Central and Peripheral Nervous System Sequelae Following Blast Exposures: A Multi-Organ System Analysis of a Complex Full-Body Injury

Britahny M Baskin

A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2023

Reading Committee:

Abigail Schindler, Chair

Rebecca Hendrickson

David Cook

Program Authorized to Offer Degree:

Neuroscience

University of Washington

Abstract

Central and Peripheral Nervous System Sequelae Following Blast Exposures: A Multi-Organ System Analysis of a Complex Full-Body Injury

Britahny M Baskin

Chair of the Supervisory Committee:

Abigail Schindler

Department of Pharmacology

Many injuries have the capacity to impact the entire body, even if the inciting incident was something seemingly localized to one body part, like a concussion. Injuries sustained following blast overpressure waves (such as in the case of exposure to improvised explosive devices (IED), building collapses, etc.) however, due to their mechanism of action, don't just start in one location and spread to the rest of the body, but occur as the result of the overpressure wave hitting the entire body at the same time. Thus, the focus of my dissertation is to examine the impact of blast injuries on the entire body instead of narrowing down one particular portion of the system. I accomplish this by 1) analyzing a broad range of central and peripheral biomarkers and behavioral changes in both male and female mice at multiple timepoints following blast exposures 2) analyze higher level cognitive

functioning following blast exposure at a chronic time point and 3) use our mouse model of blast injuries to gain further insight into a clinical disorder seen in a large portion of blast-exposed veterans. Taken together, these experiments detail the full-body sequelae that often co-occurs with blast-induced mild traumatic brain injuries (mTBI).

The main conclusion of all the studies presented in this dissertation is the finding that TBIs caused by blast overpressure waves can be particularly detrimental to daily functioning. Both males and females exhibited changes in pro- and anti-inflammatory cytokine expression and gut microbiome flora (some changes were similar and others dissimilar) with specific bacteria tracking with increased blood-brain behavior permeability, cytokine expression, and behaviors following blasts, suggesting potential targets for biomarkers. Chronic deficits in PTSD-related phenotypes occurred only in males at the chronic timepoint and in other experiments done in males alone, blast male mice expressed increased motivation for natural rewards, cognitive inflexibility and increased risky alcohol consumption, the latter of which mimicked risky drinking behaviors in male Veterans with a history of blast mTBI diagnoses. Therefore, this dissertation demonstrates that the impact of a blast exposure can be seen across a broad range of behaviors and blast has a negative impact on multiple different organ systems in the body, from the brain to vasculature structure, circulating inflammatory cytokines, to the gastrointestinal tract.

TABLE OF CONTENTS

LIST OF FIGURES

ACKNOWLEDGEMENTS

Thank you to first and foremost to Abbie (Dr. Abigail Schindler). You are the PI everyone wishes they had. There is no contest. I will forever be grateful for the incredible amount of support and guidance when I needed it most. Nothing was ever just a checkmark to you. You truly are the PI that I aspire to be; someone who both embodies work-life balance and encourages everyone in the lab to do the same. You are the change we all want to see in academia, and I can only hope that we can continue our friendship and mentorship long past my defense.

Thank you to my committee Drs David Cook, John Neumaier, Rebecca Hendrickson, and Ben Land. Dave, you always pushed me to defend my ideas and to consider practical obstacles that could occur in my research which made me stronger at arguing my cases. John, thank you for allowing me to learn cellular and molecular techniques in your lab when I had no background in them and for giving me a side job, a car to borrow, and introducing Yoshi to his best friend in Seattle. We will surely miss Fritz. Rebecca, thank you not only for being the person I knew that I could count on to make sure I was aware of the clinical work, but also for all the covid research you do. You make a difference in so many ways. And thank you Ben for being the two-in-one GSR aka a GSR that ends up asking the hard questions. From our first chat, you gave me so many things to think about and really pressed me to think about what reviewers will think of which is a skill I'm grateful for.

I'd also like to thank the Neumaier lab members, past and present, in particular Marjorie, Alec, Atom, and David; you were like a second lab to me in many ways. To my favorite people in Seattle, Rachael and Ellen. You were ears when I needed to rant, were supportive when I needed a push, helped me to make hard decisions, and showed up for me in more ways than I could count.

To my home base on the east coast: Mom, Dad, Emily, Chloe and Drew. Thank you for supporting me throughout this journey. Chloe, you've truly been with me from day one and I can't imagine falling in love with research in the same way if I hadn't been working with you as an undergrad. Emily, you have always been like family to me and have given me the support that only chosen family could.
And finally, thank you to all the doctors, therapists, and specialists who truly kept me going throughout my dissertation.

DEDICATION

This dissertation is dedicated to two groups.

First, it is dedicated to anyone living with the effects of trauma regardless of the cause or how it manifests. I conducted this research during a point at which my mental health was at an all-time low. My only hope is that this research moves us forward and that those who are in a dark place, including those during their PhD, are able to get the help and support they deserve.

It is also dedicated to the animals; the ones I worked with prior to grad school, the ones I worked with at UW, and the ones I will work with in the future. None of our work would be possible without them. They deserve to be treated with care and respect and all animal researchers should conduct our work constantly keeping this in mind.

Chapter 1.

Introduction to Blast Traumatic Brain Injuries, Outcomes of Blast Exposures, and The Biochemical Changes that Follow Blast mTBIs

Traumatic Brain Injuries

Most people know someone who has had a minor concussion, whether it be caused by a fall, a sports injury, or a car accident. In most cases, these people will recover and return to life as normal, not focusing on how this injury has impacted them. In fact, as is the case with many people who sustain sub-concussions or minor concussions, many won't even seek medical treatment because they may perceive the injury as minor, disclosure may impact their ability to play a sport or work, or they are impacted by social constructs related to attributes like "toughness" (Redmond et al., 2015; Ruff et al., 2009; Sanderson et al., 2017; Tallapragada & Cranmer, 2022). However, concussions, also known as traumatic brain injuries (TBI), are currently a leading cause of death and disability not just in the United States but globally (Johnson & Griswold, 2017; A. I. Maas et al., 2008; Taylor et al., 2017). TBIs affect every segment of the population and can often lead to significantly decreased quality of life and increased financial burden for both the person with a TBI and their caregiver(s) (Di Battista et al., 2012; Hendrickson et al., 2018; Malec et al., 2017; Ozga et al., 2018). The majority of TBIs (70-90%) are classified as mild (Gardner & Yaffe, 2015; Narayan et al., 2002) using the Glasgow Coma Scale, a tool typically used to assess the extent of impaired consciousness immediately following a TBI which bases it's score on three

aspects of responsiveness: eye-opening, motor, and verbal responses. Yet mild comes off as a misnomer in the case of TBIs because mild traumatic brain injuries (mTBI) often result in a constellation of physical, cognitive, sensorimotor, affective, and behavioral disturbances that may persist months to years after the initial injury. Though awareness of mTBI and the resulting adverse health outcomes has increased in the past few decades, particularly through research articles, movies and news focusing on concussions sustained during sports injuries (Beidler et al., 2020; Bell et al., 2019; Sarmiento et al., 2014; Tallapragada & Cranmer, 2022), few interventions exist that address post-concussive symptoms and behavioral dysfunction either acutely following injury, and for subsequent chronic health problems that which may be hard to identify and treat.

Blast Traumatic Brain Injuries

Blast overpressure waves are the propagation of a sudden strong change in atmospheric pressure such as those caused by improvised explosive devices (IEDs), explosions related to building collapses and failing infrastructure, and industrial accidents, and are becoming an increasingly common cause of TBI (Salem et al., 2017; Song et al., 2018; Wolf et al., 2009). In fact, blast exposure (via detonation of high explosives; most commonly repetitive in nature) is the primary source of mTBI in Veterans, a significant driver of PTSD in this group, and a major source of morbidity among Veterans enrolled in the VA health care system (*DOD TBI Worldwide Numbers*, n.d.; Elder & Cristian, 2009; Peskind et al., 2011) due to increased use of IEDs in war.

Additionally, since improvements in body armor have occurred over time, more soldiers that may have once died in combat are coming home with mTBI sustained from repetitive blast exposures. Futhermore, even if their armor enables them to survive deployment, the helmets designed to protect Servicemembers, were designed to protect from projectile head trauma not blast-related injuries, and may even have been exacerbating blast-related injuries (Ganpule et al., 2012; Grujicic et al., 2011; Kulkarni et al., 2013; Moss et al., 2009).

This has led to mTBI being referred to as the “signature injury” in the conflicts in Iraq and Afghanistan (OEF/OIF/OND) (*DOD TBI Worldwide Numbers*, n.d.; Hendrickson et al., 2018; Tanielian et al., 2008). Rates of mTBI post-deployment are estimated at 10-25% (approximately 380,000 Veterans diagnosed with mTBI since 2000), with PTSD comorbidity estimated at 50-75% (*DOD TBI Worldwide Numbers*, n.d.; Hendrickson et al., 2018; Owens et al., 2008). Further, war does not only impact or even target soldiers, and for every police or soldier injured or killed by a blast exposure, ~4 civilians are injured or killed (Edwards et al., 2012; Overton, 2020) many of which are women and children, a group underrepresented in blast TBI research (Edwards et al., 2012; Tovar et al., 2021). Thus, the research in pre-clinical models of blast exposures have relevance both to military personnel impacted by war but also, as mentioned previously, civilians exposed to events including explosions from failing infrastructure and industrial accidents. Additionally, women have been serving in military positions with increased risk of blast trauma since 2016 yet there are no published reports examining sex as a biological variable in models of repetitive blast mTBI, potentially limiting diagnosis and treatment. Finally, because of the mechanism

of blast TBIs, where the primary injury is caused by the blast overpressure wave and not the skull coming into contact with another physical object, many who sustain blast TBI may not even know it, especially if it is “mild” in terms of the Glascow Coma Scale. This may cause people to not seek treatment right away and once they do seek treatment, might not know to bring it up. Further, in the case of veterans, if a blast exposure is mild, they likely will not take a moment of their day to care for it, and just continue as planned, particularly since military culture and values may dissuade Servicemembers and Veterans (Lindberg et al., 2021; Redmond et al., 2015). Additionally, many brief screening assessments such as the Brief Traumatic Brain Injury Screen (BTBIS) only ask about recent or deployment related TBIs and may miss a prior history of TBI during the assessment (Betthauser et al., 2019) which is concerning because repetitive TBIs are likely to cause more neurological damage. Additionally, the odds of being diagnosed with a deployment-related TBI is 8 times higher during the first 4 weeks post-deployment when compared to the subsequent 32 weeks, and the likelihood, decreases even more after 32 weeks (Regasa et al., 2019). Yet, the majority of veterans that participate in clinical research received their blast exposures years before enrollment; all of which makes it difficult for us to determine what is taking place acutely following blast and prevents early interventions from occurring.

Alcohol Use

TBI has been historically linked to alcohol use however previously, the general view of comorbid TBIs and substance use is that substance use predates the

existence of a TBI and can be a cause of TBIs, primarily through violence, motor vehicle accidents and falls (Bogner et al., 2001; Corrigan, 1995). Conversely, now we know that a history of mTBI is associated with increased health risk behaviors (e.g., sensation/novelty seeking, impulsivity, risk taking, irritability/aggression, (Elder & Cristian, 2009; Halbauer et al., 2009; Hendrickson et al., 2018; Miller et al., 2013; Olson-Madden et al., 2012; Peskind et al., 2011; Petrie et al., 2014; A. G. Schindler et al., 2017; Seidl et al., 2015; Tanielian et al., 2008) and substance use/misuse (e.g., alcohol use disorder (AUD) (Adams et al., 2012; Grossbard et al., 2017; Miller et al., 2013; Petrie et al., 2014; A. G. Schindler et al., 2017). Comorbid alcohol use could potentially compound negative outcomes following blast mTBI yet much research does not address this relationship however because clinical studies examining the effects of TBI on cognition often exclude applicants with a history of a substance use disorder and vice versa (Bjork & Grant, 2009; Saxon, 2011).

In fact, a common outcome of blast mTBI is increased alcohol use, problematic alcohol use is increased in Veterans with TBI than those without TBI (Grossbard et al., 2017), and this alcohol use can exacerbate the previously mentioned negative effect mTBI have on executive function and memory. Servicemembers are already at a higher risk of adverse consequences from substance use (Bray et al., 2010; Norman et al., 2014) with 84.5% of those in active-duty reporting alcohol use and over 25% reporting moderate to heavy use (Barlas et al., 2013), whereas only 16% of civilians reporting moderate to heavy use. This heavy drinking isn't limited to active duty Servicemembers but Veterans continue to drink significantly following their military term (Balan et al., 2013; Chermack et al., 2012) sometimes using alcohol to self-

medicate symptoms such as headache, fatigue, and sleep, (Derefinko et al., 2018), symptoms that overlap with mTBI sequelae. In fact, in a study looking at mTBI in almost two million discharged military personnel, the relative risk of discharge due to AUD was 2.6 times higher for those who had sustained a mTBI (n=1778) (Ommaya et al., 1996).

What complicates the role that alcohol plays in mTBI recovery is that there exists a bidirectional relationship between heavy alcohol use and mTBI. First those who have a history of heavy alcohol use are more likely to sustain a mTBI and those who sustain a mTBI are likely to have heavy alcohol use post-injury, with one longitudinal study of subjects sustaining a mTBI at age 5 or younger, finding that there was 3.6 times more likely to experience heavy substance use in adolescence, many years following the injury (McKinlay et al., 2009). Further, a history of risky alcohol use prior to mTBI correlates with poorer outcomes following mTBI (Dikmen et al., 1993; Ruff et al., 1990) and alcohol use post-injury negatively impacts recovery from cognitive impairment (Gontkovsky et al., 2006). The research within, offers a controlled way to look at repetitive blast mTBIs in animal without a prior history of heavy alcohol use or previous mTBIs so that we can analyze if blast mTBI alone will increase alcohol intake, and compare this alcohol use to that reported in Veteran populations with or without blast mTBI.

Executive Dysfunction

Beyond risky behaviors, the most common complaints that Veterans have when presenting to the VA are related to executive dysfunction. The umbrella term "executive function" refers to a broad group of cognitive processes that are often considered "higher order capabilities" necessary for cognitive control and motivated behavior. Although there is some debate over the number of cognitive functions that make up executive function, the following are examples of capabilities that are considered executive functions: working memory, self-monitoring, planning and prioritizing, organization, initiating tasks, cognitive flexibility, and impulsive control. These functions allow people to continually adapt to a changing environment, make and pursue goals, and are integral to productivity. Thus, proper executive function is necessary for daily life including but not limited to, work-related activities, independent living, functioning at all levels of schooling, and social interactions, and impaired executive function (executive dysfunction or dysexecutive syndrome), can negatively impact life and well-being. The capabilities related to executive function are primarily controlled by the prefrontal cortex but also involve areas such as the nucleus accumbens and cerebellum, all of which are often impacted following blast exposures.

Executive dysfunction, i.e. problems with the following higher order capabilities, has been widely documented in patients with a history of mTBI, including patients with a history of blast-induced mTBI (Amick et al., 2013; Hendrickson et al., 2018; Karr et al., 2019; Pagulayan et al., 2018, 2020; A. G. Schindler et al., 2017; Sullivan et al., 2018), and are one of the most commonly reported persistent symptoms in patients with a history of mTBI (McDonald et al., 2002; McInnes et al., 2017; Rabinowitz & Levin, 2014). Some commonly reported issues with executive dysfunction that are

reported to follow TBI include impulsively, working memory, and learning difficulties. Executive dysfunction is considered one of the most debilitating outcomes following mTBI injuries, because as previously mentioned, executive dysfunction often leads to impaired daily functioning (Alexander, 1995) and decreased quality of life (Hendrickson et al., 2018; McDonald et al., 2002; McInnes et al., 2017; Ozga et al., 2018). Even in those who do not present with comorbid PTSD, prolonged stress, such as stress that would be present during deployment, negatively impacts neuropsychological functioning (Vasterling et al., 2006; Vasterling & Proctor, 2011), and prolonged stress combined with TBI has been associated with poorer recovery. Further, in age-matched groups with similar TBI severities (~90% mild, ~10% moderate), military participants with blast TBIs performed more poorly on a task of inhibitory control than civilians who had sustained blunt TBIs and had exhibited fMRI hyperactivation in areas related to inhibition and attention modulation of visual cues when they were unable to inhibit a response (Fischer et al., 2014).

Although behavioral deficits related to executive dysfunction have been studied in rodent models of moderate and severe TBI (Modrak et al., 2020; Ozga et al., 2018; Vonder Haar et al., 2014; Vonder Haar & Winstanley, 2016) the research described in this thesis is the first to examine executive dysfunction following repetitive blasts that induced TBI in the mild range.

Biochemical Biomarkers

A large focus of the research on mTBI following blast exposure has focused on damage done directly to the brain itself. One study using diffusion tensor imaging to determine white matter integrity found that a history of blast mTBI was associated with a diffuse, global pattern of lower white matter integrity with increased blast exposures correlating to larger white matter disruption when compared to deployed controlled without history of blast exposures (Davenport et al., 2012). An fMRI study comparing participants in the military with a history of blast mTBI to civilians with blunt TBIs found that the blast TBI cohort exhibited hyperactivation in bilateral inferior temporal and fusiform gyri inactivation compared to non-TBI military controls when failing to inhibit responses on an inhibition task (Stop Signal Task) and under activation when they were able to correctly inhibit a response compared to when they correctly inhibited (Fischer et al., 2014). Yet a review on neuroimaging in patients with a history of blast mTBI found mixed results due to difference in samples, design, and measurements (Caplan et al., 2017).

Because of the technology necessary to examine the pathophysiology associated with mTBI in patients presenting to clinicians and clinical researchers (Caplan et al., 2017; Salat et al., 2017) and their limitations, a lot of the evidence we have on anatomical dysfunction, and biochemical changes comes from animal models or analyzing post-mortem tissue. Animal models have replicated some of the results and expanded on the imaging results in humans finding evidence of axonal injury and neuronal death, increased BBB permeability, and mitochondrial degeneration in areas such as the cortex, hippocampus, and striatum (Garman et al., 2011; Song et al., 2018). Although there have been attempts to find a biomarker for blast mTBI to create

a biosensor to monitor troops for concussive effects following blast, so far, no biomarkers for blast mTBI exist (Engel et al., 2019).

One mechanism believed to contribute to the development of post-concussive symptoms (PCS; e.g., headaches, dizziness, fatigue, sensorimotor impairment), and behavioral dysfunction (e.g., anxiety, depression, impulsivity) following mTBI is mTBI-induced autonomic system dysfunction, or dysautonomia, which is expressed by changes in heart rate variability (HRV), blood pressure, pupillary dynamics, and problems with the gastrointestinal (GI) system (Esterov & Greenwald, 2017; Howard et al., 2018; Swai et al., 2019). Likewise, acute and chronic inflammatory responses have been demonstrated in mild to severe TBI (Fan et al., 1996; Perez-Polo et al., 2013b; Shohami et al., 1999), and correlate with autonomic and behavioral dysfunction (Khalid et al., 2019; Perez-Polo et al., 2013a; Ramlackhansingh et al., 2011). While much work has focused on developing and evaluating therapeutics that target the central nervous system following blast exposure and mTBI, less attention has been placed on understanding and developing treatments that target peripheral systems for potential therapeutic application.

Chapter 2.

Pre-Clinical Models of Repetitive Blast Exposures Give Insight Into Mechanisms Underlying Risky Drinking Behaviors in Blast Exposed Combat Veterans*

*This chapter was formatted for this thesis from the following peer-reviewed publication:

"Repetitive blast mild traumatic brain injury increases ethanol sensitivity in male mice and risky drinking behavior in male combat veterans." Schindler, A. G., Baskin, B., Juarez, B., Lee, S. J., Hendrickson, R., Pagulayan, K., Zweifel, L.S., Raskind, M.A., Phillips, P.E., Peskind, E.R. & Cook, D. G. (2021) *Alcoholism: clinical and experimental research. 45(5), pp.1051-1064.*

B.M.B helped designed, performed, and analyzed the experiments done on the mice in the paper and helped write the paper. (Work started as part of rotation)

Introduction

Traumatic brain injury (TBI) affects every segment of the population and can lead to significantly decreased quality of life and social and occupational functioning. This is particularly important for Veterans, who experience disproportionate rates of physical and mental illness compared with civilian counterparts (Hoerster et al., 2012; Trivedi et al., 2020). Critically, US Veterans of the conflicts in Iraq and Afghanistan (Operation Enduring Freedom/Operation Iraqi Freedom/Operation New Dawn (OEF/OIF/OND)) exhibit especially high rates of mild traumatic brain injury (mTBI; labeled the "signature injury" of these conflicts)(*DOD TBI Worldwide Numbers*, n.d.; Hoge et al., 2008; Hoge & Cotting, 2004; Tanielian et al., 2008). OEF/OIF/OND mTBI rates are estimated at 10–25% (approximately 400,000 Veterans diagnosed since 2000); most commonly repetitive in nature, blast exposure (via detonation of high explosives) is the primary source of mTBI (blast-mTBI) in this population, and a major source of morbidity among Veterans enrolled in the VA health care (Hendrickson et al., 2018; Tanielian et al., 2008; Wenger et al., 2018). A history of mTBI is associated with increased health risk behaviors (e.g., sensation/novelty seeking, impulsivity, risk taking, irritability/aggression, (Elder & Cristian, 2009; Halbauer et al., 2009; Hendrickson et al., 2018; Miller et al., 2013; Olson-Madden et al., 2012; Peskind et al., 2011; Petrie et al., 2014; A. G. Schindler et al., 2017; Seidl et al., 2015; Tanielian et al., 2008) and substance use/misuse (e.g., alcohol use disorder (AUD) (Adams et al., 2012; Grossbard et al., 2017; Miller et al., 2013; Petrie et al., 2014), potentially compounding negative outcomes following injury/trauma, but how mTBI and substance use might interact to

promote addiction risk remains poorly understood. Likewise, potential differences in single vs. repetitive mTBI in relation to alcohol use/abuse have not been previously examined. Previous work in animal models supports the notion of brain injury as a risk factor for adverse health risk behaviors, including substance misuse/abuse (Cernak et al., 2001; Lim et al., 2015; Lowing et al., 2014; Muelbl et al., 2018; Nawarawong et al., 2019; Perez-Garcia et al., 2018; Schindler et al., 2020; Schindler et al., 2017; Vonder Haar et al., 2019). Indeed, using our established mouse model of blast exposure (Huber et al., 2013; Logsdon et al., 2020; Meabon et al., 2016; A. Schindler et al., 2020; A. G. Schindler et al., 2017), we previously demonstrated increased novelty-seeking behavior at acute (7 days) and chronic (1 month) time points following injury (Schindler et al., 2017). Likewise, increased risk-like behavior has also been reported following mild impact TBI (Krukowski et al., 2020; Mouzon et al., 2014). Results from animal models are more varied in relation to potential addiction risk following brain injury and depend on the type and severity of TBI exposure. For example, a moderate impact TBI increased ethanol (EtOH)-induced sedation and decreased voluntary EtOH consumption (Lowing et al., 2014), whereas mild impact or blast with body shielding resulted in increased cocaine, oxycodone, and alcohol intake and/or seeking behaviors (Lim et al., 2015; Muelbl et al., 2018; Nawarawong et al., 2019; Vonder Haar et al., 2019).

Critically, no study to date has compared single vs. repetitive blast exposure nor examined potential mTBI-induced differences in EtOH-induced stimulation vs. sedation vs. voluntary intake. Here, we investigated in male mouse potential effects of single (1×) and repetitive (3×) blast exposure on low-dose EtOH locomotor stimulation, high-dose

EtOH sedation, tolerance, and metabolism, and finally, voluntary EtOH intake using intermittent 2-bottle choice (herein referred to collectively as measures of “EtOH responsivity”). Using an electronically controlled pneumatic shock tube that models battlefield-relevant open-field blast forces generated by detonation of high explosives (Huber et al., 2013; Logsdon et al., 2020; Meabon et al., 2016; A. Schindler et al., 2020; A. G. Schindler et al., 2017), we found that while both single and repetitive blast exposure increased the sedative properties of high-dose EtOH (with no change in tolerance nor metabolism), only repetitive blast exposure resulted in potentiation of EtOH-induced locomotor stimulation and a shift in EtOH intake patterns (i.e., increased consumption “front-loading” and decreased total daily intake) during intermittent 2-bottle choice (2BC). Next, we examined self-report responses to the Alcohol Use Disorders Identification Test-Consumption Questions (AUDIT- C; a widely used measure to identify risky drinking and potential AUD) (Bush et al., 1998; Crawford et al., 2013; Dawson et al., 2005) in male OEF/OIF/OND Veterans and used a novel unsupervised machine learning approach to investigate whether a history of repetitive blast exposure and mTBI affected drinking behaviors. As predicted, AUDIT-C scores were increased in OEF/OIF/OND Veterans with a history of acute symptomatic blast. Subsequent AUDIT-C cluster analysis identified a 3-cluster solution: “low” (low intake and low frequency), “frequent” (low intake but high frequency), and “risky” (high intake and high frequency) and revealed a significant increase in assignment to the “risky” drinking cluster in Veterans with a history of blast exposure, where the “risky” cluster was characterized by a higher level of combat exposure and numbers of mTBIs with loss of consciousness (LOC). Together, these results suggest disparate trauma effects of single vs. repetitive

blast exposure, where only repetitive blast-mTBI is characterized by increased risky drinking self-report in male Veterans and increased EtOH-induced stimulation and consumption front-loading in male mice.

Materials and Methods

Animals and mouse model of blast overpressure Male C57BI/6 mice (Jackson Laboratory) aged 3–4 months (weight 25–33 g; mean 28.5 ± 0.17 g) were used. All animal experiments were carried out in accordance with Association for Assessment and Accreditation of Laboratory Animal Care guidelines and were approved by the VA Puget Sound Institutional Animal Care and Use Committees. The shock tube (Baker Engineering and Risk Consultants) was designed to generate blast overpressures that mimic open-field high explosive detonations encountered by military service members in combat, and the design and modeling characteristics have been described in detail elsewhere (Huber et al., 2013; Logsdon et al., 2020; Meabon et al., 2016; A. Schindler et al., 2020; A. G. Schindler et al., 2017). Briefly, mice were anesthetized with isoflurane (induced at 5% and maintained at 2–3%), secured against a gurney, and placed into the shock tube oriented perpendicular to the oncoming blast wave (ventral body surface toward blast). Sham (control) animals received anesthesia only for a duration matched to blast animals. Repeated blast/sham exposures occurred successively over the course of 3 days (1 per day). The blast overpressure (BOP) peak intensity (psi), initial pulse duration (ms), and impulse (psi/ms) used were in keeping with mild blast TBI (19.9 psi +/− 0.14 psi). Under these experimental conditions, the overall survival rate exceeded 95%, with blast-exposed mice appearing comparable to sham-exposed mice by inspection 2–4 h postblast exposure as previously reported (Huber et al., 2013;

Logsdon et al., 2020; Meabon et al., 2016; A. Schindler et al., 2020; A. G. Schindler et al., 2017). All behavioral tests were conducted starting at 1 month postsham/blast exposure, a time point that allows for the development of blast-induced neuropathology (Goldstein et al., 2014; Rubovitch et al., 2011) and that correlates to a time period where enduring functional and behavioral deficits are detected (A. Schindler et al., 2020; A. G. Schindler et al., 2017). Separate groups of mice were used for EtOH locomotor stimulation, EtOH sedation, and voluntary EtOH consumption (see below for specific details of each procedure), 1× and 3× sham and blast mice were run in parallel, and at least 2 cohorts of mice were used in each behavioral paradigm.

Low-dose EtOH-induced locomotor stimulation

Ethanol-induced locomotor stimulation was investigated over the course of 3 days. On day 1, each animal was preexposed/habituated to the behavioral arena (clean rat cage) for 15 min. On the second day, animals were injected with saline (1.0 ml/kg w/v, i.p.) and were again allowed to explore the behavioral arena for 15 min. Finally, on day 3, animals were injected with EtOH (2.0 g/kg, i.p., 20% w/v) and allowed to explore the behavioral area for 15 min. Activity was recorded from above and analyzed using ANY-maze (Wood Dale, IL). EtOH-induced locomotor stimulation was expressed as a percent increase in distance traveled (EtOH/saline).

High-dose EtOH-induced sedation, tolerance, and metabolism

Ethanol-induced sedation and tolerance were investigated using the loss of righting reflex (LORR) paradigm. Each animal was injected with EtOH (4 g/kg, i.p., 40% w/v) and upon sedation (1–3 minutes later) was placed on its back in a V-shaped trough (any

animal that did not lose LORR within 5 min was excluded from the study (n = 2)). Animals were then observed undisturbed for up to 5 hours. When the animal was able to right itself twice within 30 seconds (i.e., flipped from back to stomach in the V-shaped trough), total sedation time was recorded (LORR duration). The identical procedure was repeated 24 h later to assess EtOH tolerance. Blood was collected from the submandibular vein at 10 min and 4 h following EtOH injection to determine blood EtOH concentrations. Blood EtOH concentrations were determined using Bioassay Systems (Hayward, CA) EnzyChrom EtOH Assay Kit as per manufacturer instructions.

Intermittent 2-bottle choice (I2BC)

Starting 4 days prior to the commencement of the I2BC procedure, mice were singly housed, and their usual water bottle removed and replaced with 2 bottles filled each with water. The bottles were 50-mL centrifuge tubes with a #6.5 neoprene rubber stopper and 2.5” ball sipper tube (Antrin). Bottles were weighed immediately prior to placement in the cage and then again at 24-h timepoints (at approximately 5:30 pm) throughout the duration of the study. Each time the bottles were weighed, their position in the cage was reversed to prevent any side bias. One night each week (Wednesday), bottles were also weighed at 8 pm (2 h into the dark cycle) to assess drinking patterns during the acute phase of access. Following 4 days of baseline exposure to the water bottles, animals had access to 1 water bottle and 1 EtOH bottle every Monday, Wednesday, and Friday, and access to only water every Tuesday, Thursday, Saturday, and Sunday. During the first week of EtOH exposure, the EtOH dose was slowly increased (3% on Monday, 6% on Wednesday, and 9% on Friday). For the remaining

duration of the study (21 more days), the 20% EtOH dose was used. Body weight was recorded for each animal on Sundays and used to express EtOH intake in g/kg.

Human subjects

These studies were approved by the VA Puget Sound Health Care System Human Subjects Committee and conformed to institutional regulatory guidelines and principles of human subject protection in the Declaration of Helsinki. All participants provided written informed consent prior to any study procedures. The current study used the self-reports of 105 Veterans with a history of blast exposure with acute symptoms and 34 deployed controls with no lifetime history of TBI of any severity (Table 1). As previously reported (Meabon et al., 2016; Peskind et al., 2011; Petrie et al., 2014), blast exposure(s) were reported as mild in nature and ranging from 1 (10%) to 50 or greater (15%) blast exposures. All participants in this report were male. Both males and females were eligible for enrollment and study inclusion. However, in this study we currently have no available data from blast-mTBI women because most mTBI Veteran participants were from combat military occupational specialties. Inclusion criteria for the blast group were as follows: documented hazardous duty in Iraq and/or Afghanistan with the U.S. Armed Forces and at least 1 blast exposure with acute symptoms (e.g., nausea, ringing in ears, blurry vision, hearing loss, unsteady on feet, eyes sensitive to light, headache, alteration of consciousness; occurring within 30 minutes of blast exposure). The VA/Department of Defense/American Congress of Rehabilitation Medicine criteria was used to establish mTBI (for the blast group), with 98% of Veterans within these group meeting criteria for at least 1 mTBI. Exclusion criteria were as follows: moderate-severe TBI, seizure disorder, insulin-dependent diabetes, current

Study participant demographics and blast exposures

	Deployed control	Blast exposed	
	Mean ± SEM, (Range)	**Mean ± SEM, (Range)**	***p* Value**
Demographics			
Number of participants, *n*	34	105	
Age (years)	32.7 ± 1.1 (22–46)	33.6 ±0.8 (21–60)	0.588
Education (years)	14.2 ± 0.3 (12–18)	14.1 ± 0.2 (11–20)	0.805
Race, nonwhite, *n* (%)	9 (26%)	21 (20%)	0.476
Blast exposure			
Estimated number of blast exposures with		19 ± 3 (1 to >100)	
Acute symptoms during military service (lifetime)		Median = 7	
Estimated number of mTBI with loss of		0.98 ± 0.2 (0 to 8)	
Consciousness (lifetime)		Median = 1	
Time since last blast-related		5.4 ± 0.3 (0.25–13)	
mTBI (years)		Median = 5	

Table 2.1. Study participant demographics and blast exposures

diagnosis of alcohol or other substance abuse or dependence (excluding nicotine), schizophrenia or other psychotic disorders, bipolar disorder, dementia, and taking medications likely to affect cognition performance. Inclusion and exclusion criteria for deployed control Veterans were identical with the exception that controls had no lifetime history of TBI of any severity. Exclusionary psychiatric disorders were ruled out by SCID- IV interview (First et al., 1995). Self-report alcohol use was examined using the Alcohol Use Disorders Identification Test-Consumption Questions (AUDIT-C) (Bush et al., 1998; Crawford et al., 2013; Dawson et al., 2005).

Unsupervised machine learning (cluster analysis)

Individual AUDIT-C question responses were used as features in an unsupervised machine learning model. Using Python and common scientific computing libraries (e.g., scipy, pandas, sk-learn), hierarchical clustering algorithm (Ward method) and k-means (Euclid) were used to assist in optimal cluster number selection. Silhouette, Davies Bouldin, and Calinski Harabasz scores were computed to assess k-means cluster assignments. Cluster stability was assessed using the scores for homogeneity, completeness, and mutual information criterion and a bootstrap approach with repeated random assignment of initial cluster centroids. K = 3 clusters were chosen based on above evaluation metrics. Finally, cluster assignment was compared between deployed controls and Veterans with a history of blast exposure and potential effects of combat and blast exposure on cluster assignment was examined.

Data analysis

As appropriate, data were analyzed using: (i) 2-tailed Student's t-tests; (ii) 1-way or 2-way (between/within-subjects design) repeated-measures analysis of variance (RM ANOVA), followed by Newman–Keuls multiple comparison tests or Bonferroni post hoc tests, respectively; (iii) chi-square tests were conducted for categorical data (e.g., AUDIT-C). Reported p values denote 2-tailed probabilities of $p < 0.05$, and nonsignificance (n.s.) indicates $p > 0.05$. Statistical analyses were conducted using GraphPad Prism 4.0 (GraphPad Software, Inc.) and Python.

Results

EtOH-induced locomotor stimulation is prolonged following repetitive blast exposure. Depending on dose, EtOH can have stimulating and/or sedative effects and can be assessed in mice via EtOH-induced locomotion and loss of righting reflex paradigms, respectively. To investigate whether blast exposure modulates the stimulating effects of EtOH, we first examined the ability of low-dose EtOH (2 g/kg) to increase locomotion (over saline) in C57BL/6 male mice 1 month after they received either 1 (1×) or 3 (3×, 1 per day) blast exposures. EtOH stimulation was expressed as a percent change in distance traveled (EtOH/ saline) and was examined in 5-minute bins (15 min total). There were no statistically significant differences between 1× sham-(n = 8) and 3× sham-treated (n = 9) mice, 2-way RM ANOVA: interaction effect $F(2, 30) = 0.554$, $p > 0.05$, and thus, 1× and 3× sham animals were pooled together for subsequent analyses related to EtOH stimulation. Figure 2.1A shows a significant effect of time postethanol injection, 2-way RM ANOVA: main effect of time $F(2, 68) = 19.54$, $p = 0.0001$, Bonferroni multiple comparison test post hoc: sham = 17, blast 1× = 12, blast 3× = 8, and while post hoc analysis revealed no difference in locomotor stimulation at 5

min postethanol injection, a significantly prolonged EtOH effect in 3× but not 1× animals was apparent when examined at 15 min postethanol injection (Figure 1A). We next examined raw distance traveled during the entire 15-min tests across the 3 days of behavioral testing (habituation/baseline, saline, and EtOH) and found a significant effect across groups, 2-way RM ANOVA: interaction effect $F(4,68) = 3.359$, $p = 0.014$, Bonferroni multiple comparison test post hoc: sham = 17, blast 1× = 12, blast 3× = 8, Figure 2.1B). Post hoc analyses demonstrated no differences during the initial habituation/baseline phase (base) but did reveal a significant increase in distance traveled during the final EtOH phase (EtOH) in 3× but not 1× animals. Finally, we examined raw distance traveled in 5-min bins during the each of the behavioral testing days. At baseline, there was a significant effect of time, 2-way RM ANOVA: time effect $F(2, 68) = 10.12$, $p = 0.0005$, Bonferroni multiple comparison test post hoc: sham = 17, blast 1× = 12, blast 3× = 8, Figure 2.1C, but post hoc analyses revealed no differences between groups in any time bin. Likewise, following saline injection, there was a significant interaction effect, 2-way RM ANOVA: interaction effect, $F(4, 68) = 4.91$, $p = 0.0043$, Bonferroni multiple comparison test post hoc: sham = 17, blast 1× = 12, blast 3× = 8, Figure 2.1D, but post hoc analyses revealed no differences between groups in any time bin. Conversely, following EtOH injection, there was a significant group effect, 2-way RM ANOVA: group effect $F(2, 68) = 7.762$, $p = 0.0017$, Bonferroni multiple comparison test post hoc: sham = 17, blast 1× = 12, blast 3× =8, Figure 2.1E. Post hoc analyses demonstrated increased distance traveled at 5 min in 1× blast mice and at 10 and 15 min in 3× blast mice.

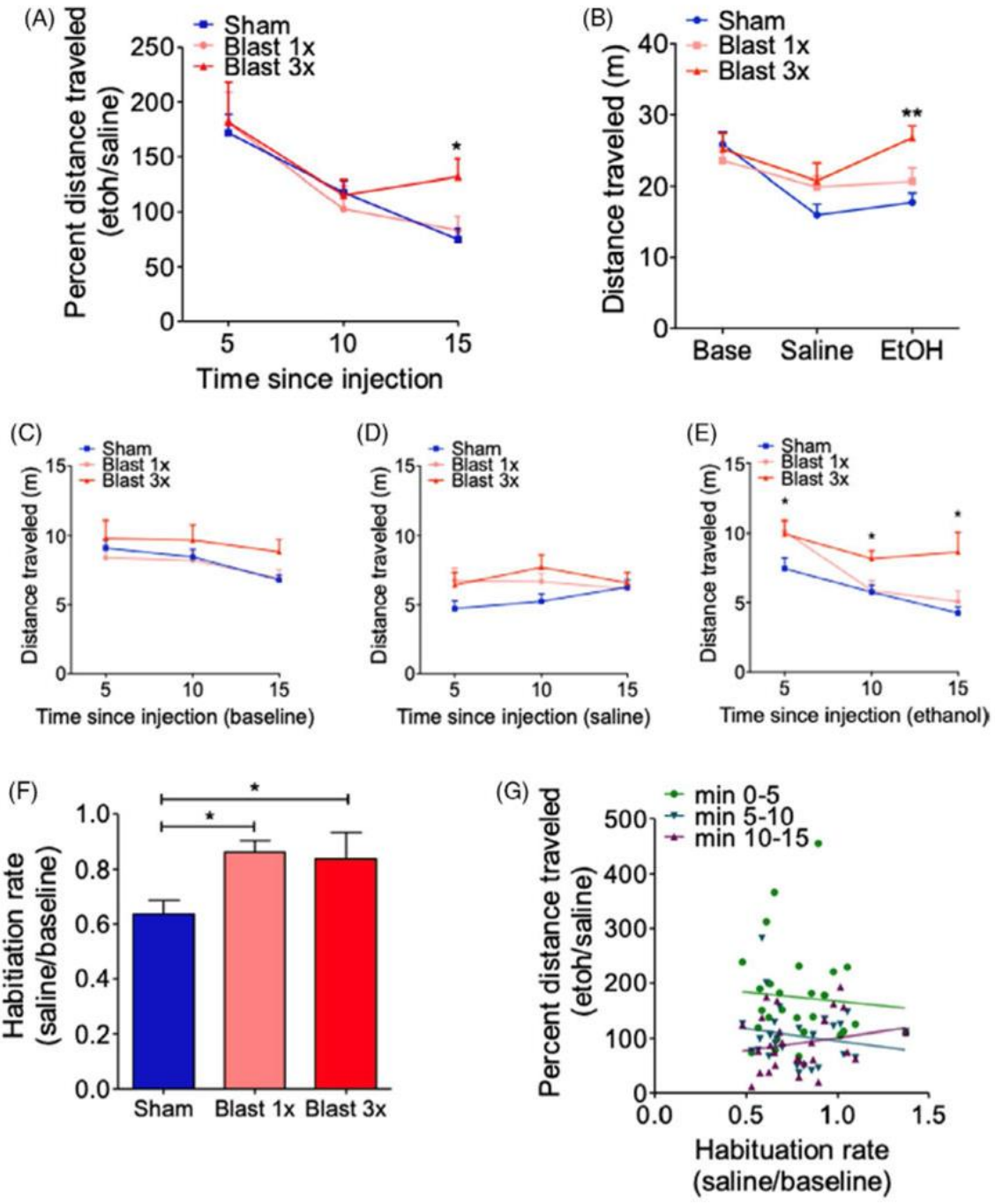

Figure 2.1. Repetitive blast exposure increases ethanol (EtOH)-induced locomotor stimulation (A) Locomotor stimulating effects of EtOH as expressed by percent distance traveled. Two-way RM ANOVA post hoc Bonferroni multiple comparison test. (B) Distance traveled at baseline and following saline or EtOH administration. Two-way RM

ANOVA post hoc Bonferroni multiple comparison test. (C) Distance traveled at baseline in 5-min bins. Two-way RM ANOVA post hoc Bonferroni multiple comparison test. (D) Distance traveled following saline administration in 5-min bins. Two-way RM ANOVA post hoc Bonferroni multiple comparison test. (E) Distance traveled following EtOH administration in 5-min bins. Two-way RM ANOVA post hoc Bonferroni multiple comparison test. (F) Locomotor habituation. One-way ANOVA post hoc Newman–Keuls comparison test. (G) Correlation between EtOH locomotor stimulation and locomotor habituation rate. Spearman correlation. *$p \leq 0.05$ **$p \leq 0.001$: sham vs. blast. Values represent mean ± SEM

Differences in locomotor habituation can potentially confound EtOH investigations and are especially relevant in paradigms where locomotion is repeatedly measured over a number of days, as is the case here. Indeed, when we computed a habituation rate (saline/base), we found a significant difference in both 1× and 3× blast-exposed mice, 1-way ANOVA: $F(2, 36) = 4.946$, $p = 0.013$, Newman–Keuls comparison test post hoc: sham = 17, blast 1× = 12, blast 3× = 8, Figure 2.1C. Critically, we did not find any significant correlation between habituation rate (saline/base) and EtOH stimulation (EtOH/saline) at any of the 5-min bins (min 0–5: Spearman $\rho = 0.153$, n.s, $n = 27$; min 5–10: Spearman $\rho = 0.749$, n.s, $n = 27$; min 10–15: Spearman $\rho = 0.945$, n.s, $n = 27$), suggesting that our blast effects on EtOH stimulation were not mediated by potentially unrelated differences in locomotor habituation rates. Together, these results demonstrate that repetitive blast exposure increases the duration of the stimulatory effects of EtOH, which is not related to blast-induced deficits in habituation.

Blast exposure increases EtOH-induced sedation without affecting tolerance or metabolism

To investigate whether blast exposure modulates the sedative effects of EtOH, we examined the ability of high-dose EtOH (4 g/kg) to cause loss of righting reflex (LORR) on 2 consecutive days in C57BL/6 male mice 1 month after they received either 1 (1×) or 3 (3×, 1 per day) blast exposures. There were no statistically significant differences between 1× sham- (n = 8) and 3× sham-treated (n = 11) mice, LORR day 1: Student’s unpaired t-test, $t(17) = 0.463$, $p > 0.05$; LORR day 2: Student’s unpaired t-

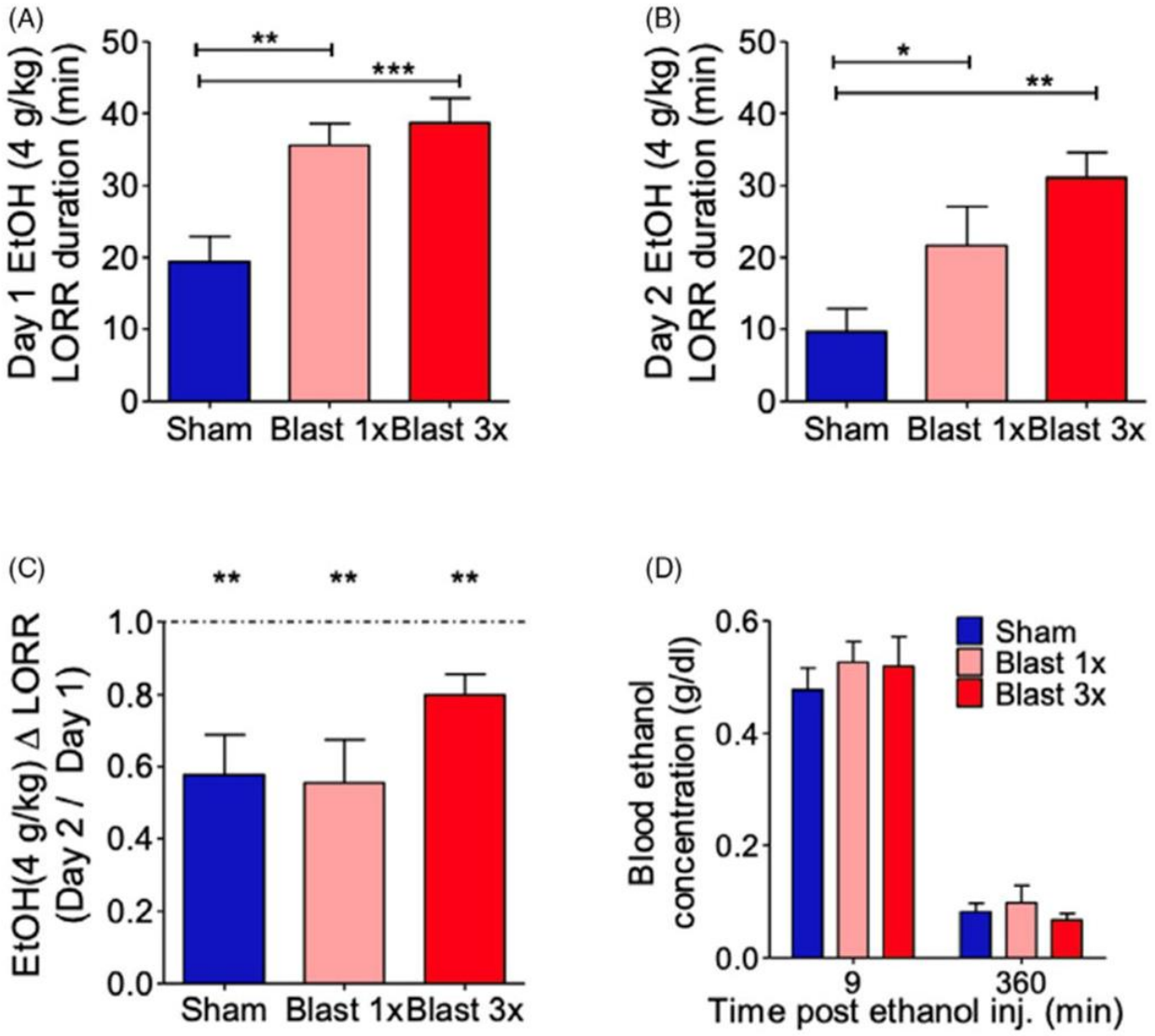

Figure 2.2. Blast exposure increases ethanol (EtOH)-induced loss of righting reflex. (A, B) EtOH-induced LORR duration on day 1 (A) and day 2 (B) of EtOH administration. One-way ANOVA post hoc Newman–Keuls comparison test. (C) Tolerance to EtOH-induced LORR. One-way ANOVA post hoc Newman–Keuls comparison test. (D) Blood EtOH concentration 9 min and 4 h after EtOH administration. Two-way RM ANOVA post hoc Bonferroni multiple comparison test. *$p \leq 0.05$, **$p \leq 0.001$, and ***$p \leq 0.0001$. Error bars are mean ± SEM

test, t(17) = 0.056, p > 0.05, and thus, 1× and 3× sham animals were pooled together for subsequent analyses related to EtOH sedation.

In accordance with previous results [21], a significant increase in LORR duration on the first day of testing was found in both 1× and 3× blast-exposed mice, 1-way ANOVA: F(2, 42) = 9.854, p = 0.0003, Newman–Keuls multiple comparison test post hoc: sham = 19, blast 1× = 13, blast 3× = 13, Figure 2A. A similar increase was found on the second day of repeat testing, 1-way ANOVA: F(2, 42) = 7.413, p = 0.001, Newman–Keuls multiple comparison test post hoc: sham = 19, blast 1× = 13, blast 3× = 13, Figure 2B. We next assessed EtOH tolerance by examining LORR change (day 2 / day 1). Figure 2C shows significant tolerance to repeated EtOH injections for all groups, 1-sample t-test vs. a theoretical mean of 1.0 (no tolerance): sham: t(16) = 3.788, p = 0.001, blast 1×: t(12) = 3.709, p = 0.003, blast 3×: t(12) = 3.66, p = 0.003, and no significant difference in tolerance rate across groups, 1-way ANOVA: F(2, 42) = 1.615, p = 0.211, sham = 17 blast 1× = 13, blast 3× = 13.

Finally, we examined potential blast-induced changes to EtOH metabolism by measuring blood EtOH concentrations (BEC) at 10 min and 4 hours postinjection. There were no statistically significant differences between 1× sham- (n = 4) and 3× sham-treated (× = 5) mice, 10 min: Student’s unpaired t-test, t(7) = 1.667, p > 0.05; 4 h: Student’s unpaired t-test, t(7) = 1.098, p > 0.05, and thus, 1× and 3× sham animals were pooled together for subsequent analyses related to EtOH metabolism. In accordance with previous results [21], no significant differences were found in EtOH metabolism at time point in either 1× or 3× blast-exposed mice, 2-way RM ANOVA:

interaction effect $F(2, 15) = 0.427$, $p > 0.05$, sham = 8, blast 1× = 4, blast 3× = 6, Figure 2D.

Repetitive, but not single, blast exposure decreases 24-hour EtOH intake but increases consumption "front-loading"

Previous results have suggested seemingly disparate trauma outcomes in rodents when EtOH self-administration is measured in short-access vs. long-access paradigms. To further investigate these phenomena, we conducted intermittent 2-bottle choice testing with 20% EtOH in C57BL/6 male mice 1 month after they received either 1 (1×) or 3 (3×, 1 per day) blast exposures, where intake measurements were repeatedly taken at time points corresponding to short access (after approximately 2 h of access) and long access (after 24 h of access). Consumption front-loading describes the tendency for some groups to consume significantly more in the acute phase of a self-administration paradigm after a period of forced abstinence and has implications for health risk behaviors such as binge drinking.

Using this paradigm, blast exposure caused a significant decrease in daily EtOH intake across the 3 weeks of testing, 2-way RM ANOVA: main effect of group $F(2,192) = 4.417$, $p = 0.023$, sham = 10, blast 1× = 9, blast 3× = 8, Figure 2.3A, and a significant decrease in average daily intake, 1-way ANOVA: $F(2, 24) = 4.632$, $p = 0.02$, Newman–Keuls multiple comparison test post hoc: sham = 10, blast 1× = 9, blast 3× = 8, Figure 2.3B. Conversely, blast exposure caused a significant increase in daily water intake across the 3 weeks of testing, 2-way RM ANOVA: main effect of group $F(2, 192) =$

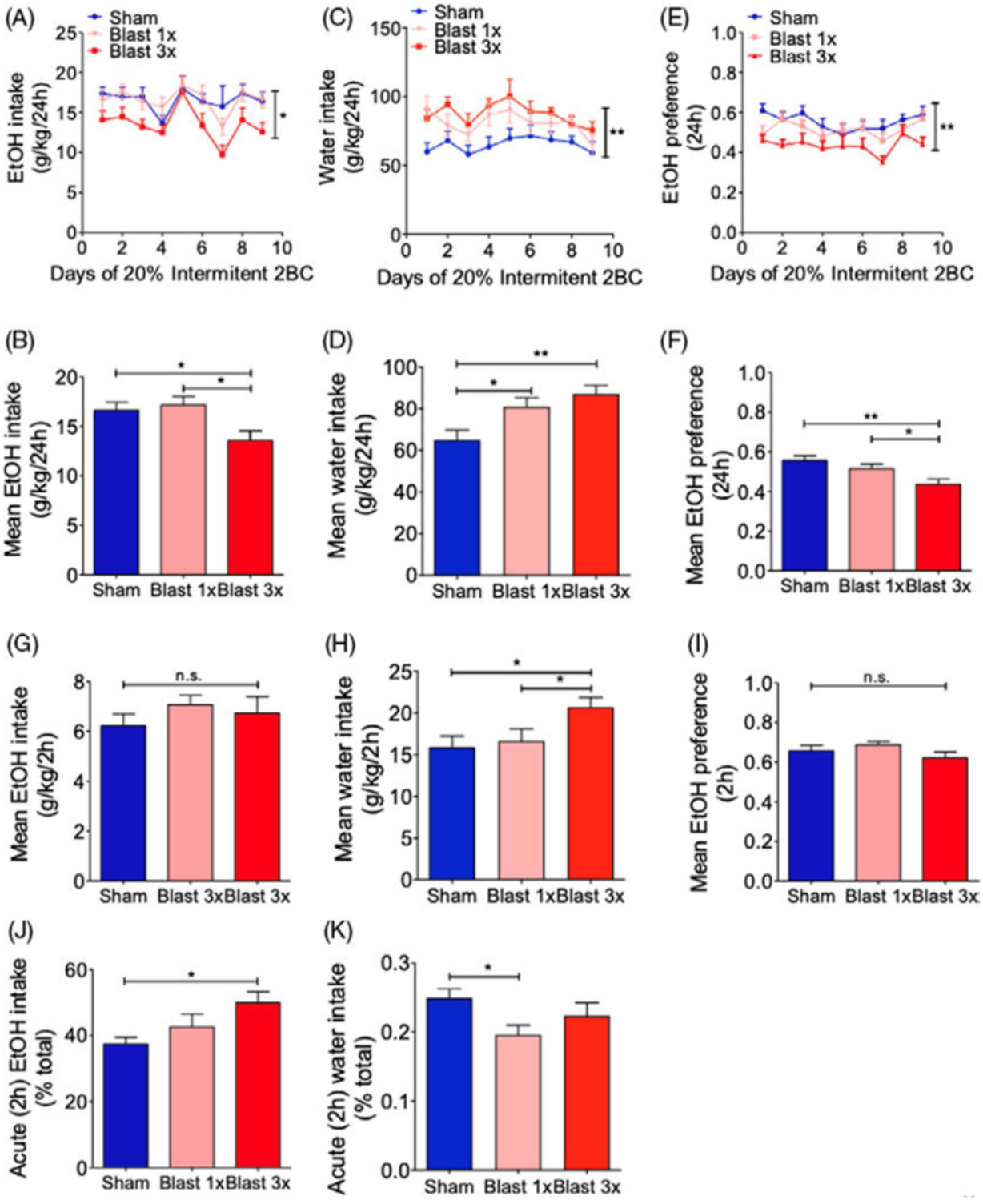

Figure 2.3. Blast exposure increases ethanol (EtOH) consumption “front-loading.” (A, B) Daily intermittent 2-bottle choice 20% EtOH intake. Two-way RM ANOVA post hoc

Bonferroni multiple comparison test (daily) and 1-way ANOVA post hoc Newman–Keuls comparison test (average). (C, D) Daily intermittent water intake. Two-way RM ANOVA post hoc Bonferroni multiple comparison test (daily) and 1-way ANOVA post hoc Newman–Keuls comparison test (average). (E, F) Daily intermittent 2-bottle choice 20% EtOH preference. Two-way RM ANOVA post hoc Bonferroni multiple comparison test (daily) and 1-way ANOVA post hoc Newman–Keuls comparison test (average). (G) EtOH consumption during initial 2 hours of access. One-way ANOVA post hoc Newman–Keuls comparison test. (H) Water consumption during initial 2 hours of access. One-way ANOVA post hoc Newman–Keuls comparison test. (I) EtOH preference during initial 2 hours of access. One-way ANOVA post hoc Newman–Keuls comparison test. (J) EtOH “front-loading” (percent total intake). One-way ANOVA post hoc Newman–Keuls comparison test. (K) Water “front-loading” (percent total intake). One-way ANOVA post hoc Newman–Keuls comparison test. *$p \leq 0.05$ **$p \leq 0.001$: sham vs. blast. Values represent mean ± SEM

6.711, p = 0.0048, sham = 10, blast 1× = 9, blast 3× = 8, Figure 2.3C, and a significant increase in average daily intake, 1-way ANOVA: F(2, 24) = 6.711, p = 0.0048, Newman–Keuls multiple comparison test post hoc: sham = 10, blast 1× = 9, blast 3× = 8, Figure 3D. In accordance with these intake results, we found a similar pattern of effects when examining daily EtOH preference (EtOH intake/water intake), 2-way RM ANOVA: main effect of group F(2, 192) = 5.866, p = 0.008, sham = 10, blast 1× = 9, blast 3× = 138, Figure 2.3E, and average daily preference, 1-way ANOVA: F(2, 24) = 6.038, p = 0.007, Newman–Keuls multiple comparison test post hoc: sham = 10, blast 1× = 9, blast 3× = 138, Figure 3F, across the 3 weeks of testing.

Conversely, when we measured intake levels approximately 2.5 h after reaccess to EtOH (i.e., each Wednesday evening at 6 pm, 2 h into the dark cycle). We found no significant difference between sham- and blast-exposed animals in average EtOH intake, 1-way ANOVA: F(2, 24) = 0.744, p = 0.485; sham = 10, blast 1× = 9, blast 3× = 8, Figure 2.3G, a significant difference between sham- and blast-exposed animals in average water intake, 1-way ANOVA: F(2, 24) = 3.549, p = 0.045; sham = 10, blast 1× = 9, blast 3× = 138, Figure 2.3H, and no significant difference in EtOH preference, 1-way ANOVA: F(2, 24) = 1.714, p = 0.201; n = 8–10, Figure 3I, measured after approximately 2.5 h of access. To assess potential changes in consumption “front-loading” (e.g., the tendency to consume higher amounts immediately following a period of forced abstinence), we computed the percent of EtOH consumed within the first 2.5 h of exposure and found that repetitive, but not single, blast exposure caused a significant increase in “front-loading” consumption, 1-way ANOVA: F(2, 24) = 3.975, p = 0.032, Newman–Keuls multiple comparison test post hoc: sham = 10, blast 1× = 9, blast 3× =

138, Figure 2.3J. Conversely, percent of water consumed within the first 2.5 hours of exposure was significantly decreased in 1× but not 3× blast mice, 1-way ANOVA: F(2, 24) = 3.509, p = 0.046, Newman–Keuls multiple comparison test post hoc: sham = 10, blast 1× = 9, blast 3× = 138, Figure 2.3K. Together, these results suggest that repetitive blast exposure modifies the pattern of voluntary EtOH intake resulting in increased consumption “front-loading.”

Cluster analysis reveals a change in drinking patterns in Veterans with a history of repetitive blast exposure

A cohort of 105 OEF/OIF/OND Veterans with previous history of blast exposure with acute symptoms (BE) and 34 OEF/OIF/OND Deployed Control (DC) Veterans with no lifetime history of TBI of any severity were studied. Table 2.1 shows that the BE and DC groups were statistically comparable in terms of age at time of evaluation, education level, and race distribution (nonwhite/white ratio). Veterans in the BE group had an average of 19 ± 3 blast exposures (median = 7) where the average time from their last blast exposure to evaluation was 5.4 ± 0.3 years. Current alcohol use was assessed as previously shown (Peskind et al., 2011) with the Alcohol Use Disorders Identification Test-Consumption Questions (AUDIT-C), a screening measure used to identify individuals who are at risk for problematic drinking (Olson-Madden et al., 2012). Scores on this measure range from 0 to 12, with 0 indicating no alcohol use and higher scores indicating more risk of unhealthy alcohol use. Previously established study criteria (Elder & Cristian, 2009; Hendrickson et al., 2018; Nawarawong et al., 2019)required individuals meeting DSM-IV criteria for alcohol abuse or dependence to be excluded

from study. Nonetheless, AUDIT-C scores were significantly higher in BE as compared to DC ($\chi 2 = 20.95$, $p = 0.021$) (Figure 2.4A).

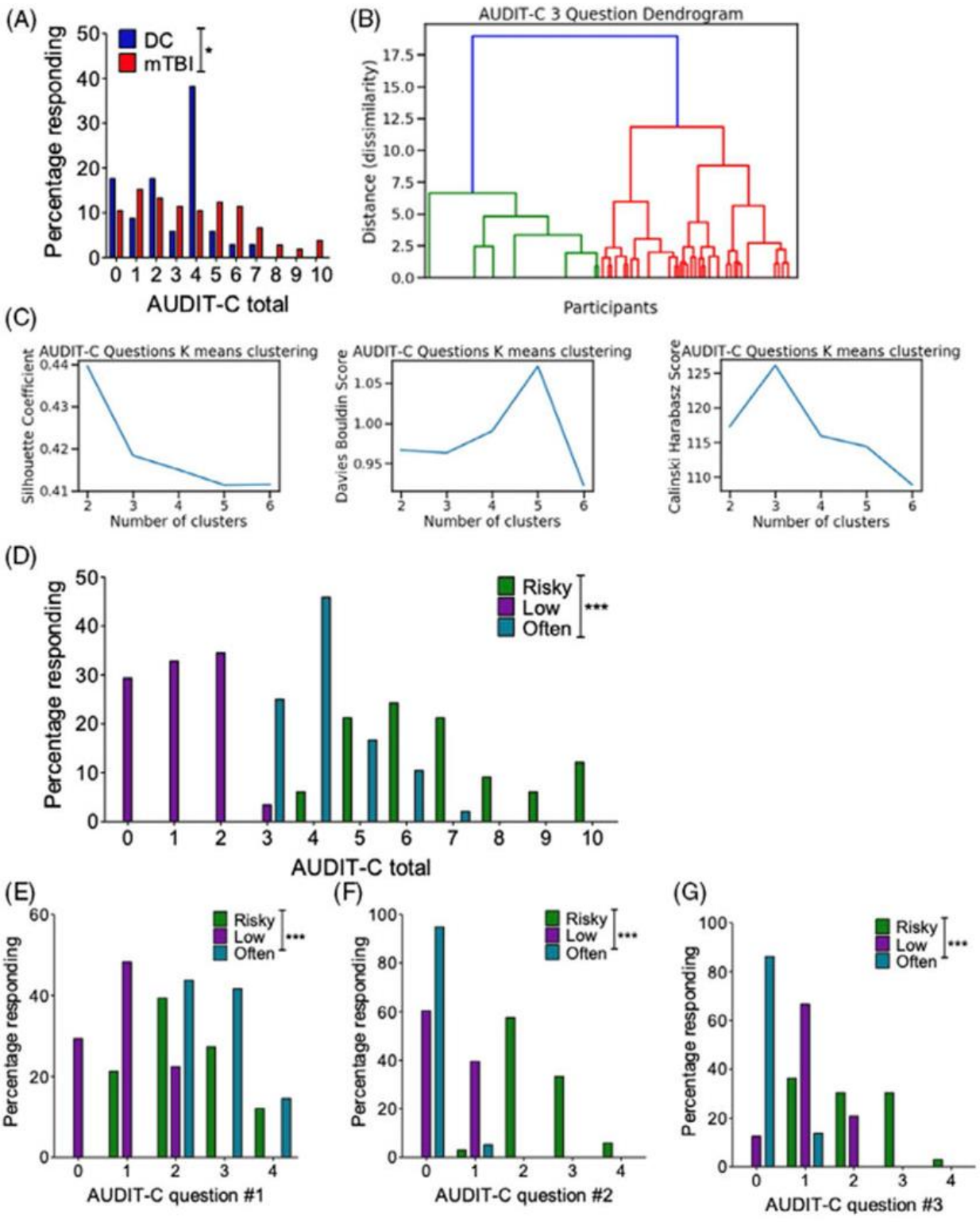

Figure 2.4. AUDIT-C cluster analysis. (A) Self-report AUDIT-C total scores in Veterans with/without a history of blast exposure with acute symptoms. Chi-square. (B)

Hierarchical clustering dendrogram visualization. (C) K-means cluster metrics highlight an optimal 3-cluster optimal. (D) AUDIT-C total scores across the 3 clusters. Chi-square. (D) AUDIT-C total scores across the 3 clusters. Chi-square. (E) AUDIT-C question 1 (i.e., drinking frequency) scores across the 3 clusters. Chi-square. (F) AUDIT-C question 2 (i.e., drinking amount) scores across the 3 clusters. (G) AUDIT-C question 3 (i.e., binge-like drinking) scores across the 3 clusters. Chi-square. *$p \leq 0.05$ and ***$p \leq 0.0001$. Values represent mean ± SEM

The AUDIT-C consists of 3 Likert-scale questions; while all 3 questions are related to intake patterns, each question probes a different drinking modality—the first question focuses on drinking frequency, the second on drinking quantity, and the third on “binge”-like drinking behavior. Here, we used an unsupervised machine learning approach to examine potential subgroup differences across the AUDIT-C questions. Distance (dissimilarity) from a hierarchical clustering algorithm suggested an optimal cluster number of 3 (Figure 2.4B), and this was further supported by examining cluster metrics using a K-means clustering algorithm (Figure 2.4C). Finally, analysis of cluster stability also supported a 3-cluster solution for this data set (Table 2.2). Using k = 3 with k-means clustering, Veterans were assigned to a single cluster based on their AUDIT-C responses. In support of this cluster assignment, total AUDIT-C scores were significantly different across cluster groups ($\chi 2 = 201.3$, $p = 0.0001$) (Figure 4D). Likewise, individual question AUDIT-C scores were also significantly different across cluster groups (question 1 (drinking frequency): $\chi 2 = 82.99$, $p = 0.0001$; question 2 (drinking quantity): $\chi 2 = 157.7$, $p = 0.0001$; question 3 (binge-like frequency): $\chi 2 = 123.4$, $p = 0.001$). Based on the distribution of scores within each cluster, we labeled the clusters as “low” (n = 58), “often” (n = 48), and “risky” (n = 33). Figure 4D–G shows the outcomes of the cluster assignments: The “low” drinking cluster is characterized by low responses across the 3 AUDIT-C questions, the “often” drinking cluster is characterized by a high response on question 1 (i.e., the question related to drinking frequency) but low responses on questions 2 (i.e., quantity) and 3 (i.e., binge), and the “risky” drinking

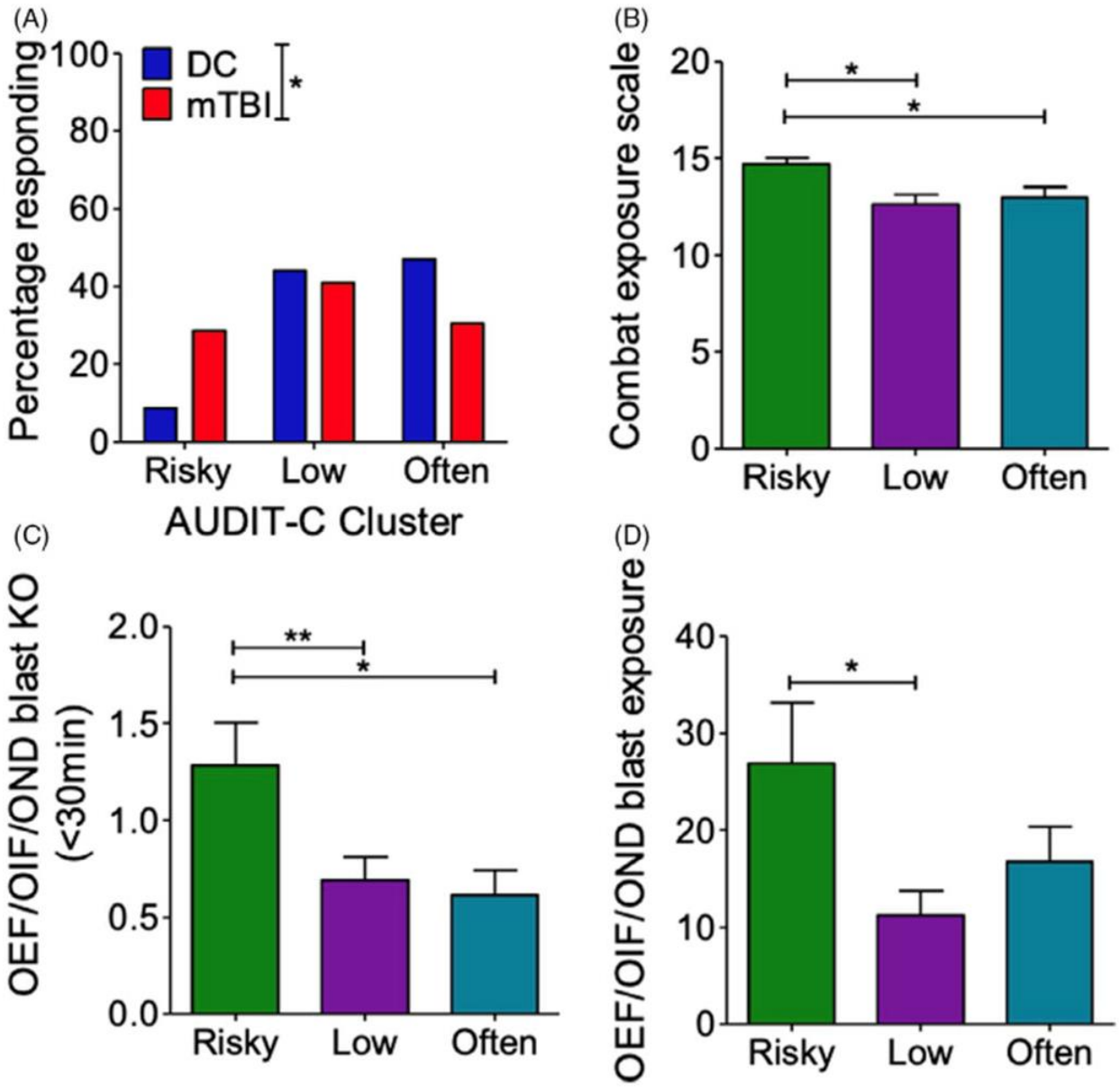

Figure 2.5. Blast-mTBI results in shift to “risky” drinking cluster. (A) AUDIT-C k-means cluster assignment in Veterans with/without a history of blast exposure with acute symptoms. Chi-square. (B) Combat exposure by cluster assignment in Veterans with a history of blast exposure with acute symptoms. One-way ANOVA post hoc Newman–Keuls comparison test. (C) Blast-mTBI with loss of consciousness (KO) count by cluster assignment in Veterans with a history of blast exposure with acute symptoms. One-way

ANOVA post hoc Newman–Keuls comparison test. (D) Blast exposure count by cluster assignment in Veterans with a history of blast exposure with acute symptoms. One-way ANOVA post hoc Newman–Keuls comparison test. *$p \leq 0.05$ and **$p \leq 0.01$. Values represent mean ± SEM

cluster is characterized by high responses on questions 2 (i.e., the question related to drinking quantity) and 3 (i.e., the question related to binge-like consumption) and intermediate responses on question 1 (i.e., frequency).

Comparing cluster assignment between DC and BE Veterans, we found a significant change in cluster assignment ($\chi 2 = 6.326$, $p = 0.042$) toward an increased "risky" group membership in the BE group (Figure 2.5A). To further understand potential drivers of cluster assignment, we examined whether there were significant differences in combat exposure and/or blast number across the clusters (focusing now only on BE Veterans). We found a significant increase in reported combat exposure, 1-way ANOVA: $F(2,103) = 4.725$, $p = 0.012$, Newman–Keuls post hoc: "low" n = 43, "often" n = 32, and "risky" n = 29) (Figure 5B) within the "risky" drinking cluster. Likewise, we found that the risky drinking cluster was also associated with significantly greater number of blast-mTBIs resulting in loss of consciousness, 1-way ANOVA: $F(2, 100) = 5.21$, $p = 0.007$, Newman–Keuls comparison test post hoc: "low" n = 42, "often" n = 31, and "risky" n = 29) (Figure 5C), and greater number of blast-mTBIs, 1-way ANOVA: $F(2, 101) = 3.895$, $p = 0.023$, Newman–Keuls comparison test post hoc: "low" n = 43, "often" n = 32, and "risky" n = 29, Figure 5D). Together, these results suggest that repetitive blast-mTBI increases alcohol intake and potentially risky drinking behaviors and are in correspondence with current results from our animal model of blast.

K-means cluster stability

k	Homogeneity	Completeness	Adj. mutual info.
2	0.93	0.94	0.93
3	0.98	0.98	0.98
4	0.85	0.86	0.85

Table 4.2. K-means cluster stability

Discussion

The armed conflicts of OEF/OIF/OND have resulted in an estimated mTBI rate of 10–25% (*DOD TBI Worldwide Numbers*, n.d.; Hoge et al., 2008; Tanielian et al., 2008). In these conflicts, an estimated 75% of all TBIs reported by service members are a result of blast caused by detonation of high explosives (Tanielian et al., 2008) and multiple deployments are common (2.77 million service members have served on 5.4 million deployments since 2011) (Wenger et al., 2018), resulting in the increased potential for repetitive blast exposure. As such, blast exposure represents a major potential source of physical and psychological trauma, with implications for subsequent health risk behaviors (e.g., sensation/novelty seeking, impulsivity, risk taking, irritability/aggression). Indeed, mTBI can worsen preexisting psychiatric disorders such as depression and increase and/or exacerbate substance misuse/addiction and other health risk behaviors (Adams et al., 2012; Elder & Cristian, 2009; Grossbard et al., 2017; Halbauer et al., 2009; Hendrickson et al., 2018; Miller et al., 2013; Olson-Madden et al., 2012; Peskind et al., 2011; Petrie et al., 2014; A. G. Schindler et al., 2017; Seidl et al., 2015; Tanielian et al., 2008) potentially compounding negative outcomes following injury and trauma. We previously reported increased PTSD and depression symptoms as well as alcohol use in OEF/OIF/OND Veterans with a history of blast-mTBI as compared to deployed control Veterans with no lifetime history of TBI (Peskind et al., 2011; Petrie et al., 2014), and more recently reported increased Veteran self-report of disinhibition and risk taking behaviors chronically following blast-mTBI (A. G. Schindler et al., 2017). Here, we provide evidence demonstrating blast-dose effects in relation to EtOH responsivity in mice and risky drinking behavior in Veterans,

highlighting the importance of understanding blast-mTBI history in OEF/OIF/OND Veterans that might be at heightened risk for health risk behaviors related to substance misuse and/or abuse.

Animal models support the notion of brain injury as a risk factor for adverse health risk behaviors, including substance abuse and addiction (Cernak, 2001; Lim et al., 2015; Lowing et al., 2014; Muelbl et al., 2018; Nawarawong et al., 2019; Perez-Garcia et al., 2019; A. Schindler et al., 2020; A. G. Schindler et al., 2017; Vonder Haar et al., 2019). Using our established mouse model of repetitive blast exposure (Huber et al., 2013; Logsdon et al., 2020; Meabon et al., 2016; A. Schindler et al., 2020; A. G. Schindler et al., 2017) we previously demonstrated increased novelty seeking in blast-exposed mice (A. G. Schindler et al., 2017) and more recently demonstrated acute (30 minutes post) stress responses and chronic (3 months post) PTSD-like outcomes following repetitive blast exposure (A. Schindler et al., 2020). Likewise, a variety of anxiety and depression-related behavioral outcomes have been demonstrated in rodent models of blast (Cernak, 2001; Krukowski et al., 2020; Mouzon et al., 2014; Perez-Garcia et al., 2018, 2019). In relation to potential substance misuse and abuse, repetitive blast exposure with body shielding in rats increased voluntary EtOH intake during a short-access challenge session and increased oxycodone seeking following extinction and a period of forced abstinence (Lim et al., 2015; Nawarawong et al., 2019). Likewise, a single mild frontal impact injury resulted in increased cocaine self-administration in rats (Vonder Haar et al., 2019), whereas a single moderate impact injury increased EtOH sedation and decreased voluntary consumption in mice (Krukowski et al., 2020). Finally, we report here that both single blast and repetitive

blast increased the sedative properties of EtOH (with no change in tolerance or metabolism), but only repetitive blast potentiated EtOH-induced locomotor stimulation and shifted EtOH intake patterns (i.e., increased consumption “front-loading,” decreased total daily intake) during intermittent 2-bottle choice. As is common for home cage drinking studies, mice were single housed during the intermittent 2-bottle choice procedure, creating a potential confound warranting future investigation to determine the potential for blast x housing interaction effects. Together, these results demonstrate important similarities and differences across TBI models in respect to TBI number, severity, and injury method. Our result of decreased daily EtOH intake 1 month following repetitive blast-mTBI in mice might seem contrary to our clinical data, suggesting increased EtOH intake self-report in Veterans with a history of blast-mTBI. We interpret these findings to suggest that increased EtOH stimulation and sensitivity following repetitive blast exposure in mice act to limit the overall amount of EtOH consumed (e.g., blast-mTBI mice reach reward and/or intoxication more quickly than shams, thus limiting subsequent opportunities for consumption). In line with this idea, we found that EtOH consumption “front-loading” was significantly increased in our repetitive blast-mTBI mice, highlighting a potentially more “binge”-like intake pattern following repetitive blast exposure. Such “front-loading” behavior is linked to “binge”-like consumption and has been previously reported in other animal models (Salling et al., 2018; Wilcox et al., 2014) and observed in humans with increased vulnerability to developing problem drinking and alcohol use disorder (Gowin et al., 2017). While the current studies were not originally designed to specifically investigate EtOH binge intake, back translation of the results from our AUDIT-C analysis in Veterans will be

enhanced by the specific use of an established animal model of binge drinking (i.e., drinking in the dark) and will be a focus of future investigations. The findings reported here from our unsupervised cluster analysis of AUDIT-C self-report in Veterans with/without a history of blast-mTBI are in keeping with results from animal models of mTBI. Specifically, frequency of assignment to the “risky” drinking cluster was higher in Veterans reporting a history of repetitive blast-mTBI with loss of consciousness (as compared to blast-mTBI with only altered consciousness). Our cluster analysis findings are a Iso in line with a previous report demonstrating increased odds of frequent binge drinking in Veterans with a history of TBI with loss of consciousness as compared to Veterans with no history of TBI or Veterans with history of TBI without loss of consciousness (Adams et al., 2012). It is important to note that while we tested drinking acquisition in mice using the intermittent 2-bottle choice procedure 1 month following blast-mTBI, it is likely that many of the Veteran study participants had a history of alcohol intake prior to deployment and blast exposure. Likewise, alcohol consumption and binge drinking have been commonly reported by active-duty military personnel and correlate with combat exposure, with increased rates seen in Iraq/Afghanistan (Santiago et al., 2010; Seal et al., 2011; Stahre et al., 2009), so it is possible that the Veteran participants consumed alcohol starting at a more acute time point (e.g., hours or days) post blast than the mice examined at 1 month after in the current study (mice are estimated to mature 45× faster than humans during early adulthood (Fox et al., 2007). Thus, it will be important to investigate how prior history of alcohol affects subsequent blast-induced changes to alcohol intake patters and sensitivity in future studies. Indeed, alcohol intake occurring peri-TBI exposure in male rats resulted in worse outcomes as

compared to rats with TBI but no previous history of alcohol (Fucich et al., 2019; Mayeux et al., 2015; Teng et al., 2015), supporting the potential for alcohol to impair recovery and/or exacerbate injury.

An AUDIT-C total score of 5 or higher in male Veterans is recommended as a positive screen for potential alcohol misuse and/or dependence (a score of 4 or higher is used for males in the general population), requiring follow-up with a healthcare provider (Bradley et al., 1998; Bush et al., 1998). When examined for its predictive ability, a cutoff of 5 exhibits a high rate of specificity but lower sensitivity in its ability to properly identify patients with substance misuse; indeed, this number was optimized to minimize the burden of false-positive rates on VA providers (Bradley et al., 1998; Bush et al., 1998). Using our cluster-based approach, the “frequent” and “risky” clusters share overlapping AUDIT-C scores of 4–7, raising the possibility that this approach might provide useful additional information to aid in the assessment of whether self-reported drinking behavior should be of concern for the medical provider. These results now require external validation in a larger sample without exclusion criteria related to substance abuse/dependence and/or in a population outside of the VA to determine potential merit of using such a cluster-based approach in a clinical care setting.

How repetitive blast-mTBI might drive subsequent health risk behaviors and addiction risk at the mechanistic level remains unknown. Null results from the current study discount blast- induced changes to EtOH tolerance and metabolism as potential underlying mechanisms. Conversely, we and others have demonstrated mTBI-induced changes to both tonic and phasic dopamine release patterns, as well as neuropathological/inflammatory changes within the mesolimbic system (Sajja et al.,

2013, 2015; A. G. Schindler et al., 2017; Vonder Haar et al., 2019). We previously demonstrated a blast-mTBI-induced increase in stimulated phasic dopamine release within the nucleus accumbens, and other reports demonstrate blast- mTBI-induced neuroinflammation and tissue damage within the mesolimbic system (Sajja et al., 2013, 2015). Likewise, mild-to-moderate impact TBI models in rats and mice demonstrate alterations in mesolimbic dopamine receptors and related signal-transduction proteins (Lowing et al., 2014; Vonder Haar et al., 2019). Damage to the mesolimbic system has been associated with deficits in executive function and emotional control, potentially leading to increased health risk behaviors and addiction risk. Blast-induced changes to the structure and/or function of mesolimbic circuits thus pose a potential underlying mechanism related to blast-induced changes in EtOH and drug sensitivity and intake. While the adverse outcomes of trauma are thought to be mediated at least in part though maladaptive changes to the mesolimbic dopamine system, a causal role for mesolimbic dopamine dysfunction in blast-induced behavioral pathology has yet to be established and will be the focus of future investigations.

Together, our results highlight that while a single blast-mTBI can result in changes to the sedating properties of alcohol (without changes in tolerance or metabolism), only repetitive blast-mTBI results in prolonged EtOH-induced locomotor stimulation and “binge”-like consumption “front-loading” in mice and a shift to the “risky” drinking cluster in Veterans. Binge drinking specifically is associated with increased risk for negative consequences related to addiction, criminality, and chronic adverse health outcomes (e.g., obesity, liver damage). Over 400,000 OEF/OIF/OND Veterans have a history of blast exposure and mTBI (most often repetitive), highlighting the potential for

significant costs related to blast-mTBI- induced increases in risky drinking patterns and binging. While we are not able to draw causal inferences from our Veteran cohort, these data in combination with data from our animal model strongly support the notion of repetitive blast-mTBI as a driver of health vrisk behaviors such as risky drinking. Together, these results highlight the importance of understanding blast trauma history in OEF/OIF/OND Veterans who might also be at risk for substance misuse and/or abuse. Additional studies are warranted to further explore potential underlying mechanisms and treatment targets (e.g., the mesolimbic dopamine system) for those with a history of repetitive mTBI and higher potential likelihood of health risk behaviors and addiction risk.

Chapter 3.

Blast Exposures Have a Complex Impact on Executive Function, Simultaneously Increasing Appetitive Motivation While Also Negatively Impacting Behavioral Flexibility*

*This chapter was formatted for this thesis from the following peer-reviewed publication:

"Repetitive Blast Exposure Increases Appetitive Motivation and Behavioral Inflexibility in Male Mice". Baskin B., Lee, S. J., Skillen, E., Wong, K., Rau, H., Hendrickson, R. C., Pagulayan, K., Raskind, M. A., Peskind, E. R., Phillips, P. E. M., Cook, D. G. & Schindler, A. G. (2021) *Frontiers in Behavioral Neuroscience,. 15:792648.*

B.M.B designed, performed, and analyzed the experiments in the paper and wrote the paper.

INTRODUCTION

Deficits in cognitive control and flexibility are common following mild traumatic brain injury (mTBI) and can significantly contribute to decreased quality of life (Hendrickson et al., 2018; McInnes et al., 2017; Ozga et al., 2018). Blast exposure is a leading cause of mTBI in Servicemembers and Veterans of the Iraq and Afghanistan War and can also occur in urban terrorist attacks and industrial accidents (Hendrickson et al., 2018; Hoge et al., 2008; Rosenfeld et al., 2013; Tanielian et al., 2008). Cognitive impairments commonly reported by Veterans with a history of blast exposure include alterations in memory, deficits in mental flexibility, and difficulty with adaptability (e.g., executive dysfunction) (Amick et al., 2013; Hendrickson et al., 2018; Karr et al., 2019; Pagulayan et al., 2018; A. G. Schindler et al., 2017; Sullivan et al., 2018). Posttraumatic stress disorder (PTSD) and depression are also highly comorbid with blast-related mTBI, leading to complications and difficulty in diagnosis and treatment development (Amick et al., 2013; Hendrickson et al., 2018; Karr et al., 2019; Neipert et al., 2014; Rau et al., 2018; Verfaellie et al., 2014).While an estimated 400,000 Veterans have experienced blast mTBI, prophylactic approaches and treatment options remain limited and are not universally effective.

We and others have previously reported acute and chronic maladaptive outcomes related to PTSD and depression following blast mTBI exposure in animal models (Baskin et al., 2021; Elder et al., 2010, 2012; Goldstein et al., 2014; Logsdon et al., 2020; Muelbl et al., 2018; Perez-Garcia et al., 2019; A. G. Schindler et al., 2017; A. G. Schindler, Terry, et al., 2021, 2021). Using a variety of behavioral paradigms, collective

results demonstrate blast mTBI-induced deficits in working memory, sensorimotor performance, and motivation. These results raise the possibility that deficits in executive function arise indirectly because of anxiety, hyperarousal, and/or motivation deficits. More sophisticated operant based paradigms aimed at assessing discrete aspects of flexible goal directed behavior in animal models are now required to further uncover how repetitive blast exposure contributes to executive dysfunction and is the focus of the current study.

Here we utilized our well-established pneumatic shock tube that models battlefield-relevant open-field blast forces generated by detonation of high explosives (Logsdon et al., 2020; Schindler et al., 2021; Schindler et al., 2017; Schindler, et al., 2021), behavioral measures related to anxiety, compulsivity, and hyperarousal, and operant reward learning, motivation, and flexibility paradigms in adult male mice. We hypothesized that a history of blast exposure would result in anxiety/compulsivity-like outcomes and corresponding performance deficits in operant-based reward learning and behavioral flexibility assays. Results instead demonstrate that repetitive blast exposure results in enhanced motivation and goal directed behavior with a corresponding increase in behavioral inflexibility and compulsive-like responding. Together, these data highlight a unique constellation of adverse behavioral outcomes related to executive dysfunction following repetitive blast mTBI and highlight new areas for future research aimed at diagnosis and treatment development.

MATERIALS AND METHODS

Animals and mouse model of blast overpressure:

All animal experiments were carried out in accordance with Association for Assessment and Accreditation of Laboratory Animal Care guidelines and were approved by the VA Puget Sound Institutional Animal Care and Use Committees. Male C57Bl/6 mice (Jackson Laboratory) were aged 9 weeks upon arrival and allowed to acclimate for a week followed by an additional week of handling habituation prior to any blast or sham exposures. The shock tube (Baker Engineering and Risk Consultants) was designed to generate blast overpressures to induce blast TBIs in mice that mimic open-field high explosive detonations encountered by military service members in combat, and the design and modeling characteristics have been described in detail elsewhere (Logsdon et al., 2020; Schindler, et al., 2021; Schindler et al., 2017; Schindler, et al., 2021). Briefly, mice were anesthetized with isoflurane (induced at 5% and maintained at 2-3%), secured against a gurney, and placed into the shock tube oriented perpendicular to the oncoming blast wave (ventral body surface toward blast). Sham (control) animals received anesthesia for a duration matched to blast animals. All mice had repeated blast/sham exposures which occurred successively over the course of 3 days (1 per day). The blast overpressure (BOP) peak intensity (psi), initial pulse duration (ms), and impulse (psi▪ms) used were in keeping with mild blast TBI (20.1 psi +/- 0.13 psi). Under these experimental conditions, the overall survival rate exceeded 95%, with blast-exposed mice comparable to sham-exposed mice on inspection 2–4 h following exposure (e.g., responsive to stimuli, normal posture and breathing). All behavioral tests were conducted starting at 1 month post-sham/blast exposure, a time point that allows

for the development of blast-induced neuropathology (Elder et al., 2010; Goldstein et al., 2014; Huber et al., 2013, 2016; Meabon et al., 2016) and that correlates to a time period where enduring functional and behavioral deficits are detected (Logsdon et al., 2020; Schindler, et al., 2021; Schindler et al., 2017; Schindler et al., 2021)Separate sets of mice were used for the behavioral battery (marble burying, elevated zero maze, acoustic startle) and the operant tasks (lever press discrimination, progressive ratio break point, lever alternation) and at least 2 cohorts of mice were used in each behavioral paradigm. Mice were housed on a 12:12 light:dark cycle (lights on at 6am) and were given ad libitum food and water, except during operant behaviors where their food was restricted to maintain 83%-90% of their ad libitum body weight.

Behavioral Battery (Figure 3.1A)

The behavioral battery consisted of three testing paradigms conducted over one week (one test paradigm per day). The order of behavioral tests was specifically chosen to go from the least stressful to the most stressful task in order to prevent carryover distress from one behavior to the next. *Marble burying:* Animals were allowed to explore an open field (clean rat cage) filled with 5 cm of bedding and 18 marbles for 30 min. Marbles were counted as buried if at least 2/3rd of the height of the marble was covered with bedding. *Elevated zero maze:* Animals were allowed to explore an elevated zero maze for 5 minutes. Movement was recorded to video from above and analyzed using Anymaze (Wood Dale, IL). *Acoustic startle:* Conducted using SR-LAB acoustic startle boxes (San Diego Instruments, San Diego, CA). Following a 5-min acclimation period, startle habituation testing consisted of fifty trials of 120-dB pulses delivered with an

inter-trial interval of 7-23 s. Prepulse inhibition (PPI) was next assessed and consisted of forty trials of 81-dB prepulse followed by a 120-dB pulse with varying interstimulus interval (*ISI*) of 2-1000 ms (five trials each). Blast exposure can result in hearing loss; we use the within subject analysis approaches of startle habituation and PPI in attempts to mitigate potential confounds of hearing loss on startle outcome measures and interpretation.

Operant testing (Figure 3.2A)

Operant testing was conducted in chambers (ENV-307W; Med Associates, Inc.) outfitted with a feeder situated in between two retractable levers, cue light above each lever, a houselight, and a white noise fan. Head entries were recorded during all sessions by breaking an infrared photobeam within the pellet feeder. Mice were food restricted one week prior and throughout the duration of operant behaviors. Initially, mice were trained to retrieve food pellets in a single 15-minute magazine training session in which 10 food pellets (20 mg; BIO-SERV) were delivered randomly and all mice consumed a minimum of 2 pellets. *Lever press discrimination (LPD):* Mice underwent six one-hour fixed-ratio one (FR1) discrimination sessions (one per day) during which both levers were inserted into the chamber and a response on the active lever (counterbalanced across exposure conditions and cohorts of mice), indicated by a blinking cue light above the lever, earned them a pellet. Lever presses on the inactive lever were recorded but had no consequences. After a successful press on the active lever, both levers retracted for an average inter-trial interval of 30 seconds (range 15-45 s). Mice were able to earn up to 120 pellets per session. *Progressive ratio (PR):* Next,

animals were tested for motivation and willingness to work to earn pellets in a standard progressive ratio break point task. The progressive ratio increased by a factor of square root of two across trials (rounded down integers: 1, 2, 2, 3, 4, 6, 8, 11, 16, 23, 32, 45, 63, 89, 125, 176, etc.). Sessions were terminated when an animal failed to complete a PR trial within 15 min. Animals were assessed in separate sessions where either 1 or 3 pellets served as the reinforcer. Animals were first trained in a FR3 where three lever presses were required to obtain one sucrose pellet. Animals were further trained following the 1 pellet PR session in a FR3 where three lever presses were required to obtain 3 sucrose pellets. *Lever press alternation (LPA):* Finally, animals were tested for behavioral flexibility over the course of 3 days using a 60-minute lever switching paradigm during which each session the active lever alternated between their learned active lever and the previously inactive lever every 5 trials (i.e., lever contingency switched after every 5 pellets earned).

Data analysis:

As appropriate, data were analyzed using: (*i*) two-tailed Student's t-tests; (*ii*) two-way (between/within subjects design) repeated measures analysis of variance (RM ANOVA), followed by Bonferroni-Šídák Post-hoc tests. Reported significant *p* values denote two-tailed probabilities of $p \leq 0.05$ and non-significance (n.s.) indicates $p > 0.05$. Med associated data were analyzed using custom Python scripts. Statistical analyses were conducted using Python and Graph Pad Prism 4.0 (GraphPad Software, Inc., La Jolla, CA).

RESULTS

Repetitive blast exposure increases behavioral measures of anxiety-like behavior and sensory reactivity

One month following repetitive sham or blast exposure, male mice were tested in the marble burying assay (anxiety/compulsivity), elevated zero maze (anxiety/risk-taking), acoustic startle habituation (non-associated learning), and acoustic startle prepulse inhibition (sensory gating) (Figure 3.1a), as behavioral dysfunction in these paradigms have previously been linked to deficits in executive function and motivation (Ozga et al., 2018). Repetitive blast exposure resulted in acute weight loss that resolved to sham levels by one month

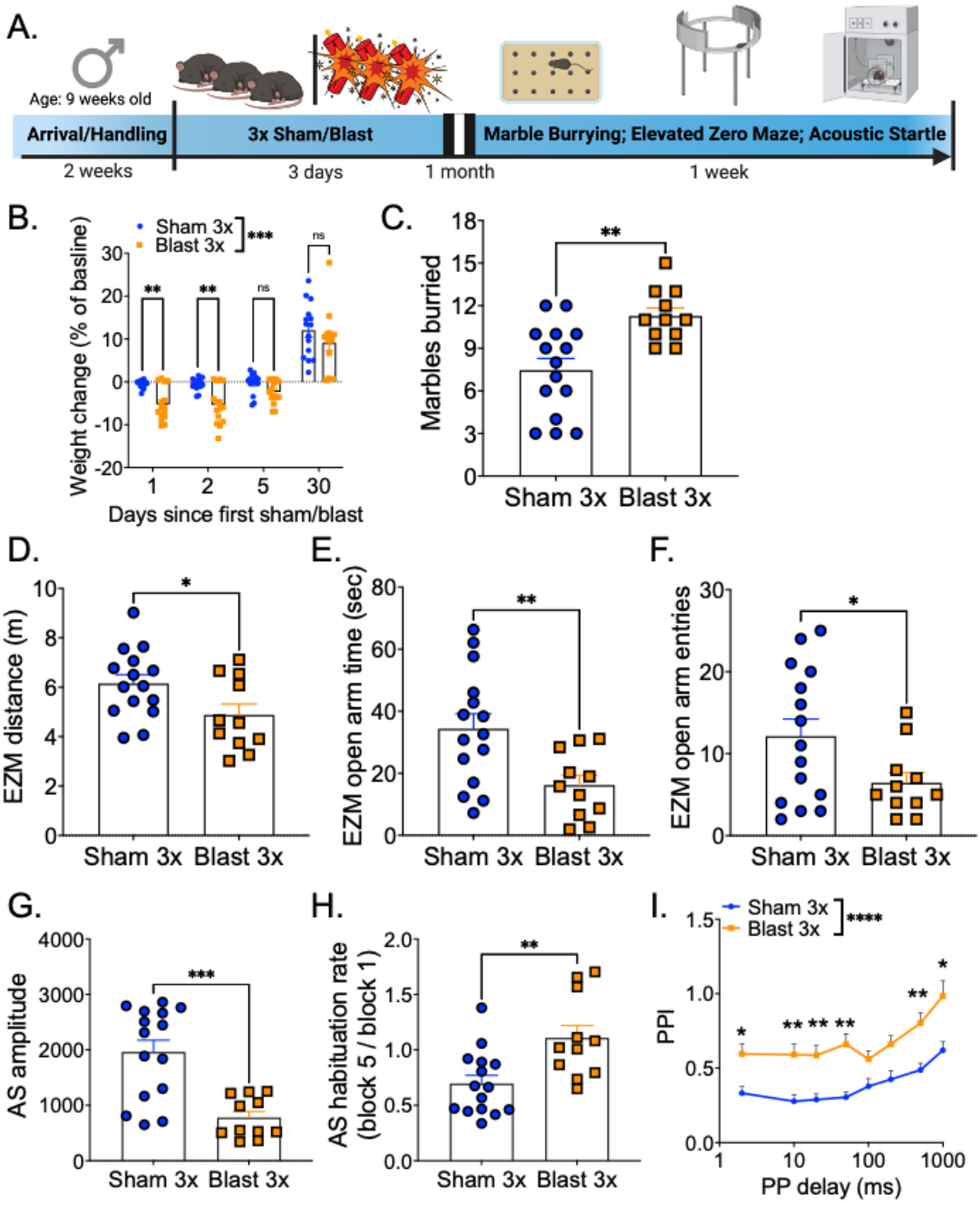

Figure 3.1. Repetitive blast exposure increases behavioral indices of anxiety/compulsivity and hyperreactivity. **(A)** Timeline schematic. **(B)** Weight change as % baseline weight. **(C)** Number of marbles buried. **(D)** Distance traveled in the elevated

zero maze. **(E)** Time spent in the open arm of the elevated zero maze. **(F)** Entries into the open arms of the elevated zero maze. **(G)** Raw acoustic startle amplitude. **(H)** Acoustic startle habituation rate. **(I)** Pre-pulse inhibition (PPI). Student's *t*-test **(C–H)**, two-way RM ANOVA Bonferroni-Šídák *post hoc* **(B,E)**. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$, ns, not significant. Values represent mean ± SEM.

post exposure (two-way RM ANOVA: main effect of group F[1,28]=21.11, p=0.0001, main effect of time F[3,28]=164.3, p<0.0001, interaction effect F[3,84]=7.884, p=0.0001, Bonferroni-Šídák; n=11-15) (Figure 3.1b). At the one month time point, repetitive blast exposure increased the number of marbles buried (Student's unpaired t-test, t[24]=3.541, *p*=0.002, n=11-15) (Figure 3.1c), decreased the distance traveled in the elevated zero maze (Student's unpaired t-test, t[24]=2.267, *p*=0.03, n=11-15) (Figure 1d), decreased the time spent in the open arms of the elevated zero maze (Student's unpaired t-test, t[24]=2.914, *p*=0.008, n=11-15) (Figure 1e), and decreased the number of entries into the open arms of the elevated zero maze (Student's unpaired t-test, t[24]=2.115, *p*=0.04, n=11-15) (Figure 1f). Likewise, repetitive blast exposure resulted in acoustic startle deficits as evidenced by a decrease in raw startle amplitude (Student's unpaired t-test, t[24]=4.417, *p*=0.0002, n=11-15) (Figure 3.1g), inhibited acquisition of acoustic startle habituation (Student's unpaired t-test, t[24]=3.183, *p*=0.004, n=11-15) (Figure 3.1h), and impaired prepulse inhibition (two-way RM ANOVA: main effect of group F[1,28]=21.2, p<0.0001, main effect of delay F[5,140]=17.65, p<0.0001, interaction effect F[7,196]=0.437, p>0.05, Bonferroni-Šídák; n=11-15) (Figure 3.1i). Together these results identify affective and sensorimotor deficits that might negatively impact executive function and highlight the need for further research aimed at assessing discrete aspects of these behaviors using more sophisticated operant paradigms.

Repetitive blast exposure increases goal directed behavior

In a separate set of male mice, we conducted a series of operant paradigms one month following repetitive sham or blast exposure (Figure 3.2a). Mice were first tested for goal-

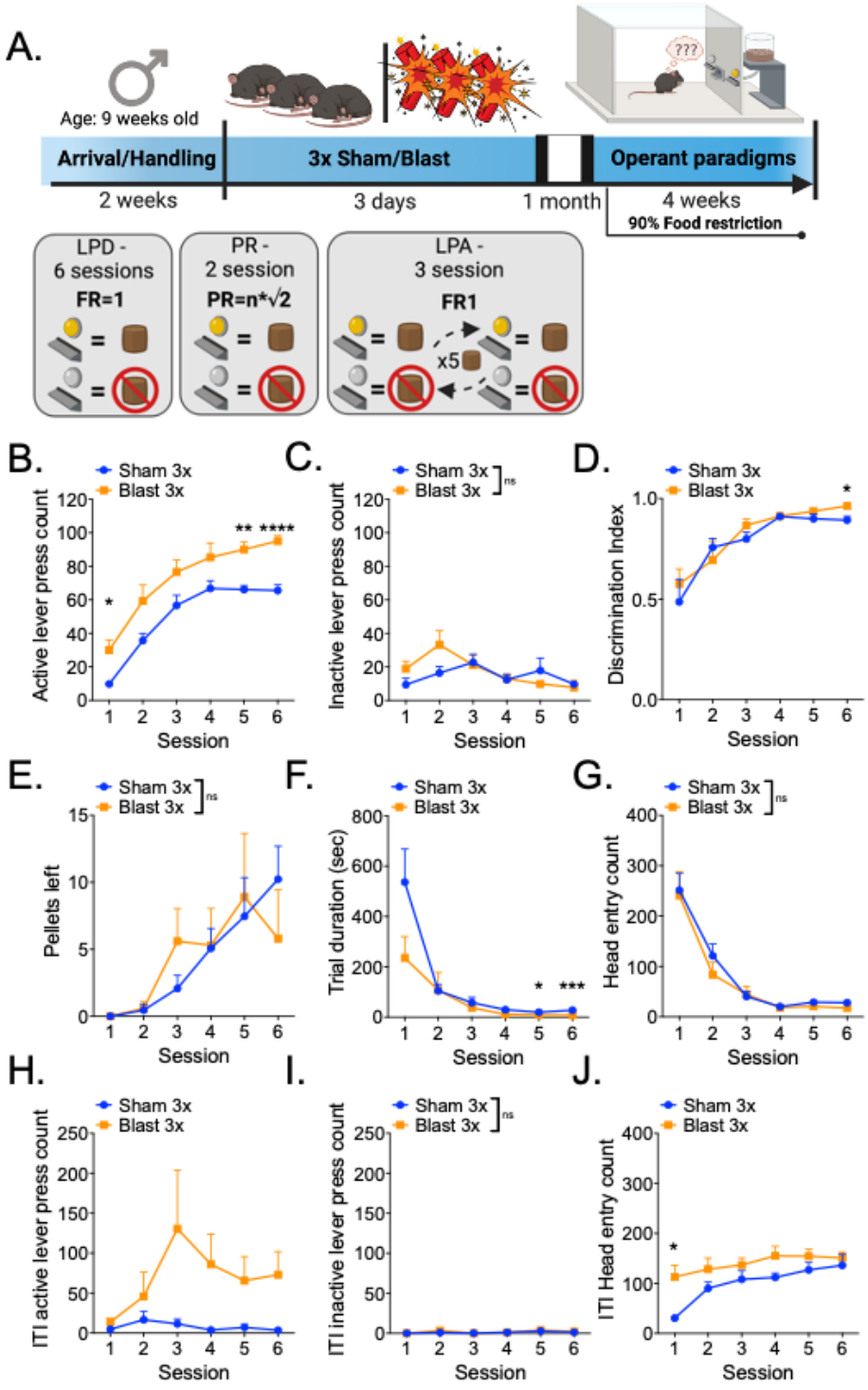

Figure 3.2. Repetitive blast exposure increases appetitive operant behavior. **(A)** Timeline schematic. **(B)** Number of active lever presses. **(C)** Number of inactive lever

presses. **(D)** Discrimination Index (active − inactive/active + inactive). **(E)** Number of pellets left uneaten. **(F)** Trial duration in seconds. **(G)** Number of head entries during lever out. **(H)** Number of active lever presses during ITI. **(I)** Number of inactive lever presses during ITI. **(J)** Number of head entries during ITI. Two-way RM ANOVA Bonferroni-Šídák *post hoc* **(B–J)**. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$, ns, not significant. Values represent mean ± SEM. ITI, inter-trial-interval.

directed lever press discrimination over six days in standard operant conditioning boxes using a fixed-ratio 1 (FR1) where a press on the active, but not on the inactive, lever yielded sucrose-pellet delivery. Blast mTBI mice displayed enhanced goal directed behavior as characterized by increased active lever presses (two-way RM ANOVA: main effect of group $F[1,22]=16.77$, $p=0.0005$, main effect of session $F[3,63]=63.96$, $p<0.0001$, interaction effect $F[5,110]=0.46$, $p>0.05$ Bonferroni-Šídák; n=11-13) (Figure 3.2b), no difference in inactive lever presses (two-way RM ANOVA: main effect of group $F[1,22]=0.87$, $p>0.05$, main effect of session $F[4,80]=3.3$, $p=0.018$, interaction effect $F[5,110]=1.8$, $p>0.05$ Bonferroni-Šídák; n=11-13) (Figure 3.2c), and no difference in discrimination index (two-way RM ANOVA: main effect of group $F[1,22]=0.812$, $p>0.05$, main effect of session $F[5,22]=19.55$, $p<0.0001$, interaction effect $F[5,110]=0.65$, $p>0.05$ Bonferroni-Šídák; n=11-13) (Figure 3.2d). Furthermore, sham and blast mice had no difference in the number of pellets left uneaten (two-way RM ANOVA: main effect of group $F[1,22]=0.005$, $p>0.05$, main effect of session $F[5,22]=7.06$, $p=.001$, interaction effect $F[5,110]=0.96$, $p>0.05$ Bonferroni-Šídák; n=11-13) (Figure 3.2e) but blast exposed mice exhibited a shorter trial duration (two-way RM ANOVA: main effect of group $F[1,22]=2.7$, $p=0.11$, main effect of session $F[5,22]=17.39$, $p<0.0001$, interaction effect $F[5,110]=2.87$, $p=0.012$ Bonferroni-Šídák; n=11-13) (Figure 3.2f) and no difference in the number of head entries (two-way RM ANOVA: main effect of group $F[1,22]=0.47$, $p>0.05$, main effect of session $F[5,22]=46.66$, $p<0.0001$, interaction effect $F[5,110]=0.31$, $p>0.05$ Bonferroni-Šídák; n=11-13) (Figure 3.2g). Blast mTBI mice also displayed enhanced perseverative/compulsive-like reward seeking behavior, as characterized by increased active lever presses during the inter-trial intervals when the

levers were retracted and unrewarded (two-way RM ANOVA: main effect of group $F[1,22]=5.65$, $p=0.023$, main effect of session $F[5,22]=1.933$, $p>0.05$, interaction effect $F[5,110]=1.825$, $p>0.05$ Bonferroni-Šídák; n=11-13) (Figure 3.2h), but not inactive lever presses (two-way RM ANOVA: main effect of group $F[1,22]=0.36$, $p>0.05$, main effect of session $F[5,22]=1.4$, $p>0.05$, interaction effect $F[5,110]=0.19$, $p>0.05$ Bonferroni-Šídák; n=11-13) (Figure 3.2i), and increased head entries (two-way RM ANOVA: main effect of group $F[1,22]=6.314$, $p=0.02$, main effect of session $F[5,22]=8.45$, $p<0.0001$, interaction effect $F[5,110]=1.6$, $p>0.05$ Bonferroni-Šídák; n=11-13) (Figure 2j).

Repetitive blast exposure increases willingness to work for reward

To further examine motivation, mice were next tested in a progressive ratio schedule during which the ratio requirement to obtain reward (either 1 or 3 sucrose pellets) increased across trials (Figure 3.3a). In line with enhanced motivation, repetitive blast exposure resulted in a higher break point (two-way RM ANOVA: main effect of group $F[1,22]=9.01$, $p=0.007$, main effect of session $F[1,22]=0.2$, $p>0.05$, interaction effect $F[1,22]=3.67$, $p>0.05$ Bonferroni-Šídák multiple comparison method; n=11-13) (Figure 3.3b), increased reinforcers earned (two-way RM ANOVA: main effect of group $F[1,22]=10.07$, $p=0.004$, main effect of session $F[1,22]=1.41$, $p>0.05$, interaction effect $F[1,22]=2.03$, $p>0.05$ Bonferroni-Šídák; n=11-13) (Figure 3.3c), a shorter inter-response interval time (IRT) (two-way RM ANOVA: main effect of group $F[1,22]=8.08$, $p=0.009$, main effect of session $F[1,22]=10.6$, $p=.004$, interaction effect $F[1,22]=1.266$, $p>0.05$ Bonferroni-Šídák; n=11-13) (Figure 3d), no difference in inactive lever presses (two-way RM ANOVA: main effect of group $F[1,22]=1.23$, $p>0.05$, main effect of session

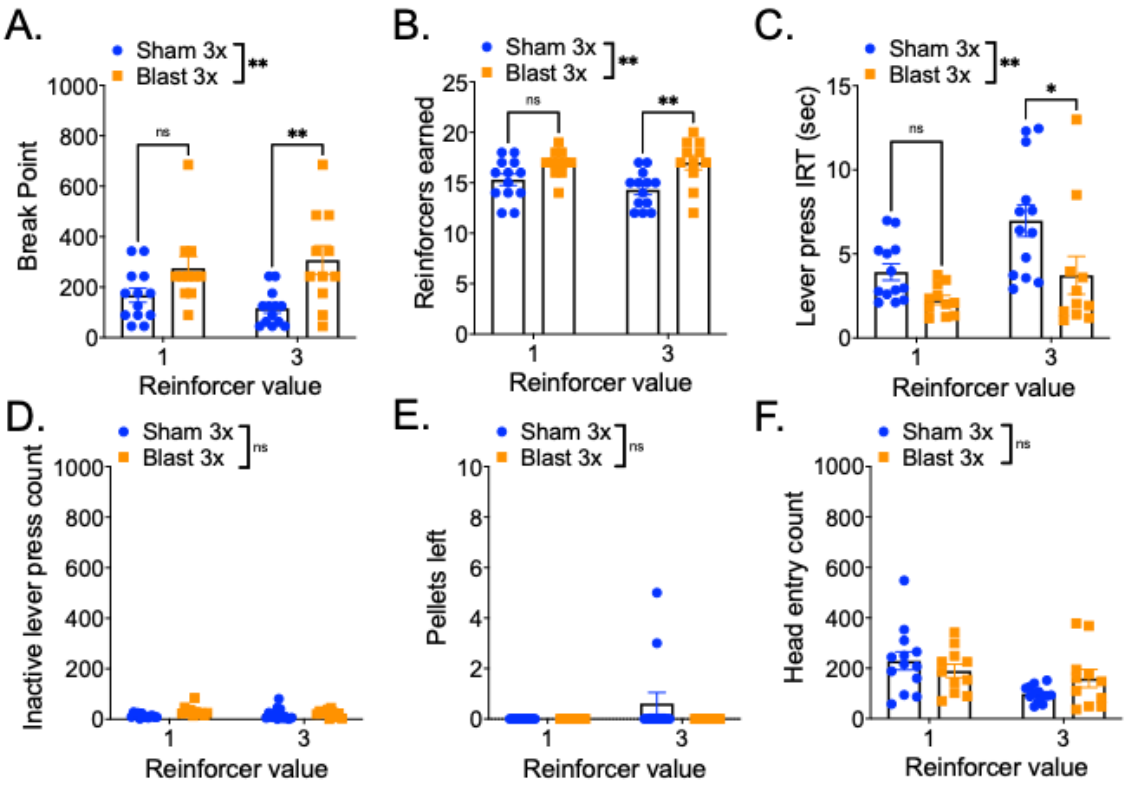

Figure 3.3. Repetitive blast exposure increases motivation and willingness to work for reward. **(A)** Progressive ratio break point (last ratio completed). **(B)** Number of reinforcers earned. **(C)** Active lever press inter-response time. **(D)** Number of inactive lever presses. **(E)** Number of pellets left uneaten. **(F)** Number of head entries during lever out. Two-way RM ANOVA Bonferroni-Šídák *post hoc* **(A–F)**. *$p \leq 0.05$, **$p \leq 0.01$, ns, not significant. Values represent mean ± SEM.

F[1,22]=1.45, p>0.05, interaction effect F[1,22]=0.05, p>0.05 Bonferroni-Šídák; n=11-13) (Figure 3.3e), no difference in the number of pellets left unwanted (two-way RM ANOVA: main effect of group F[1,22]=1.71, p>0.05, main effect of session F[1,22]=1.71, p>0.05, interaction effect F[1,22]=1.17, p>0.05 Bonferroni-Šídák; n=11-13) (Figure 3.3f), and no difference in the number of head entries (two-way RM ANOVA: main effect of group F[1,22]=0.16, p>0.05, main effect of session F[1,22]=6.85, p=0.02, interaction effect F[1,22]=2.66, p>0.05 Bonferroni-Šídák; n=11-13) (Figure 3g).

Repetitive blast exposure reduces behavioral flexibility

Finally, to study reward-related behavioral flexibility, mice were tested in a lever press alternation paradigm where the active/inactive lever contingencies switched every five correct trials (Figure 3.4a).

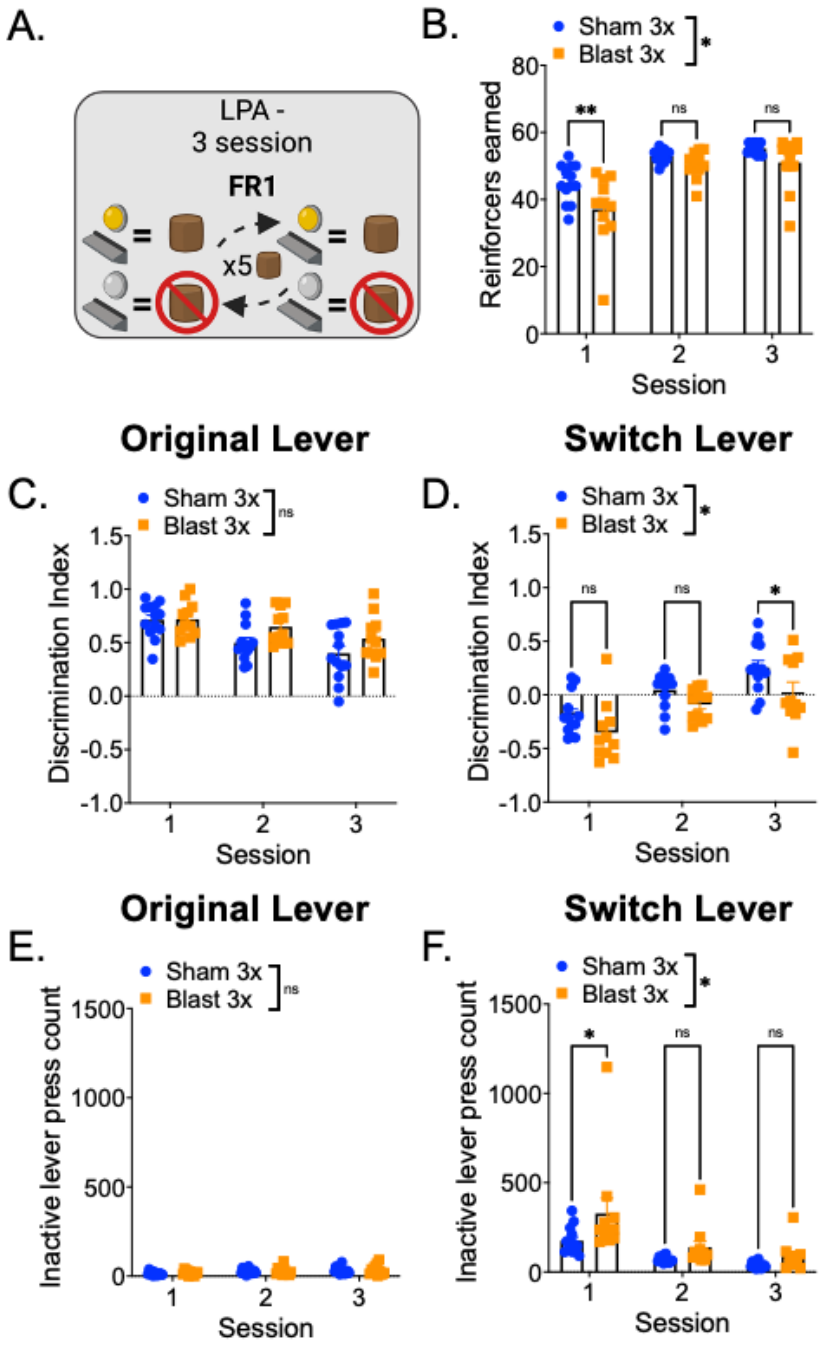

Figure 3.4. Repetitive blast exposure results in behavioral inflexibility and perseverative-like responding. **(A)** Lever press alternation (LPA) schematic—lever contingencies switch every five reinforcers obtained. **(B)** Number of reinforcers earned. **(C,D)** Discrimination Index (active−inactive/active+inactive) on original **(C)** and switch **(D)**

trials. **(E,F)** Number of inactive lever presses on original **(E)** and switch **(F)** trials. Two-way RM ANOVA Bonferroni-Šídák *post hoc* **(A–F)**. *$p \leq 0.05$, **$p \leq 0.01$, ns, not significant. Values represent mean ± SEM.

In line with increased compulsive/perseverative behavior and a decrease in behavioral flexibility, repetitive blast exposure impaired performed on this task, as evidenced by a significant decrease in the number of reinforcers earned (two-way RM ANOVA: main effect of group $F[1,22]=5.374$, $p=0.03$, main effect of session $F[2,44]=72.32$, $p=0.0001$, interaction effect $F[2,44]=2.82$, $p>0.05$ Bonferroni-Šídák; n=11-13) (Figure 3.4b). We next analyzed separately trials where the current active lever was the lever used in initial training as the active lever (original lever) and where the current active lever was the lever used in initial training as the inactive lever (switch lever) and found that while blast mice performed similarly well on the trials with the original lever, they performed significantly worse on the switch trials (Figure 3.4c-h). Specifically, we found no difference in lever discrimination on trials when the original lever was active (two-way RM ANOVA: main effect of group $F[1,22]=2.89$, $p>0.05$, main effect of session $F[2,44]=15.0$, $p<0.0001$, interaction effect $F[2,44]=1.57$, $p>0.05$ Bonferroni-Šídák; n=11-13) (Figure 3.4c) but worse lever discrimination on trials when the alternative lever was active (two-way RM ANOVA: main effect of group $F[1,22]=7.31$, $p=0.01$, main effect of session $F[2,44]=31.15$, $p<0.0001$, interaction effect $F[2,44]=0.71$, $p>0.05$ Bonferroni-Šídák; n=11-13) (Figure 3.4d). Likewise, while we found no group difference in the number of inactive lever presses on the original lever trials (two-way RM ANOVA: main effect of group $F[1,22]=0.2$, $p>0.05$, main effect of session $F[2,44]=6.27$, $p=.004$, interaction effect $F[2,44]=0.09$, $p>0.05$ Bonferroni-Šídák; n=11-13) (Figure 3.4e) but an increased number of inactive lever presses on the switch lever trials (two-way RM ANOVA: main effect of group $F[1,22]=6.84$, $p=0.01$, main effect of session $F[2,44]=17.81$, $p<0.0001$, interaction effect $F[2,44]=1.35$, $p>0.05$ Bonferroni-Šídák;

n=11-13) (Figure 4d). Together, these results suggest that repetitive blast exposure in male mice reduces behavioral flexibility, resulting in enhanced perseverative/compulsive-like responding.

DISCUSSION

Changes in affective processing, learning, and motivation are commonly reported in patients with a history of mTBI and are hallmarks of PTSD, depression, and addiction (McInnes et al., 2017; Ozga et al., 2018). Failure to properly use contextual information to modify conditioned responses and goal-directed behavior may underlie heightened fear generalization, increased impulsivity/compulsivity, and deficits in updating of stimulus-response and action-outcome contingencies (i.e., executive dysfunction) (Berridge, 2004; María-Ríos & Morrow, 2020; Treadway & Zald, 2013). Critically, while behavioral deficits related to executive function have been well studied in animal models of moderate-to-severe TBI (Modrak et al., 2020; Ozga et al., 2018; Vonder Haar et al., 2013, 2014; Vonder Haar & Winstanley, 2016), no previous animal studies have focused on examining the effects of repetitive blast mTBI on executive function. Based on clinical reports of increased executive dysfunction in Veterans with a history of blast mTBI, we hypothesized that repetitive blast exposure in male mice would result in anxiety/compulsivity-like outcomes and performance deficits in operant-based reward learning and behavioral flexibility paradigms. Instead, here we provide evidence for an increase in reward seeking and a congruent decrease in behavioral flexibility. Furthermore, we report chronic adverse behavioral changes related to anxiety, compulsivity, and hyperreactivity. In combination, these data suggest that blast mTBI

reduces behavioral flexibility and cognitive control that strengthens appetitive responding because of enhanced compulsivity/hyperreactivity while at the same time reducing the ability to update performance and adapt to new task structures.

Here we utilized a series of goal-oriented operant tasks where mice were rewarded when they successfully completed an appropriate lever press. In contrast to our original hypothesis, we found that blast mice were not delayed in acquiring lever pressing for sucrose pellets relative sham animals and did not have an impairment in operant discrimination learning. Further, blast mice completed trials faster, suggesting they displayed increased motivation when compared to sham mice. Blast mice also had increased lever pressing in the inter-trial-intervals (on the active lever alone) which suggests blast mice had higher levels of goal-directed reward seeking that they were not able to inhibit in between trials (i.e., perseveration). When mice were moved to the more demanding, progressive ratio task, blast mice reached higher breakpoints (defined as the last FR in the progressive ratio sequence that was earned) and exhibited decreased inter-response interval times, indicating higher levels of motivation and willingness to work for reward. These behavioral outcomes are in line with our reported results from the marble burying and acoustic startle assays demonstrating increased hyperreactivity and anxious/compulsive-like outcomes and are largely consistent with data from the clinical literature (McInnes et al., 2017; Ozga et al., 2018). Indeed, when animals were finally tested in the lever press alternation task, blast mice had increased difficulty in correctly completing trials in which the active lever was ‘switch’ (the lever that they were originally trained on as the inactive lever), highlighting a deficit in

behavioral flexibility with maladaptive outcomes related to task performance (i.e., fewer reinforcers earned). Importantly, although blast mice showed decreases in body weight during blast/sham exposure days, weights between groups were not different at the time of behavioral testing, suggesting motivation differences were not confounded by blast-induced weight changes.

Only two previous studies thus far have examined operant responding following blast exposure in rodent models. Muelbl et al. (2018) demonstrated that a single blast exposure with body shielding in male rats resulted in more errors during acquisition of a cue discrimination task but no differences in extradimensional set shifting or delayed matching to sample. Genovese et al. (2013) demonstrated that repetitive low-level blast exposure resulted in a decrease in inhibitory behavioral control in a conditioned fear suppression task. Likewise, results from more moderate-to-severe impact TBI exposure in animal models suggest higher levels of appetitive motivation, perseveration, and behavioral inflexibility (McInnes et al., 2017; Ozga et al., 2018). In combination with our current results, these data suggest that potential deficits in executive function following mTBI are at least in part related to maladaptive changes in perseveration/compulsivity and behavioral inflexibility and not simply due to a lack of motivation or inability to acquire task parameters, with important implications for subsequent diagnosis and treatment management.

Though our results are in line with clinical reports of Veterans with blast mTBI, there are several limitations to our current findings. As only male mice were used in these experiments, these findings may not extend to biological females. While most blast-related research has been conducted only in male animal models, a growing number of females are now serving with military occupational specialty codes which, like their male colleagues, can entail increased risk for blast exposure (Gray et al., 2020; Iverson et al., 2011). Sex differences in relation to a single blast exposure have been previously reported (Kawa et al., 2020; Russell, Handa, et al., 2018; Russell, Richardson, et al., 2018) and ongoing research is focused on comparing repetitive blast exposure in male and female mice. Further, here we assess behavioral outcomes to ~2 months post-blast, but these results may not extrapolate to more extreme timepoints and will require additional follow-up work focused on understanding blast mTBI outcomes in more aged populations. Likewise, here we focus on appetitive learning and motivation for sucrose reward, future work should also include the investigation of aversive stimuli and/or other rewarding substances such as alcohol.

Finally, while our current results highlight potential behavioral mechanisms underlying executive dysfunction post blast mTBI, potential underlying molecular mechanisms were not investigated. Brain network disruption, axonal sheering, white matter damage, and inflammation are blast associated outcomes and might contribute to adverse cognitive symptoms (Elder et al., 2010; Goldstein et al., 2014; Huber et al., 2013, 2016; Ivanov et al., 2017; Meabon et al., 2016; Petrie et al., 2014; Sponheim et al., 2011; Taber et al., 2015; Yeh et al., 2014). Future efforts should be placed on evaluating these outcomes as potential mechanisms underlying blast-induced executive

dysfunction. Likewise, dopaminergic neurotransmission within mesocorticolimbic circuits is critical for executive functioning and damage to these brain regions can occur because of blast exposure (Lim et al., 2015; Sajja et al., 2013; Schindler et al., 2017). Indeed, we previously demonstrated a blast mTBI-induced increase in stimulated phasic dopamine release within the nucleus accumbens (Schindler et al., 2017), and hypothesize that dopamine dysfunction may contribute to increased hyperreactivity and compulsive/perseverative behaviors exhibited post blast mTBI. Future research will thus focus on connecting potential blast mTBI-induced mesocorticolimbic dopamine dysfunction causally to adverse behavioral outcomes related to executive dysfunction.

Chapter 4.

Timing and Testing Matters: Sex Differences May Occur in Part Due to Age of Injury, and Analysis Methods*

*This chapter was formatted for this thesis from the following article that is currently being edited for submission.

"Timing matters: Sex differences in acute and chronic outcomes following repetitive blast trauma". Baskin, B., Logsdon A., Lee, S. J., Peskind E., Banks, W., Cook, D. G., Schindler, A. G. (in prep)

B.M.B designed, performed, and analyzed the experiments in the paper and wrote the paper.

Introduction

Traumatic brain injury (TBI) is currently a leading cause of death and disability not just in the United States but globally (Johnson & Griswold, 2017; A. I. R. Maas et al., 2017; Taylor et al., 2017). Affecting every segment of the population, TBI often leads to significant decreased quality of life and increased financial burden for both the person who sustained a TBI and caregivers of those with a TBI (Di Battista et al., 2012; Malec et al., 2017; Ozga et al., 2018; Taylor et al., 2017). Diagnosis and treatment options are not universally consistent nor effective, at least in part due to wide symptom variety post-TBI. The vast majority of preclinical TBI research has focused only on male research animals, which may express different symptom trajectories than female animals, resulting in a critical knowledge gap in the field at a time when women are at increasingly high risk for repetitive TBI exposure (Gupte et al., 2019; McCabe & Tucker, 2020).

Blast overpressure (BOP) waves, such as those caused by improvised explosive devices (IEDs), building collapses, failing infrastructure, and industrial accidents are becoming an increasingly common cause of TBI. Referred to as the “signature injury” of the conflicts in Iraq/Afghanistan (OEF/OIF/OND), repetitive blast exposure is the primary source of mTBI in warfighters, a significant driver of comorbid posttraumatic stress disorder (PTSD), and a major source of morbidity among Veterans enrolled in the VA health care system (Hendrickson et al., 2018; O’Neil et al., 2013; Owens et al., 2008; Tanielian et al., 2008; Wenger et al., 2018). In these conflicts, an estimated 75% of all mTBI reported by Servicemembers are a result of blast exposure caused by detonation of high explosives (Owens et al., 2008; Tanielian et al., 2008), and multiple

deployments are common (2.77 million Servicemembers have served on 5.4 million deployments since 2011), resulting in high potential for repetitive blast exposures. Currently, there is a lack of research on how these injuries may differentially impact people identifying as male vs. female, yet females currently represent ~15% of active duty Servicemembers and ~20% of the United States Reserve and Guard (Dye et al., 2016; Iverson et al., 2011; Kamarck, 2015; Street et al., 2013). Indeed, few preclinical studies have included both male and female animals and the few that have, have exclusively focused on impact TBI, a single TBI exposure, and/or only examined acute timepoints. To date there have only five reports examining potential sex differences following single blast mTBI (Hubbard et al., 2022; Kawa et al., 2020; McNamara et al., 2022; Russell, Handa, et al., 2018; Russell, Richardson, et al., 2018) and no reports following repetitive blast mTBI.

Given the lack of preclinical research using models of repetitive blast exposure in female rodents, this study was envisioned as a first-of-its-kind survey of adverse outcomes commonly seen following blast trauma in warfighters, with a specific focus on delineating acute and chronic outcomes related to inflammatory, blood brain barrier, microbiome, and behavioral pathology. Results highlight both similar and disparate outcomes in female vs. male mice at acute timepoints and an effect only in male mice at chronic time points following repetitive blast mTBI. Together, these results highlight new targets for diagnosis and treatment development and confirm the need for increased research dedicated to understanding how repetitive blast trauma affects diverse populations.

MATERIALS AND METHODS

Animals

All experiments utilized female and male (as determined by genital appearance at weaning) C57Bl/6 mice aged 9-11 weeks of age at time of arrival to VA Puget Sound. Mice were housed by sex in cages of three on a 12:12 light:dark cycle (lights on at 06:00), and were given *ad libitum* food and water. All animal experiments were carried out in accordance with Association for Assessment and Accreditation of Laboratory Animal Care guidelines and were approved by the VA Puget Sound Institutional Animal Care and Use Committees. Mice were acclimated to the housing room for a week following arrival and subsequently handled for an additional week prior to sham or blast exposure. Three experimental timelines were employed (Figure 4.1a) in separate sets of mice. To increase rigor and reproducibility, each experimental timeline included at least two cohorts of mice each run at separate times.

Model of Blast Trauma

The shock tube (Baker Engineering and Risk Consultants, San Antonio, TX) was designed to generate blast overpressures that mimic open field high explosive detonations encountered by military Servicemembers in combat, and the design and modeling characteristics have been described in detail elsewhere(Baskin et al., 2021; Huber et al., 2013, 2016; Logsdon et al., 2020; Meabon et al., 2016; Schindler et al., 2021; A. G. Schindler et al., 2017; Schindler, et al., 2021). Briefly, mice were weighed and then anesthetized with isoflurane (induced at 5% and maintained at 2-3% for the duration of the blast), secured against a gurney, and placed into the shock tube oriented perpendicular to the oncoming blast wave (ventral body surface towards Utilizing

condensed helium, pressurized air is built up on one end of the tube and released in a way that creates a blast overpressure wave that induces neuropathological and behavioral changes in line with blast mild traumatic brain injuries (mTBI) in warfighters (Ghai et al., 2020; Meabon et al., 2020; Peskind et al., 2011; Petrie et al., 2014; Schindler et al., 2021; Schindler et al., 2017; Wilkinson et al., 2012). Sham (control) animals received anesthesia only for a duration matched to blast animals and repeated blast/sham exposures occurred successively over the course of three days (one per day). The blast overpressure (BOP) peak intensity (psi), initial pulse duration (ms), and impulse (psi▪ms) used were in keeping with mild blast injury (19.1 +/- 0.09 psi). Under these experimental conditions, the overall survival rate exceeded 97%, with blast-exposed mice presenting comparable to sham-exposed mice by inspection 2-4 hours-post blast exposure as previously reported (Baskin et al., 2021; Huber et al., 2013, 2016; Logsdon et al., 2018, 2020; Meabon et al., 2016; Schindler et al., 2021; Schindler et al., 2017; Schindler et al., 2021). Following sham/blast exposure and removal from isoflurane, loss of righting reflex (LORR) was recorded as the amount of time it took for animals to right themselves. Once animals were able to regain full sternal recumbency, they were weighed and then returned to their cages for recovery.

Cytokine Measurement:

Mice were euthanized via cervical decapitation 4 hours after their last sham or blast exposure. Trunk blood was collected in 1.5-mL capacity serum-separator tubes containing EDTA, allowed to clot at room temperature for 30-40 minutes, and then centrifuged at 3,000 x g for 10 minutes. Serum was then aliquoted and stored at -80°C until analyzed. Whole brains were also collected at the time of euthanasia,

hemisectioned, and flash frozen at -80°C. One hemisphere of brain tissue was then lysed in a 0.02% Triton-X homogenization buffer of 10mM HEPES, 1.5mM MgCl2, and 10mM KCl, with fresh protease/phosphatase inhibitor cocktail (Sigma) in a bead homogenizer. The samples were then centrifuged at 4°C at 18,000 x g for 10 minutes and the supernatant was aliquoted and stored at -80°C. Pro- and anti-inflammatory cytokine level were then analyzed using the IDEXX (Columbia, MO) Cytokine Mouse 25-Plex Panel and values were normalized by protein concentration quantified with a BCA Protein Assay Kit.

Fecal Microbiome:

Fresh fecal pellets were collected 24 hours following final sham or blast exposure. Collection occurred in the morning between 09:00 and 11:00 using sterile technique. Fecal pellets were flash frozen in liquid nitrogen and stored at -80°C until shipment to Diversigen for downstream processing and analysis. Briefly, the DNA of fecal pellets were extracted and sequenced by Diversigen using their BoosterShot Shallow Shotgun Sequencing. DNA sequences were aligned to a curated database containing all representative genomes in RefSeq for bacteria. Alignments were made at 97% identity against all reference genomes. Every input sequence was compared to every reference sequence in the Diversigen Venti database using fully gapped alignment with BURST. For taxonomy assignment, each input sequence was assigned the lowest common ancestor that was consistent across at least 80% of all reference sequences tied for best hit. Samples with fewer than 10,000 sequences were discarded. OTUs accounting for less than one millionth of all strain-level markers and those with less than 0.01% of their unique genome regions covered (and < 0.1% of the whole genome) at the species

level were discarded. The number of counts for each OTU was normalized to the OTU's genome length.

Blood-brain barrier:

Following established procedures (Banks et al., 2015; Logsdon et al., 2018, 2020), albumin (Sigma, St. Louis MO), (~66.44 kDa), a blood-borne molecule not normally localized in the CNS was labeled with 99mTc (GE Healthcare, Piscataway, NJ). A mixture of 240 mg/ml stannous tartrate and 1 mg/ml albumin was adjusted to pH 3.0 with HCl and one millicurie of 99mTc-NaOH4 was added to this mixture and incubated for 20 min. The 99mTc-albumin was purified on a column of G-10 Sephadex (GE Healthcare) in 0.1 ml fractions of phosphate buffer (0.25 M). Radioactivity in the purified 99mTc-albumin peak was more than 90% acid precipitable in an equal volume of 1% bovine serum albumin (BSA) and trichloroacetic acid (30%). 5 × 106 cpm/mouse of purified 99mTc-albumin fraction was prepared in a final volume (0.2 ml/mouse) of lactated Ringer's solution containing 1% BSA.

In keeping with previous experiments, 72 hours after the final blast/sham exposure, mice were anesthetized with urethane (4 g/kg; 0.2 ml; ip); and jugular veins were exposed and injected with 99mTc-albumin (5 × 106 Counts per minute (cpm)) in 0.2 ml of lactated Ringer's solution with 1% BSA for 10 min. The descending abdominal aorta was clamped with hemostats, severed, and blood collected in 1.5-mL capacity serum-separator tubes containing EDTA, allowed to clot at room temperature for 30-40 minutes, and then centrifuged at room temp at 3,200 x g for 10 minutes. Serum was then aliquoted and stored at -80°C until analyzed. Next, the brain was perfused with 20 ml of lactated Ringer's solution through the left ventricle of the heart in less than

1 min and collected and flash frozen in liquid nitrogen. 99mTc radioactivity in the serum and brain was measured by a gamma counter. Brain tissue radioactivity was calculated by dividing the cpm in the brain by the weight of the brain to yield cpm/g. Serum radioactivity was calculated by dividing the cpm in the serum by the microliters of serum counted to yield cpm/microliter. The brain tissue radioactivity was then divided by the corresponding serum radioactivity and the results given in units of microliters/gram of brain tissue.

Behavioral assays:

Acute (48 hours post): To probe potential locomotor deficits and anxiety-like behavior acutely following sham/blast exposure, mice were tested in an open field assay. Mice were allowed 5 minutes to explore a large circular open space (1 meter diameter) and their movements were recorded from above and analyzed with Anymaze (Wood Dale, IL). On this test, decreases in the amount of time spent in the middle of the environment is indicative of an anxiety-like phenotype. The total distance traveled, the delay to first enter the center of the field, time spent in the center, and entries into the center were recorded and analyzed.

Chronic (1 month post): A behavioral battery consisting of three testing paradigms was conducted over 1 week (one test paradigm per day) starting one month after the last sham/blast exposure. The order of behavioral tests was specifically chosen to go from the least stressful to the most stressful task to prevent carryover distress from one behavior to the next.

Elevated zero maze (EZM): Animals were allowed to explore an elevated zero maze (Maze Engineers, Skokie, IL) for 5 min. Decreased time spent exploring the open

arms is thought to reflect an anxiety-like behavior. Movement was recorded from above and analyzed using Anymaze (Stoelting, Wood Dale, IL).

Acoustic startle (AS): In accordance with previous reports (Baskin et al., 2021) acoustic startle habituation and prepulse inhibition was measured using SR-LAB acoustic startle boxes (San Diego Instruments, San Diego, CA). Following a 5-min acclimation period, startle habituation testing consisted of 50 trials of 120-dB pulses delivered with an inter-trial interval of 7–23 s. Following a two min break period, prepulse inhibition (PPI) was next assessed and consisted of forty trials of 81-dB prepulse followed by a 120-dB pulse with varying interstimulus interval (ISI) of 2–1,000 ms (five trials each). Due to the established effects of blast exposure on hearing loss, we use these established within-subject analysis approaches of startle habituation and PPI in attempts to mitigate potential confounds of hearing loss on startle outcome measures and interpretation.

Conditioned odorant aversion (COA): As previously described (Schindler et al., 2021) mice were first exposed to a neutral almond scent starting five minutes prior to sham/blast exposure by placing a mesh tea ball containing a quarter nestlet with 20 ul almond extract into the home cage. The tea ball and the nestlet with almond scent was refreshed daily and remained in the home cage until 24 hours after the final sham/blast exposure. To measure blast-induced aversion at the chronic testing time point, a tea ball with almond extract nestlet was placed in the left arm of a tea maze and a tea ball with a clean nestlet was placed in the right arm of the t-maze. Animals were then placed in the long arm of the T-maze, equidistant away from either tea ball and given 5 min to explore the entire maze. Latency to enter and time spent in each of the two distal ends

of the short arms was recorded and analyzed using Anymaze (Stoelting, Wood Dale, IL).

Analysis:

Data are expressed as mean ± SEM. Differences between groups were determined using two-way (between-subjects design: sex and exposure factors) analysis of variance (ANOVA) followed by posthoc testing using Sidak's Multiple Comoparison. Microbiome bioinformatics (alpha (Shannon) and beta diversity (Bray Curtis), relative abundance) were performed by Diversigen. Shannon diversity and relative abundance at the taxonomic order level were compared across all groups using Kruskal-Wallis test followed by Mann-Whitney *U* test for individual group by group comparison if Kruskal-Wallis was significant at FDR $p<0.1$. Bray Curtis dissimilarity was used to estimate beta diversity among samples and represented using PCoA plot. Analysis of similarities (ANOSIM) was used to compare beta diversity across groups (999 permutations). Spearman correlation with FDR correction was used to examine associations between microbial abundance and behavioral parameters. Hierarchical clustering was performed using Ward agglomeration method. Differentially expressed taxa were identified using Lefse (Huttentower Galaxy). Reported p values denote two-tailed probabilities of $p \leq 0.05$ and non-significance (n.s.) indicates $p > 0.05$. Statistical analysis and visualization were conducted using Graph Pad Prism 4.0 (GraphPad Software, Inc., La Jolla, CA) and with custom Python scripts.

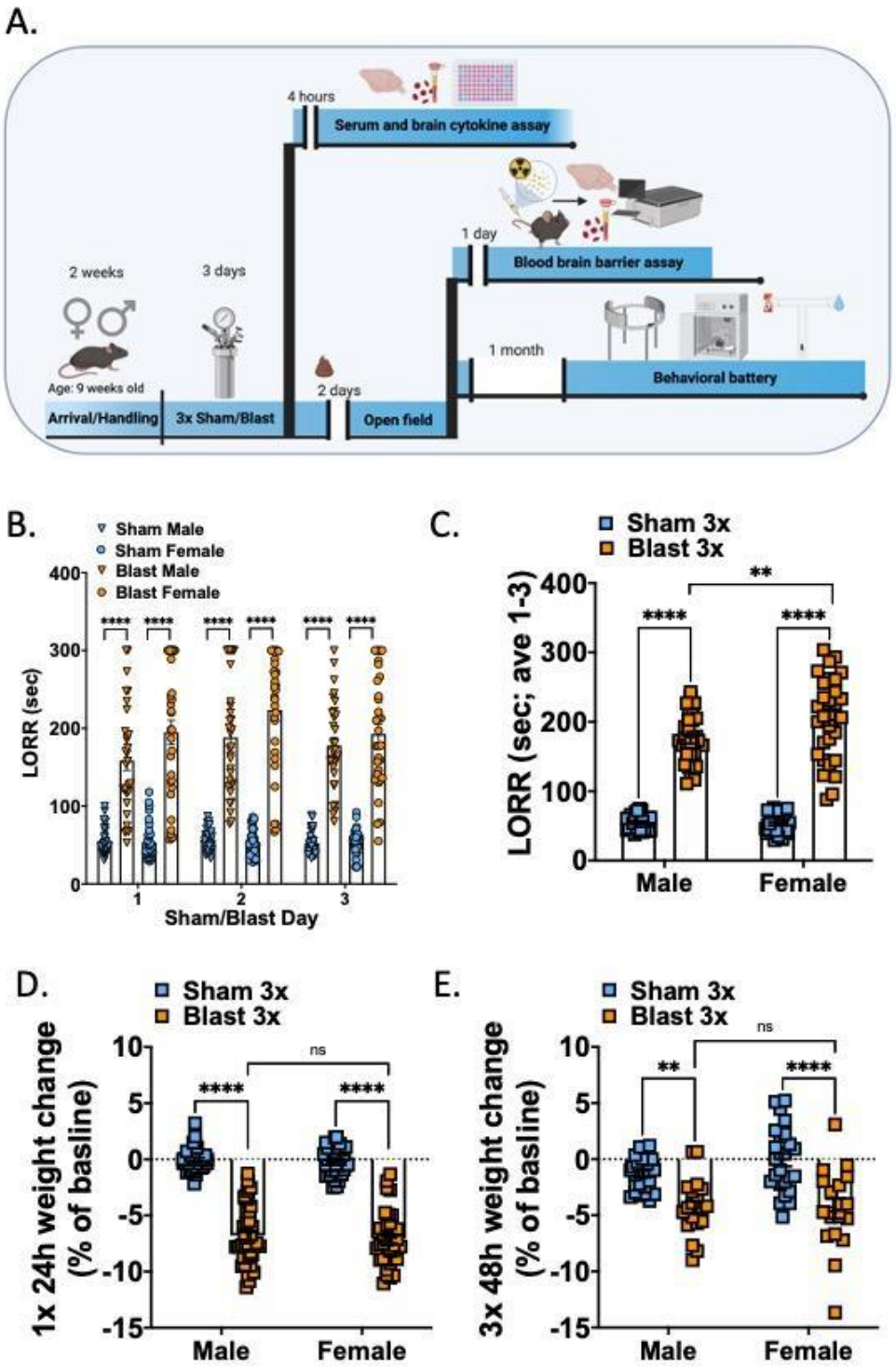

Figure 4.1. Female and male mice both display increased LORR and weight loss acutely following repetitive blast exposure. (A) Experimental timelines. Three groups of

female and male mice were used. (B) LORR is increased acutely following each blast exposure in female and male mice. (C) Mean LORR across days 1-3 of blast exposure is increased in female and male mice and female blast mice are significantly higher than male blast mice. (D) Both female and male mice lose weight to a similar extend 24 the blast).

RESULTS

Repetitive blast exposure increases loss of righting reflex and acute weight loss in both female and male mice.

Using well-established methods adult female and male mice were exposed to repetitive BOPs (1/day for 3 consecutive days) using a pneumatic shock tube. Loss of righting reflex (LORR; the time it takes for a mouse to right itself following sedation) was measured starting immediately upon removal from isoflurane and consisted of placing the mouse on its back and waiting until it rights itself two times within 15 seconds. Following LORR, mice were weighed and then returned to their home cage to recover.

Repetitive blast exposure resulted in increased LORR in both male and female mice (Fig 4.1b) immediately following each sham/blast exposure (two-way RM ANOVA: main effect of exposure $F(3,124) = 171.5$, $p<0.0001$), but was not affected by sham/blast day (two-way RM ANOVA: main effect of time $F(2,217) = 3.19$, ns) and there was no interaction effect (two-way RM ANOVA: main effect of time $F(6,248) = 1.30$, ns). When examining the mean LORR across all three exposure days, there was a significant interaction effect between exposure and sex (two-way ANOVA: interaction effect $F(1,124) = 6.674$, $p=0.01$) and main effects of exposure (two-way ANOVA: main effect of exposure $F(1,124) = 482.6$, $p<0.0001$) and time (two-way ANOVA: main effect of time $F(1,124) = 5.384$, $p=0.022$). Šídák's multiple comparison post-hoc test revealed that male blast mice (n=32) took longer to right themselves when compared to male sham mice (n=33; $p<0.0001$) and female blast mice (n=27) had longer LORR than both female shams (n=38; $p<0.0001$) and male blast mice ($p<0.01$).

Acute weight loss was also seen in both male and female blast mice (Fig 4.1d, e). Following one blast (Fig 1d), blast mice lost more weight (represented as a decrease in percent of their total baseline weight) than their sham controls (two-way RM ANOVA: main effect of exposure $F(1,144) = 466.5$, $p<0.0001$). There was no significant interaction effect between exposure and sex (two-way ANOVA: interaction effect $F(1,144) = 0.07$, ns) or main effect of sex (two-way ANOVA: main effect of sex $F(1,144) = 0.346$, ns). Šídák's multiple comparison post-hoc found that both male and female blast mice lost weight when compared to their sham controls but there was no difference between male and female blast mice. When examining weight loss at 48 hours post (day of open field testing), there remained a main effect of blast (two-way ANOVA $F(1,84) = 39.72$, $p<0.0001$) and no significant interaction effect between exposure and sex (two-way ANOVA: interaction effect $F(1,84) = 1.45$, ns) or main effect of sex (two-way ANOVA: main effect of sex $F(1,84) = 0.927$, ns) (Fig 1e). Šídák's multiple comparison post-hoc found that both male and female blast mice lost weight when compared to their sham controls but there was no difference between male and female blast mice.

Repetitive blast exposure results in both similar and disparate acute cytokine changes in serum and brain of female and male mice.

To determine if acute changes in serum and brain cytokine levels were different between female and male mice following repetitive blast, we euthanized a subgroup of mice (Fig 4.1a) 4 hours following their final sham/blast exposure. Analysis revealed both blast and sex differences dependent on cytokine sample type (Fig 4.2-3). Results from two-way ANOVA (exposure, sex factors) followed by Šídák's multiple comparisons tests

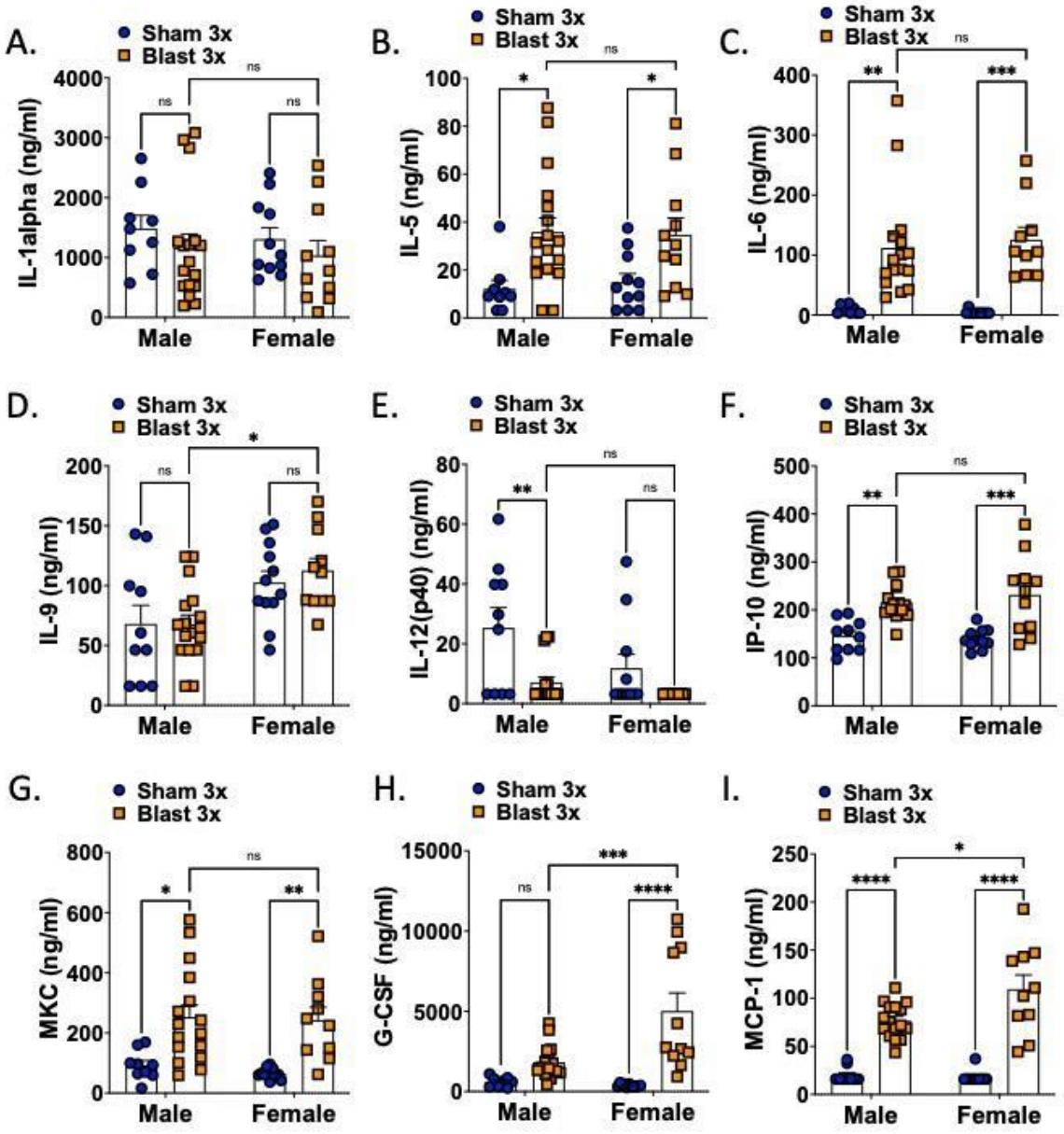

Figure 2.2. Serum cytokine levels are acutely affected by blast in both female and male mice. Two-way ANOVA Šídák's multiple comparison post-hoc. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, ns = not significant. Values represent mean ± SEM.

Table 1: Serum Cytokine Statistics						
Analyte	Interaction F(df)	Interaction P	Sex F(df)	Sex P	Exposure F(df)	Exposure P
IL-1α	F[1,44] = 0.008	0.927	F[1,44] = 0.415	0.523	F[1,44] = 1.53	0.223
IL-5	F[1,44] = 0.113	0.739	F[1,44] = 0.019	0.89	F[1,44] = 13.91	0.0005
IL-6	F[1,43] = 0.266	0.608	F[1,43] = 0.06	0.807	F[1,43] = 38.62	<0.0001
IL-9	F[1,46] = 0.242	0.625	F[1,46] = 14.28	0.0005	F[1,46] = 0.218	0.643
IL-12 (p40)	F[1,44] = 1.611	0.211	F[1,44] = 5.324	0.026	F[1,44] = 12.66	0.0009
G-CSF	F[1,42] = 8.429	0.006	F[1,42] = 6.36	0.016	F[1,42] = 24.99	<0.0001
IP-10	F[1,43] = 0.637	0.429	F[1,43] = 0.116	0.735	F[1,44] = 34.02	<0.0001
MKC	F[1,43] = 0.063	0.803	F[1,43] = 0.289	0.594	F[1,43] = 24.29	<0.0001
MCP-1	F[1,42] = 5.602	0.023	F[1,42] = 4.302	0.044	F[1,42] = 99.65	<0.0001

Table 4.1. Statistical analysis of cytokine levels in blood serum four hours after the final blast. All analysis used two-way ANOVA and Šídák post-hoc analysis.

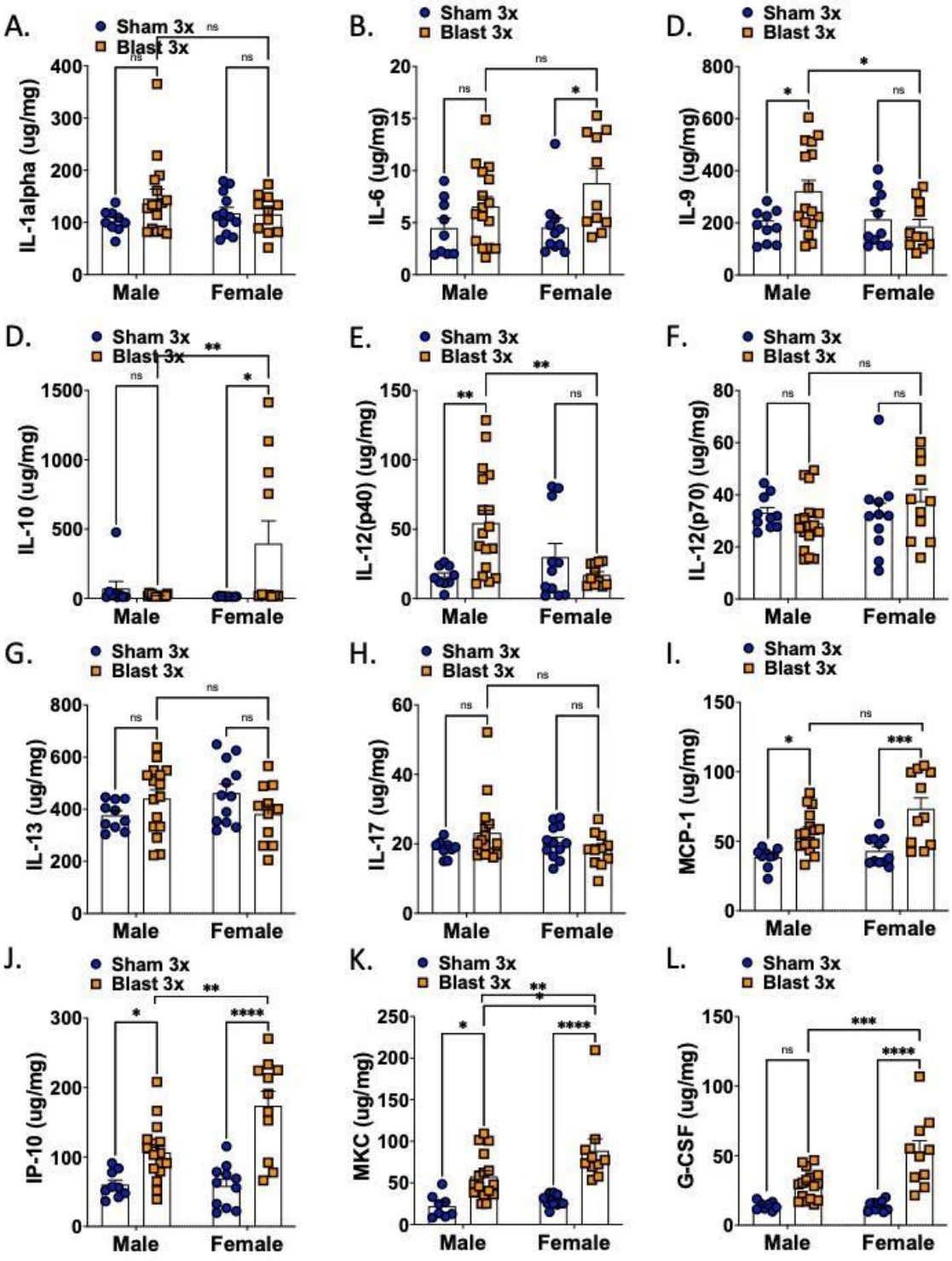
A.
Sham 3x
Blast 3x
IL-1alpha (ug/mg)
Male
Female
B.
IL-6 (ug/mg)
D.
IL-9 (ug/mg)
D.
IL-10 (ug/mg)
E.
IL-12(p40) (ug/mg)
F.
IL-12(p70) (ug/mg)
G.
IL-13 (ug/mg)
H.
IL-17 (ug/mg)
I.
MCP-1 (ug/mg)
J.
IP-10 (ug/mg)
K.
MKC (ug/mg)
L.
G-CSF (ug/mg)

Figure 4.3. Brain cytokine levels are acutely affected by blast in both female and male mice. Two-way ANOVA Šídák's multiple comparison post-hoc. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, ns = not significant. Values represent mean ± SEM.

Table 1: Brain Cytokine Statistics						
Analyte	Interaction F(df)	Interaction P	Sex F(df)	Sex P	Exposure F(df)	Exposure P
IL-1α	F[1,44] = 2.212	0.144	F[1,44] = 0.249	0.62	F[1,44] = 1.850	0.1807
IL-2	F[1,43] = 0.351	0.557	F[1,43]= 0.885	0.352	F[1,43] = 1.298	0.261
IL-6	F[1,43]= 1.040	0.314	F[1,43] = 1.107	0.299	F[1,43] = 8.612	0.005
IL-9	F[1,44] = 5.247	0.027	F[1,44] = 2.422	0.127	F[1,44] = 2.256	0.14
IL-10	F[1,42] = 6.909	0.012	F[1,42] = 3.38	0.062	F[1,42] = 3.999	0.052
IL-12 (p40)	F[1,44] = 9.873	0.003	F[1,44]= 2.02	0.162	F[1,44] = 2.48	0.123
IL-12 (p70)	F[1,45] = 1.706	0.198	F[1,45] = 1.143	0.291	F[1,45] = 0.039	0.844
IL-13	F[1,45] = 4.977	0.031	F[1,45] = 0.157	0.694	F[1,45] = 0.061	0.805
IL-15	F[1,44]= 0.304	0.584	F[1,44] = 1.594	0.214	F[1,44] = 1.669	0.203
IL-17	F[1,44] = 3.216	0.08	F[1,44]= 0.619	0.436	F[1,44] = 0.363	0.55
G-CSF	F[1,41] = 8.167	0.007	F[1,41] = 7.932	0.007	F[1,41] = 40.83	<0.0001
INFγ	F[1,43] = 0.235	0.631	F[1,43] = 0.732	0.397	F[1,43] = 0.038	0.846
IP-10	F[1,43] = 6.567	0.014	F[1,43] = 5.877	0.02	F[1,43] = 36.15	<0.0001
MKC	F[1,42] = 2.2	0.146	F[1,42] = 5.502	0.031	F[1,42] = 33.37	<0.0001
MCP-1	F[1,43] = 1.585	0.215	F[1,43] = 4.919	0.032	F[1,43] = 25.01	<0.0001

Table 4.2. Statistical analysis of cytokine levels in brain lysate four hours after the final blast. All analysis used two-way ANOVA and Šídák post-hoc analysis.

for each analyze measured in appreciable concentration are reported in Tables 4.1 and 4.2 (for serum and brain respectively).

In the serum (Fig 4.2, Table 1), there was a main effect of blast on IL-6, G-CSF, IP-10, MKC, and MCP-1. There was main effect of sex on G-CSF and MCP-1. Only G-CSF and MCP-1 showed an interaction effect. Blast male and female mice only significantly differed in two cytokines, G-CSF and MCP-1, with blast males expressing significantly lower levels than blast females. In samples taken from whole brain (Fig 3, Table 2), there was a main effect of blast on IL-6, G-CSF, IL-10, MKC, and MCP-1. There were main effects of sex on G-CSF, IP-10, MKC, and MCP-1 and an interaction effect on IL-9, IL-10, IL-12(p40), IL-13, G-CSF, and IP-10. Blast males showed higher levels of IL-9 and IL-12(p40) as compared to blast females whereas blast females showed higher levels of IP-10, G-CSF, IP-10, and MKC as compared to blast males.

Repetitive blast exposure differentially affects gut microbiota in female vs. male mice.

To determine if repetitive blast exposure differentially affected the gut microbiome in female vs. male mice, fecal samples were collected 24 hours post final sham/blast exposure and analyzed using shallow shotgun sequencing. Alpha diversity measured using the Shannon Index (Figure 4.4a) was not different when comparing across groups (Kruskal Wallis $F(4,94) = 2.47$, $p=0.57$). Conversely, beta diversity measured using Bray Curtis distance (Figure 44.b) was significantly different across groups (ANOSIM, 999 permutations, $p=0.001$). Furthermore, we found significant differences in abundance of nine bacteria orders (Figure 4.4c). Finally, we examined relative abundance differences

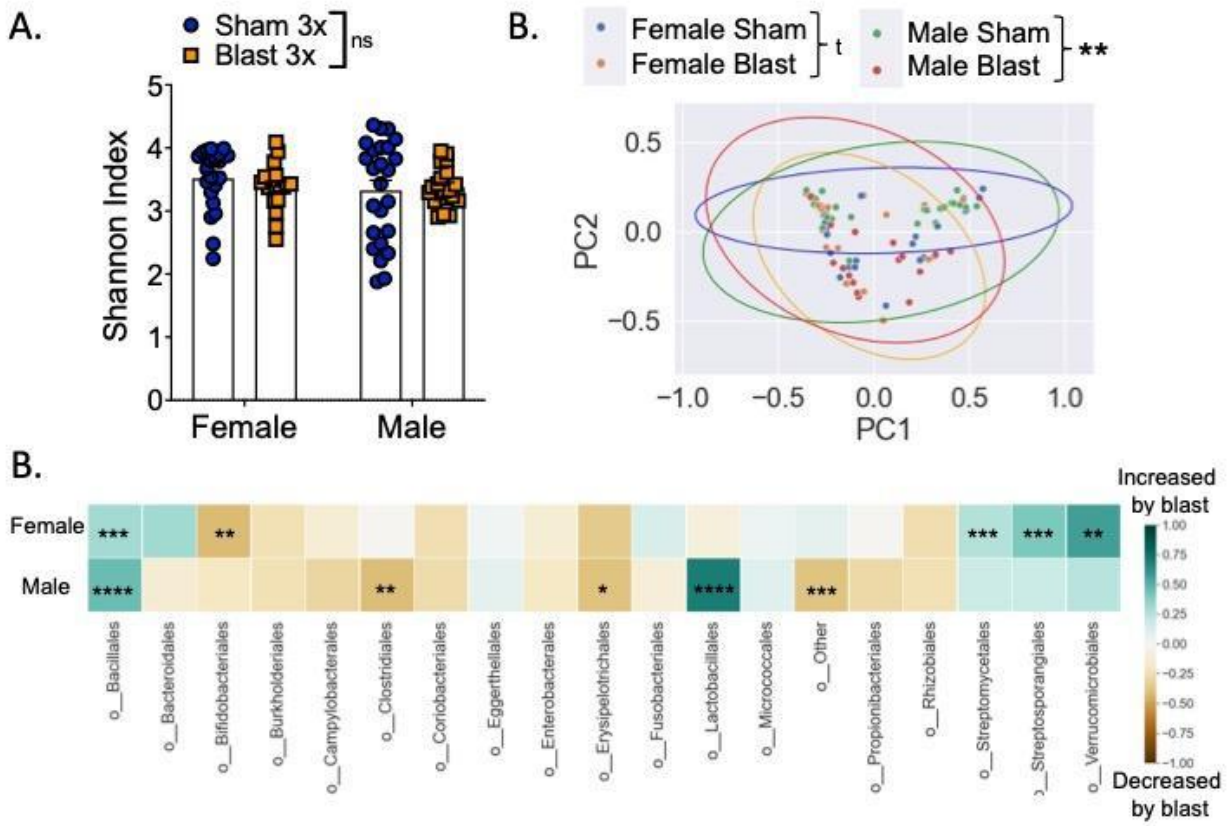

Figure 4.4. Differential effects of repetitive blast on acute gut microbiome changes in female vs. male mice. (A). No difference in Shannon (alpha) diversity as a function of exposure type or sex. (B) PCoA on Bray-Curtis dissimilarity distances (beta diversity) among the four groups examined. Each point represents an individual sample colored according to group. Ellipses represent 95% CI around cluster centroid for each experimental group. (C) Differences between female sham vs. blast mice (top row) and male sham vs. blast mice (bottom row) at the order level, expressed as mean relative abundance z score (z score computed separately for female and male mice). Kruskal-Wallis test followed by Mann-Whitney *U* test for individual group by group comparison if Kruskal-Wallis was significant at FDR $p<0.1$ (A, C). Analysis of similarities (ANOSIM) (B). *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$, ns = not significant. Values represent mean ± SEM.

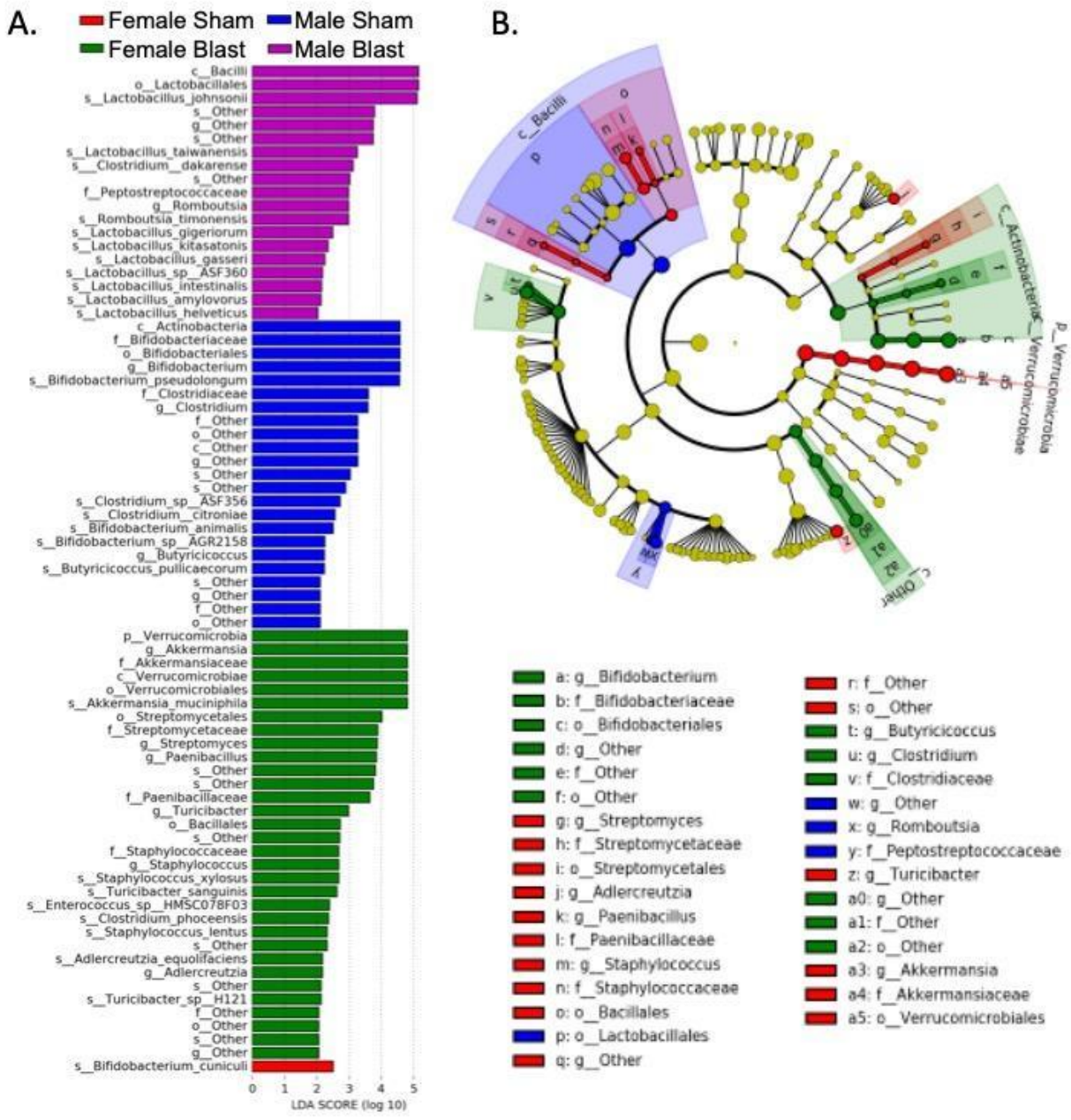

Figure 4.5. LEfSe analysis of the gut microbial taxonomy. (A). Enriched species (LDA score > 2) in female sham (red), female blast (green), male sham (blue), and male blast (purple). (B) Taxonomic representation of statistically and biologically consistent differences in the four groups. Differences are represented by the color of the most abundant class. Circle diameter is in proportion to that taxon's abundance.

the species level using LeFSe (Huttentower Lab Galaxy) and found unique species characterizing each of the four experimental groups (Figure 4.5).

Repetitive blast exposure decreases locomotion and increase anxiety-like behavior acutely in both male and female mice.

To probe potential locomotor deficits and anxiety-like behavior acutely following blast exposure, male and female mice were tested in a large circular open field 48 hours following their last sham/blast exposure. In distance traveled, there was a significant main effect of blast (two-way ANOVA $F(1,85) = 43.15$, $p<0.0001$) and no significant interaction effect between exposure and sex (two-way ANOVA: interaction effect $F(1,85) = 2.573$, ns) or main effect of sex (two-way ANOVA: main effect of sex $F(1,85) = 0.158$, ns) (Fig 4.6a). In entries to the center of the open field, there was a significant main effect of blast (two-way ANOVA $F(1,85) = 35.71$, $p=0.0001$) and a main effect of sex (two-way ANOVA: main effect of sex $F(1,85) = 6.591$, ns), but no significant interaction effect between exposure and sex (two-way ANOVA: interaction effect $F(1,85) = 0.641$, ns) (Fig 4.6b). In time spent in the center of the open field, there was a significant main effect of blast (two-way ANOVA $F(1,85) = 15.05$, $p=0.0002$) and a main effect of sex (two-way ANOVA: main effect of sex $F(1,85) = 8.736$, $p=0.004$), but no significant interaction effect between exposure and sex (two-way ANOVA: interaction effect $F(1,85) = 0.081$, ns) (Fig 4.6c). Finally, in delay to first center entry, there was a significant main effect of blast (two-way ANOVA $F(1,85) = 11.57$, $p=0.001$) and no significant interaction effect between exposure and sex (two-way ANOVA: interaction

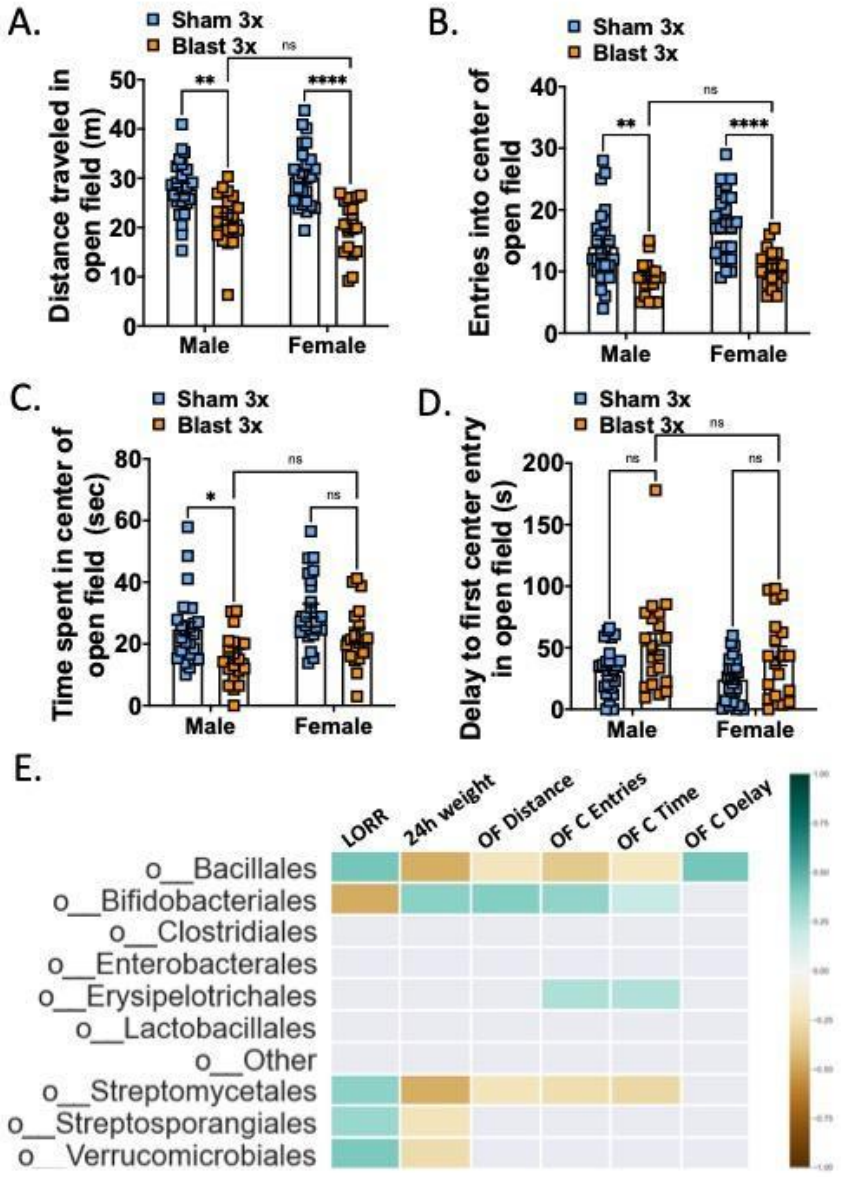

Figure 4.6. Blast exposure increases acute anxiety-like open field behaviors in both female and male mice. (A) Distance traveled in open field. (B) Entries into center of open field. (C) Time spent in center of open field. (D) Delay to first entry into center of open field. (E) Pearson correlation between bacterial taxa (taxa order that were significantly different between groups from Figure 4) and open field parameters. Two-

way ANOVA Šídák's multiple comparison post-hoc. *p ≤ 0.05, **p ≤ 0.01, ****p ≤ 0.0001, ns = not significant. Values represent mean ± SEM.

effect $F(1,85) = 1.99$, ns) or main effect of sex (two-way RM ANOVA: main effect of sex $F(1,85) = 0.045$, ns) (Fig 4.6d).

To further examine potential interactions between acute behavioral effects and fecal microbiome changes, we computed Pearson correlations and corrected for multiple comparison using FDR (Figure 6e). The bacterial orders bacillales, bifidobacteriales, and streptomycetales were all correlated with multiple open field parameters as well as LORR and 24-hour weight loss.

Blood brain barrier disruption is apparent in both female and male mice acutely following repetitive blast exposure.

We previously reported that repetitive (2X or 3X), but not single (1X) blast exposure results in delayed-onset (72 h) brain BBB permeability to albumin (Logsdon et al., 2018, 2020). Consistent with these prior reports, we found a blast effect on BBB permeability to albumin 72 hours following the last blast exposure (two-way ANOVA $F(1,33) = 5.607$, p=0.024) but there were no differences between sexes (Figure 4.7a). To further examine potential interactions between blood brain barrier disruption and fecal microbiome changes, we computed Pearson correlations and corrected for multiple comparison using FDR (Figure 4.7b). Only the bacterial order enterobacterales was correlated with albumin levels after correcting for multiple comparisons.

Male but not female mice exhibit anxious/aversive-like outcomes one moth following repetitive blast exposure.

To examine more chronic effects of repeated blast exposure on mTBI and PTSD-like outcomes, one month following their last sham/blast exposure, mice were tested in

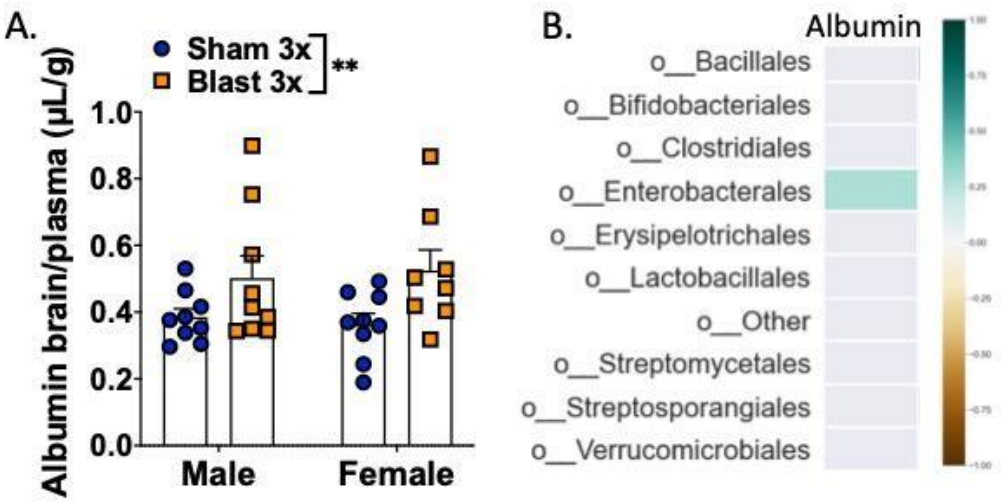

Figure 4.7. BBB disruption in female and male mice acutely following repetitive blast exposure. (A) Blast increases brain/serum (µl/g) radiolabeled albumin 72 h after final blast. Brain/serum ratios were calculated by dividing the cpm per brain by the cpm per microliter in the corresponding serum and then by the weight of the brain. (B) Pearson correlation between bacterial taxa (taxa order that were significantly different between groups from Figure 4) and albumin BBB permeability. Two-way ANOVA Šídák post-hoc analysis. **$p \leq 0.01$, ns = not significant. Values represent means ± SEM and are expressed as microliters per gram of brain tissue.

a behavioral battery consisting of elevated zero maze, acoustic startle, and blast-odorant aversion (Fig 4.8).

Mice were exposed to an elevated zero maze to probe anxiety-like behaviors with less time spent exploring the open arms indicative of a more anxious phenotype. For distance traveled, there was no significant main effect of exposure (two-way ANOVA $F(1,48) = 2.518$, ns) and no significant interaction effect between exposure and sex (two-way ANOVA: interaction effect $F(1,48) = 0.209$, ns), but there was a significant main effect of sex (two-way ANOVA: main effect of sex $F(1,48) = 5.95$, $p=0.018$) (Fig 8a). Conversely, on time spent in the open arm of the maze, there was a significant main effect of exposure (two-way ANOVA $F(1,48) = 5.354$, $p=0.025$), significant main effect of sex (two-way ANOVA: main effect of sex $F(1,48) = 10.10$, $p=0.003$), and a significant interaction effect between exposure and sex (two-way ANOVA: interaction effect $F(1,48) = 4.317$, $p=0.043$) (Fig 4.8b). Post-hoc analysis revealed that blast males spent significantly less time in the open arms than sham males ($p<0.05$) and whereas female sham and blast mice did not differ in open arm time.

To test a PTSD-like startle response in the mice, mice went through an acoustic startle session during which decreases in the ability to habituate to repeated presentation of the same stimuli is representative of a higher startle magnitude/deficit in non-associative learning and decreases in prepulse inhibition is indicative of a difficulty in sensory gating (Valsamis & Schmid, 2011). In line with previous reports in male mice (Baskin et al., 2021), there was a main effect of exposure (two-way ANOVA $F(1,37) = 8.764$, $p=0.005$), but no significant main effect of sex (two-way ANOVA: main effect of sex $F(1,37) = 0.769$, ns) or interaction effect between exposure and sex

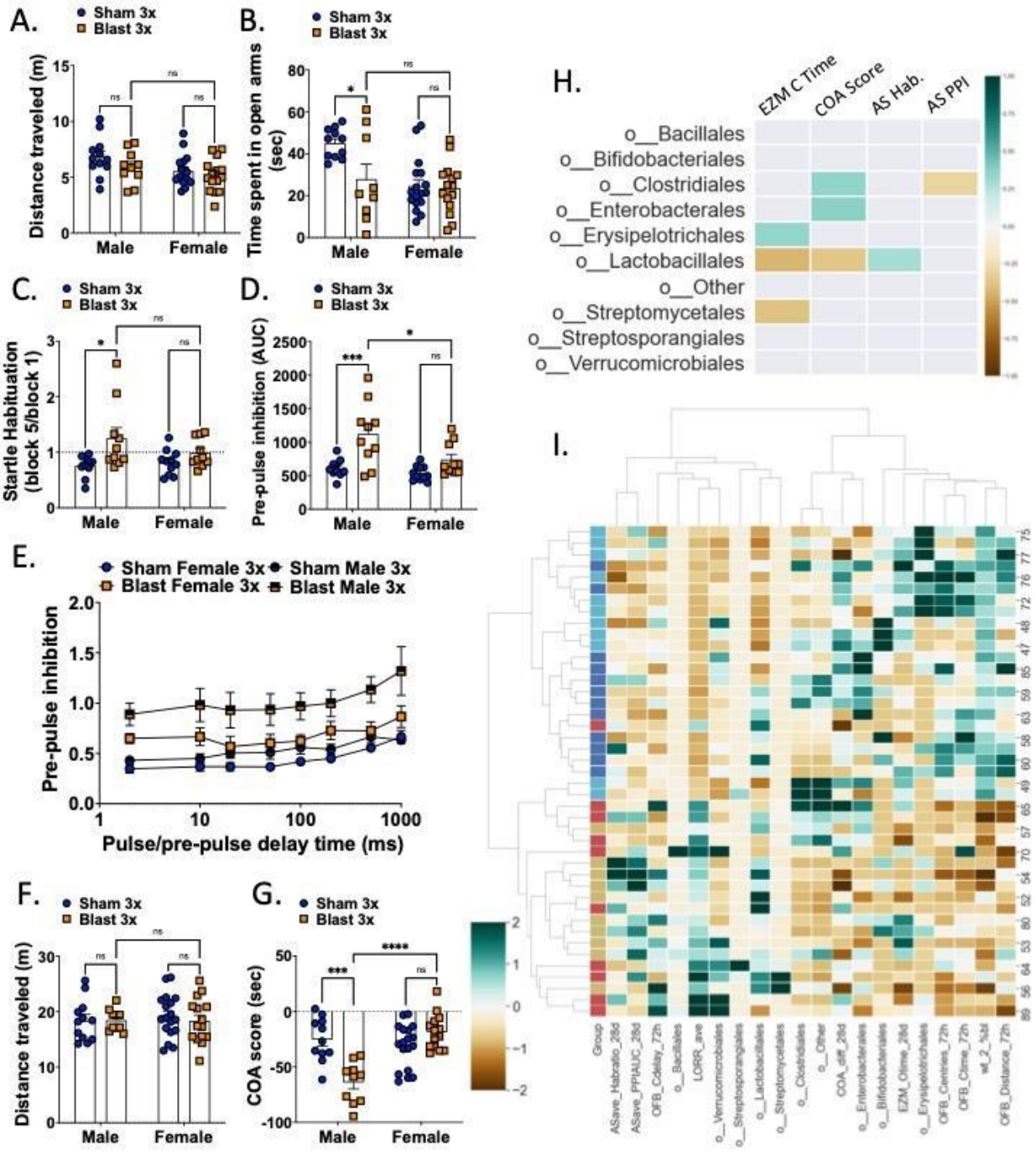

Figure 4.8. Male but not female mice exhibit chronic blast-induced behavioral deficits. Male and female mice did not differ on total distance traveled on the elevated zero maze 1-month post-blast exposure (A) but male blast mice spent significantly less time than their sham controls in the open arms (B). Only male blast mice showed impaired

startle habituation (C) and prepulse inhibition (D, E) on the acoustic startle task. Male and female mice did not differ on total distance traveled in the conditioned odorant aversion posttest (F) but male blast showed an aversion to an odorant previously paired with blast-exposures (G). (H) Pearson correlation between bacterial taxa (taxa order that were significantly different between groups from Figure 4) and chronic behavioral parameters. (I) Heatmap of hierarchical clustering between individual mice vs. behavioral and microbiota composition (taxa order that were significantly different between groups from Figure 4). Each row is a mouse, each column is a parameter. Group column colors: sham female – dark blue; blast female – red; sham male – light blue; blast male yellow. Heatmap colors represent z-score for each parameter computed from all mice. Two-way ANOVA Šídák post-hoc analysis (A-G). *p ≤ 0.05, **p ≤ 0.01, ****p ≤ 0.0001, ns = not significant. Values represent mean ± SEM.

(two-way ANOVA: interaction effect $F(1,37) = 2.04$, ns) (Fig 4.8c). Prepulse inhibition was measured following a five minute rest period after the habituation procedure. The area under the curve of the PPI vs. time delay showed a main effect of exposure (two-way ANOVA $F(1,37) = 17.63$, $p=0.0002$) and main effect of sex (two-way ANOVA: main effect of sex $F(1,37) = 7.074$, $p=0.011$), but no interaction effect between exposure and delay (two-way ANOVA: interaction effect $F(1,37) = 3.218$, ns) (Fig 4.8d). We also examined PPI across time delays and found a main effect of exposure (two-way ANOVA $F(3,37) = 9.96$, $p<0.0001$) and main effect of pulse-prepulse delay time (two-way ANOVA: main effect of delay $F(5,154) = 13.37$, $p<0.0001$) but no interaction effect between exposure and delay (two-way ANOVA: interaction effect $F(21,259) = 0.688$, ns) (Fig 4.8e). Post-hoc analysis revealed that only blast males demonstrated acoustic startle deficits as compared to sham controls but there was no blast effect in females.

Finally, to test aversion-like behaviors in mice, prior to every sham/blast exposure, a fresh almond-scented nestlet in a tea diffuser was placed in the home cages of the mice to associate the scent with the experience of the sham or blast trauma. We measured aversion to the almond-scented nestlet in a posttest conducted in a T-maze where the almond scent was placed in the left arm of the T and a clean nestlet with no scent was placed in the right arm of the T. When examining distance traveled, there was no significant main effect of exposure (two-way ANOVA $F(1,48) = 0.0178$, ns), no significant main effect of sex (two-way ANOVA: main effect of sex $F(1,48) = 0.117$, ns), and no significant interaction effect between exposure and sex (two-way ANOVA: interaction effect $F(1,48) = 0.209$, ns) (Fig 4.8f). Conversely, in the difference between time spent with the odor vs. without the odor (COA), there was a

significant main effect of exposure (two-way ANOVA $F(1,48) = 6.148$, $p=0.017$), significant main effect of sex (two-way ANOVA: main effect of sex $F(1,48) = 14.11$, $p=0.0005$), and a significant interaction effect between exposure and sex (two-way ANOVA: interaction effect $F(1,48) = 24.00$, $p<0.0001$) (Fig 8g). Post-hoc analysis revealed that blast males spent significantly less time with the almond odor than sham males ($p<0.05$), whereas females did not different between sham and blast groups.

To further examine potential interactions between chronic behavioral effects and fecal microbiome changes, we computed Pearson correlations and corrected for multiple comparisons using FDR (Figure 8h). The bacterial orders clostridales and lactobacillales were each correlated with distinct behavioral parameters. Finally, hierarchical clustering across acute and chronic parameters revealed distinct clusters of sham male vs. sham female mice but more mixed clusters consisting of male and female blast mice.

DISCUSSION

While the effects of repetitive blast mTBI have been well studied using rodent models, these studies almost exclusively used male research subjects. Thus, almost nothing is known regarding potential adverse outcomes of repetitive blast exposure in female rodents, resulting in a critical knowledge gap at a time when women are increasingly seen in combat roles with high probability of repetitive blast exposure. Here we present novel data demonstrating acute and chronic behavioral and inflammatory changes in male and female mice; intriguingly, while acute behavioral outcomes were relatively consistent between male and female blast mice, chronic adverse behavioral

outcomes were only found in male mice. These results highlight the importance of examining potential blast effects across sexes and at multiple time points. Furthermore, differential effects of blast in male vs. female mice in cytokine and gut microbiome changes identify potential diagnostic and therapeutic targets for future development and precision medicine efforts.

Understanding the effects of repetitive blast exposure across sexes is critical for diagnostic and treatment development (McCabe & Tucker, 2020). Due in large part to increases in body armor and battlefield medicine, multiple deployments are exceedingly common, greatly increasing the potential for repetitive blast exposure resulting in mTBI comorbid with PTSD (O'Neil et al., 2013; Owens et al., 2008; Tanielian et al., 2008; Wenger et al., 2018). Indeed, following blast exposure, mTBI-related persistent post-concussive symptoms (e.g., cognitive difficulties, auditory and vestibular dysfunction, sleep disturbances, negative affect) appreciably overlap with PTSD symptoms (e.g., hypervigilance, aversion to trauma cues, cognitive difficulties, sleep disturbances, negative affect) (Elder & Cristian, 2009; Owens et al., 2008; Petrie et al., 2014; Schindler et al., 2021; Schindler et al., 2017; Tanielian et al., 2008). Likewise, both mTBI and PTSD can worsen pre-existing psychiatric disorders such as depression and increase and/or exacerbate substance misuse/addiction and other health risk behaviors (e.g., sensation/novelty seeking, impulsivity, risk taking, irritability/aggression) (María-Ríos & Morrow, 2020; McFarlane, 2010; Miller et al., 2013; Olson-Madden et al., 2012; Schindler et al., 2021; Schindler et al., 2017), potentially compounding negative outcomes following injury and trauma. Indeed, in Veterans we previously reported worse outcomes in relation to 'risky' drinking behavior as a function of combat exposure

and blast mTBI number (Schindler et al., 2021) and significant correlation between blast mTBI number and neuroimaging results (Meabon et al., 2016; Piantino et al., 2021; Schindler et al., 2021). Results from animal models further highlight the importance of studying repetitive blast exposure; we previously demonstrated PTSD-like outcomes in male mice only following repetitive but not single blast mTBI (Schindler et al., 2021) and more recently demonstrated a blast-dose effect in relation to ethanol sensitivity and 'binge'-like intake patterns in male mice (Schindler et al., 2021). Critically, to date there has been no reports of repetitive blast TBI research in female rodent models.

Acute increase in pro-inflammatory cytokines and blood brain barrier disruption has been well documented in male TBI rodents but has not yet been investigated in females following repetitive blast trauma. Here we demonstrate robust blast-induced changes in serum and brain cytokine levels that display similar and disparate patterns in male vs. female mice. In line with these results, models of single moderate to severe impact TBI have demonstrated disparate inflammatory outcomes in male vs. female mice acutely following injury (Bromberg et al., 2020; Doran et al., 2019; Krukowski, 2021; Krukowski et al., 2020; Späni et al., 2018; Villapol et al., 2017). Likewise, a recent study using a single mild blast exposure with body shielding reported worse acute and sub-acute neuroinflammatory and blood brain barrier outcomes in male vs. female rats (Hubbard et al., 2022). The discrepancy between this study and our current results of similar BBB disruption in male vs. female blast mice is likely due to the repetitive nature of our blast model and suggests that while female rodents are protected from acute

single TBI effects, repetitive blast exposure is sufficient to result in cytokine changes and blood brain barrier disruption in females as well as males.

To more fully understand potential differences in adverse outcomes as an effect of sex, we analyzed fecal microbial abundance acutely following repetitive blast exposure. Intestinal (gut) microbiota and the genes they produce (collectively referred to as the gut metagenome) help regulate homeostasis and benefit the host through a range of physiological functions (e.g., protection against pathogens, digestion and assimilation of nutrients, regulation of immune system) (Moloney et al., 2014; Thursby & Juge, 2017). Likewise, the 'microbiota-gut-brain-axis' (MGBA) represents a critical bidirectional communication system between the gut and the brain, involving metabolic, endocrine, neural, and immune pathways critical for brain health and cognition (Cryan et al., 2019; Moloney et al., 2014; Thursby & Juge, 2017; Wiley et al., 2017). Conversely, altered microbial abundance and composition (i.e., dysbiosis) can have detrimental effects on health and wellness, including implications for cognitive functioning, mental health, and neurodegeneration (Ceppa et al., 2020; Liu et al., 2020; Ticinesi et al., 2018; Tremlett et al., 2017). Importantly, alterations in gut microbiota have been documented years post injury in individuals with a history of moderate/severe TBI (Brenner et al., 2020; Urban et al., 2020), and preclinical work using animal models also supports the microbiome as playing a mechanistic role in adverse outcomes following stress and trauma(Angoa-Pérez et al., 2020; Matharu et al., 2019; Opeyemi et al., 2021), but no studies thus far have specifically utilized animal models of blast (either single or repetitive). In line with our cytokine results, we find similar and disparate effects of repetitive blast on gut microbiome in male vs. female mice. Linear discriminant analysis

of fecal samples demonstrated bifidobacteria as significantly increased in sham vs. blast mice, which is already being tested as a potential probiotic for PTSD and depression, raising the possibility of this bacteria as a potential treatment target for further development.

In relation to potential sex differences in adverse behavioral outcomes following blast exposure, only three studies thus far has been reported (Hubbard et al., 2022; McNamara et al., 2022; Russell, Handa, et al., 2018). Hubbard et al. (2022) found increased anxiety-like behavior in male but not female rats in the open field (at 2 days post) and elevated plus maze (at 14 days post) following single blast mTBI with body shielding. Conversely, McNamara et al. (2022) found no injury effects in either female or male mice on the elevated plus and zero mazes when tested at 2-4 weeks post single blast exposure. Finally, Russell et al (2018) found increased anxiety-like behavior in male, and to a lesser extent in female, mice at 6 days post single blast exposure. Differences in reported results are likely due to differences in injury severity and specifically the repetitive nature of our injury model. Our behavioral outcomes at the one-month time point are also in line with recent preclinical research looking at animal models of PTSD. One preclinical model of PTSD based on the ability of rats to extinguish fear conditioning, found that female mice were less likely to show long-term fear and anxiety-like behaviors on a variety of behaviors when compared to males with similar deficits in fear extinguishing (Emtyazi et al., 2022).

There are several drawbacks and limitations to our current study, including the lack of brain region specific cytokine measurement and/or biochemical/histochemical assays aimed at understanding potential chances in microglia and astrocytes. Likewise,

the behavioral tests conducted were relatively simple and warrant future investigation focused on using more sophisticated operant behavioral paradigms as previously reported in male mice following repetitive blast (Baskin et al., 2021). Finally, we did not measure estrus cycle or gonadal hormone levels, but it is important to note that we consistently find less variability in our female mice as compared to their male counterparts. Likewise, research on sex differences in preclinical models of PTSD repeatedly have indicated no association between estrous stage and development of PTSD-like phenotypes (Emtyazi et al., 2022; Zoladz et al., 2019).

Despite TBI being a leading cause of morbidity and mortality worldwide, as well as a common outcome of modern-day warfare, understanding sex as a biological variable in TBI is still in its infancy (Cogan et al., 2020; Gupte et al., 2019). Results from existing human literature are mixed, and overwhelmingly these reports were not powered to examine potential sex differences. Here we report on a series of translationally relevant outcome measures known to be impacted by TBI in humans, with the goal of providing a survey comparison of male and female mice at acute and chronic timepoint following repetitive blast mTBI. Together, our results demonstrate that adverse effects of repetitive blast are dependent on interactions between gonadal sex, timepoint, sample time, and/or behavioral test, and highlight new targets for diagnosis and treatment development aimed at understanding how repetitive blast trauma affects diverse populations.

Chapter 5.

Discussion and Recommendations for Future Research

Traumatic brain injury is a leading cause of death and disability globally (Johnson & Griswold, 2017; A. I. R. Maas et al., 2017, 2017) affecting millions of people a year, leading to some people referring to the rates of TBIs as a pandemic. TBIs affects every portion of the population, leading to significant decreased quality of life for those injured and those supporting them (Di Battista et al., 2012; A. I. R. Maas et al., 2017; Malec et al., 2017; Ozga et al., 2018). Research on the risk and outcomes of TBI has increased in the past few decades but primarily the research on TBIs has focused on contact TBIs, or trauma to the brain when something solid physically comes into contact with the skull and/or causes the brain to rapidly shift inside the head, particularly severe contact TBIs. Further research has 1. either excluded crucial portions of the population that experience TBIs, either through exclusion criteria that removes clinical participants with co-existing diagnoses or preclinical research utilizing only male animals; 2. has focused on prevention or treating the more well-defined symptomology that follows or co-occurs with TBIs such as headaches, dizziness, and nausea and 3. has focused on analyzing the central nervous system to examine sequelae and treatment options.

In order to address these gaps in the literature, this dissertation focused on blast-induced mTBI, an increasingly common injury in both servicemembers and civilians through exposures to explosions, building collapses, failing infrastructure, and industrial

accidents, which can all cause blast overpressure waves. They are particularly common in Servicemembers though due to the increasing use of high explosives such as IEDs in warfare and are considered both a “invisible illness” and the “signature injury” of current conflicts since many of the symptoms that follow a blast mTBI are not readily apparent or immediately linked to the injury. This, along with the military culture of “toughness”, and some of the self-reports used to diagnose mTBI lead to missed and inconsistent diagnosis. Common through all the studies presented in this dissertation is the finding that TBIs caused solely by blast overpressure waves without any additional physical impact to the skull can be particularly detrimental to functioning. This impact can be seen across a broad range of comorbid behaviors and through the negative impact that blast injuries have on multiple different systems in the body, from the brain to vasculature structure, circulating inflammatory cytokines, to the gastrointestinal tract.

One group of people with a history of mTBI (blast or otherwise) that is overlooked is those with comorbid disorders, in particular those that have risky drinking behaviors or show signs of alcohol use disorder. Mild TBIs have been found to increase risky health behaviors and addiction risk, so I conducted an experiment where I exposed mice to either a single (1) or repetitive (3) blasts and then examined how this impacted ethanol induced behaviors. I found that there was a dose-dependent (blast being the dose) effect on locomotor stimulation and intake patterns. In collaboration with clinical researchers at the VA Seattle MIRECC, we found similar changes in drinking behaviors in combat veterans exposed to blasts. Consistent with other papers, the most severe outcomes were associated with repetitive blast exposures, and the majority of veterans report high numbers of blast exposures.

To understand more complex behavioral changes that occur following blast mTBI, I sought to analyze how blast mTBI may impact executive dysfunction and cognitive flexibility and how these changes may influence the resulting blast mTBI negative sequelae such as affective disorders, risky drinking behaviors, and quality of life. In this, I found blast mTBI animals exhibited more reward-seeking and a goal-directed phenotype yet decreased behavioral flexibility while also exhibiting increased anxiety- and compulsive-like phenotypes as well as hyperarousal, suggesting important implications for diagnosis and treatment management. Future work on blast mTBI should consider how complex the injury is, how it manifests differently in different populations of people, and how a treatment may need to impact both the central and peripheral nervous systems and symptoms.

The final aim of this dissertation was to further our understanding of the biochemical and behavioral impact that blast exposures have on females, and to compare that to males. Because there are no published reports examining sex as a biological variable in models of repetitive blast mTBI, almost nothing is known regarding potential adverse outcomes of repetitive blast exposure in female rodents, resulting in a critical knowledge gap at a time when women are increasingly seen in combat roles with high probability of repetitive blast exposure. Therefore, I exposed both male and female mice to repetitive (3x) blast exposures to examine differences, if any, in males and females following repetitive blast exposures at both an acute and chronic time point. In keeping with analyzing the effects on the entire system, and to identify potential diagnostic and therapeutic targets for future development and precision medicine efforts, I looked at behavioral, inflammatory, microbiome, and vascular dysfunction.

First, of particular importance, I found that while acute behavioral outcomes were found to be consistent in male and female mice, at the chronic timepoint (1 month post-blast) adverse behavioral outcomes were only detected in male mice, demonstrating the importance of using not just both male and female rodents in pre-clinical experiments, but enough of each sex to be able to detect differences, if any, between the two sexes. Specifically, we found that both males and females showed similar decreases in weight loss and increases in their loss of righting reflexes on blast days and increased anxiety-like behaviors compared to their sham controls 48 hours post-blast. At the 1 month post-blast timepoint however, males but not females, showed increased PTSD-adjacent behaviors as determined through increased anxiety-like behaviors on the elevated zero maze, increased startling behaviors on the acoustic startle, and increased aversion of a scent previously paired with blast exposures. Since multiple deployments are increasing common, leading to repetitive blast exposure resulting in mTBI comorbid with PTSD (O'Neil et al., 2013; Owens et al., 2008; Tanielian et al., 2008; Wenger et al., 2018), many post-concussive symptoms overlap with PTSD symptoms (Elder & Cristian, 2009; Owens et al., 2008; Petrie et al., 2014; Schindler et al., 2021; Schindler et al., 2017; Tanielian et al., 2008), co-occurring mTBI and PTSD can synergistically worsen quality of life, and compound negative outcomes following injury and trauma. Results from animal models further highlight the importance of studying repetitive blast exposure; we previously demonstrated PTSD-like outcomes in male mice only following repetitive but not single blast mTBI (Schindler et al., 2021) and in this dissertation demonstrated a blast-dose effect in relation to ethanol sensitivity and 'binge'-like intake patterns in male

mice. Yet this is the first study to date looking at repetitive blast-injuries in female mice and it indicates that the trajectory of TBI sequelae may differ between sexes.

Even in the cases that both sexes appear similar on behavioral tasks, the takeaway from those experiments are at least more applicable to a wider population, however as shown here, there may be differences that are time-dependent. This finding underscores the value in more longitudinal studies, particularly for chronic diseases and disorders. Thus, in order to more accurately represent disease/disorder states and more reliably find targets for biomarkers and treatments, more resources should be allocated for researchers to do studies in multiple sexes spanning a longer, more translationally valuable, timeline.

To target potential biomarkers of mTBI and in an effort to examine the peripheral nervous system changes, we also analyzed serum and brain cytokine expression (4 hours post-blast) gut microbiome changes (24 hours post-blast), and blood brain barrier disruption (72 hours post-blast). Here we demonstrate robust blast-induced changes in serum and brain cytokine levels some of which were in males and females, while others differed, with some changes dependent on whether the sample was from blood serum or whole-brain. The changes in blood brain barrier disruption (an increase in permeability to albumin) didn't differ between males and females. The changes found in inflammatory cytokine expression is in line with models of single moderate to severe impact TBI have demonstrated disparate inflammatory outcomes in male vs. female mice acutely following injury (Bromberg et al., 2020; Doran et al., 2019; Krukowski, 2021; Krukowski et al., 2020; Späni et al., 2018; Villapol et al., 2017) but is the first to examine the effect of repetitive blasts.

Finally, I analyzed the intestinal microbiota in order to analyze changes acutely following blast and potentially find a biomarker of mTBI/or target for treatment. Sometimes called our “second brain” the gut microbiota helps us to regulate homeostasis can improve protection against pathogens, digestion, and assimilation of nutrients, along with impacting our immune system (Moloney et al., 2014; Thursby & Juge, 2017). The ‘microbiota-gut-brain-axis’ (MGBA) represents a critical bidirectional communication system between the gut and the brain, involving metabolic, endocrine, neural, and immune pathways critical for brain health and cognition (Cryan et al., 2019; Moloney et al., 2014; Thursby & Juge, 2017; Wiley et al., 2017). Altered microbial abundance and composition can have detrimental effects on health and wellness, including implications for cognitive functioning, mental health, and neurodegeneration (Ceppa et al., 2020; Liu et al., 2020; Ticinesi et al., 2018; Tremlett et al., 2017) and alterations in gut microbiota have been documented years post injury in individuals with a history of moderate/severe TBI (Brenner et al., 2020; Urban et al., 2020). Preclinical work using animal models also supports the microbiome as playing a mechanistic role in adverse outcomes following stress and trauma (Angoa-Pérez et al., 2020; Matharu et al., 2019; Opeyemi et al., 2021), but this is the first study analyzing gut microbiota in a model of blast (either single or repetitive), and the first of it’s kind to compare gut microbiota following blasts across sexes. Similar to our cytokine results, I found both similar and disparate effects of repetitive blast on gut microbiome in male vs. female mice. Of particular note however, linear discriminant analysis of fecal samples demonstrated bifidobacteria as significantly increased in sham vs. blast mice, which is

already being tested as a potential probiotic for PTSD and depression, raising the possibility of this bacteria as a potential treatment target for further development.

Works Cited

Adams, R. S., Corrigan, J. D., & Larson, M. J. (2012). Alcohol Use After Combat-Acquired Traumatic Brain Injury: What We Know and Don't Know. *Journal of Social Work Practice in the Addictions*, *12*(1), 28–51. https://doi.org/10.1080/1533256X.2012.647580

Alexander, M. P. (1995). Mild traumatic brain injury: Pathophysiology, natural history, and clinical management. *Neurology*, *45*(7), 1253–1260. https://doi.org/10.1212/WNL.45.7.1253

Amick, M. M., Clark, A., Fortier, C. B., Esterman, M., Rasmusson, A. M., Kenna, A., Milberg, W. P., & McGlinchey, R. (2013). PTSD Modifies Performance on a Task of Affective Executive Control among Deployed OEF/OIF Veterans with Mild Traumatic Brain Injury. *Journal of the International Neuropsychological Society*, *19*(7), 792–801. https://doi.org/10.1017/S1355617713000544

Angoa-Pérez, M., Zagorac, B., Anneken, J. H., Briggs, D. I., Winters, A. D., Greenberg, J. M., Ahmad, M., Theis, K. R., & Kuhn, D. M. (2020). Repetitive, mild traumatic brain injury results in a progressive white matter pathology, cognitive deterioration, and a transient gut microbiota dysbiosis. *Scientific Reports*, *10*(1), Article 1. https://doi.org/10.1038/s41598-020-65972-4

Balan, S., Widner, G., Shroff, M., van den Berk-Clark, C., Scherrer, J., & Price, R. K. (2013). Drug use disorders and post-traumatic stress disorder over 25 adult years: Role of psychopathology in relational networks. *Drug and Alcohol Dependence*, *133*(1), 228–234. https://doi.org/10.1016/j.drugalcdep.2013.04.030

Banks, W. A., Gray, A. M., Erickson, M. A., Salameh, T. S., Damodarasamy, M., Sheibani, N., Meabon, J. S., Wing, E. E., Morofuji, Y., Cook, D. G., & Reed, M. J. (2015). Lipopolysaccharide-induced blood-brain barrier disruption: Roles of cyclooxygenase, oxidative stress, neuroinflammation, and elements of the neurovascular unit. *Journal of Neuroinflammation*, *12*(1), 223. https://doi.org/10.1186/s12974-015-0434-1

Barlas, F. M., Higgins, W. B., Pflieger, J. C., & Diecker, K. (2013). *2011 Health Related Behaviors Survey of Active Duty Military Personnel:* Defense Technical Information Center. https://doi.org/10.21236/ADA582287

Baskin, B., Lee, S. J., Skillen, E., Wong, K., Rau, H., Hendrickson, R. C., Pagulayan, K., Raskind, M. A., Peskind, E. R., Phillips, P. E. M., Cook, D. G., & Schindler, A. G. (2021). Repetitive Blast Exposure Increases Appetitive Motivation and Behavioral Inflexibility in Male Mice. *Frontiers in Behavioral Neuroscience*, *15*, 792648. https://doi.org/10.3389/fnbeh.2021.792648

Beidler, E., Wallace, J., Alghwiri, A. A., & O'Connor, S. (2020). Collegiate Athletes' Concussion Awareness, Understanding, and -Reporting Behaviors in Different Countries With Varying Concussion Publicity. *Journal of Athletic Training*, *56*(1), 77–84. https://doi.org/10.4085/1062-6050-0575.19

Bell, T. R., Applequist, J., & Dotson-Pierson, C. (2019). *CTE, Media, and the NFL: Framing a Public Health Crisis as a Football Epidemic*. Rowman & Littlefield.

Berridge, K. C. (2004). Motivation concepts in behavioral neuroscience. *Physiology & Behavior*, *81*(2), 179–209. https://doi.org/10.1016/j.physbeh.2004.02.004

Betthauser, L. M., Adams, R. S., Hostetter, T. A., Scher, A. I., Schwab, K., & Brenner, L. A. (20190801). Characterization of lifetime TBIs in a cohort of recently deployed soldiers: The warrior strong study. *Rehabilitation Psychology*, *64*(4), 398. https://doi.org/10.1037/rep0000286

Bjork, J. M., & Grant, S. J. (2009). Does traumatic brain injury increase risk for substance abuse? *Journal of Neurotrauma*, *26*, 1077–1082. https://doi.org/10.1089/neu.2008.0849

Bogner, J. A., Corrigan, J. D., Mysiw, W. J., Clinchot, D., & Fugate, L. (2001). A comparison of substance abuse and violence in the prediction of long-term rehabilitation outcomes after traumatic brain injury. *Archives of Physical Medicine and Rehabilitation*, *82*(5), 571–577. https://doi.org/10.1053/apmr.2001.22340

Bradley, K. A., McDonell, M. B., Bush, K., Kivlahan, D. R., Diehr, P., & Fihn, S. D. (1998). The AUDIT alcohol consumption questions: Reliability, validity, and responsiveness to change in older male primary care patients. *Alcoholism, Clinical and Experimental Research*, *22*(8), 1842–1849. https://doi.org/10.1111/j.1530-0277.1998.tb03991.x

Bray, R. M., Pemberton, M. R., Lane, M. E., Hourani, L. L., Mattiko, M. J., & Babeu, L. A. (2010). Substance Use and Mental Health Trends Among U.S. Military Active Duty Personnel: Key Findings From the 2008 DoD Health Behavior Survey. *Military Medicine*, *175*(6), 390–399. https://doi.org/10.7205/MILMED-D-09-00132

Brenner, L. A., Stamper, C. E., Hoisington, A. J., Stearns-Yoder, K. A., Stanislawksi, M. A., Brostow, D. P., Hoffmire, C. A., Forster, J. E., Schneider, A. L., Postolache, T. T., &

Lowry, C. A. (2020). Microbial Diversity and Community Structures Among Those With Moderate to Severe TBI: A United States-Veteran Microbiome Project Study. *The Journal of Head Trauma Rehabilitation*, *35*(5), 332. https://doi.org/10.1097/HTR.0000000000000615

Bromberg, C. E., Condon, A. M., Ridgway, S. W., Krishna, G., Garcia-Filion, P. C., Adelson, P. D., Rowe, R. K., & Thomas, T. C. (2020). Sex-Dependent Pathology in the HPA Axis at a Sub-acute Period After Experimental Traumatic Brain Injury. *Frontiers in Neurology*, *11*. https://www.frontiersin.org/articles/10.3389/fneur.2020.00946

Bush, K., Kivlahan, D. R., McDonell, M. B., Fihn, S. D., Bradley, K. A., & for the Ambulatory Care Quality Improvement Project (ACQUIP). (1998). The AUDIT Alcohol Consumption Questions (AUDIT-C): An Effective Brief Screening Test for Problem Drinking. *Archives of Internal Medicine*, *158*(16), 1789–1795. https://doi.org/10.1001/archinte.158.16.1789

Caplan, B., Bogner, J., Brenner, L., Mu, W., Catenaccio, E., & Lipton, M. L. (2017). Neuroimaging in Blast-Related Mild Traumatic Brain Injury. *Journal of Head Trauma Rehabilitation*, *32*(1), 55–69. https://doi.org/10.1097/HTR.0000000000000213

Ceppa, F. A., Izzo, L., Sardelli, L., Raimondi, I., Tunesi, M., Albani, D., & Giordano, C. (2020). Human Gut-Microbiota Interaction in Neurodegenerative Disorders and Current Engineered Tools for Its Modeling. *Frontiers in Cellular and Infection Microbiology*, *10*. https://www.frontiersin.org/articles/10.3389/fcimb.2020.00297

Cernak, Z. W., Jianxin Jiang, Xiuwu Bian, Jovan Savic Ibolja. (2001). Cognitive deficits following blast injury-induced neurotrauma: Possible involvement of nitric oxide. *Brain Injury*, *15*(7), 593–612. https://doi.org/10.1080/02699050119009

Chermack, S. T., Bohnert, A. S. B., Price, A. M., Austin, K., & Ilgen, M. A. (2012). Substance Use Disorders and Homicide Death in Veterans. *Journal of Studies on Alcohol and Drugs*, *73*(1), 10–14. https://doi.org/10.15288/jsad.2012.73.10

Cogan, A. M., McCaughey, V. K., & Scholten, J. (2020). Gender Differences in Outcomes after Traumatic Brain Injury among Service Members and Veterans. *PM&R*, *12*(3), 301–314. https://doi.org/10.1002/pmrj.12237

Corrigan, J. D. (1995). Substance abuse as a mediating factor in outcome from traumatic brain injury. *Archives of Physical Medicine and Rehabilitation*, *76*(4), 302–309. https://doi.org/10.1016/S0003-9993(95)80654-7

Crawford, E. F., Fulton, J. J., Swinkels, C. M., Beckham, J. C., & Calhoun, P. S. (2013). Diagnostic efficiency of the AUDIT-C in U.S. veterans with military service since September 11, 2001. *Drug and Alcohol Dependence*, *132*(1), 101–106. https://doi.org/10.1016/j.drugalcdep.2013.01.012

Cryan, J. F., O'Riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., Codagnone, M. G., Cussotto, S., Fulling, C., Golubeva, A. V., Guzzetta, K. E., Jaggar, M., Long-Smith, C. M., Lyte, J. M., Martin, J. A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., … Dinan, T. G. (2019). The Microbiota-Gut-Brain Axis. *Physiological Reviews*, *99*(4), 1877–2013. https://doi.org/10.1152/physrev.00018.2018

Davenport, N. D., Lim, K. O., Armstrong, M. T., & Sponheim, S. R. (2012). Diffuse and spatially variable white matter disruptions are associated with blast-related mild traumatic brain injury. *NeuroImage*, *59*(3), 2017–2024. https://doi.org/10.1016/j.neuroimage.2011.10.050

Dawson, D. A., Grant, B. F., Stinson, F. S., & Zhou, Y. (2005). Effectiveness of the Derived Alcohol Use Disorders Identification Test (AUDIT-C) in Screening for Alcohol Use Disorders and Risk Drinking in the US General Population. *Alcoholism: Clinical and Experimental Research*, *29*(5), 844–854. https://doi.org/10.1097/01.ALC.0000164374.32229.A2

Derefinko, K. J., Hallsell, T. A., Isaacs, M. B., Salgado Garcia, F. I., Colvin, L. W., Bursac, Z., McDevitt-Murphy, M. E., Murphy, J. G., Little, M. A., Talcott, G. W., & Klesges, R. C. (2018). Substance Use and Psychological Distress Before and After the Military to Civilian Transition. *Military Medicine*, *183*(5–6), e258–e265. https://doi.org/10.1093/milmed/usx082

Di Battista, A., Soo, C., Catroppa, C., & Anderson, V. (2012). Quality of Life in Children and Adolescents Post-TBI: A Systematic Review and Meta-Analysis. *Journal of Neurotrauma*, *29*(9), 1717–1727. https://doi.org/10.1089/neu.2011.2157

Dikmen, S. S., Donovan, D. M., Løberg, T., Machamer, J. E., & Temkin, N. R. (1993). Alcohol use and its effects on neuropsychological outcome in head injury. *Neuropsychology*, *7*(3), 296–305. https://doi.org/10.1037/0894-4105.7.3.296

DOD TBI Worldwide Numbers. (n.d.). Military Health System. Retrieved July 15, 2022, from https://www.health.mil/Military-Health-Topics/Centers-of-Excellence/Traumatic-Brain-Injury-Center-of-Excellence/DOD-TBI-Worldwide-Numbers

Doran, S. J., Ritzel, R. M., Glaser, E. P., Henry, R. J., Faden, A. I., & Loane, D. J. (2019). Sex Differences in Acute Neuroinflammation after Experimental Traumatic Brain Injury Are Mediated by Infiltrating Myeloid Cells. *Journal of Neurotrauma*, *36*(7), 1040–1053. https://doi.org/10.1089/neu.2018.6019

Dye, J. L., Eskridge, S. L., Tepe, V., Clouser, M. C., & Galarneau, M. (2016). Characterization and Comparison of Combat-Related Injuries in Women During OIF and OEF. *Military Medicine*, *181*(suppl_1), 92–98. https://doi.org/10.7205/MILMED-D-15-00237

Edwards, M. J., Lustik, M., Eichelberger, M. R., Elster, E., Azarow, K., & Coppola, C. (2012). Blast injury in children: An analysis from Afghanistan and Iraq, 2002–2010. *Journal of Trauma and Acute Care Surgery*, *73*(5), 1278–1283. https://doi.org/10.1097/TA.0b013e318270d3ee

Elder, G. A., & Cristian, A. (2009). Blast-related mild traumatic brain injury: Mechanisms of injury and impact on clinical care. *The Mount Sinai Journal of Medicine, New York*, *76*(2), 111–118. https://doi.org/10.1002/msj.20098

Elder, G. A., Dorr, N. P., De Gasperi, R., Gama Sosa, M. A., Shaughness, M. C., Maudlin-Jeronimo, E., Hall, A. A., McCarron, R. M., & Ahlers, S. T. (2012). Blast Exposure Induces Post-Traumatic Stress Disorder-Related Traits in a Rat Model of Mild

Traumatic Brain Injury. *Journal of Neurotrauma*, *29*(16), 2564–2575. https://doi.org/10.1089/neu.2012.2510

Elder, G. A., Mitsis, E. M., Ahlers, S. T., & Cristian, A. (2010). Blast-induced mild traumatic brain injury. *The Psychiatric Clinics of North America*, *33*(4), 757–781. https://doi.org/10.1016/j.psc.2010.08.001

Emtyazi, D., Rabelo, T. K., Katzman, H., Campos, A. C., Diwan, M., Gidyk, D., Rabelo dos Santos, P., Giacobbe, P., Lipsman, N., Aubert, I., & Hamani, C. (2022). Sex differences in long-term fear and anxiety-like responses in a preclinical model of PTSD. *Journal of Psychiatric Research*, *151*, 619–625. https://doi.org/10.1016/j.jpsychires.2022.05.015

Engel, C. C., Hoch, E., Simmons, M., Arroyo Center, Rand Corporation, & United States (Eds.). (2019). *The neurological effects of repeated exposure to military occupational blast: Implications for prevention and health: proceedings, findings, and expert recommendations from the Seventh Annual Department of Defense State-of-the-Science Meeting, 12-14 March, 2018*. RAND.

Esterov, D., & Greenwald, B. D. (2017). Autonomic Dysfunction after Mild Traumatic Brain Injury. *Brain Sciences*, *7*(8), Article 8. https://doi.org/10.3390/brainsci7080100

Fan, L., Young, P. R., Barone, F. C., Feuerstein, G. Z., Smith, D. H., & McIntosh, T. K. (1996). Experimental brain injury induces differential expression of tumor necrosis factor-α mRNA in the CNS. *Molecular Brain Research*, *36*(2), 287–291. https://doi.org/10.1016/0169-328X(95)00274-V

First, M. S. R., Gibbon, M., & Williams, J. (1995). Structured Clinical Interview for DSM-IV Axis II Disorders-Patient Edition (SCID-IVP, Version 2.0). Ed. *NYP Institute*.

Fischer, B. L., Parsons, M., Durgerian, S., Reece, C., Mourany, L., Lowe, M. J., Beall, E. B., Koenig, K. A., Jones, S. E., Newsome, M. R., Scheibel, R. S., Wilde, E. A., Troyanskaya, M., Merkley, T. L., Walker, M., Levin, H. S., & Rao, S. M. (2014). Neural Activation during Response Inhibition Differentiates Blast from Mechanical Causes of Mild to Moderate Traumatic Brain Injury. *Journal of Neurotrauma*, *31*(2), 169–179. https://doi.org/10.1089/neu.2013.2877

Fox, J. G., Barthold, S. W., Davisson, M. T., Newcomer, C. E., Quimby, F. W., & Smith, A. L. (2007). The mouse in biomedical research. American College of Laboratory Animal Medicine series. *Elsevier, Amsterdam, Ed*, *2*, 2–4.

Fucich, E. A., Mayeux, J. P., McGinn, M. A., Gilpin, N. W., Edwards, S., & Molina, P. E. (2019). A Novel Role for the Endocannabinoid System in Ameliorating Motivation for Alcohol Drinking and Negative Behavioral Affect after Traumatic Brain Injury in Rats. *Journal of Neurotrauma*, *36*(11), 1847–1855. https://doi.org/10.1089/neu.2018.5854

Ganpule, S., Gu, L., Alai, A., & Chandra, N. (2012). Role of helmet in the mechanics of shock wave propagation under blast loading conditions. *Computer Methods in Biomechanics and Biomedical Engineering*, *15*(11), 1233–1244. https://doi.org/10.1080/10255842.2011.597353

Gardner, R. C., & Yaffe, K. (2015). Epidemiology of mild traumatic brain injury and neurodegenerative disease. *Molecular and Cellular Neuroscience*, *66*, 75–80. https://doi.org/10.1016/j.mcn.2015.03.001

Garman, R. H., Jenkins, L. W., Switzer, R. C., Bauman, R. A., Tong, L. C., Swauger, P. V., Parks, S. A., Ritzel, D. V., Dixon, C. E., Clark, R. S. B., Bayır, H., Kagan, V., Jackson, E. K., & Kochanek, P. M. (2011). Blast Exposure in Rats with Body Shielding Is Characterized Primarily by Diffuse Axonal Injury. *Journal of Neurotrauma*, *28*(6), 947–959. https://doi.org/10.1089/neu.2010.1540

Genovese, R. F., Simmons, L. P., Ahlers, S. T., Maudlin-Jeronimo, E., Dave, J. R., & Boutte, A. M. (2013). Effects of mild TBI from repeated blast overpressure on the expression and extinction of conditioned fear in rats. *Neuroscience*, *254*, 120–129. https://doi.org/10.1016/j.neuroscience.2013.09.021

Ghai, V., Fallen, S., Baxter, D., Scherler, K., Kim, T.-K., Zhou, Y., Meabon, J. S., Logsdon, A. F., Banks, W. A., Schindler, A. G., Cook, D. G., Peskind, E. R., Lee, I., & Wang, K. (2020). Alterations in Plasma microRNA and Protein Levels in War Veterans with Chronic Mild Traumatic Brain Injury. *Journal of Neurotrauma*, *37*(12), 1418–1430. https://doi.org/10.1089/neu.2019.6826

Goldstein, L. E., McKee, A. C., & Stanton, P. K. (2014). Considerations for animal models of blast-related traumatic brain injury and chronic traumatic encephalopathy. *Alzheimer's Research & Therapy*, *6*(5), 64. https://doi.org/10.1186/s13195-014-0064-3

Gontkovsky, S. T., Sherer, M., Nick, T. G., Nakase-Thompson, R., & Yablon, S. A. (2006). Effect of urbanicity of residence on TBI outcome at one year post-injury. *Brain Injury*, *20*(7), 701–709. https://doi.org/10.1080/02699050600744103

Gowin, J. L., Sloan, M. E., Stangl, B. L., Vatsalya, V., & Ramchandani, V. A. (2017). Vulnerability for Alcohol Use Disorder and Rate of Alcohol Consumption. *American*

Journal of Psychiatry, *174*(11), 1094–1101. https://doi.org/10.1176/appi.ajp.2017.16101180

Gray, M., Adamson, M. M., Thompson, R. C., Kapphahn, K. I., Han, S., Chung, J. S., & Harris, O. A. (2020). Sex differences in symptom presentation and functional outcomes: A pilot study in a matched sample of veterans with mild TBI. *Brain Injury*, *34*(4), 535–547. https://doi.org/10.1080/02699052.2020.1725979

Grossbard, J., Malte, C. A., Lapham, G., Pagulayan, K., Turner, A. P., Rubinsky, A. D., Bradley, K. A., Saxon, A. J., & Hawkins, E. J. (2017). Prevalence of Alcohol Misuse and Follow-Up Care in a National Sample of OEF/OIF VA Patients With and Without TBI. *Psychiatric Services (Washington, D.C.)*, *68*(1), 48–55. https://doi.org/10.1176/appi.ps.201500290

Grujicic, M., Bell, W. C., Pandurangan, B., & Glomski, P. S. (2011). Fluid/Structure Interaction Computational Investigation of Blast-Wave Mitigation Efficacy of the Advanced Combat Helmet. *Journal of Materials Engineering and Performance*, *20*(6), 877–893. https://doi.org/10.1007/s11665-010-9724-z

Gupte, R., Brooks, W., Vukas, R., Pierce, J., & Harris, J. (2019). Sex Differences in Traumatic Brain Injury: What We Know and What We Should Know. *Journal of Neurotrauma*, *36*(22), 3063–3091. https://doi.org/10.1089/neu.2018.6171

Halbauer, J. D., Ashford, J. W., Zeitzer, J. M., Adamson, M. M., Lew, H. L., & Yesavage, J. A. (2009). Neuropsychiatric diagnosis and management of chronic sequelae of war-related mild to moderate traumatic brain injury. *The Journal of Rehabilitation Research and Development*, *46*(6), 757. https://doi.org/10.1682/JRRD.2008.08.0119

Hendrickson, R. C., Schindler, A. G., & Pagulayan, K. F. (2018). Untangling PTSD and TBI: Challenges and Strategies in Clinical Care and Research. *Current Neurology and Neuroscience Reports*, *18*(12), 106. https://doi.org/10.1007/s11910-018-0908-5

Hoerster, K. D., Lehavot, K., Simpson, T., McFall, M., Reiber, G., & Nelson, K. M. (2012). Health and Health Behavior Differences: U.S. Military, Veteran, and Civilian Men. *American Journal of Preventive Medicine*, *43*(5), 483–489. https://doi.org/10.1016/j.amepre.2012.07.029

Hoge, C. W., & Cotting, D. I. (2004). Combat Duty in Iraq and Afghanistan, Mental Health Problems, and Barriers to Care. *The New England Journal of Medicine*.

Hoge, C. W., McGurk, D., Thomas, J. L., Cox, A. L., Engel, C. C., & Castro, C. A. (2008). Mild Traumatic Brain Injury in U.S. Soldiers Returning from Iraq. *New England Journal of Medicine*, *358*(5), 453–463. https://doi.org/10.1056/NEJMoa072972

Howard, L., Dumkrieger, G., Chong, C. D., Ross, K., Berisha, V., & Schwedt, T. J. (2018). Symptoms of Autonomic Dysfunction Among Those With Persistent Posttraumatic Headache Attributed to Mild Traumatic Brain Injury: A Comparison to Migraine and Healthy Controls. *Headache: The Journal of Head and Face Pain*, *58*(9), 1397–1407. https://doi.org/10.1111/head.13396

Hubbard, W. B., Velmurugan, G. V., Brown, E. P., & Sullivan, P. G. (2022). Resilience of females to acute blood–brain barrier damage and anxiety behavior following mild blast traumatic brain injury. *Acta Neuropathologica Communications*, *10*(1), 93. https://doi.org/10.1186/s40478-022-01395-8

Huber, B. R., Meabon, J. S., Hoffer, Z. S., Zhang, J., Hoekstra, J. G., Pagulayan, K. F., McMillan, P. J., Mayer, C. L., Banks, W. A., Kraemer, B. C., Raskind, M. A., McGavern, D. B., Peskind, E. R., & Cook, D. G. (2016). Blast exposure causes dynamic microglial/macrophage responses and microdomains of brain microvessel dysfunction. *Neuroscience*, *319*, 206–220. https://doi.org/10.1016/j.neuroscience.2016.01.022

Huber, B. R., Meabon, J. S., Martin, T. J., Mourad, P. D., Bennett, R., Kraemer, B. C., Cernak, I., Petrie, E. C., Emery, M. J., Swenson, E. R., Mayer, C., Mehic, E., Peskind, E. R., & Cook, D. G. (2013). Blast Exposure Causes Early and Persistent Aberrant Phospho- and Cleaved-Tau Expression in a Murine Model of Mild Blast-Induced Traumatic Brain Injury. *Journal of Alzheimer's Disease*, *37*(2), 309–323. https://doi.org/10.3233/JAD-130182

Ivanov, I., Fernandez, C., Mitsis, E. M., Dickstein, D. L., Wong, E., Tang, C. Y., Simantov, J., Bang, C., Moshier, E., Sano, M., Elder, G. A., & Hazlett, E. A. (2017). Blast Exposure, White Matter Integrity, and Cognitive Function in Iraq and Afghanistan Combat Veterans. *Frontiers in Neurology*, *8*, 127. https://doi.org/10.3389/fneur.2017.00127

Iverson, K. M., Hendricks, A. M., Kimerling, R., Krengel, M., Meterko, M., Stolzmann, K. L., Baker, E., Pogoda, T. K., Vasterling, J. J., & Lew, H. L. (2011). Psychiatric diagnoses and neurobehavioral symptom severity among OEF/OIF VA patients with deployment-related traumatic brain injury: A gender comparison. *Women's Health Issues: Official Publication of the Jacobs Institute of Women's Health*, *21*(4 Suppl), S210-217. https://doi.org/10.1016/j.whi.2011.04.019

Johnson, W. D., & Griswold, D. P. (2017). Traumatic brain injury: A global challenge. *The Lancet Neurology*, *16*(12), 949–950. https://doi.org/10.1016/S1474-4422(17)30362-9

Kamarck, K. N. (2015, September 1). *Women in Combat: Issues for Congress* (United States) [Report]. UNT Digital Library; Library of Congress. Congressional Research Service. https://digital.library.unt.edu/ark:/67531/metadc743572/

Karr, J. E., Rau, H. K., Shofer, J. B., Hendrickson, R. C., Peskind, E. R., & Pagulayan, K. F. (2019). Variables associated with subjective cognitive change among Iraq and Afghanistan war Veterans with blast-related mild traumatic brain injury. *Journal of Clinical and Experimental Neuropsychology*, *41*(7), 680–693. https://doi.org/10.1080/13803395.2019.1611740

Kawa, L., Arborelius, U. P., Hökfelt, T., & Risling, M. (2020). Sex-Specific Differences in Rodents Following a Single Primary Blast Exposure: Focus on the Monoamine and Galanin Systems. *Frontiers in Neurology*, *11*, 540144. https://doi.org/10.3389/fneur.2020.540144

Khalid, F., Yang, G. L., McGuire, J. L., Robson, M. J., Foreman, B., Ngwenya, L. B., & Lorenz, J. N. (2019). Autonomic dysfunction following traumatic brain injury: Translational insights. *Neurosurgical Focus*, *47*(5), E8. https://doi.org/10.3171/2019.8.FOCUS19517

Krukowski, K. (2021). Short review: The impact of sex on neuroimmune and cognitive outcomes after traumatic brain injury. *Brain, Behavior, & Immunity - Health*, *16*, 100327. https://doi.org/10.1016/j.bbih.2021.100327

Krukowski, K., Nolan, A., Frias, E. S., Grue, K., Becker, M., Ureta, G., Delgado, L., Bernales, S., Sohal, V. S., Walter, P., & Rosi, S. (2020). Integrated Stress Response Inhibitor Reverses Sex-Dependent Behavioral and Cell-Specific Deficits after Mild Repetitive Head Trauma. *Journal of Neurotrauma*, *37*(11), 1370–1380. https://doi.org/10.1089/neu.2019.6827

Kulkarni, S. G., Gao, X.-L., Horner, S. E., Zheng, J. Q., & David, N. V. (2013). Ballistic helmets – Their design, materials, and performance against traumatic brain injury. *Composite Structures*, *101*, 313–331. https://doi.org/10.1016/j.compstruct.2013.02.014

Lim, Y. W., Meyer, N. P., Shah, A. S., Budde, M. D., Stemper, B. D., & Olsen, C. M. (2015). Voluntary Alcohol Intake following Blast Exposure in a Rat Model of Mild Traumatic Brain Injury. *PLOS ONE*, *10*(4), e0125130. https://doi.org/10.1371/journal.pone.0125130

Lindberg, M. A., Sloley, S. S., Ivins, B. J., Marion, D. W., & Moy Martin, E. M. (2021). Military TBI—What civilian primary care providers should know. *Journal of Family Medicine and Primary Care*, *10*(12), 4391–4397. https://doi.org/10.4103/jfmpc.jfmpc_98_21

Liu, S., Gao, J., Zhu, M., Liu, K., & Zhang, H.-L. (2020). Gut Microbiota and Dysbiosis in Alzheimer's Disease: Implications for Pathogenesis and Treatment. *Molecular Neurobiology*, *57*(12), 5026–5043. https://doi.org/10.1007/s12035-020-02073-3

Logsdon, A. F., Meabon, J. S., Cline, M. M., Bullock, K. M., Raskind, M. A., Peskind, E. R., Banks, W. A., & Cook, D. G. (2018). Blast exposure elicits blood-brain barrier disruption and repair mediated by tight junction integrity and nitric oxide dependent

processes. *Scientific Reports*, *8*(1), Article 1. https://doi.org/10.1038/s41598-018-29341-6

Logsdon, A. F., Schindler, A. G., Meabon, J. S., Yagi, M., Herbert, M. J., Banks, W. A., Raskind, M. A., Marshall, D. A., Keene, C. D., Perl, D. P., Peskind, E. R., & Cook, D. G. (2020). Nitric oxide synthase mediates cerebellar dysfunction in mice exposed to repetitive blast-induced mild traumatic brain injury. *Scientific Reports*, *10*(1), Article 1. https://doi.org/10.1038/s41598-020-66113-7

Lowing, J. L., Susick, L. L., Caruso, J. P., Provenzano, A. M., Raghupathi, R., & Conti, A. C. (2014). Experimental Traumatic Brain Injury Alters Ethanol Consumption and Sensitivity. *Journal of Neurotrauma*, *31*(20), 1700–1710. https://doi.org/10.1089/neu.2013.3286

Maas, A. I. R., Menon, D. K., Adelson, P. D., Andelic, N., Bell, M. J., Belli, A., Bragge, P., Brazinova, A., Büki, A., Chesnut, R. M., Citerio, G., Coburn, M., Cooper, D. J., Crowder, A. T., Czeiter, E., Czosnyka, M., Diaz-Arrastia, R., Dreier, J. P., Duhaime, A.-C., … Zumbo, F. (2017). Traumatic brain injury: Integrated approaches to improve prevention, clinical care, and research. *The Lancet Neurology*, *16*(12), 987–1048. https://doi.org/10.1016/S1474-4422(17)30371-X

Maas, A. I., Stocchetti, N., & Bullock, R. (2008). Moderate and severe traumatic brain injury in adults. *The Lancet Neurology*, *7*(8), 728–741. https://doi.org/10.1016/S1474-4422(08)70164-9

Malec, J. F., Van Houtven, C. H., Tanielian, T., Atizado, A., & Dorn, M. C. (2017). Impact of TBI on caregivers of veterans with TBI: Burden and interventions. *Brain Injury*, *31*(9), 1235–1245. https://doi.org/10.1080/02699052.2016.1274778

María-Ríos, C. E., & Morrow, J. D. (2020). Mechanisms of Shared Vulnerability to Post-traumatic Stress Disorder and Substance Use Disorders. *Frontiers in Behavioral Neuroscience*, *14*, 6. https://doi.org/10.3389/fnbeh.2020.00006

Matharu, D., Dhotre, D., Balasubramanian, N., Pawar, N., Sagarkar, S., & Sakharkar, A. (2019). Repeated mild traumatic brain injury affects microbial diversity in rat jejunum. *Journal of Biosciences*, *44*(5), 120. https://doi.org/10.1007/s12038-019-9940-0

Mayeux, J. P., Teng, S. X., Katz, P. S., Gilpin, N. W., & Molina, P. E. (2015). Traumatic brain injury induces neuroinflammation and neuronal degeneration that is associated with escalated alcohol self-administration in rats. *Behavioural Brain Research*, *279*, 22–30. https://doi.org/10.1016/j.bbr.2014.10.053

McCabe, J. T., & Tucker, L. B. (2020). Sex as a Biological Variable in Preclinical Modeling of Blast-Related Traumatic Brain Injury. *Frontiers in Neurology*, *11*. https://www.frontiersin.org/articles/10.3389/fneur.2020.541050

McDonald, B. C., Flashman, L. A., & Saykin, A. J. (2002). Executive dysfunction following traumatic brain injury: Neural substrates and treatment strategies. *NeuroRehabilitation*, *17*(4), 333–344. https://doi.org/10.3233/NRE-2002-17407

McFARLANE, A. C. (2010). The long-term costs of traumatic stress: Intertwined physical and psychological consequences. *World Psychiatry*, *9*(1), 3–10. https://doi.org/10.1002/j.2051-5545.2010.tb00254.x

McInnes, K., Friesen, C. L., MacKenzie, D. E., Westwood, D. A., & Boe, S. G. (2017). Mild Traumatic Brain Injury (mTBI) and chronic cognitive impairment: A scoping review. *PLOS ONE*, *12*(4), e0174847. https://doi.org/10.1371/journal.pone.0174847

McKinlay, A., Grace, R., Horwood, J., Fergusson, D., & MacFarlane, M. (2009). Adolescent Psychiatric Symptoms Following Preschool Childhood Mild Traumatic Brain Injury: Evidence From a Birth Cohort. *The Journal of Head Trauma Rehabilitation*, *24*(3), 221–227. https://doi.org/10.1097/HTR.0b013e3181a40590

McNamara, E. H., Tucker, L. B., Liu, J., Fu, A. H., Kim, Y., Vu, P. A., & McCabe, J. T. (2022). Limbic Responses Following Shock Wave Exposure in Male and Female Mice. *Frontiers in Behavioral Neuroscience*, *16*. https://www.frontiersin.org/articles/10.3389/fnbeh.2022.863195

Meabon, J. S., Cook, D. G., Yagi, M., Terry, G. E., Cross, D. J., Muzi, M., Pagulayan, K. F., Logsdon, A. F., Schindler, A. G., Ghai, V., Wang, K., Fallen, S., Zhou, Y., Kim, T.-K., Lee, I., Banks, W. A., Carlson, E. S., Mayer, C., Hendrickson, R. C., … Peskind, E. R. (2020). Chronic elevation of plasma vascular endothelial growth factor-A (VEGF-A) is associated with a history of blast exposure. *Journal of the Neurological Sciences*, *417*. https://doi.org/10.1016/j.jns.2020.117049

Meabon, J. S., Huber, B. R., Cross, D. J., Richards, T. L., Minoshima, S., Pagulayan, K. F., Li, G., Meeker, K. D., Kraemer, B. C., Petrie, E. C., Raskind, M. A., Peskind, E. R., & Cook, D. G. (2016). Repetitive blast exposure in mice and combat veterans causes persistent cerebellar dysfunction. *Science Translational Medicine*, *8*(321), 321ra6-321ra6. https://doi.org/10.1126/scitranslmed.aaa9585

Miller, K. J., Ivins, B. J., & Schwab, K. A. (2013). Self-reported mild TBI and postconcussive symptoms in a peacetime active duty military population: Effect of multiple TBI history versus single mild TBI. *The Journal of Head Trauma Rehabilitation*, *28*(1), 31–38. https://doi.org/10.1097/HTR.0b013e318255ceae

Modrak, C. G., Giesler, L. P., & Vonder Haar, C. (2020). Traumatic brain injury substantially reduces the conditioned reinforcing effects of environmental cues in rats. *Brain Research*, *1748*, 147084. https://doi.org/10.1016/j.brainres.2020.147084

Moloney, R. D., Desbonnet, L., Clarke, G., Dinan, T. G., & Cryan, J. F. (2014). The microbiome: Stress, health and disease. *Mammalian Genome*, *25*(1), 49–74. https://doi.org/10.1007/s00335-013-9488-5

Moss, W. C., King, M. J., & Blackman, E. G. (2009). Skull Flexure from Blast Waves: A Mechanism for Brain Injury with Implications for Helmet Design. *Physical Review Letters*, *103*(10), 108702. https://doi.org/10.1103/PhysRevLett.103.108702

Mouzon, B. C., Bachmeier, C., Ferro, A., Ojo, J.-O., Crynen, G., Acker, C. M., Davies, P., Mullan, M., Stewart, W., & Crawford, F. (2014). Chronic neuropathological and neurobehavioral changes in a repetitive mild traumatic brain injury model. *Annals of Neurology*, *75*(2), 241–254. https://doi.org/10.1002/ana.24064

Muelbl, M. J., Slaker, M. L., Shah, A. S., Nawarawong, N. N., Gerndt, C. H., Budde, M. D., Stemper, B. D., & Olsen, C. M. (2018). Effects of Mild Blast Traumatic Brain Injury on Cognitive- and Addiction-Related Behaviors. *Scientific Reports*, *8*(1), Article 1. https://doi.org/10.1038/s41598-018-28062-0

Narayan, R. K., Michel, M. E., Ansell, B., Baethmann, A., Biegon, A., Bracken, M. B., Bullock, M. R., Choi, S. C., Clifton, G. L., Contant, C. F., Coplin, W. M., Dietrich, W. D., Ghajar, J., Grady, S. M., Grossman, R. G., Hall, E. D., Heetderks, W., Hovda, D. A., Jallo, J., … Yurkewicz, L. (2002). Clinical Trials in Head Injury. *Journal of Neurotrauma*, *19*(5), 503–557. https://doi.org/10.1089/089771502753754037

Nawarawong, N. N., Slaker, M., Muelbl, M., Shah, A. S., Chiariello, R., Nelson, L. D., Budde, M. D., Stemper, B. D., & Olsen, C. M. (2019). Repeated blast model of mild traumatic brain injury alters oxycodone self-administration and drug seeking. *European Journal of Neuroscience*, *50*(3), 2101–2112. https://doi.org/10.1111/ejn.14281

Neipert, L., Pastorek, N. J., Troyanskaya, M., Scheibel, R. S., Petersen, N. J., & Levin, H. S. (2014). Effect of clinical characteristics on cognitive performance in service members and veterans with histories of blast-related mild traumatic brain injury. *Brain Injury*, *28*(13–14), 1667–1674. https://doi.org/10.3109/02699052.2014.947623

Norman, S. B., Schmied, E., & Larson, G. E. (2014). Predictors of Continued Problem Drinking and Substance Use Following Military Discharge. *Journal of Studies on Alcohol and Drugs*, *75*(4), 557–566. https://doi.org/10.15288/jsad.2014.75.557

Olson-Madden, J. H., Forster, J. E., Huggins, J., & Schneider, A. (2012). Psychiatric diagnoses, mental health utilization, high-risk behaviors, and self-directed violence among veterans with comorbid history of traumatic brain injury and substance use disorders. *The Journal of Head Trauma Rehabilitation*, *27*(5), 370–378. https://doi.org/10.1097/HTR.0b013e318268d496

Ommaya, A. K., Salazar, A. M., Dannenberg, A. L., Ommaya, A. K., Chervinsky, A. B., & Schwab, K. (1996). Outcome after Traumatic Brain Injury in the U.S. Military Medical System. *Journal of Trauma and Acute Care Surgery*, *41*(6), 972.

O'Neil, M. E., Carlson, K., Storzbach, D., Brenner, L., Freeman, M., Quiñones, A., Motu'apuaka, M., Ensley, M., & Kansagara, D. (2013). *Complications of Mild Traumatic Brain Injury in Veterans and Military Personnel: A Systematic Review*. Department of Veterans Affairs (US). https://www.ncbi.nlm.nih.gov/books/NBK189785/

Opeyemi, O. M., Rogers, M. B., Firek, B. A., Janesko-Feldman, K., Vagni, V., Mullett, S. J., Wendell, S. G., Nelson, B. P., New, L. A., Mariño, E., Kochanek, P. M., Bayır, H., Clark, R. S. B., Morowitz, M. J., & Simon, D. W. (2021). Sustained Dysbiosis and Decreased Fecal Short-Chain Fatty Acids after Traumatic Brain Injury and Impact on Neurologic Outcome. *Journal of Neurotrauma*, *38*(18), 2610–2621. https://doi.org/10.1089/neu.2020.7506

Overton, I. (2020, October 15). A decade of global IED harm reviewed. *AOAV*. https://aoav.org.uk/2020/a-decade-of-global-ied-harm-reviewed/

Owens, B. D., Kragh, J. F. J., Wenke, J. C., Macaitis, J., Wade, C. E., & Holcomb, J. B. (2008). Combat Wounds in Operation Iraqi Freedom and Operation Enduring Freedom. *Journal of Trauma and Acute Care Surgery*, *64*(2), 295–299. https://doi.org/10.1097/TA.0b013e318163b875

Ozga, J. E., Povroznik, J. M., Engler-Chiurazzi, E. B., & Haar, C. V. (2018). Executive (dys)function after traumatic brain injury: Special considerations for behavioral

pharmacology. *Behavioural Pharmacology*, *29*(7), 617–637. https://doi.org/10.1097/FBP.0000000000000430

Pagulayan, K. F., Petrie, E. C., Cook, D. G., Hendrickson, R. C., Rau, H., Reilly, M., Mayer, C., Meabon, J. S., Raskind, M. A., Peskind, E. R., & Kleinhans, N. (2020). Effect of blast-related mTBI on the working memory system: A resting state fMRI study. *Brain Imaging and Behavior*, *14*(4), 949–960. https://doi.org/10.1007/s11682-018-9987-9

Pagulayan, K. F., Rau, H., Madathil, R., Werhane, M., Millard, S. P., Petrie, E. C., Parmenter, B., Peterson, S., Sorg, S., Hendrickson, R., Mayer, C., Meabon, J. S., Huber, B. R., Raskind, M., Cook, D. G., & Peskind, E. R. (2018). Retrospective and Prospective Memory Among OEF/OIF/OND Veterans With a Self-Reported History of Blast-Related mTBI. *Journal of the International Neuropsychological Society*, *24*(4), 324–334. https://doi.org/10.1017/S1355617717001217

Perez-Garcia, G., De Gasperi, R., Gama Sosa, M. A., Perez, G. M., Otero-Pagan, A., Tschiffely, A., McCarron, R. M., Ahlers, S. T., Elder, G. A., & Gandy, S. (2018). PTSD-Related Behavioral Traits in a Rat Model of Blast-Induced mTBI Are Reversed by the mGluR2/3 Receptor Antagonist BCI-838. *ENeuro*, *5*(1). https://doi.org/10.1523/ENEURO.0357-17.2018

Perez-Garcia, G., Gama Sosa, M. A., De Gasperi, R., Tschiffely, A. E., McCarron, R. M., Hof, P. R., Gandy, S., Ahlers, S. T., & Elder, G. A. (2019). Blast-induced “PTSD”: Evidence from an animal model. *Neuropharmacology*, *145*, 220–229. https://doi.org/10.1016/j.neuropharm.2018.09.023

Perez-Polo, J. R., Rea, H. C., Johnson, K. M., Parsley, M. A., Unabia, G. C., Xu, G., Infante, S. K., DeWitt, D. S., & Hulsebosch, C. E. (2013a). Inflammatory Consequences in a Rodent Model of Mild Traumatic Brain Injury. *Journal of Neurotrauma*, *30*(9), 727–740. https://doi.org/10.1089/neu.2012.2650

Perez-Polo, J. R., Rea, H. C., Johnson, K. M., Parsley, M. A., Unabia, G. C., Xu, G., Infante, S. K., DeWitt, D. S., & Hulsebosch, C. E. (2013b). Inflammatory Consequences in a Rodent Model of Mild Traumatic Brain Injury. *Journal of Neurotrauma*, *30*(9), 727–740. https://doi.org/10.1089/neu.2012.2650

Peskind, E. R., Petrie, E. C., Cross, D. J., Pagulayan, K., McCraw, K., Hoff, D., Hart, K., Yu, C.-E., Raskind, M. A., Cook, D. G., & Minoshima, S. (2011). Cerebrocerebellar hypometabolism associated with repetitive blast exposure mild traumatic brain injury in 12 Iraq war Veterans with persistent post-concussive symptoms. *NeuroImage*, *54*, S76–S82. https://doi.org/10.1016/j.neuroimage.2010.04.008

Petrie, E. C., Cross, D. J., Yarnykh, V. L., Richards, T., Martin, N. M., Pagulayan, K., Hoff, D., Hart, K., Mayer, C., Tarabochia, M., Raskind, M. A., Minoshima, S., & Peskind, E. R. (2014). Neuroimaging, behavioral, and psychological sequelae of repetitive combined blast/impact mild traumatic brain injury in Iraq and Afghanistan war veterans. *Journal of Neurotrauma*, *31*(5), 425–436. https://doi.org/10.1089/neu.2013.2952

Piantino, J., Schwartz, D. L., Luther, M., Newgard, C., Silbert, L., Raskind, M., Pagulayan, K., Kleinhans, N., Iliff, J., & Peskind, E. (2021). Link between Mild Traumatic Brain Injury, Poor Sleep, and Magnetic Resonance Imaging: Visible Perivascular

Spaces in Veterans. *Journal of Neurotrauma*, *38*(17), 2391–2399. https://doi.org/10.1089/neu.2020.7447

Rabinowitz, A. R., & Levin, H. S. (2014). Cognitive Sequelae of Traumatic Brain Injury. *Psychiatric Clinics*, *37*(1), 1–11. https://doi.org/10.1016/j.psc.2013.11.004

Ramlackhansingh, A. F., Brooks, D. J., Greenwood, R. J., Bose, S. K., Turkheimer, F. E., Kinnunen, K. M., Gentleman, S., Heckemann, R. A., Gunanayagam, K., Gelosa, G., & Sharp, D. J. (2011). Inflammation after trauma: Microglial activation and traumatic brain injury. *Annals of Neurology*, *70*(3), 374–383. https://doi.org/10.1002/ana.22455

Rau, H. K., Hendrickson, R. C., Roggenkamp, H. C., Peterson, S., Parmenter, B., Cook, D. G., Peskind, E., & Pagulayan, K. F. (2018). Fatigue—But not mTBI history, PTSD, or sleep quality—Directly contributes to reduced prospective memory performance in Iraq and Afghanistan era Veterans. *The Clinical Neuropsychologist*, *32*(7), 1319–1336. https://doi.org/10.1080/13854046.2017.1381277

Redmond, S. A., Wilcox, S. L., Campbell, S., Kim, A., Finney, K., Barr, K., & Hassan, A. M. (2015). A brief introduction to the military workplace culture. *Work (Reading, Mass.)*, *50*(1), 9–20. https://doi.org/10.3233/WOR-141987

Regasa, L. E., Agimi, Y., & Stout, K. C. (2019). Traumatic Brain Injury Following Military Deployment: Evaluation of Diagnosis and Cause of Injury. *The Journal of Head Trauma Rehabilitation*, *34*(1), 21–29. https://doi.org/10.1097/HTR.0000000000000417

Rosenfeld, J. V., McFarlane, A. C., Bragge, P., Armonda, R. A., Grimes, J. B., & Ling, G. S. (2013). Blast-related traumatic brain injury. *The Lancet. Neurology*, *12*(9), 882–893. https://doi.org/10.1016/S1474-4422(13)70161-3

Rubovitch, V., Ten-Bosch, M., Zohar, O., Harrison, C. R., Tempel-Brami, C., Stein, E., Hoffer, B. J., Balaban, C. D., Schreiber, S., Chiu, W.-T., & Pick, C. G. (2011). A mouse model of blast-induced mild traumatic brain injury. *Experimental Neurology*, *232*(2), 280–289. https://doi.org/10.1016/j.expneurol.2011.09.018

Ruff, R. M., Iverson, G. L., Barth, J. T., Bush, S. S., Broshek, D. K., & the NAN Policy and Planning Committee. (2009). Recommendations for Diagnosing a Mild Traumatic Brain Injury: A National Academy of Neuropsychology Education Paper. *Archives of Clinical Neuropsychology*, *24*(1), 3–10. https://doi.org/10.1093/arclin/acp006

Ruff, R. M., Marshall, L. F., Klauber, M. R., Blunt, B. A., Grant, I., Foulkes, M. A., Eisenberg, H., Jane, J., & Marmarou, A. (1990). Alcohol abuse and neurological outcome of the severely head injured. *The Journal of Head Trauma Rehabilitation*, *5*(3), 21.

Russell, A. L., Handa, R. J., & Wu, T. J. (2018). Sex-Dependent Effects of Mild Blast-induced Traumatic Brain Injury on Corticotropin-releasing Factor Receptor Gene Expression: Potential Link to Anxiety-like Behaviors. *Neuroscience*, *392*, 1–12. https://doi.org/10.1016/j.neuroscience.2018.09.014

Russell, A. L., Richardson, M. R., Bauman, B. M., Hernandez, I. M., Saperstein, S., Handa, R. J., & Wu, T. J. (2018). Differential Responses of the HPA Axis to Mild Blast Traumatic Brain Injury in Male and Female Mice. *Endocrinology*, *159*(6), 2363–2375. https://doi.org/10.1210/en.2018-00203

Sajja, V. S. S. S., Galloway, M., Ghoddoussi, F., Kepsel, A., & VandeVord, P. (2013). Effects of blast-induced neurotrauma on the nucleus accumbens. *Journal of Neuroscience Research*, *91*(4), 593–601. https://doi.org/10.1002/jnr.23179

Sajja, V. S. S. S., Hubbard, W. B., Hall, C. S., Ghoddoussi, F., Galloway, M. P., & VandeVord, P. J. (2015). Enduring deficits in memory and neuronal pathology after blast-induced traumatic brain injury. *Scientific Reports*, *5*(1), Article 1. https://doi.org/10.1038/srep15075

Salat, D. H., Robinson, M. E., Miller, D. R., Clark, D. C., & McGlinchey, R. E. (2017). Neuroimaging of deployment-associated traumatic brain injury (TBI) with a focus on mild TBI (mTBI) since 2009. *Brain Injury*, *31*(9), 1204–1219. https://doi.org/10.1080/02699052.2017.1327672

Salem, S., Campidelli, M., El-Dakhakhni, W., & Tait, M. (2017). Blast Resilient Design of Infrastructure Subjected to Ground Threats. *Volume 4: Fluid-Structure Interaction*, V004T04A018. https://doi.org/10.1115/PVP2017-65205

Salling, M. C., Skelly, M. J., Avegno, E., Regan, S., Zeric, T., Nichols, E., & Harrison, N. L. (2018). Alcohol Consumption during Adolescence in a Mouse Model of Binge Drinking Alters the Intrinsic Excitability and Function of the Prefrontal Cortex through a Reduction in the Hyperpolarization-Activated Cation Current. *Journal of Neuroscience*, *38*(27), 6207–6222. https://doi.org/10.1523/JNEUROSCI.0550-18.2018

Sanderson, J., Weathers, M., Snedaker, K., & Gramlich, K. (2017). “I Was Able to Still Do My Job on the Field and Keep Playing”: An Investigation of Female and Male

Athletes' Experiences With (Not) Reporting Concussions. *Communication & Sport*, *5*(3), 267–287. https://doi.org/10.1177/2167479515623455

Santiago, P. N., Wilk, J. E., Milliken, C. S., Castro, C. A., Engel, C. C., & Hoge, C. W. (2010). Screening for Alcohol Misuse and Alcohol-Related Behaviors Among Combat Veterans. *Psychiatric Services*, *61*(6), 575–581. https://doi.org/10.1176/ps.2010.61.6.575

Sarmiento, K., Hoffman, R., Dmitrovsky, Z., & Lee, R. (2014). A 10-year review of the Centers for Disease Control and Prevention's Heads Up initiatives: Bringing concussion awareness to the forefront. *Journal of Safety Research*, *50*, 143–147. https://doi.org/10.1016/j.jsr.2014.05.003

Saxon, A. J. (2011). Returning veterans with addictions: Issues and implications for clinical practice. *Psychiatric Times*, *28*(7), 49–49.

Schindler, A. G., Baskin, B., Juarez, B., Janet Lee, S., Hendrickson, R., Pagulayan, K., Zweifel, L. S., Raskind, M. A., Phillips, P. E. M., Peskind, E. R., & Cook, D. G. (2021). Repetitive blast mild traumatic brain injury increases ethanol sensitivity in male mice and risky drinking behavior in male combat veterans. *Alcoholism, Clinical and Experimental Research*, *45*(5), 1051–1064. https://doi.org/10.1111/acer.14605

Schindler, A. G., Meabon, J. S., Pagulayan, K. F., Hendrickson, R. C., Meeker, K. D., Cline, M., Li, G., Sikkema, C., Wilkinson, C. W., Perl, D. P., Raskind, M. R., Peskind, E. R., Clark, J. J., & Cook, D. G. (2017). Blast–related disinhibition and risk seeking in mice and combat Veterans: Potential role for dysfunctional phasic dopamine release. *Neurobiology of Disease*, *106*, 23–34. https://doi.org/10.1016/j.nbd.2017.06.004

Schindler, A. G., Terry, G. E., Wolden-Hanson, T., Cline, M., Park, M., Lee, J., Yagi, M., Meabon, J. S., Peskind, E. R., Raskind, M. M., Phillips, P. E. M., & Cook, D. G. (2021). Repetitive Blast Promotes Chronic Aversion to Neutral Cues Encountered in the Peri-Blast Environment. *Journal of Neurotrauma*, *38*(7), 940–948. https://doi.org/10.1089/neu.2020.7061

Schindler, A., Terry, G. E., Wolden-Hanson, T., Cline, M. M., Park, M., Lee, S. J., Yagi, M., Meabon, J., Raskind, M., Peskind, E., Phillips, P. E., & Cook, D. (2020). Repetitive blast promotes chronic aversion to neutral cues encountered in the peri-blast environment. *Journal of Neurotrauma*. https://doi.org/10.1089/neu.2020.7061

Seal, K. H., Cohen, G., Waldrop, A., Cohen, B. E., Maguen, S., & Ren, L. (2011). Substance use disorders in Iraq and Afghanistan veterans in VA healthcare, 2001–2010: Implications for screening, diagnosis and treatment. *Drug and Alcohol Dependence*, *116*(1), 93–101. https://doi.org/10.1016/j.drugalcdep.2010.11.027

Seidl, J. N. T., Pastorek, N. J., Lillie, R., Rosenblatt, A., Troyanskaya, M., Miller, B. I., Romesser, J., Lippa, S., Sim, A. H., & Linck, J. (2015). Factors related to satisfaction with life in veterans with mild traumatic brain injury. *Rehabilitation Psychology*, *60*(4), 335–343. https://doi.org/10.1037/rep0000064

Shohami, E., Ginis, I., & Hallenbeck, J. M. (1999). Dual role of tumor necrosis factor alpha in brain injury. *Cytokine & Growth Factor Reviews*, *10*(2), 119–130. https://doi.org/10.1016/S1359-6101(99)00008-8

Song, H., Cui, J., Simonyi, A., Johnson, C. E., Hubler, G. K., DePalma, R. G., & Gu, Z. (2018). Linking blast physics to biological outcomes in mild traumatic brain injury:

Narrative review and preliminary report of an open-field blast model. *Behavioural Brain Research*, *340*, 147–158. https://doi.org/10.1016/j.bbr.2016.08.037

Späni, C. B., Braun, D. J., & Van Eldik, L. J. (2018). Sex-related responses after traumatic brain injury: Considerations for preclinical modeling. *Frontiers in Neuroendocrinology*, *50*, 52–66. https://doi.org/10.1016/j.yfrne.2018.03.006

Sponheim, S. R., McGuire, K. A., Kang, S. S., Davenport, N. D., Aviyente, S., Bernat, E. M., & Lim, K. O. (2011). Evidence of disrupted functional connectivity in the brain after combat-related blast injury. *NeuroImage*, *54 Suppl 1*, S21-29. https://doi.org/10.1016/j.neuroimage.2010.09.007

Stahre, M. A., Brewer, R. D., Fonseca, V. P., & Naimi, T. S. (2009). Binge Drinking Among U.S. Active-Duty Military Personnel. *American Journal of Preventive Medicine*, *36*(3), 208–217. https://doi.org/10.1016/j.amepre.2008.10.017

Street, A. E., Gradus, J. L., Giasson, H. L., Vogt, D., & Resick, P. A. (2013). Gender Differences Among Veterans Deployed in Support of the Wars in Afghanistan and Iraq. *Journal of General Internal Medicine*, *28*(2), 556–562. https://doi.org/10.1007/s11606-013-2333-4

Sullivan, D. R., Hayes, J. P., Lafleche, G., Salat, D. H., & Verfaellie, M. (2018). Functional Brain Alterations Associated With Cognitive Control in Blast-Related Mild Traumatic Brain Injury. *Journal of the International Neuropsychological Society*, *24*(7), 662–672. https://doi.org/10.1017/S1355617718000279

Swai, J., Hu, Z., Zhao, X., Rugambwa, T., & Ming, G. (2019). Heart rate and heart rate variability comparison between postural orthostatic tachycardia syndrome versus

healthy participants; a systematic review and meta-analysis. *BMC Cardiovascular Disorders*, *19*. https://doi.org/10.1186/s12872-019-01298-y

Taber, K. H., Hurley, R. A., Haswell, C. C., Rowland, J. A., Hurt, S. D., Lamar, C. D., & Morey, R. A. (2015). White matter compromise in veterans exposed to primary blast forces. *The Journal of Head Trauma Rehabilitation*, *30*(1), E15-25. https://doi.org/10.1097/HTR.0000000000000030

Tallapragada, M., & Cranmer, G. A. (2022). Media Narratives About Concussions: Effects on Parents' Intention to Inform Their Children About Concussions. *Communication & Sport*, *10*(3), 517–540. https://doi.org/10.1177/2167479520944549

Tanielian, T. L., Tanielian, T., & Jaycox, L. (2008). *Invisible Wounds of War: Psychological and Cognitive Injuries, Their Consequences, and Services to Assist Recovery*. Rand Corporation.

Taylor, C. A., Bell, J. M., Breiding, M. J., & Xu, L. (2017). Traumatic Brain Injury–Related Emergency Department Visits, Hospitalizations, and Deaths—United States, 2007 and 2013. *Morbidity and Mortality Weekly Report: Surveillance Summaries*, *66*(9), 1–16.

Teng, S. X., Katz, P. S., Maxi, J. K., Mayeux, J. P., Gilpin, N. W., & Molina, P. E. (2015). Alcohol exposure after mild focal traumatic brain injury impairs neurological recovery and exacerbates localized neuroinflammation. *Brain, Behavior, and Immunity*, *45*, 145–156. https://doi.org/10.1016/j.bbi.2014.11.006

Thursby, E., & Juge, N. (2017). Introduction to the human gut microbiota. *Biochemical Journal*, *474*(11), 1823–1836. https://doi.org/10.1042/BCJ20160510

Ticinesi, A., Tana, C., Nouvenne, A., Prati, B., Lauretani, F., & Meschi, T. (2018). Gut microbiota, cognitive frailty and dementia in older individuals: A systematic review. *Clinical Interventions in Aging*, *13*, 1497–1511. https://doi.org/10.2147/CIA.S139163

Tovar, M. A., Bell, R. S., & Neal, C. J. (2021). Epidemiology of Blast Neurotrauma: A Meta-analysis of Blast Injury Patterns in the Military and Civilian Populations. *World Neurosurgery*, *146*, 308-314.e3. https://doi.org/10.1016/j.wneu.2020.11.093

Treadway, M. T., & Zald, D. H. (2013). Parsing Anhedonia: Translational Models of Reward-Processing Deficits in Psychopathology. *Current Directions in Psychological Science*, *22*(3), 244–249. https://doi.org/10.1177/0963721412474460

Tremlett, H., Bauer, K. C., Appel-Cresswell, S., Finlay, B. B., & Waubant, E. (2017). The gut microbiome in human neurological disease: A review. *Annals of Neurology*, *81*(3), 369–382. https://doi.org/10.1002/ana.24901

Trivedi, R. B., Post, E. P., Piegari, R., Simonetti, J., Boyko, E. J., Asch, S. M., Mori, A., Arnow, B. A., Fihn, S. D., Nelson, K. M., & Maynard, C. (2020). Mortality Among Veterans with Major Mental Illnesses Seen in Primary Care: Results of a National Study of Veteran Deaths. *Journal of General Internal Medicine*, *35*(1), 112–118. https://doi.org/10.1007/s11606-019-05307-w

Urban, R. J., Pyles, R. B., Stewart, C. J., Ajami, N., Randolph, K. M., Durham, W. J., Danesi, C. P., Dillon, E. L., Summons, J. R., Singh, C. K., Morrison, M., Kreber, L. A., Masel, B., Miller, A. L., Wright, T. J., & Sheffield-Moore, M. (2020). Altered Fecal Microbiome Years after Traumatic Brain Injury. *Journal of Neurotrauma*, *37*(8), 1037–1051. https://doi.org/10.1089/neu.2019.6688

Valsamis, B., & Schmid, S. (2011). Habituation and Prepulse Inhibition of Acoustic Startle in Rodents. *Journal of Visualized Experiments : JoVE*, *55*, 3446. https://doi.org/10.3791/3446

Vasterling, J. J., & Proctor, S. P. (2011). Understanding the Neuropsychological Consequences of Deployment Stress: A Public Health Framework. *Journal of the International Neuropsychological Society*, *17*(1), 1–6. https://doi.org/10.1017/S1355617710001116

Vasterling, J. J., Proctor, S. P., Amoroso, P., Kane, R., Heeren, T., & White, R. F. (2006). Neuropsychological Outcomes of Army Personnel Following Deployment to the Iraq War. *JAMA*, *296*(5), 519–529. https://doi.org/10.1001/jama.296.5.519

Verfaellie, M., Lafleche, G., Spiro, A., & Bousquet, K. (2014). Neuropsychological outcomes in OEF/OIF veterans with self-report of blast exposure: Associations with mental health, but not MTBI. *Neuropsychology*, *28*(3), 337–346. https://doi.org/10.1037/neu0000027

Villapol, S., Loane, D. J., & Burns, M. P. (2017). Sexual dimorphism in the inflammatory response to traumatic brain injury. *Glia*, *65*(9), 1423–1438. https://doi.org/10.1002/glia.23171

Vonder Haar, C., Ferland, J.-M. N., Kaur, S., Riparip, L.-K., Rosi, S., & Winstanley, C. A. (2019). Cocaine self-administration is increased after frontal traumatic brain injury and associated with neuroinflammation. *European Journal of Neuroscience*, *50*(3), 2134–2145. https://doi.org/10.1111/ejn.14123

Vonder Haar, C., Friend, D. M., Mudd, D. B., & Smith, J. S. (2013). Successive bilateral frontal controlled cortical impact injuries show behavioral savings. *Behavioural Brain Research*, *240*, 153–159. https://doi.org/10.1016/j.bbr.2012.11.029

Vonder Haar, C., Smith, T. R., French, E. J., Martens, K. M., Jacobs, E. A., & Hoane, M. R. (2014). Simple tone discriminations are disrupted following experimental frontal traumatic brain injury in rats. *Brain Injury*, *28*(2), 235–243. https://doi.org/10.3109/02699052.2013.860473

Vonder Haar, C., & Winstanley, C. A. (2016). Minor Functional Deficits in Basic Response Patterns for Reinforcement after Frontal Traumatic Brain Injury in Rats. *Journal of Neurotrauma*, *33*(20), 1892–1900. https://doi.org/10.1089/neu.2015.4276

Wenger, J., O'Connell, C., & Cottrell, L. (2018). *Examination of Recent Deployment Experience Across the Services and Components*. RAND Corporation. https://doi.org/10.7249/RR1928

Wilcox, M. V., Carlson, V. C. C., Sherazee, N., Sprow, G. M., Bock, R., Thiele, T. E., Lovinger, D. M., & Alvarez, V. A. (2014). Repeated Binge-Like Ethanol Drinking Alters Ethanol Drinking Patterns and Depresses Striatal GABAergic Transmission. *Neuropsychopharmacology*, *39*(3), Article 3. https://doi.org/10.1038/npp.2013.230

Wiley, N. C., Dinan, T. G., Ross, R. P., Stanton, C., Clarke, G., & Cryan, J. F. (2017). The microbiota-gut-brain axis as a key regulator of neural function and the stress response: Implications for human and animal health1,2. *Journal of Animal Science*, *95*(7), 3225–3246. https://doi.org/10.2527/jas.2016.1256

Wilkinson, C., Pagulayan, K., Petrie, E., Mayer, C., Colasurdo, E., Shofer, J., Hart, K., Hoff, D., Tarabochia, M., & Peskind, E. (2012). High Prevalence of Chronic Pituitary and Target-Organ Hormone Abnormalities after Blast-Related Mild Traumatic Brain Injury. *Frontiers in Neurology*, *3*. https://www.frontiersin.org/articles/10.3389/fneur.2012.00011

Wolf, S. J., Bebarta, V. S., Bonnett, C. J., Pons, P. T., & Cantrill, S. V. (2009). Blast injuries. *The Lancet*, *374*(9687), 405–415. https://doi.org/10.1016/S0140-6736(09)60257-9

Yeh, P.-H., Wang, B., Oakes, T. R., French, L. M., Pan, H., Graner, J., Liu, W., & Riedy, G. (2014). Postconcussional disorder and PTSD symptoms of military-related traumatic brain injury associated with compromised neurocircuitry. *Human Brain Mapping*, *35*(6), 2652–2673. https://doi.org/10.1002/hbm.22358

Zoladz, P. R., D'Alessio, P. A., Seeley, S. L., Kasler, C. D., Goodman, C. S., Mucher, K. E., Allison, A. S., Smith, I. F., Dodson, J. L., Stoops, T. S., & Rorabaugh, B. R. (2019). A predator-based psychosocial stress animal model of PTSD in females: Influence of estrous phase and ovarian hormones. *Hormones and Behavior*, *115*, 104564. https://doi.org/10.1016/j.yhbeh.2019.104564