MECHANISMS OF NEUROPATHIC PAIN FOLLOWING MILD BLAST TRAUMATIC BRAIN INJURY AND CHRONIC STRESS

by

Marcela Cruz Haces

A Dissertation

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Doctor of Philosophy

School of Biomedical Engineering

West Lafayette, Indiana

August 2019

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Riyi Shi, Chair
Department of Biomedical Engineering

Dr. Brad Duerstock
Department of Biomedical Engineering

Dr. Amy Brewster
Department of Biomedical Engineering

Dr. Chongli Yuan
Department of Chemical Engineering

Approved by:

Dr. George R. Wodicka
Head of the Graduate Program

To my family, to my wonderful and loving husband Enrique Aldana
and to my three golden retriever dogs Mandy, Trixie and Logan,
for brightening my world with their beautiful selves,
with their limitless love, endless support and radiant joy.

ACKNOWLEDGMENTS

Here I would like to thank the persons who supported me to get to Purdue, to start my Ph.D., to achieve all the milestones of the program successfully, and for making my life better during the Ph.D. In this section, I will start with the names of the persons who have helped me in my professional life, and I will finalize with my personal life, for simplicity. Although I would really like to name all the people who have contributed to my success, I will only name a few. Otherwise, I would need a whole new thesis just for this. In any case, none of this would have been possible nor equivalently successful without each and every one of you, thank you.

I would like to start by thanking my advisor, Dr. Shi, who let me be part of his laboratory and gave me his support with great ideas, feedback, resources to run all the studies described here and more and helped me achieve all the milestones of my studies on time. Thank you, Dr. Shi, for also inviting us to your home during Thanksgiving and making us feel welcome with your wife and daughter playing ping-pong.

I would like to thank the members of my committee as well, Dr. Amy Brewster, Dr. Brad Duerstock and Dr. Chongli Yuan for agreeing to be part of my committee, for being always kind to me and providing me with great feedback to help me learn, explore and grow. Thank you, professors, for sharing your experiences and wisdom with me, as well as your time despite your multiple commitments.

I am also grateful to Dr. Ernesto Marinero and Dr. Hazel Marinero who helped me and Enrique get here through the Mexican-government scholarship program, CONACYT. For insisting us to come when we were not sure, and for making everything possible in our departments and with our student visas. Without you, we would not be here. Thank you for all.

From the BME department, I would like to thank Sandy May, for being outstanding and going above and beyond to help us achieve our milestones and answering our questions even from Spain. Thank you, Sandy, I wish all the schools had Sandy to make our lives better as students.

I am forever grateful for being able to share this journey with the love of my life, Enrique, who is the best man I know, who supports me, makes me laugh more than ever, and for showing me that we can achieve anything together. Thank you so much for sharing your beautiful self with me, for giving me all your love and support, and for helping me become a better person on all this time we have been together. Thank you for always being up to new adventures and places with me and for having shared our life as a couple in Mexico, Germany, USA, and soon in Finland too. You are the kindest, smartest, most patient, fun, creative and most loving person I know, and I am blessed for being able to share my life with you. My eternal thanks and love go to you.

I would also like to thank my family in Mexico, my mom, my grandmother, my aunts, cousins, my mother-in-law, my brothers-in-law, and my uncles-in-law for always supporting me and Enrique despite the distance, and who always make us feel that we are not alone in this world and that we can always count on them. Thank you so much for all your love, you are an example to us, and we feel blessed for having you as family.

I would like to thank my lab mates, specially Glen, Jonathan and Seth for also making the life in the lab more fun and enjoyable. I would like to thank Glen for teaching me the western blotting technique, Jonathan for always being up for discussion and helping us improve techniques, and Seth for being such a fun friend, always helping me keep the lab clean, talking about our experiments, and laughing even when things do not work as we expect. Thank you all for your friendship and companionship.

Last, but not least, I would like to thank my golden retriever dogs, Mandy, Trixie and Logan, who were adopted from Mexico and have traveled the world with us sharing their immense joy of life and love. Thank you for always making me laugh, for warming my heart and for making me a better person.

Thank you all who shared instances with me throughout the Ph.D. for sharing your time, experiences, and love, and for helping me learn, laugh and become a better person. This would not have been possible without you.

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

Author: Cruz Haces, Marcela. Ph.D.
Institution: Purdue University
Degree Received: August 2019
Title: Mechanisms of Neuropathic Pain Following Mild Blast Traumatic Brain Injury and Stress
Committee chair: Dr. Riyi Shi, M.D., Ph.D.

The incidence of mild blast traumatic brain injury has risen due to the increased use of improvised explosive devices (IEDs) in military conflicts. Mild blast TBI (mbTBI) is especially relevant due to its lack of acutely observable symptoms, and to its association with long-term neurodegenerative and neuropsychiatric disorders. Predominantly, TBI patients often suffer from chronic stress, neuropathic pain and headaches, which greatly compromise the health and quality of life of these individuals. Treatments for neuropathic pain have been empirically found and produce little effect in lessening neuropathic pain, likely due to the lack of targeted therapies. This highlights the need for better understanding of the molecular mechanisms underlying neuropathic pain, TBI and chronic stress that could lead to mechanistic therapeutic targets. Oxidative stress is an important mechanism of the pathophysiology of neuropathic pain, TBI and chronic stress. We hypothesize that acrolein, an endogenously formed neurotoxin, is able to stay active in the body for up to 10 days, is involved in the pathophysiology of neuropathic pain in TBI and chronic stress. This study aims to correlate acrolein elevation in the body with neuropathic pain, deepen the understanding of underlying mechanisms of pain in TBI and chronic stress, and mitigate this pain with acrolein scavenging. The ultimate goal of this research is to provide therapies for TBI and chronic stress patients that can eliminate pain and significantly improve their health and quality of life.

CHAPTER 1. INTRODUCTION

1.1 Blast injury definitions, epidemiology, impact and symptoms

Traumatic brain injury (TBI) is one of the most common causes of death and disability in young people,[101,148] and is the leading cause of death in individuals under 45 years of age in the United States and Europe.[158] TBI can result from a physical blow to the head in traumatic events such as falls,[205] motor vehicle collisions,[91] sports related injuries,[123] or by exposure to explosive blasts.[49]

In military conflicts, one out of five injured soldiers suffer from TBI[253] and 52% of these cases are due to explosion.[93] For this reason, blast traumatic brain injury (bTBI) is known as the signature injury of modern warfare.[253] Of these cases, mild blast traumatic brain injury (mbTBI) is the most common type.[203] It was long thought that the skull would act as a protecting shield preventing injuries to the brain from the blast waves, but it is now well know that the brain is vulnerable to blast wave injuries at thresholds even below of other organs.[19,20,195]

Mild blast traumatic brain injury is particularly relevant due to its lack of acute symptoms, since there is an inconsistency in diagnosis.[47,79,226] It is usually believed that no injury has been sustained if the individual has not lost consciousness or has had any memory or cognitive issues after the incident; however, in most cases, there is no need of any of these symptoms to show to have suffered from a mild blast traumatic brain injury that can initiate a long-term neurodegenerative cascade. This lack of acute symptoms can lead the physicians and the individual to believe that no injury has been sustained, diminishing the importance of the injury. When the importance of the injury is diminished, it is common that the injured individuals lack of preventative measures that might therefore lead to repetitive TBI[222] promoting even more damage to the brain.

Mild blast traumatic brain injury has also been closely associated to long term severe neurodegenerative and neuropsychiatric disorders.[30,75,107] Some of the most common neurodegenerative diseases associated with TBI include Alzheimer's disease,[97] Parkinson's Disease,[106] amyotrophic lateral sclerosis,[53] and chronic traumatic encephalopathy.[21] Among other reasons, this can be due to the fact that protein accumulation and oxidative stress, which are important characteristics of many neurodegenerative disorders, have been found to occur after TBI

as well. Particularly, amyloid-beta,[122] tau,[204] alfa-synuclein,[105] and other protein accumulations have been observed after TBI, which are the same hallmark protein accumulations observed in Alzheimer's disease, tauopathies, including CTE, and Parkinson's disease respectively.

Blast traumatic brain injury also has significant comorbidity and symptoms that commonly overlap with the combat-related neurological dysfunction post-traumatic stress disorder (PTSD),[19] and chronic stress.[209] These disorders often coexist because mild blast traumatic brain injuries are commonly sustained during military conflicts, which are also often associated with traumatic experiences. Evidence suggests that mild TBI can promote PTSD and chronic stress, and that stress reactions after TBI attribute to secondary impairment after mild-TBI. This indicates a synergy where stress can exacerbate impairment post-mild TBI, and where also mild-TBI can lead to PTSD and chronic stress.[42] Importantly, some of the most common symptoms post-TBI include neuropathic pain, migraine and headache;[220] which have been also linked to PTSD and chronic stress.[34]

Pain is quite frequent in TBI and neurotrauma, where patients experience long-lasting and persistent pain after the traumatic injury.[212] Pain after trauma is difficult to treat, hinders the ability of participating in rehabilitation programs, delays the acquisition of an optimal level of activity and independence, is burdensome and negatively affects the patients' mood. In TBI, even after a minor injury in both children and adults, a major subset of patients develop chronic headache that hinders the ability to concentrate and chronic pain, with about 43% of veteran patients and 58% civilian populations developing chronic posttraumatic headache (PTH) after mild-TBI, where the majority of combat veterans report the headache intensity as moderate to severe.[166] Neuropathic pain, low back pain, extremity pains and complex regional pain syndrome are also present post-TBI.[166] For these reasons, pain is consistently rated as one of the most difficult problems associated with traumatic injuries.[166]

Although chronic stress and pain are common comorbid conditions after TBI, the exact molecular mechanisms underlying and linking these conditions are still poorly understood, which makes the identification of therapeutic targets for the development of effective treatments for these conditions harder to achieve. These disorders greatly compromise the health and quality of life of injured individuals, are costly to treat and manage, and are burdensome to the health care providers and loved ones of TBI victims. Thus, it is imperative that more research efforts are focused on

understanding the mechanisms underlying these conditions to reduce the economic burden and improve the health and quality of life of individuals suffering from these conditions.

Importantly, as of today TBI is still poorly understood, and therefore, there are no current treatments that can prevent, slow down or stop the neurodegenerative processes involved in TBI or the symptoms associated with TBI. This highlights the importance of focusing research efforts on understanding the molecular mechanisms involved in TBI that could potentially lead to therapeutic targets and treatments that can stop the neurodegeneration and alleviate symptoms post-TBI.

1.2 Chronic stress definitions, epidemiology, impact and symptoms

Emotionally and physiologically challenging experiences can cause stress, and the brain is the key organ of the response to stress since it determines what is threatening.[157] There are two types of stress: acute, where an event occurs with a foreseeable end and it is expected that the stress shall pass after the end of the event or experience is accomplished (e.g. an exam); or chronic, where there is uncertainty of the duration of the event, or the event does not end (e.g. losing a job, caring for a loved one with dementia).[210] During acute stress the cellular immunity has been observed to be suppressed, while the humoral immunity is preserved; and during chronic stress both the cellular and humoral immunity have been observed to be suppressed.[210] The cellular immunity involves the activation of phagocytes, cytotoxic T-lymphocytes and release of cytokines; while the humoral immunity is mediated by antigen responses involving B cells. Particularly, chronic stress has the potential to induce disease,[129] and has been linked to multiple disorders that greatly increase the mortality rate and compromise the health and quality of life of the individuals who suffer from them, such as cardiovascular diseases,[259] obesity,[64] depression,[201] cancer,[197] among other disorders.

Chronic stress is a very common condition[38] that is experienced by numerous individuals due to different circumstances and situations, such as social stressors,[241] work,[51] environmental conditions,[22] among others. Chronic stress situations occur when the stress lasts for months or years. Specifically, chronic stress has been highly reported post-TBI and has been linked to burdensome fatigue and to a decreased quality of life.[26] Prominently, a significant symptom involved with chronic stress is pain.[263] An example is fibromyalgia, a disorder where the painful sensations are amplified leading to widespread musculoskeletal pain, that has been reported after

significant psychological stress and has been called a "stress-related disorder".[264] Commonly, TBI victims have experienced situations of extreme stress in military conflicts that can lead to chronic stress, which in turn can lead to persistent pain.

Stress and pain have significant conceptual and physiological overlaps since both phenomena challenge the body's homeostasis and share the behavioral model of failure to extinguish negative memories or emotions. In normal situations, the organism is able to adapt to stress or pain by regulating the internal milieu and maintaining stability, which ability is termed allostasis. Stress and pain are adaptive in protecting the organism in short term situations. However, persistence of either stress or pain leads to an allostatic overload that is maladaptive and can lead to suffering and compromised well-being. Importantly, physiological and structural remodeling of the learning circuitry including the hippocampus, amygdala and ventromedial pre-frontal cortex, also known as the limbic brain, have been observed in chronic pain and stress conditions and are also linked to risk the onset of one another.[3]

Stress has been linked to pain by different mechanisms. One of them is by changing the dopaminergic activity that can either inhibit pain in acute stress situations or promote pain in chronic stress situations. On the one hand, acute stress has been observed to activate a pain-suppression system that includes the release of endogenous opioids and substance P mediated by the activation of mesolimbic dopamine neurons that arise from the cell bodies of the ventral tegmental area and project to the nucleus accumbens.[264] On the other hand, in situations of persistent stress, pain or hyperalgesia has been observed to occur due to a reduction of the dopamine output in the nucleus accumbens; thus, chronic stress appears to lead to a dysfunction of the mesolimbic dopaminergic activity that promotes pain.[264]

Chronic pain and chronic stress have also been linked via chronic stress-induced hypothalamo-pituitary-adrenal (HPA) dysfunction, where chronic pain activates the hypothalamo-pituitary-adrenal axis acting as an inescapable stressor.[34] Moreover, chronic stress and neuropathic pain have also been closely related due to the response of the hippocampus to both chronic and neuropathic pain,[156] in that chronic stress induces an increased efflux of norepinephrine in the hippocampus,[4,174] and neuropathic pain suppresses the hippocampal norepinephrine neurotransmission through elevated local production of the inflammatory cytokine TNF-alfa.[60,118] Thus, chronic stress and neuropathic pain induce important responses of the hippocampus by producing changes in neurotransmission of norepinephrine, which is an important neurotransmitter

in the fight-or-flight response of the body, with high concentrations of norepinephrine in situations of stress or danger, and low concentrations of norepinephrine in relaxed states of the body such as those observed during sleep.

The hippocampal formation is vulnerable to damages such as TBI and is also particularly sensitive to the adrenal glucocorticoids secreted during the diurnal rhythm and chronic stress. Adrenal glucocorticoids have adaptive or protective effects in the short term but promote pathophysiology when there is repeated stress or hypothalamo-pituitary-adrenal axis (HPA) dysregulation, which is also induced by chronic stress. Repeated stress or elevated levels of glucocorticoids in the CA3 region have been observed to promote changes in the structural plasticity of the hippocampus causing dendritic remodeling.[156] Importantly, it has been shown that chronic stress induces morphological changes in the hippocampus that can lead to changes in pain perception.[156]

Although chronic stress and pain are often comorbid conditions, the exact mechanisms that link chronic stress to pain are still incompletely understood. For this reason, a better understanding of the overlapping and distinguishing features of chronic stress and pain could provide greater insight into the neurobiology of these processes and contribute to the development of rational drug treatments.[3]

Up to date, chronic stress is a very common condition that compromises the health and quality of life of individuals who suffer from it. The health deterioration effects associated to chronic stress are costly to treat and manage, and burdensome to the individual, health care providers, and their loved ones. Moreover, since the molecular mechanisms associated to chronic stress are still incompletely understood, the treatments available for the health deterioration experienced by individuals suffering from chronic stress only superficially and temporarily improve the symptoms but do not treat the root problem, which is chronic stress. For this reason, a better understanding of the molecular mechanisms involved in chronic stress is necessary to potentially provide treatments that prevent the health deterioration cascade that occurs due to chronic stress.

1.3 Neuropathic pain definitions, epidemiology and impact

Pain can become a disabling chronic, burdensome, and intractable state that interferes with the normal social or occupational functioning of people who experience it.[34] Chronic pain conditions, such as neuropathic pain, affect every aspect of a patient's life, the society, and the lives of their

loved ones in many ways. It contributes to a loss of physical and emotional function that affects the patient's ability to work at home and job and engage in social and recreational pursuits.[240] These conditions also affect the patients and the world economically due to the care expenses involved and the potential decrease of financial income due to the disabling characteristic of chronic pain conditions. Chronic pain conditions have also been associated with an increase in risk of mortality, independent of sociodemographic factors.[234] Pain is a global cause of disability in both developed and developing countries.[199]

Although there is a general lack of awareness, pain is a global health problem in urgent need of new specific therapeutic approaches.[196] Due to the enormous global burden of pain, this condition has been considered as a disease, and chronic pain conditions have been defined as a pathological state.[196] Pain has a valuable role in medical action as the symptom par excellence and as a precious and meaningful tool; however, as stated by the founding father of pain medicine, John J Bonica, in 1953, pain "in its late phases, when it becomes intractable, it no longer serves as a useful purpose and then becomes, through its mental and physical effects, a destructive force".[37] Thus, pain can be a biologically protective tool, or it can lose its adaptive function and become a pathological condition that greatly reduces the quality of life of individuals. Due to this complex nature of pain, the attempt to understand pain remains one of the oldest challenges in history of medicine.[196]

Approximately 10% of the population experience neuropathic pain.[112,235] However, the true incidence of neuropathic pain is unknown since it is commonly under-diagnosed and treated inadequately.[28,111] Neuropathic pain occurs due to damage to the nervous system, causing central molecular changes such as impaired inhibitory transmission.[266]

Neuropathic pain elicits different types of symptoms, such as anesthesia, dysesthesia, hyperalgesia, hyperpathia, hypoesthesia, paresthesia, phantom pain, referred pain, and allodynia.[95] Anesthesia is a loss of pain detection,[94] dysesthesia is an unpleasant abnormal sensation,[61] hyperalgesia is an exacerbated pain response,[153] hyperpathia is a delayed and prolonged response to a stimulus,[116] hypoesthesia is a reduced tactile detection of a stimulus that would normally cause pain,[151] paresthesia is an abnormal non-painful sensation,[65] phantom pain occurs by perceiving pain in a limb that has been amputated,[87] and referred pain consists of pain that does not reflect the area of tissue damage, but a different area that is not damaged.[247] The typical examinations to assess neuropathic pain include allodynia evaluations such as touch, pressure, cold, heat, vibration,

pinprick, and the summation, in which there would be pain responses to stimuli that are normally not painful.[41]

Headache is considered to be a mixture of neuropathic and non-neuropathic pain because it prospectively involves a complex of musculoskeletal, inflammatory and neuropathic mechanisms.[36] Musculoskeletal pain is caused by involuntary muscle contractions triggered by demyelinated neurons,[36] but is different from neuropathic pain because, despite the demyelination of neurons, the nerve damage does not appear to affect the somatosensory pathways.[236] On the other hand, inflammatory pain refers to pain that occurs after tissue injury and the resulting inflammatory process[117] that normally resolves as the tissue heals,[268] although some of the mechanisms of chronic inflammation overlap with neuropathic pain.[55]

1.3.1 Mechanisms of neuropathic pain

The type of pain that is perceived when the tissue is damaged is called nociceptive pain, which has an assistive role of wound repair by generating avoidance of contact until the damaged tissue has repaired.[267] This type of pain is distinct from neuropathic pain, which is "initiated or caused by a lesion or dysfunction in the nervous system".[40] Neuroinflammation and neuroimmune activation has been suggested to play a role in acute and chronic pain, since both occur following nervous system injuries.[257] Neuroimmune activation involves microglia, endothelial cells and astrocytes; and neuroinflammation can be defined as the migration of immune cells to the site of nervous injury.[67] Microglia are known to keep the homeostatic balance in the CNS, and after an acute insult, microglia activate and work as CNS immune cells;[63] but if the balance is compromised by the insult, microglia promote excitotoxic and inflammatory cascades, such as extracellular glutamate increase, that contribute to chronic neuroinflammation and neurodegeneration.[67,225] A group of proteins that have been observed to have modulation properties in the neuroinflammatory cascades and neuropathic pain, are cytokines.[140,249] Cytokines can induce the production of arachidonic acid, which exacerbates the injury by increasing extracellular levels of glutamate, and can also lead to the formation of superoxide free radicals, which can have a direct effect in sensitization, and even cellular death.[67]

The sensory neurons terminate as free nerve endings on the skin, near to blood vessels and hair follicles; accordingly, being able to recognize temperature, mechanical and chemical changes in the environment.[185] These groups of neurons that allow identification of external stimuli are

located in the dorsal root ganglia (DRG), which are positioned in the vertebral column lateral to the spinal cord, and in cranial nerves, such as the trigeminal nerves in the trigeminal ganglia.[185] Interestingly, correlations have been found between trigeminal nerve demyelination and trigeminal neuropathic pains, such as trigeminal neuralgia in multiple sclerosis. [231] Also, neuropathic pain has been correlated to inflammation of the DRG,[152] but the mechanisms are still to be elucidated.

These facts highlight the importance to understand the molecular mechanisms that also occur within the sensory neurons after the nerves have been damaged, such as in TBI, or the homeostatic balance has been compromised, such as in situations of chronic stress, that can lead to neuropathic pain.

1.3.2 Current treatments for neuropathic pain

Neuropathic pain is more difficult to treat than many other types of chronic pain, and is often misdiagnosed and poorly managed.[180] Neuropathic pain is a debilitating disease state that is commonly resistant to currently available therapeutic agents. People suffering from neuropathic pain often receive temporary pain relief with prescribed analgesics, such as morphine, nonsteroidal anti-inflammatory drugs and anticonvulsants, which can bring long-term complications and their limited efficacy leaves a significant number of people untreated and in a constant pain that also constitutes a largely inescapable stress.[34]

Existing treatments include opioids,[131] antidepressants,[217] anticonvulsants,[262] NMDA antagonists,[84] and various others like capsaicin,[73] lidocaine,[11] mexiletine,[85] and cannabinoids.[177] Current medications, such as anticonvulsants and antidepressants, have empirically showed effectiveness for neuropathic pain;[198] consequently being non-specific for neuropathic pain.[115] Hence, many of the actual medications are not tolerated by patients due to adverse side effects, such as addiction,[92] cardiac arrhythmia,[120] psychotomimetic effects.[138] burning sensations,[71] drug interactions,[160] tremors,[67] loss of motor coordination,[113] and sudden death.[218] These treatments also fail to relieve pain entirely,[206] and in the long term,[160] causing neuropathic pain treatments to continue to be unsatisfactory.[12] This is due to the still unclear exact pathophysiological mechanisms underlying most types of chronic pain.[219] Remarkably, the pathophysiological mechanisms underlying pain post-TBI and in chronic stress have not been specifically identified.[103,244] Thus, it is imperative that mechanisms underlying neuropathic pain in TBI and chronic stress are elucidated, that might lead to the development of new long term pain relieving

drugs with mechanistic therapeutic targets that will be able to relieve pain entirely without undesired side effects.

1.4 The role of oxidative stress in blast injury, chronic stress, and neuropathic pain

In order to find a mechanistic therapeutic target for neuropathic pain in TBI and chronic stress, it is necessary to understand the overlapping pathophysiological mechanisms underlying these conditions. It is well known that oxidative stress plays a major role in the secondary injury cascade that occurs after TBI and promotes long-term neurodegeneration that leads to neurodegenerative and neuropsychiatric disorders.[62] Similarly, people experiencing chronic stress have been found to exhibit high levels of oxidative stress by increases in plasma superoxide anions and malondialdehyde, and modified antioxidant defense.[50,100] Moreover, the role of oxidative stress in neuropathic pain has been widely reported with the presence of superoxide free radicals as previously mentioned, and in different diseases, including diabetes,[82,139,172,179,246,248] cancer,[10,125] injuries,[171] etc.

Despite the involvement of oxidative stress in TBI, chronic stress, neuropathic pain, and many other diseases that are considered incurable as of today, antioxidant treatments have proven ineffective.[208] Significant efforts have been made to apply pharmaceuticals that target reactive oxygen species (ROS), but little success has been achieved, possibly due to the short half-life and instability of ROS,[104,178] and the short-term duration of the oxidative stress markers.[9] This has pointed to the pursuit of a longer-lasting oxidative stress compound that could serve as a therapeutic target for all TBI, chronic stress and neuropathic pain, where acrolein could serve this purpose.

1.4.1 Acrolein's role in secondary injury process and neuropathic pain

Acrolein, also known as 2-propenal, is a relatively electrophilic alfa-beta unsaturated aldehyde; and consequently, extremely reactive,[77] and highly toxic.[27] It is found as a pollutant in the environment, as an exhaust from gas combustion, and vapor from extremely heated cooking oils.[77] The permissible exposure limit is 0.1 ppm in a period of time of eight hours.[127] Acrolein exposure commonly occurs by inhaling tobacco smoke, being correlated to lung cancer;[80] and by ingesting foods with burnt fats, where it is not believed to cause any health risk.[5]

Acrolein is a product of lipid peroxidation, a process that is generated naturally in small amounts in the body, mainly by the effect of several reactive oxygen species, in which the reactive oxygen species attack the polyunsaturated fatty acids of the fatty acid cell membrane, instigating a self-propagating chain reaction that is dangerous for the viability of cells and tissues, and that has been identified as a crucial step in the pathogenesis of several disease states.[167] Acrolein is able to induce the production of reactive oxygen species, which are necessary for the lipid peroxidation chain reaction in which acrolein is formed; thus, instigating a vicious cycle of lipid peroxidation and formation of acrolein and reactive oxygen species.[228]

Acrolein is formed in the metal-catalyzed oxidation of polyunsaturated fatty acids including arachidonic acid,[242] and metabolized by the conjugation with gluthatione.[223] Acrolein's extreme reactivity allows it to bind to phospholipids and proteins, damaging the phospholipidic cell membrane, and potentially inhibiting many enzimes;[110] and to modify DNA bases,[56] promoting DNA damage, and inhibiting DNA repair;[80] accordingly being genotoxic and causing cytopathic effects.[108] It is also able to generate free radicals,[146,147] and to stay active in the body for seven to ten days, much longer compared to other oxidative species that decay within fractions of a second.[102]

Protein-bound acrolein has been suggested as a potent biomarker of oxidative stress and protein damage in the long term;[243,269] and acrolein has proven to induce inflammation, [39,183,256] and to play a role in tissue damage at sites of inflammation.[7] Moreover, oxidative stress caused by acrolein has demonstrated to be a crucial factor in the neurotoxicity mechanisms that lead to neurodegeneration and neuronal death in Alzheimer's disease.[45,145]

Acrolein has the ability to damage lipids and proteins, which are major components of the myelin sheath;[164] therefore, being able to induce demyelination.[216] The acrolein-mediated myelin retraction exposes voltage-gated potassium channels, which causes an outward potassium current that results in failure of action potential conduction.[119,193,227,35,211,213,258] The demyelination permits the exposition of axons that allows acrolein to damage the lipid membrane, causing breakage of the membrane and axonal permeability.[214] Since mitochondria have the ability to combat of oxidative stress by producing non-pathological ROS through the electron transport chain, the demyelination and axonal membrane compromise allow acrolein to attack mitochondria, which causes mitochondrial dysfunction by oxidative stress; this instigates deficient energy production, and release of ROS that result in cell death, and axonal loss.[32,44,76,86,141,190,245,251,252]

Several studies have suggested that acrolein has the ability to contribute to neurogenic inflammatory pathways of pain through the activation and upregulation of the TRPA1 channel, which in turn elicits inflammatory pain and neurogenic inflammation.[23,46,54,237] Neurogenic inflammation consists of vasodilation and is triggered by the release of proinflammatory neuropeptides such as the substance P, and calcitonin gene-related peptide (CGRP).[99] Importantly, acrolein plays such a crucial role in inflammatory processes, that it is used to induce chronic or acute inflammation in animal models that experience associated pain.[161] Likewise, acrolein can induce pain by glutamate release,[17] which is known to be an excitatory neurotransmitter that is released by pain-transmitting afferent neurons;[202,267] and which can be stimulated by TRPA1 channel activation,[137,254] that can be induced by acrolein. Moreover, acrolein is able to induce pain by increasing the proinflammatory chemokine MCP1, which is also related to pain and able to transactivate TRPA1 inducing pain directly and indirectly.[134]

Hitherto, our lab has proven that acrolein plays a significant role in the neurological disability process that occurs in multiple sclerosis (MS), and that injections of acrolein scavengers in experimental autoimmune encephalomyelitis (EAE) mice, a model of MS, provides an effective sequestration of acrolein that results in reduced demyelination, neuroprotection, dampened symptom severity and slowed disease progression.[142] These observations indicate that acrolein is a pathological factor in MS.[238]

Furthermore, our lab has also proposed and demonstrated that acrolein plays an important role in the secondary injury process that contributes to motor and sensory deficits, particularly paralysis and neuropathic pain, after spinal cord injury (SCI);[215] specifically, acrolein and TRPA1 concentrations have shown an elevation for 14 days after SCI. Moreover, the pronociceptive role of acrolein has been confirmed by an increased thermal and tactile sensitivity for up to 10 days after the application of an intrathecal injection of acrolein in uninjured rats.[72] These observations lead us to propose a role of acrolein in neuropathic pain in TBI and chronic stress.

1.4.2 The role of TRPA1 in neuropathic pain

Nerve damage has been observed to upregulate the expression of different types of transient receptor potential ion channels (TRP channels), that are involved in neuropathic pain and in the pathway in these ganglia.[18] The TRP channels are generally activated by changes in the temperature and send the information through the nervous system.[159] The TRP cation channels are

classified by ankyrin repeats in subfamilies that include TRPC, TRPV, TRPM, TRPN, TRPP, and TRPML.[162] Six TRP channels have been identified to contribute to pain, which include TRPV1, TRPV2, TRPV3, TRPV4, TRPM8 and TRPA1.[143] Of these six channels, TRPV1, TRPV2, TRPV3, TRPV4, TRPM8, and TRPA1 are considered as thermoreceptors;[25,109,163,192,265,272] TRPV1, TRPV3, TRPM8, and TRPA1 are considered as chemoreceptors;[15,29,150,191] and TRPV4, and TRPA1 are considered as mechanoreceptors.[144,189] Of these channels, the two channels that have been closely associated to neuropathic pain or pain after trauma are the TRPV1 and TRPA1 channels.

The TRPV1 channel is known to transduce nociceptive currents, since it activates at pain threshold temperatures of 42ºC or higher, it also activates at low pH, and responds to capsaicin, the chili peppers ingredient that causes the burning sensation.[170,232] However, in animal studies it has been observed that mice lacking TRV1 do respond to noxious mechanical stimuli, indicating that this channel might not be involved in instigating neuropathic pain due to noxious mechanical stimuli.[48] Interestingly, it was observed that in the presence of extracellular ATP, the TRPV1 activation threshold was reduced from 42ºC to 35ºC, suggesting that ATP released by damaged cells post-trauma might contribute to allodynia.[233] On the other hand, the channel that has been observed to transduce noxious currents in response to painful cold, is the ANKTM1 channel, also known as TRPA1, which is activated at temperatures below 17ºC.[224] Both TRPV1 and TRPA1 are expressed by the same unmyelinated nociceptors,[23] but the TRPA1 is particularly relevant since it is involved in pain and responds to all three types of stimuli as a thermoreceptor,[272] chemoreceptor,[15] and mechanoreceptor,[189] making it a specially interesting receptor target for the treatment of neuropathic pain.

The TRPA1 channel is a Ca(2+)-permeable non-selective cation channel that depolarizes the plasma membrane and causes calcium influx.[128] It was first cloned from human fetal lung fibroblasts, and it is the sole representative of the A subfamily of TRP channels. The TRPA1 channel consist of six putative transmembrane spanning segments (S1-6), a pore forming loop between S6 and S6, and intracellularly located NH_2 and COOH termini, exhibiting an unusual structural feature of 14 NH_2-terminal ankyrin repeats, from which the channel derives its name as transient receptor potential ankyrin 1 channel.[16] On these ankyrin repeated domain, calcium ions can bind and activate the TRPA1 channel. In addition, the N-terminal cysteine residues are also responsible for electrophile-mediated TRPA1 channel activation.

The TRPA1 channel is expressed in neurons of the dorsal root ganglia (DRG), vagal ganglia, trigeminal ganglia, and hair cells,[169] where it is believed to be vital for the proper function of the auditory system. TRPA1 has been shown to be activated by the pungent components of mustard oil and garlic, to be related to pain and neurogenic inflammation, and to cause hypersensitivity to thermal and mechanical stimuli.[24,124,149] As well, the TRPA1 channel is activated by the pro-inflammatory factor bradykinin, an inflammatory compound that plays a role in pain after tissue damage by being released from the damaged cells and acting as a neurotransmitter for pain signaling.[255,270] After nerve injury, the TRPA1 expression has been shown to increase.[88] Remarkably, TRPA1 has been proven to promote hyperalgesia in neuropathic pain models,[194] and to play an important role in inflammatory and neuropathic pain models.[74]

Nociceptive sensory neurons express TRPA1 that can transmit noxious stimuli, or pain signals into electrical activity to the CNS.[58,59,230] Acrolein is a known ligand that directly excites the TRPA1 channel in DRG nociceptive neurons to transmit pain sensation.[23] Due to the electrophilic properties of acrolein, it acts as an agonist to the TRPA1 channel and is able to gate this channel by reacting with the nucleophilic thiol group of cysteine and lysine residues in the N-terminal of the TRPA1 channel via covalent modification to activate the channel.[14] TRPA1 can also be activated by byproducts of oxidative stress by forming disulfide bonds via oxidation by hydrogen peroxide,[173,186] which results in a modification of the N-terminal cysteine in TRPA1 that results in widening of the pore of the channel that leads to a much larger influx of calcium ions compared to normal physiological conditions.[8] Additionally, acrolein is also able to promote TRPA1 upregulation that can also lead to hypersensitivity.[181]

TRPA1 can be activated indirectly through the G-protein coupled receptor (GPCR) mediated pathway such as the bradykinin-induced signaling pathway, where bradykinin binds to the bradykinin 2 receptor and the intracellular calcium level is increased through a phospholipase C (PLC)-mediated down-stream signaling; thus, allowing these calcium ions to bind to the N-terminal of the TRPA1 channel and activating the channel.[15]

Similarly, TRPA1 can be activated via TRPV1. As mentioned above, both channels are expressed by the same unmyelinated nociceptors, which allow the TRPV1 channel to indirectly activate TRPA1. The TRPV1 channel can be sensitized by high temperatures or by binding to its agonists such as capsaicin or secondary messengers such as PKC. The activation of the TRPV1 channel leads to an increase in influx of calcium ions into the cytoplasm, and these calcium ions

can bind to TRPA1 resulting in an activation of the TRPA1 channel via an indirect TRPV1 activation.[31,114]

Interestingly, anesthetic agents such as lidocaine can promote the activation and sensitization of the TRPA1 channel.[155] These anesthetic agents depress the CNS, but also activate peripheral nociceptive sensory neurons by activating the TRPA1 channel. Thus, the lack of effective therapies for the treatment of neuropathic pain and the general addiction associated with anesthetic agents might be explained by this phenomenon in which the agents depress the CNS but also sensitize TRPA1, promoting an unsatisfactory treatment of pain and neuropathic pain conditions where TRPA1 is involved.

After trauma, such as after spinal cord injury, the TRPA1 channel gene expression level has been observed to be increased in the spinal dorsal horn and dorsal root ganglia. Interestingly, TRPA1 messenger RNA (mRNA) levels have been observed to be increased seven days after spinal cord injury in the lumbar level of DRG cells. This suggests that acrolein could contribute to generation of neuropathic pain even after the acrolein levels have returned to normal physiological concentrations after trauma; thus, indicating that even after acrolein levels return to normal, the TRPA1 channels might have been upregulated by acrolein and might be hypersensitized to acrolein. In this case, possibly an even lower concentration of acrolein might be necessary to be maintained post-trauma to be able to reduce neuropathic pain. In other words, since acrolein is able to activate and upregulate TRPA1, even after normal physiological concentrations of acrolein have been achieved post-trauma, even a small physiological concentration of acrolein in the body might be sufficient to generate neuropathic pain post-trauma; thus, since there are more TRPA1 channels post-trauma and these are hypersensitized, acrolein might be needed to be maintained at even lower physiological concentrations than normal to prevent the instigation of neuropathic pain post-trauma.

Another pathway through which the TRPA1 channel can be activated is through inflammation. The same nociceptive neurons that are colonized with TRPA1 channels, also express the chemokine monocyte chemoattractant protein-1 (MCP1). Remarkably, previous studies have also shown that the chemokine MCP1 produced by pro-inflammatory factors such as acrolein could activate the TRPA1 channel.[126] Thus, acrolein can act both as a TRPA1 agonist and as a pro-inflammatory factor, being able to activate TRPA1 directly and indirectly through chemokine-mediated signaling pathways.

1.4.3 The role of MCP1 in neuropathic pain

The monocyte chemoattractant protein-1 (MCP1), also known as CCL2, is one of the key chemokines that regulate migration and infiltration of monocytes and macrophages, being highly relevant to the immune and inflammatory responses.[68] MCP1 has been closely related to several disease states, including neuropathic pain.

Neuroimmune activation involves microglia, endothelial cells and astrocytes; and neuroinflammation can be defined as the migration of immune cells to the site of nervous injury.[67] Microglia are known to keep the homeostatic balance in the central nervous system (CNS), and after an acute insult, microglia activate and work as CNS immune cells;[63] but if the imbalance is compromised by the insult, microglia promote excitotoxic and inflammatory cascades, such as extracellular glutamate increase, that contribute to chronic neuroinflammation and neurodegeneration.[67,225]

Cytokines, specially chemokines, have modulation properties in the neuroinflammatory cascades and neuropathic pain,[140,249] and can induce the production of arachidonic acid, which exacerbates the injury by increasing extracellular levels of glutamate, and can also lead to the formation of superoxide free radicals, which can have a direct effect in sensitization, and even cellular death.[67] Interestingly, the lipid peroxidation of arachidonic acid generates acrolein, which in turn promotes neuroinflammation; and neuroinflammation can induce the production of arachidonic acid leading to increased oxidative stress, making a vicious loop between neuroinflammation and lipid peroxidation with acrolein formation in neurodegenerative processes and neuropathic pain.

In the nervous system, MCP1 is released at the postsynaptic spinal dorsal horn to promote the activity of neuronal glial cells that express MCP-1 receptor CCR2.[260,261] MCP1 is expressed in the same nociceptive sensory neurons which are densely colocalized with TRPA1 channels.[1] Studies have shown a significant involvement of MCP1 in neuropathic pain states.[2,96] Additionally, it has been demonstrated that acrolein is able to stimulate the release of MCP1, and that MCP1 is able to transactivate the TRPA1 channel,[132,133,136] being involved in direct and indirect instigation of neuropathic pain.

Studies have shown that the activation of the MCP1 receptor CCR-2 by MCP1, which can be activated by acrolein, can elicit membrane depolarization, trigger action potentials and sensitize nociceptors through the transactivation of the TRPA1 channels to generate neuropathic pain after

injury.[135] Thus, due to the reactive nature of acrolein, as well as its diffusive, stable and prolonged presence in the body and its ability to promote the release of MCP1, acrolein also plays an important role in an inflammation-associated pathway that leads to the chronic nature of neuropathic pain.

Thus, MCP1 is not only an important inflammatory marker, but is also related to pain conditions in different diseases. MCP1 is closely related to inflammatory cascades that promote oxidative stress, its release is stimulated by acrolein, and is able to transactivate the TRPA1 channel.

1.5 Thesis objectives

In summary, the exact mechanisms underlying neuropathic pain in TBI and chronic stress are still incompletely understood. All TBI, chronic stress and neuropathic pain greatly compromise the health and quality of life of individuals who suffer from these conditions, and there are currently no treatments that can stop the neurodegeneration in TBI, to treat chronic stress to avoid the long-term disorders associated with it, and to treat neuropathic pain mechanistically and effectively.

We propose that acrolein, due to its' long half-life compared to other reactive oxygen species and important involvement in oxidative stress pathological mechanisms, might play a significant role in instigating neuropathic pain in TBI and chronic stress by directly upregulating and activating the TRPA1 channel, and indirectly by stimulating the release of MCP1, which also transactivates the TRPA1 channel, where both TRPA1 and MCP1 have been correlated to neuropathic pain.

The proposed study will examine a globally relevant issue using an animal model of mild blast traumatic brain injury (mbTBI), and an animal model of chronic stress where neuropathic pain behaviors will be investigated as well as their relationship with acrolein, TRPA1 and MCP1. This study also aims to provide a method of acrolein sequestering to treat and mitigate neuropathic pain in both conditions, mbTBI and chronic stress, with the purpose of reducing acrolein levels *in-vivo* and consequently reducing MCP1 and TRPA1 levels, which are notably involved in neuropathic pain.

The objectives of this thesis are: 1) demonstrate the role of acrolein in neuropathic pain post-TBI, 2) demonstrate the role of acrolein in neuropathic pain in chronic stress, 3) evaluate the

efficacy of acrolein scavengers in mitigating neuropathic pain post-TBI and in chronic stress. The knowledge gained by this study will influence the lives of many patients suffering from neuropathic pain, TBI and chronic stress, and will potentially offer an easily translational therapy that will improve the health and quality of life of these patients.

The applications of this study are not limited to patients suffering from TBI and chronic stress given that neuropathic pain is present in many other conditions, and that oxidative stress and acrolein have been implicated in many disease and trauma pathological states. Our laboratory is currently investigating the role of acrolein in multiple sclerosis, Parkinson's disease, and spinal cord injury, and this study can easily be extended to any animal model of those disease states in the future.

CHAPTER 2. CORRELATION OF NEUROPATHIC PAIN-LIKE BEHAVIORS AND CHANGES IN ACROLEIN, TRPA1 AND MCP1 PROTEIN EXPRESSION POST-MBTBI

2.1 Allodynia behavioral experiments

2.1.1 Rationale

Cogitating that neuropathic pain in the body and headaches are common symptoms post-mbTBI in humans, it was important to determine whether an induced-mbTBI rat model would produce similar effects, so we could further study the mechanisms underlying neuropathic pain after mbTBI in a laboratory setting.

Central neuropathic pain is associated in human patients to thermal and mechanical hyperalgesia and allodynia.[229] Allodynia refers to cutaneous sensitivity to stimuli that are innocuous under normal conditions.[75] The typical examinations to assess neuropathic pain include allodynia evaluations such as touch, pressure, cold, heat, vibration, pinprick, and the summation; in which there would be pain responses to stimuli that are normally not painful.[41] Previous studies in TBI patients using von Frey stimuli for allodynia have shown that an approximate 40% of these patients experience chronic head and face pain after head injury.[66] In humans and rodents, headache pain involves an abnormal activation of the trigeminovascular system; and peripheral axons of the trigeminal ganglion innervate the periorbital skin,[75] which is why periorbital allodynia measurements with von Frey filaments are commonly used to test for headache pain. Additionally, changes in pain perception in rodents are assessed by measuring thresholds of hind paw withdrawal from mechanical pressure with von Frey filaments.[154] Thus, we conducted periorbital and hind limb mechanical allodynia measurements with the well-known and validated method of von Frey sensory testing.[90]

2.1.2 Brief methods

The mbTBI was induced to live male sprague dawley rats of approximately 350 g of body weight. As a first approach to understand whether neuropathic pain behaviors were present in rats post-mbTBI and to understand whether acrolein, TRPA1 and MCP1 were involved in this model, only male rats were used for these first chapters. This was only as a first approach and to avoid variation between genders in revealing neuropathic pain and molecular involvement of acrolein,

TRPA1 and MCP1. However, in the studies included in the Chapter 6 and Chapter 7, male and female rats were included to better understand the differences and effects of these studies.

The rats were deeply anesthetized with a mixture of ketamine and hydralazine (80 mg/kg and 10 mg/kg respectively) though intraperitoneal (IP) injection, restrained by the nose and the sides of the head, and placed 3.5 cm under the shock tube nozzle directed towards the rat's brain. A shield mimicking the soldiers' armor was placed over the rat's body for protection during the blast injury. All procedures were in accordance to the Purdue Animal Care and Use Committee (PACUC) animal protocol #111000280. For these experiments, the n=4 for sham and n=4 for blast, with a total of n=8 for these preliminary experiments.

To deliver the shock wave, a validated blast wave model was used, which consists of an open-ended shock tube where nitrogen gas is compressed into a chamber with one end sealed by a thin (0.25") PET membrane.[253] The pressure inside the chamber exceeds the mechanical strength of the membrane, breaking the membrane and releasing the nitrogen through the shock tube generating a shock wave that mimics the ideal Friedlander waveform used to describe shock wave phenomena.[13] The blast wave intensity is of 150 kPa with a duration of 2 msec. The blast wave is directed to the rat's brain from above onto the dorsal surface of the prone animal, which applies downward pressure onto the work table. After the blast wave is delivered, the animals are allowed to recover from anesthesia with maintenance of core temperature on a thermostatic heating pad.

Previously, our lab has demonstrated that this blast model is capable of causing deformation of the brain inside the skull *in-vivo*, which was tested by implanting a soft implantable elastomeric polymeric magnet and three external 3-axis giant magnetoresistance sensors (GMR) on the skull. The change in relative position due to a brain deformation under a blast wave was measured by a change in the magnetic field.[220] The schematics of the blast model setting and the sensor system used to validate brain deformation under a blast wave is shown in Figure 1.

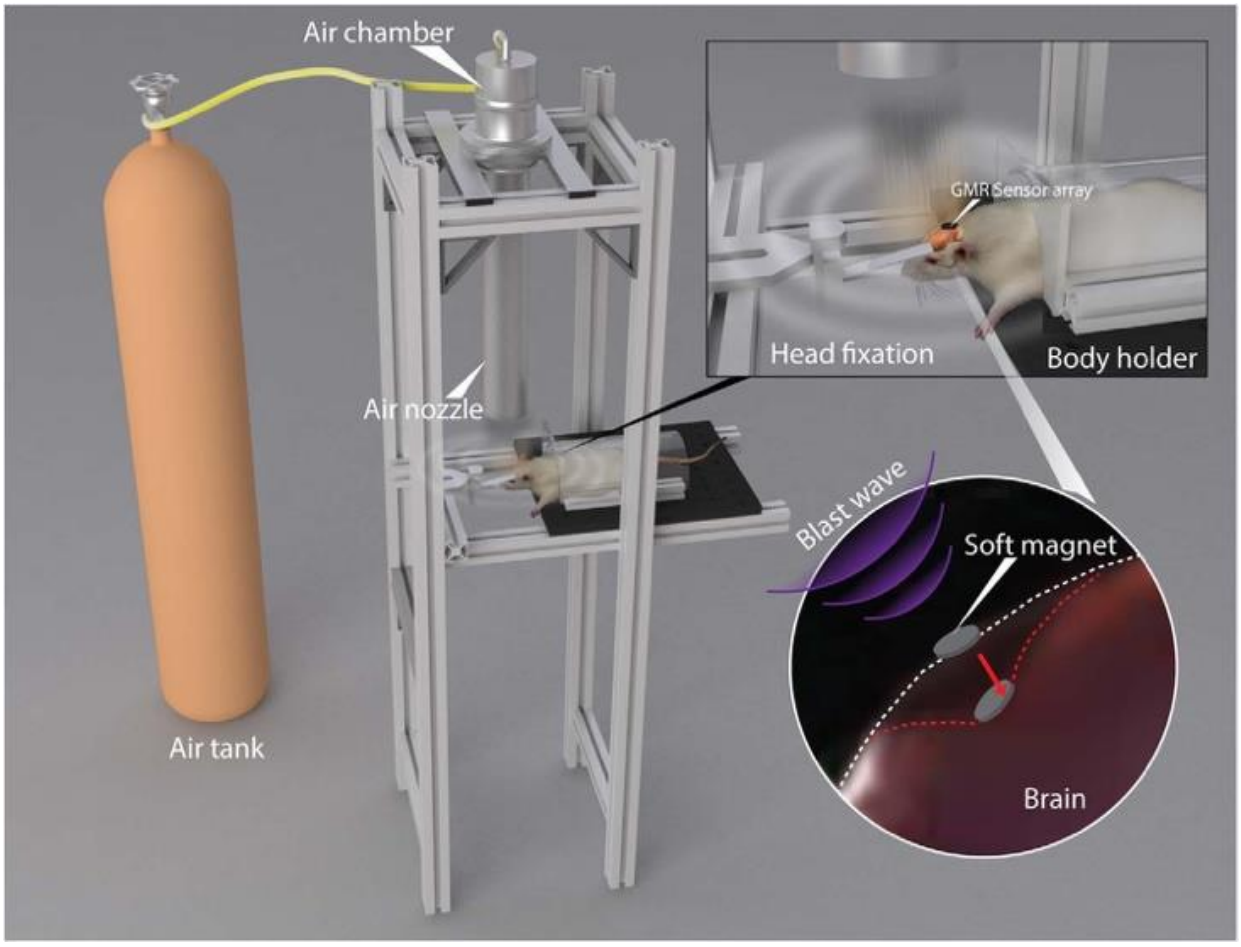

Figure 1 Schematic of blast model setting and intracranial brain deformation sensor system. The air tank containing nitrogen is shown on the left, the air chamber and the air nozzle are divided by the thin PET membrane that breaks when the pressure inside the chamber exceeds the mechanical strength of the membrane, delivering the blast wave over the rats' head, which is fixed by the nose and sides of the head. The figure on the right shows the rat's head fixed, the body holder that mimics the body armor worn by soldiers protecting the body from the blast wave, and the GMR sensor array used to measure the brain deformation under the blast wave. The image on the bottom right represents the mechanics of the magnet, measuring the distance between the soft magnet implanted on the dura matter and the GMR fixed on the skull while the brain is deformed under the blast wave.[220]

Allodynia measurements were performed on the periorbital areas and hind limbs for testing of headache and general body pain respectively. Before starting the tests, rats were allowed to acclimate in the behavioral room for 30 min to 2 hours to permit acclimation of differences in temperature and noise, to avoid stress, and allow for more robust measurements. The tests were performed in three different days within two weeks before inducing the blast injury to obtain three baselines, and on days 1, 4, 8, 11, 15, and 22 post-mbTBI starting with periorbital allodynia measurements following by hind limb measurements.

The hind limb measurements are performed by placing the rat in a plastic cage with a wire mesh bottom that allows complete access to the hind paws. The rats were acclimated for approximately 15 min or until cage exploration or grooming activities ceased. Both left and right paws were tested on the mid-plantar area. The paws were touched with 1 von Frey filament at a time, with logarithmically incremental stiffness, perpendicular to the paw. Stimuli were presented at intervals of 10-15 sec, allowing for resolution of any behavioral responses to previous stimuli. A positive response was recorded when there was a sharp withdrawal of the paw, as well as flinching immediately upon removal of the filament. The highest force used to test rats in hind limb regions was 15 g.[52] Each rat was stimulated 10 times bilaterally with each filament ascendingly until 15 g were reached or until a filament caused a 100% response. The 50% withdrawal threshold was determined by using the up-down method,[69] where in the absence of a positive response of the filament selected, a stronger filament is presented; and in the event of paw withdrawal, a weaker filament is presented. The optimal threshold calculation requires 6 responses in the immediate vicinity of the 50% threshold. The 50% threshold is intrapolated using the following formula, where X_f is the last filament used in value of log units, k is a tabular value for the pattern of positive and negative responses, and δ is the mean difference in log units between stimuli (here, 0.252 for the use of all filaments 1.65-5.18).[52]

$$50\%\ g\ threshold = (10^{[X_f + k\delta]})/10{,}000$$

For the periorbital measurements, rats were placed in a universal acrylic restrainer tube designed for rodents of up to 500 g that allowed access to the face and tail. They entered the restrainer uncoaxed and were restrained without force.

Rats were allowed to acclimate in the tube for 5 min or no longer than 10 min, and tested for no longer than 5 min; thus, the rats were in the restrainer tube for no longer than 15 min to avoid stress.

The periorbital measurements were performed by touching the upper part of the face right above each eye using calibrated von Frey filaments (Stoelting, Wood Dale, IL, USA) as shown in Figure 2,[75] with the difference that rats were not inclined in the tube for testing. The mechanical threshold was determined by applying the von Frey monofilaments to the periorbital region on the right and left side of the face on the rostral portion of the eye. Similar to the method described above for hind limb allodynia assessments, a firm perpendicular contact with the skin was applied causing the filament to bend at the precise bending force that was calibrated by the manufacturer. When the rat stroked its face vigorously with the forepaw, shook its head or withdrew its head from the stimulus, it was considered as a positive response; otherwise, it was considered as a negative response. The highest force used to test rats in periorbital regions was 10 g.[75] Each rat was stimulated 10 times bilaterally with each filament ascendingly. Force thresholds were defined as greater than 50% response frequency and are presented as mean threshold (g) ± standard error of the mean (SEM).

The data were analyzed statistically by using an ANOVA test and a Tukey post-hoc test. The mechanical threshold is presented by group as mean g ± standard error of the mean.

2.1.3 Results and Discussion

The hind limb mechanical allodynia tests showed an increase in pain-like behaviors on post-mbTBI rats starting one day after injury and persisting with increased sensitivity compared to day 1 throughout days 4, 8, 11, 15, and 22 as shown in Figure 3A. The results with * indicate a p-value < 0.05.

Sham rats, which were just subject to anesthesia and to the noise of the blast wave at the same distance from the nozzle but without receiving the blast wave, showed no significant changes in allodynia and showed results consisting to allodynia behaviors observed in healthy rats.[52] On the other hand, rats that received the blast wave showed increased pain by becoming sensitive and responsive to stimuli that were innocuous to sham healthy rats.

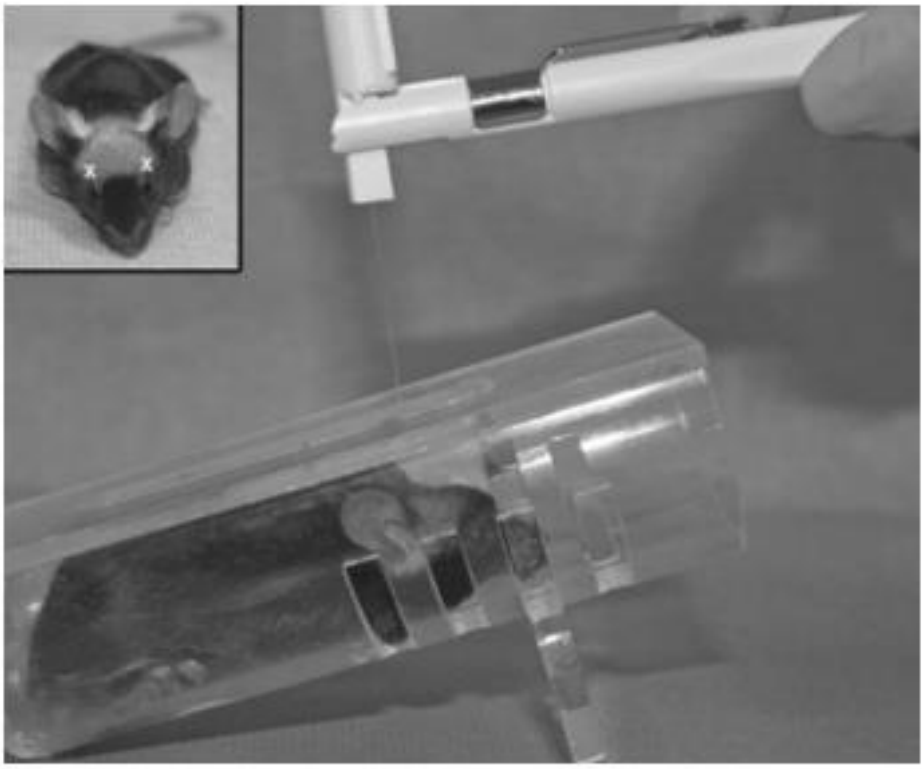

Figure 2 Representation of periorbital testing from [75]. The rat is placed in a universal cylindrical restrainer parallel to the floor rather than inclined, and is tested with von Frey monofilaments ascendingly with a maximum force of 10 g. The periorbital areas tested are shown in white crosses over the eyes, which consists of the right and left side of the face on the rostral portion of the eye. The rats are stimulated 10 times bilaterally with each filament.

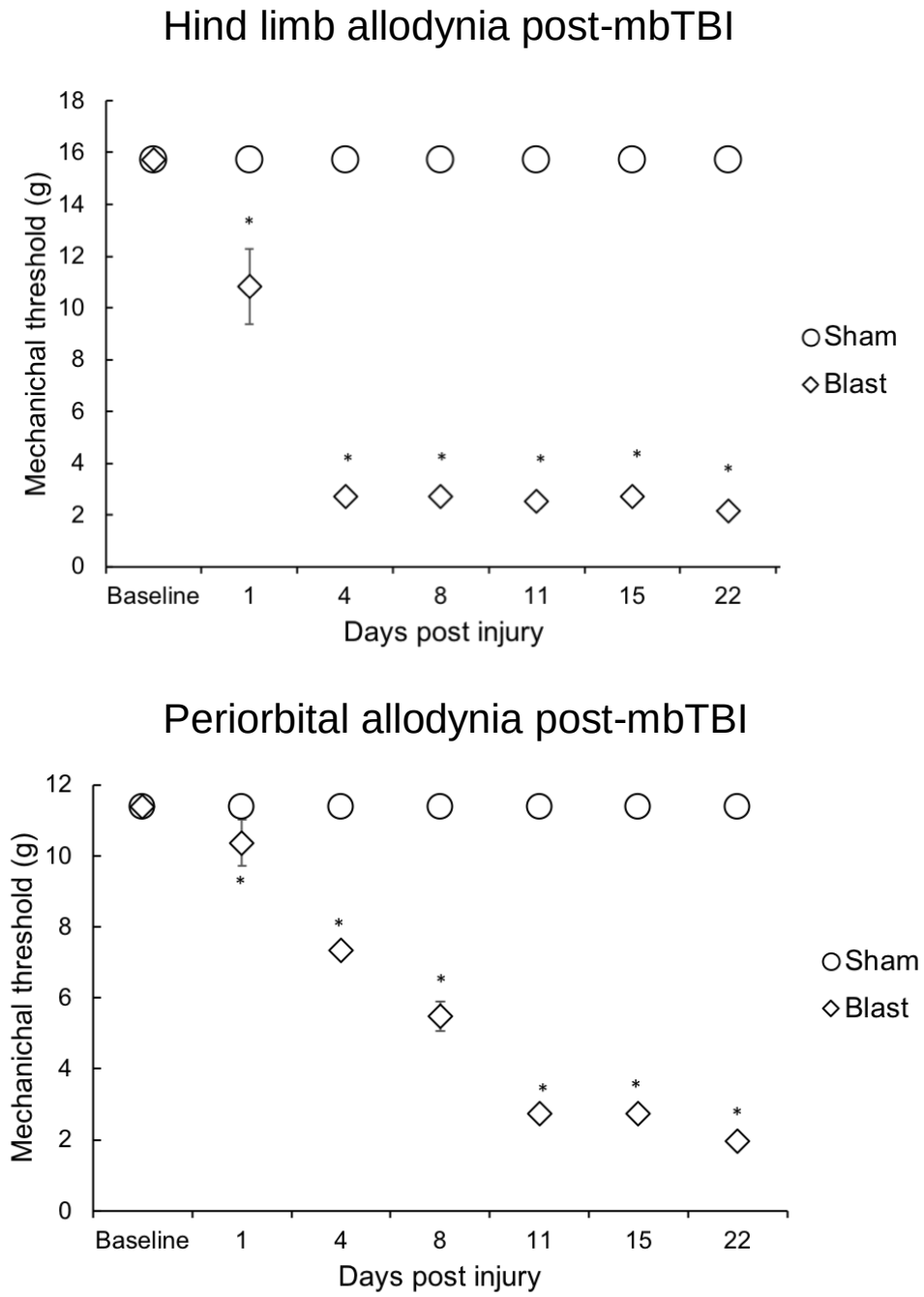

Figure 3 Allodynia post mild blast TBI. (A) Hind limb mechanical allodynia post-mbTBI. Sham rats, subjected to anesthesia and the noise of the blast at the same distance than the mbTBI rats but without receiving the blast wave, did not show any changes throughout the study and behaved as healthy rats. The mbTBI rats, however, showed an increase in pain behaviors by a reduction of mechanical thresholds since day 1 and with even more sensitivity throughout the study. (B) Periorbital allodynia post-mbTBI. The mbTBI rats showed an increase in headache behaviors by a reduction of mechanical thresholds since day 1 and with even more sensitivity throughout the study, while sham rats did not show headache behaviors. N=4 per group statistically analyzed with Anova and post-hoc Tukey test, $p<0.05$.

Since day 1, mbTBI rats showed a reduced threshold of 10.83 g, which was even further reduced to approximately 2.7 g of force thresholds throughout the study. In other words, on day 1 the rats became 30% more sensitive than healthy rats and became 82% more sensitive than healthy rats throughout the rest of the study. Moreover, the length of the persistent pain of two months correlates with chronic pain behaviors in humans.[43] Therefore, we can cognize that mbTBI rats became highly sensitive and displayed chronic pain behaviors post-mbTBI.

Since the typical evaluations to assess neuropathic pain in rodents consist of allodynia measurements with von Frey filaments, these results indicate that rats that receive a mild blast traumatic brain injury experience general body pain that can relate to the neuropathic pain experienced in different areas of the body by post-mbTBI patients.

Thus, these results validate that this model is appropriate for the study of neuropathic pain post-mbTBI, and that the results, consisting with behaviors observed in humans, can be a plausible method for translational treatments.

The periorbital mechanical allodynia tests showed an increase in headache-like behaviors on post-mbTBI rats starting one day after injury and persisting with increased sensitivity throughout days 4, 8, 11, 15, and 22 as shown in Figure 3B. Sham rats showed no significant changes in allodynia and showed results consisting to allodynia behaviors observed in healthy rats.[75] On the other hand, rats that received the blast wave showed increased pain by becoming sensitive and responsive to stimuli that were innocuous to sham healthy rats. Throughout the study, rats showed gradually increasing headache, which was even further reduced to approximately 1.97 g of force thresholds. In other words, the rats became 80% more sensitive than healthy rats throughout the rest of the study. Therefore, we can cognize that mbTBI rats became highly sensitive and displayed headache behaviors post-mbTBI.

These results indicate that rats that receive a mild blast traumatic brain injury experience headache pain that can relate to the headaches and migraines experienced by post-mbTBI patients. Thus, these results validate that this model is appropriate for the study of headache and neuropathic pain post-mbTBI, and that the results, consisting with behaviors observed in humans, can be a plausible method for translational treatments.

2.2 Biochemical experiments

2.2.1 Rationale

As a first approach to better understand whether the behavioral changes observed in the mbTBI rats would correlate with molecular changes in acrolein, TRPA1 and MCP1 as previously proposed, the tissue of sham and blast rats, including the brain, and spinal cords, were collected 8 weeks post-mbTBI. Moreover, since acrolein western blotting was only restricted to the end of the study as a first approach, we decided to collect urine throughout the study to further analyze the acrolein metabolite, 3-HPMA, and understand how acrolein would change at different time points *in-vivo*.

2.2.2 Brief methods

At week 8 post-mbTBI, rats were deeply anesthetized with a mixture of ketamine and xylazine (80 mg/kg and 20 mg/kg respectively) and were transcardially perfused with freshly prepared oxygenated Kreb's solution. The whole brain and the whole spinal cord were excised immediately and were frozen and stored at 80ºC.

For the western blotting procedure, the tissue was homogenized and sonicated in 1X RIPA buffer (Sigma, St. Louis, MO) with protease inhibitor cocktail at 1:100 final concentration (Sigma). Samples were centrifuged at 14,000 g at 4ºC for 30 min. The supernatant was saved for protein concentration quantification via the biochoninic acid protein kit (Pierce, Rockford, IL USA) and SPECTRAmax (Molecular Devices, Sunnyvale, CA). A mass of 30 ug of protein with 20% SDS, β-mercaptoethanol, and 2x laemmli buffer were loaded to individual wells of a 15% tris-HCl gels and electrophoresed at 80 Volts for around 2.5 hours. Proteins were subsequently transferred to a nitrocellulose membrane via electro-blotting in an ice bath for 60 minutes at 75 volts in 1X transfer buffer (Tris-glycine buffer, BioRad Hercules) in 20% methanol. The membrane was then blocked in 1X casein (Vector) and incubated with anti-actin, anti-acrolein, anti-TRPA1 or anti-MCP1 at 4ºC overnight. Membranes were washed in casein and incubated with a biotinylated anti-rabbit secondary antibody. Chemiluminescent signal acquisition was performed with the DuoLux substrate immunodetection kit (Vector). Membranes were imaged using an Azure c300 Western blot imaging system with 5-minute exposure (Azure Biosystems, Dublin, CA). AlphaView software (Protein Simple, San Jose, CA) was used to quantify the relative

signal of each band with local background analysis. Data were normalized to actin levels and further normalized to control.

Urine was collected in standard metabolic collection cages prior to mbTBI to obtain three baselines within a two-week period, and after the injury, and quantified as previously described.[271] Urine was prepared using solid phase extraction before liquid chromatography with tandem mass spectrometry (LC/MS/MS) analysis,[271] and 3-HPMA levels were normalized to urine creatinine levels under the assumption of normal kidney function.

2.2.3 Results and Discussion

The western blots of acrolein shown in Figure 4 indicate a significant increase in acrolein concentrations in the brain of mbTBI rats 2 days and 7 days post injury compared to sham rats. These results correlate with the increase in headache and neuropathic pain-like behaviors 1 and 8 days post-mbTBI, indicating that as pain behaviors increased, acrolein increased as well.

TRPA1 is also elevated 8-weeks post-mbTBI in the brain compared to sham rats, as shown in Figure 5. This indicates that more pain channels are expressed in the brain that can correlate with the pain behaviors observed in mbTBI rats. Thus, TRPA1 appears to also be involved in the neuropathic pain behaviors post-mbTBI.

MCP1 shows a significant elevation in the brain 8-weeks post-mbTBI compared to sham rats, as shown in Figure 6. This indicates that more inflammation is present in the brain that can contribute to more pain behaviors. Therefore, this suggests that MCP1 may also be involved in the neuropathic pain behaviors post-mbTBI.

Additionally, analysis of urine of rats before injury and 1, 2, 5 and 7 days after injury shows an increased 3-HPMA metabolite in the urine post-blast, as shown in Figure 7. This indicates that acrolein is systemically elevated in the body and persists at least for up to 7 days post-injury according to the results. This is very relevant considering that most oxidative stress markers just last up to seconds or minutes in the body; however, according to our results, acrolein is able to stay elevated in the body for up to a week after the injury, suggesting that this neurotoxin might still be affecting and contributing to the secondary injury process for a long time after injury.

These results suggest that acrolein appears to be elevated both in the brain and the body after mbTBI and possibly contributes to the upregulation of both TRPA1 and MCP1, which are directly

related to increased pain. Thus, acrolein might be generating the secondary injury cascade that contributes to increased TRPA1 and MCP1 and therefore to neuropathic pain post-mbTBI.

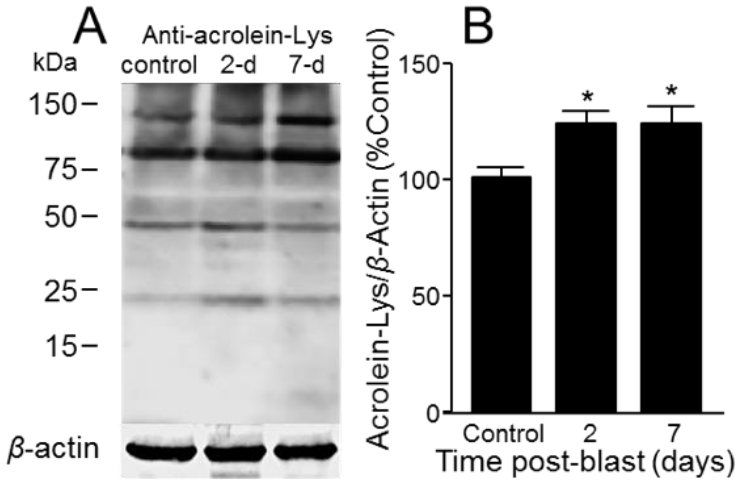

Figure 4 Acrolein protein expression is increased in the brain at 2 and 7 days post-mbTBI as compared to healthy sham rats as shown by the western blotting results. N=4 per group statistically analyzed with Anova and post-hoc Tukey test, *p<0.05. From Acosta, Race et al. unpublished date.

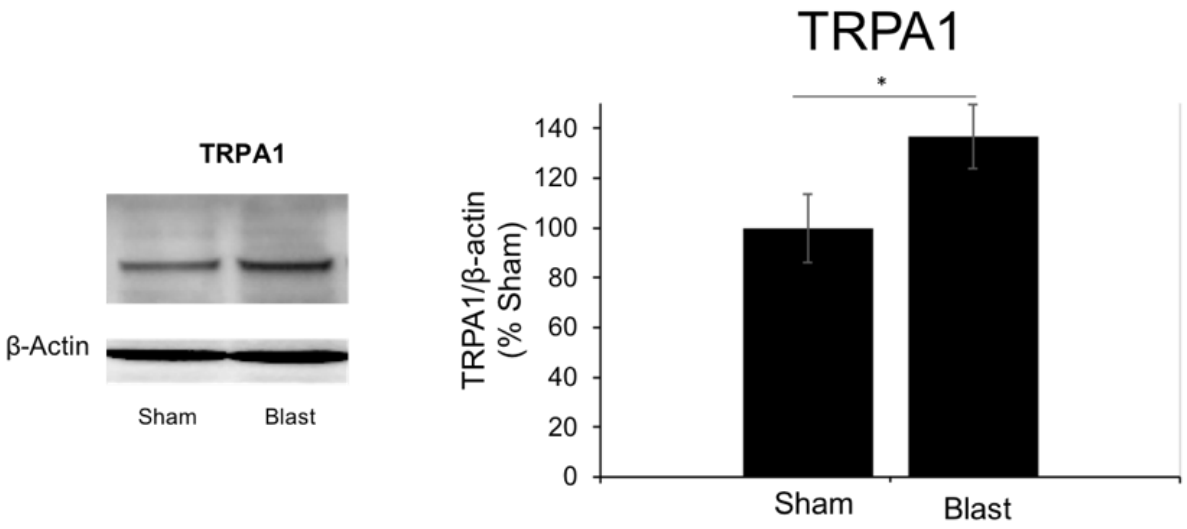

Figure 5 TRPA1 protein expression is increased in the brain 8 weeks post-mbTBI as compared to healthy sham rats as shown by the western blotting results. N=4 per group statistically analyzed with Anova and post-hoc Tukey test, *p<0.05.

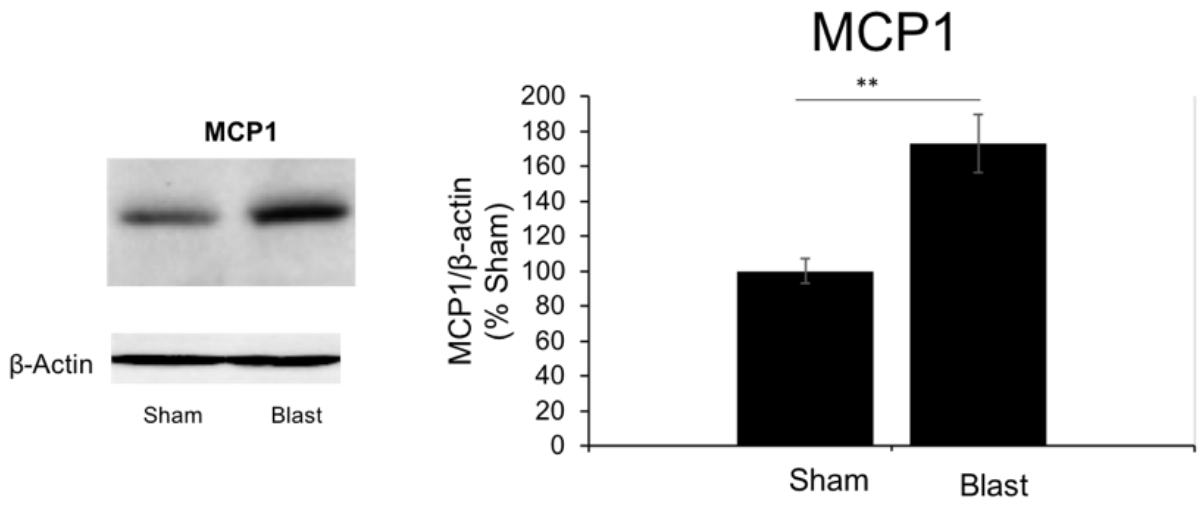

Figure 6 MCP1 protein expression is increased in the brain 8 weeks post-mbTBI as compared to healthy sham rats as shown by the western blotting results. N=4 per group statistically analyzed with Anova and post-hoc Tukey test, **p<0.01

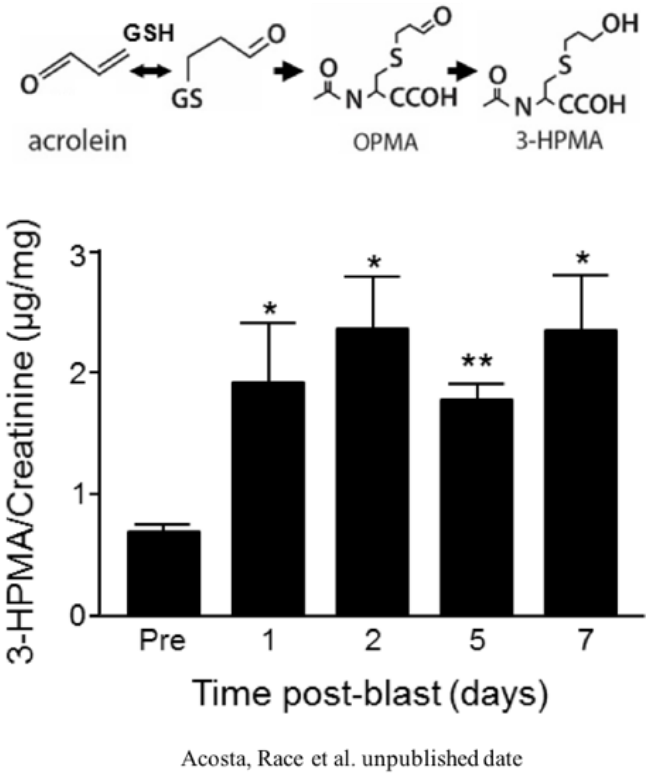

Figure 7 3-HPMA is increased in urine 1, 2, 5 and 7 post-mbTBI as compared to baseline. N=4 per group statistically analyzed with Anova and post-hoc Tukey test, *p<0.05, **p<0.01.

CHAPTER 3. CORRELATION OF NEUROPATHIC PAIN-LIKE BEHAVIORS AND CHANGES IN ACROLEIN, TRPA1 AND MCP1 PROTEIN EXPRESSION AFTER CHRONIC STRESS

3.1 Allodynia behavioral experiments

3.1.1 Rationale

To better understand chronic stress and its relationship with neuropathic pain, it seemed necessary to establish a chronic stress animal model that would enable us to control parameters and determine whether neuropathic pain was reproducible after chronic stress in an animal model. For this purpose, we used sprague dawley male rats of approximately 200 g of body weight and implemented a chronic stress animal model to determine whether this model would lead to neuropathic pain throughout the study mimicking the neuropathic pain observed in humans in chronic stress.

3.1.2 Brief methods

The chronic stress induction was achieved by restraining the rats for 2 hours every day in a universal cylindrical restrainer throughout the length of the study.[273] Periorbital and hind limb allodynia assessments were performed on days 1, 4, 8, 11, 15, 18, 22 and 29 as described on section 2.1.2. For these experiments, we expected a higher variation within groups, so our preliminary studies had an n=5.

As a first approach to understand whether neuropathic pain behaviors were present in rats in chronic stress and to understand whether acrolein, TRPA1 and MCP1 were involved in this model, only male rats were used for these first chapters. This was only as a first approach and to avoid variation between genders in revealing neuropathic pain and molecular involvement of acrolein, TRPA1 and MCP1. However, in the studies included in the Chapter 6 and Chapter 7, male and female rats were included to better understand the differences and effects of these studies and different disease models.

3.1.3 Results and Discussion

The allodynia measurements of the hind limbs of restrained rats showed increased neuropathic pain behaviors starting on day 1 of stress and being maintained throughout the study as shown in

Figure 8A. On day 1 the rats showed a mechanical threshold of 4.5 g and by the end of the experiment of 1.5 g. This indicates that the hypersensitivity of the stressed rats increased by 70% on day 1 and was maintained at 90% throughout the study. These results indicate that chronic stress alone can induce general body neuropathic pain behaviors.

The periorbital allodynia measurements of restrained rats showed increased headache behaviors starting on day 1 of stress and gradually increasing throughout the study as shown in Figure 8B. On day 1 restrained rats showed a mechanical threshold of 7.3 g that decreased to 0.03 g by the end of the study. In other words, headache behaviors of stressed rats increased 27% on day 1 and were maintained at 99.7% throughout the study. This indicates that chronic stress alone can induce headache behaviors and increase headache hypersensitivity significantly.

Moreover, these results also indicate that the chronic stress rat model is appropriate for the study of neuropathic pain in chronic stress since it shows similar neuropathic pain and headache behaviors as those observed in humans with chronic stress.

3.2 Biochemical experiments

3.2.1 Rationale

To further determine whether acrolein, TRPA1 and MCP1 were involved in the neuropathic pain behaviors observed in the chronic stress rat model, as proposed in our theory, and similar to the results obtained of mbTBI rats, the brains and spinal cords of control and stress rats were collected on day 30 and submitted to western blots.

3.2.2 Brief methods

The animals were submitted to stress daily as described on section 3.1.2, and the tissue collection and western blotting techniques were as described on section 2.2.2.

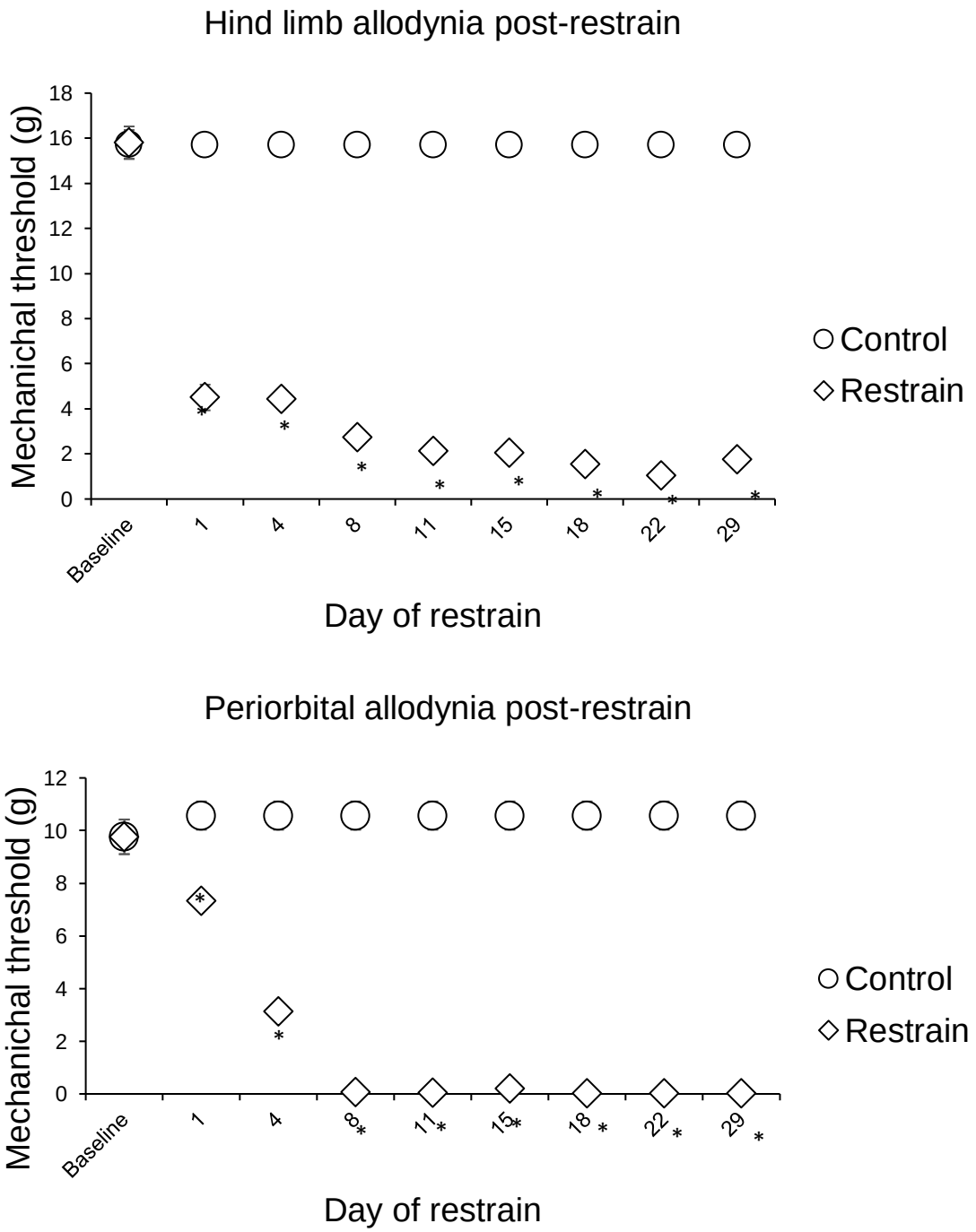

Figure 8 Allodynia post restrain. (A) Hind limb mechanical allodynia during stress. Control rats did not show any changes throughout the study and behaved as healthy rats. The restrained rats, however, showed an increase in pain behaviors by a reduction of mechanical thresholds since day 1 and with even more sensitivity throughout the study. (B) Periorbital mechanical allodynia during stress. Control rats did not show any changes throughout the study and behaved as healthy rats. The restrained rats, however, showed an increase in pain behaviors by a reduction of mechanical thresholds since day 1 and with even more sensitivity throughout the study. N=4 per group statistically analyzed with Anova and post-hoc Tukey test, *$p < 0.05$

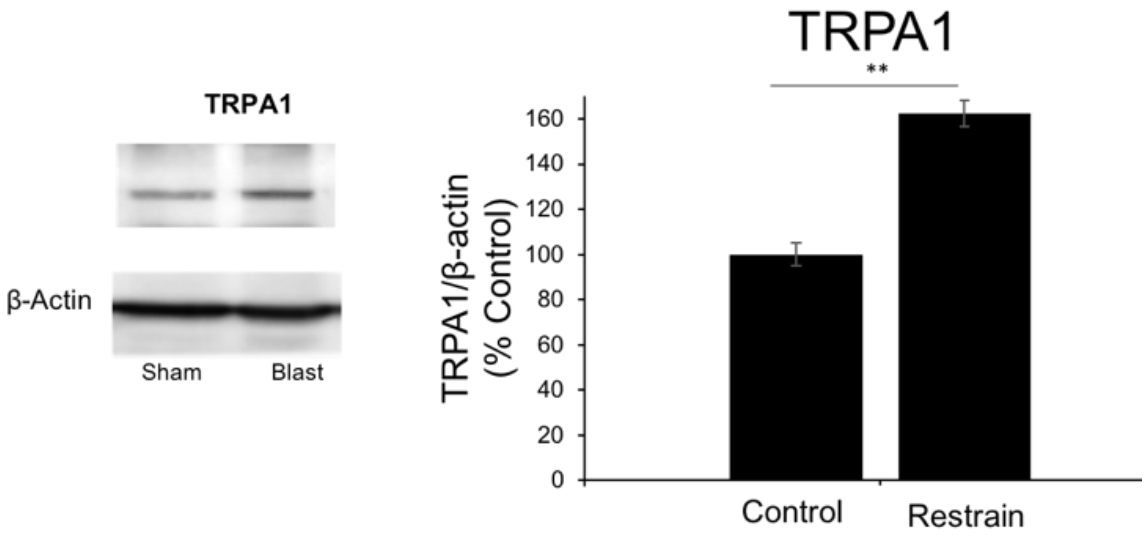

Figure 9 TRPA1 protein expression is increased in the brain at 4 weeks after chronic stress as compared to control rats as shown by the western blotting results. N=4 per group statistically analyzed with Anova and post-hoc Tukey test, **p<0.01.

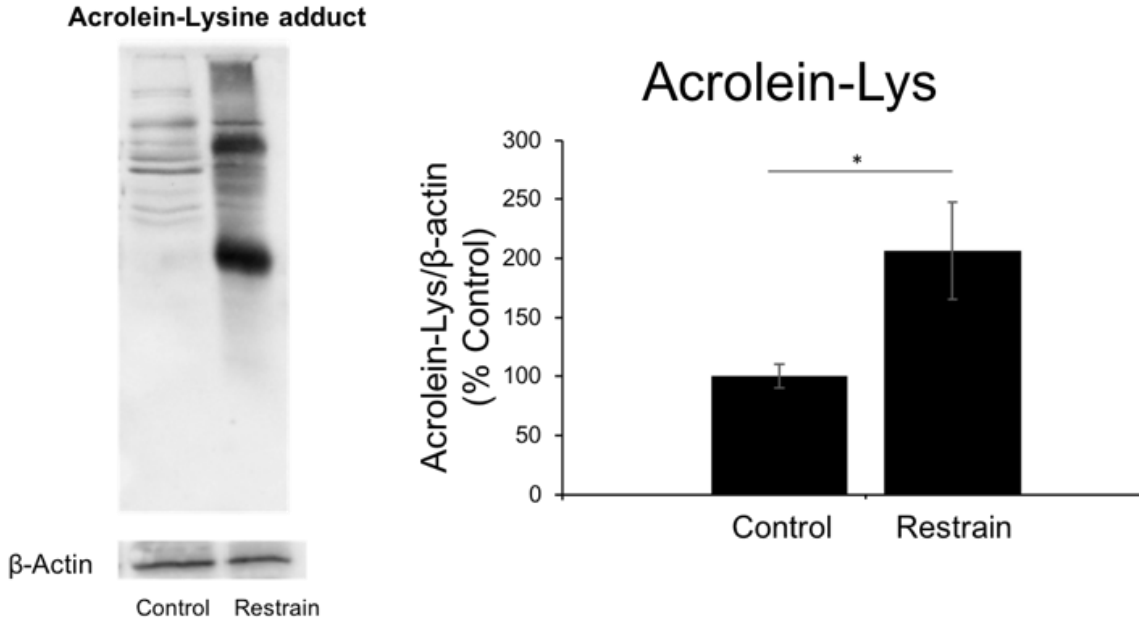

Figure 10 Acrolein protein expression is increased in the spinal cord at 4 weeks after chronic stress as compared to control rats as shown by the western blotting results. N=4 per group statistically analyzed with Anova and post-hoc Tukey test, *p<0.05.

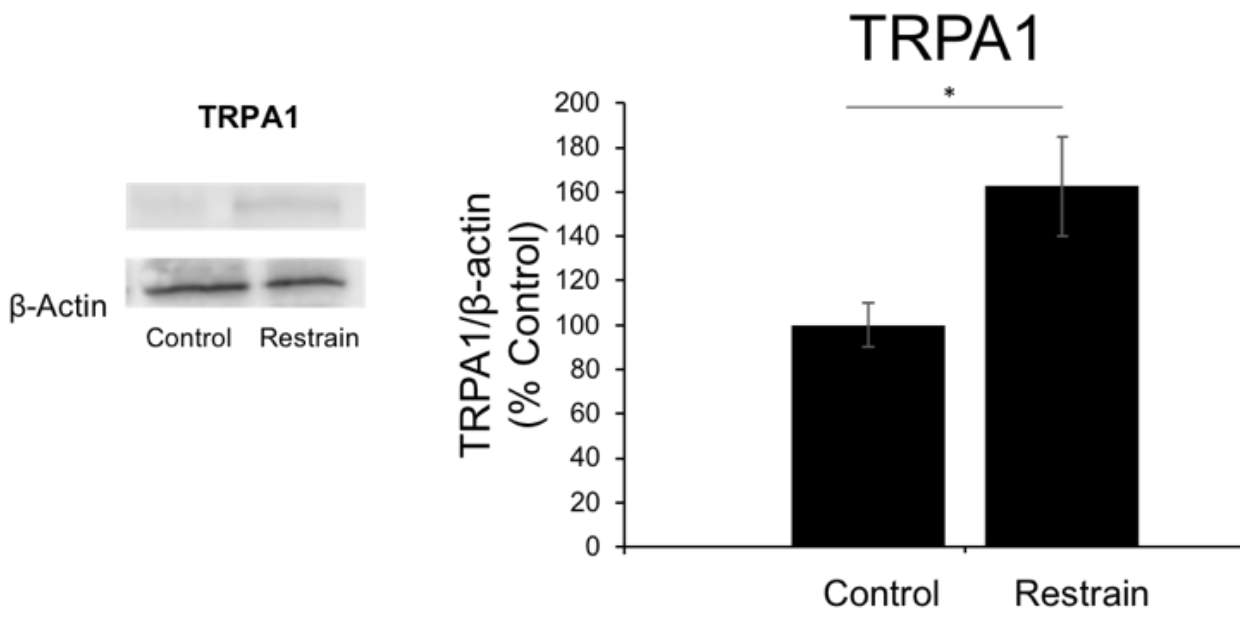

Figure 11 TRPA1 protein expression is increased in the spinal cord at 4 weeks after chronic stress as compared to control rats as shown by the western blotting results. N=4 per group statistically analyzed with Anova and post-hoc Tukey test, *p<0.05.

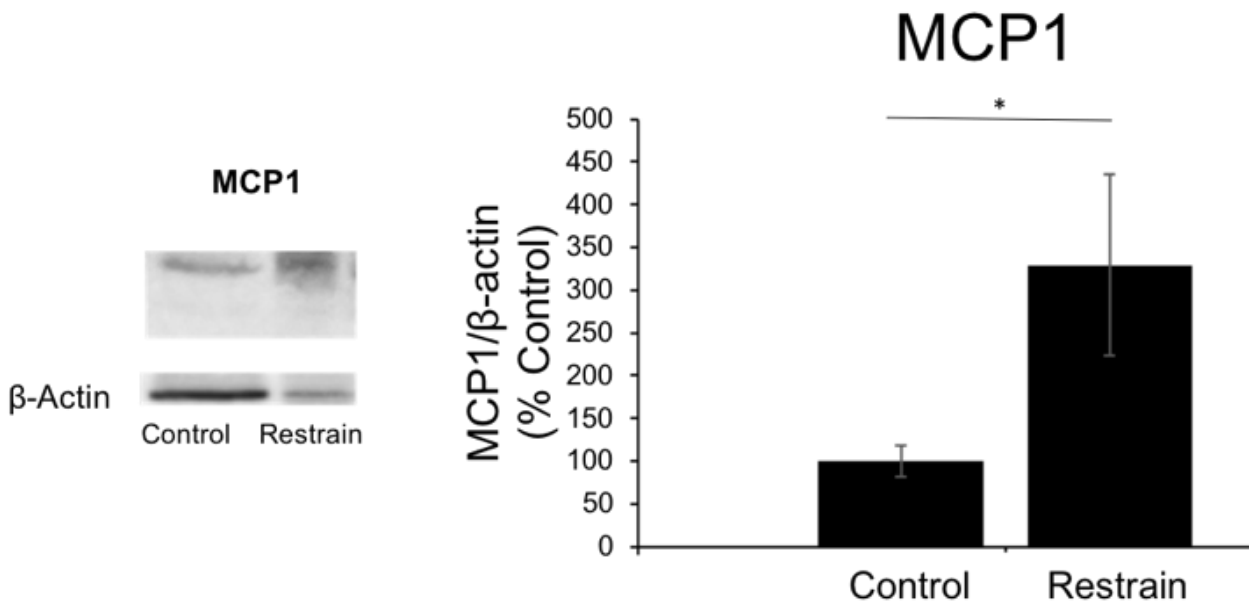

Figure 12 MCP1 protein expression is increased in the spinal cord at 4 weeks after chronic stress as compared to control rats as shown by the western blotting results. N=4 per group statistically analyzed with Anova and post-hoc Tukey test, *p<0.05.

3.2.3 Results and Discussion

The western blots of the brains of restrain rats showed a significant increase in TRPA1 channel expression as compared to healthy controls 4 weeks after chronic stress as shown in Figure 9. These results indicate that more pain channels are expressed in the brain that could contribute to the high sensitivity observed in headache behaviors shown in the previous section. Thus, this suggests that the TRPA1 channel might be involved in the headache mechanisms in chronic stress.

The acrolein-lysine adducts in the western blots showed a significant increase in acrolein 4 weeks post-stress in the spinal cords of restrained rats as compared to healthy controls as shown in Figure 10. This acrolein increase correlates with the changes in hind limb neuropathic pain behaviors observed in restrained rats after 4 weeks of chronic stress. This suggests that acrolein might be involved in the neuropathic pain observed in chronic stress.

The protein expression of TRPA1 was also elevated in the spinal cords of stressed rats 4 weeks post-stress as compared to healthy controls as shown in Figure 11. This indicates that more TRPA1 pain channels are expressed in the spinal cord 4 weeks after chronic stress and that this elevation correlates with hind limb neuropathic pain behaviors observed 4 weeks post-stress.

Thus, these results suggest that the TRPA1 channel might also be involved in the neuropathic pain in chronic stress.

Additionally, the protein expression of the chemokine MCP1 was also elevated 4 weeks post-stress in restrained rats compared to control rats as shown in Figure 12. This is an indicative of inflammation that correlates with inflammatory pain and with hind limb neuropathic pain behaviors observed in restrained rats 4 weeks post-stress. Therefore, these results indicate that MCP1 might also be involved in neuropathic pain behaviors in chronic stress.

In summary, since acrolein, TRPA1 and MCP1 are elevated either in the brain or the spinal cord in chronic stress rats as compared to controls in the same way that headache and neuropathic pain behaviors are present in chronic stress rats compared to controls, this suggests that all acrolein, TRPA1 and MCP1 appear to be involved in headache and neuropathic pain in chronic stress.

CHAPTER 4. REDUCTION IN NEUROPATHIC PAIN-LIKE BEHAVIOR AND ACROLEIN, TRPA1 AND MCP1 PROTEIN EXPRESSION BY SEQUESTERING ACROLEIN IN MBTBI

4.1 Allodynia behavioral experiments

4.1.1 Rationale

Based on the preliminary results of increased headache and neuropathic pain behaviors in rats post-mbTBI, and based on the fact that acrolein, TRPA1 and/or MCP1 were elevated in the spinal cord and/or brains of the mbTBI rats, we considered potential mechanisms that could decrease such behaviors and protein expression elevations.

Oxidative stress plays a major role in the secondary injury cascade, and acrolein is a lipid peroxidation product that lasts for at least up to 7 days according to the studies shown here. Moreover, acrolein is able to upregulate and transactivate the TRPA1 channel, and it is also able to stimulate the release of chemokines such as MCP1, which are both directly related in neuropathic pain. For these reasons, we considered the role of acrolein in neuropathic pain post-mbTBI. Thus, the use of acrolein scavengers to sequester acrolein seemed as an efficient option to decrease acrolein elevation post-mbTBI, reduce the oxidative stress post-injury, discontinue the secondary injury cascade, potentially also reduce TRPA1 and MCP1 expressions assuming that these were elevated in this injury model due to acrolein, and alleviate headaches and neuropathic pain.

Previously our lab has shown that the acrolein scavenger hydralazine is able to reach the CNS within 2 hours after IP injection; as well as to provide neuroprotection against acrolein in a spinal cord injury rat model and alleviate acute neuropathic pain after spinal cord injury.[182] Consequently, we decided to test whether acrolein also played a significant role in neuropathic pain post-mbTBI by sequestering acrolein with the acrolein scavenger hydralazine.

4.1.2 Brief methods

The mbTBI induction was performed exactly as described on section 2.1.2 with the addition of a new mbTBI group that received the hydralazine treatment. Rats were injected intraperitoneally with hydralazine (5 mg/kg) within 1 hour after injury, and every day before allodynia testing

thereafter. The allodynia testing was performed exactly as described on section 2.1.2 for periorbital and hind limb assessments. For this set of experiments n=7.

4.1.3 Results and Discussion

The hind limb allodynia assessments after the hydralazine treatment showed a significant improvement in alleviation of neuropathic pain as compared to mbTBI rats that was statistically comparable to healthy rats, as shown in Figure 13A. This shows that hydralazine is able to sequester acrolein providing an alleviation of general body neuropathic pain, demonstrating that acrolein appears to be directly involved in neuropathic pain post-mbTBI.

The periorbital allodynia assessments showed a significant alleviation of headache behaviors post-mbTBI with the hydralazine treatment, as shown in Figure 13B. These results, similar to the hind limb assessments, demonstrate that acrolein might be directly involved in headache post-mbTBI and that the sequestering of acrolein is able to provide an alleviation of headache post-mbTBI.

In both cases, these results indicate that hydralazine, an FDA approved drug,[81] is able to alleviate neuropathic pain and headaches post-mbTBI *in-vivo*, and might represent a potential therapy for the treatment of neuropathic pain in TBI patients.

4.2 Biochemical experiments

4.2.1 Rationale

In order to confirm the efficacy of hydralazine in sequestering acrolein in the animal tissue, and to confirm that acrolein reduction would also lead to a reduction in TRPA1 and MCP1 expressions, and therefore alleviate pain, western blot experiments were conducted in the brains, trigeminal nerves, spinal cords, and dorsal root ganglia (DRG) of the three different groups. The trigeminal nerves were particularly important for the analysis of headaches since changes in the trigeminovascular system are directly involved in headache mechanisms.[75] Moreover, the analysis of DRG was also particularly important for the general body neuropathic pain since it is known that sensory afferent impulses originate from the DRG,[250] and since changes in channel expression have been observed in the DRG in different pain models.[33]

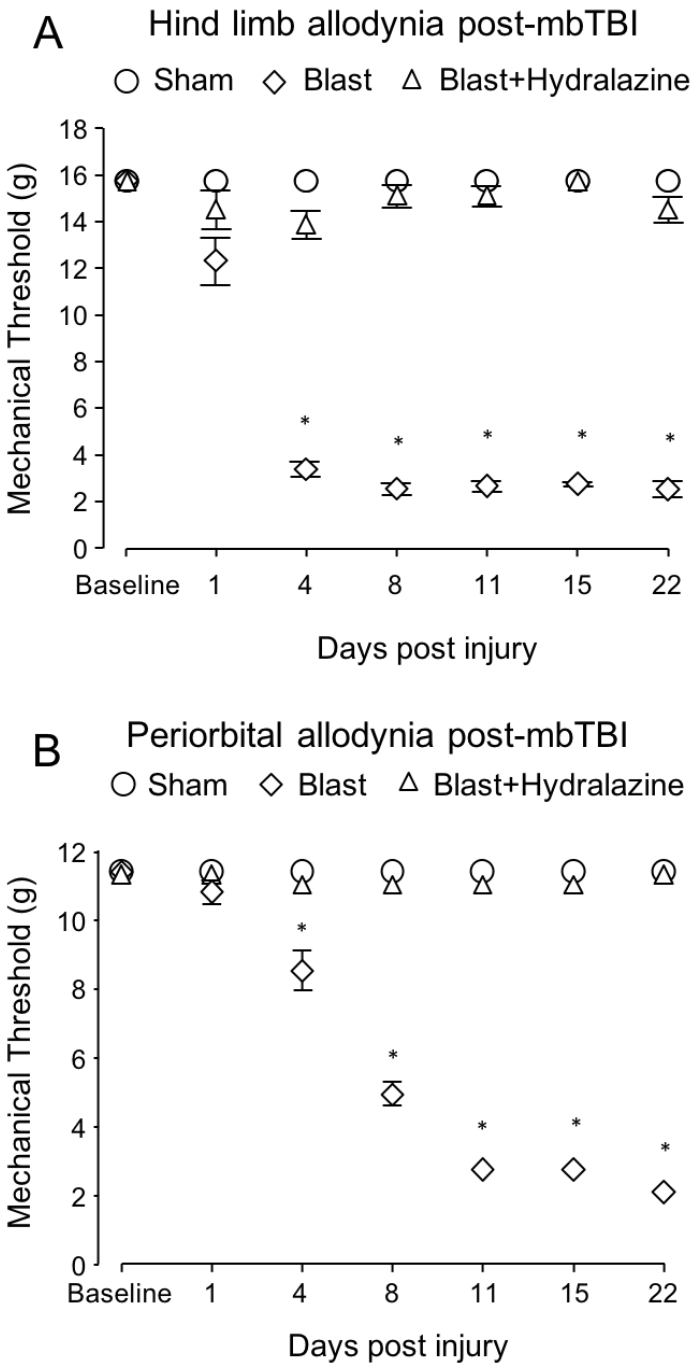

Figure 13 Hind limb and periorbital allodynia after a mild blast traumatic brain injury. In both hind limb (A) and periorbital (B) allodynia tests, sham, blast, and blast rats treated with the acrolein scavenger hydralazine show similar trends. The high mechanical threshold of sham rats indicate there is no hypersensitivity after blast noise exposure. Starting at 4 days post-mbTBI, blast rats indicate a significant hypersensitivity that persists throughout the observation period. Blast rats treated with hydralazine show no hypersensitivity and significant differences from the blast rats, indicating that the hydralazine treatment is able to mitigate hypersensitivity post-mbTBI. N=7 per group statistically analyzed with Anova and post-hoc Tukey test, * $p<0.05$.

4.2.2 Brief methods

On day 60 after mbTBI induction and hydralazine treatment, brains, trigeminal nerves, spinal cords and DRGs of all sham, mbTBI treated with hydralazine, and mbTBI rats were collected. Tissue harvesting, and western blotting techniques were performed exactly as described on section 2.2.2. Additionally, immunohistochemistry staining with acrolein on the amygdala and basal ganglia slices of the brain was performed.

4.2.3 Results and Discussion

The brain analysis with the western blotting technique showed a significant increase of acrolein, TRPA1 and MCP1 in mbTBI rats that were significantly reduced to healthy levels with the hydralazine treatment, as shown in Figure 15 and Figure 16. Moreover, immunohistochemistry staining with acrolein showed a darker staining on the blast group that was similar to the control group after the hydralazine treatment was applied, as shown on Figure 14. These results indicate that hydralazine treatment is able to reduce acrolein, TRPA1 and MCP1 levels in the brain post-mbTBI. These reductions in acrolein, TRPA1 and MCP1 with hydralazine treatment correlate with the decreased headaches observed in the periorbital allodynia assessments of the hydralazine treated group. Thus, this suggests that acrolein plays a significant role in upregulating TRPA1 and MCP1 levels in the brain post-mbTBI, which are involved in neuropathic pain and headache; and that hydralazine treatment is able to sequester acrolein significantly, therefore reducing TRPA1 and MCP1 in the brain, and consequently providing alleviation of headaches post-mbTBI.

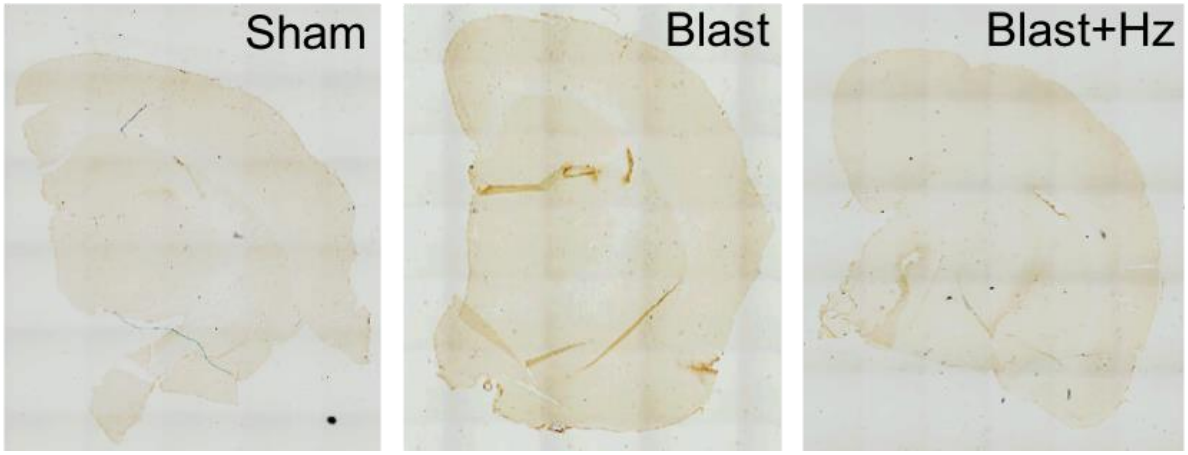

Figure 14 Immunohistochemical staining of the basal ganglia and amygdala with acrolein in sham, blast, and blast rats treated with hydralazine.

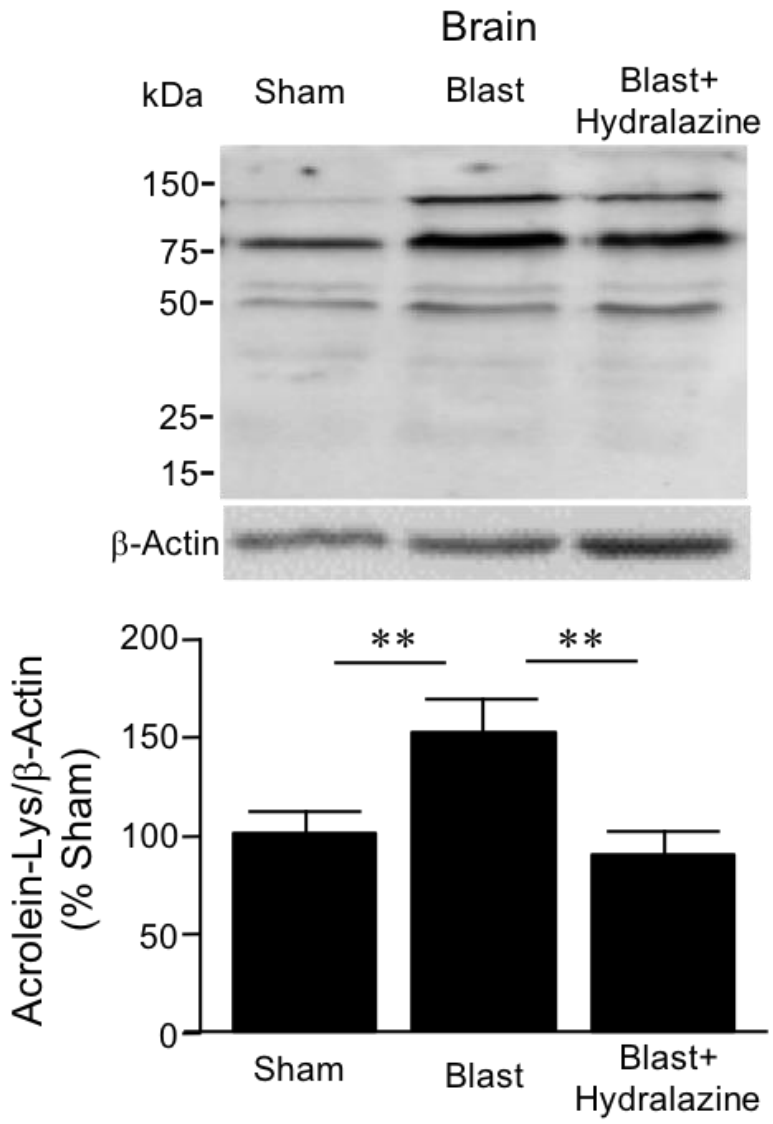

Figure 15 Acrolein concentrations in the brain after mb-TBI. Acrolein is significantly elevated in the brains of blast rats compared to sham rats and blast rats treated with the acrolein scavenger hydralazine. N=7 per group statistically analyzed with Anova and post-hoc Tukey test, **$p<0.01$.

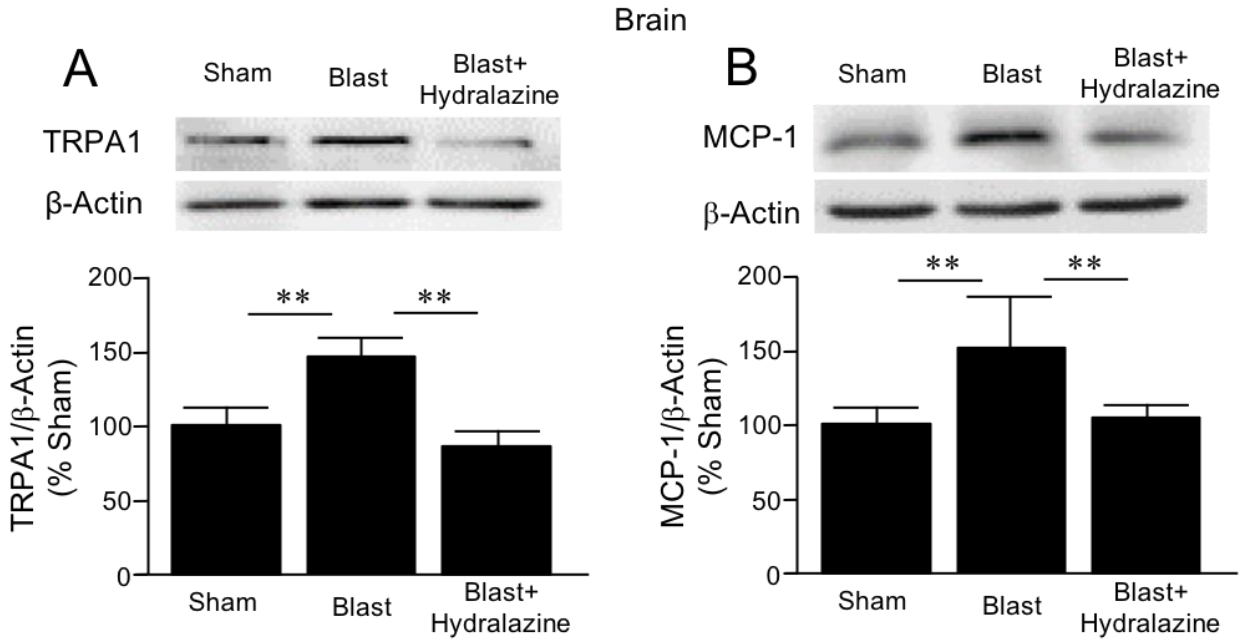

Figure 16 TRPA1 and MCP1 concentrations in the brain after mb-TBI. TRPA1 (A) and MCP-1 (B) are significantly elevated in the brains of blast rats compared to sham rats and blast rats treated with the acrolein scavenger hydralazine. N=7 per group statistically analyzed with Anova and post-hoc Tukey test, **p<0.01.

The trigeminal nerves' analysis showed an increase in acrolein, TRPA1 and MCP1 in mbTBI rats that was significantly reduced to healthy levels with hydralazine treatment, as shown in Figure 17 and Figure 18. These reductions in acrolein, TRPA1 and MCP1 correlate with reductions in headache with the hydralazine treatment. Thus, the results suggest that acrolein is significantly involved in headache post-mbTBI by elevating TRPA1 and MCP1, which play a major role in headache and neuropathic pain. Nevertheless, sequestering acrolein with hydralazine is able to reduce acrolein levels, therefore reducing TRPA1 and MCP1 in the trigeminal nerves, and consequently providing headache alleviation post-mbTBI.

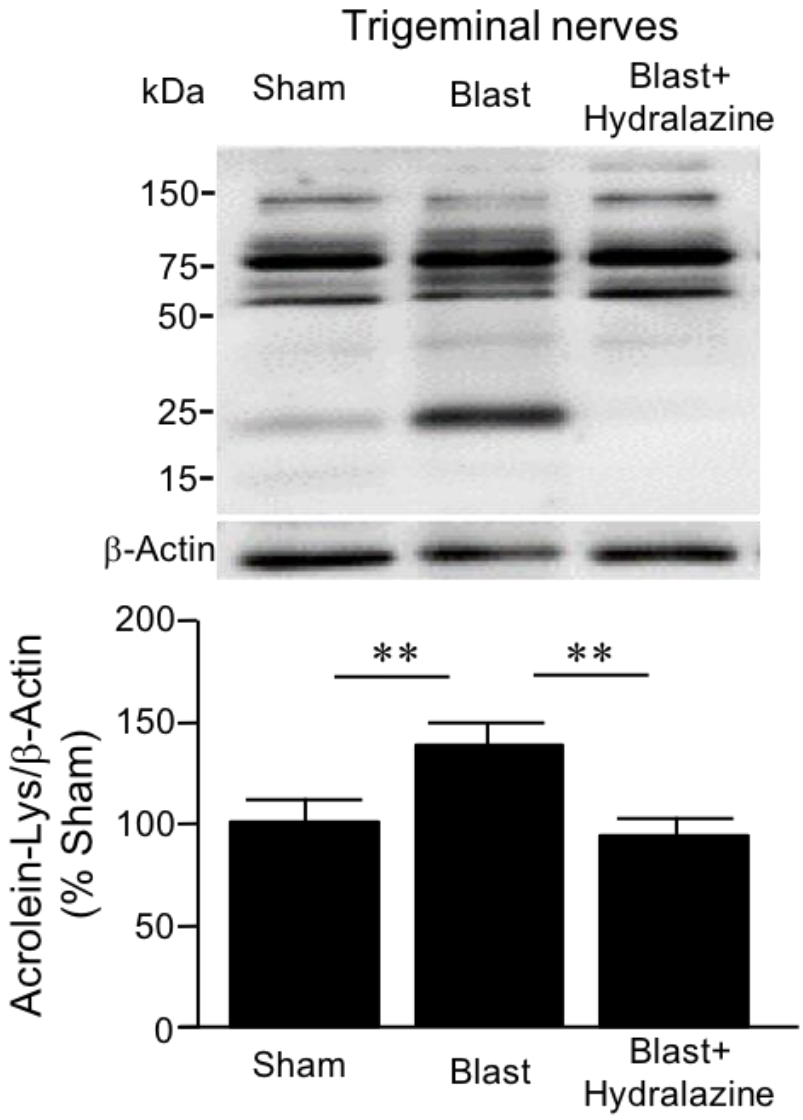

Figure 17 Acrolein concentrations in the trigeminal nerves after mb-TBI. Acrolein is significantly elevated in the trigeminal nerves of blast rats compared to sham rats and blast rats treated with the acrolein scavenger hydralazine. N=7 per group statistically analyzed with Anova and post-hoc Tukey test, **$p<0.01$.

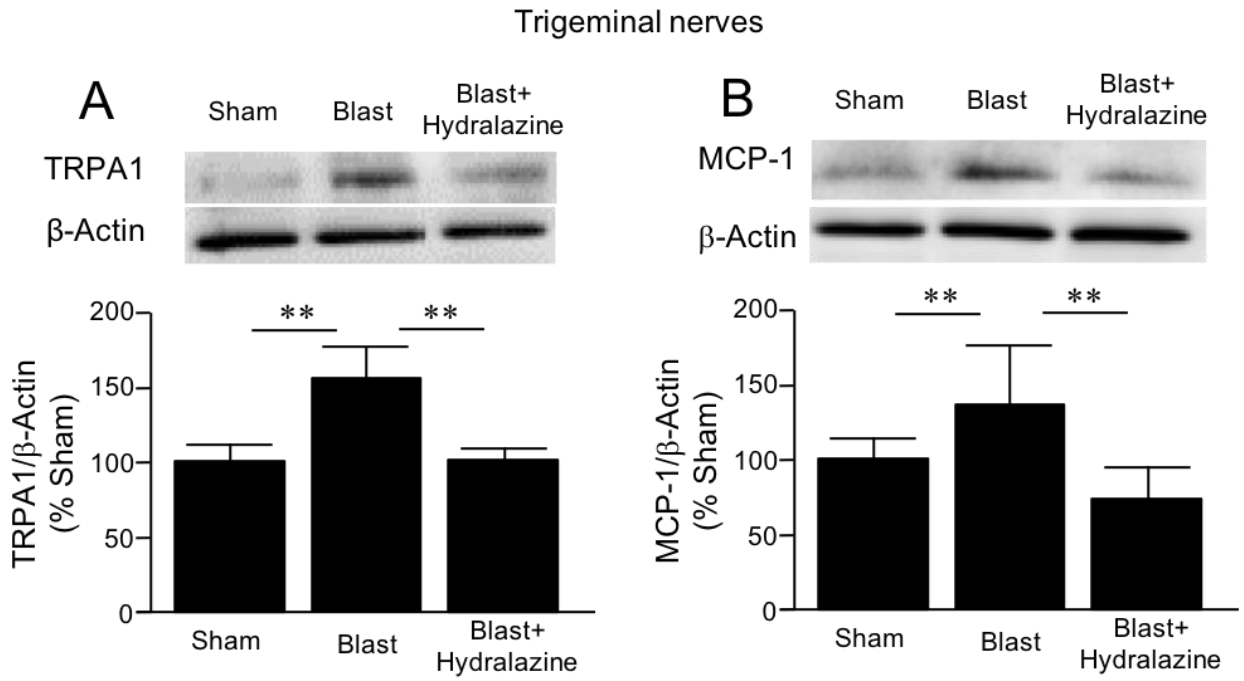

Figure 18 TRPA1 and MCP1 concentrations in the trigeminal nerves after mb-TBI. TRPA1 (A) and MCP-1 (B) are significantly elevated in the trigeminal nerves of blast rats compared to sham rats and blast rats treated with the acrolein scavenger hydralazine. N=7 per group statistically analyzed with Anova and post-hoc Tukey test, **$p<0.01$.

In the spinal cords, acrolein, TRPA1 and MCP1 were elevated in mbTBI rats, but were significantly decreased with hydralazine treatment, as shown in Figure 19 and Figure 20. Acrolein continued to be elevated in the spinal cord too, which is consistent with previous results that showed acrolein's metabolite, 3-HPMA, is elevated in the body up to 7 days post-mbTBI. Therefore, this suggests that hydralazine treatment is able to downregulate TRPA1 and MCP1 by sequestering acrolein post-mbTBI in the spinal cord, which correlates with reductions in hind limb neuropathic pain with hydralazine treatment post-mbTBI.

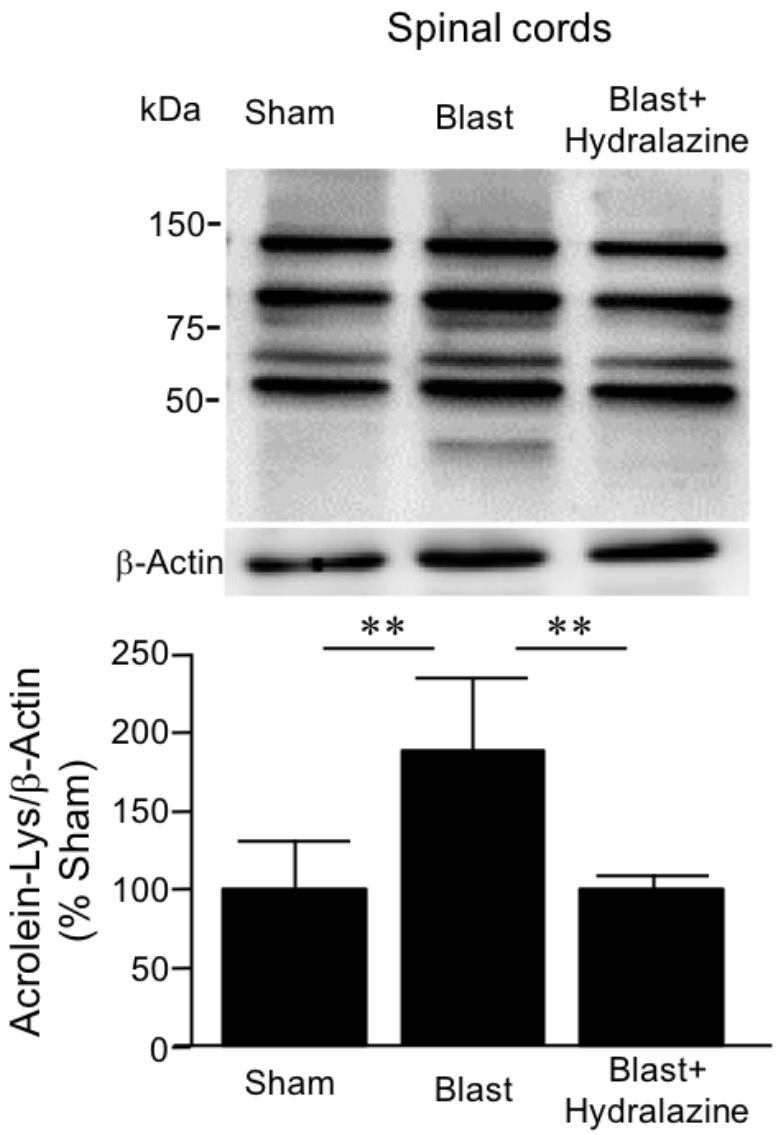

Figure 19 Acrolein concentrations in the spinal cord after mb-TBI. Acrolein is significantly elevated in the spinal cord of blast rats compared to sham rats and blast rats treated with the acrolein scavenger hydralazine. N=7 per group statistically analyzed with Anova and post-hoc Tukey test, **$p<0.01$.

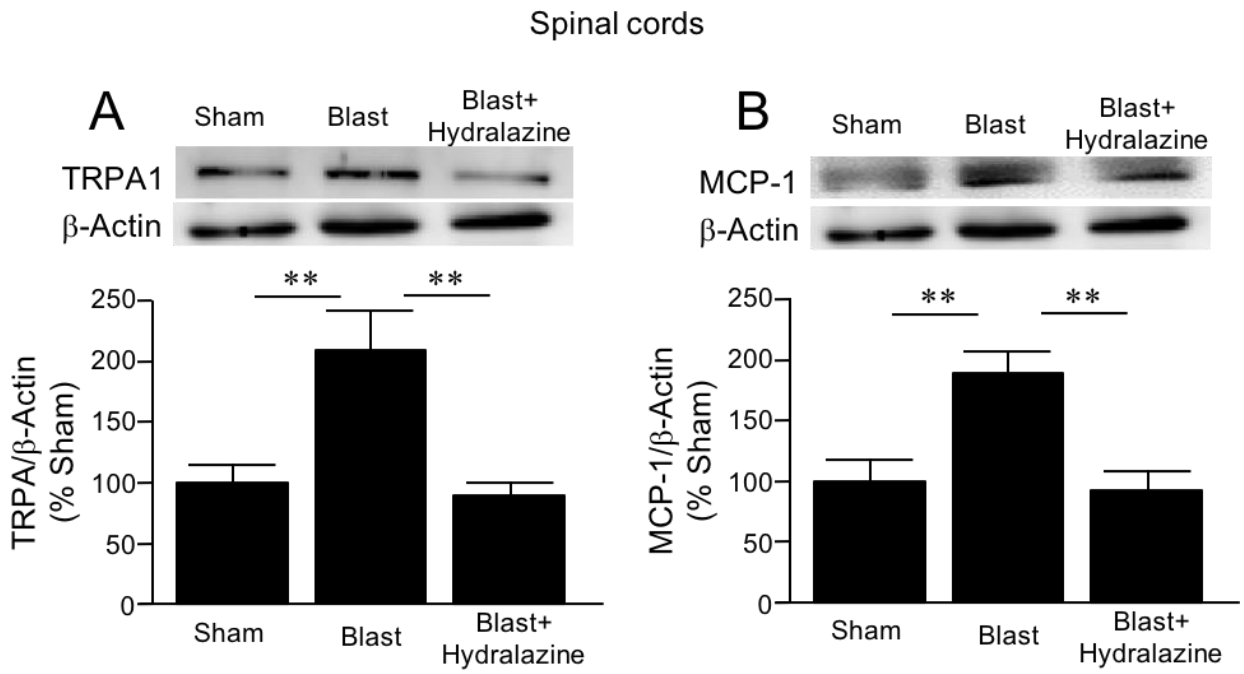

Figure 20 TRPA1 and MCP1 concentrations in the spinal cord after mb-TBI. TRPA1 (A) and MCP-1 (B) are significantly elevated in the spinal cord of blast rats compared to sham rats and blast rats treated with the acrolein scavenger hydralazine. N=7 per group statistically analyzed with Anova and post-hoc Tukey test, **$p<0.01$.

The DRG showed a significant increase in acrolein, TRPA1 and MCP1 protein expression that was significantly reduced to healthy levels with hydralazine treatment, as shown in Figure 21 and Figure 22. This indicates that acrolein is significantly involved in general body neuropathic pain, and that hydralazine treatment is able to sequester acrolein, therefore reducing TRPA1 levels in the DRG post-mbTBI that alleviate neuropathic pain.

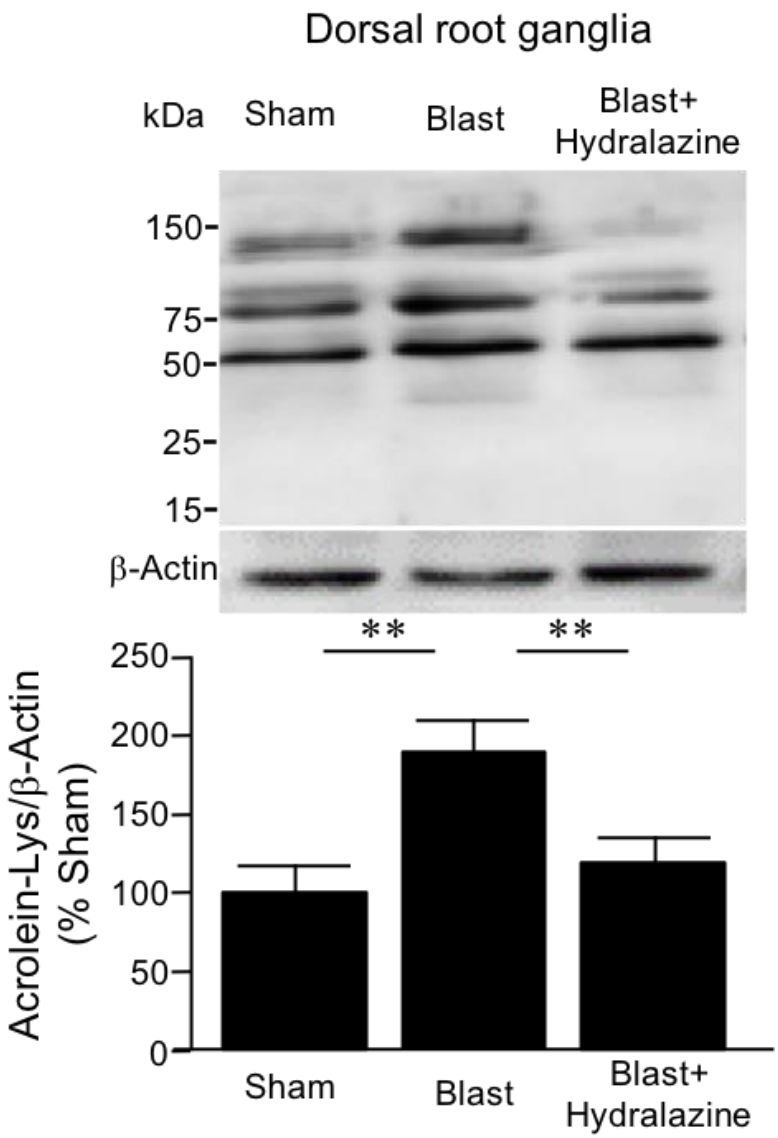

Figure 21 Acrolein concentrations in the dorsal root ganglia after mb-TBI. Acrolein is significantly elevated in the dorsal root ganglia of blast rats compared to sham rats and blast rats treated with the acrolein scavenger hydralazine. N=7 per group statistically analyzed with Anova and post-hoc Tukey test, **$p<0.01$.

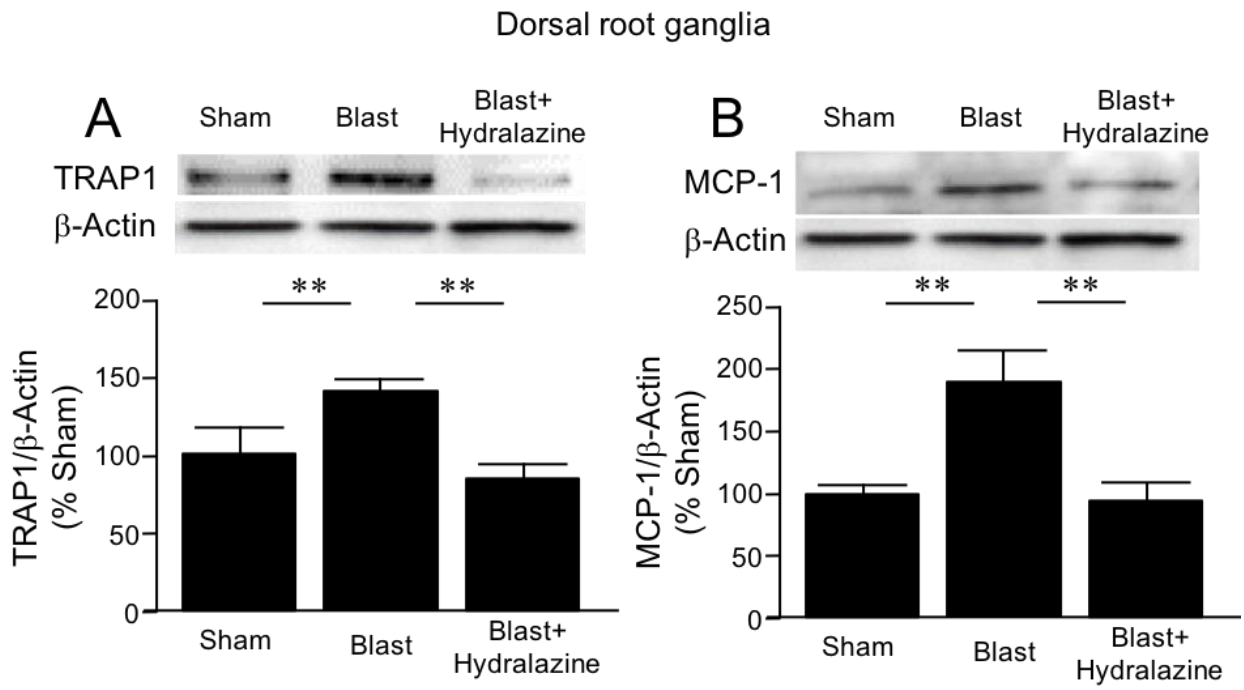

Figure 22 TRPA1 and MCP1 concentrations in the dorsal root ganglia after mb-TBI. TRPA1 (A) and MCP-1 (B) are significantly elevated in the dorsal root ganglia of blast rats compared to sham rats and blast rats treated with the acrolein scavenger hydralazine. N=7 per group statistically analyzed with Anova and post-hoc Tukey test, **$p<0.01$.

In summary, the biochemical results indicate that hydralazine is able to sequester acrolein significantly, and therefore provide a significant decrease in TRPA1 and MCP1. This cascade reduction correlates with alleviation of headaches and neuropathic pain and indicates that hydralazine treatment might be an effective option to treat neuropathic pain, and even potentially reduce the secondary injury cascade post-mbTBI.

CHAPTER 5. REDUCTION IN NEUROPATHIC PAIN-LIKE BEHAVIOR AND ACROLEIN, TRPA1 AND MCP1 PROTEIN EXPRESSION BY SEQUESTERING ACROLEIN IN CHRONIC STRESS

5.1 Allodynia behavioral experiments

5.1.1 Rationale

Previous results showed a significant increase in neuropathic pain in chronic stress that correlated with acrolein, TRPA1, and/or MCP1 elevations in the spinal cord and/or brain. Therefore, we decided to test acrolein's direct involvement in neuropathic pain in chronic stress by treating restrained rats with the acrolein scavenger hydralazine, expecting that by sequestering acrolein, TRPA1 and MCP1 concentrations would be reduced and consequently neuropathic pain and headache would be alleviated.

5.1.2 Brief methods

Chronic stress was induced by restraining the rats every day as described on section 3.1.2. Hind limb and periorbital allodynia measurements were performed as described on section 2.1.2. Hydralazine was injected intraperitoneally before induction of chronic stress on day 1, and daily as described on section 4.1.2. For this set of experiments n=7 per group.

5.1.3 Results and Discussion

Hind limb mechanical allodynia assessments showed an increase in neuropathic pain in restrain rats that was alleviated by the hydralazine treatment, as shown in Figure 23A. This indicates that acrolein is directly involved in neuropathic pain in chronic stress as sequestering of acrolein by the application of the hydralazine treatment can attenuate neuropathic pain behaviors.

Periorbital allodynia assessments similarly showed an increase in pain in restrained rats that was alleviated by the hydralazine treatment, as shown in Figure 23B. This indicates that acrolein is also involved in headache in chronic stress, and that hydralazine alone can attenuate such pain by sequestering acrolein.

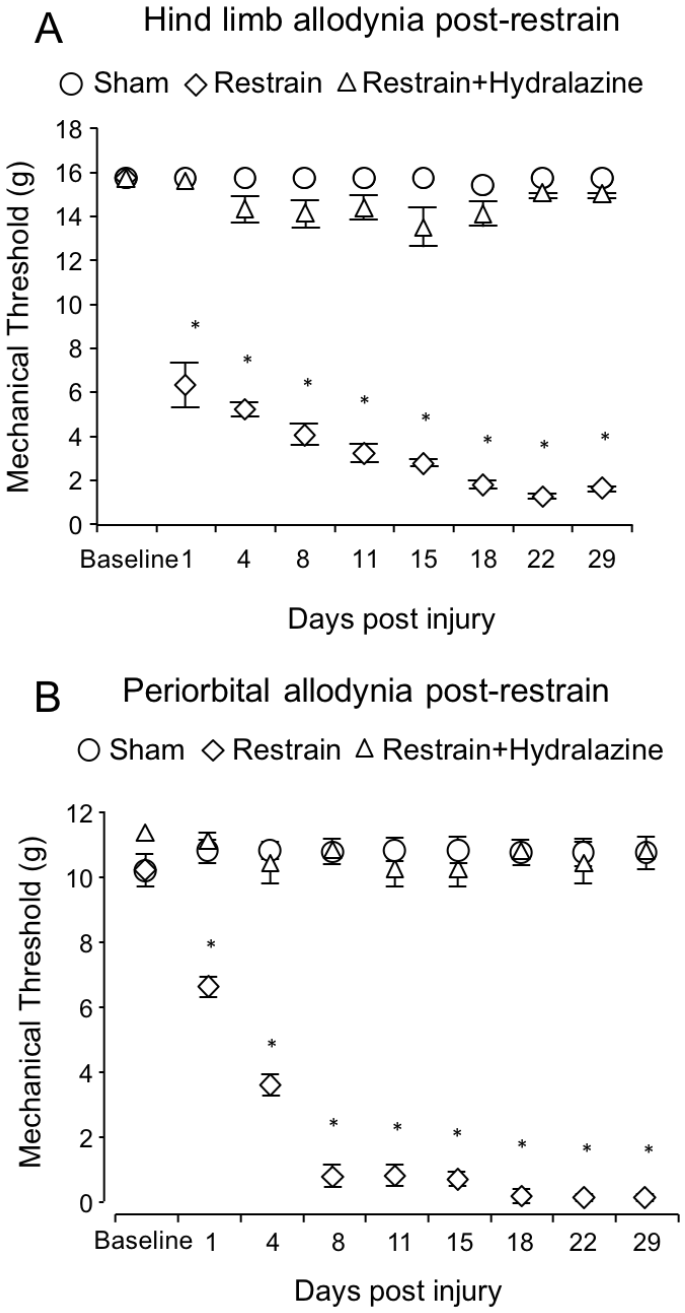

Figure 23 Hind limb and periorbital allodynia after a restrain. In both hind limb (A) and periorbital (B) allodynia tests, control, restrain, and restrain rats treated with the acrolein scavenger hydralazine show similar trends. The high mechanical threshold of control rats indicate there is no hypersensitivity. Starting at 1 days post-restrain, restrain rats indicate a significant hypersensitivity that persists throughout the observation period. Restrain rats treated with hydralazine show no hypersensitivity and significant differences from the restrain rats, indicating that the hydralazine treatment is able to mitigate hypersensitivity post-restrain. N=7 per group statistically analyzed with Anova and post-hoc Tukey test, * $p<0.05$.

These results suggest a direct involvement of acrolein in headache and neuropathic pain in chronic stress and demonstrate that treatment with hydralazine can alleviate these symptoms. Thus, hydralazine treatment could be a potent treatment for headache and neuropathic pain for patients suffering from chronic stress.

5.2 Biochemical experiments

5.2.1 Rationale

The section above demonstrated that acrolein sequestering could alleviate pain in chronic stress. Hence, it was expected that hydralazine treatment would be able to reduce acrolein levels at a molecular level, in synchrony with reducing headache and neuropathic pain. In this case, considering that acrolein is able to promote the upregulation of TRPA1 and release of MCP1, it was also expected that acrolein depletion by hydralazine treatment would at least partially reduce the elevated expression of TRPA1 and MCP1 and consequently reduce headaches and neuropathic pain. Therefore, it was necessary to confirm that acrolein elevation in the tissue had a direct relationship with headache and neuropathic pain in chronic stress. For this purpose, the rats' tissue was collected and submitted to western blots.

5.2.2 Brief methods

As described on section 2.2.2, rats were deeply anesthetized and the brains, trigeminal nerves, spinal cords and DRG of all sham, restrained, and hydralazine treated rats were collected. The western blotting technique was performed as described in the same section. Additionally, immunohistochemistry with acrolein staining of the basal ganglia and amygdala was performed on all 3 groups.

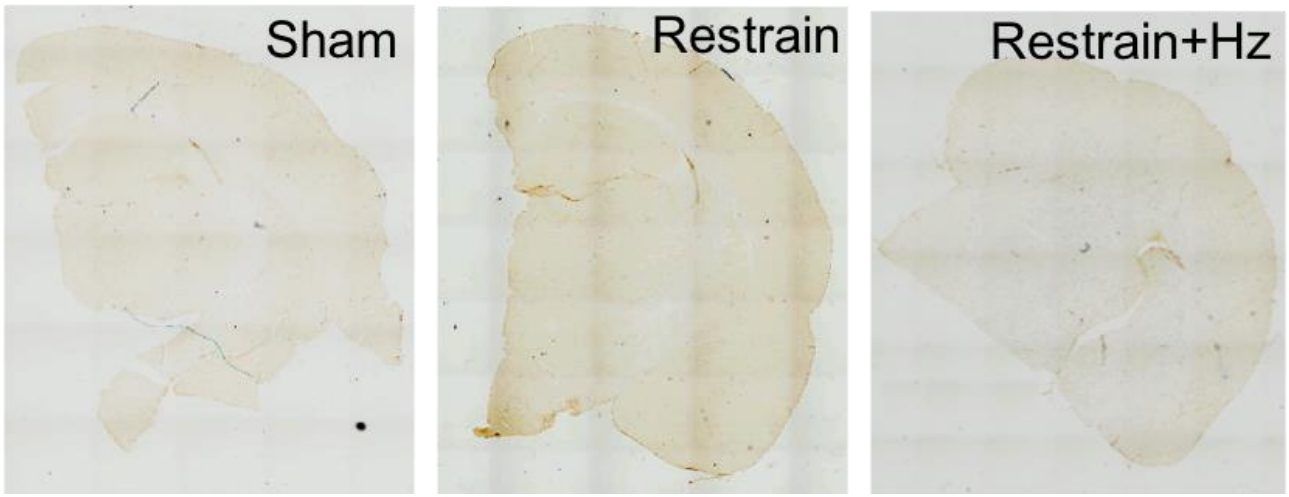

Figure 24 Immunohistochemical staining of the basal ganglia and amygdala with acrolein in sham, blast, and blast rats treated with hydralazine.

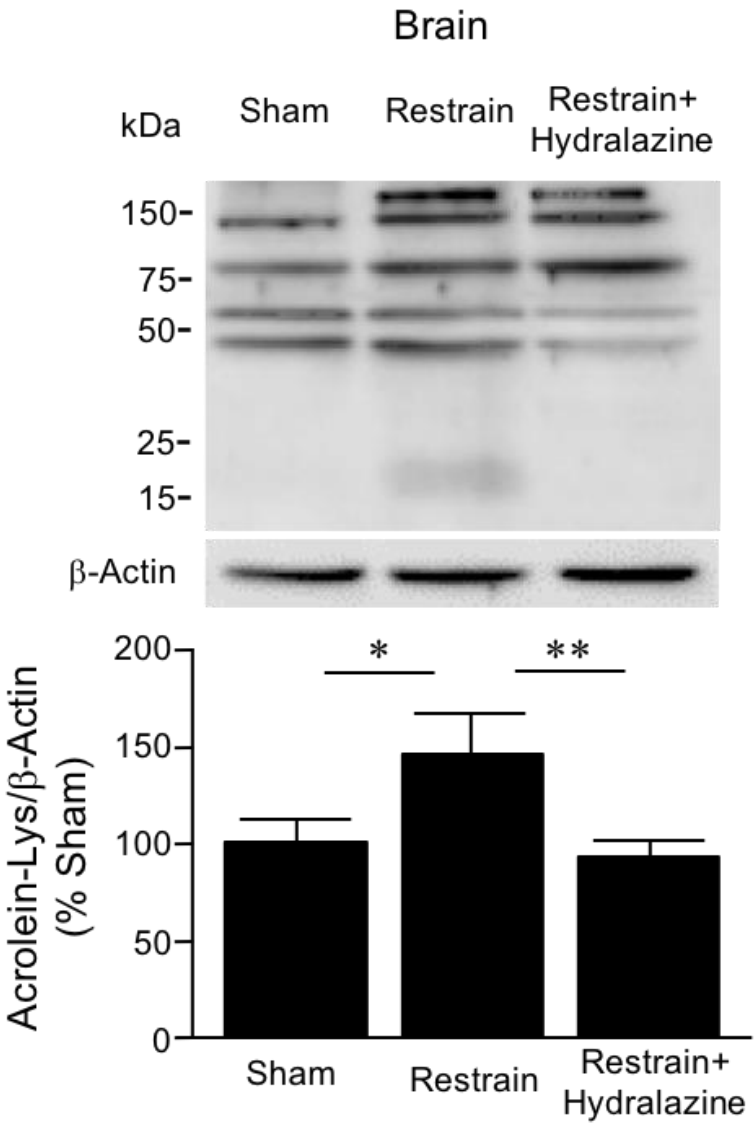

Figure 25 Acrolein concentrations in the brain after restrain. Acrolein is significantly elevated in the brain of restrain rats compared to control rats and restrain rats treated with the acrolein scavenger hydralazine. N=7 per group statistically analyzed with Anova and post-hoc Tukey test, **$p<0.01$.

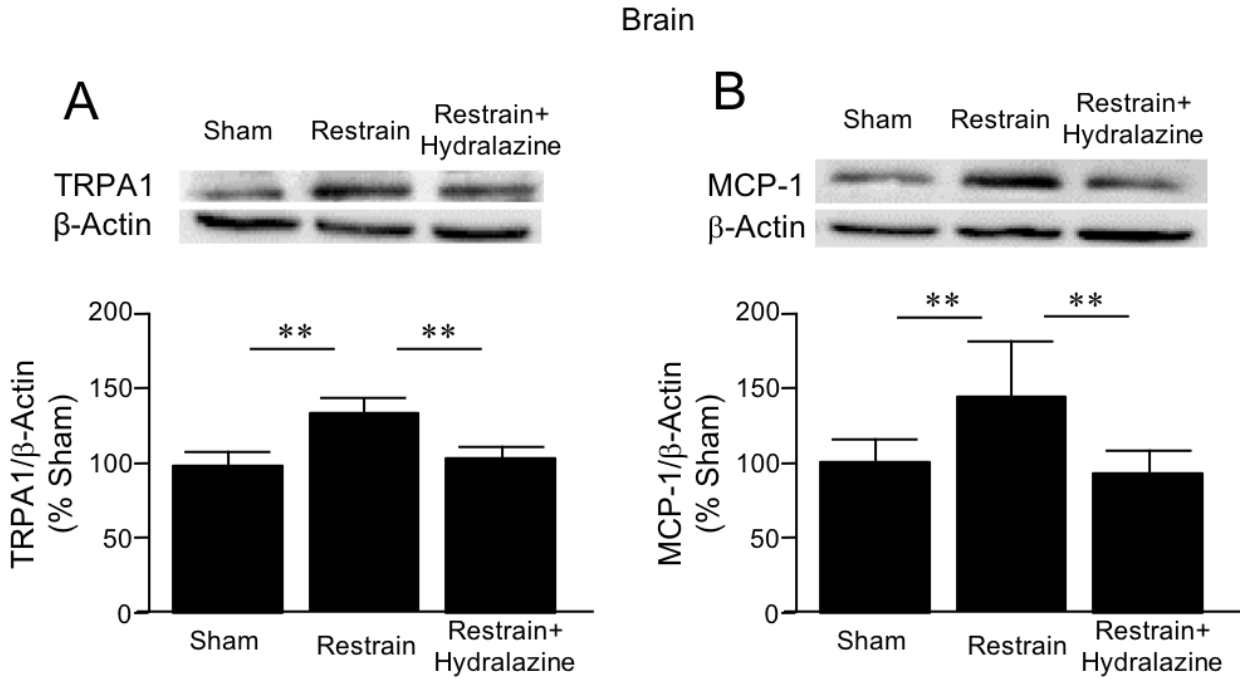

Figure 26 TRPA1 and MCP1 concentrations in the brain after restrain. TRPA1 (A) and MCP-1 (B) are significantly elevated in the brain of restrain rats compared to control rats and restrain rats treated with the acrolein scavenger hydralazine. N=7 per group statistically analyzed with Anova and post-hoc Tukey test, **p<0.01.

5.2.3 Results and Discussion

A significant increase in acrolein, TRPA1 and MCP1 concentrations were observed in the brain of restrained rats. These molecular changes were back to normal after application of hydralazine, as shown in Figure 25 and Figure 26. Moreover, the immunohistochemical staining of the basal ganglia and amygdala of control, restain and restrain rats treated with hydralazine, showed a darker staining of acrolein on the restrain group that got as healthy after treated with hydralazine, as shown in Figure 24. This indicates a direct relationship between acrolein concentration and headache behaviors in the brain, as the hydralazine treatment was able to attenuate headaches in chronic stress, as well as decrease acrolein, TRPA1 and MCP1 levels. Due to the importance of TRPA1 and MCP1 in neuropathic pain, we can also deduce that acrolein sequestering reduces TRPA1 and MCP1 levels attenuating headaches in chronic stress.

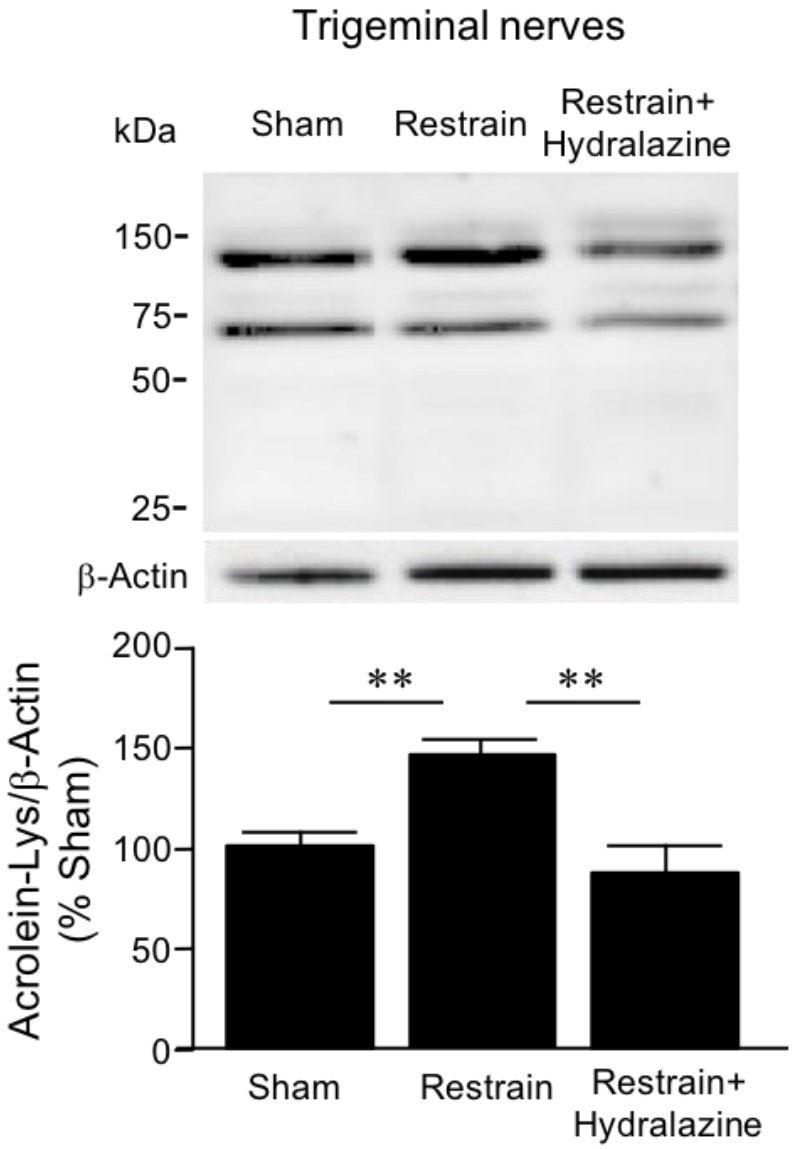

Figure 27 Acrolein concentrations in the trigeminal nerves after restrain. Acrolein is significantly elevated in the trigeminal nerves of restrain rats compared to control rats and restrain rats treated with the acrolein scavenger hydralazine. N=7 per group statistically analyzed with Anova and post-hoc Tukey test, **$p<0.01$.

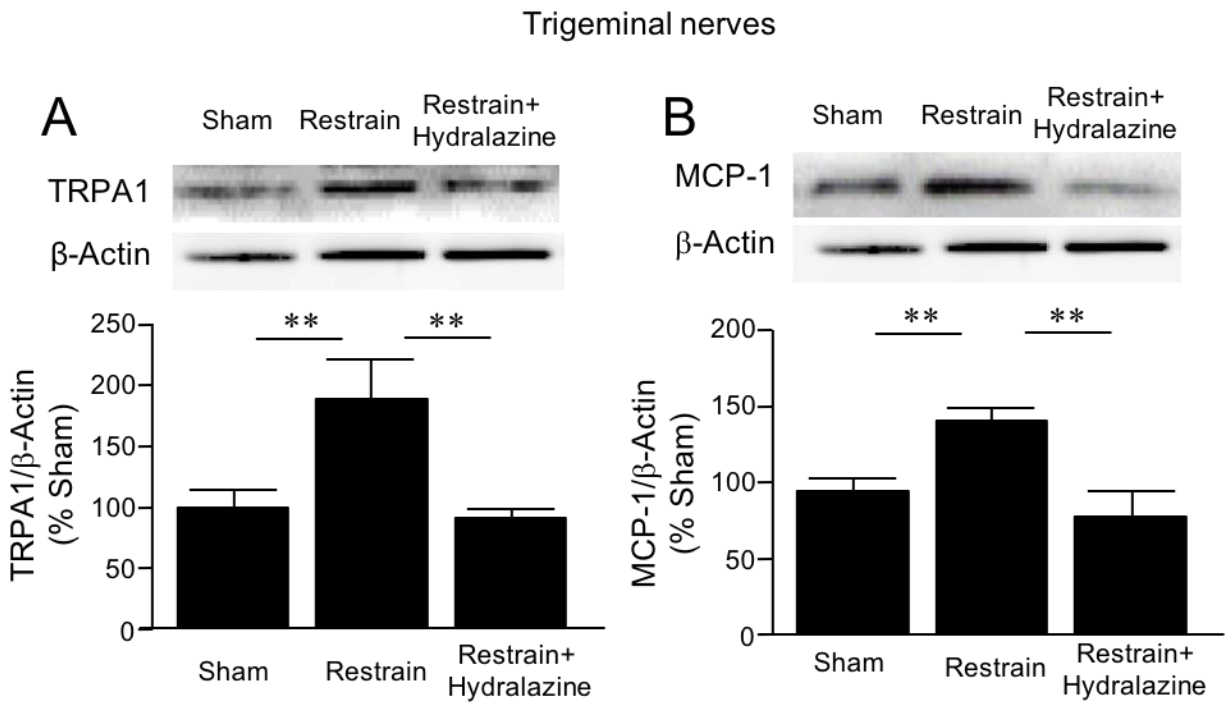

Figure 28 TRPA1 and MCP1 concentrations in the trigeminal nerves after restrain. TRPA1 (A) and MCP-1 (B) are significantly elevated in the trigeminal nerves of restrain rats compared to control rats and restrain rats treated with the acrolein scavenger hydralazine. N=7 per group statistically analyzed with Anova and post-hoc Tukey test, **$p<0.01$.

The analysis of the trigeminal nerves showed an increase in acrolein, TRPA1 and MCP1 levels that was attenuated to healthy levels by the hydralazine treatment in chronic stress, as shown in Figure 27 and Figure 28. This correlates with the reduced headaches observed in restrained rats that received the hydralazine treatment. Thus, acrolein appears to have a significant role in headaches as the sequestering of such decreases both TRPA1 and MCP1 and alleviates headaches in chronic stress.

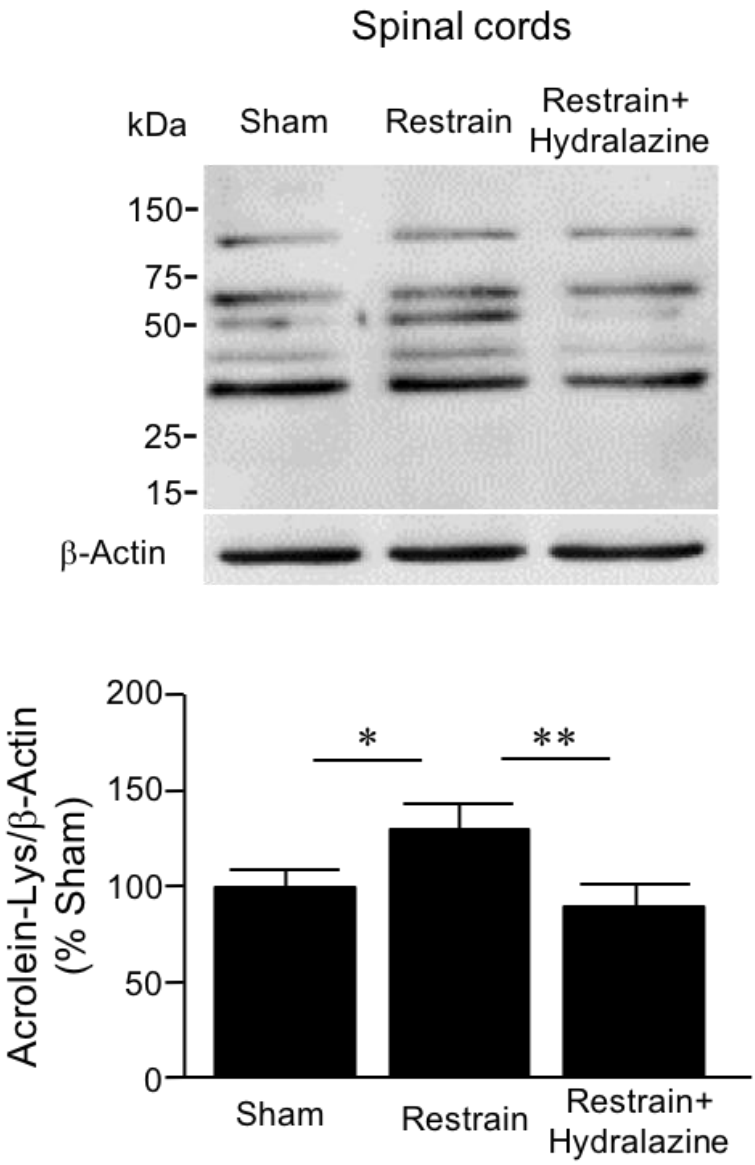

Figure 29 Acrolein concentrations in the spinal cords after restrain. Acrolein is significantly elevated in the spinal cords of restrain rats compared to control rats and restrain rats treated with the acrolein scavenger hydralazine. N=7 per group statistically analyzed with Anova and post-hoc Tukey test, **$p<0.01$.

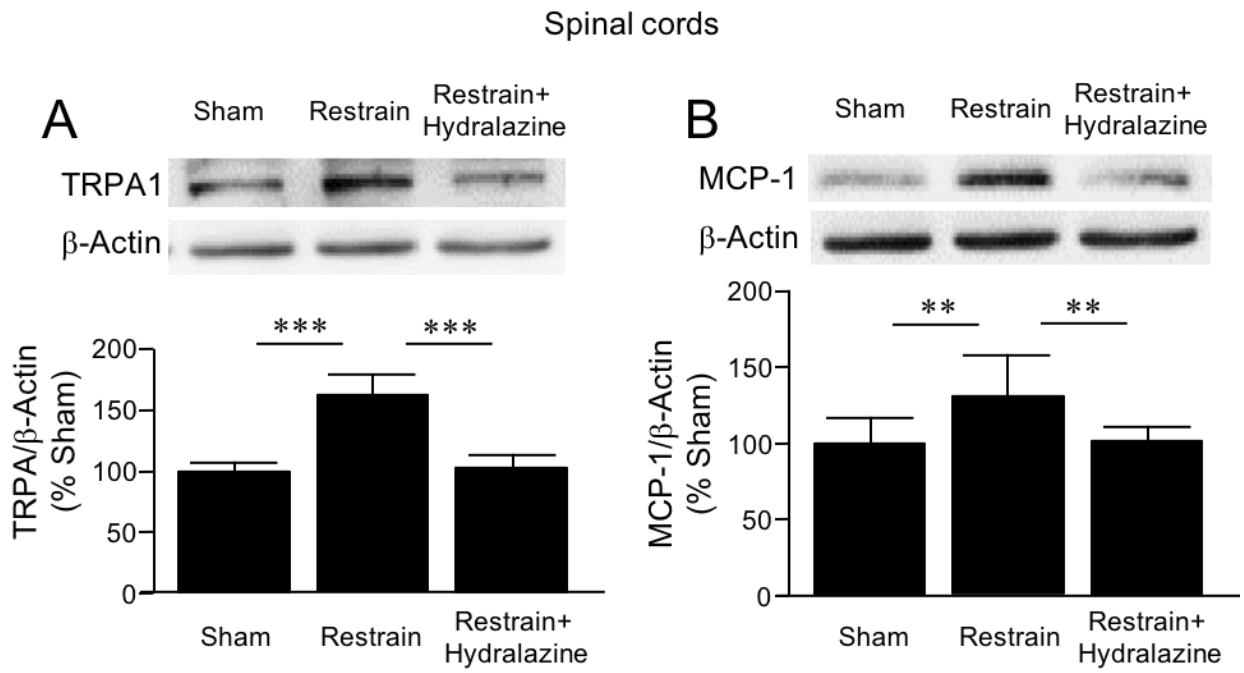

Figure 30 TRPA1 and MCP1 concentrations in the spinal cords after restrain. TRPA1 (A) and MCP-1 (B) are significantly elevated in the spinal cords of restrain rats compared to control rats and restrain rats treated with the acrolein scavenger hydralazine. N=7 per group statistically analyzed with Anova and post-hoc Tukey test, **p<0.01.

Acrolein, TRPA1 and MCP1 were also significantly elevated in the spinal cords of the restrained rats, and the hydralazine treatment reduced these elevations significantly, as shown in Figure 29 and Figure 30. These changes in molecular expression corelate with the hind limb neuropathic pain behaviors observed in these groups, suggesting an important role of acrolein in neuropathic pain in chronic stress.

The western blots of DRG showed a significant reduction of acrolein, TRPA1 and MCP1 in hydralazine treated rats compared to restrained rats, as shown in Figure 31 and Figure 32. This also correlates with the hind limb allodynia assessments of all three groups and demonstrates a significant role of acrolein in the perpetuation of neuropathic pain in chronic stress.

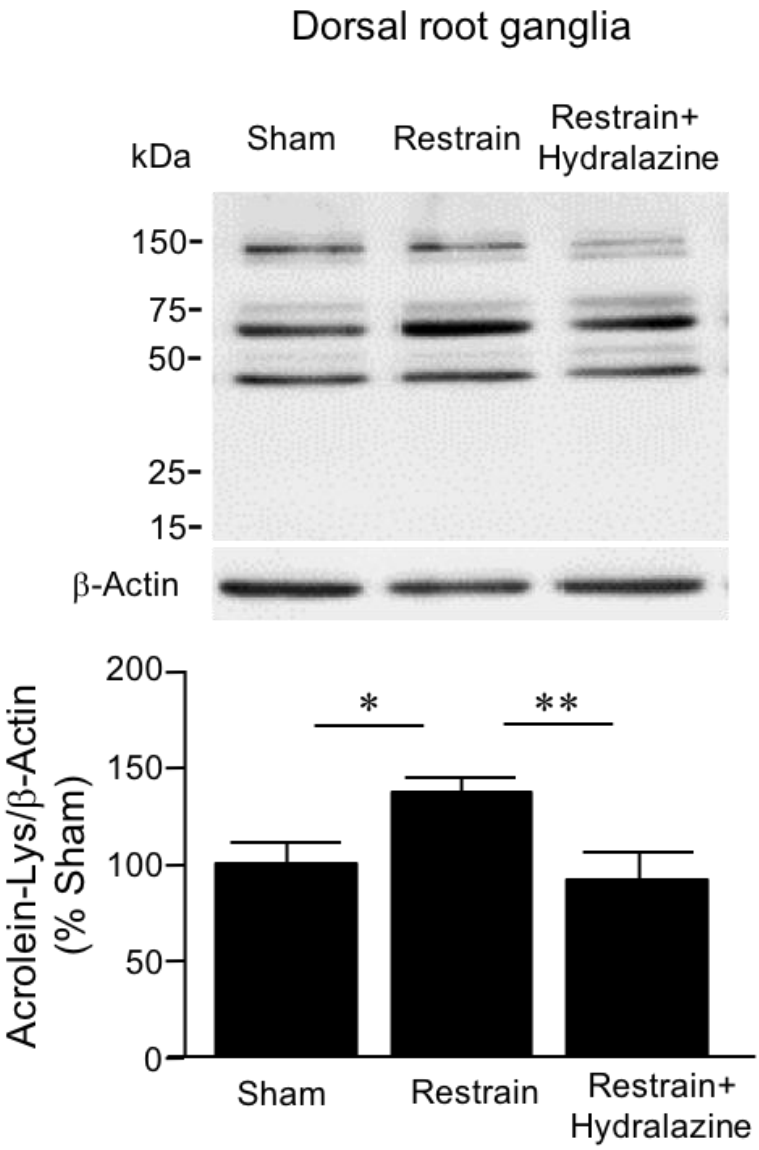

Figure 31 Acrolein concentrations in the dorsal root ganglia after restrain. Acrolein is significantly elevated in the dorsal root ganglia of restrain rats compared to control rats and restrain rats treated with the acrolein scavenger hydralazine. N=7 per group statistically analyzed with Anova and post-hoc Tukey test, **$p<0.01$.

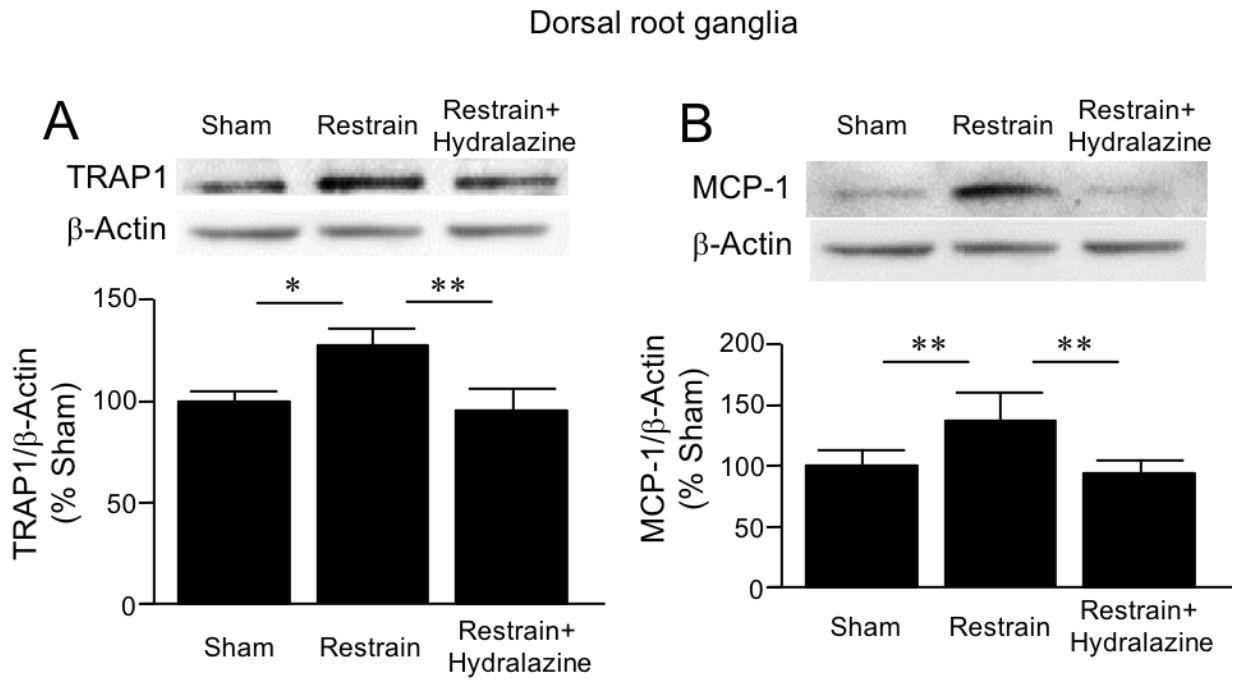

Figure 32 TRPA1 and MCP1 concentrations in the dorsal root ganglia after restrain. TRPA1 (A) and MCP-1 (B) are significantly elevated in the dorsal root ganglia of restrain rats compared to control rats and restrain rats treated with the acrolein scavenger hydralazine. N=7 per group statistically analyzed with Anova and post-hoc Tukey test, **p<0.01.

Altogether, these results demonstrate that acrolein is present in brains, trigeminal nerves, spinal cords and DRG of chronically stressed rats, and that acrolein appears to be responsible of the headache and neuropathic pain in chronic stress. Hydralazine treatment alone is able to decrease these molecular expressions back to normal levels, and to alleviate pain. Hence, hydralazine treatment acts as a potent treatment against oxidative stress and the neuropathic pain cascade that occurs in chronic stress and could be easily translated into treatments for human patients with chronic stress.

CHAPTER 6. STUDY OF THE COMORBIDITY OF A MILD BLAST TRAUMATIC BRAIN INJURY AND CHRONIC STRESS

6.1 Allodynia behavioral experiments

6.1.1 Rationale

Previous results demonstrated that chronic stress and mbTBI alone were able to promote neuropathic pain and headache. It is well known that military populations experience excessive stress before, during and after military conflicts; however, it is unknown whether chronic stress before, after and during battle is able to exacerbate headache and neuropathic pain in mbTBI. Given the high incidence of chronic stress in comorbidity with mbTBI, we designed a clinically relevant comorbidity model of chronic stress and mbTBI to elucidate the effect of chronic stress and mbTBI in instigating neuropathic pain and headache.

To understand whether acrolein-scavenging still proves effective in mitigating pain even in the comorbidity of both conditions, a comorbidity group treated with hydralazine was included and tested for allodynia.

6.1.2 Brief methods

In order to generate a comorbidity model of mbTBI and chronic stress, we decided to generate similar situations in rats that could reflect the experiences of military populations in respect of these two conditions. Thus, we submitted the rats to stress as previously described for 1 week before the blast to reflect the stress that military populations might encounter during training, after leaving the family and before the combat occurs. After 1 week of restrain, the rats were exposed to mbTBI as described on previous sections. The rats were allowed to recover that day after mbTBI and were not restrained. One day after mbTBI the rats were subjected to stress again for 2 weeks after mbTBI.

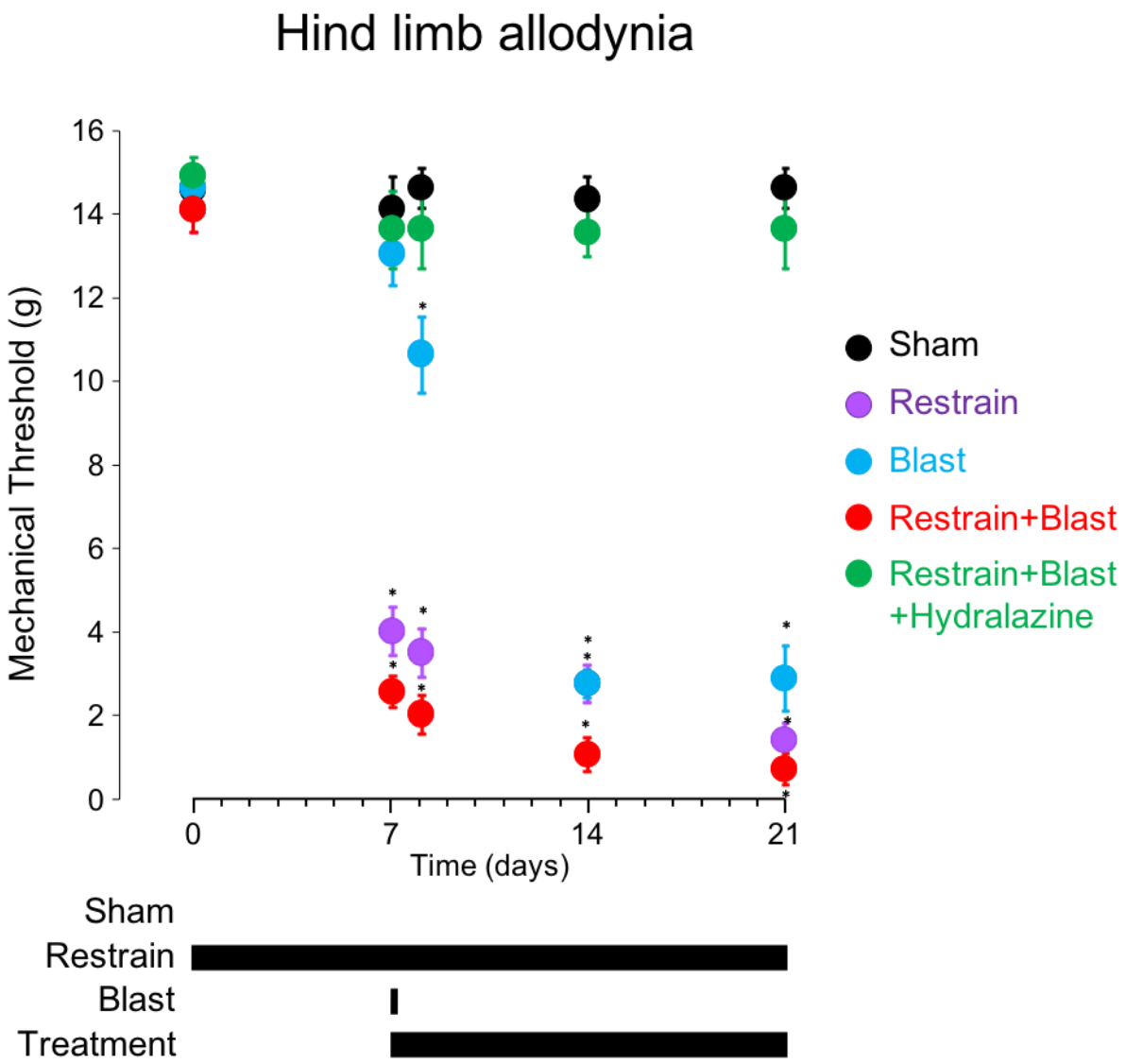

Figure 33 Hind limb allodynia in sham, blast, restrain, comorbidity and comorbidity rats treated with hydralazine. Restrain, blast and comorbidity rats show significantly more hypersensitivity than sham and hydralazine treated rats. N=8 per group from an average of four female and four male rats, statistically analyzed with Anova and post-hoc Fisher test, * $p<0.05$.

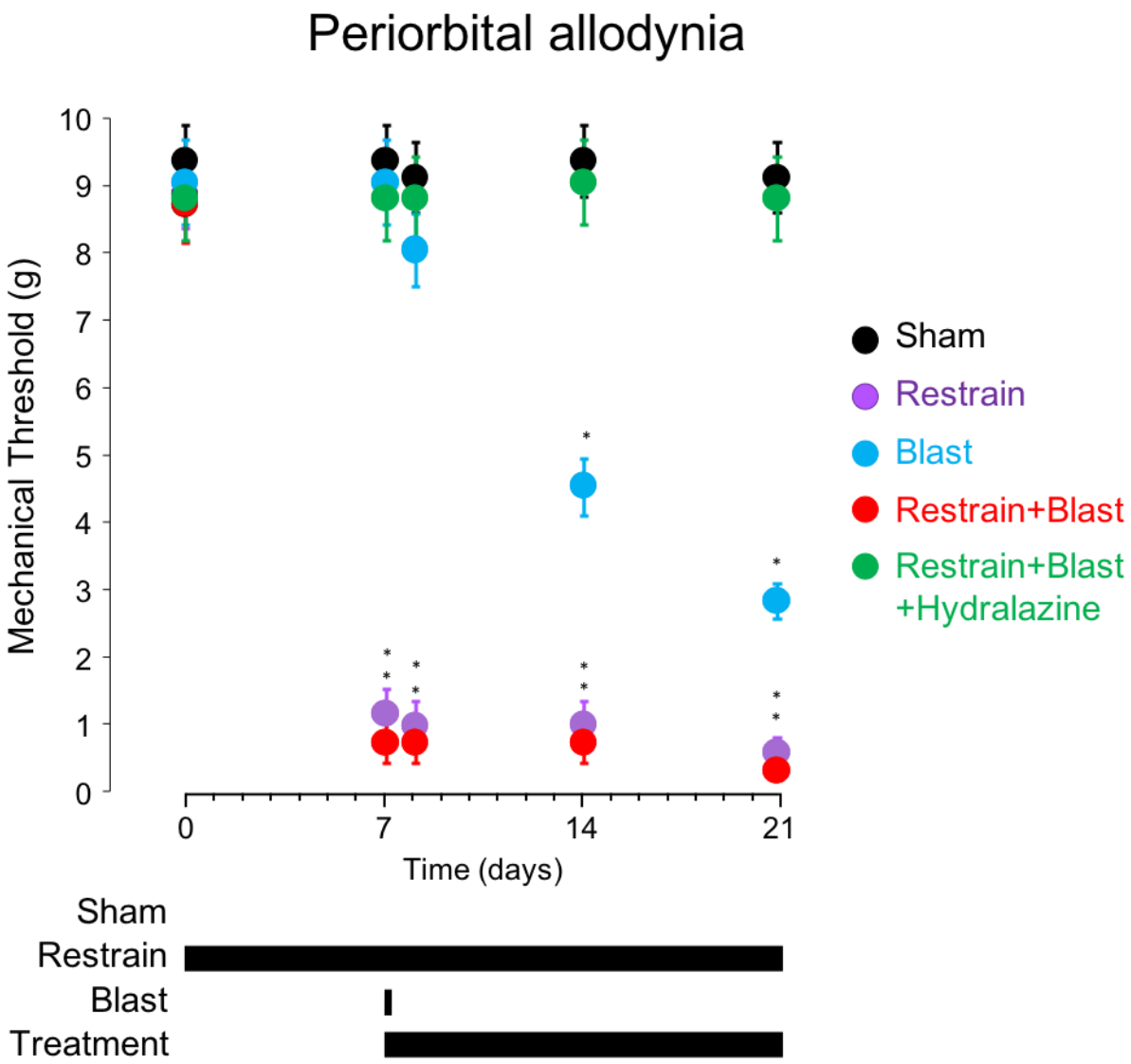

Figure 34 Hind limb allodynia in sham, blast, restrain, comorbidity and comorbidity rats treated with hydralazine. Restrain, blast and comorbidity rats show significantly more hypersensitivity than sham and hydralazine treated rats. N=8 per group from an average of four female and four male rats, statistically analyzed with Anova and post-hoc Fisher test, * $p<0.05$.

In this set of experiments, female and male rats were included to better reflect the military populations and variations between females and males. Additionally, sham, blast, restrain and

comorbidity rats treated with hydralazine were included with an N=4 per group per gender with a total N=40 for the whole experiment with the 5 groups and both genders.

6.1.3 Results and Discussion

In both hind limb and periorbital allodynia measurements, as shown in Figures 33 and 34 respectively, the comorbidity group showed an even lower mechanical threshold than the blast and restrain groups, indicating even more hypersensitivity in the comorbidity of both conditions. Despite the increased hypersensitivity of the comorbidity group, the hydralazine treatment still proved effective even at the same concentrations used before, indicating that hydralazine is a potent treatment for neuropathic pain in the comorbidity of mbTBI and chronic stress.

6.2 Biochemical experiments

6.2.1 Rationale

To test whether the neuropathic pain behaviors in the comorbidity group and the comorbidity group treated with hydralazine coincide, the brains, trigeminal nerves, spinal cords and DRG of all five groups were collected and biochemically analyzed with western blotting for acrolein, TRPA1 and MCP1 concentrations.

6.2.2 Brief methods

The blots were designed to show all five groups in one same gel from the same gender to block the gender and only show variations of disease model. The results were analyzed and averaged considering both genders to have a total N=8 per group including female and male rats.

6.2.3 Results and discussion

In most cases acrolein, TRPA1 and MCP1 was elevated in the comorbidity group compared with the blast and restrain groups. The hydralazine treatment in the comorbidity group was able to significantly reduce protein concentrations to healthy levels. These results coincide with the allodynia results and indicate that the comorbidity of these conditions instigates more protein accumulation and more neuropathic pain than the conditions alone, and despite this, the hydralazine treatment is able to mitigate these effects and restore normal levels.

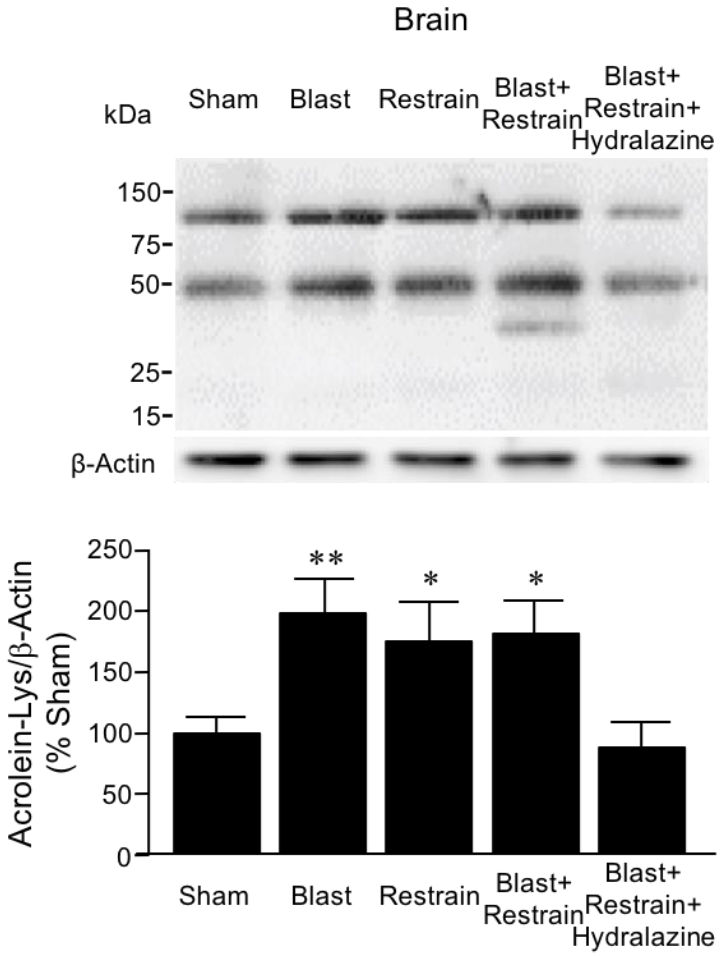

Figure 35 Acrolein in the brain of sham, blast, restrain, comorbidity and comorbidity rats treated with hydralazine. The brains of the comorbidity group showed an increase in acrolein concentrations compared to the blast and restrain groups. The treatment with hydralazine was able to reduce acrolein levels that were significantly similar to the sham. N=8 per group from an average of four female and four male rats, statistically analyzed with Anova and post-hoc Fisher test, * $p<0.05$.

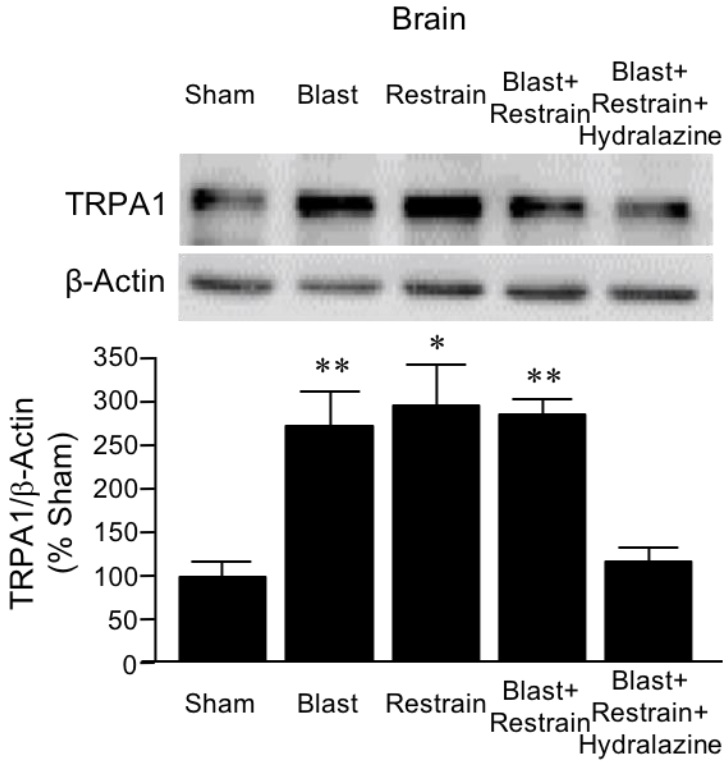

Figure 36 TRPA1 in the brain of sham, blast, restrain, comorbidity and comorbidity rats treated with hydralazine. The brains of the blast, restrain and comorbidity groups showed an increase in protein concentrations. The treatment with hydralazine was able to reduce TRPA1 levels that were significantly similar to the sham. N=8 per group from an average of four female and four male rats, statistically analyzed with Anova and post-hoc Fisher test, * $p<0.05$.

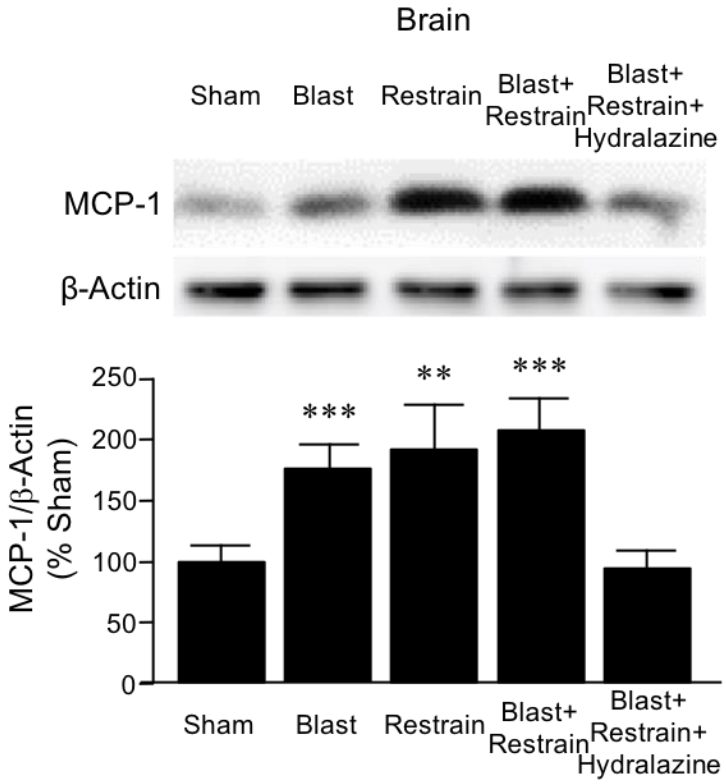

Figure 37 MCP1 in the brain of sham, blast, restrain, comorbidity and comorbidity rats treated with hydralazine. The brains of the comorbidity group showed an increase in protein concentrations compared to the blast and restrain groups. The treatment with hydralazine was able to reduce MCP1 levels that were significantly similar to the sham. N=8 per group from an average of four female and four male rats, statistically analyzed with Anova and post-hoc Fisher test, * $p<0.05$.

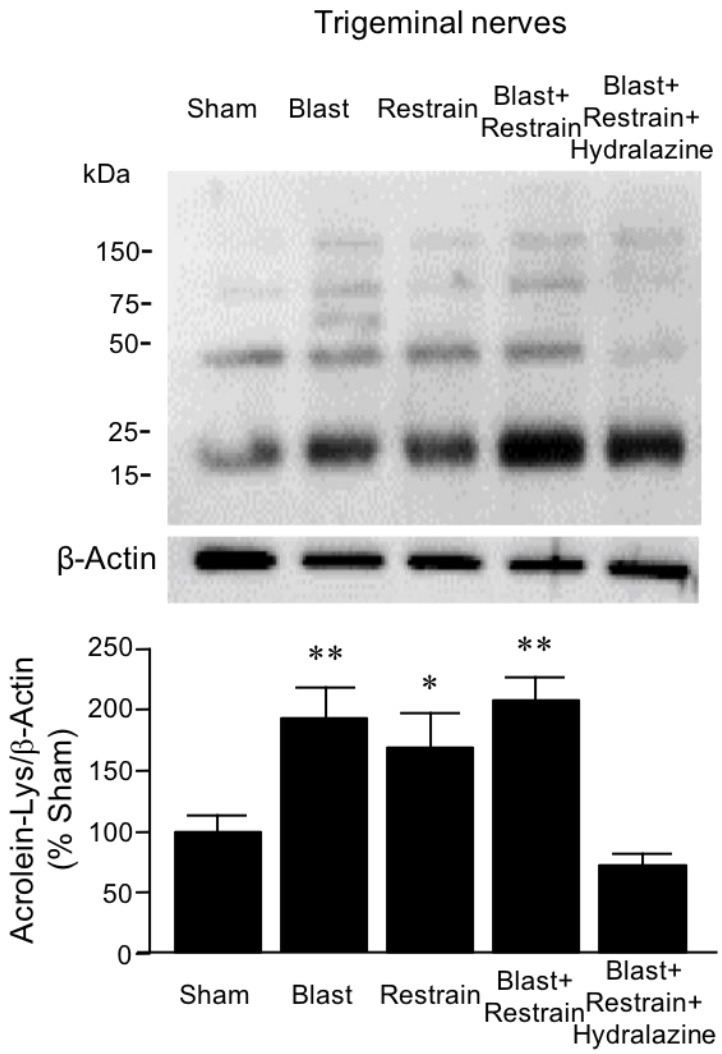

Figure 38 Acrolein in the trigeminal nerves of sham, blast, restrain, comorbidity and comorbidity rats treated with hydralazine. The trigeminal nerves of the comorbidity group showed an increase in protein concentrations compared to the blast and restrain groups. The treatment with hydralazine was able to reduce acrolein levels that were significantly similar to the sham. N=8 per group from an average of four female and four male rats, statistically analyzed with Anova and post-hoc Fisher test, * $p<0.05$.

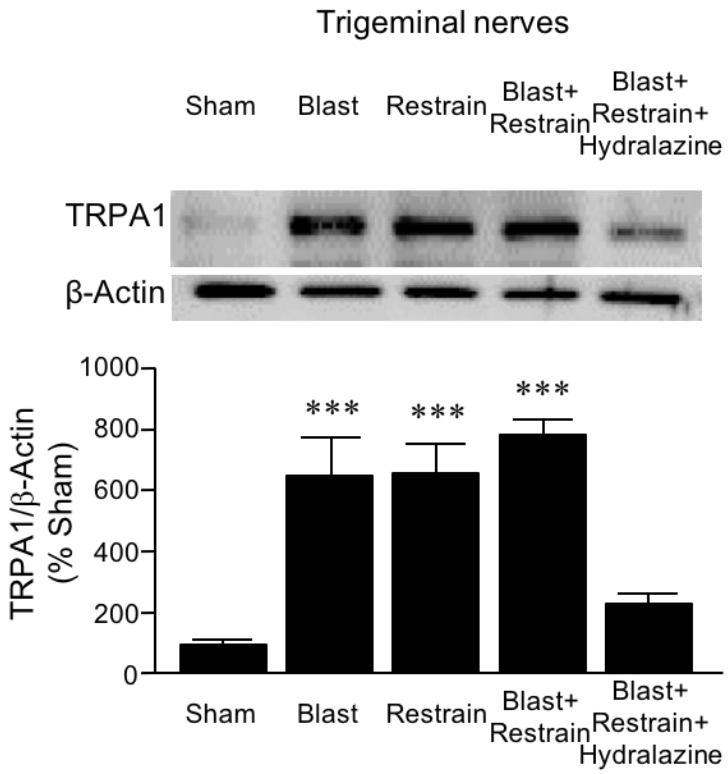

Figure 39 TRPA1 in the trigeminal nerves of sham, blast, restrain, comorbidity and comorbidity rats treated with hydralazine. The trigeminal nerves of the comorbidity group showed an increase in protein concentrations compared to the blast and restrain groups. The treatment with hydralazine was able to reduce TRPA1 levels that were significantly similar to the sham. N=8 per group from an average of four female and four male rats, statistically analyzed with Anova and post-hoc Fisher test, * $p<0.05$.

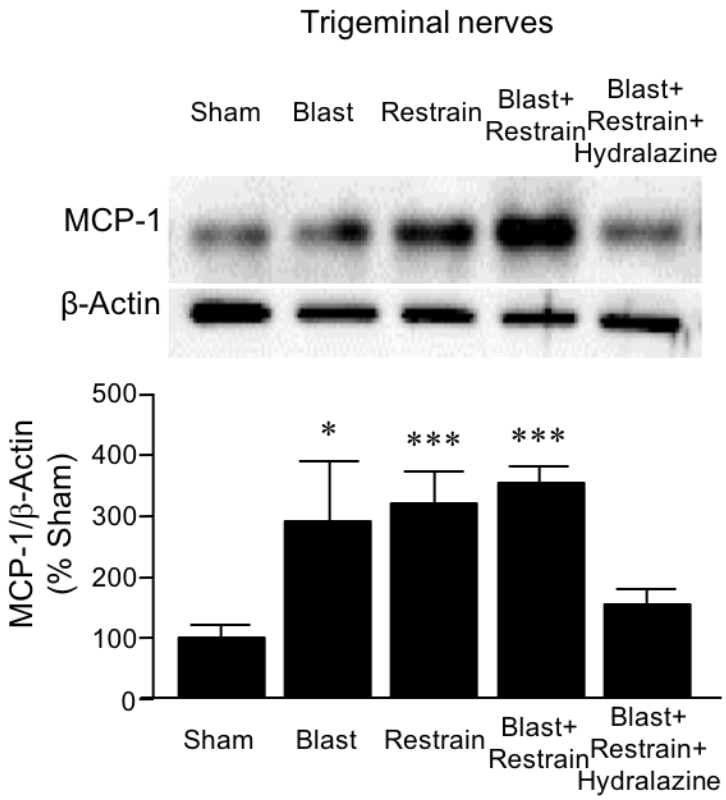

Figure 40 MCP1 in the trigeminal nerves of sham, blast, restrain, comorbidity and comorbidity rats treated with hydralazine. The trigeminal nerves of the comorbidity group showed an increase in protein concentrations compared to the blast and restrain groups. The treatment with hydralazine was able to reduce MCP1 levels that were significantly similar to the sham. N=8 per group from an average of four female and four male rats, statistically analyzed with Anova and post-hoc Fisher test, * $p<0.05$.

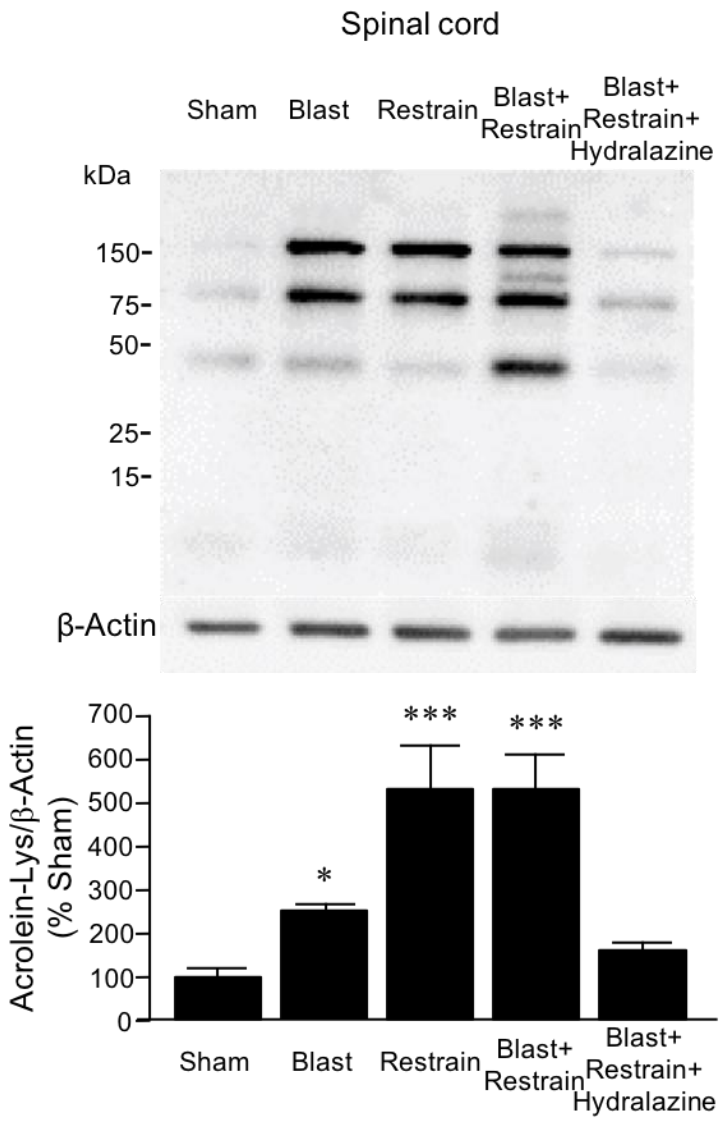

Figure 41 Acrolein in the spinal cords of sham, blast, restrain, comorbidity and comorbidity rats treated with hydralazine. The spinal cords of the comorbidity group showed an increase in protein concentrations compared to the blast and restrain groups. The treatment with hydralazine was able to reduce acrolein levels that were significantly similar to the sham. N=8 per group from an average of four female and four male rats, statistically analyzed with Anova and post-hoc Fisher test, * $p<0.05$.

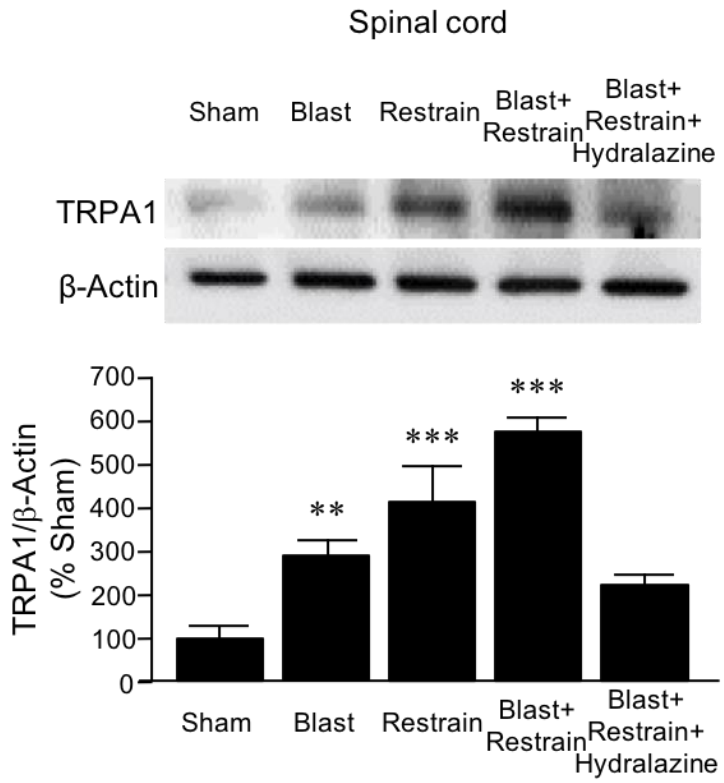

Figure 42 TRPA1 in the spinal cords of sham, blast, restrain, comorbidity and comorbidity rats treated with hydralazine. The spinal cords of the comorbidity group showed an increase in protein concentrations compared to the blast and restrain groups. The treatment with hydralazine was able to reduce TRPA1 levels that were significantly similar to the sham. N=8 per group from an average of four female and four male rats, statistically analyzed with Anova and post-hoc Fisher test, * $p<0.05$.

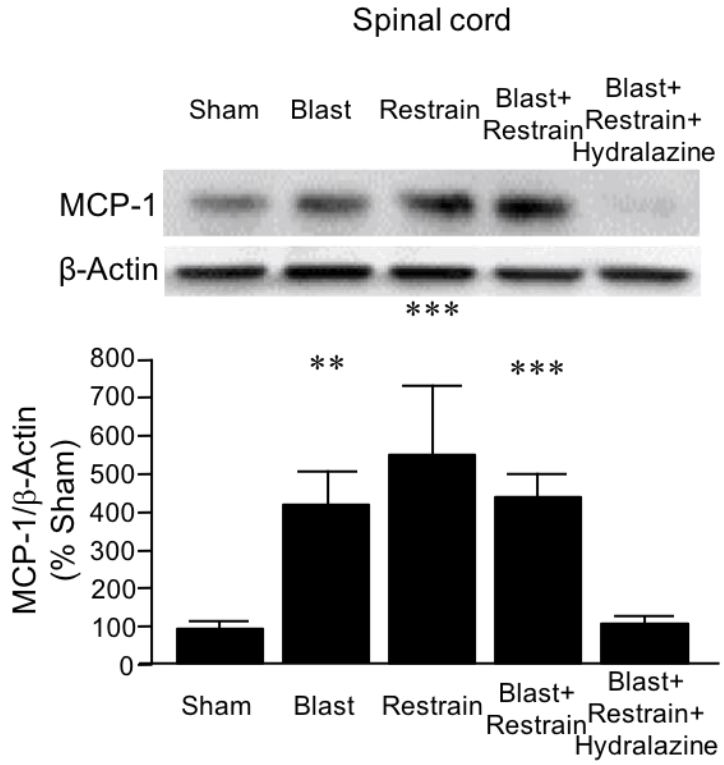

Figure 43 MCP1 in the spinal cords of sham, blast, restrain, comorbidity and comorbidity rats treated with hydralazine. The spinal cords of the comorbidity group showed an increase in protein concentrations compared to the blast group. The treatment with hydralazine was able to reduce MCP1 levels that were significantly similar to the sham. N=8 per group from an average of four female and four male rats, statistically analyzed with Anova and post-hoc Fisher test, * $p<0.05$.

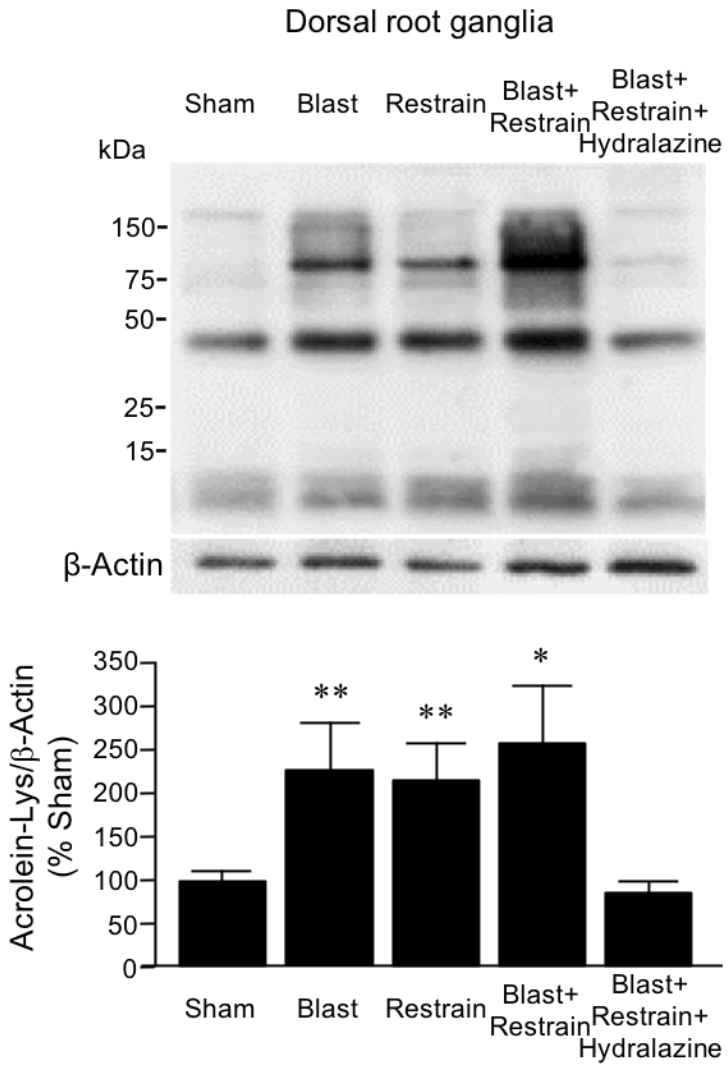

Figure 44 Acrolein in the dorsal root ganglia of sham, blast, restrain, comorbidity and comorbidity rats treated with hydralazine. The dorsal root ganglia of the comorbidity group showed an increase in protein concentrations compared to the blast and restrain groups. The treatment with hydralazine was able to reduce acrolein levels that were significantly similar to the sham. N=8 per group from an average of four female and four male rats, statistically analyzed with Anova and post-hoc Fisher test, * $p<0.05$.

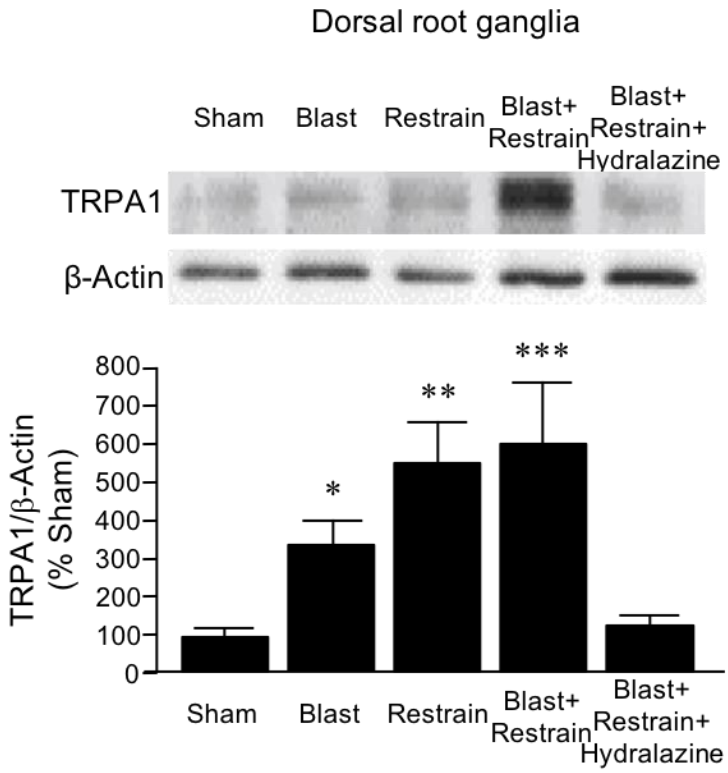

Figure 45 TRPA1 in the dorsal root ganglia of sham, blast, restrain, comorbidity and comorbidity rats treated with hydralazine. The dorsal root ganglia of the comorbidity group showed an increase in protein concentrations compared to the blast and restrain groups. The treatment with hydralazine was able to reduce TRPA1 levels that were significantly similar to the sham. N=8 per group from an average of four female and four male rats, statistically analyzed with Anova and post-hoc Fisher test, * $p<0.05$.

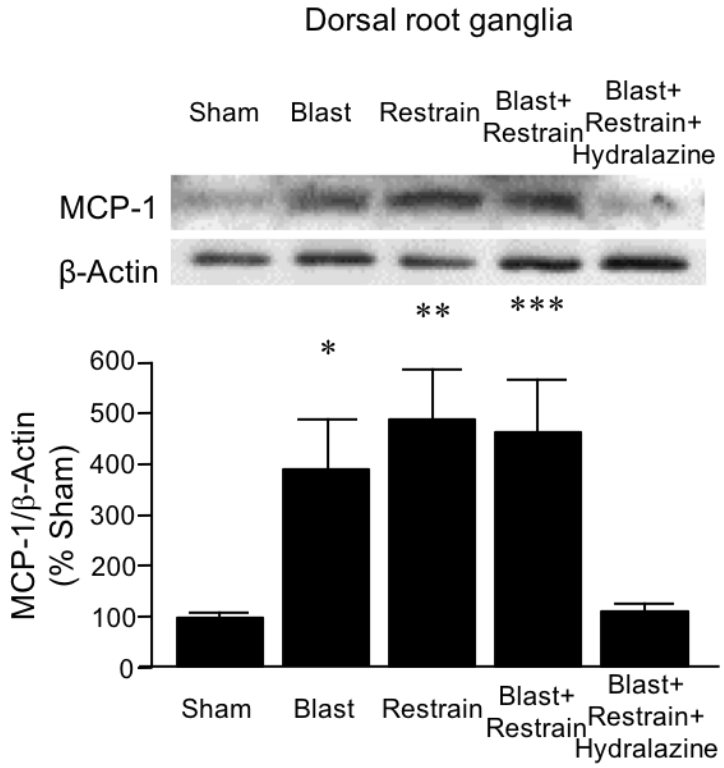

Figure 46 MCP1 in the dorsal root ganglia of sham, blast, restrain, comorbidity and comorbidity rats treated with hydralazine. The dorsal root ganglia of the comorbidity group showed an increase in protein concentrations compared to the blast and restrain groups. The treatment with hydralazine was able to reduce MCP1 levels that were significantly similar to the sham. N=8 per group from an average of four female and four male rats, statistically analyzed with Anova and post-hoc Fisher test, * $p<0.05$.

CHAPTER 7. NEUROPATHIC PAIN-LIKE BEHAVIORS AND ACROLEIN, TRPA1 AND MCP1 EXPRESSION DIFFERENCES BETWEEN FEMALE AND MALE RATS

7.1 Allodynia behavioral experiments

7.1.1 Rationale

Due to the relevance of many disease and condition differences observed in humans and animals between different genders, we decided to analyze the allodynia responses of female and male rats of each group, including sham, blast, restrain, blast with combination of restrain, and blast with combination of restrain with the addition of the hydralazine. In other words, this chapter includes all the groups included on the Chapter 6, which are the main disease states included with the hydralazine treatment on the group with the comorbidity of blast and stress. The purpose of this study was to better understand whether female or male rats were more sensitive to allodynia measurements in healthy conditions, and to understand whether a specific disease model would affect more one gender or the other.

7.1.2 Brief methods

Female and male age-matched rats were used for this study. Male rats of approximately 350 g of body weight and female rats of approximately 320 g were included in the study. The same time points and same experimental procedures as described on the section 6.1.2 were used for this study. The sham, blast, comorbidity and hydralazine treated comorbidity rats were exposed to blast under anesthesia of a mixture of ketamine and xylazine as previously described.

Sham rats were only exposed to the blast noise at the same distance from the overpressure explosion as the blast rats but at a different angle to avoid receiving the blast pressure wave. The blast rats were placed under anesthesia and exposed to the blast pressure wave as previously described. The restrain rats were not placed under anesthesia and were just placed on a universal restrainer for 2 hours every day throughout the length of the study. The comorbidity group was restrained for 1 week before blast, with no restrain on the day of blast to allow for recovery of anesthesia and restrained for other 2 weeks thereafter. The hydralazine treated comorbidity group received a hydralazine treatment of 5 mg/kg daily as previously described. N=4 per group with a total N=40 rats considering all 5 groups with female and male rats.

Allodynia was measured with von Frey filaments as previously described before the experiments were conducted for baseline, and 1 week thereafter for the sham and restrain groups. The blast group was measured before the experiments were conducted for baseline, 1 day, 1 week and 2 weeks after blast. And the comorbidity and hydralazine treated comorbidity groups were measured for baseline, 1 week after restrain, 1 day, 1 week and 2 weeks after blast.

7.1.3 Results and Discussion

The allodynia measurements showed no significant differences either in hind limb (A) or periorbital (B) areas between females and males in any of the analyzed groups including sham in Figure 47, blast in Figure 48, restrain in Figure 49, comorbidity in Figure 50 and comorbidity rats treated with hydralazine in Figure 51.

These results indicate a general similarity in response to pain between females and males, although a small difference was observed, with female rats being slightly more sensitive to allodynia measurements in all groups but not significant. This might be due to the small exploratory N=4 per group used for this set of experiments. Perhaps if the N increases per group, the small differences observed might become significant and female rats might be statistically more sensitive to allodynia measurements.

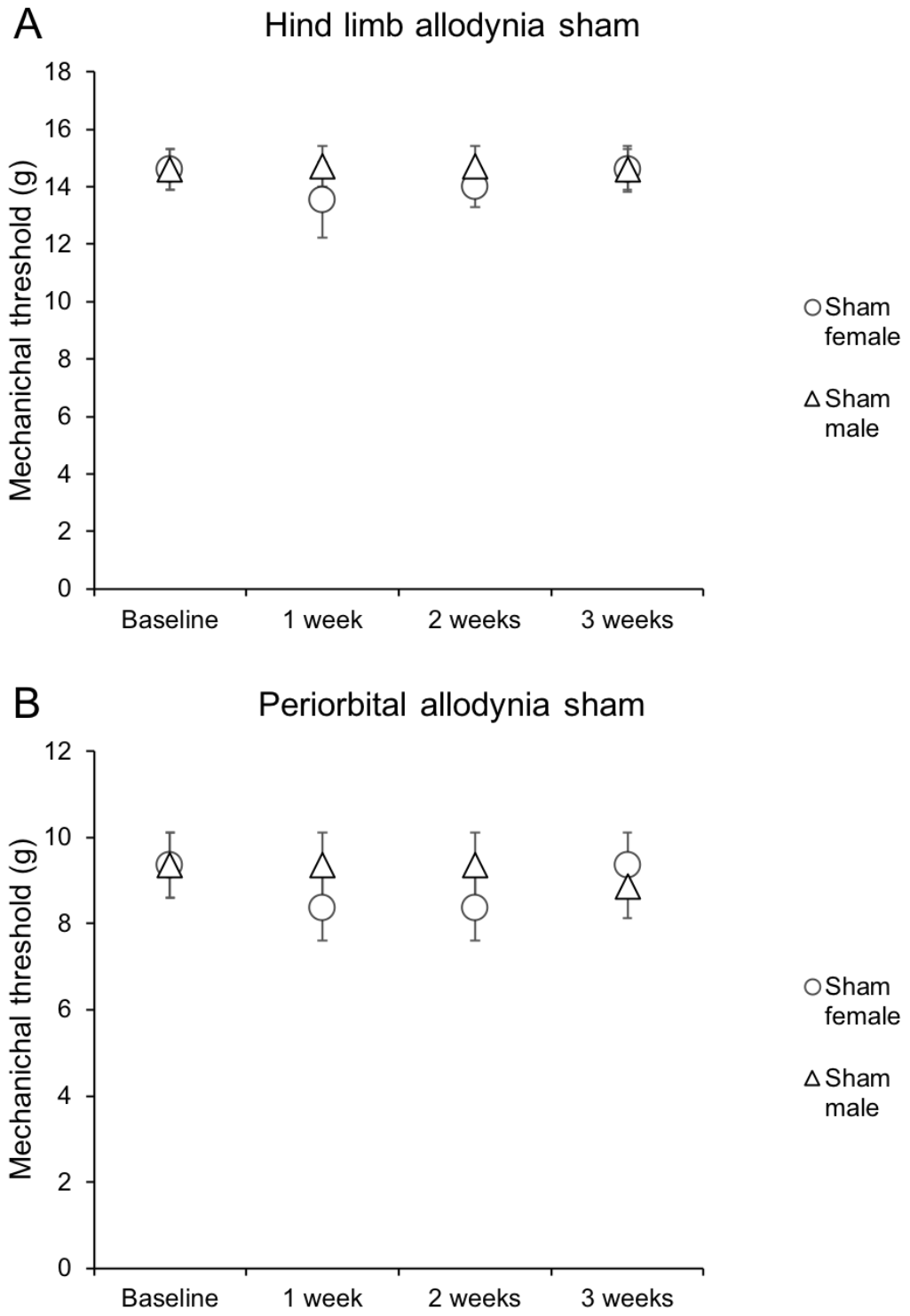

Figure 47 Hind limb (A) and periorbital allodynia (B) in female and male sham rats. Both genders show similar trends with no statistically significant differences. N=4 per group statistically analyzed with Anova and post-hoc Fisher test, *$p<0.05$.

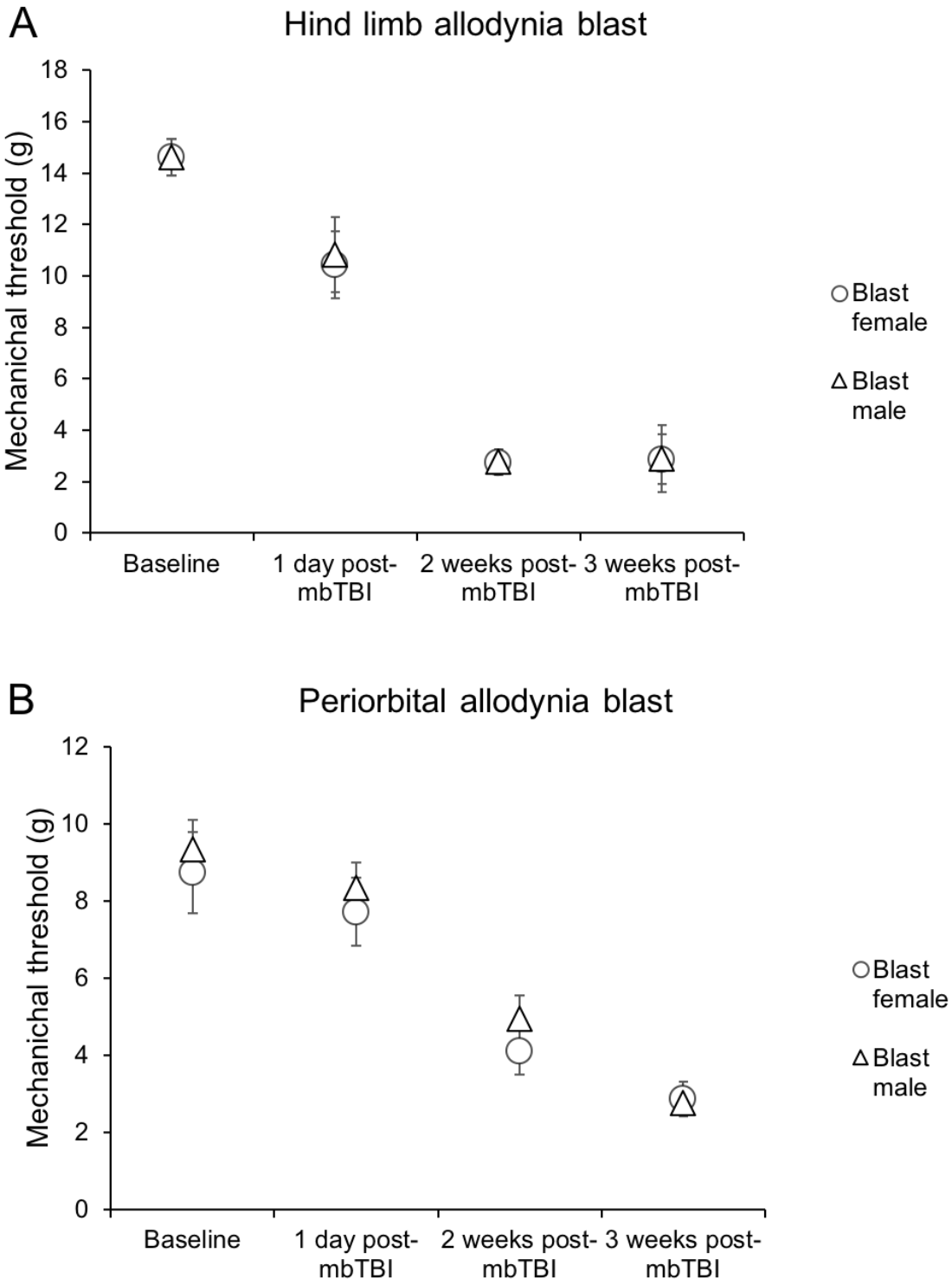

Figure 48 Hind limb (A) and periorbital allodynia (B) in female and male mbTBI rats. Both genders show similar trends with no statistically significant differences. N=4 per group statistically analyzed with Anova and post-hoc Fisher test, *$p<0.05$.

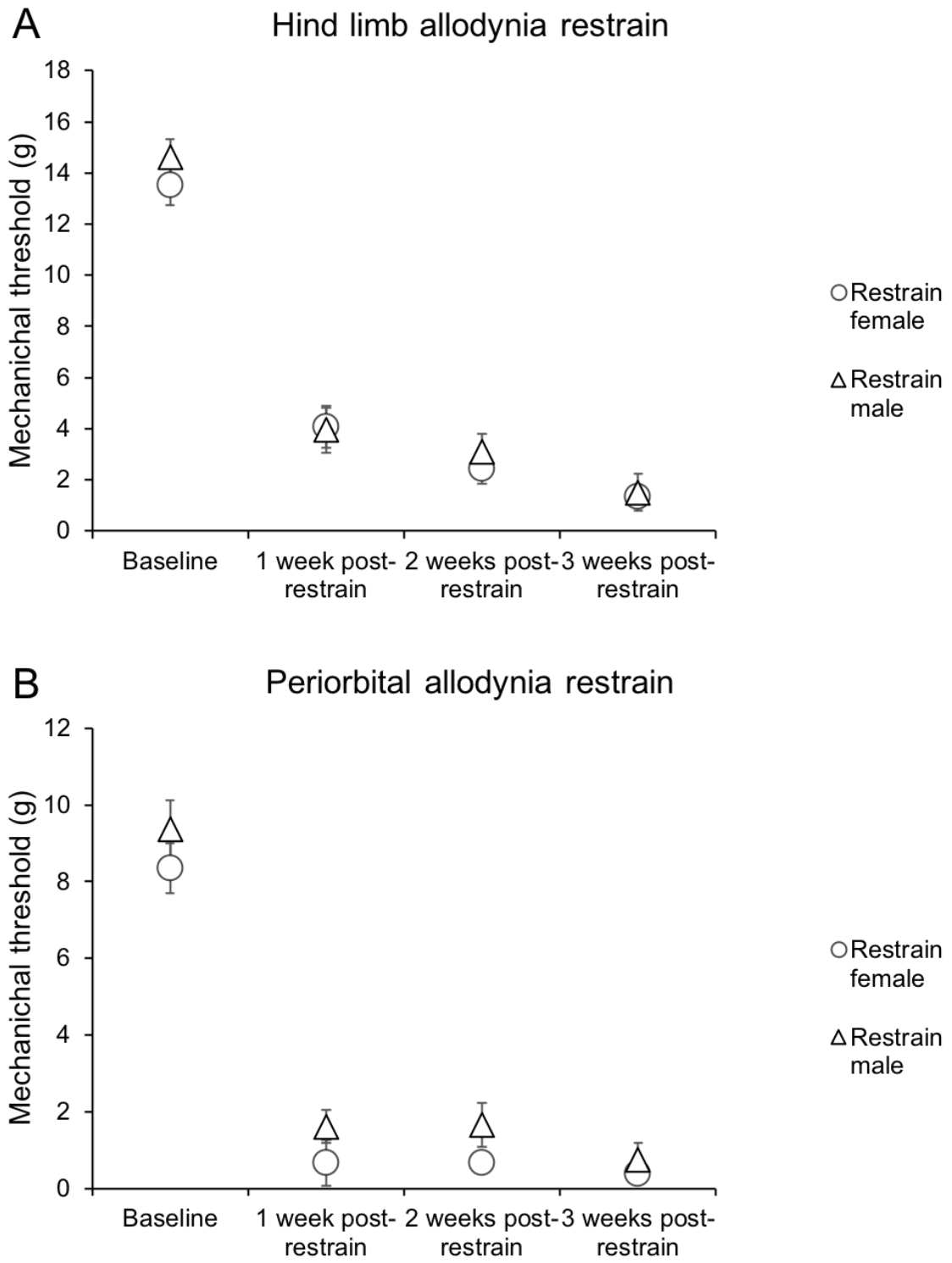

Figure 49 Hind limb (A) and periorbital allodynia (B) in female and male chronic stress rats. Both genders show similar trends with no statistically significant differences. N=4 per group statistically analyzed with Anova and post-hoc Fisher test, *$p<0.05$.

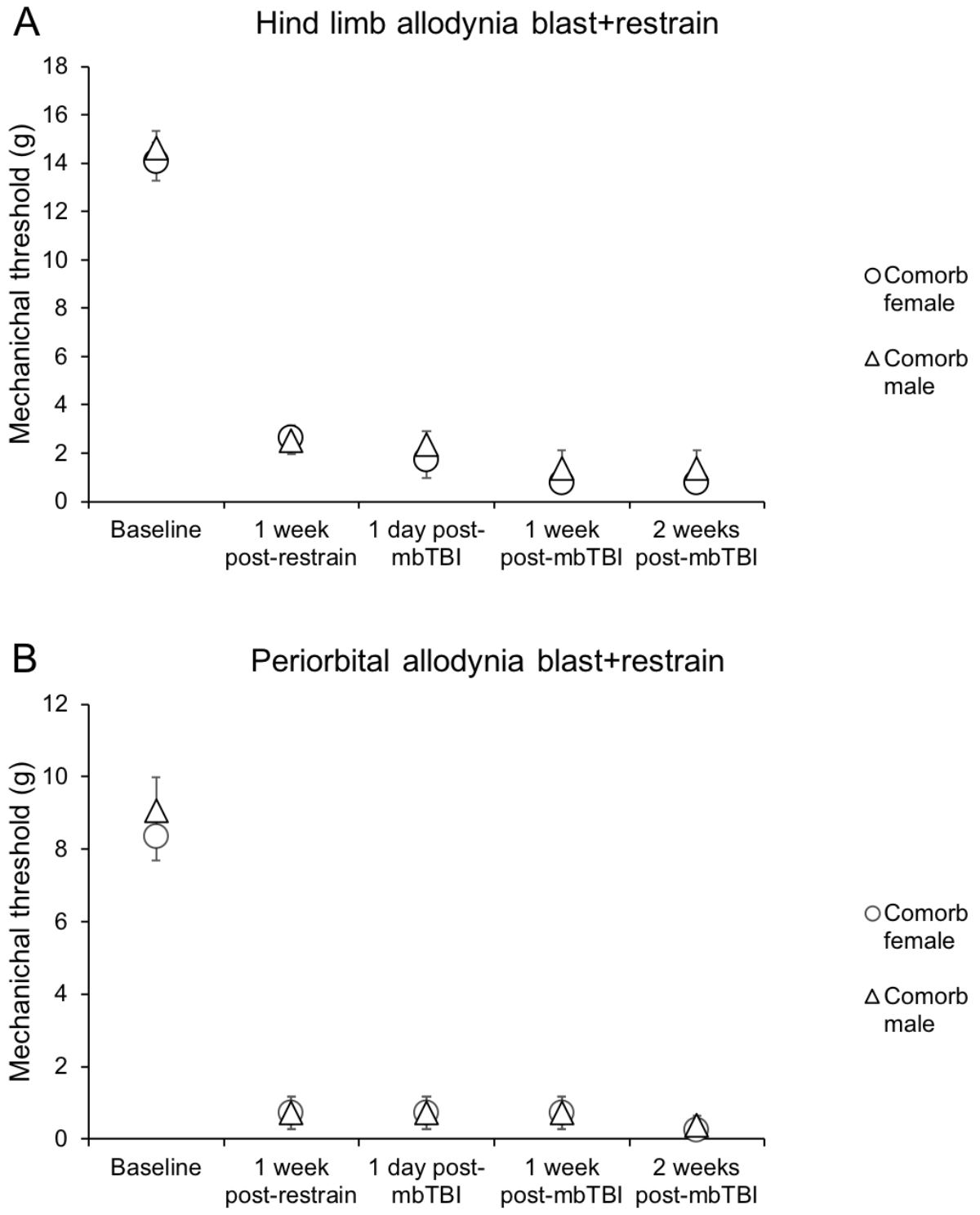

Figure 50 Hind limb (A) and periorbital allodynia (B) in female and male comorbidity rats. Both genders show similar trends with no statistically significant differences. N=4 per group statistically analyzed with Anova and post-hoc Fisher test, *$p<0.05$.

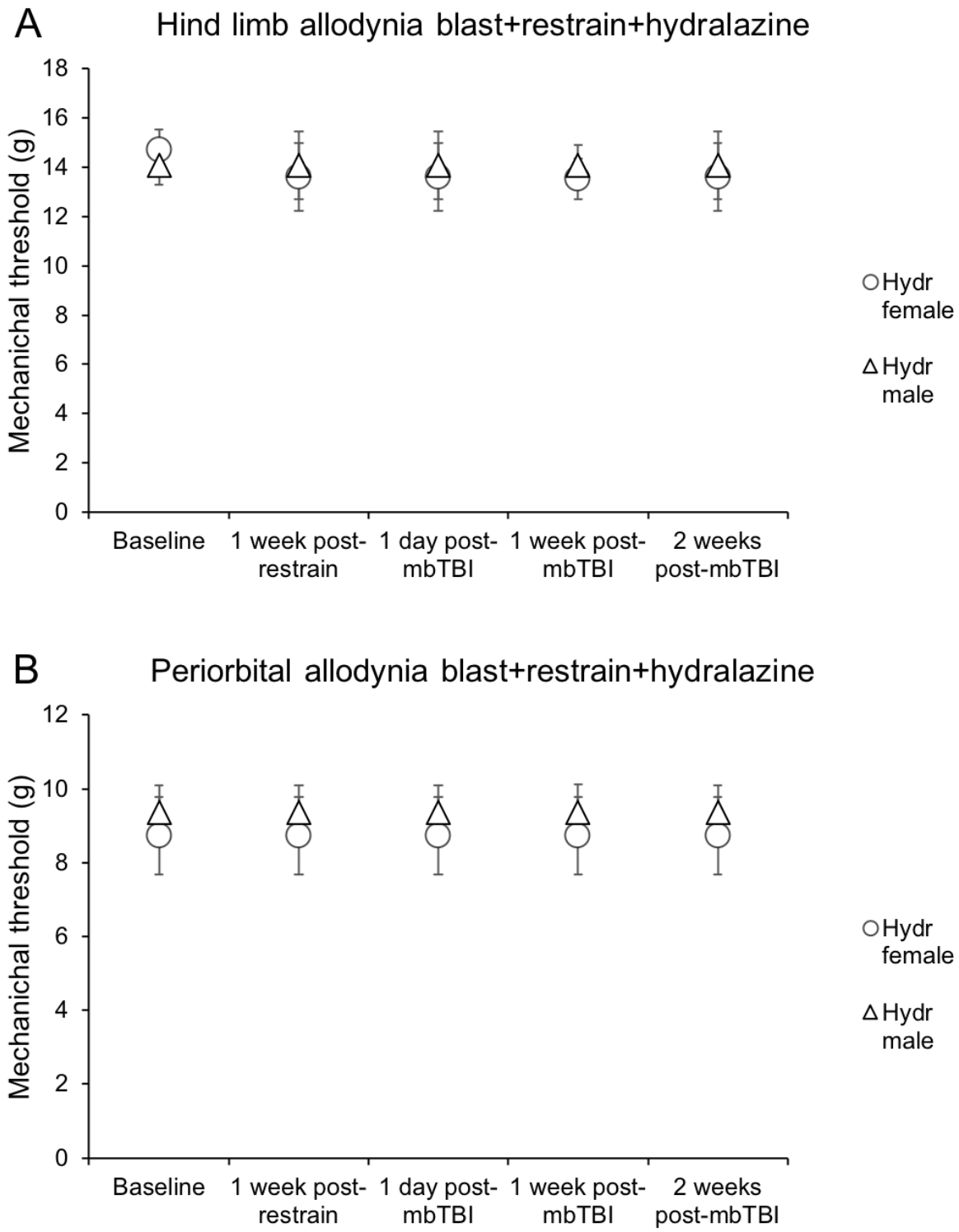

Figure 51 Hind limb (A) and periorbital allodynia (B) in female and male comorbidity rats treated with hydralazine. Both genders show similar trends with no statistically significant differences. N=4 per group statistically analyzed with Anova and post-hoc Fisher test, *$p<0.05$.

7.2 Biochemical experiments

7.2.2 Rationale

The purpose of this study was to understand whether female or male rats expressed different acrolein, TRPA1 and MCP1 concentrations in the brain, trigeminal nerves, spinal cords and DRG and to analyze these results and compare them with the allodynia measurements to better understand whether allodynia and protein concentrations match between genders and between disease models.

7.2.3 Brief methods

Female and male age-matched rats were used for this study as described on section 7.1.2. Male rats of approximately 350 g of body weight and female rats of approximately 320 g were included in the study. The same time points and same experimental procedures as described on the section 6.2.2 were used for this study, with the study lasting approximately 3 weeks for all groups, and with the tissue including brains, trigeminal nerves, spinal cords and DRG collected after the 3 weeks of the study. The tissue was collected as previously described.

For this specific section of the chapter, the data is presented as the average of the group with SEM without including representative western blot images. This is since all representative images are shown on Chapter 6 and blocked by gender to avoid variation between gender and groups at the same time, so on Chapter 6 only the disease model causes variation on the images. This decision was made to be able to show differences between disease models on Chapter 6, but also be able to quantify these differences between genders and show them on this Chapter in the spirit to reduce animals use as accorded with the Animal Care and Use Committee.

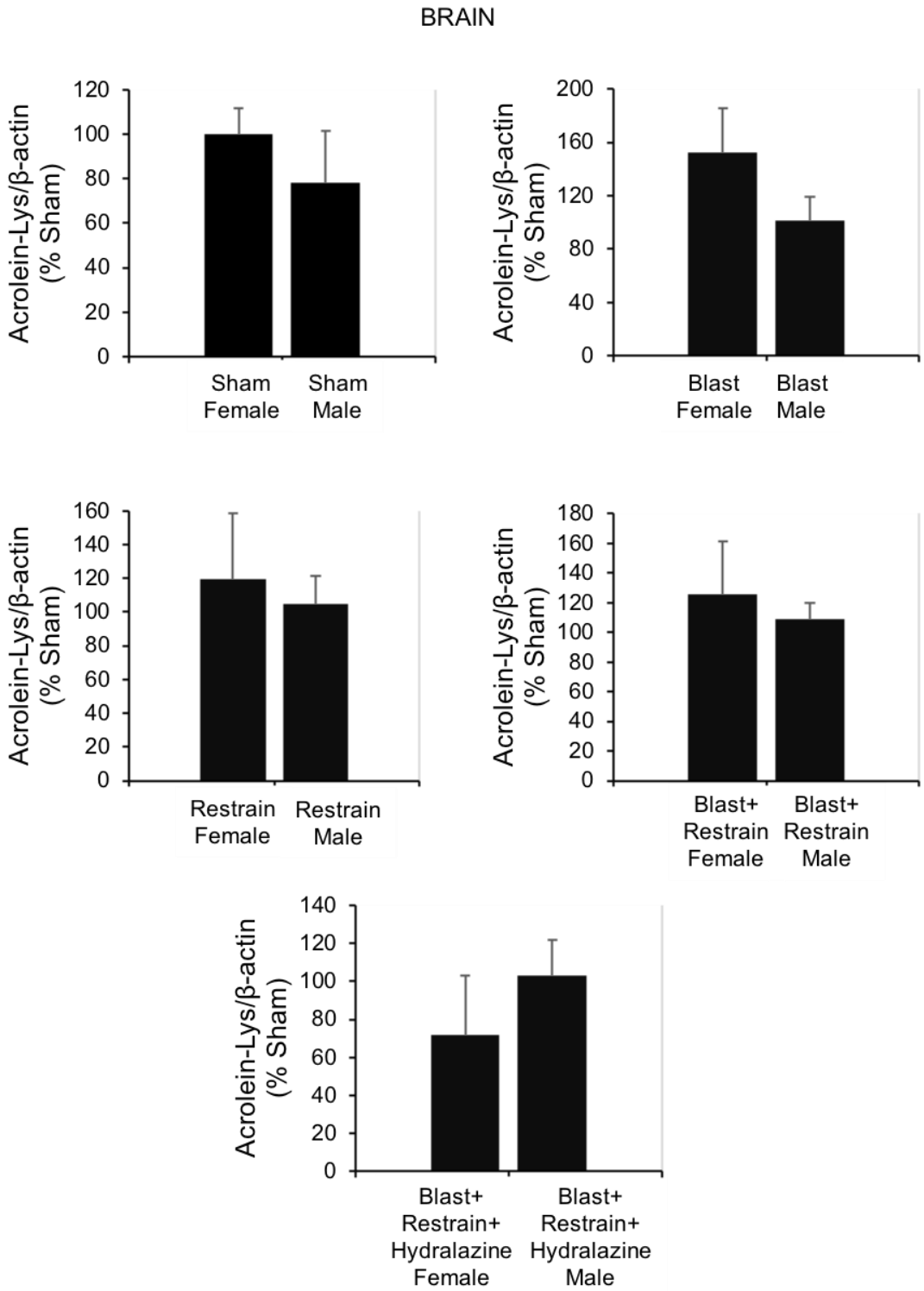

Figure 52 Acrolein concentrations in the brain of female and male rats. N=4 per group statistically analyzed with Anova and post-hoc Fisher test, *$p<0.05$.

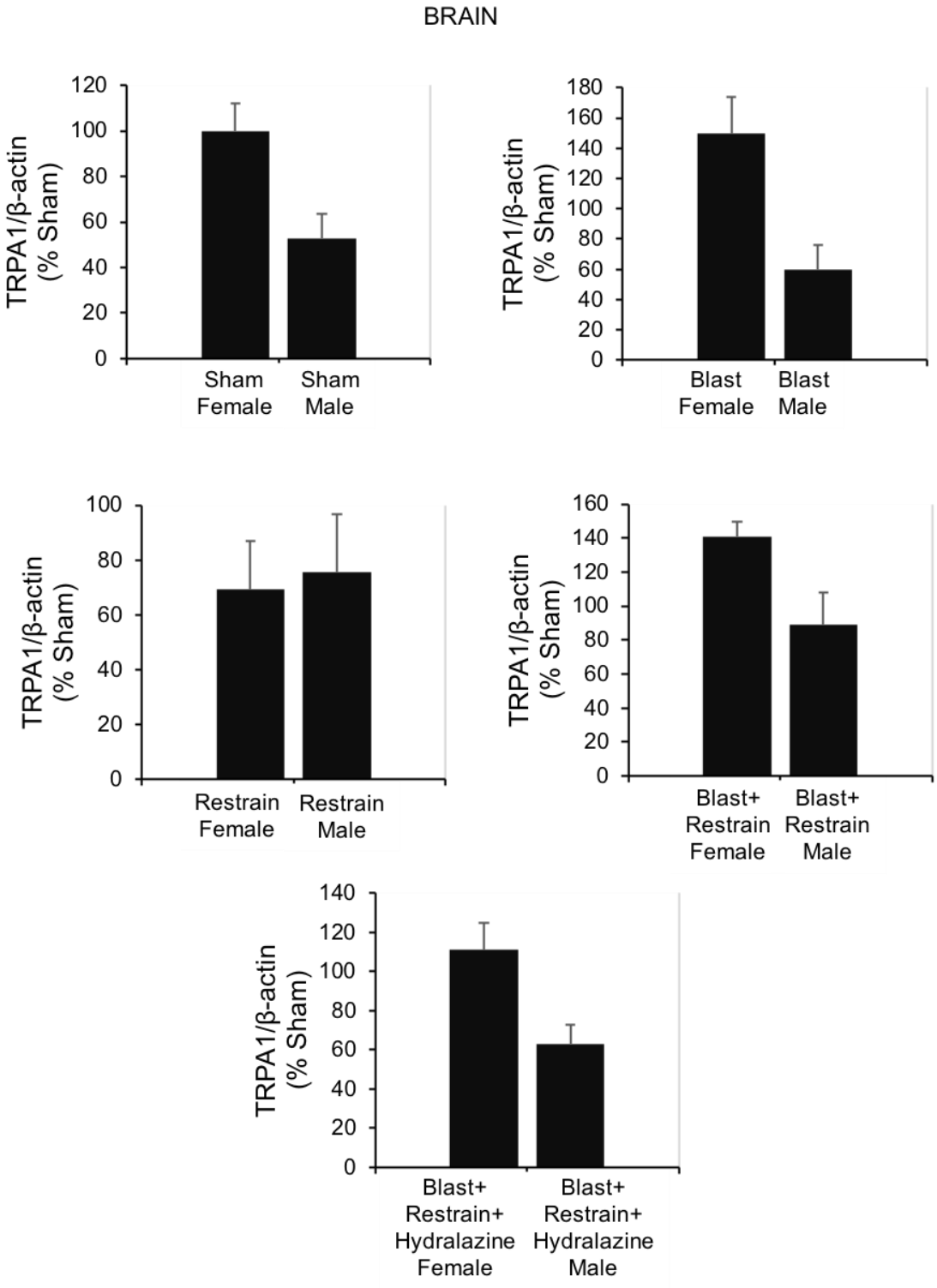

Figure 53 TRPA1 concentrations in the brain of female and male rats. N=4 per group statistically analyzed with Anova and post-hoc Fisher test, *$p<0.05$.

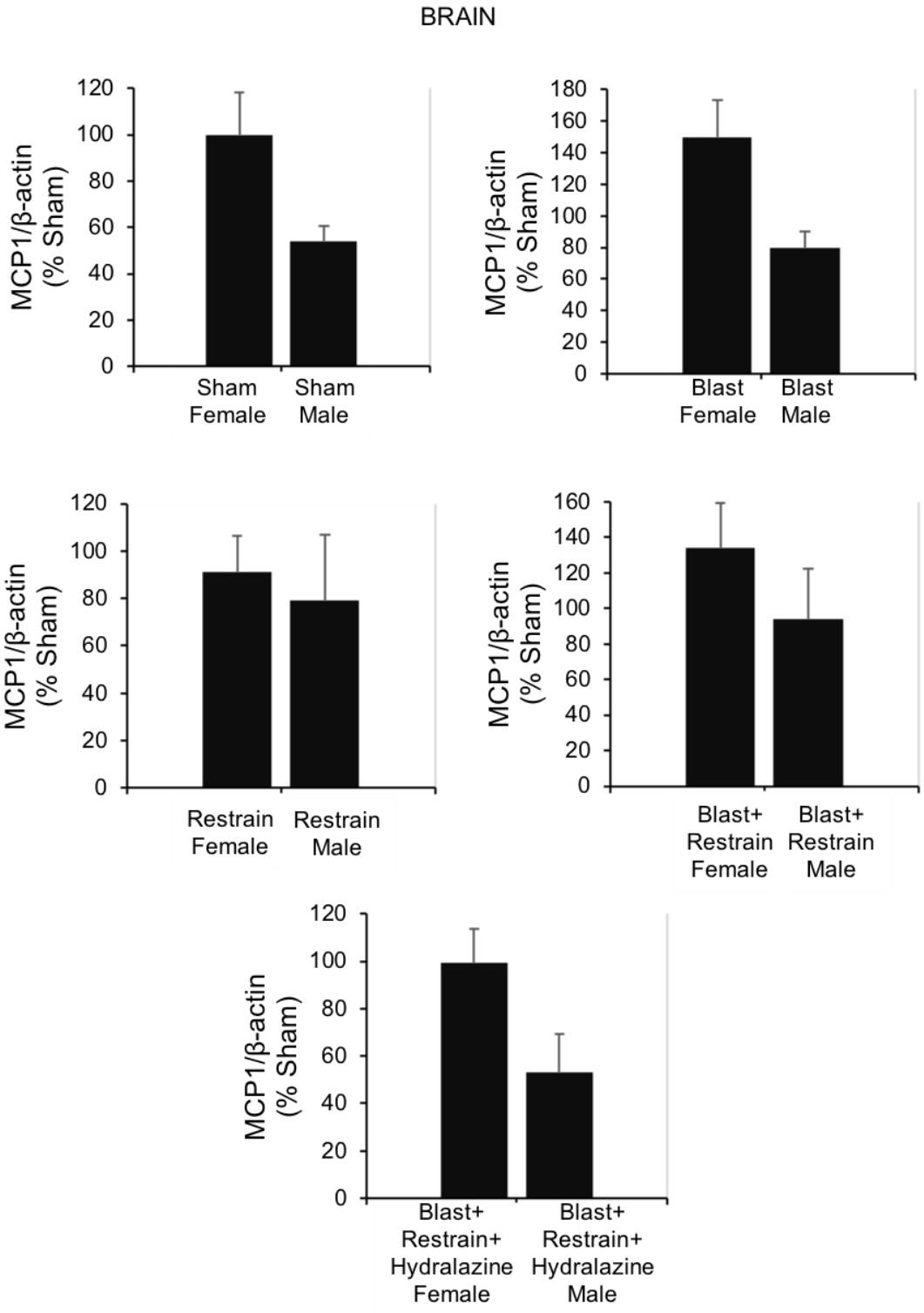

Figure 54 MCP1 concentrations in the brain of female and male rats. N=4 per group statistically analyzed with Anova and post-hoc Fisher test, *$p<0.05$.

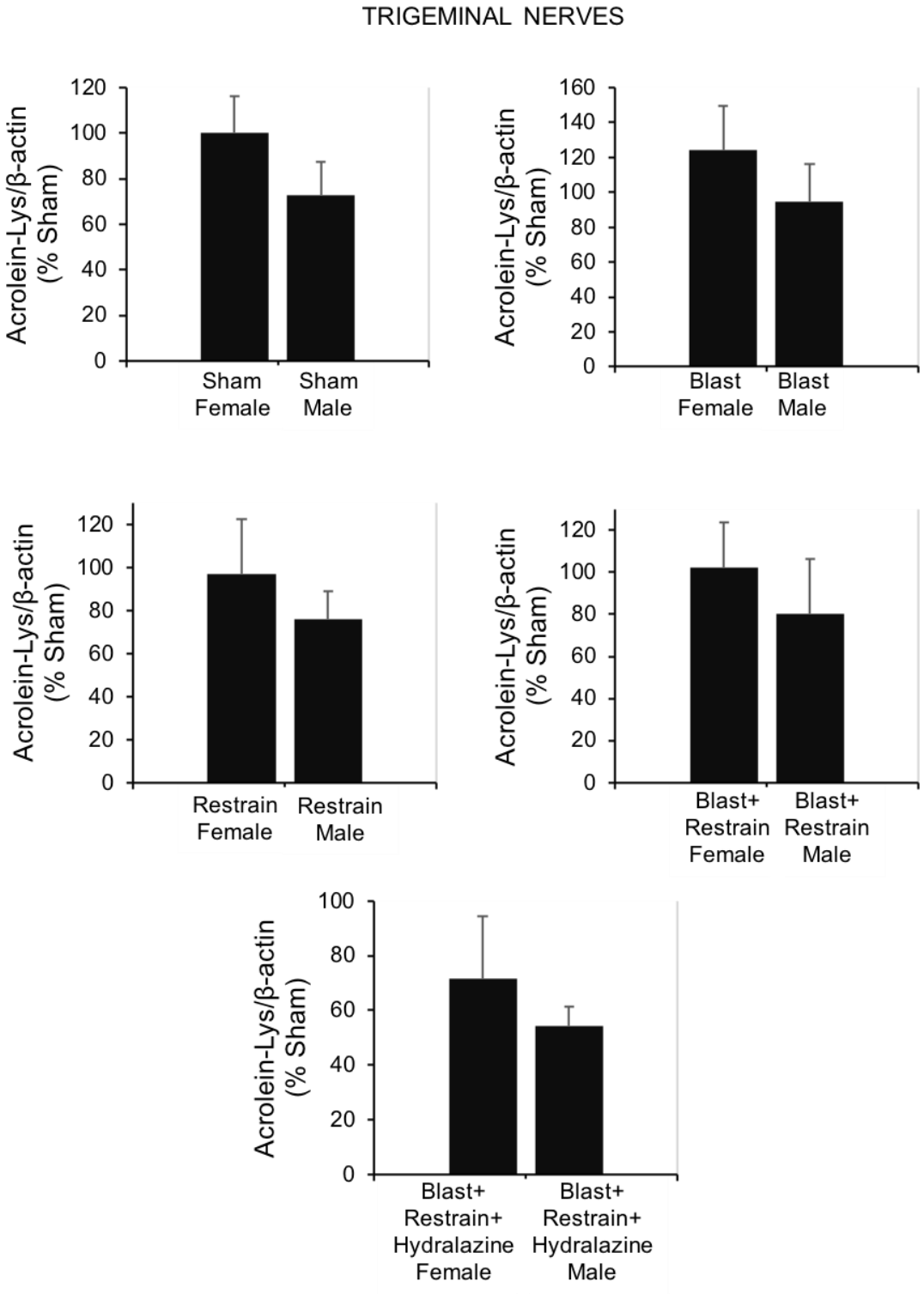

Figure 55 Acrolein concentrations in the trigeminal nerves of female and male rats. N=4 per group statistically analyzed with Anova and post-hoc Fisher test, *p<0.05.

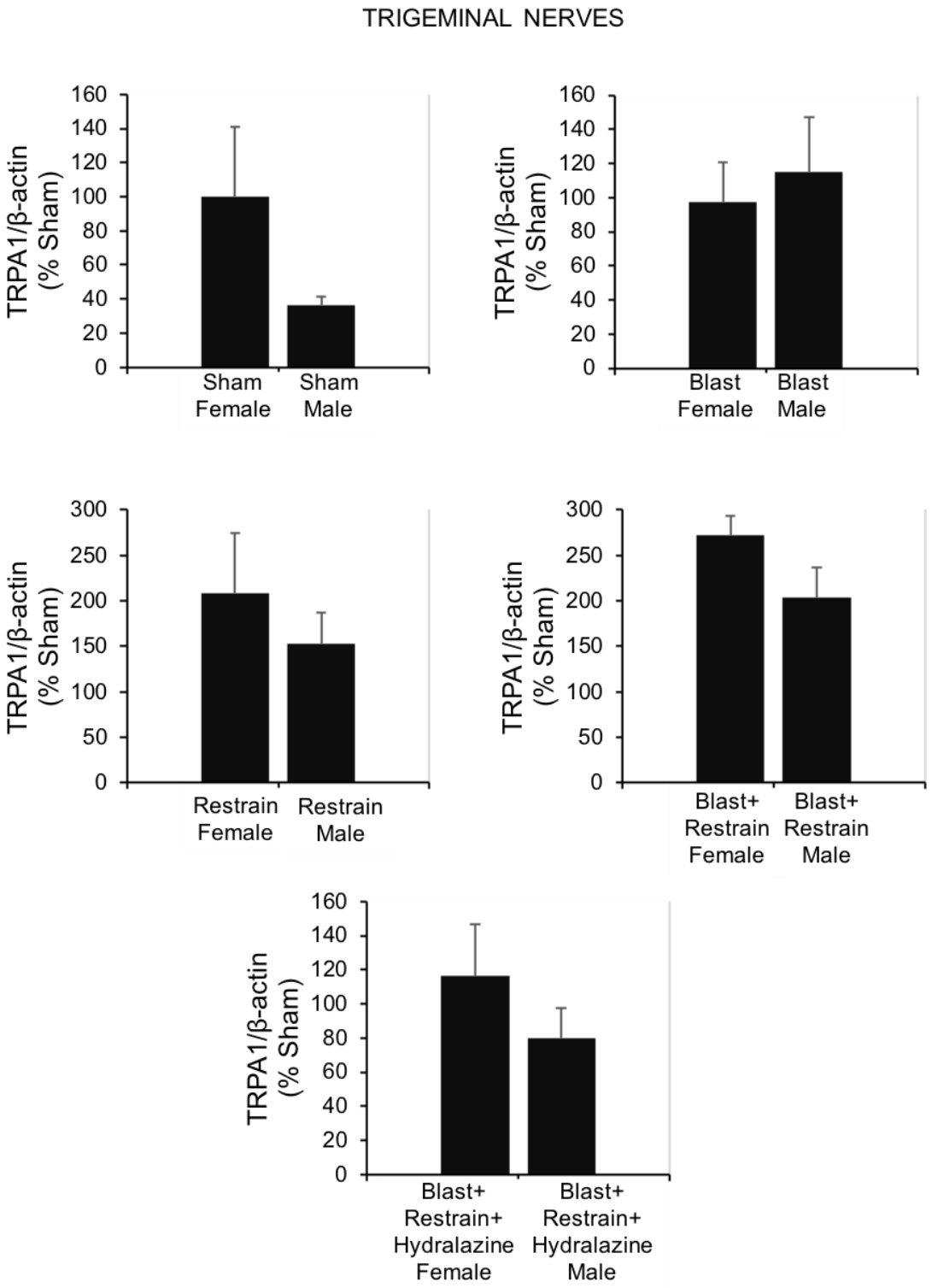

Figure 56 TRPA1 concentrations in the trigeminal nerves of female and male rats. N=4 per group statistically analyzed with Anova and post-hoc Fisher test, *$p<0.05$.

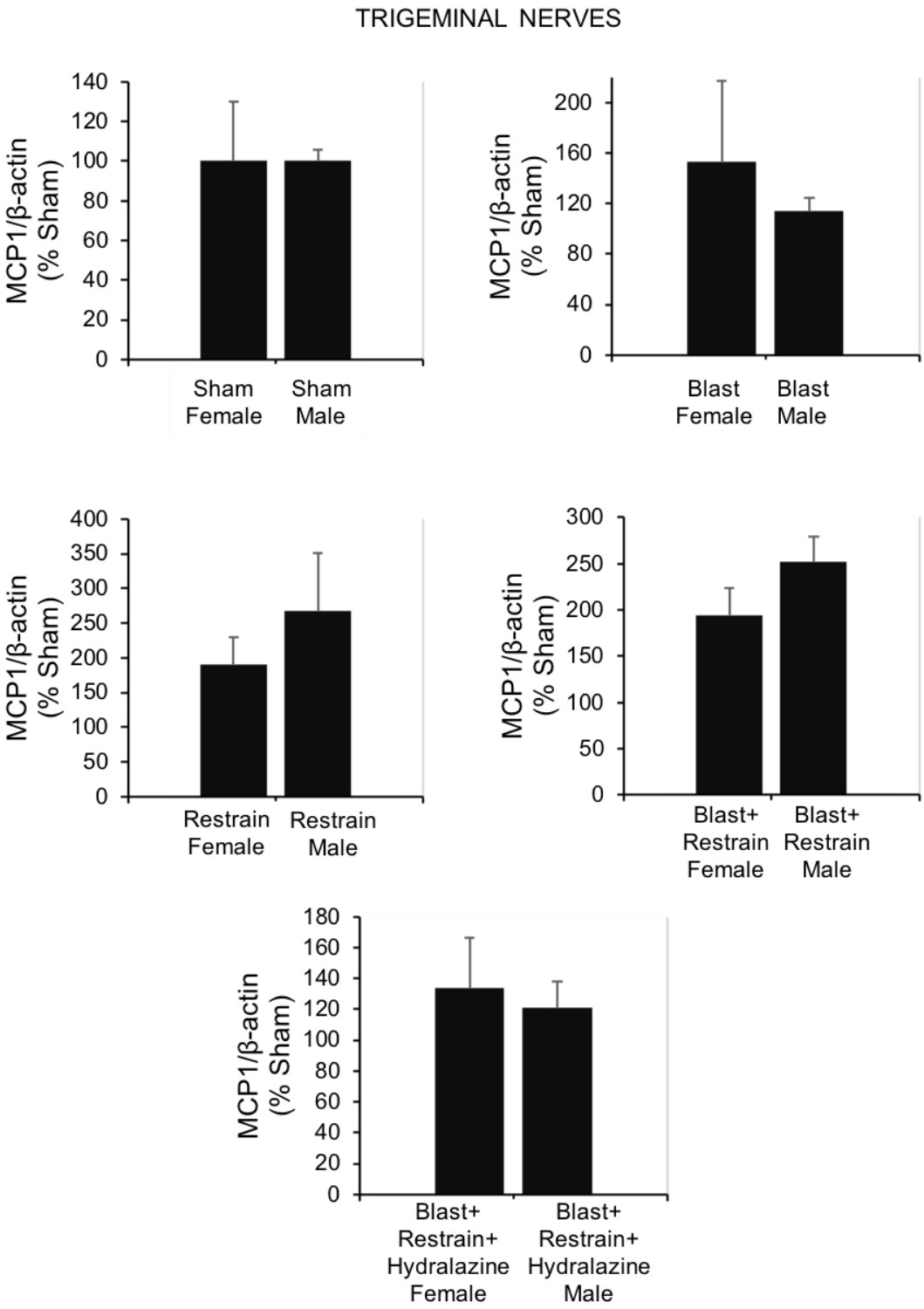

Figure 57 MCP1 concentrations in the trigeminal nerves of female and male rats. N=4 per group statistically analyzed with Anova and post-hoc Fisher test, *$p<0.05$.

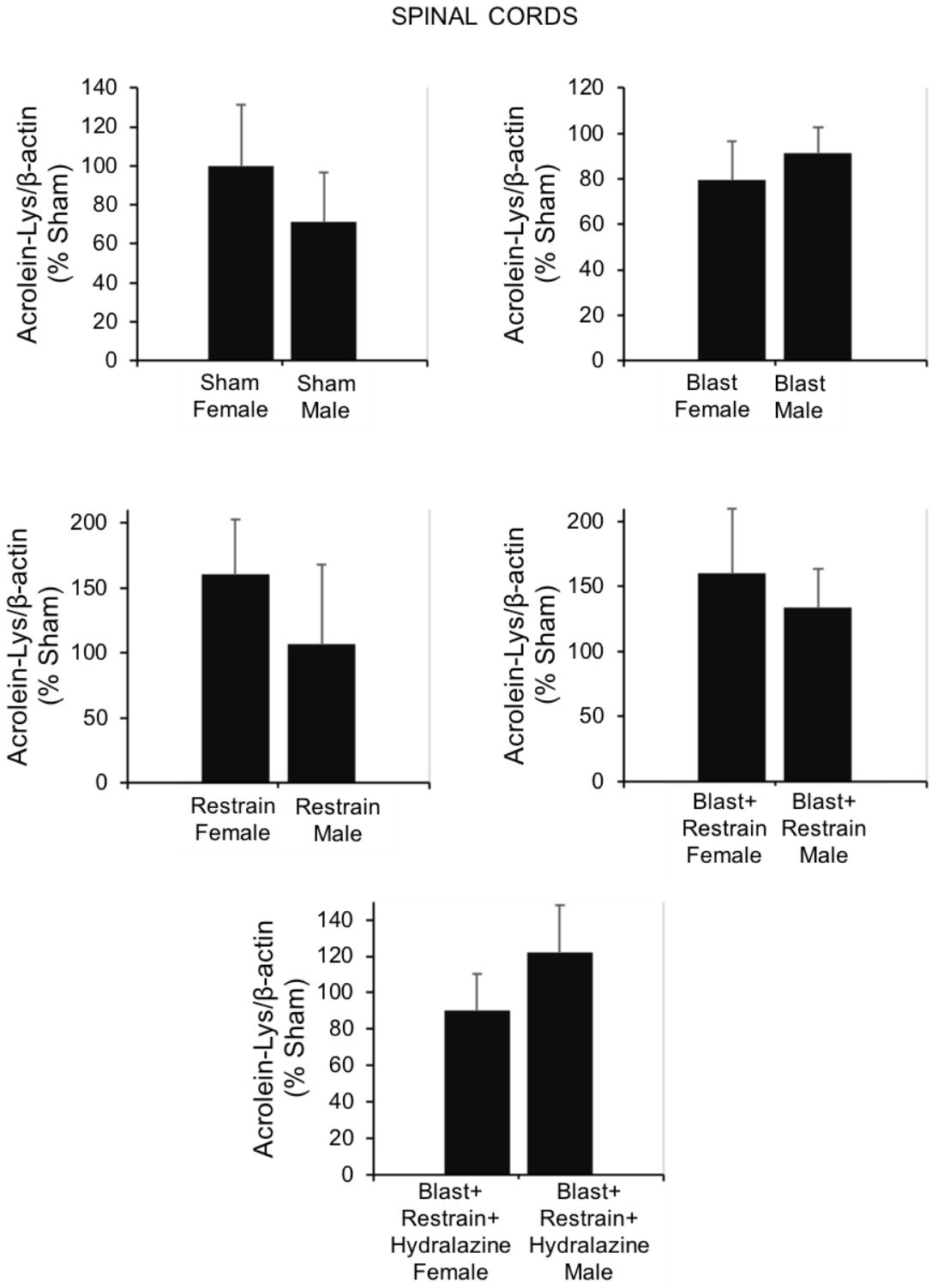

Figure 58 Acrolein concentrations in the spinal cords of female and male rats. N=4 per group statistically analyzed with Anova and post-hoc Fisher test, *$p<0.05$.

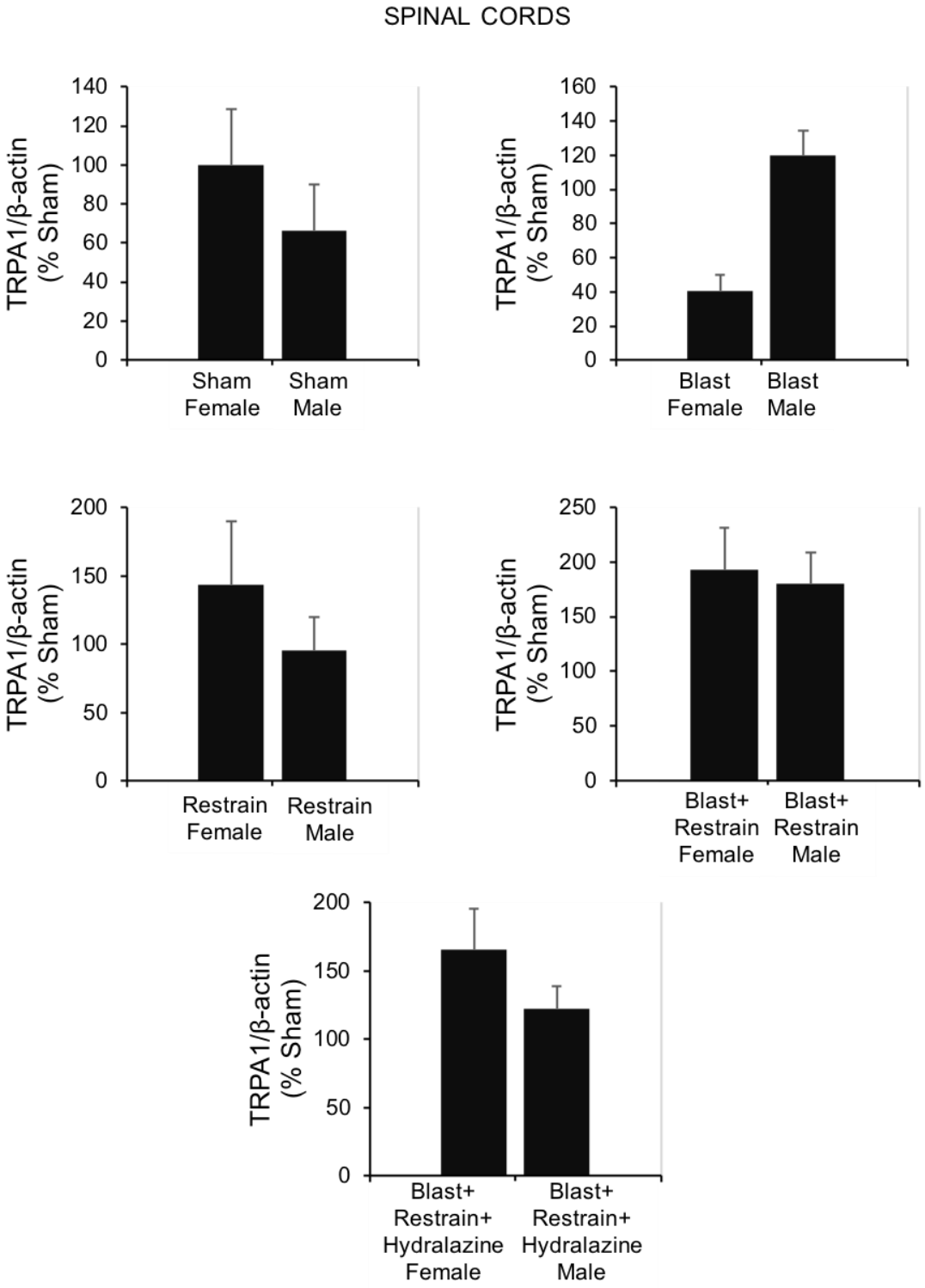

Figure 59 TRPA1 concentrations in the spinal cords of female and male rats. N=4 per group statistically analyzed with Anova and post-hoc Fisher test, *$p<0.05$.

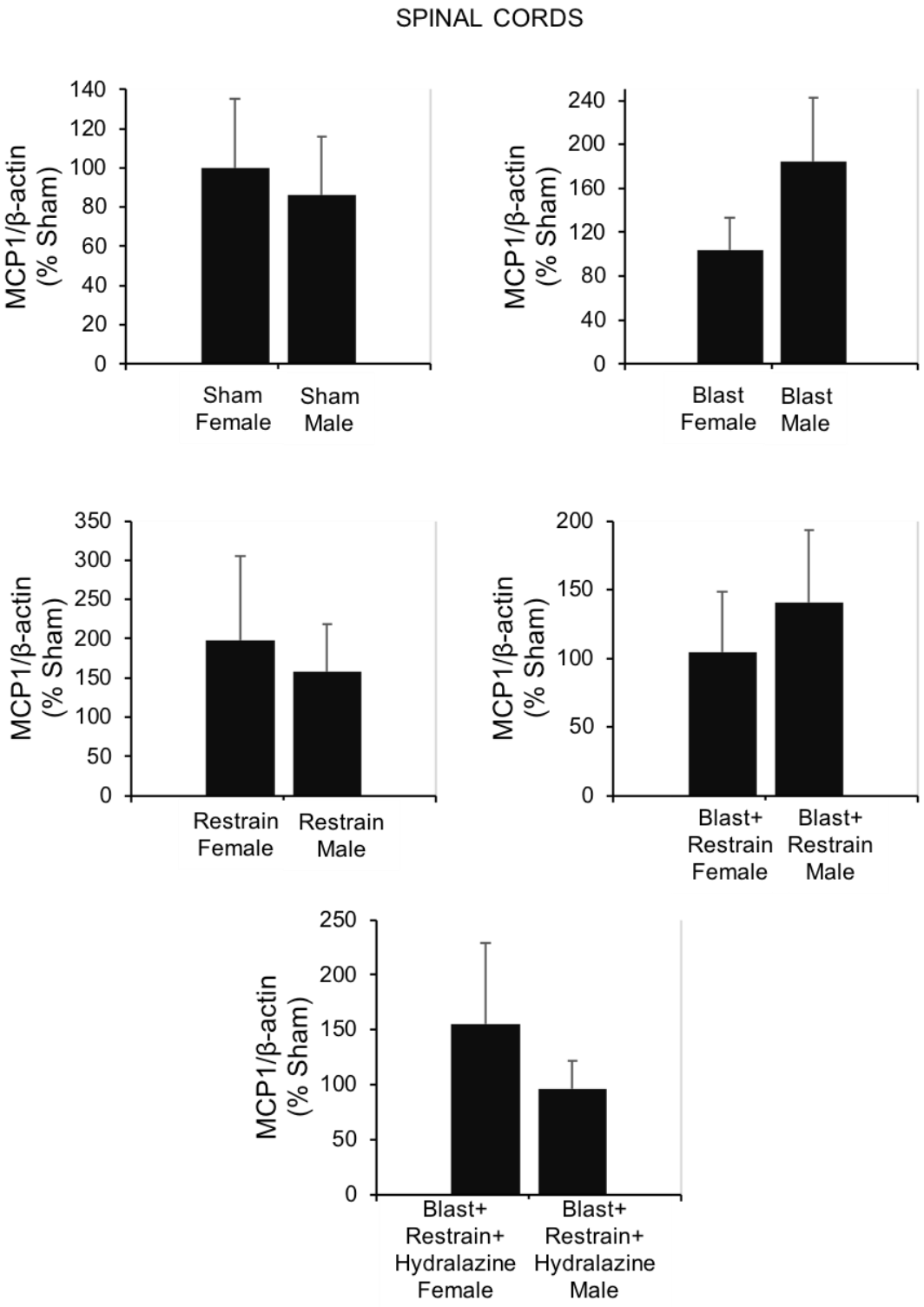

Figure 60 MCP1 concentrations in the spinal cords of female and male rats. N=4 per group statistically analyzed with Anova and post-hoc Fisher test, *$p<0.05$.

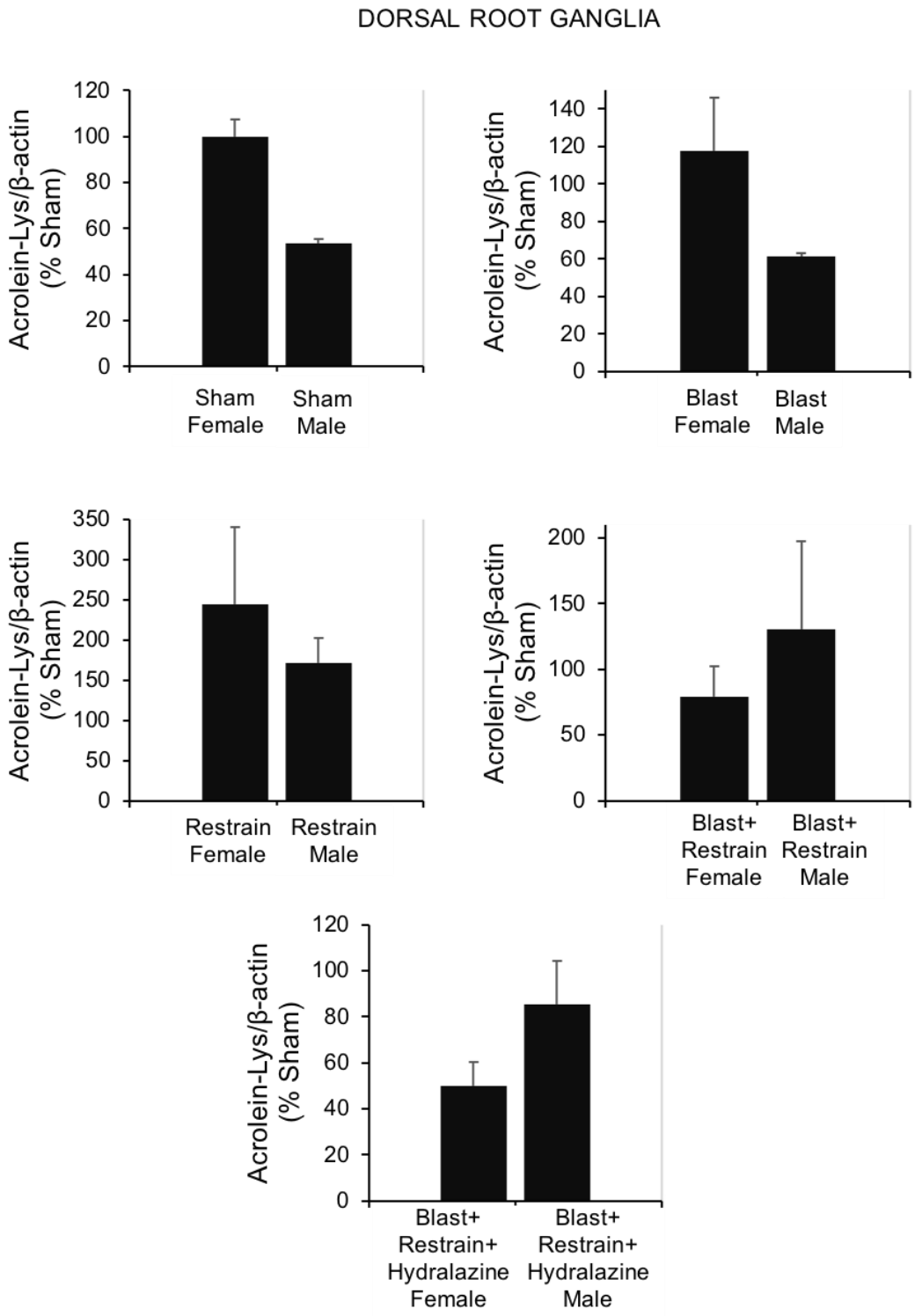

Figure 61 Acrolein concentrations in the DRG of female and male rats. N=4 per group statistically analyzed with Anova and post-hoc Fisher test, *$p<0.05$.

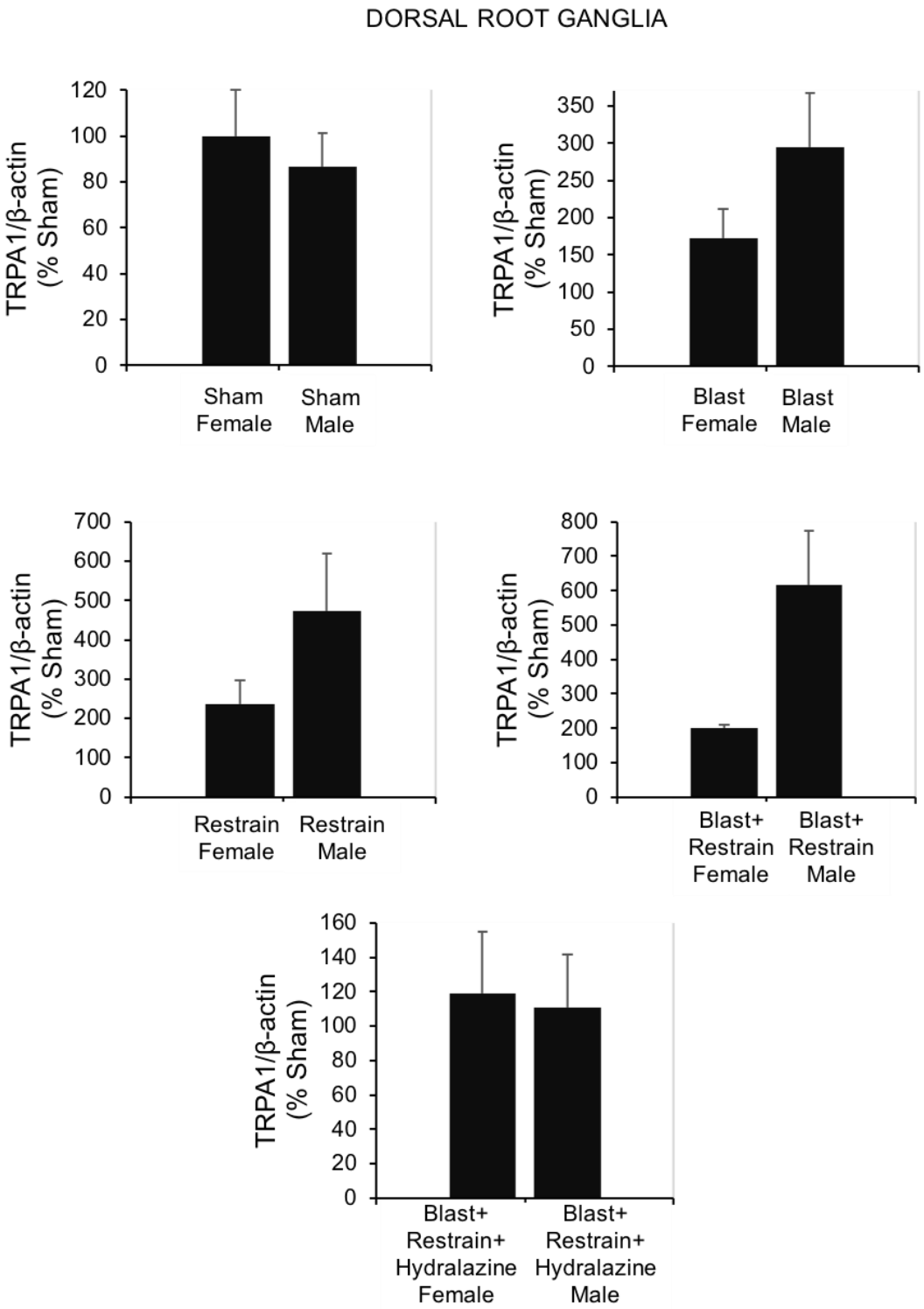

Figure 62 TRPA1 concentrations in the DRG of female and male rats. N=4 per group statistically analyzed with Anova and post-hoc Fisher test, *$p<0.05$.

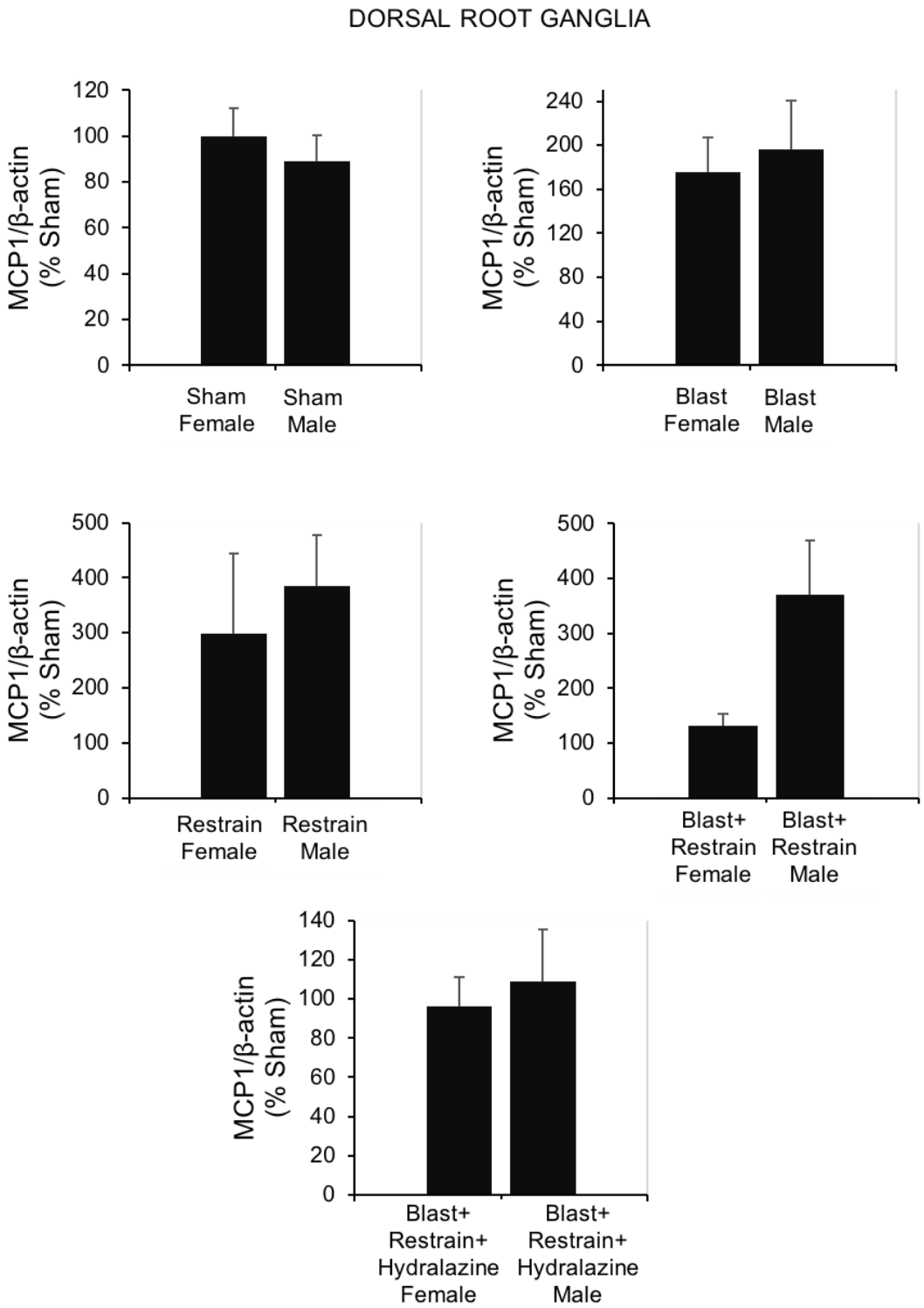

Figure 63 MCP1 concentrations in the DRG of female and male rats. N=4 per group statistically analyzed with Anova and post-hoc Fisher test, *$p<0.05$.

7.2.1 Results and Discussion

The acrolein, TRPA1 and MCP1 protein concentration analyses between females and males in the brains, trigeminal nerves, spinal cords and DRG within disease groups showed no statistically significant differences. Brain protein concentrations are shown in Figures 52-54; trigeminal nerves in Figures 55-57; spinal cords in Figures 58-60; and DRG in Figures 61-63. These results coincide with the allodynia measurements of the previous section where no statistically significant differences were observed between genders within the same disease models.

These results indicate a general similarity in protein expression between females and males, although a small difference was observed, with female rats usually showing a higher average of protein quantification compared to male rats in most tissue samples in most disease groups. This might be due to the small exploratory N=4 per group used for this set of experiments. Perhaps if the N increases per group, the small differences observed might become significant and female rats might have statistically higher protein concentration of acrolein, TRPA1 and MCP1 in all brain, trigeminal nerves, spinal cords and DRG within disease group.

CHAPTER 8. CONCLUSIONS

This study clearly demonstrates the involvement of acrolein in neuropathic pain post-mbTBI and in chronic stress given that increased headache and neuropathic pain was observed along with increases in acrolein, TRPA1 and MCP1 in both conditions and in the comorbidity of these two conditions. Moreover, the headache and neuropathic pain observed in mbTBI, chronic stress, and the comorbidity of both conditions, was mitigated after the injection of the acrolein-scavenger hydralazine, and all acrolein, TRPA1 and MCP1 were significantly reduced in brains, trigeminal nerves, spinal cords and DRG of mbTBI, chronic stress and even comorbidity rats after the injection of the acrolein-scavenger hydralazine.

These results suggest that acrolein is able to promote headache and neuropathic pain in mbTBI and chronic stress, and in the comorbidity of these conditions via upregulation and activation of TRPA1, and by stimulating the release of MCP1, which also transactivates TRPA1. Additionally, this study demonstrates that acrolein is present in the CNS in mbTBI, chronic stress, and the comorbidity of these conditions which coincides with the literature that indicates the important involvement of oxidative stress in both conditions.

Thus, since oxidative stress has been involved in the neurodegenerative conditions post-TBI, and in the health deterioration due to chronic stress, acrolein-scavenging has the potential to not only mitigate the headache and neuropathic pain in both conditions, but also to mitigate the neurodegenerative processes and secondary injury cascade in TBI, and to prevent the health consequences observed in chronic stress. In that case, acrolein scavenging could prevent the long term neurodegenerative and neuropsychiatric disorders associated with TBI, such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, chronic traumatic encephalopathy, PTSD, etc; and could prevent the health complications observed in chronic stress, such as cardiac diseases, obesity, and even cancer. These are all great long-term implications, but much more detailed research in each of these areas is needed to confirm this; and possibly, these negative health effects need to be treated not only by acrolein scavenging, but by a combination of different mechanistic therapies.

Acrolein scavenging by hydralazine could be an excellent treatment that could be easily translated for human patients given that hydralazine is an FDA approved drug. Remarkably, the

hydralazine treatment was also efficient in mitigating neuropathic pain and headache behaviors in the comorbidity of both mbTBI and chronic stress together, suggesting that hydralazine is a potent acrolein-scavenger that might be effective not only for the treatment of neuropathic pain and headache in the comorbidity of mbTBI and chronic stress, but also on the comorbidity of different conditions where oxidative stress plays a role. Thus, this study has the potential to provide an easily translational treatment for neuropathic pain post-mbTBI and chronic stress that could greatly improve the health and quality of life of these patients.

CHAPTER 9. FUTURE DIRECTIONS

As discussed above, soldiers are exposed to enormous stress in the battlefield, and many mbTBI patients suffer from PTSD and chronic stress post-injury. From the results presented here, it is clear that chronic stress alone can induce headache and neuropathic pain, and that stress before and after a mild blast TBI exacerbates neuropathic pain, and acrolein, TRPA1, and MCP1 concentrations in the brain, trigeminal nerves, spinal cords and DRG. Although it is very likely that exposure to stress could also exacerbate more severe or repetitive TBI neuropathic pain and protein concentrations in the nervous system, these implications were not analyzed in these experiments but would be interested to pursue given the commonality of repetitive TBI due to misdiagnosis of mild TBI. Naturally, the studies presented here could also be extended to human studies as a clinical trial for neuropathic pain after mbTBI or during chronic stress.

Additionally, this research studies focused on the effect of the hydralazine treatment on neuropathic pain but not on the neurodegeneration promoted by mbTBI and by chronic stress. Thus, in a future study more neurodegenerative markers could be analyzed in these disease models to analyze first whether chronic stress can promote mild neurodegeneration markers and second, whether the treatment of acrolein-scavenging with hydralazine is able to reverse such effects in the nervous system. The experimental procedures are explained in more detail in the Part 1 of the proposed future studies.

Moreover, it has been previously reported that cigarette smoking tends to be predominant in military populations, specifically post-TBI patients.[207] Similarly, individuals under chronic stress have also been found to smoke cigarettes in higher proportion.[221] Cigarette smoke has been linked to many diseases, such as emphysema, cardiovascular disease, microbial infections like meningitis, lung diseases, and cancer; where it has been linked to increased risk of morbidity and mortality.[70,98,168,175,176] In 1999 more than a million deaths were linked to tobacco smoke in China, predicting a large increase in mortality on the decades to come.[188]

Cigarette smoke has been known to produce toxicological consequences due to its effect on complex lipid peroxidation processes,[57] where acrolein can be produced. For this reason, the carcinogenesis of acrolein in cigarette smoke in lung cancer has been tested, finding that acrolein is able to mutate DNA and also impair the DNA repair capacity.[80] Acrolein has also been proven

to be a major mediator of cigarette smoke-induced macrophage activation and inflammation.[78,83,130,165] Hence, acrolein in cigarette smoke is a major component that relates to the morbidity caused by smoking.[121]

It has been previously demonstrated that acrolein inhalation in similar concentrations to those of cigarette smoke is able to penetrate the CNS, and be detected in the spinal cord in the EAE mouse model of multiple sclerosis.[239] Cigarette smoke has also been shown to cause neuropathic pain in spinal cord injury patients.[200] Thus, considering this evidence, bearing in mind that acrolein is a major component of cigarette smoke, and that acrolein played a major role in neuropathic pain in TBI and chronic stress based on the results provided, we propose that acrolein in cigarette smoke can exacerbate headache and neuropathic pain in TBI and chronic stress.

Although smoking cessation would be a natural option to reduce acrolein levels and neuropathic pain in the body of smoker TBI and chronic stress patients, this might not be so simple. Disease-related issues might hinder smoking cessation for patients with neurodegenerative and inflammatory dieases.[6] It has been demonstrated that smoker patients of neurodegenerative disorders tend to be heavy smokers and find it difficult to quit due to the pleasurable effects of smoking, and because smoking helps them cope with their conditions.[89] Additionally, smoker individuals under chronic stress report that cigarette smoke help them relieve feelings of stress.[184] Thereupon, it might be useful to offer these patients another option that does not affect their lifestyle choices, such as an anti-acrolein treatment. This treatment would potentially reduce endogenous and exogenous sources of acrolein that lead to neuropathic pain in TBI and chronic stress, which would improve the quality of life of both non-smoker patients, and patients that have difficulties with smoking cessation. Accordingly, the proposed Part 2 will determine the effects of cigarette smoke in neuropathic pain post-mbTBI, as well as the efficacy of the acrolein-scavenger hydralazine in mitigating pain despite the exogenous input of acrolein through cigarette smoking. And finally, Part 3 will determine the effects of cigarette smoke in neuropathic pain in chronic stress, as well as the efficacy of hydralazine treatment in mitigating such pain. Additionally, the comorbidity of these two conditions along with cigarette smoking and acrolein-scavenging with hydralazine could also be tested.

Part 1. Determine the neurotoxicity in mbTBI and chronic stress, and the efficacy of the acrolein-scavenger hydralazine in reversing these effects

It is well known that mbTBI is associated with long-term neurodegenerative and neuropsychiatric disorders, and that chronic stress has been associated with several disease states. For this reason, understanding whether mbTBI and chronic stress promote neurotoxic changes in the nervous system is important to prevent these conditions in the long-term. To test this, changes in myelin could be observed in healthy conditions, in mbTBI, chronic stress, and if changes are observed in these disease states, the hydralazine treatment could be used to further investigate whether acrolein sequestering is able to reduce this neurotoxicity in these two conditions. Similarly, changes in neuronal populations could be analyzed, as well as microglial activation, blood brain barrier disruption, mitochondrial disfunction, among others could be analyzed in both conditions. These studies could provide more insight on the exact pathophysiological mechanisms occurring in the nervous system in these two conditions that can lead to a deteriorated state in the long-term, and possibly even ameliorate these with acrolein-scavenging with the hydralazine treatment.

Part 2. Determine the effects of cigarette smoke in neuropathic pain post-mbTBI, and the efficacy of the acrolein-scavenger hydralazine

Similar to the studies described above, we will examine neuropathic pain behaviors by testing for periorbital and hind limb allodynia, as well as examining the brains, trigeminal nerves, spinal cords, and DRG for biochemical analysis of acrolein, TRPA1 and MCP1. As previously, baseline mechanical allodynia will be assessed in three different days within a 2-week period before mbTBI induction.

This animal model will be similar to the mbTBI model described above with the addition of cigarette smoke inhalation starting on the day of mbTBI induction. Reference cigarettes (3R4F, University of Kentucky Reference Cigarette Program, KY, USA) will be kept frozen until use. The cigarettes will be conditioned at room temperature for 30 min prior to ignition and will be coupled to a vacuum pump that will be in line with a custom-built translucent 6”x12”x24” air-tight chamber, where a maximum of 8 rats will be placed. Inhalation sessions will occur once daily for 1 hour throughout the study.[187] Rats will be tested for periorbital and hind limb allodynia 1 day post-injury and weekly thereafter for 4 weeks. Rats will be sacrificed 1 day after the last allodynia test and tissue will be harvested for biochemical analyses as previously described. Rats will be

divided into three groups: control, cigarette smoke-blast, and cigarette smoke-blast with hydralazine treatment (n=7 per group). Hydralazine treatment will be applied through IP injection on the day of the mbTBI induction.

We hypothesize that headache and neuropathic pain will be exacerbated by the cigarette smoke in this mbTBI model, and that the hydralazine treatment will be able to mitigate this effect.

Part 3. Determine the effects of cigarette smoke in neuropathic pain in chronic stress, and the efficacy of the acrolein-scavenger hydralazine

As previously described, headache and neuropathic pain will be examined by testing for periorbital and hind limb allodynia, as well as examining the brains, trigeminal nerves, spinal cords, and DRG for changes in protein expression of acrolein, TRPA1 and MCP1. As previously, baseline mechanical allodynia will be assessed in three different days within a 2-week period before the start of chronic stress induction.

This animal model will be similar to the chronic stress model described above with the addition of cigarette smoke inhalation starting on the first day of restrain. Cigarette smoke inhalation will be performed as described above with 1 hour of inhalation daily throughout the length of the study. Rats will be tested for periorbital and hind limb allodynia 1 day post-restrain and weekly thereafter for 4 weeks. The rats will be sacrificed 1 day after the last allodynia test, and the tissue will be harvested for biochemical analyses as previously described. Rats will be divided into three groups: control, cigarette smoke-restrain, and cigarette smoke-restrain with hydralazine treatment (n=7 per group). Hydralazine treatment will be applied through IP injection on day 1 of restrain.

We hypothesize that headache and neuropathic pain will be exacerbated by the cigarette smoke in this chronic stress model, and that the hydralazine treatment will be able to mitigate this effect.

Potential difficulties and alternative solutions

No complications are expected for Parts 1, 2 and 3, given that these animal models have already been successfully tested for the experiments described here, and given that cigarette smoke inhalation has previously been performed successfully in our laboratory with minor complications such as cigarette tar getting attached to the pump, which can be easily addressed by detaching and cleaning the tubes with paper towels. In a scenario where the buildup is impossible to be cleaned, the tubes and pump can be replaced.

Additionally, the comorbidity study might induce very high endogenous levels of acrolein that could potentially not be entirely sequestered by the hydralazine dose proposed here. For that case,

the hydralazine dose could be safely increased, or a different acrolein scavenger, such as phenelzine could be tested. Even, if safe and possible, both hydralazine and phenelzine could be used for the sequestering of acrolein in this comorbidity condition.

In a similar way, Part 2 and 3 intend to evaluate the effect of the exogenous input of acrolein through cigarette smoking. This will very likely increase acrolein concentrations causing very low pain thresholds, but this should not be a major complication given the large range of von Frey filaments. However, the input of endogenous and exogenous acrolein in the body, through TBI or chronic stress, and through cigarette smoke, respectively, might cause the levels of acrolein in the body to be too high for the hydralazine treatment to be effective. In this case, we could also use a higher dose that would still be safe for the rats or evaluate the use of phenelzine for acrolein scavenging. Phenelzine has previously been used in our laboratory, so the application of this treatment should not be a complication either. In case that none of these proved effective, or were just partially effective, we could also investigate the safe use of both for a complete mitigation of neuropathic pain and acrolein scavenging.

REFERENCES

1. Abbadie, C., S. Bhangoo, Y. De Koninck, M. Malcangio, S. Melik-Parsadaniantz, and F. A. White. Chemokines and pain mechanisms. *Brain Res. Rev.* 60:125–134, 2009.
2. Abbadie, C., J. A. Lindia, A. M. Cumiskey, L. B. Peterson, J. S. Mudgett, E. K. Bayne, J. A. DeMartino, D. E. MacIntyre, and M. J. Forrest. Impaired neuropathic pain responses in mice lacking the chemokine receptor CCR2. *Proc. Natl. Acad. Sci.* 100:7947–7952, 2003.
3. Abdallah, C. G., and P. Geha. Chronic Pain and Chronic Stress: Two Sides of the Same Coin? *Chronic Stress Thousand Oaks Calif* 1:, 2017.
4. Abercrombie, E. D., R. W. Keller, and M. J. Zigmond. Characterization of hippocampal norepinephrine release as measured by microdialysis perfusion: Pharmacological and behavioral studies. *Neuroscience* 27:897–904, 1988.
5. Abraham, K., S. Andres, R. Palavinskas, K. Berg, K. E. Appel, and A. Lampen. Toxicology and risk assessment of acrolein in food. *Mol. Nutr. Food Res.* 55:1277–1290, 2011.
6. Aimer, P., L. Stamp, S. Stebbings, N. Valentino, V. Cameron, and G. J. Treharne. Identifying Barriers to Smoking Cessation in Rheumatoid Arthritis: Smoking Cessation and Rheumatoid Arthritis. *Arthritis Care Res.* 67:607–615, 2015.
7. Anderson, M. M., S. L. Hazen, F. F. Hsu, and J. W. Heinecke. Human neutrophils employ the myeloperoxidase-hydrogen peroxide-chloride system to convert hydroxy-amino acids into glycolaldehyde, 2-hydroxypropanal, and acrolein. A mechanism for the generation of highly reactive alpha-hydroxy and alpha,beta-unsaturated aldehydes by phagocytes at sites of inflammation. *J. Clin. Invest.* 99:424–432, 1997.
8. Andersson, D. A., C. Gentry, S. Moss, and S. Bevan. Transient receptor potential A1 is a sensory receptor for multiple products of oxidative stress. *J. Neurosci. Off. J. Soc. Neurosci.* 28:2485–2494, 2008.
9. Ansari, M. A., K. N. Roberts, and S. W. Scheff. A Time Course of Contusion-Induced Oxidative Stress and Synaptic Proteins in Cortex in a Rat Model of TBI. *J. Neurotrauma* 25:513–526, 2008.
10. Areti, A., V. G. Yerra, V. G. M. Naidu, and A. Kumar. Oxidative stress and nerve damage: role in chemotherapy induced peripheral neuropathy. *Redox Biol.* 2:289–295, 2014.
11. Attal, N., G. Cruccu, R. Baron, M. Haanpää, P. Hansson, T. S. Jensen, and T. Nurmikko. EFNS guidelines on the pharmacological treatment of neuropathic pain: 2010 revision: Treatment of neuropathic pain. *Eur. J. Neurol.* 17:1113-e88, 2010.
12. Attal, N., G. Cruccu, M. Haanpää, P. Hansson, T. S. Jensen, T. Nurmikko, C. Sampaio, S. Sindrup, and P. Wiffen. EFNS guidelines on pharmacological treatment of neuropathic pain. *Eur. J. Neurol.* 13:1153–1169, 2006.
13. Baker, W. E. Explosions in air. University of Texas press, 1973.
14. Bandell, M., L. J. Macpherson, and A. Patapoutian. From chills to chilis: Mechanisms for thermosensation and chemesthesis via thermoTRPs. *Curr. Opin. Neurobiol.* 17:490–497, 2007.
15. Bandell, M., G. M. Story, S. W. Hwang, V. Viswanath, S. R. Eid, M. J. Petrus, T. J. Earley, and A. Patapoutian. Noxious Cold Ion Channel TRPA1 Is Activated by Pungent Compounds and Bradykinin. *Neuron* 41:849–857, 2004.

16. Baraldi, P. G., D. Preti, S. Materazzi, and P. Geppetti. Transient Receptor Potential Ankyrin 1 (TRPA1) Channel as Emerging Target for Novel Analgesics and Anti-Inflammatory Agents. *J. Med. Chem.* 53:5085–5107, 2010.
17. Barger, S. W., M. E. Goodwin, M. M. Porter, and M. L. Beggs. Glutamate release from activated microglia requires the oxidative burst and lipid peroxidation. *J. Neurochem.* 101:1205–1213, 2007.
18. Baron, R. Mechanisms of Disease: neuropathic pain—a clinical perspective. *Nat. Clin. Pract. Neurol.* 2:95–106, 2006.
19. Bass, C. R., M. B. Panzer, K. A. Rafaels, G. Wood, J. Shridharani, and B. Capehart. Brain injuries from blast. *Ann. Biomed. Eng.* 40:185–202, 2012.
20. Bass, C. R., K. A. Rafaels, and R. S. Salzar. Pulmonary injury risk assessment for short-duration blasts. *J. Trauma Acute Care Surg.* 65:604–615, 2008.
21. Baugh, C. M., J. M. Stamm, D. O. Riley, B. E. Gavett, M. E. Shenton, A. Lin, C. J. Nowinski, R. C. Cantu, A. C. McKee, and R. A. Stern. Chronic traumatic encephalopathy: neurodegeneration following repetitive concussive and subconcussive brain trauma. *Brain Imaging Behav.* 6:244–254, 2012.
22. Baum, A., J. P. Garofalo, and A. M. Yali. Socioeconomic Status and Chronic Stress: Does Stress Account for SES Effects on Health? *Ann. N. Y. Acad. Sci.* 896:131–144.
23. Bautista, D. M., S.-E. Jordt, T. Nikai, P. R. Tsuruda, A. J. Read, J. Poblete, E. N. Yamoah, A. I. Basbaum, and D. Julius. TRPA1 Mediates the Inflammatory Actions of Environmental Irritants and Proalgesic Agents. *Cell* 124:1269–1282, 2006.
24. Bautista, D. M., P. Movahed, A. Hinman, H. E. Axelsson, O. Sterner, E. D. Hogestatt, D. Julius, S.-E. Jordt, and P. M. Zygmunt. Pungent products from garlic activate the sensory ion channel TRPA1. *Proc. Natl. Acad. Sci.* 102:12248–12252, 2005.
25. Bautista, D. M., J. Siemens, J. M. Glazer, P. R. Tsuruda, A. I. Basbaum, C. L. Stucky, S.-E. Jordt, and D. Julius. The menthol receptor TRPM8 is the principal detector of environmental cold. *Nature* 448:204–208, 2007.
26. Bay, E., and M. B. de-Leon. Chronic Stress and Fatigue-Related Quality of Life after Mild-to-Moderate Traumatic Brain Injury (TBI). *J. Head Trauma Rehabil.* 26:355–363, 2011.
27. Beauchamp, R. O., D. A. Andjelkovich, A. D. Kligerman, K. T. Morgan, H. d'A Heck, and V. J. Feron. A critical review of the literature on acrolein toxicity. *CRC Crit. Rev. Toxicol.* 14:309–380, 1985.
28. Becker, N., A. B. Thomsen, A. K. Olsen, P. Sjøgren, P. Bech, and J. Eriksen. Pain epidemiology and health related quality of life in chronic non-malignant pain patients referred to a Danish multidisciplinary pain center. *Pain* 73:393–400, 1997.
29. Behrendt, H.-J., T. Germann, C. Gillen, H. Hatt, and R. Jostock. Characterization of the mouse cold-menthol receptor TRPM8 and vanilloid receptor type-1 VR1 using a fluorometric imaging plate reader (FLIPR) assay. *Br. J. Pharmacol.* 141:737–745, 2004.
30. Belanger, H. G., T. Kretzmer, R. Yoash-Gantz, T. Pickett, and L. A. Tupler. Cognitive sequelae of blast-related versus other mechanisms of brain trauma. *J. Int. Neuropsychol. Soc.* 15:1–8, 2009.
31. Bessac, B. F., and S.-E. Jordt. Breathtaking TRP channels: TRPA1 and TRPV1 in airway chemosensation and reflex control. *Physiol. Bethesda Md* 23:360–370, 2008.
32. Biagini, R., M. Toraason, D. Lynch, and G. Winston. Inhibition of rat heart mitochondrial electron transport in vitro: implications for the cardiotoxic action of allylamine or its primary metabolite, acrolein. *Toxicology* 62:95–106, 1990.

33. Black, J. A., S. Liu, M. Tanaka, T. R. Cummins, and S. G. Waxman. Changes in the expression of tetrodotoxin-sensitive sodium channels within dorsal root ganglia neurons in inflammatory pain. *Pain* 108:237–247, 2004.
34. Blackburn-Munro, G., and R. E. Blackburn-Munro. Chronic Pain, Chronic Stress and Depression: Coincidence or Consequence?: HPA axis involvement in comorbidity of chronic pain and depression. *J. Neuroendocrinol.* 13:1009–1023, 2001.
35. Blight, A. R. Effect of 4-aminopyridine on axonal conduction-block in chronic spinal cord injury. *Brain Res. Bull.* 22:47–52, 1989.
36. Bond, M., H. Breivik, T. S. Jensen, W. Scholten, O. Soyannwo, and R. D. Treede. Pain associated with neurological disorders. *Neurol. Disord. Public Health Chall.* 127–139, 2006.
37. Bonica, J. J., and J. F. Hoffman. The Management of Pain with Special Emphasis on the Use of Analgesic Blocks in Diagnosis, Prognosis, and Therapy. *Anesth. Analg.* 34:57, 1954.
38. Boonstra, R. Reality as the leading cause of stress: rethinking the impact of chronic stress in nature. *Funct. Ecol.* 27:11–23.
39. Borchers, M. T., S. Wesselkamper, S. E. Wert, S. D. Shapiro, and G. D. Leikauf. Monocyte inflammation augments acrolein-induced Muc5ac expression in mouse lung. *Am. J. Physiol. - Lung Cell. Mol. Physiol.* 277:L489–L497, 1999.
40. Bouhassira, D., N. Attal, H. Alchaar, F. Boureau, B. Brochet, J. Bruxelle, G. Cunin, J. Fermanian, P. Ginies, A. Grun-Overdyking, H. Jafari-Schluep, M. Lantéri-Minet, B. Laurent, G. Mick, A. Serrie, D. Valade, and E. Vicaut. Comparison of pain syndromes associated with nervous or somatic lesions and development of a new neuropathic pain diagnostic questionnaire (DN4): *Pain* 114:29–36, 2005.
41. Bouhassira, D., N. Attal, J. Fermanian, H. Alchaar, M. Gautron, E. Masquelier, S. Rostaing, M. Lanteri-Minet, E. Collin, J. Grisart, and F. Boureau. Development and validation of the Neuropathic Pain Symptom Inventory. *Pain* 108:248–257, 2004.
42. Bryant, R. Post-traumatic stress disorder vs traumatic brain injury. *Dialogues Clin. Neurosci.* 13:251–262, 2011.
43. Butler, S. H., F. Godefroy, J.-M. Besson, and J. Weil-Fugazza. A limited arthritic model for chronic pain studies in the rat. *Pain* 48:73–81, 1992.
44. Cadenas, E., and K. J. A. Davies. Mitochondrial free radical generation, oxidative stress, and aging11This article is dedicated to the memory of our dear friend, colleague, and mentor Lars Ernster (1920–1998), in gratitude for all he gave to us. *Free Radic. Biol. Med.* 29:222–230, 2000.
45. Calingasan, N. Y., K. Uchida, and G. E. Gibson. Protein-Bound Acrolein: A Novel Marker of Oxidative Stress in Alzheimer's Disease. *J. Neurochem.* 72:751–756, 1999.
46. del Camino, D., S. Murphy, M. Heiry, L. B. Barrett, T. J. Earley, C. A. Cook, M. J. Petrus, M. Zhao, M. D'Amours, N. Deering, G. J. Brenner, M. Costigan, N. J. Hayward, J. A. Chong, C. M. Fanger, C. J. Woolf, A. Patapoutian, and M. M. Moran. TRPA1 Contributes to Cold Hypersensitivity. *J. Neurosci.* 30:15165–15174, 2010.
47. Carroll, L., J. D. Cassidy, P. Peloso, J. Borg, H. Von Holst, L. Holm, C. Paniak, and M. Pépin. Prognosis for mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. *J. Rehabil. Med.* 36:84–105, 2004.
48. Caterina, M. J. Impaired Nociception and Pain Sensation in Mice Lacking the Capsaicin Receptor. *Science* 288:306–313, 2000.

49. Cernak, I., and L. J. Noble-Haeusslein. Traumatic brain injury: an overview of pathobiology with emphasis on military populations. *J. Cereb. Blood Flow Metab.* 30:255–266, 2010.
50. Cernak, I., V. Savic, J. Kotur, V. Prokic, B. Kuljic, D. Grbovic, and M. Veljovic. Alterations in magnesium and oxidative status during chronic emotional stress. *Magnes. Res.* 13:29–36, 2000.
51. Chandola, T., E. Brunner, and M. Marmot. Chronic stress at work and the metabolic syndrome: prospective study. *Bmj* 332:521–525, 2006.
52. Chaplan, S. R., F. W. Bach, J. W. Pogrel, J. M. Chung, and T. L. Yaksh. Quantitative assessment of tactile allodynia in the rat paw. *J. Neurosci. Methods* 53:55–63, 1994.
53. Chen, H., M. Richard, D. P. Sandler, D. M. Umbach, and F. Kamel. Head Injury and Amyotrophic Lateral Sclerosis. *Am. J. Epidemiol.* 166:810–816, 2007.
54. Chen, J., S. K. Joshi, S. DiDomenico, R. J. Perner, J. P. Mikusa, D. M. Gauvin, J. A. Segreti, P. Han, X.-F. Zhang, W. Niforatos, B. R. Bianchi, S. J. Baker, C. Zhong, G. H. Simler, H. A. McDonald, R. G. Schmidt, S. P. McGaraughty, K. L. Chu, C. R. Faltynek, M. E. Kort, R. M. Reilly, and P. R. Kym. Selective blockade of TRPA1 channel attenuates pathological pain without altering noxious cold sensation or body temperature regulation: *Pain* 152:1165–1172, 2011.
55. Chessell, I. P., J. P. Hatcher, C. Bountra, A. D. Michel, J. P. Hughes, P. Green, J. Egerton, M. Murfin, J. Richardson, and W. L. Peck. Disruption of the P2X7 purinoceptor gene abolishes chronic inflammatory and neuropathic pain. *Pain* 114:386–396, 2005.
56. Chung, F.-L., R. Young, and S. S. Hecht. Formation of Cyclic 1,N2-Propanodeoxyguanosine Adducts in DNA upon Reaction with Acrolein or Crotonaldehyde. *Cancer Res.* 44:990–995, 1984.
57. Church, D. F., and W. A. Pryor. Free-radical chemistry of cigarette smoke and its toxicological implications. *Environ. Health Perspect.* 64:111, 1985.
58. Clapham, D. E. TRP channels as cellular sensors. *Nature* 426:517–524, 2003.
59. Clapham, D. E., L. W. Runnels, and C. Strübing. The TRP ion channel family. *Nat. Rev. Neurosci.* 2:387–396, 2001.
60. Covey, W. C., T. A. Ignatowski, P. R. Knight, and R. N. Spengler. Brain-derived TNFα: involvement in neuroplastic changes implicated in the conscious perception of persistent pain. *Brain Res.* 859:113–122, 2000.
61. Cruccu, G., P. Anand, N. Attal, L. Garcia-Larrea, M. Haanpaa, E. Jorum, J. Serra, and T. S. Jensen. EFNS guidelines on neuropathic pain assessment. *Eur. J. Neurol.* 11:153–162, 2004.
62. Cruz-Haces, M., J. Tang, G. Acosta, J. Fernandez, and R. Shi. Pathological correlations between traumatic brain injury and chronic neurodegenerative diseases. *Transl. Neurodegener.* 6:20, 2017.
63. Cws Kincaid-Colton. The brain's immune system. *Sci. Am.* 273:54.
64. Dallman, M. F., N. Pecoraro, S. F. Akana, S. E. La Fleur, F. Gomez, H. Houshyar, M. E. Bell, S. Bhatnagar, K. D. Laugero, and S. Manalo. Chronic stress and obesity: a new view of "comfort food." *Proc. Natl. Acad. Sci.* 100:11696–11701, 2003.
65. De Ridder, D., S. Vanneste, M. Plazier, E. van der Loo, and T. Menovsky. Burst Spinal Cord Stimulation: Toward Paresthesia-Free Pain Suppression. *Neurosurgery* 66:986–990, 2010.

66. Defrin, R., H. Gruener, S. Schreiber, and C. G. Pick. Quantitative somatosensory testing of subjects with chronic post-traumatic headache: Implications on its mechanisms. *Eur. J. Pain* 14:924–931.
67. DeLeo, J. A., and R. P. Yezierski. The role of neuroinflammation and neuroimmune activation in persistent pain: *Pain* 90:1–6, 2001.
68. Deshmane, S. L., S. Kremlev, S. Amini, and B. E. Sawaya. Monocyte Chemoattractant Protein-1 (MCP-1): An Overview. *J. Interferon Cytokine Res.* 29:313–326, 2009.
69. Dixon, W. J. Efficient analysis of experimental observations. *Annu. Rev. Pharmacol. Toxicol.* 20:441–462, 1980.
70. Doll, R., and R. Peto. Mortality in relation to smoking: 20 years' observations on male British doctors. *Br. Med. J.* 2:1525–1536, 1976.
71. Donofrio, P. D. Treatment of Painful Diabetic Neuropathy with Topical Capsaicin: A Multicenter, Double-blind, Vehicle-Controlled Study. *Arch. Intern. Med.* 151:2225, 1991.
72. Due, M. R., J. Park, L. Zheng, M. Walls, Y. M. Allette, F. A. White, and R. Shi. Acrolein involvement in sensory and behavioral hypersensitivity following spinal cord injury in the rat. *J. Neurochem.* 128:776–786, 2014.
73. Dworkin, R. H., A. B. O'Connor, M. Backonja, J. T. Farrar, N. B. Finnerup, T. S. Jensen, E. A. Kalso, J. D. Loeser, C. Miaskowski, T. J. Nurmikko, R. K. Portenoy, A. S. C. Rice, B. R. Stacey, R.-D. Treede, D. C. Turk, and M. S. Wallace. Pharmacologic management of neuropathic pain: Evidence-based recommendations: *Pain* 132:237–251, 2007.
74. Eid, S. R., E. D. Crown, E. L. Moore, H. A. Liang, K.-C. Choong, S. Dima, D. A. Henze, S. A. Kane, and M. O. Urban. HC-030031, a TRPA1 selective antagonist, attenuates inflammatory- and neuropathy-induced mechanical hypersensitivity. *Mol. Pain* 4:48, 2008.
75. Elliott, M. B., M. L. Oshinsky, P. S. Amenta, O. O. Awe, and J. I. Jallo. Nociceptive Neuropeptide Increases and Periorbital Allodynia in a Model of Traumatic Brain Injury. *Headache J. Head Face Pain* 52:966–984.
76. Esposito, L. A., S. Melov, A. Panov, B. A. Cottrell, and D. C. Wallace. Mitochondrial disease in mouse results in increased oxidative stress. *Proc. Natl. Acad. Sci.* 96:4820–4825, 1999.
77. Esterbauer, H., R. J. Schaur, and H. Zollner. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic. Biol. Med.* 11:81–128, 1991.
78. Facchinetti, F., F. Amadei, P. Geppetti, F. Tarantini, C. Di Serio, A. Dragotto, P. M. Gigli, S. Catinella, M. Civelli, and R. Patacchini. α,β-Unsaturated Aldehydes in Cigarette Smoke Release Inflammatory Mediators from Human Macrophages. *Am. J. Respir. Cell Mol. Biol.* 37:617–623, 2007.
79. Faul, M., and V. Coronado. Epidemiology of traumatic brain injury. In: Handbook of Clinical Neurology. Elsevier, 2015, pp. 3–13.
80. Feng, Z., W. Hu, Y. Hu, and M. -s. Tang. Acrolein is a major cigarette-related lung cancer agent: Preferential binding at p53 mutational hotspots and inhibition of DNA repair. *Proc. Natl. Acad. Sci.* 103:15404–15409, 2006.
81. Ferdinand, K. C. Isosorbide dinitrate and hydralazine hydrochloride: a review of efficacy and safety. *Expert Rev. Cardiovasc. Ther.* 3:993–1001, 2005.
82. Figueroa-Romero, C., M. Sadidi, and E. L. Feldman. Mechanisms of disease: the oxidative stress theory of diabetic neuropathy. *Rev. Endocr. Metab. Disord.* 9:301–314, 2008.

83. Finkelstein, E. I., M. Nardini, and A. van der Vliet. Inhibition of neutrophil apoptosis by acrolein: a mechanism of tobacco-related lung disease? *Am. J. Physiol. - Lung Cell. Mol. Physiol.* 281:L732–L739, 2001.
84. Finnerup, N. B., M. Otto, H. J. McQuay, T. S. Jensen, and S. H. Sindrup. Algorithm for neuropathic pain treatment: An evidence based proposal: *Pain* 118:289–305, 2005.
85. Finnerup, N. B., S. H. Sindrup, and T. S. Jensen. The evidence for pharmacological treatment of neuropathic pain: *Pain* 150:573–581, 2010.
86. Fiore, C., V. Trézéguet, A. Le Saux, P. Roux, C. Schwimmer, A. C. Dianoux, F. Noel, G. J.-M. Lauquin, G. Brandolin, and P. V. Vignais. The mitochondrial ADP/ATP carrier: Structural, physiological and pathological aspects. *Biochimie* 80:137–150, 1998.
87. Flor, H., T. Elbert, S. Knecht, C. Wienbruch, C. Pantev, N. Birbaumer, W. Larbig, and E. Taub. Phantom-limb pain as a perceptual correlate of cortical reorganization following arm amputation. *Nature* 375:482–484, 1995.
88. Frederick, J., M. E. Buck, D. J. Matson, and D. N. Cortright. Increased TRPA1, TRPM8, and TRPV2 expression in dorsal root ganglia by nerve injury. *Biochem. Biophys. Res. Commun.* 358:1058–1064, 2007.
89. Friend, K. B., S. T. Mernoff, P. Block, and G. Reeve. Smoking rates and smoking cessation among individuals with multiple sclerosis. *Disabil. Rehabil.* 28:1135–1141, 2006.
90. Fruhstorfer, H., W. Gross, and O. Selbmann. von Frey hairs: new materials for a new design. *Eur. J. Pain* 5:341–342, 2001.
91. Fu, T. S., R. Jing, S. R. McFaull, and M. D. Cusimano. Health & Economic Burden of Traumatic Brain Injury in the Emergency Department. *Can J Neuro Sci* , 2015.at <http://www.academia.edu/download/43593917/Fu_et_al_2016_Canadian_Journal_of_Neurological_Sciences.pdf>
92. Furlan, A. D. Opioids for chronic noncancer pain: a meta-analysis of effectiveness and side effects. *Can. Med. Assoc. J.* 174:1589–1594, 2006.
93. Galarneau, M. R., S. I. Woodruff, J. L. Dye, C. R. Mohrle, and A. L. Wade. Traumatic brain injury during Operation Iraqi Freedom: findings from the United States Navy–Marine Corps Combat Trauma Registry. , 2008.
94. Galluzzi, K. E. Management of Neuropathic Pain. *J. Am. Osteopath. Assoc.* 105:12–19, 2005.
95. Galluzzi, K. E. Managing neuropathic pain. *J. Am. Osteopath. Assoc.* 107:39–48, 2007.
96. Gao, Y.-J., L. Zhang, O. A. Samad, M. R. Suter, K. Yasuhiko, Z.-Z. Xu, J.-Y. Park, A.-L. Lind, Q. Ma, and R.-R. Ji. JNK-induced MCP-1 production in spinal cord astrocytes contributes to central sensitization and neuropathic pain. *J. Neurosci.* 29:4096–4108, 2009.
97. Gardner, R. C., and K. Yaffe. Epidemiology of mild traumatic brain injury and neurodegenerative disease. *Mol. Cell. Neurosci.* 66:75–80, 2015.
98. General, S. The health consequences of smoking. *Rockv. MD Public Health Serv.* , 1982.
99. Geppetti, P., R. Nassini, S. Materazzi, and S. Benemei. The concept of neurogenic inflammation. *BJU Int.* 101:2–6, 2008.
100. Ghadrdoost, B., A. A. Vafaei, A. Rashidy-Pour, R. Hajisoltani, A. R. Bandegi, F. Motamedi, S. Haghighi, H. R. Sameni, and S. Pahlvan. Protective effects of saffron extract and its active constituent crocin against oxidative stress and spatial learning and memory deficits induced by chronic stress in rats. *Eur. J. Pharmacol.* 667:222–229, 2011.
101. Ghajar, J. Traumatic brain injury. *The Lancet* 356:923–929, 2000.

102. Ghilarducci, D. P., and R. S. Tjeerdema. Fate and Effects of Acrolein. In: Reviews of Environmental Contamination and Toxicology, edited by G. W. Ware. New York, NY: Springer New York, 1995, pp. 95–146.
103. Gironda, R. J., M. E. Clark, R. L. Ruff, S. Chait, M. Craine, R. Walker, and J. Scholten. Traumatic brain injury, polytrauma, and pain: challenges and treatment strategies for the polytrauma rehabilitation. *Rehabil. Psychol.* 54:247, 2009.
104. Gold, R. Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. *Brain* 129:1953–1971, 2006.
105. Goldman, S. M., F. Kamel, G. W. Ross, S. A. Jewell, G. S. Bhudhikanok, D. Umbach, C. Marras, R. A. Hauser, J. Jankovic, S. A. Factor, S. Bressman, K. E. Lyons, C. Meng, M. Korell, D. F. Roucoux, J. A. Hoppin, D. P. Sandler, J. W. Langston, and C. M. Tanner. Head injury, alpha-synuclein Rep1, and Parkinson's disease. *Ann. Neurol.* 71:40–48, 2012.
106. Goldman, S. M., C. M. Tanner, D. Oakes, G. S. Bhudhikanok, A. Gupta, and J. W. Langston. Head injury and Parkinson's disease risk in twins. *Ann. Neurol.* 60:65–72, 2006.
107. Goldstein, L. E., A. M. Fisher, C. A. Tagge, X.-L. Zhang, L. Velisek, J. A. Sullivan, C. Upreti, J. M. Kracht, M. Ericsson, and M. W. Wojnarowicz. Chronic traumatic encephalopathy in blast-exposed military veterans and a blast neurotrauma mouse model. *Sci. Transl. Med.* 4:134ra60–134ra60, 2012.
108. Grafström, R. C., J. M. Dypbukt, J. C. Willey, K. Sundqvist, C. Edman, L. Atzori, and C. C. Harris. Pathobiological Effects of Acrolein in Cultured Human Bronchial Epithelial Cells. *Cancer Res.* 48:1717–1721, 1988.
109. Güler, A. D., H. Lee, T. Iida, I. Shimizu, M. Tominaga, and M. Caterina. Heat-Evoked Activation of the Ion Channel, TRPV4. *J. Neurosci.* 22:6408–6414, 2002.
110. Haenen, G. R. M. M., N. P. E. Vermeulen, J. N. L. Tai Tin Tsoi, H. M. N. Ragetli, H. Timmerman, and A. Bast. Activation of the microsomal glutathione-s-transferase and reduction of the glutathione dependent protection against lipid peroxidation by acrolein. *Biochem. Pharmacol.* 37:1933–1938, 1988.
111. Hall, G. C., D. Carroll, D. Parry, and H. J. McQuay. Epidemiology and treatment of neuropathic pain: the UK primary care perspective. *Pain* 122:156–162, 2006.
112. van Hecke, O., S. K. Austin, R. A. Khan, B. H. Smith, and N. Torrance. Neuropathic pain in the general population: A systematic review of epidemiological studies: *Pain* 155:654–662, 2014.
113. Herzberg, U., E. Eliav, G. J. Bennett, and I. J. Kopin. The analgesic effects of R(+)-WIN 55,212–2 mesylate, a high affinity cannabinoid agonist, in a rat model of neuropathic pain. *Neurosci. Lett.* 221:157–160, 1997.
114. Hinman, A., H.-H. Chuang, D. M. Bautista, and D. Julius. TRP channel activation by reversible covalent modification. *Proc. Natl. Acad. Sci. U. S. A.* 103:19564–19568, 2006.
115. Honore, P., K. Kage, J. Mikusa, A. T. Watt, J. F. Johnston, J. R. Wyatt, C. R. Faltynek, M. F. Jarvis, and K. Lynch. Analgesic profile of intrathecal P2X3 antisense oligonucleotide treatment in chronic inflammatory and neuropathic pain states in rats: *Pain* 99:11–19, 2002.
116. Hopkins, A., and P. Rudge. Hyperpathia in the central cervical cord syndrome. *J. Neurol. Neurosurg. Psychiatry* 36:637–642, 1973.
117. Hunskaar, S., and K. Hole. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain* 30:103–114, 1987.

118. Ignatowski, T. A., W. C. Covey, P. R. Knight, C. M. Severin, T. J. Nickola, and R. N. Spengler. Brain-derived TNFα mediates neuropathic pain. *Brain Res.* 841:70–77, 1999.
119. Jensen, J. M. Effects of 4-Aminopyridine on Stretched Mammalian Spinal Cord: The Role of Potassium Channels in Axonal Conduction. *J. Neurophysiol.* 90:2334–2340, 2003.
120. Jensen, T. S. Anticonvulsants in neuropathic pain: rationale and clinical evidence. *Eur. J. Pain* 6:61–68, 2002.
121. Jia, L., Z. Liu, L. Sun, S. S. Miller, B. N. Ames, C. W. Cotman, and J. Liu. Acrolein, a Toxicant in Cigarette Smoke, Causes Oxidative Damage and Mitochondrial Dysfunction in RPE Cells: Protection by α-Lipoic Acid. *Investig. Opthalmology Vis. Sci.* 48:339, 2007.
122. Johnson, V. E., W. Stewart, and D. H. Smith. Traumatic brain injury and amyloid-β pathology: a link to Alzheimer's disease? *Nat. Rev. Neurosci.* 11:361–370, 2010.
123. Jordan, B. D. The clinical spectrum of sport-related traumatic brain injury. *Nat. Rev. Neurol.* 9:222–230, 2013.
124. Jordt, S.-E., D. M. Bautista, H. Chuang, D. D. McKemy, P. M. Zygmunt, E. D. Högestätt, I. D. Meng, and D. Julius. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature* 427:260–265, 2004.
125. Joseph, E. K., X. Chen, O. Bogen, and J. D. Levine. Oxaliplatin acts on IB4-positive nociceptors to induce an oxidative stress-dependent acute painful peripheral neuropathy. *J. Pain* 9:463–472, 2008.
126. Jung, H., P. T. Toth, F. A. White, and R. J. Miller. Monocyte chemoattractant protein-1 functions as a neuromodulator in dorsal root ganglia neurons. *J. Neurochem.* 104:254–263, 2008.
127. Kane, L. E., and Y. Alarie. Sensory irritation to formaldehyde and acrolein during single and repeated exposures in mice. *Am. Ind. Hyg. Assoc. J.* 38:509–522, 1977.
128. Karashima, Y., J. Prenen, V. Meseguer, G. Owsianik, T. Voets, and B. Nilius. Modulation of the transient receptor potential channel TRPA1 by phosphatidylinositol 4,5-biphosphate manipulators. *Pflugers Arch.* 457:77–89, 2008.
129. Kiecolt-Glaser, J. K., L. McGuire, T. F. Robles, and R. Glaser. Emotions, morbidity, and mortality: new perspectives from psychoneuroimmunology. *Annu. Rev. Psychol.* 53:83–107, 2002.
130. Kim, G.-D., S. E. Lee, T.-H. Kim, Y.-H. Jin, Y. S. Park, and C.-S. Park. Melatonin suppresses acrolein-induced IL-8 production in human pulmonary fibroblasts: Melatonin inhibits acrolein-induced IL-8. *J. Pineal Res.* 52:356–364, 2012.
131. Kingery, W. S. A critical review of controlled clinical trials for peripheral neuropathic pain and complex regional pain syndromes: *Pain* 73:123–139, 1997.
132. Knerlich-Lukoschus, F., M. Juraschek, U. Blömer, R. Lucius, H. M. Mehdorn, and J. Held-Feindt. Force-dependent development of neuropathic central pain and time-related CCL2/CCR2 expression after graded spinal cord contusion injuries of the rat. *J. Neurotrauma* 25:427–448, 2008.
133. Knerlich-Lukoschus, F., M. Noack, B. von der Ropp-Brenner, R. Lucius, H. M. Mehdorn, and J. Held-Feindt. Spinal cord injuries induce changes in CB1 cannabinoid receptor and CC chemokine expression in brain areas underlying circuitry of chronic pain conditions. *J. Neurotrauma* 28:619–634, 2011.
134. Knerlich-Lukoschus, F., B. von der Ropp-Brenner, R. Lucius, H. M. Mehdorn, and J. Held-Feindt. Chemokine expression in the white matter spinal cord precursor niche after force-defined spinal cord contusion injuries in adult rats. *Glia* 58:916–931, 2010.

135. Knerlich-Lukoschus, F., B. von der Ropp-Brenner, R. Lucius, H. M. Mehdorn, and J. Held-Feindt. Chemokine expression in the white matter spinal cord precursor niche after force-defined spinal cord contusion injuries in adult rats. *Glia* 58:916–931, 2010.
136. Knerlich-Lukoschus, F., B. von der Ropp-Brenner, R. Lucius, H. M. Mehdorn, and J. Held-Feindt. Spatiotemporal CCR1, CCL3 (MIP-1α), CXCR4, CXCL12 (SDF-1α) expression patterns in a rat spinal cord injury model of posttraumatic neuropathic pain. *J. Neurosurg. Spine* 14:583–597, 2011.
137. Kosugi, M., T. Nakatsuka, T. Fujita, Y. Kuroda, and E. Kumamoto. Activation of TRPA1 Channel Facilitates Excitatory Synaptic Transmission in Substantia Gelatinosa Neurons of the Adult Rat Spinal Cord. *J. Neurosci.* 27:4443–4451, 2007.
138. Kristensen, J. D., B. Svensson, and T. Gordh. The NMDA-receptor antagonist CPP abolishes neurogenic 'wind-up pain' after intrathecal administration in humans: *Pain* 51:249–253, 1992.
139. Kumar, A., R. K. Kaundal, S. Iyer, and S. S. Sharma. Effects of resveratrol on nerve functions, oxidative stress and DNA fragmentation in experimental diabetic neuropathy. *Life Sci.* 80:1236–1244, 2007.
140. Laughlin, T. M., J. R. Bethea, R. P. Yezierski, and G. L. Wilcox. Cytokine involvement in dynorphin-induced allodynia: *Pain* 84:159–167, 2000.
141. Lenaz, G., C. Bovina, M. D'Aurelio, R. Fato, G. Formiggini, M. L. Genova, G. Giuliano, M. M. Pich, U. Paolucci, G. P. Castelli, and B. Ventura. Role of Mitochondria in Oxidative Stress and Aging. *Ann. N. Y. Acad. Sci.* 959:199–213, 2002.
142. Leung, G., W. Sun, L. Zheng, S. Brookes, M. Tully, and R. Shi. Anti-acrolein treatment improves behavioral outcome and alleviates myelin damage in experimental autoimmune enchephalomyelitis mouse. *Neuroscience* 173:150–155, 2011.
143. Levine, J. D., and N. Alessandri-Haber. TRP channels: Targets for the relief of pain. *Biochim. Biophys. Acta BBA - Mol. Basis Dis.* 1772:989–1003, 2007.
144. Liedtke, W., D. M. Tobin, C. I. Bargmann, and J. M. Friedman. Mammalian TRPV4 (VR-OAC) directs behavioral responses to osmotic and mechanical stimuli in Caenorhabditis elegans. *Proc. Natl. Acad. Sci.* 100:14531–14536, 2003.
145. Lovell, M. A., C. Xie, and W. R. Markesbery. Acrolein is increased in Alzheimer's disease brain and is toxic to primary hippocampal cultures. *Neurobiol. Aging* 22:187–194, 2001.
146. Luo, J., and R. Shi. Acrolein induces axolemmal disruption, oxidative stress, and mitochondrial impairment in spinal cord tissue. *Neurochem. Int.* 44:475–486, 2004.
147. Luo, J., and R. Shi. Acrolein induces oxidative stress in brain mitochondria. *Neurochem. Int.* 46:243–252, 2005.
148. Maas, A. I., N. Stocchetti, and R. Bullock. Moderate and severe traumatic brain injury in adults. *Lancet Neurol.* 7:728–741, 2008.
149. Macpherson, L. J., B. H. Geierstanger, V. Viswanath, M. Bandell, S. R. Eid, S. Hwang, and A. Patapoutian. The Pungency of Garlic: Activation of TRPA1 and TRPV1 in Response to Allicin. *Curr. Biol.* 15:929–934, 2005.
150. Macpherson, L. J., S. W. Hwang, T. Miyamoto, A. E. Dubin, A. Patapoutian, and G. M. Story. More than cool: Promiscuous relationships of menthol and other sensory compounds. *Mol. Cell. Neurosci.* 32:335–343, 2006.
151. Magerl, W., and R.-D. Treede. Secondary tactile hypoesthesia: a novel type of pain-induced somatosensory plasticity in human subjects. *Neurosci. Lett.* 361:136–139, 2004.

152. Malinow, K., G. D. Yannakakis, S. M. Glusman, D. W. Edlow, J. Griffin, A. Pestronk, D. L. Powell, R. Ramsey-Goldman, B. H. Eidelman, T. A. Medsger, and E. L. Alexander. Subacute sensory neuronopathy secondary to dorsal root ganglionitis in primary Sjögren's syndrome: Ganglionitis in Sjögren's Syndrome. *Ann. Neurol.* 20:535–537, 1986.
153. Malmberg, A., and T. Yaksh. Hyperalgesia mediated by spinal glutamate or substance P receptor blocked by spinal cyclooxygenase inhibition. *Science* 257:1276–1279, 1992.
154. Martinov, T., M. Mack, A. Sykes, and D. Chatterjea. Measuring Changes in Tactile Sensitivity in the Hind Paw of Mice Using an Electronic von Frey Apparatus. *J. Vis. Exp. JoVE* , 2013.doi:10.3791/51212
155. Matta, J. A., P. M. Cornett, R. L. Miyares, K. Abe, N. Sahibzada, and G. P. Ahern. General anesthetics activate a nociceptive ion channel to enhance pain and inflammation. *Proc. Natl. Acad. Sci. U. S. A.* 105:8784–8789, 2008.
156. McEwen, B. S. Plasticity of the hippocampus: adaptation to chronic stress and allostatic load. *Ann. N. Y. Acad. Sci.* 933:265–277, 2001.
157. McEwen, B. S. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol. Rev.* 87:873–904, 2007.
158. McIntosh, T. K., D. H. Smith, D. F. Meaney, M. J. Kotapka, T. A. Gennarelli, and D. I. Graham. Neuropathological sequelae of traumatic brain injury: relationship to neurochemical and biomechanical mechanisms. *Lab. Investig. J. Tech. Methods Pathol.* 74:315–342, 1996.
159. McKemy, D. D., W. M. Neuhausser, and D. Julius. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature* 416:52–58, 2002.
160. Meier, T., G. Wasner, M. Faust, T. Kuntzer, F. Ochsner, M. Hueppe, J. Bogousslavsky, and R. Baron. Efficacy of lidocaine patch 5% in the treatment of focal peripheral neuropathic pain syndromes: a randomized, double-blind, placebo-controlled study: *Pain* 106:151–158, 2003.
161. Merriam, F. V., Z. Wang, S. D. Guerios, and D. E. Bjorling. Cannabinoid receptor 2 is increased in acutely and chronically inflamed bladder of rats. *Neurosci. Lett.* 445:130–134, 2008.
162. Montell, C. Physiology, Phylogeny, and Functions of the TRP Superfamily of Cation Channels. *Sci. Signal.* 2001:re1–re1, 2001.
163. Moqrich, A. Impaired Thermosensation in Mice Lacking TRPV3, a Heat and Camphor Sensor in the Skin. *Science* 307:1468–1472, 2005.
164. Morell, P., and R. H. Quarles. Myelin Formation, Structure and Biochemistry. , 1999.
165. Moretto, N., F. Facchinetti, T. Southworth, M. Civelli, D. Singh, and R. Patacchini. Unsaturated aldehydes contained in cigarette smoke elicit IL-8 release in pulmonary cells through mitogen-activated protein kinases. *AJP Lung Cell. Mol. Physiol.* 296:L839–L848, 2009.
166. Moshourab, R. A., M. Schäfer, and E. D. Al-Chaer. Chronic Pain in Neurotrauma: Implications on Spinal Cord and Traumatic Brain Injury. In: Brain Neurotrauma: Molecular, Neuropsychological, and Rehabilitation Aspects, edited by F. H. Kobeissy. Boca Raton (FL): CRC Press/Taylor & Francis, 2015.at <http://www.ncbi.nlm.nih.gov/books/NBK299176/>
167. Mylonas, C., and D. Kouretas. Lipid peroxidation and tissue damage. *Vivo Athens Greece* 13:295–309, 1999.
168. Nagai, S., Y. Hoshino, M. Hayashi, and I. Ito. Smoking-related interstitial lung diseases. *Curr. Opin. Pulm. Med.* 6:415–419, 2000.

169. Nagata, K. Nociceptor and Hair Cell Transducer Properties of TRPA1, a Channel for Pain and Hearing. *J. Neurosci.* 25:4052–4061, 2005.
170. Nagy, I., and H. Rang. Noxious heat activates all capsaicin-sensitive and also a sub-population of capsaicin-insensitive dorsal root ganglion neurons. *Neuroscience* 88:995–997, 1999.
171. Naik, A. K., S. K. Tandan, S. P. Dudhgaonkar, S. H. Jadhav, M. Kataria, V. R. Prakash, and D. Kumar. Role of oxidative stress in pathophysiology of peripheral neuropathy and modulation by N-acetyl-l-cysteine in rats. *Eur. J. Pain* 10:573–573, 2006.
172. Nazıroğlu, M., D. M. Dikici, and Ş. Dursun. Role of oxidative stress and Ca 2+ signaling on molecular pathways of neuropathic pain in diabetes: focus on TRP channels. *Neurochem. Res.* 37:2065–2075, 2012.
173. Nilius, B., G. Appendino, and G. Owsianik. The transient receptor potential channel TRPA1: from gene to pathophysiology. *Pflugers Arch.* 464:425–458, 2012.
174. Nisenbaum, L. K., M. J. Zigmond, A. F. Sved, and E. D. Abercrombie. Prior exposure to chronic stress results in enhanced synthesis and release of hippocampal norepinephrine in response to a novel stressor. *J. Neurosci.* 11:1478–1484, 1991.
175. Nuorti, J. P., J. C. Butler, M. M. Farley, L. H. Harrison, A. McGeer, M. S. Kolczak, and R. F. Breiman. Cigarette Smoking and Invasive Pneumococcal Disease. *N. Engl. J. Med.* 342:681–689, 2000.
176. Obeid, P., and P. Bercy. Effects of smoking on periodontal health: a review. *Adv. Ther.* 17:230–237, 2000.
177. O'Connor, A. B., and R. H. Dworkin. Treatment of Neuropathic Pain: An Overview of Recent Guidelines. *Am. J. Med.* 122:22–32, 2009.
178. Offen, D., Y. Gilgun-Sherki, and E. Melamed. The role of oxidative stress in the pathogenesis of multiple sclerosis: The need for effective antioxidant therapy. *J. Neurol.* 251:261–268, 2004.
179. Ozkul, A., M. Ayhan, C. Yenisey, A. Akyol, E. Guney, and F. A. Ergin. The role of oxidative stress and endothelial injury in diabetic neuropathy and neuropathic pain. *Neuro Endocrinol. Lett.* 31:261–264, 2010.
180. O'Connor, A. B. Neuropathic Pain: Quality-of-Life Impact, Costs and Cost Effectiveness of Therapy. *PharmacoEconomics* 27:95–112, 2009.
181. Park, J., L. Zheng, G. Acosta, S. Vega-Alvarez, Z. Chen, B. Muratori, P. Cao, and R. Shi. Acrolein contributes to TRPA1 up-regulation in peripheral and central sensory hypersensitivity following spinal cord injury. *J. Neurochem.* 135:987–997, 2015.
182. Park, J., L. Zheng, A. Marquis, M. Walls, B. Duerstock, A. Pond, S. Vega-Alvarez, H. Wang, Z. Ouyang, and R. Shi. Neuroprotective role of hydralazine in rat spinal cord injury-attenuation of acrolein-mediated damage. *J. Neurochem.* 129:339–349.
183. Park, Y. S., and N. Taniguchi. Acrolein Induces Inflammatory Response Underlying Endothelial Dysfunction. *Ann. N. Y. Acad. Sci.* 1126:185–189, 2008.
184. Parrott, A. C. Does cigarette smoking cause stress? *Am. Psychol.* 54:817–820, 1999.
185. Patapoutian, A., A. M. Peier, G. M. Story, and V. Viswanath. Sensory systems: ThermoTRP channels and beyond: mechanisms of temperature sensation. *Nat. Rev. Neurosci.* 4:529–539, 2003.
186. Patapoutian, A., S. Tate, and C. J. Woolf. Transient receptor potential channels: targeting pain at the source. *Nat. Rev. Drug Discov.* 8:55–68, 2009.

187. Peltola, V., E. Mäntylä, I. Huhtaniemi, and M. Ahotupa. Lipid Peroxidation and Antioxidant Enzyme Activities in the Rat Testis after Cigarette Smoke Inhalation or Administration of Polychlorinated Biphenyls or Polychlorinated Naphthalenes. *J. Androl.* 15:353–361.
188. Peto, R., Z.-M. Chen, and J. Boreham. Tobacco-the growing epidemic. *Nat. Med.* 5:15–17, 1999.
189. Petrus, M., A. M. Peier, M. Bandell, S. Hwang, T. Huynh, N. Olney, T. Jegla, and A. Patapoutian. A role of TRPA1 in mechanical hyperalgesia is revealed by pharmacological inhibition. *Mol. Pain* 3:40, 2007.
190. Picklo, M. J., and T. J. Montine. Acrolein inhibits respiration in isolated brain mitochondria. *Biochim. Biophys. Acta BBA - Mol. Basis Dis.* 1535:145–152, 2001.
191. Pingle, S. C., J. A. Matta, and G. P. Ahern. Capsaicin Receptor: TRPV1 A Promiscuous TRP Channel. In: Transient Receptor Potential (TRP) Channels, edited by V. Flockerzi, and B. Nilius. Berlin, Heidelberg: Springer Berlin Heidelberg, 2007, pp. 155–171.
192. Planells-Cases, R., P. Valente, A. Ferrer-Montiel, F. Qin, and A. Szallasi. Complex Regulation of TRPV1 and Related Thermo-TRPs: Implications for Therapeutic Intervention. In: Transient Receptor Potential Channels, edited by Md. S. Islam. Dordrecht: Springer Netherlands, 2011, pp. 491–515.
193. Poliak, S., and E. Peles. The local differentiation of myelinated axons at nodes of Ranvier. *Nat. Rev. Neurosci.* 4:968–980, 2003.
194. Proudfoot, C. J., E. M. Garry, D. F. Cottrell, R. Rosie, H. Anderson, D. C. Robertson, S. M. Fleetwood-Walker, and R. Mitchell. Analgesia Mediated by the TRPM8 Cold Receptor in Chronic Neuropathic Pain. *Curr. Biol.* 16:1591–1605, 2006.
195. Rafaels, K., C. R. "Dale" Bass, R. S. Salzar, M. B. Panzer, W. Woods, S. Feldman, T. Cummings, and B. Capehart. Survival risk assessment for primary blast exposures to the head. *J. Neurotrauma* 28:2319–2328, 2011.
196. Raffaeli, W., and E. Arnaudo. Pain as a disease: an overview. *J. Pain Res.* 10:2003–2008, 2017.
197. Reiche, E. M. V., S. O. V. Nunes, and H. K. Morimoto. Stress, depression, the immune system, and cancer. *Lancet Oncol.* 5:617–625, 2004.
198. Rice, A. S. C., and R. G. Hill. New Treatments for Neuropathic Pain. *Annu. Rev. Med.* 57:535–551, 2006.
199. Rice, A. S. C., B. H. Smith, and F. M. Blyth. Pain and the global burden of disease. *Pain* 157:791–796, 2016.
200. Richards, J. S., S. C. Kogos, T. J. Ness, and C. V. Oleson. Effects of smoking on neuropathic pain in two people with spinal cord injury. *J. Spinal Cord Med.* 28:330, 2005.
201. Robles, T. F., R. Glaser, and J. K. Kiecolt-Glaser. Out of balance: A new look at chronic stress, depression, and immunity. *Curr. Dir. Psychol. Sci.* 14:111–115, 2005.
202. Roman, K., M. Yang, and R. L. Stephens. GLT-1 over-expression attenuates bladder nociception and cross-organ sensitization. *FASEB J.* 25:651–3, 2011.
203. Rosenfeld, J. V., A. C. McFarlane, P. Bragge, R. A. Armonda, J. B. Grimes, and G. S. Ling. Blast-related traumatic brain injury. *Lancet Neurol.* 12:882–893, 2013.
204. Rubenstein, R., B. Chang, N. Grinkina, E. Drummond, P. Davies, M. Ruditzky, D. Sharma, K. Wang, and T. Wisniewski. Tau phosphorylation induced by severe closed head traumatic brain injury is linked to the cellular prion protein. *Acta Neuropathol. Commun.* 5:, 2017.

205. Rutland-Brown, W., J. A. Langlois, K. E. Thomas, Y. L. Xi, and others. Incidence of traumatic brain injury in the United States, 2003. *J. Head Trauma Rehabil.* 21:544, 2006.
206. Samkoff, L. M., M. Daras, A. J. Tuchman, and B. S. Koppel. Amelioration of refractory dysesthetic limb pain in multiple sclerosis by gabapentin. *Neurology* 49:304–305, 1997.
207. Schmidt, S., L. C. Kwee, K. D. Allen, and E. Z. Oddone. Association of ALS with head injury, cigarette smoking and APOE genotypes. *J. Neurol. Sci.* 291:22–29, 2010.
208. Scott, J. A., and G. L. King. Oxidative Stress and Antioxidant Treatment in Diabetes. *Ann. N. Y. Acad. Sci.* 1031:204–213.
209. Seal, K. H., D. Bertenthal, D. E. Barnes, A. L. Byers, I. Strigo, and K. Yaffe. Association of Traumatic Brain Injury With Chronic Pain in Iraq and Afghanistan Veterans: Effect of Comorbid Mental Health Conditions. *Arch. Phys. Med. Rehabil.* 98:1636–1645, 2017.
210. Segerstrom, S. C., and G. E. Miller. Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. *Psychol. Bull.* 130:601, 2004.
211. Sg, W. Demyelination in spinal cord injury and multiple sclerosis: what can we do to enhance functional recovery? *J. Neurotrauma* 9 Suppl 1:S105-17, 1992.
212. Sherman, K. B., M. Goldberg, and K. R. Bell. Traumatic brain injury and pain. *Phys. Med. Rehabil. Clin. N. Am.* 17:473–490, viii, 2006.
213. Shi, R., and A. R. Blight. Differential effects of low and high concentrations of 4-aminopyridine on axonal conduction in normal and injured spinal cord. *Neuroscience* 77:553–562, 1997.
214. Shi, R., J. Luo, and M. Peasley. Acrolein inflicts axonal membrane disruption and conduction loss in isolated guinea-pig spinal cord. *Neuroscience* 115:337–340, 2002.
215. Shi, R., B. Muratori, and J. Park. Acrolein as a novel therapeutic target for motor and sensory deficits in spinal cord injury. *Neural Regen. Res.* 9:677, 2014.
216. Shi, Y., W. Sun, J. J. McBride, J.-X. Cheng, and R. Shi. Acrolein induces myelin damage in mammalian spinal cord: Acrolein-mediated myelin damage. *J. Neurochem.* 117:554–564, 2011.
217. Sindrup, S. H., and T. S. Jensen. Efficacy of pharmacological treatments of neuropathic pain: an update and effect related to mechanism of drug action: *Pain* 83:389–400, 1999.
218. Sindrup, S. H., M. Otto, N. B. Finnerup, and T. S. Jensen. Antidepressants in the Treatment of Neuropathic Pain. *BCPT* 96:399–409, 2005.
219. Sn, R., M. Ra, and C. Jn. Peripheral mechanisms of somatic pain. *Anesthesiology* 68:571–590, 1988.
220. Song, S., N. S. Race, A. Kim, T. Zhang, R. Shi, and B. Ziaie. A Wireless Intracranial Brain Deformation Sensing System for Blast-Induced Traumatic Brain Injury. *Sci. Rep.* 5:, 2015.
221. Steptoe, A., and P. J. Feldman. Neighborhood problems as sources of chronic stress: development of a measure of neighborhood problems, and associations with socioeconomic status and health. *Ann. Behav. Med.* 23:177–185, 2001.
222. Stern, R. A., D. O. Riley, D. H. Daneshvar, C. J. Nowinski, R. C. Cantu, and A. C. McKee. Long-term Consequences of Repetitive Brain Trauma: Chronic Traumatic Encephalopathy. *PM&R* 3:S460–S467, 2011.
223. Stevens, J. F., and C. S. Maier. Acrolein: Sources, metabolism, and biomolecular interactions relevant to human health and disease. *Mol. Nutr. Food Res.* 52:7–25, 2008.
224. Story, G. M., A. M. Peier, A. J. Reeve, S. R. Eid, J. Mosbacher, T. R. Hricik, T. J. Earley, A. C. Hergarden, D. A. Andersson, S. W. Hwang, P. McIntyre, T. Jegla, S. Bevan, and A.

Patapoutian. ANKTM1, a TRP-like Channel Expressed in Nociceptive Neurons, Is Activated by Cold Temperatures. *Cell* 112:819–829, 2003.
225. Stret, W. J., R. E. Mrak, and W. S. T Griffin. Microglia and neuroinflammation: a pathological perspective. *Journal of Neuroinflammation* 1–14, 2004.
226. Summers, C. R., B. Ivins, and K. A. Schwab. Traumatic brain injury in the United States: an epidemiologic overview. *Mt. Sinai J. Med. J. Transl. Pers. Med.* 76:105–110, 2009.
227. Sun, W., D. Smith, Y. Fu, J.-X. Cheng, S. Bryn, R. Borgens, and R. Shi. Novel Potassium Channel Blocker, 4-AP-3-MeOH, Inhibits Fast Potassium Channels and Restores Axonal Conduction in Injured Guinea Pig Spinal Cord White Matter. *J. Neurophysiol.* 103:469–478, 2010.
228. Sun, Y., S. Ito, N. Nishio, Y. Tanaka, N. Chen, L. Liu, and K. Isobe. Enhancement of the acrolein-induced production of reactive oxygen species and lung injury by GADD34. *Oxid. Med. Cell. Longev.* 2015:170309, 2015.
229. Svendsen, K. B., T. S. Jensen, H. J. Hansen, and F. W. Bach. Sensory function and quality of life in patients with multiple sclerosis and pain. *Pain* 114:473–481, 2005.
230. Tai, C., S. Zhu, and N. Zhou. TRPA1: The Central Molecule for Chemical Sensing in Pain Pathway? *J. Neurosci.* 28:1019–1021, 2008.
231. Thorburn, K. C., J. W. Paylor, C. A. Webber, I. R. Winship, and B. J. Kerr. Facial hypersensitivity and trigeminal pathology in mice with experimental autoimmune encephalomyelitis: *PAIN* 157:627–642, 2016.
232. Tominaga, M., M. J. Caterina, A. B. Malmberg, T. A. Rosen, H. Gilbert, K. Skinner, B. E. Raumann, A. I. Basbaum, and D. Julius. The Cloned Capsaicin Receptor Integrates Multiple Pain-Producing Stimuli. *Neuron* 21:531–543, 1998.
233. Tominaga, M., M. Wada, and M. Masu. Potentiation of capsaicin receptor activity by metabotropic ATP receptors as a possible mechanism for ATP-evoked pain and hyperalgesia. *Proc. Natl. Acad. Sci.* 98:6951–6956, 2001.
234. Torrance, N., A. M. Elliott, A. J. Lee, and B. H. Smith. Severe chronic pain is associated with increased 10 year mortality. A cohort record linkage study. *Eur. J. Pain Lond. Engl.* 14:380–386, 2010.
235. Torrance, N., B. H. Smith, M. I. Bennett, and A. J. Lee. The epidemiology of chronic pain of predominantly neuropathic origin. Results from a general population survey. *J. Pain* 7:281–289, 2006.
236. Treede, R.-D., T. S. Jensen, J. N. Campbell, G. Cruccu, J. O. Dostrovsky, J. W. Griffin, P. Hansson, R. Hughes, T. Nurmikko, and J. Serra. Neuropathic pain: Redefinition and a grading system for clinical and research purposes. *Neurology* 70:1630–1635, 2008.
237. Trevisani, M., J. Siemens, S. Materazzi, D. M. Bautista, R. Nassini, B. Campi, N. Imamachi, E. Andre, R. Patacchini, G. S. Cottrell, R. Gatti, A. I. Basbaum, N. W. Bunnett, D. Julius, and P. Geppetti. 4-Hydroxynonenal, an endogenous aldehyde, causes pain and neurogenic inflammation through activation of the irritant receptor TRPA1. *Proc. Natl. Acad. Sci.* 104:13519–13524, 2007.
238. Tully, M., and R. Shi. New Insights in the Pathogenesis of Multiple Sclerosis—Role of Acrolein in Neuronal and Myelin Damage. *Int. J. Mol. Sci.* 14:20037–20047, 2013.
239. Tully, M., L. Zheng, G. Acosta, R. Tian, and R. Shi. Acute systemic accumulation of acrolein in mice by inhalation at a concentration similar to that in cigarette smoke. *Neurosci. Bull.* 30:1017–1024, 2014.

240. Turk, D. C., H. D. Wilson, and A. Cahana. Treatment of chronic non-cancer pain. *Lancet Lond. Engl.* 377:2226–2235, 2011.
241. Turner, R. J., B. Wheaton, and D. A. Lloyd. The epidemiology of social stress. *Am. Sociol. Rev.* 104–125, 1995.
242. Uchida, K., M. Kanematsu, Y. Morimitsu, T. Osawa, N. Noguchi, and E. Niki. Acrolein Is a Product of Lipid Peroxidation Reaction: formation of free acrolein and its conjugate with lysine residues in oxidized low density lipoproteins. *J. Biol. Chem.* 273:16058–16066, 1998.
243. Uchida, K., M. Kanematsu, K. Sakai, T. Matsuda, N. Hattori, Y. Mizuno, D. Suzuki, T. Miyata, N. Noguchi, E. Niki, and others. Protein-bound acrolein: potential markers for oxidative stress. *Proc. Natl. Acad. Sci.* 95:4882–4887, 1998.
244. Uum, S. H. M. V., B. Sauvé, L. A. Fraser, P. Morley-Forster, T. L. Paul, and G. Koren. Elevated content of cortisol in hair of patients with severe chronic pain: A novel biomarker for stress. *Stress* 11:483–488, 2008.
245. Vaishnav, R. A., I. N. Singh, D. M. Miller, and E. D. Hall. Lipid Peroxidation-Derived Reactive Aldehydes Directly and Differentially Impair Spinal Cord and Brain Mitochondrial Function. *J. Neurotrauma* 27:1311–1320, 2010.
246. Valsecchi, A. E., S. Franchi, A. E. Panerai, A. Rossi, P. Sacerdote, and M. Colleoni. The soy isoflavone genistein reverses oxidative and inflammatory state, neuropathic pain, neurotrophic and vasculature deficits in diabetes mouse model. *Eur. J. Pharmacol.* 650:694–702, 2011.
247. Verne, I. T. Referred pain from skeletal structures. *LWW* 99:660–667, 1944.
248. Vincent, A. M., J. W. Russell, P. Low, and E. L. Feldman. Oxidative stress in the pathogenesis of diabetic neuropathy. *Endocr. Rev.* 25:612–628, 2004.
249. Vitkovic, L., J. Bockaert, and C. Jacque. "Inflammatory" Cytokines: Neuromodulators in Normal Brain? *J. Neurochem.* 74:457–471, 2001.
250. Wall, P. D., and M. Devor. Sensory afferent impulses originate from dorsal root ganglia as well as from the periphery in normal and nerve injured rats. *Pain* 17:321–339, 1983.
251. Wallace, D. C. Mitochondrial Diseases in Man and Mouse. *Science* 283:1482–1488, 1999.
252. Wallace, D. C. Mouse models for mitochondrial disease. *Am. J. Med. Genet.* 106:71–93, 2001.
253. Walls, M. K., N. Race, L. Zheng, S. M. Vega-Alvarez, G. Acosta, J. Park, and R. Shi. Structural and biochemical abnormalities in the absence of acute deficits in mild primary blast-induced head trauma. *J. Neurosurg.* 124:675–686, 2016.
254. Wang, H. Bradykinin Produces Pain Hypersensitivity by Potentiating Spinal Cord Glutamatergic Synaptic Transmission. *J. Neurosci.* 25:7986–7992, 2005.
255. Wang, S., Y. Dai, T. Fukuoka, H. Yamanaka, K. Kobayashi, K. Obata, X. Cui, M. Tominaga, and K. Noguchi. Phospholipase C and protein kinase A mediate bradykinin sensitization of TRPA1: a molecular mechanism of inflammatory pain. *Brain* 131:1241–1251, 2007.
256. Wang, T., Y. Liu, L. Chen, X. Wang, X.-R. Hu, Y.-L. Feng, D.-S. Liu, D. Xu, Y.-P. Duan, J. Lin, X.-M. Ou, and F.-Q. Wen. Effect of sildenafil on acrolein-induced airway inflammation and mucus production in rats. *Eur. Respir. J.* 33:1122–1132, 2009.
257. Watkins, L. R., S. F. Maier, and L. E. Goehler. Immune activation: the role of pro-inflammatory cytokines in inflammation, illness responses and pathological pain states: *Pain* 63:289–302, 1995.

258. Waxman, S. G., D. A. Utzschneider, and J. D. Kocsis. Chapter 29 Enhancement of action potential conduction following demyelination: experimental approaches to restoration of function in multiple sclerosis and spinal cord injury. In: Progress in Brain Research. Elsevier, 1994, pp. 233–243.
259. Weis, F., E. Kilger, B. Roozendaal, J.-F. Dominique, P. Lamm, M. Schmidt, M. Schmölz, J. Briegel, and G. Schelling. Stress doses of hydrocortisone reduce chronic stress symptoms and improve health-related quality of life in high-risk patients after cardiac surgery: a randomized study. *J. Thorac. Cardiovasc. Surg.* 131:277–282, 2006.
260. White, F. A., S. K. Bhangoo, and R. J. Miller. Chemokines: integrators of pain and inflammation. *Nat. Rev. Drug Discov.* 4:834, 2005.
261. White, F. A., P. Feldman, and R. J. Miller. Chemokine signaling and the management of neuropathic pain. *Mol. Interv.* 9:188, 2009.
262. Wiffen, P. J., S. Collins, H. J. McQuay, D. Carroll, A. Jadad, and R. A. Moore. Anticonvulsant drugs for acute and chronic pain. In: Cochrane Database of Systematic Reviews, edited by The Cochrane Collaboration. Chichester, UK: John Wiley & Sons, Ltd, 2005.
263. Wood, P. B. Stress and dopamine: implications for the pathophysiology of chronic widespread pain. *Med. Hypotheses* 62:420–424, 2004.
264. Wood, P. B. Stress and dopamine: implications for the pathophysiology of chronic widespread pain. *Med. Hypotheses* 62:420–424, 2004.
265. Woodbury, C. J. Nociceptors Lacking TRPV1 and TRPV2 Have Normal Heat Responses. *J. Neurosci.* 24:6410–6415, 2004.
266. Woolf, C. J. Somatic pain--pathogenesis and prevention. *Br. J. Anaesth.* 75:169–176, 1995.
267. Woolf, C. J., and R. J. Mannion. Neuropathic pain: aetiology, symptoms, mechanisms, and management. *The Lancet* 353:1959–1964, 1999.
268. Xu, Q., and T. L. Yaksh. A brief comparison of the pathophysiology of inflammatory versus neuropathic pain. *Curr. Opin. Anaesthesiol.* 24:400–407, 2011.
269. Yoshida, M., H. Tomitori, Y. Machi, M. Hagihara, K. Higashi, H. Goda, T. Ohya, M. Niitsu, K. Kashiwagi, and K. Igarashi. Acrolein toxicity: Comparison with reactive oxygen species. *Biochem. Biophys. Res. Commun.* 378:313–318, 2009.
270. Yu, S., and A. Ouyang. TRPA1 in bradykinin-induced mechanical hypersensitivity of vagal C fibers in guinea pig esophagus. *AJP Gastrointest. Liver Physiol.* 296:G255–G265, 2008.
271. Zheng, L., J. Park, M. Walls, M. Tully, A. Jannasch, B. Cooper, and R. Shi. Determination of Urine 3-HPMA, a Stable Acrolein Metabolite in a Rat Model of Spinal Cord Injury. *J. Neurotrauma* 30:1334–1341, 2013.
272. Zhong, L., A. Bellemer, H. Yan, K. Honjo, J. Robertson, R. Y. Hwang, G. S. Pitt, and W. D. Tracey. Thermosensory and Nonthermosensory Isoforms of Drosophila melanogaster TRPA1 Reveal Heat-Sensor Domains of a ThermoTRP Channel. *Cell Rep.* 1:43–55, 2012.
273. Zimprich, A., L. Garrett, J. M. Deussing, C. T. Wotjak, H. Fuchs, V. Gailus-Durner, M. H. de Angelis, W. Wurst, and S. M. Hölter. A robust and reliable non-invasive test for stress responsivity in mice. *Front. Behav. Neurosci.* 8:125, 2014.

VITA

Personal information

Last names	Cruz Haces
First name	Marcela
Nationality	Mexican
Marital status	Married, no children

Education

2015-Aug 2019	Ph.D. in Biomedical Engineering at Purdue University. Thesis topic: Mechanisms of Neuropathic Pain following mild blast Traumatic Brain Injury and Chronic Stress. Expected graduation on August 2019.
2014	B.S. Biotechnology Engineer from Instituto Tecnológico de Estudios Superiores de Monterrey, Campus Monterrey, Grade 91/100, Book 32 Folio 82408.
2014	"International Undergraduate Program in B.S. Biotechnology Engineering" Diploma from Instituto Tecnológico de Estudios Superiores de Monterrey, with more than 33% of the lectures in a language different than Spanish, one year of study abroad, a score higher than 600 in the TOEFL Test and a constant grade greater than 87/100, Book 32 Folio 82408.
2014	Honorable Mention from Instituto Tecnológico de Estudios Superiores de Monterrey due to academic distinction in the B.S. Biotechnology Engineering, Number 1413 Folio 172.

2014 Honors Diploma from Instituto Tecnológico de Estudios Superiores de Monterrey, as distinguished student of the B.S. Biotechnology Engineering, Code 1413 Folio 005.

2014 Certificate of Molecular Biology from Instituto Tecnológico de Estudios Superiores de Monterrey, Number 1413 Folio 046.

2014 Student Development Diploma from Instituto Tecnológico de Estudios Superiores de Monterrey, for constant participation in formative activities such as cultural diffusion and sports.

2013-2014 Study Abroad Experience in Process Engineering at the Hochschule Offenburg University; Offenburg, Germany.

2013 High Performance Academic Program "Innovation Sphere" Diploma from Instituto Tecnológico de Estudios Superiores de Monterrey and The DOW Chemical Company.

Academic conferences

2017, 2018 IU/PU Joint Symposium on Spinal Cord Injury and Brain Injury Research attendance.

2017 Purdue Institute for Integrative Neuroscience Summer Retreat. Poster: "Mechanisms of Neuropathic Pain following a Mild Blast Traumatic Brain Injury".

2017 Purdue Biomedical Engineering Summer Seminar. Presentation: "Mechanisms of Neuropathic Pain following Physical and Psychological Trauma".

2017 Mass Spectrometry Applications to the Clinical Lab Conference. Poster: "Discriminating Lipid and Metabolite Distribution from Mild Blast Traumatic Brain Injury using DESI and High-Resolution Mass Spectrometry".

2017	American Society for Mass Spectrometry Conference. Poster: "Identification of Biomarkers for Mild Traumatic Brain Injury in Tissue and Biological Fluids using DESI and PaperSpray Ionization with HRAM".
2016	National Neurotrauma Society Conference, Lexington, KY. Poster: "Dimercaperol provides neuroprotection in acrolein-exposed PC-12 cells and rats contusive spinal cord injury".
2015-2016	Purdue Biomedical Engineering Seminar attendance.

Work experience

2014	Internship in BASF; Ludwigshafen, Germany. Activity: Investigation Project in fermenting and mixing for a patent.
2012-2013	Innovation Project with DOW and Proactiva; Monterrey, Nuevo León, México. Activity: Use of solar energy in land fields for a patent.

Languages

Spanish	Native Language
English	620/670 in the TOEFL Test
German	TestDaF: 4 Reading, 5 Listening, 5 Writing, 4 Speaking (Level C1)
French	B1

Skills

Animal handling, injections, pain testing with von Frey, heat sensitivity and cold sensitivity with acetone, rotarod test, urine collection, anesthesia, surgery, mild blast traumatic brain injury induction, spinal cord injury surgery, stress induction, euthanasia, tissue collection, Western Blotting, Immunohistochemistry, RT-PCR, mass spectrometry, Microsoft Word, Excel, PPT, Flash Player, Corel Draw, Visual Basic, C++, HTML.

PUBLICATIONS

Cruz-Haces, M., J. Tang, G. Acosta, J. Fernandez, and R. Shi. Pathological correlations between traumatic brain injury and chronic neurodegenerative diseases. *Translational Neurodegeneration* 6, 2017.

Garcia-Gonzalez, D., N. Race, N. Voets, D. Jenkins, S. Sotiropoulos, G. Acosta, M. Cruz-Haces, J. Tang, R. Shi, and A. Jérusalem. Cognition based bTBI mechanistic criteria; a tool for preventive and therapeutic innovations. *Scientific Reports* 8, 2018.

Cruz-Haces, M., S. Herr, J. Tang and R. Shi. Hydralazine treatment reduces hypersensitivity, acrolein, TRPA1 and MCP1 protein expressions in a rat model of stress. Manuscript in preparation, 2019.

Cruz-Haces, M., S. Herr, J. Tang and R. Shi. Hydralazine treatment reduces hypersensitivity, acrolein, TRPA1 and MCP1 protein expressions in a rat model of mild blast traumatic brain injury. Manuscript in preparation, 2019.

Cruz-Haces, M., S. Herr, J. Tang and R. Shi. Hydralazine treatment reduces hypersensitivity, acrolein, TRPA1 and MCP1 protein expressions in a comorbidity rat model of mild blast traumatic brain injury and stress. Manuscript in preparation, 2019.