretzmer T, Kim M, French LM, Vanderploeg RD. 2011. Symptom complaints following reports of blast versus non-blast mild TBI: does mechanism of injury matter? *The Clinical Neuropsychologist* 25:702-15

12. Berry A, Capone F, Giorgio M, Pelicci P, de Kloet E, et al. 2007. Deletion of the life span determinant $p66^{Shc}$ prevents age-dependent increases in emotionality and pain sensitivity in mice. *Experimental Gerontology* 42:37-45

13. Bombardier CH, Fann JR, Temkin NR, Esselman PC, Barber J, Dikmen SS. 2010. Rates of major depressive disorder and clinical outcomes following traumatic brain injury. *JAMA: the journal of the American Medical Association* 303:1938-45

14. Bowen DJ, Eury S, Grunberg NE. 1986. Nicotine's effects on female rats' body weight: Caloric intake and physical activity. *Pharmacology Biochemistry & Behavior* 25:1131-6

15. Brown K. 1997. *Examination of acute sensitivity to morphine and morphine self-administration following physical and environmental stressors in Fischer-344 and Lewis female rat*. Uniformed Services University of the Health Sciences, Bethesda, MD

16. Brown K, Grunberg NE. 1995. Effects of housing on male and female rats: crowding stresses males but calms females. *Physiology & Behavior* 58:1085-9

17. Brown K, Grunberg NE. 1996. Effects of environmental conditions on food consumption in female and male rats. *Physiology & Behavior* 60:293-7

18. Bruns J, Hauser W. 2003. The epidemiology of traumatic brain injury: A review. *Epilepsia* 44:2-10

19. Cannon WB. 1929. Bodily changes in pain, hunger, fear and rage.

20. Cernak I. 2005. Animal models of head trauma. *NeuroRx* 2:410-22

21. Chandran R, Sharma A, Barry ES, Balakathiresan NS, Hutchison MA, et al. 2013, April. Altered serum microRNA expression and neurobehavioral deficits following mild closed head injury in mice. In *National Capital Area TBI Research Symposium*. Bethesda, MD

22. Chavko M, Watanabe T, Adeep S, Lankasy J, Ahlers S, McCarron R. 2011. Relationship between orientation to a blast and pressure wave propagation inside the rat brain. *Journal of Neuroscience Methods* 195:61-6

23. Craft R, Stratmann J, Bartok R, Walpole T, King S. 1999. Sex differences in the development of morphine tolerance and dependence in the rat. *Psychopharmacology* 143:1-7

24. Darwin C. 1859. *The origin of species*. London: Murray

25. Defense and Veterans Brain Injury Center. 2012. *TBI Basics*. http://www.dvbic.org/tbi-basics

26. Department of Veterans Affairs/Department of Defense. 2009. VA/DOD clinical practice guidelines for management of concussion/mild traumatic brain injury (mTBI), Version 1.0. Washington, DC: Veterans Health Administration, Department of Defense

27. Dixon CE, Clifton GL, Lighthall JW, Yaghmai AA, Hayes RL. 1991. A controlled cortical impact model of traumatic brain injury in the rat. *Journal of Neuroscience Methods* 39:253-62

28. DoD/VA Definition and Symptomatic Taxonomy Working Group. 2008. DoD/VA Code Proposal Final - 508 Compliant. Washington, DC: Department of Defense, Veterans Health Administration

29. Dubinsky TJ, Cuevas C, Dighe MK, Kolokythas O, Hwang JH. 2008. High-intensity focused ultrasound: Current potential and oncologic applications. *American Journal of Roentgenology* 190:191-9

30. Elder GA, Dorr NP, De Gasperi R, Gama Sosa MA, Shaughness MC, et al. 2012. Blast exposure induces post-traumatic stress disorder-related traits in a rat model of mild traumatic brain injury. *Journal of Neurotrauma* 29:2564-75

31. Elliott B. 2004. Environmental Enrichment, Performance, and Brain Injury in Male and Female Rats, DTIC Document

32. Elliott B, Faraday MM, Phillips J, Grunberg NE. 2004. Effects of nicotine on elevated-plus maze and locomotor activity in male and female adolescent and adult rats. *Pharmacology Biochemistry & Behavior* 77:21-8

33. Elliott B, Grunberg NE. 2005. Effects of social and physical enrichment on open field activity differ in male and female Sprague–Dawley rats. *Behavioural brain research* 165:187-96

34. Emanuelson I, Andersson Holmkvist E, Björklund R, Stålhammar D. 2003. Quality of life and post-concussion symptoms in adults after mild traumatic brain injury: a population-based study in western Sweden. *Acta Neurologica Scandinavica* 108:332-8

35. Ettenhofer ML, Melrose RJ, Delawalla Z, Castellon SA, Okonek A. 2012. Correlates of Functional Status Among OEF/OIF Veterans With a History of Traumatic Brain Injury. *Military Medicine* 177:1272-8

36. Faraday MM, Elliott B, Phillips J, Grunberg NE. 2003. Adolescent and adult male rats differ in sensitivty to nicotine's activity effects. *Pharmacology Biochemistry & Behavior* 74:917-31

37. Faraday MM, O'Donoghue VA, Grunberg NE. 2003. Effects of nicotine and stress on locomotion in Sprague-Dawley and Long-Evans male and female rats. *Pharmacology Biochemistry & Behavior* 74:325-33

38. Faraday MM, Scheufele P, Rahman M, Grunberg NE. 1999. Effects of chronic nicotine administration on locomotion depend on rat sex and housing condition. *Nicotine and Tobacco Research* 1:143-51

39. Fischer H. 2013. U.S. Military Casualty Statistics: Operation New Dawn, Operation Iraqi Freedom, and Operation Enduring Freedom. ed. CR Service. Washington D.C.

40. Gad S, ed. 2006. *Animal models in toxicology*: Informa HealthCare.

41. Goldberg M. 2010. Death and injury rates of US military personnel in Iraq. *Military Medicine* 175:220-6

42. Green RE, Melo B, Christensen B, Ngo L-A, Monette G, Bradbury C. 2008. Measuring premorbid IQ in traumatic brain injury: An examination of the validity of the Wechsler Test of Adult Reading (WTAR). *Journal of Clinical and Experimental Neuropsychology* 30:163-72

43. Grunberg NE. 1982. The effects of nicotine and cigarette smoking on food consumption and taste preferences. *Addictive Behaviors* 7:317-31

44. Grunberg NE, Bowen DJ. 1985. The role of physical activity in nicotine's effects on body weight. *Pharmacology Biochemistry & Behavior* 23:851-4

45. Grunberg NE, Bowen DJ, Winders SE. 1986. Effects of nicotine on body weight and food consumption in female rats. *Psychopharmacology* 90:101-5

46. Grunberg NE, Winders SE, Popp KA. 1987. Sex differences in nicotine's effects on consummatory behavior and body weight in rats. *Psychopharmacology* 91:221-5

47. Grunberg NE, Winders SE, Wewers ME. 1991. Gender differences in tobacco use. *Health Psychology* 10:143-53

48. Grunberg NE, Yarnell AM, Chwa A, Hutchison MA, Barry ES. A revised neurological severity scale (NSS-R and mNSS-R) for rodents. *Proc. Society for Neuroscience Annual Meeting*, *Washington, D.C.*, *2011*:

49. Gyorgy A, Ling G, Wingo D, Walker J, Tong L, et al. 2011. Time-dependent changes in serum biomarker levels after blast traumatic brain injury. *Journal of Neurotrauma* 28:1121-6

50. Haar GT, Coussios C. 2007. High intensity focused ultrasound: Physical principles and devices. *International Journal of Hyperthermia* 23:89-104

51. Hamm R. 2001. Neurobehavioral assessment of outcome following traumatic brain injury in rats: An evaluation of selected measures. *Journal of Neurotrauma* 18:1207-16

52. Hau J, Van Hoosier G, eds. 2002. *Handbook of laboratory animal science: Essential principles and practices*, Vols. 1: CRC.

53. Hauret K, Clennin M, Taylor B, Jones BH. 2012. Deployment Surveillance Report: Traumatic Brain Injury Identified from Hospitalization and Air Evacuation Records-Army, 2004-2009, DTIC Document

54. Hendricks AM, Amara J, Baker E, Charns MP, Gardner JA, et al. 2013. Screening for mild traumatic brain injury in OEF-OIF deployed US military: An empirical assessment of VHA's experience. *Brain Injury* 27:125-34

55. Hesdorffer DC, Rauch SL, Tamminga CA. 2009. Long-term Psychiatric Outcomes Following Traumatic Brain Injury: A Review of the Literature. *The Journal of Head Trauma Rehabilitation* 24:452-9

56. Holmes A. 2001. Targeted gene mutation approaches to the study of anxiety-like behavior in mice. *Neuroscience and Biobehavioral Reviews* 25:261-73

57. Hu Z, Yu D, Almeida-Suhett C, Tu K, Marini AM, et al. 2012. Expression of miRNAs and their cooperative regulation of the pathophysiology in traumatic brain injury. *PloS one* 7:e39357

58. Institute of Laboratory Animal Resources (US). 1996. Guide for the care and use of laboratory animals. National Academies Press

59. Jacobowitz DM, Cole JT, McDaniel DP, Pollard HB, Watson WD. 2012. Microglia activation along the corticospinal tract following traumatic brain injury in the rat: a neuroanatomical study. *Brain Research* 1465:80-9

60. Jorge RE, Robinson RG, Moser D, Tateno A, Crespo-Facorro B, Arndt S. 2004. Major depression following traumatic brain injury. *Archives of General Psychiatry* 61:42-50

61. Kamnaksh A, Kovesdi E, Kwon S, Wingo D, Farid A, et al. 2011. Factors affecting blast traumatic brain injury. *Journal of Neurotrauma* 28:2145-53

62. Katz RJ, Roth KA, Carroll BJ. 1981. Acute and chronic stress effects on open field activity in the rat: implications for a model of depression. *Neuroscience & Biobehavioral Reviews* 5:247-51

63. Kennedy JE, Haar GT, Cranston D. 2003. High intensity focused ultrasound: Surgery of the future? *The British Journal of Radiology* 76:590-9

64. Ling G, Lee E, Kalehua A. 2004. Traumatic brain injury in the rat using the fluid-percussion model. *Current Protocols in Neuroscience* 9.2.1–9.2.11

65. Mahmood A, Lu D, Wang L, Li Y, Lu M, Chopp M. 2001. Treatment of traumatic brain injury in female rats with intravenous administration of bone marrow stromal cells. *Neurosurgery* 49:1196-204

66. Marmarmou A, Foda M, Brink W, Campbell J, Kita H, Demetriadou K. 1994. A new model of diffuse brain injury in rats. *Journal of Neurosurgery* 80:291-300

67. McCabe JT, Moratz C, Liu Y, Egan R, Chen H, et al. 2010. Animal models for the study of military-related, blast-induced traumatic brain injury. In *Biomedical Sciences and Engineering Conference*. Oak Ridge, TN

68. Mesiwala AH, Farrell L, Wenzel HJ, Silbergeld DL, Crum LA, et al. 2002. High-intensity focused ultrasound selectively disrupts the blood-brain barrier *in vivo*. *Ultrasound in Medicine and Biology* 28:389-400

69. Morse DE, Davis H, Popke E, Brown K, O'Donoghue VA, Grunberg NE. 1997. Effects of ddC and AZT on locomotion and acoustic startle, I: Acute effects in female rats. *Pharmacology Biochemistry & Behavior* 56:221-8

70. Nampiaparampil D. 2008. Prevalence of chronic pain after traumatic brain injury: A systematic review. *JAMA* 300:711-9

71. National Institutes of Health. 2007. Definitions under Subsection 1 (Research Objectives), Section I (Funding Opportunity Description). Institutional Clinical and Translational Science Award

72. Ojeda SR, Urbanski HF. 1994. Puberty in the rat. In *The physiology of reproduction*, ed. E Knobil, JD Neill:363-409. New York: Raven Press. Number of 363-409 pp.

73. Ottochian M, Salim A, Berry C, Chan L, Wilson M, Margulies D. 2009. Severe traumatic brain injury: Is there a gender difference in mortality? *The American Journal of Surgery* 197:155-8

74. Petit-Demouliere B, Chenu F, Bourin M. 2005. Forced swimming test in mice: A review of antidepressant activity. *Psychopharmacology* 177:245-55

75. Prager EM, Bergstrom HC, Grunberg NE, Johnson LR. 2011. The importance of reporting housing and husbandry in rat research. *Frontiers in Behavioral Neuroscience* 5:1-4

76. Prut L, Belzung C. 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *European journal of pharmacology* 463:3-33

77. Rose C. 2011. *The Effects of Recurrent Stress and a Music Intervention on Tumor Progression and Indices of Distress in an MNU-induced Mammary Cancer in Rats*. Uniformed Services University of the Health Sciences

78. Shanks N, Greek R, Greek J. 2009. Are animal models predictive for humans? *Philosophy, Ethics, and Humanities in Medicine* 4:1-20

79. Sharma A, Chandran C, Barry ES, Hutchison MA, Nagaraja B, et al. under review. Mild closed head injury causes acute behavioral and serum microRNA expression alterations in mice. *Journal of Neurotrauma*

80. Sharma P, Su YA, Barry ES, Grunberg NE, Lei Z. 2012. Mitochondrial targeted neuron focused genes in hippocampus of rats with traumatic brain injury. *International Journal of Critical Illness and Injury Science* 2:172-9

81. Shohami E, Novikov M, Bass R. 1995. Long-term effect of HU-211, a novel non-competitive NMDA antagonist, on motor and memory function after closed head injury in the rat. *Brain Research* 647:55-62

82. Skinner BF. 1969. *Contingencies of reinforcement: A theoretical analysis*. Appleton-Century-Crofts New York

83. Sonmez U, Sonmez A, Erbil G, Tekmen I, Baykara B. 2007. Neuroprotective effect of resveratrol against traumatic brain injury in immature rats. *Neuroscience Letters* 420:133-7

84. Spear LP, Brake SC. 1983. Periadolescence: Age-dependent behavior and psychopharmacological responsivity in rats. *Developmental Psychobiology* 16:83-109

85. Tanter M, Pernot M, Aubry J, Montaldo G, Marquet F, Fink M. 2007. Compensating for bone interfaces and respiratory motion in high-intensity focused ultrasound. *International Journal of Hyperthermia* 23:141-51

86. Taylor S, Klein L, Lewis B, Gruenewald T, Gurung R, Updegraff J. 2000. Biobehavioral responses to stress in females: Tend-and-befriend, no fight-or-flight. *Psychological Review* 107:411-29

87. Thompson HL, Lifshitz J, Marklund N, Grady MS, Graham DI, et al. 2005. Lateral fluid percussion brain injury: A 15-year review and evaluation. *Journal of Neurotrauma* 22:47-75

88. Tkacs N, Thompson HL. 2006. From bench to bedside and back again: Research issues in animal models of human disease. *Biological Research for Nursing* 8:78-88

89. Tucker L, Fu A, Fortin LA, Malkesman O, McCabe JT. Behavioral changes after traumatic brain injury induced by high-intensity focused ultrasound in the laboratory mouse. *Proc. Neuroscience*, *New Orleans, LA*, *2012*:

90. U.S. Department of Defense. 2012. *Traumatic Brain Injury: Department of Defense Special Report*. http://www.defense.gov/home/features/2012/0312_tbi/

91. U.S. Department of Defense. 2013. *DoD Worldwide Numbers for Traumatic Brain Injury*. http://www.health.mil/Research/TBI_Numbers.aspx

92. U.S. Department of Health and Human Services. 2009. Blast Injuries: Radiological Diagnosis. ed. CDC. Atlanta, GA

93. VA San Diego Healthcare System. n.d. Traumatic Brain Injury: A guide for patients. ed. V Affairs

94. Van Der Stay F, Arndt S, Nordquist R. 2009. Evaluation of animal models of neurobehavioral disorders. *Behavioral and Brain Functions* 5:1-23

95. Wahab RA, Choi M, Liu Y, Krauthamer V, Zderic V, Myers MR. 2012. Mechanical bioeffects of pulsed high intensity focused ultrasound on a simple neural model. *Medical Physics* 39:4274-83

96. Watson JB, Rayner R. 1920. Conditioned emotional reactions. *Journal of Experimental Psychology* 3:1-14

97. Watts G. 2009. John Wild. *British Medical Journal* 339

98. Xing G, Ren M, O'Neill JT, Sharma P, Verma A, Watson WD. 2012. Pyruvate dehydrogenase phosphatase1 mRNA expression is divergently and dynamically regulated between rat cerebral cortex, hippocampus and thalamus after traumatic brain injury: A potential biomarker of TBI-induced hyper-and hypo-glycaemia and neuronal vulnerability. *Neuroscience Letters* 525:140-5

99. Yao X-L, Liu J, Lee E, Ling GS, McCabe JT. 2005. Progesterone differentially regulates pro-and anti-apoptotic gene expression in cerebral cortex following traumatic brain injury in rats. *Journal of Neurotrauma* 22:656-68

100. Yarnell AM. 2012. *A neurobehavioral phenotype of blast traumatic brain injury and psychological stress in male and female rats*. Uniformed Services University of the Health Science, Bethesda, MD

101. Yarnell AM, Shaughness M, Barry E, Ahlers S, McCarron R, Grunberg NE. 2013. Blast traumatic brain injury in the rat using a blast overpressure model. *Current Protocols in Neuroscience* 62:9-41

102. Zhao Z, Loane DJ, Murray MG, Stoica BA, Faden AI. 2012. Comparing the predictive value of multiple cognitive, affective, and motor tasks after rodent traumatic brain injury. *Journal of Neurotrauma* 29:2475-89