The Dissertation for Kaila N Parker certifies that this is the approved version of the following Dissertation:

Investigating the Interplay between Early Life Stress, Acute Secondary Pathogenesis, and Chronic Hippocampal Impairments in Young Mice with Traumatic Brain Injury

Committee:

Linda J. Noble-Haeusslein, Supervisor

Frances A. Champagne

James P. Curley

Andrea C. Gore

Theresa A. Jones

Laura K. Fonken

Investigating the Interplay between Early Life Stress, Acute Secondary Pathogenesis, and Chronic Hippocampal Impairments in Young Mice with Traumatic Brain Injury

By

Kaila N Parker

Dissertation

Presented to the faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

The University of Texas at Austin

August 2023

Dedication

This dedication is dedicated to my parents and sister, Hazel and Cardell Parker and Brittany R Parker, for their unconditional support, commitment, and celebration of all my successes.

This dissertation is also dedicated to my husband, Nathan Green. He has chosen and loved every version of me before, during, and after the completion of my dissertation. I am so grateful to be your teammate in life and feel honored to have your endless support and love.

To my friends who have loved, supported, and healed me in every phase of this dissertation, this is also for you. You are family I will choose time and time again and having you on my team is so invaluable and I will never be able to thank you enough for being there for me.

Lastly, but certainly not least, this dissertation is also dedicated to my grandparents, Dr. William C. Parker, Emily Parker, and Costella Robinson. Although you are not with me anymore, you showed me how to be tenacious and your wholehearted adoration for my love of curiosity and urge to excel was a formidable driving force to pursue my doctorate degree. Here's to another Dr. Parker!

Acknowledgements

I would like to thank my mentor and advisor Dr. Linda J Noble-Haeusslein for taking a chance on me for the last six years. I am so grateful that she responded to my email many years ago while I applied to graduate school. From our initial interaction, she invested in me, my experience, and my unconventional path to choosing a PhD. Linda fostered an environment of true hands-on learning and strongly emphasized that it's not always about knowing how to do something right the first time, but rather that it's about teachable moments to make me more malleable over time. As a mentor with another unconventional background, she showed me how to not only catch up to the learning curve, but how to be on the upper end of it. Over the course of my program, she challenged me like I never had before and helped me develop a backbone for standing up for myself, my research, and ultimately for science. I will never be able to thank Linda enough for the mentorship she has provided to me over the past 6 years, and I am so grateful to have had my PhD experience with her.

I would like to thank Dr. Michael D. Donovan for his endless patience and support of lab expertise. His instruction, patience, and humor were crucial to my success and comfort in the lab. I am so fortunate to have had him as a lab manager and I'm so appreciative of all that he taught me. I would like to thank former lab member Dr. Ramona E. von Leden and current lab member Kylee A Smith for your emotional support. I could not have had a better relationship with lab mates and the level of friendship and comradery is invaluable.

I would also like to thank Anna Webb for her patience and crucial support in microscope training. Without her, the quality of my images and qualitative data would not have been

possible, and for that I am so grateful. I would also like to thank Dr. Frances A Champagne and Dr. James P Curley for acting as a sounding board for the logistics behind implementing early-life stress prior to an injury, without them my whole dissertation would not be possible. I would also like to thank Dr. Tallie Z. Baram and Annabel Short for their willingness to discuss and flush out details of the Limited Bedding Nestlet model, which served as the foundation for my studies. I would also like to thank Dr. Laura K. Fonken for her assistance with the Barnes Maze paradigm, which served as a unique behavioral outcome in my study. I would also like to thank Dr. Theresa Jones for her extensive knowledge of stereology. She showed patience in answering all my questions and boosted my confidence in mastering this essential technique for my dissertation. Dr. Alex Etz, thank you so much for your expertise in statistics and your patience in consulting in statistics. Your approachable and conversational attitude towards statistics made conceptualizing my data and how I think about stats so much more attainable. I would like to thank Kimberly Terry for her absolute critical guidance in navigating fellowships, the graduate program, and literally everything about graduate school. She is the glue behind the graduate psychology program, and I would be absolutely lost without her.

I would also like to thank my undergraduate professor and mentor, Dr. Carolyn Martineau. She was the first to see the spark of a researcher in my early graduate career and encouraged me to pursue a career in research. From letters of recommendation, occasional check-ins, to an invitation to be a guest lecturer, she has continued to provide me with support and advice on my journey. Thank you for continually providing a mirror as a reminder of all my successes along the way and for guiding me towards a research career.

Undergraduate assistant Macy Fewell was an invaluable asset in completing my dissertation. Thankful does not even begin to describe the expertise she brought to the lab and

her love for learning and fearless personality made completing these studies in a timely manner possible. I am so lucky to have had her for the time I had.

Finally, I would like to thank the friends in my psychology program, especially Dr. Emma Y Siegel and Dr. M Nicole Kunkel. They have always showed me support and grace over the past six years. I'm beyond proud of each of them and grateful to have formed these lifelong friendships.

ABSTRACT

Investigating the Interplay between Early Life Stress, Acute Secondary Pathogenesis, and Chronic Hippocampal Impairments in Young Mice with Traumatic Brain Injury

Kaila N Parker, Ph.D.

The University of Texas at Austin, 2023

Supervisor: Linda J. Noble-Haeusslein, Ph.D.

While Traumatic brain injury (TBI) is the leading cause of disability in children, it is unclear how early life stress (ELS) may act as a determinant of long-term recovery in brain-injured children. A murine model of ELS preceding TBI at postnatal day (P)21 addressed the following: regionally specific acute pathogenesis of the hippocampus after ELS+TBI, are these early changes predictive of hippocampal damage and impairment at adulthood. Males and females were exposed to ELS (P2-9) with the limited bedding nestlet (LBN) model, randomized to TBI or sham, and euthanized at P22 or adulthood. At P22, ELISAs revealed an upregulation of IL-1B, IL-6, TNFα, and IFNγ in both sexes after injury. ELS+TBI elevated IL-1B, IL-10, TNFα, and IFNγ in males compared to TBI. Iba-1 and caspase-3 were evaluated in hippocampal subregions. While TBI increased microglial density in both sexes, ELS+TBI increased microglial density in male CA2 and CA3 but only in the CA3 in females compared to TBI. Quantification of caspase-3 revealed apoptosis in males and females after TBI. ELS + TBI increased apoptosis in CA1 and CA3 in males and females compared to TBI. Adulthood learning and memory were assessed with the NOR and Barnes Maze. Compared to TBI, ELS+TBI reduced novelty preference in females and increased path length to target in both sexes. Hippocampal neuron loss after ELS+TBI was evaluated at adulthood. TBI significantly reduced neurons in all subregions; ELS+TBI reduced neurons in the CA1 region in females only. These findings highlight

hippocampal vulnerability after ELS+TBI and ELS prior to a TBI may enhance acute pathogenesis in males. Correlation matrices determined hippocampal acute pathogenesis is predictive of neuronal loss at adulthood and is associated with learning and memory impairments. Males and females were assessed for all outcomes. Both sexes showed similar vulnerability to secondary pathogenesis following TBI and adulthood impairments in learning and memory; males showed greater vulnerability to acute pathogenesis and females showed greater vulnerability to adulthood outcomes. These findings may advocate for opportunities to tailor therapies specific to each sex. Thus, developing pre-clinical biomarkers to predict long-term recovery may continue to bolster care management.

Table of Contents

List of Tables:

Supplementary Tables:

Table S17: Microglial Density CA1 (Females) ANOVA Table

Table S18: Microglial Density CA2 (Females) ANOVA Table

Table S19: Microglial Density CA3 (Females) ANOVA Table

Table S20: Microglial Density Dentate Gyrus (Females) ANOVA Table

Table S21: Caspase-3+ CA1 (Males) ANOVA Table

Table S22: Caspase-3+ CA2 (Males) ANOVA Table

Table S23: Caspase-3+ CA3 (Males) ANOVA Table

Table S24: Caspase-3+ Dentate Gyrus (Males) ANOVA Table

Table S25: CA1 (Females) ANOVA Table

Table S26: CA2 (Females) ANOVA Table

Table S27: CA3 (Females) ANOVA Table

Table S28: Dentate Gyrus (Females) ANOVA Table

Table S29: Percentage of Time Inner Zone (Males) ANOVA Table

Table S30: Open Field Distance Moved (Males) ANOVA Table

Table S31: Percentage of Time Inner Zone (Females) ANOVA Table

Table S32: Open Field Distance Moved (Females) ANOVA Table

Table S33: Percentage of Time in Open Arms (Males) ANOVA Table

Table S34: Elevated Zero Maze Distance Moved (Males) ANOVA Table

Table S35: Percentage of Time in Open Arms (Females) ANOVA Table

Table S36: Elevated Zero Maze Distance Moved (Females) ANOVA Table

Table S37: Novel Object Preference Index (Males) ANOVA Table

Table S38: Novel Object Preference Index (Females) ANOVA Table

Table S39: Time in Novel Chamber (Males) ANOVA Table

List of Figures:

Supplemental Figures:

Figure S1: Barnes Maze Time Spent at Target Hole (Males and Females)

Figure S2: Barnes Maze Total Distance Moved (Males and Females)

Chapter 1: Introduction

1.1 Children and Traumatic Brain Injuries

According to the Centers for Disease Control (Centers for Disease Control, 2014), children (age 0-17) are more likely to sustain a traumatic brain injury (TBI), with those 4 years and under at highest risk. Here I focus on the developing brain, due to the high prevalence of TBIs in this age group. I address how early life stress (ELS) alters acute secondary pathogenesis and long-term recovery after an early age brain injury.

1.2 Evolution of the injury

TBI results from both a primary insult, due to the direct tearing and shearing of brain structures, and a secondary cascade of adverse events that begins within minutes post injury and includes disruption of the blood-brain barrier, vasogenic and cytotoxic edema, excitotoxicity, neuroinflammation, dysregulation of metabolism, and cell death (See reviews, Simon et al., (Simon, McGeachy et al. 2017) and Potts et al. (Potts, Koh et al. 2006)). With low antioxidant reserves, the developing brain is rendered more vulnerable to these adverse secondary events (Bayir, Kagan et al. 2002, Fan, Yamauchi et al. 2003, Bayir, Kochanek et al. 2006, Tsuru-Aoyagi, Potts et al. 2009). Moreover, injury to the developing brain disrupts normal developmental processes, including myelination, synaptogenesis, synaptic pruning, and gliogenesis, each of which contribute to long-term brain function ((Scheff, Price et al. 2005, Tasker 2006, Wilde, Chu et al. 2006, Threlkeld, Rosen et al. 2007, Domowicz, Wadlington et al. 2018) and See review, Semple et al. (Semple, Blomgren et al. 2013)). These disruptions and subsequent progressive neurodegeneration adversely affect normal progression of age-dependent behaviors, such as social cognition, social play, social interaction, working memory, and skill

acquisition. When these key stages are disrupted during early childhood, risk-taking tendencies, decreased social interactions, novelty seeking, emotional instability, and impulsivity may emerge during adolescence (Anderson and Catroppa 2005, Anderson, Catroppa et al. 2005, Catroppa, Anderson et al. 2008, Catroppa, Anderson et al. 2008, Anderson, Beauchamp et al. 2013, Ryan, Anderson et al. 2014).

1.3 The Developing Brain and TBIs

A child is more vulnerable to a TBI than an adult due to unique physical attributes of the young brain and body. With a larger head to body ratio and weak musculature of the neck (Young 1966), the child's brain is more likely to be exposed to greater acceleration/deceleration forces, resulting in a higher incidence of diffuse axonal injury and cerebral edema (Meaney, Smith et al. 1995, Tong, Ashwal et al. 2003, Tong, Ashwal et al. 2004). Additionally, the young brain may sustain greater damage from an impact due to a thin calvarium (Hirsch and Evans 1965, Cory, Jones et al. 2001). Beyond these general physical features, recovery after an early age TBI is also influenced by characteristics of the lesion, such as severity, location, focal or diffuse patterns of damage, and laterality of injury, each of which may impact outcomes (Chapman and McKinnon 2000, Anderson, Catroppa et al. 2005, Wilde, Hunter et al. 2012, Lindsey, Wilde et al. 2019). Children with large, more diffuse, and/or bilateral injuries show the poorest performance across cognitive domains (Anderson, Damasio et al. 2000, Eslinger and Biddle 2000, Anderson and Catroppa 2005, Catroppa, Anderson et al. 2008, Catroppa, Anderson et al. 2008, Lindsey, Wilde et al. 2019).

1.3.1 Biological sex is also a determinant of recovery after an early age TBI

Beyond genetic and endocrine differences (De Vries, Rissman et al. 2002), sex differences also manifest in the timing of the closure of sensitive developmental periods, which occurs earlier in males than in females (Nugent, Wright et al. 2015). Clinical studies of brain-injured children likewise identify differences between sexes. For example, females who sustain a TBI during childhood are more likely to internalize emotional problems such as depression and anxiety, whereas males may display emotional problems in the form of substance abuse and criminal behaviors (Gerring, Slomine et al. 2002, Moreno and McKerral 2015, Scott, McKinlay et al. 2015, Despins, Turkstra et al. 2016) . Similarly, other clinical studies have reported that females have an increased risk for developing emotional and psychiatric disorders after injury, while males present an increased risk for social and behavioral problems (i.e. communication, social cognition, attention/executive function) within the first year following an early age TBI (Schwartz, Taylor et al. 2003, Scott, McKinlay et al. 2015, Lindsey, Wilde et al. 2019)

1.3.2 Critical periods of brain development

A TBI during the early postnatal period adversely affects maturation of key developmental processes. Brain development spans early gestation to early adulthood (Lenroot and Giedd 2006). During early postnatal development, the brain's acquisition of new functions and capabilities is highly dependent upon experiential and environmental influences (Lenroot and Giedd 2006). Critical periods of brain development are characterized by robust synaptic pruning, myelination, programmed cell death, alterations in density of neurotransmitters, gliogenesis, and white/gray matter differentiation (Crain, Cotman et al. 1973, Huttenlocher 1979, Lidow, Goldman-Rakic et al. 1991, Giedd, Blumenthal et al. 1999, Hu, Liu et al. 2000, Anderson, Catroppa et al. 2005, Tyzio, Holmes et al. 2007). While some developmental processes, including the maturation of the immune system and the blood-brain barrier, are mostly

complete by birth (Daneman, Zhou et al. 2010), others, including synaptogenesis, myelination, and programmed cell death extend well beyond the postnatal period and into adulthood (Giedd, Blumenthal et al. 1999). In the human brain, synaptogenesis begins before birth and peaks around the age of 3 (Huttenlocher 1979). A subsequent decrease in synaptogenesis coincides with increased synaptic pruning, which continues over the next several decades (Giedd, Blumenthal et al. 1999). Programmed cell death peaks during gestation (Huttenlocher 1979) and also extends into adulthood (Huttenlocher 1979). While myelination is most prominent during years 2-3, this process also continues into early adulthood (Huttenlocher 1979, Keshavan, Diwadkar et al. 2002). Importantly, each of these developmental processes are critical for normal brain function at adulthood (Huttenlocher 1979).

The first several years of life are considered a sensitive period of growth, in which key developmental processes shape brain function and behavior at adulthood. The importance of this period of development has been demonstrated in studies of social behaviors, sensory experiences and cognition. Toddler-aged children are characterized by a high level of activity and sociability (Terranova and Laviola 2005). Early age brain injuries may alter the shaping and maturation of these behaviors. As sociability continues to develop into adolescence ((Burnett, Sebastian et al. 2011, Mills, Lalonde et al. 2014) and See review, Blakemore,2012 (Blakemore 2008)), a disruption in the toddler aged child may interfere with the proper sequence of age-appropriate social behaviors and increase the risk of psychiatric disorders (Bondar, Lepeshko et al. 2018). Children, during this critical period, are also particularly sensitive to sensory experiences as they shape neural circuits involved in basic sensory processes. For example, light and sound shape the formation of the visual and auditory cortices, respectively, and dictate visual and auditory processing (Hubel and Wiesel 1970, Jenkins and Merzenich 1984). Prolonged

deprivation of either stimulus during this period results in an impairment in sensory processing later on in life (Hubel and Wiesel 1970, Jenkins and Merzenich 1984, Fine, Wade et al. 2003, Lewis and Maurer 2005). Similarly, early age TBI may also result in poorer cognitive outcomes (Levin, Hanten et al. 2002, Anderson, Catroppa et al. 2005, Anderson, Godfrey et al. 2012, Karver, Wade et al. 2012, Semple, Canchola et al. 2012, Anderson, Spencer-Smith et al. 2014, Karver, Kurowski et al. 2014). This relationship between early age TBI and cognitive abilities is considered non-linear and is likely sensitive to injury at critical periods of plasticity and behavioral development (Kolb, Gibb et al. 1994, Kozlowski and Schallert 1998). The earlier the age of a TBI, the higher the risk for delayed or arrested development of cognitive and higher-level executive functioning (Catroppa, Anderson et al. 2008, Anderson, Godfrey et al. 2012, Karver, Wade et al. 2012).

1.4 Early life stress (ELS)

Children who are exposed to early life stress are at risk for developing long-term psychosocial impairments and chronic illnesses at adulthood (Felitti, Anda et al. 1998, Richards and Wadsworth 2004, Danese, Moffitt et al. 2009, Flaherty, Thompson et al. 2013, Kelly-Irving, Lepage et al. 2013, Giovanelli, Reynolds et al. 2016). ELS may encompass a variety of scenarios including extreme poverty, parental loss, malnutrition, domestic/school/community violence, trauma, child neglect and/or abuse, altered parental behavior (Briere and Runtz 1988, Cavaiola and Schiff 1988, Chu and Dill 1990, Riggs, Alario et al. 1990, Moeller, Bachmann et al. 1993, Gould, Stevens et al. 1994, Rorty, Yager et al. 1994, McCauley, Kern et al. 1997, Banyard 1999, Bensley, Van Eenwyk et al. 1999, Merrill, Newell et al. 1999), and institutional rearing (Tottenham, Hare et al. 2010). ELS impacts many aspects of brain health and

development, including metabolism, circadian rhythms, neuroendocrine function, neuro-immune interactions, and oxidative stress (Vandewalle, Middleton et al. 2007, Rice, Sandman et al. 2008, Kalsbeek, Yi et al. 2010, van Reedt Dortland, Giltay et al. 2010, van Reedt Dortland, Giltay et al. 2012, Wilson, Boyle et al. 2012). Children who experience ELS also have a greater risk for diabetes, obesity-related problems, cardiovascular diseases, autoimmune disease, cancer, and depression at adulthood as well as early mortality (Felitti, Anda et al. 1998, Caspi, Sugden et al. 2003, Richards and Wadsworth 2004, Danese, Moffitt et al. 2009, Dalle Molle, Portella et al. 2012, Flaherty, Thompson et al. 2013, Kelly-Irving, Lepage et al. 2013, Giovanelli, Reynolds et al. 2016).

1.4.1 The social environment and TBI

In a seminal paper, Fletcher et al. (Fletcher, Ewing-Cobbs et al. 1990) questioned why antecedent psychosocial behavioral traits, such as adaptive behavior, communication, daily living, and socialization were not considered in studies of brain-injured children. Such questioning has served as a catalyst for subsequent research to examine the moderating role of the social environment before or shortly after an early age TBI. In long term clinical studies of sociocognitive functioning after childhood TBI (Catroppa, Anderson et al. 2008, Ryan, Anderson et al. 2014), individuals at adulthood showed poorer emotional perception, as evidenced by deficits in both recognizing and interpreting emotions based upon facial and vocal cues (Ryan, Anderson et al. 2014). These findings are thought to reflect vulnerability of the immature social brain to this insult, with sociocognitive deficits resulting from disrupted brain development and inability to acquire social skills at the appropriate developmental time (Yeates, Bigler et al. 2007). Importantly, long term deficits in emotional perception may be linked to a child's socioeconomic status and levels of family intimacy at the time of injury (Catroppa, Anderson et

al. 2008). Catroppa et al., reported the first prospective study that compared pre-injury and 6 months post-injury behavioral outcomes with social participation being predicted by both the severity of the TBI and pre-injury deficits, including lower social participation (Catroppa et al., 2006). Subsequent longitudinal studies support these results; children, exposed to a poor social environment prior to a TBI, have greater impairments in psychosocial outcomes, including social cognition and communication compared to brain-injured children with higher socioeconomic status and optimal home environments prior to their injury (Yeates, Bigler et al. 2007, Anderson, Beauchamp et al. 2013, McNally, Bangert et al. 2013). The results of these early studies indicate that pre-injury demographics such as socioeconomic status and social environment are likely determinants of behavioral recovery after a TBI.

1.5 Pre-clinical models of early age brain injuries

Currently, there are two models of TBIs in rodents that have been used to study the consequences of ELS; namely, a focal cortical injury produced by a controlled cortical impactor device, and a more diffuse injury, produced by a fluid percussion device (Table 1, See reviews, Kochanek et al (Kochanek, Wallisch et al. 2017) and Thompson et al (Thompson, Lifshitz et al. 2005)). Each of these models involves a craniectomy and exposure of the brain. A focal cortical injury is produced by a pneumatically or electronically driven piston that impacts the exposed dura with tightly controlled velocity, depth of penetration and dwell time, producing a consistent injury to proximal cortical and subcortical areas. The fluid percussion model is based upon the delivery of a defined pulse of fluid against the intact dura, resulting in brief deformation of the brain and diffuse axonal injury(Thompson, Lifshitz et al. 2005). Severity of the injury is dependent upon the strength of the pressure wave, which is generated when a pendulum swings

from a variable height to strike a plunger in a saline-filled reservoir. This results in delivery of a pulse of saline against the intact dura. Depending upon the severity of the injury, each of these models may result in deficits in learning and memory, social behaviors, hyperactivity, and anxiety- and depression-like behaviors (Hamm, Dixon et al. 1992, Prins, Lee et al. 1996, Fox, Fan et al. 1998, Dixon, Kochanek et al. 1999, Fox, LeVasseur et al. 1999, Kraus, Susmaras et al. 2007, Chauhan and Gatto 2011, Byrnes, Loane et al. 2012, Semple, Canchola et al. 2012, Washington, Forcelli et al. 2012, Chen, Noble-Haeusslein et al. 2013, Kamper, Pop et al. 2013, Osier and Dixon 2016, Kochanek, Wallisch et al. 2017, Zhao, Yu et al. 2017).

Table 1

Type of Injury Model	Species	Sex	Description	Location of Injury	Type of Injury	References
Controlled Cortical Impact	Mouse, Rat	M	Craniectomy; Impactor tip is set at predetermined depth and velocity to strike cortical surface	Parietal Lobe Frontal Lobe	Focal Contusion	Cannella, et al. Hanlon, et al. Anthonymuthu, et al. Schober, et al. Arambula, et al. Jantzie, et al. Hajiaghamemar, et al. Kochanek, et al. Chen, et al.
Fluid Percussion Injury	Rat, Mouse	M, F	Craniectomy; Plastic cork is struck by pendulum dropped from a pre-defined height- saline is delivered to cortical surface	Parietal Lobe	Diffuse Injury	Cannella et al. Hajiaghamemar et al. Kochanek et al.
Weight Drop	Rat, Mouse	M, F	Craniectomy; Rod falls from a fixed height to impact cortical surface Closed head; Skull exposed, weighted impactor drops onto intact skull	Parietal Lobe	Focal Contusion	Mychasiuk et al. Kochanek et al. Hajiaghamemar et al. Bodnar et al. Marmarou et al.
Impact Acceleration	Rat	M	Closed head injury; Rod free-falls from pre-determined height onto exposed skull	Parietal Lobe	Diffuse Injury	Adelson et al. Marmarou et al. Kochanek et al.

Table 1. Preclinical models of traumatic injuries to the developing brain. While there are 4 commonly used rodent models of TBI to the developing brain, only 2 (controlled cortical impact and fluid percussion injury) have been studied following ELS. Abbreviations: Male = M; Female+ F

1.6 Preclinical models of ELS

There are two common models of ELS in rodents, the maternal separation model and the limited bedding nestlet model. These models target early brain development in rodents that spans birth to postnatal day 21 with notable variations that include the timing and duration of exposure to an impoverished environment and/or maternal separation.

One of the earliest accounts of the maternal separation paradigm used handling or non-handling of rat pups to invoke an early stress (stimulation) response (Levine 1957). This foundational model examined how neonatal handling affected plasma corticosterone levels and emotionality later in life (Levine 1957, Levine 1962). The maternal separation model subsequently evolved into the more modern paradigm of physically separating the pups from the mom, resulting in a more pronounced response of the hypothalamic-pituitary-adrenal (HPA) axis(Levine 1957, Levine 1962, Ader 1970, Spencer-Booth and Hinde 1971, Levine, Huchton et al. 1991, Walker, Scribner et al. 1991, Clarke 1993, Plotsky and Meaney 1993, Schmidt, Oitzl et al. 2002). While maternal separation is suitable for an examination of acute or repeated stressors, the model is not typically applied to chronic stress, which may result in pup exhaustion due to malnutrition and hypothermia (Rice, Sandman et al. 2008). Additionally, the maternal separation model may result in inconsistent results and includes many variations of the paradigm (i.e. timing of separation, duration of separation, measure of stress response). The Limited Bedding Nestlet (LBN) model was developed to examine the effects of chronic ELS, in which rodent pups and the nursing dam are exposed to a metal mesh cage bottom and a reduced nestlet square (Rice, Sandman et al. 2008). The LBN model produces a robust activation of the HPA axis as a result of erratic and unpredictable maternal care with minimal observer handling (Gilles, Schultz et al. 1996, Avishai-Eliner, Gilles et al. 2001, Brunson, Kramar et al. 2005, Rice, Sandman et al.

2008, Arp, Ter Horst et al. 2016, Bath, Manzano-Nieves et al. 2016, Moussaoui, Larauche et al. 2016).

1.6.1 Maternal separation model (MS)

In this rodent model of childhood neglect (Diaz-Chavez, Lajud et al. 2020, Sanchez, Titus et al. 2020), the mother is separated from her pups for a defined period of time each day during the postnatal development. The MS model is used by many groups (Levine 1957, Levine 1962, L.J. Grota 1969, Spencer-Booth and Hinde 1971, Levine, Huchton et al. 1991, Walker, Scribner et al. 1991, Clarke 1993, Plotsky and Meaney 1993, Bhatnagar and Meaney 1995, Schmidt, Oitzl et al. 2002). It results in activation of the HPA, as evidenced, in part, by elevated corticosterone and altered expression of corticotropin releasing-hormone (CRH) (Levine 1957, Ader 1970, Plotsky and Meaney 1993, Viau, Sharma et al. 1993, Bhatnagar and Meaney 1995). The MS model also results in long-term changes in psychosocial behaviors, including anxiety- and depression-related behaviors. Importantly, there are several variations of this model, including the daily duration of MS (brief versus prolonged), the timing of the first day of separation, the number of days of separation, if the mother remains in the same room as the pups, and if the pups are maintained on a warming pad while separated from the mother. In some cases, there seems to be habituation to the handling by the observer over an extended period of time (Rice, Sandman et al. 2008). It should be noted that brief maternal separation is a model of social handling and produces positive physiological and behavioral effects later in development, presumably because it replicates the repeated, short periods of separation between mom and pups in the wild, in which the nursing dam leaves her nest to forage for food (L.J. Grota 1969, Weinberg 1987). The desired adverse effects of MS seem to emerge when periods of separation exceed 15 minutes (Ogawa, Mikuni et al. 1994, Hall, Wilkinson et al. 1999, Vazquez, Penit-

Soria et al. 2005). While variation in MS methods may produce some variability in outcomes, there are some key behaviors at adulthood that are common to most models, including anxiety- and depression-like behaviors (Romeo, Mueller et al. 2003, Veenema, Bredewold et al. 2007, Mehta and Schmauss 2011, Tsuda and Ogawa 2012, Bondar, Lepeshko et al. 2018). Moreover, these models typically show an exaggerated response of the HPA axis, a hallmark of ELS, immediately after the separation period that extends well into adulthood (Biagini, Pich et al. 1998, Levine 2000, Pryce and Feldon 2003, de Kloet, Sibug et al. 2005, Schmidt 2010, Bondar, Lepeshko et al. 2018).

1.6.2 Limited bedding nestlet (LBN) model

In the LBN model, the mother rears her pups on an altered cage bottom, typically metal mesh, with a reduced amount of a nesting material during the first week of postnatal life. This model creates a stressful environment, resulting in altered maternal behavior towards her pups (i.e. neglect, abuse, and hypervigilance) (Gilles, Schultz et al. 1996, Brunson, Kramar et al. 2005, Ivy, Brunson et al. 2008, Rice, Sandman et al. 2008, Wang, Jiao et al. 2011, Dalle Molle, Portella et al. 2012, Raineki, Cortes et al. 2012, Wang, Su et al. 2013, Naninck, Hoeijmakers et al. 2015, Raineki, Sarro et al. 2015, Arp, Ter Horst et al. 2016, Bath, Manzano-Nieves et al. 2016, Rincon-Cortes and Sullivan 2016, Krugers, Arp et al. 2017, Gallo, Shleifer et al. 2019) and an exaggerated response by the HPA axis of the pups, based on changes in vasopressin (AVP), CRH and, elevated corticosterone levels, that extends into adulthood (Brunson, Kramar et al. 2005, Rice, Sandman et al. 2008, Gunn, Cunningham et al. 2013, Naninck, Hoeijmakers et al. 2015, Moussaoui, Larauche et al. 2016). This paradigm, usually applied from P2-P9, produces long-term behavioral impairments such as fear learning, anxiety-like, depression-like, reduced sociality (play behavior), and deficits in spatial learning and memory later in life (Brunson,

Kramar et al. 2005, Ivy, Brunson et al. 2008, Rice, Sandman et al. 2008, Wang, Jiao et al. 2011, Dalle Molle, Portella et al. 2012, Raineki, Cortes et al. 2012, Wang, Su et al. 2013, Naninck, Hoeijmakers et al. 2015, Raineki, Sarro et al. 2015, Arp, Ter Horst et al. 2016, Bath, Manzano-Nieves et al. 2016, Rincon-Cortes and Sullivan 2016). A key strength of this model is that there is opportunity to continuously monitor maternal care and interaction with her pups without any confounding effects, resulting from handling by the experimenter.

There is reduced pup weight during and after the period of LBN (Brunson, Kramar et al. 2005, McLaughlin, Verlezza et al. 2016, Molet, Heins et al. 2016, Moussaoui, Larauche et al. 2016), which in some cases persists into adulthood (Bath, Manzano-Nieves et al. 2016). Although the LBN model shows variability in body development, it consistently results in altered metabolism, as evidenced by changes in brown adipose tissue and in circulating leptin and glucose levels. The lasting metabolic effects of LBN may be a result of the combination of the quality and quantity of nutrition, stress hormones, and sensory stimuli from the mother (Lucassen, Naninck et al. 2013).

1.7 ELS and immune Priming

While the immune response to a TBI contributes to secondary damage (Roth, Nayak et al. 2014, Dickens, Tovar et al. 2017, Makinde, Cuda et al. 2017, Zhao, Yu et al. 2017, Russo, Latour et al. 2018), we have yet to fully understand the interaction between ELS and TBI in this context. ELS may prime the immune system, leaving it sensitized to inflammatory reactions later in life.

1.7.1 Causes and effects of immune priming

Exposure to a wide variety of early-life insults may elicit a persistent immune-sensitized condition in the brain, such that a subsequent insult produces a heightened inflammatory response. This phenomenon is referred to as "immune priming". Early life insults that have been shown to cause immune priming include infections (Bilbo, Biedenkapp et al. 2005, Williamson, Sholar et al. 2011), seizures (Somera-Molina, Nair et al. 2009), early postnatal alcohol exposure (Chastain, Franklin et al. 2019), in utero stress (Vanbesien-Mailliot, Wolowczuk et al. 2007), and, as discussed in detail below, ELS (Reus, Fernandes et al. 2017, Sagae, Zanardini et al. 2018, Wang, Levine et al. 2020, Saavedra, Hernandez-Velazquez et al. 2021).

Insults in the early period of life may produce life-long sensitization, creating immune cells that remain primed for many months in rodents and decades in humans (Frank, Baratta et al. 2007, Reus, Fernandes et al. 2017). Immune priming typically involves circulating immune cells, peripheral macrophages, astrocytes, or even neurons, but the most heavily implicated cells in immune priming of the Central Nervous System are the brain's resident immune cells, microglia. These glia undergo a phenotypic shift, that expedites robust responses to subsequent immune signals (Bilbo 2010, Mattei, Ivanov et al. 2017, De Miguel, Obi et al. 2018).

1.7.2 The HPA axis and inflammation

In response to a stressor, there is activation of the HPA axis. The hypothalamus, initially stimulated by the sympathetic nervous system, releases corticotropin-releasing hormone into the nearby pituitary gland, which in turn releases adrenocorticotropic hormone (ACTH) into the blood stream. Upon reaching the adrenal glands, ACTH stimulates release of glucocorticoids (GC), namely, corticosterone in rodents and cortisol in humans. GCs then act on glucocorticoid receptors that are distributed throughout the body, including the brain. In this way the stress signal is amplified and extended to enable a whole-animal response in the minutes and hours

following a stressor. In general, GCs have an anti-inflammatory effect, inhibiting lymphocyte proliferation and production of anti-inflammatory cytokines, and reducing expression of pro-inflammatory cytokines (Cox 1995, Liu, Cousin et al. 1999, Piemonti, Monti et al. 1999, van der Goes, Hoekstra et al. 2000, Galon, Franchimont et al. 2002). This is especially true when GC levels are high, since, of the two GC receptors, the one that predominates in response to elevated levels of GC has a distinctly more anti-inflammatory signaling profile (Bernhagen, Calandra et al. 1993, Calandra, Bernhagen et al. 1995). How then, does ELS lead to chronic inflammation and immune priming? One part of the puzzle may be that GCs elicit responses in the brain that are quite different than the primarily anti-inflammatory effect in the periphery. In addition to microglia, neurons and astrocytes in the brain also express GC receptors and elevated GCs can weaken these cells, compromising their ability to withstand further insult (Kolber, Roberts et al. 2008, Barik, Marti et al. 2013, Fitzsimons, van Hooijdonk et al. 2013, Hartmann, Dedic et al. 2017, Tertil, Skupio et al. 2018). Frank et al. have recently demonstrated that either stress or exogenous GCs produces immune-primed hippocampal microglia that, when challenged with lipopolysaccharide (LPS) *ex vivo*, secrete increased proinflammatory cytokines (Frank, Weber et al. 2016, Frank, Fonken et al. 2020). Furthermore, this effect is long-lasting; microglia exhibit a primed phenotype 28 days after exposure to a single stressor. One intriguing potential mechanism for GC-mediated priming of microglia is the nod-like receptor protein 3 (NLRP3) inflammasome. This protein complex is induced by GCs, is capable of regulating proinflammatory cytokine release, and has been implicated in microglial immune priming (Busillo, Azzam et al. 2011, Frank, Weber et al. 2016, Trojan, Chamera et al. 2019, Frank, Fonken et al. 2020, Niu, Luo et al. 2020).

1.7.3 The HPA Axis and TBI

TBI results in a suppression of the HPA axis (See review, Tapp et al., 2019,(Tapp, Godbout et al. 2019)). As described above, the HPA axis responds to stressors by releasing ACTH into the bloodstream and the subsequent release of glucocorticoids, including corticosterone (CORT). Under normal conditions, HPA axis activity is regulated by glucocorticoid receptors (GR) in the hypothalamus, pituitary, and adrenal glands. In addition to damage to subcortical areas (Rowe, Rumney et al. 2016), TBI causes a release of CORT in the brain. GR involved in the HPA axis negative feedback loop also become damaged from TBI, resulting in an excess of CORT. The pituitary is particularly vulnerable to injury-induced dysfunction, which results in a decreased release of ACTH and cannot stimulate the adrenal glands. The lack of stimulation leads to a decrease in the release in CORT, resulting in an aberrant altered stress response. Experimental models of TBI have examined HPA axis suppression in rats, where CORT was diminished in brain-injured mice at 7 and 21 days after injury (Taylor, Rahman et al. 2006, Taylor, Rahman et al. 2008). Excessive glucocorticoid release and a suppressed HPA axis response after TBI is associated with microglial priming and an increase in inflammatory cytokines, which may contribute to neuronal death (Roe, McGowan et al. 1998, Grundy, Harbuz et al. 2001). This maladaptive chronic inflammatory response contributes to the development or worsening of psychiatric disorders later in life, such as depression (Murphy, Michael et al. 2003, Keller, Gomez et al. 2017). The aberrant interaction between the persistent neuroendocrine responses and compromised psychiatric behavior illustrates the importance of HPA axis dysfunction and long-term TBI recovery.

1.7.4 ELS animal models and immune priming

To date, only a handful of studies have examined immune priming or markers of chronic inflammation in the context of either the MS or LBN model of ELS (Table 2). Most of these

studies have reported robust and long-lasting effects of ELS on cytokine expression. Reus et al. used an MS model in rats (P1-P10, 3 hr/day), and quantified multiple cytokines at P20, P30, P40, and P60 in 3 different brain regions (Reus, Fernandes et al. 2017). They found persistently increased levels of proinflammatory cytokines IL-1B, IL-6, and TNFα, as well as decreased levels of the anti-inflammatory cytokine IL-10 (see Table 2 for details). Wang et al. employed a rat MS model (P2-P20, 4 hr/day) and reported elevated pro-inflammatory IL-1B, IL-6, and TNFα protein in the hippocampus and elevated TNFα protein in the prefrontal cortex at P60 (Wang, Levine et al. 2020). Three studies, all from the same group and using an MS model in mice (P2-P14), reported similar results between P50 and P60; that is, elevated hippocampal mRNA for pro-inflammatory cytokines IL-1B and TNFα, as well as for the inflammasome protein NLRP3(Amini-Khoei, Haghani-Samani et al. 2019, Nouri, Hashemzadeh et al. 2020, Lorigooini, Boroujeni et al. 2021). Saavedra et al., using a rat MS model (P1-P14, 3hr/day) did not examine cytokines but found an increased proportion of hippocampal microglia that maintained an activated phenotype when examined between P140 and P170, long after ELS, (Saavedra, Hernandez-Velazquez et al. 2021). Sagae et al., utilizing an LBN model (P3-P9) in rats, also reported elevation in circulating pro-inflammatory cytokines TNFα and IL-6 at P98 (Sagae, Zanardini et al. 2018).

Other studies have reported more subtle and variable impacts of ELS models on cytokines. Hoejimakers et al. used a LBN model from P2-P9 in mice and reported increased hippocampal expression of IL-1B at P9, immediately after stress, but decreased hippocampal IL-6 mRNA at 4 months and no differences in any pro-inflammatory cytokines at 10 months (Hoeijmakers, Ruigrok et al. 2017). Additionally, these investigators reported an increase in CD68 immunoreactivity, characteristic of activated microglia, at 4 months after stress, but not at

10 months. Delpech et al. (Delpech, Wei et al. 2016) used a brief MS model (P1-P21, 15 min/day) in mice, following a single stressor event at P21 and at P28 and demonstrated an elevation of serum c-reactive protein, a marker of immune activation. At P28 however, there was no effect of ELS on the number and morphology of hippocampal microglia, that had been seen at P21. They also reported elevated IL-6 mRNA from microglia isolated from the hippocampus at P28.

Perhaps the variability of results from ELS models is not surprising given the differences in the paradigms to produce stress and in the methodology employed to measure cytokines and other features of immune priming. In explaining the differences between the MS studies (Reus, Fernandes et al. 2017, Amini-Khoei, Haghani-Samani et al. 2019, Nouri, Hashemzadeh et al. 2020, Wang, Levine et al. 2020, Lorigooini, Boroujeni et al. 2021, Saavedra, Hernandez-Velazquez et al. 2021), it seems that the duration of the separation may underly the stark differences in results between Delpech et al. (15 min/day) and the rest (3-4 hr/day). In the case of the two LBN studies (Hoeijmakers, Ruigrok et al. 2017, Sagae, Zanardini et al. 2018), differences may arise from the quite disparate means of cytokine quantification (protein in serum for Sagae et al. versus hippocampal mRNA for Hoeijmakers et al.). There may also be species differences in how the immune systems of mice and rats respond to ELS, as several of the studies that found the most robust signs of immune priming were in rats (Reus, Fernandes et al. 2017, Sagae, Zanardini et al. 2018, Wang, Levine et al. 2020, Saavedra, Hernandez-Velazquez et al. 2021), while other studies in mice reported weak evidence of immune priming (Delpech, Wei et al. 2016, Hoeijmakers, Ruigrok et al. 2017).

Table 2

ELS Model	Cytokines	Time of Cytokine Measurement	Findings	References
Maternal Separation (P4-11)	IL-1β IL-6 TNFα	P20, P30, P40, P60	**IL-1β** P20: ↑HPC, no change in serum or PFC P30: ↑HPC, PFC, Serum P40: ↑HPC, no change in serum or PFC P60: ↓HPC, ↑Serum, no change in PFC **IL-6** P20: ↑HPC, no change in serum or PFC P30: ↑HPC, no change in serum or PFC P40: ↑HPC, Serum, PFC P60: ↑HPC, PFC, no change in serum **TNFα** All time points: ↑HPC, Serum, PFC	Reus et al.
Maternal Separation (P2-20)	IL-1β IL-6 TNFα	P65	**IL-1β:** ↑HPC, no change in PFC **IL-6:** ↑HPC, no change in PFC **TNFα:** ↑HPC, ↑PFC	Wang et al.
Maternal Separation (P2-14)	IL-1β TNFα	P50	**IL-1β:** ↑HPC mRNA **TNFα:** no change HPC mRNA	Amini-Khoei et al.
Maternal Separation (P2-14)	IL-1β TNFα	P60	**IL-1β:** ↑HPC mRNA **TNFα:** ↑HPC mRNA	Nouri et al.
Maternal Separation (P2-14)	IL-1β TNFα	P60	**IL-1β:** ↑HPC mRNA **TNFα:** ↑HPC mRNA	Lorigooini et al.
Maternal Separation (P1-21)	C-Reactive Protein IL-6	P21, P28	**C-Reactive Protein** P21 + 28: ↑Plasma **IL-6** P28: ↑HPC mRNA	Delpech et al.
Limited Bedding Nestlet (P2-9)	IL-6 TNFα	P98	**IL-6:** ↑Serum **TNFα:** ↑Serum	Sagae et al.
Limited Bedding Nestlet (P2-9)	IL-1β IL-6 TNFα	P9, 4mo, 10mo	**IL-1β** P9: ↑HPC mRNA 4 mo: No Change 10 mo: Inflammation Resolved **IL-6** P9: No Change 4 mo: ↓HPC mRNA 10 mo: Inflammation Resolved **TNFα** All time points: No change	Hoeijmakers et al.

Table 2. Pro-inflammatory cytokines after ELS in rodents. Abbreviations: HPC= Hippocampus; P= Postnatal day; PFC= Prefrontal Cortex; P=Postnatal day; IL-1β= Interleukin-1β; IL-6= Interleukin-6; TNFα= Tumor necrosis factor alpha.

1.7.5 Immune priming by ELS in humans

In humans, childhood adversity has been linked to a chronic inflammatory state (Pace, Mletzko et al. 2006, Danese, Pariante et al. 2007, Carpenter, Gawuga et al. 2010, Ehrlich, Ross et al. 2016), as well as to diseases associated with inflammation, such as cancer, cardiovascular

disease, diabetes, and arthritis (Felitti, Anda et al. 1998, Caspi, Sugden et al. 2003, Richards and Wadsworth 2004, Danese, Moffitt et al. 2009, Dalle Molle, Portella et al. 2012, Flaherty, Thompson et al. 2013, Kelly-Irving, Lepage et al. 2013, Giovanelli, Reynolds et al. 2016). Many studies have examined the relationship between socioeconomic status during childhood and inflammation, typically measured by plasma c-reactive protein (Milaniak and Jaffee 2019). Such studies may be complicated by controlling for covariates, such as adult socioeconomic status. A recent meta-analysis examined 35 such studies and found a significant relationship between childhood socioeconomic status and the profile of inflammation at adulthood, but this relationship was not evident when adjusted to factor out adult socioeconomic status (i.e. the time of data collection) (Milaniak and Jaffee 2019). Ehrlich et al. (Ehrlich, Ross et al. 2016) examined whether scores for early-life adversity in teens, based upon interviews, were associated with differences in their inflammatory profiles. Rather than rely on cytokine or c-reactive protein expression, inflammation was quantified by *ex vivo* challenge of monocytes, obtained from blood samples, with either lipopolysaccharide alone or with lipopolysaccharide in combination with varying concentrations of GC. IL-6 secreted into the culture media was quantified, and a cluster analysis was performed. ELS was associated with higher clusters related to inflammation, suggesting persistent immune priming in this population.

1.8 ELS and Brain Injury

Despite the clinical relevance, there are few preclinical studies that have examined brain injuries after exposure to LBN, brief maternal stress (BMS) or prolonged maternal stress (PMS) (Table 3 (Craft, Zhang et al. 2006, McPherson, Mascher-Denen et al. 2009, Tata, Markostamou et al. 2015, Markostamou, Ioannidis et al. 2016, Diaz-Chavez, Lajud et al. 2020, Lajud, Roque et

al. 2020, Sanchez, Titus et al. 2020)). Thus, there is substantial opportunity to build upon what has been reported, focusing on the unanswered questions, with the end goal of optimizing recovery in brain-injured children who have experienced ELS.

1.8.1 ELS + Stroke.

Although risk of stroke increases with age, incidence of stroke may occur at any age, including children (Hall, Levant et al. 2012). To date there is only one preclinical study that has examined the relationship between ELS and stroke (table 3, (Craft, Zhang et al. 2006)). In this study, brief maternal separation (BMS) was conducted on a daily basis from P1-P14, a sensitive period of brain development. After reaching adulthood, animals were subjected to an occlusion of the middle cerebral artery followed by reperfusion. There were several findings that distinguished BMS in combination with stroke from controls. These animals showed a pronounced elevation of proinflammatory cytokines IL-1B and TNFα, vasogenic edema, and higher mortality compared to BMS alone. Such findings build upon other studies reporting enhanced expression of pro-inflammatory cytokines IL-1B, TNFα, and IL-6 as a result of ELS exposure (Delpech, Wei et al. 2016, Hoeijmakers, Ruigrok et al. 2017, Reus, Fernandes et al. 2017, Sagae, Zanardini et al. 2018). BMS in combination with stroke also resulted in an impairment of sensorimotor function compared to controls, based upon paw preference using the cylinder test (Schallert, Fleming et al. 2000, Magno, Collodetti et al. 2019). It is noteworthy that there were no changes in corticosterone, either pre- or post-injury compared to relevant controls. While others have reported elevated levels of corticosterone at adulthood after BMS alone (Rice, Sandman et al. 2008, Delpech, Wei et al. 2016, Hoeijmakers, Ruigrok et al. 2017, Reus, Fernandes et al. 2017, Sagae, Zanardini et al. 2018), the duration of maternal separation may, at least in part, account for these differences. In this stroke study, the duration of BMS was 15

min/day over a period of 2 weeks. In contrast, those studies that detected elevated levels of corticosterone at adulthood after BMS alone (Biagini, Pich et al. 1998, McIntosh, Anisman et al. 1999, Wigger and Neumann 1999, Dent, Smith et al. 2000, Levine 2000, Penke, Felszeghy et al. 2001, Suarez, Rivarola et al. 2001, Kalinichev, Easterling et al. 2002, Pryce and Feldon 2003, de Kloet, Sibug et al. 2005, Yamazaki, Ohtsuki et al. 2005, Schmidt 2010, Bodnar, Roberts et al. 2019), reported a duration of 180 min/day or longer. Collectively, these findings provide the first evidence that ELS in combination with stroke at adulthood elicits a pronounced immune response and adversely affects post-stroke sensorimotor recovery.

1.8.2 ELS +Perinatal Brain Injury

Neonatal hypoxia ischemia (HI), the most common form of perinatal brain injury, results in neonatal encephalopathy and long-term disabilities (Hagberg, David Edwards et al. 2016).

Several preclinical studies have examined the consequences of ELS in combination with HI (Table 3, (McPherson, Mascher-Denen et al. 2009, Tata, Markostamou et al. 2015, Markostamou, Ioannidis et al. 2016)). Early studies evaluated ELS using BMS (15 min/day) or PMS (180 min/day) during P3-P7, followed immediately by HI, and then studied outcomes shortly after HI or at adulthood (McPherson, Mascher-Denen et al. 2009). Prior exposure to PMS and neonatal HI resulted in elevated levels of corticosterone shortly after the time of injury. Histological findings, based upon pathological scoring of hematoxylin-stained sections, suggested enhanced damage to white matter in the thalamus and internal capsule. Studies of HI at adulthood reported altered metabolism, as evidenced by elevated levels of glucose and insulin compared to BMS or PMS alone.

A later study focused on the long-term consequences of BMS or PMS in combination with HI on hippocampal functioning at adulthood (Table 3 (Tata, Markostamou et al. 2015)).

After BMS or PMS from P1-P6, animals were exposed to HI shortly thereafter and then were evaluated at adulthood. While ELS in combination with HI showed no differences in non-spatial recognition (novel object recognition and novel placement test), there were impairments in spatial learning and memory, as measured by the Morris Water Maze, compared to either insult alone.

Lastly, a follow up study focused on the interaction of ELS and HI in the context of synaptic integrity in the hippocampus and metrics of emotionality (Table 3, (Markostamou, Ioannidis et al. 2016)). Animals were exposed to PMS and subsequent HI and evaluated at adulthood for anxiety- and depressive-like behaviors, based on performance in the elevated plus maze and the forced swim test, respectively. While HI followed by PMS resulted in a more pronounced anxiety-like phenotype, compared to either insult alone, there was no evidence of depressive-like behavior across any groups. Subsequent histological analyses of the dentate gyrus revealed altered long-term synaptic plasticity as evidenced by a reduction in levels of brain-derived neurotrophic factor and synaptophysin in the hippocampus compared to either PMS or HI alone. These results suggest that cell survival and synaptic density in the hippocampus are particularly vulnerable to the additive effect of MS and HI (Markostamou, Ioannidis et al. 2016).

1.8.3 ELS + TBI

ELS has been evaluated in pre-clinical models of TBI with variables that include the type of injury (focal versus diffuse), the age at time of injury, and the timing of outcomes. Sanchez et al. (Table 3, (Sanchez, Titus et al. 2020))\ examined how prolonged ELS influences hippocampal-related function after a TBI at adulthood. Animals were exposed to daily PMS (180 min/day) from P2-P14 followed by a mild fluid percussion injury at adulthood. Behavioral

assessments were conducted 2, 3, and 4 weeks after injury. Based on contextual fear learning (2 weeks post injury), brain-injured animals, reared in PMS, showed less freezing after the cue compared to controls. Animals were subsequently tested using the Morris Water Maze at 3-4 weeks post injury. The group with PMS in combination with TBI showed deficits in spatial learning as well as greater cortical and hippocampal atrophy compared to other conditions. At 8 weeks post injury, corticosterone levels were highest in PMS in combination with TBI.

An alternative approach examined how PMS (P1-P21) is influenced by a mild TBI at P21 (Table 3, (Diaz-Chavez, Lajud et al. 2020)). In these experiments, a mild focal injury was produced by a controlled cortical impact. Deficits in spatial learning and memory were most pronounced in brain-injured adolescent rodents exposed to both PMS and TBI. Although there was no difference in the lesion volumes across all groups, PMS prior to TBI resulted in an increase in activated microglia and a reduction in proliferation of the markers bromodexoyuridine and the nuclear protein Ki67 in the hippocampus. Taken together, these findings suggest that PMS prior to an early age mild TBI, results in more profound activation of microglia, which, in turn, adversely affects neurogenesis and hippocampal-dependent behaviors (Johnson and Kaffman 2018).

A follow-up study, using the same model of PMS and TBI, examined cognitive flexibility and then measured pro-inflammatory cytokines, IL-1B, TNFα, and IL-6 in the prefrontal cortex and hippocampus. Cognitive flexibility was measured using the attentional shift task in early adolescence, whereby mice learned how to discriminate between positive odors and associate this experience with a cue (Birrell and Brown 2000, Bondi, Jett et al. 2010, Lajud, Roque et al. 2020). Mild injury had a significant impact on the first reversal of the attentional shift task. However, this was not worsened by prior exposure to MS. IL-1B, elevated in the hippocampus,

was highest in those animals exposed to both PMS and TBI compared to controls. These findings suggest that PMS in combination with a mild TBI results in a heightened inflammatory response compared to either condition alone. Although there was no additive effect seen on cognitive flexibility or in IL-1B in the prefrontal cortex, the authors suggest that IL-1B may be involved in crosstalk between hippocampal and cortical-related cognitive impairments seen after an early age, mild TBI.

Table 3

Species & Sex	Ages at Separation & Duration	Injury Type	Age at Injury	Timing & Outcomes	ELS + Injury Behavioral Findings	Findings	References
Mouse/ F	P1-14: 15 min/day **BMS**	Stroke	P100-110	**24, 72h, 7d:** Behavior CBF **24 or 72h:** CORT **48h:** Edema, Histology **12h:** RT-PCR	**Locomotion:** No change **Paw Preference:** ↓Contralateral paw	**Histology:** ↑infarct volume **RT-PCR:** ↑IL-1B ↑TNFα **CBF:** No Change **CORT:** ↓intra-ischemia **Edema:** ↑Edema	Craft et al.
Rat/ M+F	P3-7: 30 min/day **BMS** OR 8h/day **PMS**	HI	P7 or P135	**P7-Adult:** CORT **P13+P120:** Histology **P10+P120:** Physiologic measures	Not measured	**CORT:** ↑P7, P9-17, P135 **Histology:** ↑Atrophy **Injury Scale:** Worsened score **Physiology:** ↑Hyperglycemia	McPherson et al.
Rat/ M+F	P1-6: 180min/day **PMS** OR 15 min/day **BMS**	HI	P7	**Adult:** Behavior Histology	↓Spatial acquisition memory No change in object recognition ↓No change in motor behavior	**Infarct Size:** No change **CC:** No change	Tata et al.
Rat/ M+F	P1-6: 180min/day **PMS** OR 15 min/day **BMS**	HI	P7	**Adult:** Behavior Histology	↑Anxiety ↑Spontaneous movement No change depression	**Histology:** ↓Synaptophysin in HPC ↓BDNF in HPC	Markostamou et al.
Rat/ M	P2-14: 180min/day **PMS**	TBI (Mild)	Adult	**Adulthood:** Behavior Histology CORT	↓Memory retention ↓Spatial working memory	**Histology:** ↑Cortical atrophy ↑ HPC atrophy **CORT:** ↑Level	Sanchez et al.
Mouse/ M	P1-21: 180min/day **PMS**	TBI (Mild)	P21	**Adolescence:** Behavior Histology	↓Spatial learning + memory	**Histology:** ↓Cell proliferation ↑Iba-1	Diaz-Chavez et al.
Mouse/ M	P1-21: 180min/day **PMS**	TBI (Mild)	P21	**Adolescence:** Behavior ELISA RT-PCR	No Change in executive functioning	**ELISA:** ↑IL-1β ↑CORT **RT-PCR:** ↑CRH, No Change AVP	Lajud et al.

Table 3. ELS prior to neonatal hypoxia-ischemia, stroke or TBI. Abbreviations: AVP= Vasopressin; P=Postnatal day; CC= Corpus Callosum; HPC= Hippocampus; BDNF= Brain-

derived neurotrophic factor, CBF=Cerebral blood flow; CORT= Corticosterone, HI- Hypoxia Ischemia M= Males, F+ Females, TBI= Traumatic Brain Injury; BMS= brief maternal separation, PMS= prolonged maternal separation

1.9 Where do we go from here?

There are a number of research opportunities that could contribute to our current knowledge of the interactions between ELS and recovery after a brain injury. Here we address several basic directions.

1.9.1 Consider alternative models of ELS

ELS has profound adverse effects on brain development and results in both physical and psychological sequelae at adulthood. Few preclinical studies have addressed how ELS may influence recovery after brain injury. And, of these studies, ELS has only been studied in the context of MS (Craft, Zhang et al. 2006, McPherson, Mascher-Denen et al. 2009, Tata, Markostamou et al. 2015, Markostamou, Ioannidis et al. 2016, Diaz-Chavez, Lajud et al. 2020, Lajud, Roque et al. 2020, Sanchez, Titus et al. 2020). As ELS represents a broad spectrum of adverse conditions including physical, sexual and emotional forms of abuse and neglect (Fogelman and Canli 2019), there is a need to address alternative models of ELS, including LBN, as well as others that capture a broader range of adverse exposures.

1.9.2 Injury severity as a modifier of recovery after ELS

Two of the most commonly used rodent models of TBI, controlled cortical impact and fluid percussion injury, have been studied in the context of ELS (Diaz-Chavez, Lajud et al. 2020, Lajud, Roque et al. 2020, Sanchez, Titus et al. 2020). The severity of the injury likely influences recovery after ELS. This raises the possibility that very mild forms of TBIs, such as concussions, which present with nominal changes at structural and behavioral levels, may, in fact, be sensitive to prior ELS and, as such, result in broader pathological and behavioral findings. Understanding

the relationships between ELS and mild TBIs has broad implications, including how we manage concussions in youth sports.

1.9.3 Sex as a biological variable

There are few studies of ELS in combination with TBI that include both males and females in the experimental design (Table 3). There is evidence that speaks to the complexities of TBIs, where variables such as the severity and type of insult may be differential modifiers between sexes. Thus, from simply the perspective of TBI alone, sex as a biological variable should be a key element in the experimental design (See Review, Gupte et al., 2019 (Gupte, Brooks et al. 2019)). Importantly, in a scoping review of both clinical and preclinical studies, Gupte et al. (Gupte, Brooks et al. 2019) have indicated that variables such as injury severity and nature of the injury interact differently based upon sex and that these differences influence long-term outcomes.

1.9.4 Genetics and epigenetics

Genetics, including both gene variants and epigenetics, play a central role in how a brain recovers after ELS (See review, Fogelman and Canli, 2019 (Fogelman and Canli 2019)). Similarly, genetics, and in particular epigenetics, also contribute to heterogeneity in recovery after a TBI, as evidenced in both preclinical models and in human studies (See reviews, Bennett et al., 2016; Cortes and Pera, 2021(Bennett, Reuter-Rice et al. 2016, Cortes and Pera 2021)) (Kurowski, Treble-Barna et al. 2019, Treble-Barna, Patronick et al. 2020).

1.9.5 Immune function

ELS results in persistent immune priming (Ehrlich, Ross et al. 2016) (and see reviews, Neher et al., 2019 (Neher and Cunningham 2019); Fagundes et al., 2013 (Fagundes, Glaser et al. 2013); von Leden et al., 2019 (von Leden, Parker et al. 2019)). We have yet to address how this

priming may alter the immune response after a TBI. We and others have reported that the developing brain is sensitive to early cytokine exposure and, in fact, an early age TBI results in an enhanced immune response that is, in part, related to the prolonged recruitment of leukocytes to the injured brain (von Leden, Parker et al. 2019). Thus, these collective findings support a further investigation into inflammatory responses, mediated by ELS, that may be magnified after a subsequent TBI.

1.9.6 ELS, TBI, and plasticity

There are varying thoughts regarding plasticity after an early age lesion (See review, Giza and Prins, 2006, (Giza and Prins 2006)). One viewpoint is that a younger brain is able to undergo significant reorganization and recovery after an injury and that ongoing brain development may support recovery processes. This contrasts with others who consider the vulnerability of the young brain, where growth and formation of circuitry may be compromised by injury during critical periods of brain development. To address these differing viewpoints, further studies are needed to address factors that may influence outcomes, including age at time of injury in the context of brain development, severity and location of the injury, and the type of injury (focal and/or diffuse), as well as a broader viewpoint on plasticity that takes into account both its beneficial and adverse consequences.

1.10 Conclusion

This introduction summarizes scientific efforts to better understand how ELS interacts with early age brain injuries, ranging from neonatal hypoxia ischemia, to stroke and traumatic brain injuries. The consistent finding across these diverse injuries is that ELS in combination

with a brain injury at different developmental stages or at adulthood, may potentiate progressive neurodegeneration and result in long-term neurological deficits.

The following studies contribute to gaps in knowledge that have yet to be addressed. I provide the first documentation of how ELS and TBI may differentially influence the CA1, CA2, and CA3 subfields of the pyramidal layer of the hippocampus. I focus on both the acute phase, where microglial activation is a key feature in hippocampal pathogenesis, and at adulthood, where I identify volumetric and neuronal loss. I consider how ELS in combination with an early age TBI may be influenced by sex in the context of regionally specific vulnerability of the hippocampus, defined by microglial activation and cell damage. The extent to which these events follow prior studies contribute to the breadth of the literature by examining the acute timepoints after TBI, inclusion of sex as a biological variable, hippocampal mapping, highlighting hippocampal changes after ELS and TBI, and long-term functional outcomes that target hippocampal-dependent behavior and adjacent cognitive behaviors.

Chapter 2. Materials and methods

2.1 Animals

C57BL/6 mice were purchased from Jackson Laboratories (Sacramento, CA). All animals were housed in a species-specific pathogen-free facility at the University of Texas at Austin. Standard rodent chow and water were available *ad libitum*, and housing was maintained on an automated 12 h light/dark cycle at approximately 20°C. All procedures involving animals were conducted in accordance with the National Institutes of Health, Guidelines for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the University of Texas at Austin. A total of 85 breeder mice (29 males and 56 females), were used for this study, resulting in 36 litters with approximately 6-10 mice /litter. Overall mortality was 8.68% of the 288 animals raised to adulthood. There was no maternal mortality.

Triad breeding was used to generate litters. Each male, 6-8 weeks of age, was housed, undisturbed with 2 females, 6-10 weeks of age, for 16 days and then singly housed. The females were observed daily, and litters were left undisturbed until offspring were postnatal (P) day 2.

Both blinding and randomization were used to minimize bias and both sexes were studied. Controls were included with each outcome, and biochemical assays were conducted in either duplicates or triplicate samples.

2.2 Limited bedding nestlet (LBN) paradigm and controls

ELS was induced by the LBN model, as originally described by Rice et al. 2008 (Rice, Sandman et al. 2008). A nursing dam and her pups at P2 were *a priori* randomly assigned to a cage (12 x 7.5in, One Cage™ Micro-Isolator®, Lab Products, LLC., Aberdeen, MD) with a wire mesh floor insert (12 x 7.5in, ASC Stainless Steel Mesh 304, #4, Sycamore, IL) that was

positioned on top of standard corn cob bedding, and provided with enrichment that was limited to 1/3 of a nestlet square (5cm x 1.5cm). Control animals were housed in cages with corn cob bedding and a full sized nestlet square (5cm x 5cm). The nursing dam and her pups were left undisturbed for 7 days (Figure 1a). On P9, all dams and pups were returned to standard housing. Pups were weighed (American Weigh Scales LB-501 Digital Scale; Lot No: 00-A20-173; Cumming, GA) on P2, P9, P16, and P21.

Maternal behavior was recorded for all groups using a built-in camera system (Amcrest 1080P Outdoor PoE Camera, Amazon: Amcrest, Houston, TX; catalog #: B07PYGNDG9) that was positioned in the cage topper (One Cage™ Micro-Isolator®). Video recordings, collected at were conducted during the light and dark cycle [0:00, 6:00, 11:00, 12:00 18:00, and 23:00 Zeitgeber time (ZT)]. These recordings were hand scored every other 10 minutes within each ZT block, resulting in 30-min epoch. Data collection included entries by the dam to the nest and duration of dam-pup interactions (Gunn, Cunningham et al. 2013, Gallo, Shleifer et al. 2019). These values were totaled across ZT scoring hours for both cage conditions.

2.3 Controlled cortical impact (CCI) model and controls

TBIs were generated by the CCI model, as we have previously described (Pullela, Raber et al. 2006, Semple, Noble-Haeusslein et al. 2015, Semple, O'Brien et al. 2017). At P21, male and female pups from LBN and control conditions were weaned and anesthetized with 4% isoflurane in an anesthesia chamber. The mouse was positioned in ear bars on the stereotaxic apparatus (Figure 1b). Buprenorphine (0.05 mg/kg) was administered subcutaneously (s.q.) and Neosporin was applied to each eye. After exposure of the skull, 0.25% bupivacaine was administered (s.q.). After a midline incision, a 3.0 mm circular craniotomy was made mid-way between bregma and

lambda, with the medial edge positioned 0.5mm lateral to the midline. Male and female mice from ELS and control conditions were *a priori* randomly assigned to TBI or sham-surgery (craniectomy only). A 2.0mm convex impactor tip was used to produce the injury. The parameters of the injury were the following: 4.5m/s, depth of penetration of 1.9mm, and a 150ms dwell time. *A priori* exclusion criteria were <5g weight at P21, or abnormal post-operative behavior indicative of morbidity (i.e. lethargy, inflammation around the site of incision, poor grooming, damp fur, and /or failure to gain weight over the first 7 days post-surgery).

2.4 Hypothalamic pituitary adrenal axis (HPA) activity

To address hypothalamic expression of genes involved in in the HPA stress response, freshly dissected hypothalamus samples were collected at P9 via rapid decapitation and stored at 4C in RNAlater solution (Invitrogen, Waltham, MA). Each hypothalmus was homogenized in 0.9ml Trizol (Invitrogen) using a bead-based homogenizer (Beadbug, Benchmark Scientific, Sayreville, NJ) with a single 5mm stainless steel bead, for a total of 4min at 4000RPM. RNA was extracted according to the RNeasy lipid tissue protocol (Qiagen, Hilden, Germany). Briefly, 0.18ml chloroform was added and samples were mixed and spun at 12000xg for 15min at 4C. Approximately 0.45ml of the upper phase was transferred to a fresh tube, mixed with an equal volume of 70% ethanol, and pipetted onto a mini spin column. Samples were spun through columns and washed with Qiagen wash buffers. Purified RNA was eluted with 2 x 30µL water, RNA concentration measured via Nanodrop (Thermo Fisher, Waltham, MA), and stored at -80°C. Complimentary DNA (cDNA) was reverse transcribed from 2µg RNA in 20µL reactions using random hexamers with the Applied Biosystems High-Capacity cDNA Reverse Transcription Kit (Waltham, MA). Quantitative PCR was performed on an Applied Biosystems

ViiA 7 instrument, using Power SYBR Green PCR Master Mix (Thermo Fisher). All primers were obtained from IDT (Corelville, IA), and each sample (1:30 dilution of RT reaction) was evaluated in triplicate 10μL PCR reactions for two experimental genes, corticotropin-releasing hormone (CRH; F: GAGGCATCCTGAGAGAAGTCC, R: GGGCGCTCTCTTCTCCTC) and vasopressin (AVP; F: CTGCAGCGACGAGAGCTG, R: CCAGCTGTACCAGCCTTAGC), as well as 3 control genes, ribosomal protein L13a (F: AGCAGATCTTGAGGTTACGGA, R: AGGAGTCCGTTGGTCTTGAG), cytochrome C-1 (F: GCTGAGGAGGTGGAGGTC, R: TCGAACGATGTAGCTGAGGT), and phosphoglycerate kinase 1 (F: TGCCAAAATGTCGCTTTCCA, R: GAAGTCCACCCTCATCACGA). Relative gene expression for CRH and AVP was determined by the $2^{-\Delta\Delta Ct}$ method (Kozera and Rapacz 2013) and normalized to a geometric average of the three control genes.

2.5 Histology

2.5.1 Tissue collection

Mice were anesthetized at 1d post injury or sham surgery and transcardially perfused with ice-cold 0.01M phosphate-buffered saline (PBS), followed by 4% paraformaldehyde (PFA). Brains were removed and post-fixed overnight in 4% PFA and then transferred to 30% sucrose in deionized water for 72h. Coronal sections, 40μm in thickness were cut with a sliding microtome (Leica SM2010R), from interaural 6.60mm to bregma 2.80mm (Paxinos and Franklin), were serially collected in 12-well plates (Sigma-Aldrich, St. Louis, MO) and stored at 4°C in a cryoprotectant solution (0.067M sodium phosphate, 0.03M sodium biphosphate, Mili-Q®, water, sucrose, polyvinylpyrrolidone, and ethylene glycol). This resulted in 8-10 sections per well, representing the entire hippocampus.

2.5.2 *Immunohistochemistry*

Sections were selected based on hippocampal coronal sections spanning interaural 1.86-1.50mm and bregma -1.94—2.30mm (Paxinos and Franklin), yielding 2-3 sections per well. In preparation for immunocytochemistry, free floating sections in 0.01MPBS were incubated in 1% hydrogen peroxide for 15 minutes at room temperature, followed by 10% normal donkey serum (Jackson ImmunoResearch Laboratories, Pittsburgh, PA). All antibodies were visualized in sections using Vectastain ABC kits (Vector Laboratories Inc., Newark, CA). Sections were incubated for 1 hour at room temperature, followed by 3 rinses in 0.175M sodium acetate for 5 minutes each and nickel-enhanced 3,3'-diaminobenzidine tetrahydrochloride (DAB) for 20 minutes at room temperature. Sections were mounted on glass slides (Superfrost Plus charged slides). Sections were counterstained with 0.05% methyl green (Sigma-Aldrich).

2.5.3 Localization of ionized calcium-binding adaptor molecule 1 (IBA-1)

Sections were incubated in anti-goat transmembrane protein Iba-1 (1:750; Abcam, Waltham, MA), for 1 hour at room temperature and 48 hours at 4°C. Sections were rinsed in 0.01M PBS and incubated with donkey anti-goat Biotin (1:600; Jackson ImmunoResearch Laboratories) for 1 hour at room temperature.

2.5.4 Localization of caspase-3

Sections were incubated with anti-rabbit transmembrane protein Caspase-3 (1:400; Cell Signal Technology, Danvers, MA), for 24 hours at room temperature. Sections were rinsed in 0.01M PBS and incubated in goat anti-rabbit biotin (1:600; Jackson ImmunoResearch Laboratories) for 1 hour.

2.5.5 Localization of Purkinje cell protein (PCP4)

Sections of the hippocampus were stained with PCP4 antibody to identify the CA2 area of the pyramidal cell layer. The adjacent section was used to immunolocalize either Iba-1 or Caspase-3. Sections were incubated in anti-rabbit transmembrane protein PCP4 (1:1000; Sigma Aldrich), for 1 hour at room temperature, rinsed in 0.01M PBS and then incubated in goat anti-rabbit biotin (1:600; Jackson ImmunoResearch Laboratories) for 1 hour.

2.5.6 Localization of neuronal nuclei (NeuN)

Sections were incubated in anti-mouse monoclonal transmembrane NeuN (1:1000, Abcam) for 24 hours at room temperature. Sections were rinsed 0.01M PBS and then incubated in goat anti-mouse biotin (1:600; Jackson ImmunoResearch Laboratories) for 1 hour.

2.5.7 Unbiased stereology

The unbiased optical fractionator method (Gunderson 1999, Olesen 2017) was used to estimate microglia and cell death in coronal sections of the ipsilateral dentate gyrus and pyramidal cell layer of the hippocampus that had been immunolabeled with either Iba-1+ and caspase-3+. Using StereoInvestigator (MicroBrightField Inc., Williston, VT), a sample interval of 2 was selected for quantification and regions of interest were contoured using a 4x objective. Cells were counted using a 60x oil immersion objective on a Nikon Eclipse E600 microscope (Nikon Instruments Inc., Melville, NY). Systematic random sampling was achieved by determining the number of cells within an 80 x 80µm grid and a 160 x 160µm dissector counting frame (20% of the desired structure), with a 10um dissector height and top guard zone of 1um to allow for three-dimensional quantification. The Gunderson mean coefficient of error (m=1) for

individual estimates was maintained at <0.10 for the blades of the dentate gyrus and <0.20 for the hilus. The total number of cells per contoured region was estimated by the following equation: N=EQ x (t/h)(1/asf)(1/ssf); where E is the sum, Q is the number of cells counted, t is the measured section thickness, h is the dissector height, asf is the area sampling fraction, and ssf is the section sampling fraction.

As CA2 is not easily distinguished from CA1 and CA3 (San Antonio (San Antonio, Liban et al. 2014, Paul and Limaiem 2023), coronal sections, containing the hippocampus, were immunolabeled with Iba-1, Caspase-3 or PCP4, a marker that is unique to CA2 (San Antonio, Liban et al. 2014). StereoInvestigator (MBF Biosciences, Williston, VT) was used to trace the CA2 region on sections stained with PCP4 and the trace settings were saved as a template. The template was positioned on a section stained with Iba-1, caspase-3, or NeuN. The CA1 and CA3 subzones were defined as those areas bordering CA2+ PCP4 cells.

2.5.8 Cortical and hippocampal volumes

The volume of the ipsilateral cortex and hippocampus, determined at 67-70 dpi, was determined based upon 40µm coronal sections that were stained with 0.05% methyl green. Images, spanning Bregma 1.5 to -3.8 mm, were captured with a Nikon Ni-E microscope (Nikon Instruments Inc.). This yielded 5 sections per brain, using a sampling interval of 12, a 4x objective and a grid size of 500µm. Cortical measurements were performed by an observer blinded to the experimental conditions. Cortical and hippocampal volumes were estimated as the product of the summed areas of sections and the distance between sections.

2.6 Multiplex cytokine assays

TBI and sham-operated pups from ELS and control conditions were euthanized by rapid decapitation. The ipsilateral and contralateral cortices were dissected, placed in 150µL of RIPA Buffer (ThermoFisher Pierce, Waltham, MA) and subjected to sonification (Branson Digital Sonifier Model 250, Cleanosonic, Richmond, VA). The homogenates were then centrifuged at 4^{o}C at 15,000 RPM (Multi RF Centrifuge, Thermo IEC, Needham Heights, MA) for 15 minutes. Supernatants were aliquoted and stored at -80^{o}C.

Q-Plex Array Mouse 6-Plex Cytokine Panel (Quansys Biosciences, Logan, UT) was used to detect the cytokines IL-1B, IL-6, TNFa, IL-10, IFNy, IL-1a. The manufacturer's standard protocol was followed. Briefly, an 8-point calibration curve was prepared, and homogenates were diluted 1:1.5 with the provided sample diluent. All samples and calibrators were added to the plate in duplicate and incubated for 180 minutes. This was followed by two 20-minute incubations, beginning with the detection mix containing biotinylated antibodies followed by the streptavidin-HRP solution. All steps in incubation were carried out on a shaker at 500RPM (VWR Advanced Microplate Vortex Mixer, Radnor, PA), with 3 washes between incubations and 6 washes after the last incubation. Immediately following addition of the provided substrate mixture, the Q-View Imager Pro (Quansys Biosciences) was used to capture digital images of the plate (270s exposure time). The Q-Plex image was analyzed on Q-View Software (Quansys Biosciences) and cytokine values were exported to Excel (Microsoft Inc., Redmond, WA) for quantitative? analysis. Values for cytokines were normalized to total protein in the homogenate, using a Rapid Gold® BCA assay (ThermoScientific Pierce).

2.7 Behavioral assays

Assays were conducted at 9-10 weeks of age, or 60-67 days post injury in mice exposed to ELS, TBI, and the respective controls. Behaviors were conducted in a private behavioral suite in the animal vivarium with a regulated temperature and 24.5 lux. All animals were transported from the housing room to the behavioral suite and were habituated for 30 minutes prior to staring behavioral testing. Behavioral assays were conducted in the following order: open field, novel object recognition, three chamber task, elevated zero, and the Barnes maze.

2.7.1 Open field

Performance in an open field was used to assess exploration and anxiety-like behavior (Pena, Smith et al. 2019, Popovitz, Mysore et al. 2019). An animal was placed in a black-walled arena (16 x 24 x 24in, Laird Plastics, Pflugerville, TX) with a grey bottom to minimize the glare from overhead lights in the testing room (Seibenhener and Wooten 2015). The mouse was allowed to explore the field for 10 minutes. EthoVision (Noldus, Leesburg, VA) was used to track time spent in the center and peripheral area of the open field and total distance moved.

2.7.2 Novel Object Recognition (NOR)

The NOR assay was used to assess object novelty preference and object recall (Leger, Quiedeville et al. 2013). Each mouse was placed in a custom-made open field (16 x 24 x 24in, Laird Plastics). Testing was conducted immediately after the open field test, and therefore habituation to the field was unnecessary. During trial 1, two identical, yellow Duplo® blocks (5.08 x 7.62 x 5.08cm, LEGO®, item no: 2301) were placed 59 cm, diagonally away from each other: the furthest points of the field. The mouse was placed in the center of the two objects and allowed to freely explore for 5 minutes. During trial 2, object 2 was replaced with a brass

dishwasher elbow (5.08 x 7.62cm, Ace Hardware, Oak Brook, IL item #4520961). The mouse was returned to the center of the field and allowed to freely explore for 5 minutes. Ethovision was used to track the time spent with each object in trials one and two. A discrimination (preference) index was calculated for trial two to determine if a mouse preferred the familiar (Duplo block) or novel (dishwasher elbow) object:

$$Preference\ Index = \left(\frac{b}{e2}\right) * 100$$

e2= Total exploration time between object 1 and 2 during trial 2

b= time spent with novel object 2 during trial 2

2.7.3 Three-chamber task

Social Recognition and social novelty were examined using the three-chamber task (Yang, Silverman et al. 2011). A custom-made plexiglass box (61.2 x 30.5cm; Tap Plastics, San Francisco, CA) was divided into three chambers using two plexiglass dividers, each with an opening, (30.5 x 20.4cm). Stimulus mice for the three-chamber task were purchased as virgin adults (Jackson Laboratories). Each stimulus mouse was sex matched to the test mouse and was rotated throughout testing day. The left and right chambers each contained a metal cup with vertical bars, spaced approximately 2mm apart, that were attached to a metal top (7.3cm diameter x 20.4cm x 15.5cm). Each mouse was habituated to the entire box for 5 minutes. During trial 1 a stimulus mouse (s1) was placed in a metal cup, that centered in the left chamber. The test mouse was placed in the middle chamber and allowed to explore the for the enclosure for 10 minutes. The test mouse and stimulus mouse were removed from the chamber, and the apparatus was cleaned with 70% ethanol. S1 was again placed in the metal cup located in the center of the left chamber, and a stimulus mouse 2 (s2) was placed under the metal cup in the

center of the right chamber. The test mouse was returned to the middle chamber and allowed to explore the apparatus for 10 minutes. The test mice, S1, and S2, were removed, and the chamber and cups were again cleaned with ethanol. EthoVision was used to track the time spent in each chamber and total distance moved. Analyses included time the mouse spent with the familiar mouse (s1), middle chamber, and the novel mouse (s2).

2.7.4 Elevated zero maze (EZM)

The EZM was chosen to assess anxiety-like behavior and impulsivity (Popovitz, Mysore et al. 2019). The EZM was selected over the elevated plus due to its high sensitivity to manipulations and treatments (Popovitz, Mysore et al. 2019). Each mouse was placed in a "0" shaped apparatus with two open arms and two closed arms (diameter: 60.96cm, leg height: 60.96cm, San Diego Instruments, San Diego, CA). A mouse was positioned in an open arm facing a closed arm and allowed to explore for 10 minutes. EthoVision was used to track how much time was spent in the open and closed arms and total distance moved. Analyses determined a preference for the closed versus open arms.

2.7.5 Barne's Maze

The Barnes maze was used to measure spatial learning and memory (Fonken, Gaudet et al. 2016). This maze consisted of 20 holes evenly distributed along the perimeter of a circular plane 100 cm in diameter (Laird Plastics). Testing consisted of 3 components: a training day, acquisition days, and a probe trial. Black and white shapes, 18 cm in size, (triangle with diagonal lines, a square with black dots, a circle with checkered squares, and a solid black "X") were placed on the walls or surrounding surfaces 70 cm away from the maze and aligned with each

quadrant of the maze for spatial location. On the training day, a bottomless metal box was positioned in the middle of the field and a buzzer played in the background for the duration of this test that was approximately 5 minutes in length. Each mouse was placed under the metal box for 10 sec. This box was then lifted, and the observer guided the mouse into the escape (target) hole. The hole was subsequently covered, and the buzzer was turned off. The mouse remained in the covered target hole for 120 seconds.

For acquisition, the above process was repeated with the exception that the mouse was free to explore the maze for 180 seconds. The trial ended when the mouse found the target or failed to do so after 180 seconds. Once the animal found the target or was guided to the target, the hole was covered, the buzzer was turned off and the mouse was left undisturbed in the target hole for 60 seconds. Acquisition days consisted of three trials over 4 consecutive days. The mean latency to target hole was analyzed across acquisition days.

The final component was the probe trial. At 24 hours after the last acquisition day, the target hole was occluded with a white paper towel that was flush with platform. A mouse was placed under the box, located in middle of the Barnes Maze, for 10 seconds. A buzzer was audible as the mouse explored the Barnes maze with the target hole for 90 seconds. The latency to find the target hole, number of visits to holes relative to the target hole, and path length to the target hole were then analyzed.

2.8 Statistical Analyses

2.8.1 *Study design*

We calculated our effect sizes based on the open data provided by studies examining the same as ours (ELS + TBI and then some of the same outcomes). Each experiment can be

grouped as morphology, protein expression, and behavior. All data and stats values were included in these prior studies. I used these values to find their effect sized based on their determined group sizes. The morphology and protein expression studies were N=5 and the behavior were N=8. The effect size for the former was a medium effect. The effect size for behavior was small to medium. We wanted a medium to large effect for all outcomes. After theoretical simulation of plugging in values for the following equation (below) and from some past experience with animal numbers and variability in our lab, we decided on N=6/group for all morphology and cytokine groups. For behavior the equation landed us at 12/group but with more thought on how individual differences may influence behavioral output compared to a more concrete and less varying measure like protein expression, we decided to include 15/group for behavior assays.

$$d = \frac{(Fvalue * df1)}{((Fvalue * df1) + df2)}$$

(Lakens 2013)

In which,

d= effect size in Cohen's d

F-value= Reported F-value

d*f*1= degrees of freedom between groups

d*f*2= degrees of freedom in between groups

In experiments that examined maternal observation of her pups, "N" was defined as a litter because the outcome was focused on the dam rather than the pups. The group sizes for these experiments were defined as N=3-4 dam/group.

Using the above strategy, the effect of rearing condition and injury on outcome measures were determined. Unpaired t-tests were used to analyze collapsed maternal behaviors and HPA-

Axis fold change in gene expression. Mixed effect analyses were used to evaluate differences in time, condition, and sex. Two-way ANOVAs were used to compare 2 or more groups or factors (cage condition, injury, and/or time). A significance level of 0.05 was set for all analyses. If a significant interaction was detected, main effects were evaluated where appropriate. A Pearson's r Correlation was used to analyze the following relationships: hippocampal behavior outcomes, anxiety-related and overall activity outcomes, hippocampal behaviors and stereological/volumetric analysis, microglial density/cell death and stereology at adulthood.

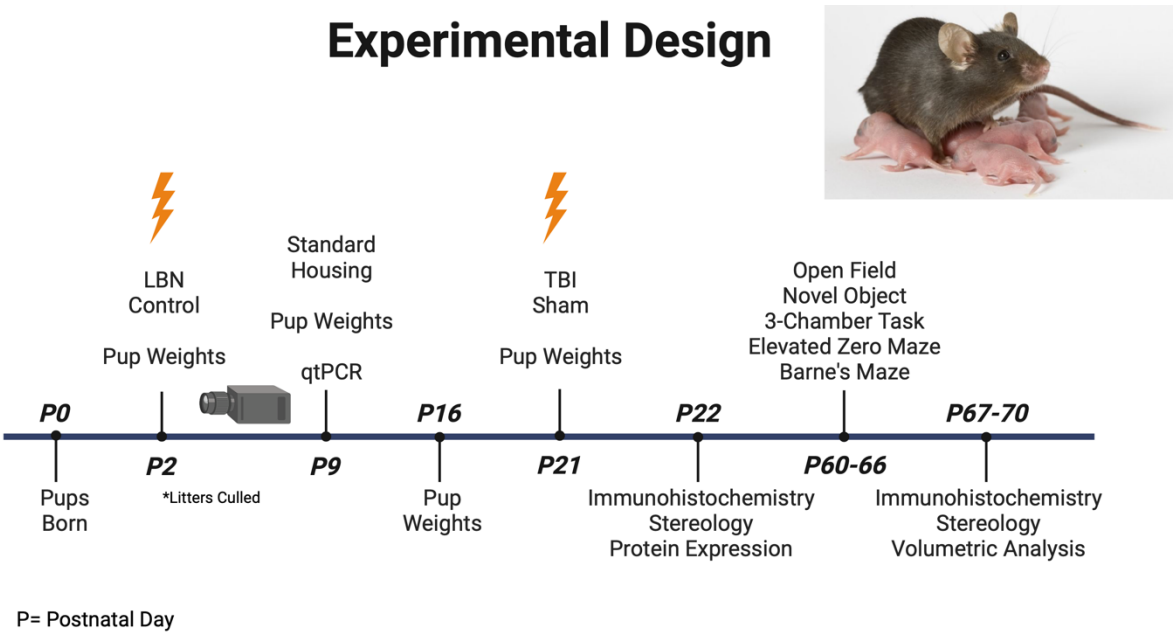


Figure 1. Experimental Design and Timeline. Mice were bred in house. On P2, litters were culled to 6-8 pups. Nursing dam and litters were randomized into LBN or Control cages until P9. Continuous home cage monitoring was used to track maternal behavior. A subset of animals was used for qTPCR to analyze collected for immunohistochemistry and protein expression. On P21, pups were weaned and randomized into TBI or Sham surgery. Brain tissue was collected for immunohistochemistry and protein expression. On 60-70, animals were analyzed for hippocampal structure and function.

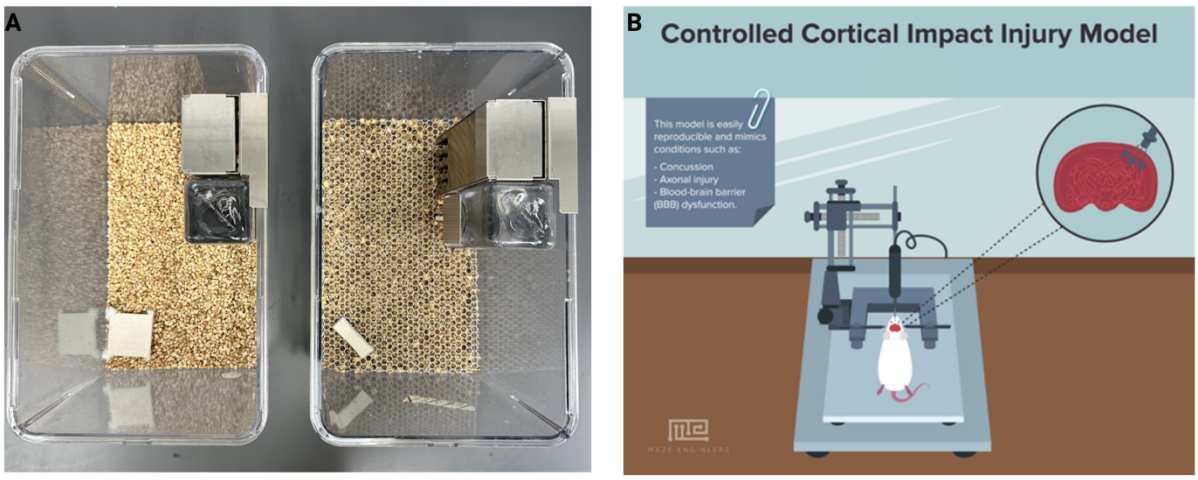


Figure 2. Standard murine models of ELS (limiting bedding nestlet, LBN) and TBI (controlled cortical impact) were used in this study.

Chapter 3: Results

3.1 Characterization of the LBN model

The rodent model has served as a foundation for understanding the impact of stress on children who experience poor maternal care that is often coupled with limited resources (Walker). As a first step in this study, the LBN model was validated in males and females by assessing maternal behaviors, activation of the hypothalamus-pituitary adrenal (HPA) axis, and body weights. After birth, dams and pups were transferred to cages with either standard bedding and a full nestlet or standard bedding that was covered by a wire grid, including 1/3 of a nestlet (Fig. 3). The behavior of the dam was recorded from on P2 to P9. The videos were then scored by a single observer. *A priori* outcomes included number of entries to the nest and time spent on the nest (Fig. 3). Time spent on the nest and dam entries to the nest were totaled across all scoring hours (0:00, 6:00, 11:00, 12:00, 18:00, 23:00ZT). While dams, exposed to an impoverished environment (termed ELS dams), spent more total time on the nest (Fig. 3A), they also made more entries to the nest (Figure 3B), an indicator of fragmented care, compared to the dams in standard caging (termed control dams). Collectively, these findings are in line with other studies that have characterized maternal behavior in the LBN model (Rice, Sandman et al. 2008, Walker, Bath et al. 2017, Gallo, Shleifer et al. 2019).

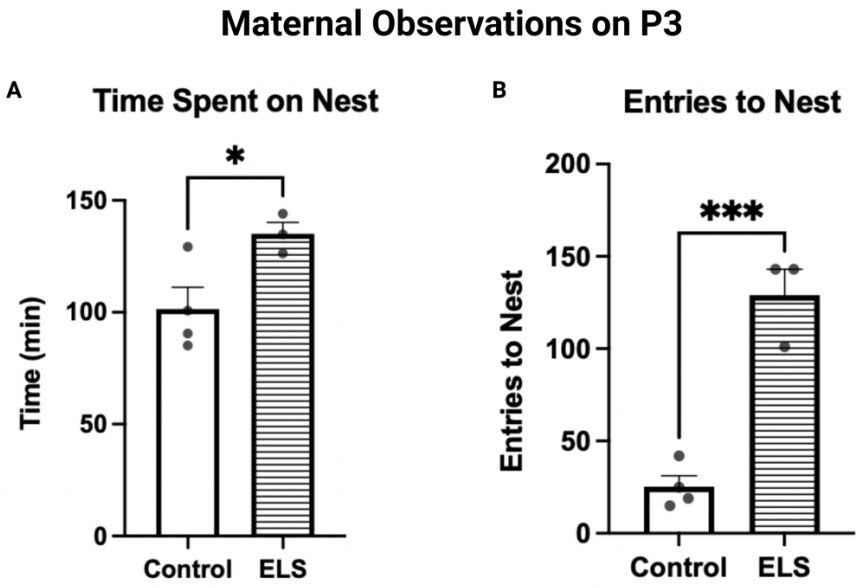


Figure 3. Maternal behaviors, measured at P3, revealed disrupted maternal behavior. Time spent on the nest and entries of dams to the nest were scored and averaged across 0:00, 6:00, 11:00, 12:00, 18:00, and 23:00 Zeitgeber time hours. A.) ELS dams spent more time on their nests (unpaired t-test, $t=2.718$, $*p<0.05$) and B.) made more entries to the nest as compared to controls (unpaired t-test, $t=7.592$ $***p<0.0001$).

To determine if ELS influenced gain in weight, each pup was weighed daily from P2-P21 (Fig. 4). While all pups gained weight, ELS pups did not gain weight at the same rate as control pups, based on a mixed effect analysis. When separated by sex, female ELS pups showed a lower gain in weight from P9-21, compared to ELS males.

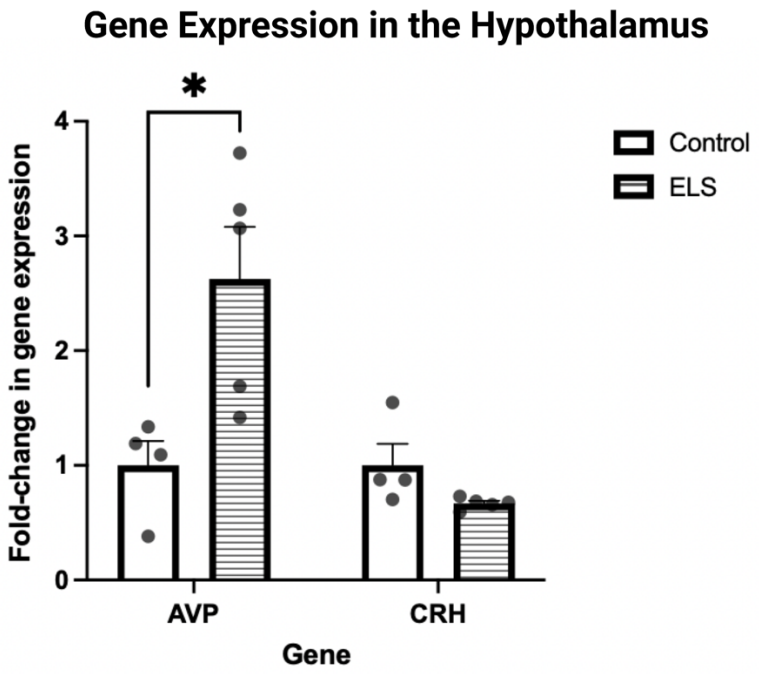


Figure 4. Arginine vasopressin (AVP) was increased in the hypothalamus after exposure to ELS. AVP and corticotropin-releasing hormone (CRH) were quantified in the hypothalamus at P9, a time point corresponding to the final day of exposure to ELS. While there was an increase in AVP (N=4-5, multiple t-test, Bonferroni Correction, t= 2.625, *p<0.05), there was no change in CRH (N=4-5, multiple t-test, Bonferroni Correction, t= 0.6694, p=0.08).

At euthanasia at P9, homogenates, prepared from the hypothalamus, were used to assess gene expression of arginine vasopressin (AVP) and corticotropin-releasing hormone (CRH). While AVP showed a significant increase compared to controls, CRH was marginally changed (p= 0.08, Figure 5). [(ANOVA tables for all outcomes are included in the supplemental data (Supplemental Tables 1-60)]

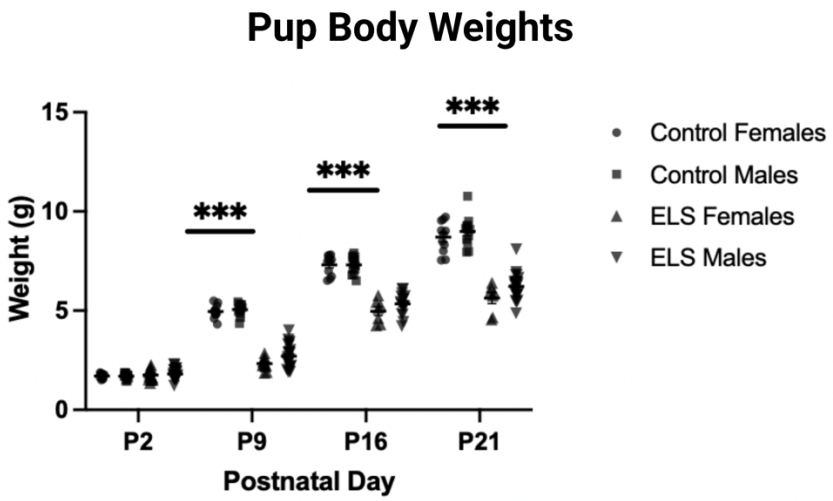
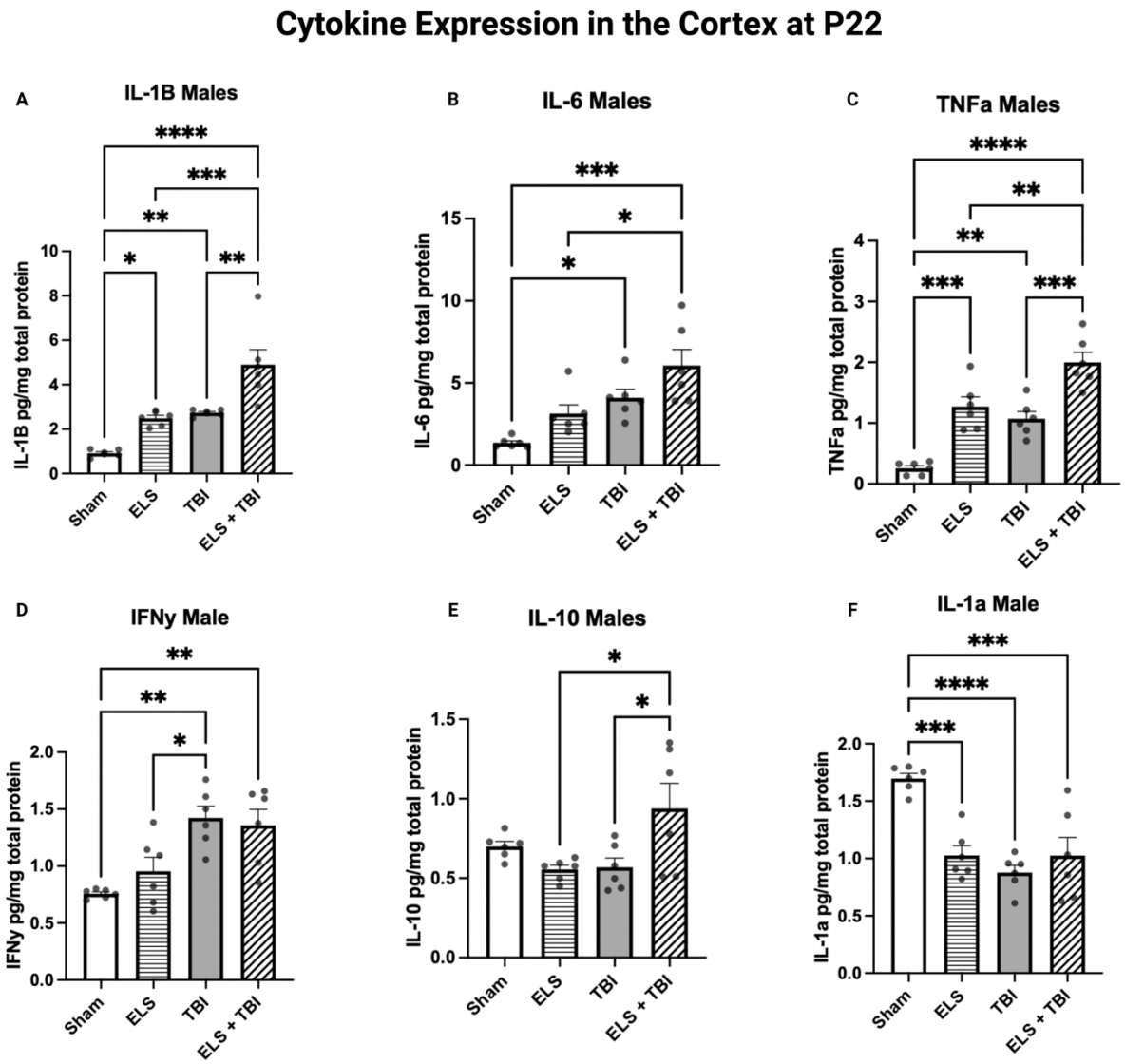


Figure 5. Body weights were tracked during and after exposure to ELS.
ELS was associated with a lower gain in weight than that of control pups (N=23-37, mixed effect analysis, $F_{3, 128}$=101.80, ***p<0.001). ELS females showed lower gains in body weights compared to ELS males (males, N=12-26, females, N=11-16; two-way ANOVA, $F_{1,129}$=10.30, **p<0.01).

3.2 Cytokines and the injured cortex

As both ELS and TBI are associated with inflammation (Potts, Koh et al. 2006, Danese, Pariante et al. 2007, Semple, Blomgren et al. 2013, Agorastos, Pervanidou et al. 2019, von Leden, Parker et al. 2019, Parker, Donovan et al. 2021), both pro- and anti-inflammatory cytokines were quantified in both males and females at P22 from homogenates prepared from the ipsilateral cortex for each condition (Figs. 6 and 7). The objectives were to determine the magnitude of the response to ELS when compared to TBI, and if ELS in combination with TBI (termed ELS+TBI), altered expression of these cytokines. TBI resulted in an increase in the pro-inflammatory cytokines IL-1B, IL-6, TNFα, and IFNγ in both males and females, compared to their respective sham controls. While IL-1α was increased in females, this anti-inflammatory cytokine was decreased in males. In response to ELS, there was a more limited profile of

cytokines as evidenced by an increase in IL-1B only, compared to shams in both males and females, and a decrease in IL-1α that was limited to male mice. When mice were exposed to ELS + TBI there was an amplified response, restricted to males, as evidenced by an increase in IL-1B, IL-10, and TNFα, compared to TBI. Cytokine expression following ELS and TBI show that pro-inflammatory cytokines are more sensitive to ELS, TBI, and ELS and that males show a greater vulnerability to cytokine expression than females.



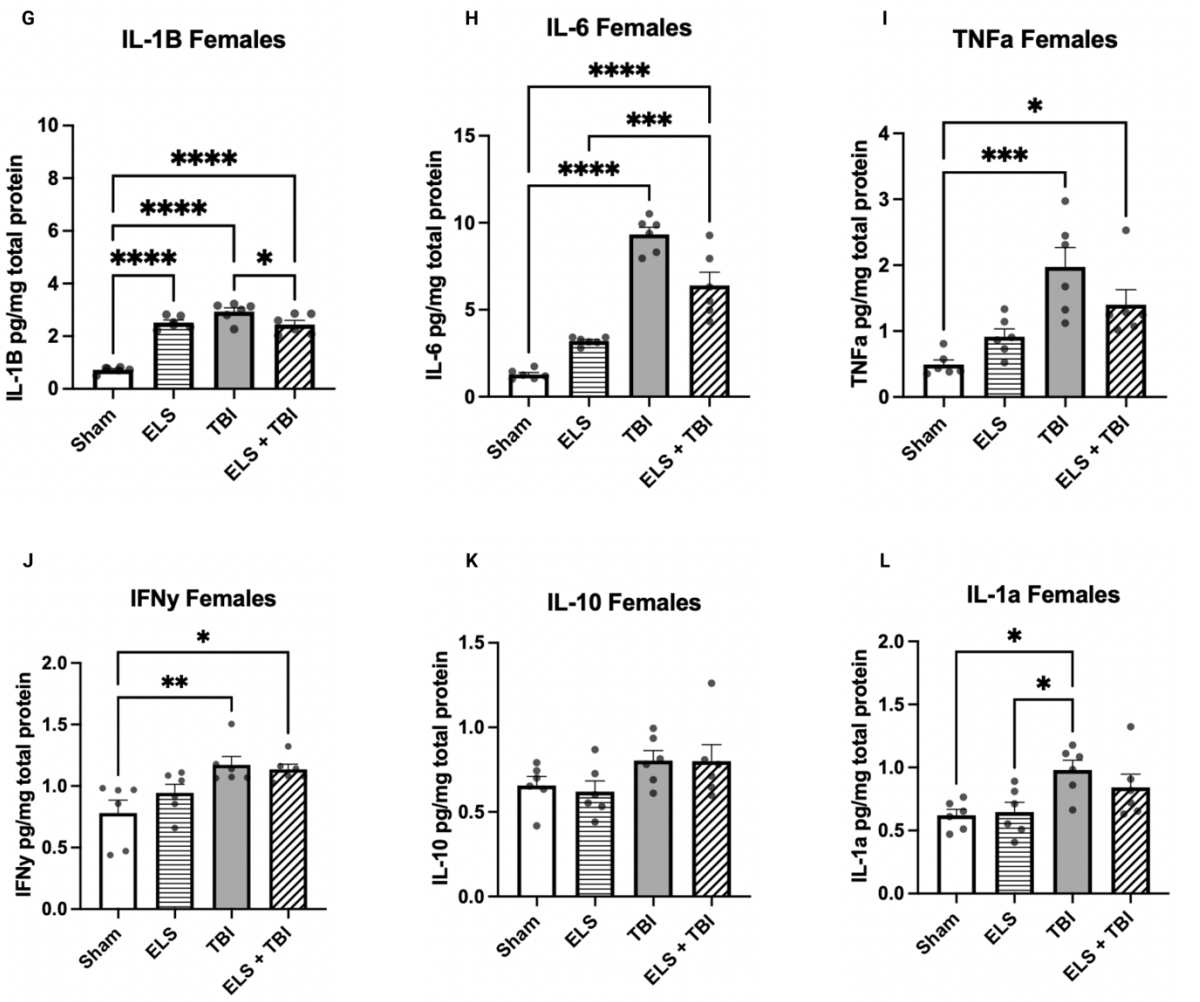


Figure 6. Cytokines, quantified in cortical homogenates at P22, revealed limited variation across experimental conditions.

Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance threshold, n.s.= not significant, one-way ANOVA). All reported stats were compared to the sham controls, unless otherwise stated.

(A-F) In males, TBI resulted in an increase in A.) IL-1B (**p<0.01), B.) IL-6 (*p<0.05), C.) TNFa (**p<0.01), and D.) IFNg (**p<0.01); there was a reduction in F.) IL-1a (****p<0.0001). Similarly, ELS resulted in increased A.) IL-1B (*p<0.05) and B.) TNFa (***p<0.001), but F.) reduced IL-1a (***p<0.001). ELS + TBI resulted in an increase in A.) IL-1B (****p<0.0001), B.) IL-6 (***p<0.001), C.) TNFa (****p<0.0001), D.) IFNg (**p<0.01), and F.) IL-1a (***p<0.001). When compared to TBI, ELS + TBI resulted in increased A.) IL-1B (**p<0.01) and E.) IL-10 (*p<0.05).

(E-F) In TBI resulted in G.) increased IL-1B (****p<0.0001), H.) IL-6 (****p<0.0001), I.) TNFa (***p<0.001), J) IFNg (**p<0.001), and L.) IL-1a (*p<0.05). ELS resulted in G). an increase that was limited to IL-1B (****p<0.0001). ELS+ TBI produced increased G.) IL-1B (****p<0.0001), H.) IL-6 (****p<0.0001), I.) TNFa (*p<0.05), and J.) IFNg (*p<0.05). When compared to TBI, ELS + TBI resulted in decreased G.) IL-1B (*p<0.05).

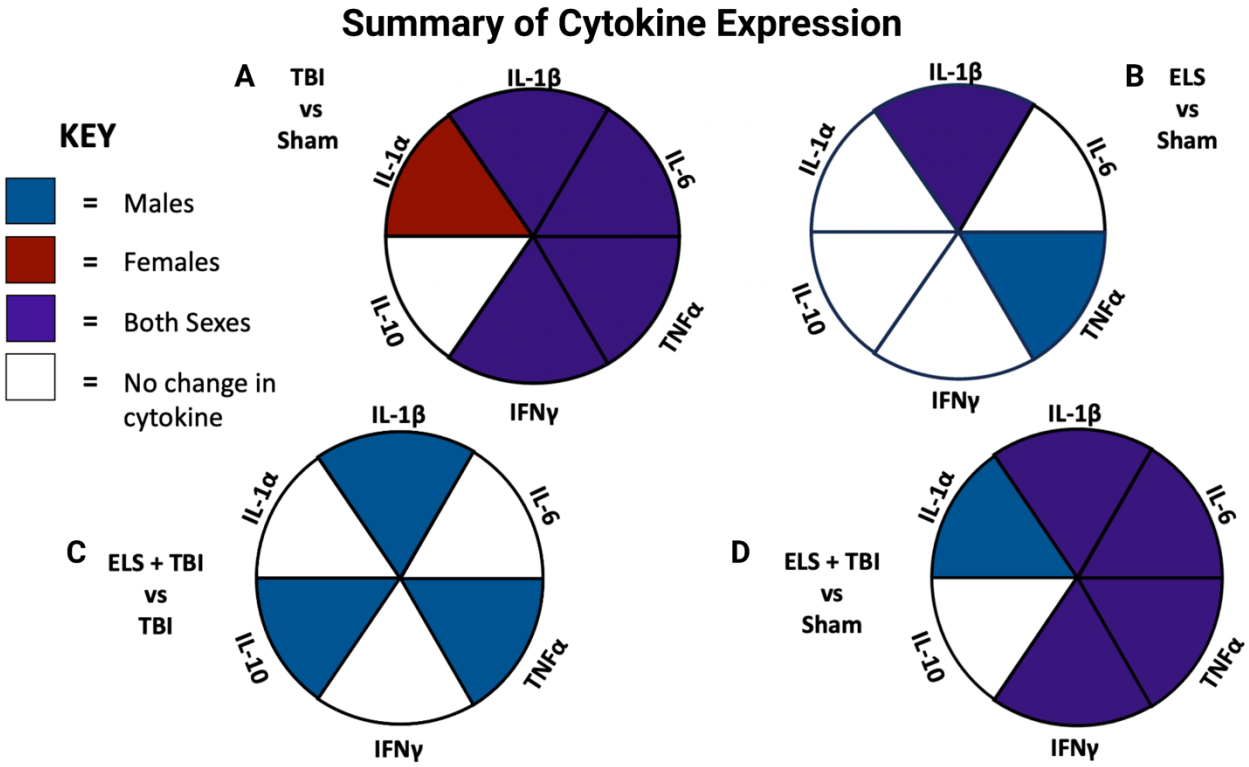


Figure 7. Summary of the differential response of cytokines in cortical homogenates at 1day post injury.
A.) With the exception of IL-10, cytokines were significantly elevated in the injured cortex, in both males and females, compared to shams.
B.) ELS resulted in a more limited increased expression of cytokines (IL-1B and TNFa only) compared to shams.
C.) Only male pups, when exposed to ELS+TBI, showed an elevation in cytokines, compared to TBI.
D.) With the exception of IL-10, cytokines were significantly elevated in the injured cortex for males and females, compared to shams.

3.3 Vulnerability of the hippocampus after ELS and/or TBI: Microglial density and apoptosis

While we have previously reported acute cell death in the hippocampus after an early age TBI (Tsuru-Aoyagi, Potts et al. 2009), no distinctions were made within hippocampal subregions. Taking advantage of an antibody that distinguishes CA2 from CA1 and CA3 (San Antonio, Liban et al. 2014), microglial density and caspase-3 were quantified within each subregion at 1 day post injury in mice exposed to ELS, TBI or ELS+ TBI (Fig. 8). There was a pronounced microglial response in all subregions after TBI in both males and females. In contrast, ELS resulted in an increase in microglial density in all subregions in males, but only CA3 in females. When ELS+TBI was compared to TBI, the findings were more variable; while both males and females showed an increase in CA3, there was no change in CA1 and an increase in microglial activation in CA2 was limited males. These findings emphasize the profound effect of TBI on CA1, CA2 and CA3 in both males and females when compared to sham controls. While ELS has a broad effect in males across all subregions, microglial activation is limited to CA3 in females.

Previous work has reported apoptosis in the corpus callosum and dorsal striatum in the acutely injured young brain (Chen, Noble-Haeusslein et al. 2013, Hanlon, Raghupathi et al. 2019). Caspase-3 was used to define apoptotic cell death in the ipsilateral hippocampal subregions (Fig. 9). While there was pronounced increase in caspase-3+ cells after TBI in CA1, CA2, CA3 in both males and females compared to sham controls, there was no evidence of apoptotic cell death in any subregion in either sex when exposed to ELS. However, ELS in combination with TBI resulted in increased cell death in CA1 and CA3 for each sex when compared to TBI only. The combined results indicate that the CA3 region is most vulnerable to the inflammation and cell death following ELS and TBI.

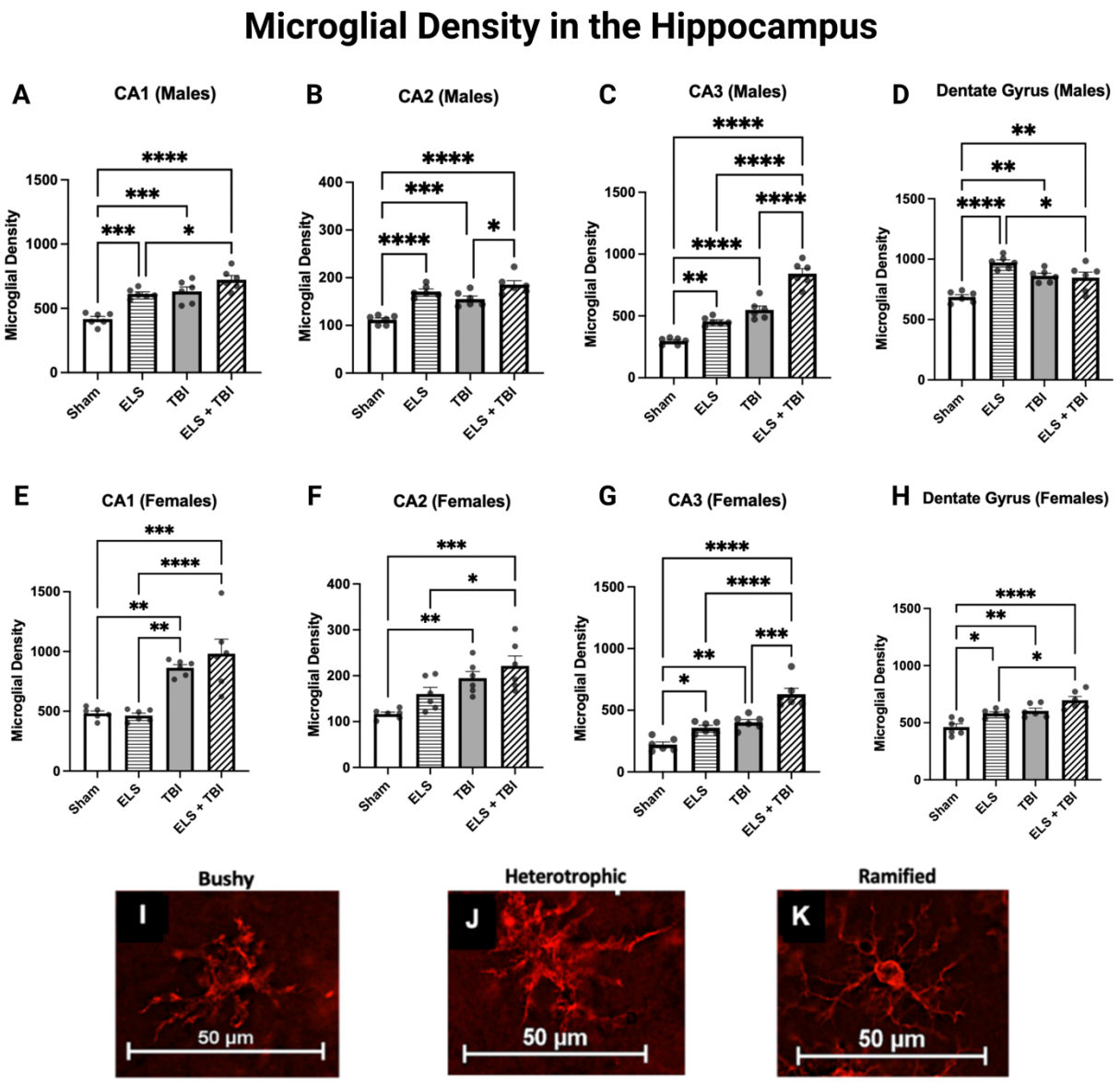


Figure 8. At 1 day post injury, there were regional differences in microglial density in the hippocampus in mice exposed to either ELS or TBI.

Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated.

(A-D) In males, there were significant changes in cytokines when compared across all groups (Supplemental tables 13-16; N= 6/group, One-Way ANOVA). TBI resulted in an increase in Iba-1+ cells in A) CA1 (***p<0.001), B) CA2 (***p<0.001), C), CA3 (****p<0.0001), and D) the dentate gyrus (**p<0.01). ELS resulted in an increase in Iba-1+ cells in A) CA1 (***p<0.001), B) CA2 (****p<0.0001), C) CA3 (**p<0.01), and D) the dentate gyrus (****p<0.0001). ELS+

TBI revealed similar findings to that of TBI alone. There was an increase in the number of Iba-1+ cells in the CA1 (****p<0.0001), CA2 (p****<0.0001), CA3 (****p<0.0001), and dentate gyrus (**p<0.01). ELS+ TBI in males, when compared to TBI, resulted in an increase in Iba-1+ cells in B.) CA2(*p<0.05) and C.) CA3(****p<0.0001) E-H.)

In females, TBI resulted in E.) an increase in Iba-1+ cells in CA1 (**p<0.01), F.) CA2 (**p<0.01), and G.) CA3 (**p<0.01). Exposure to ELS resulted in an increase in Iba-1+ cells in G.) CA3 (*p<0.05) and H.) dentate gyrus (*p<0.05). ELS + TBI resulted in an increase in Iba-1+ cells in E.) CA1 (***p<0.001), F.) CA2 (***p<0.001), G.) CA3 (****p<0.0001), and H.) dentate gyrus (****p<0.0001). ELS + TBI, when compared to TBI, resulted in an increase in Iba-1+ cells in G.) CA3 (****p<0.0001).

Distinct microglial phenotypes, randomly assessed throughout the hippocampus, were evident at 1-day post injury in the hippocampus. Representative images (I-K) were taken in the injured male hippocampus at P22.

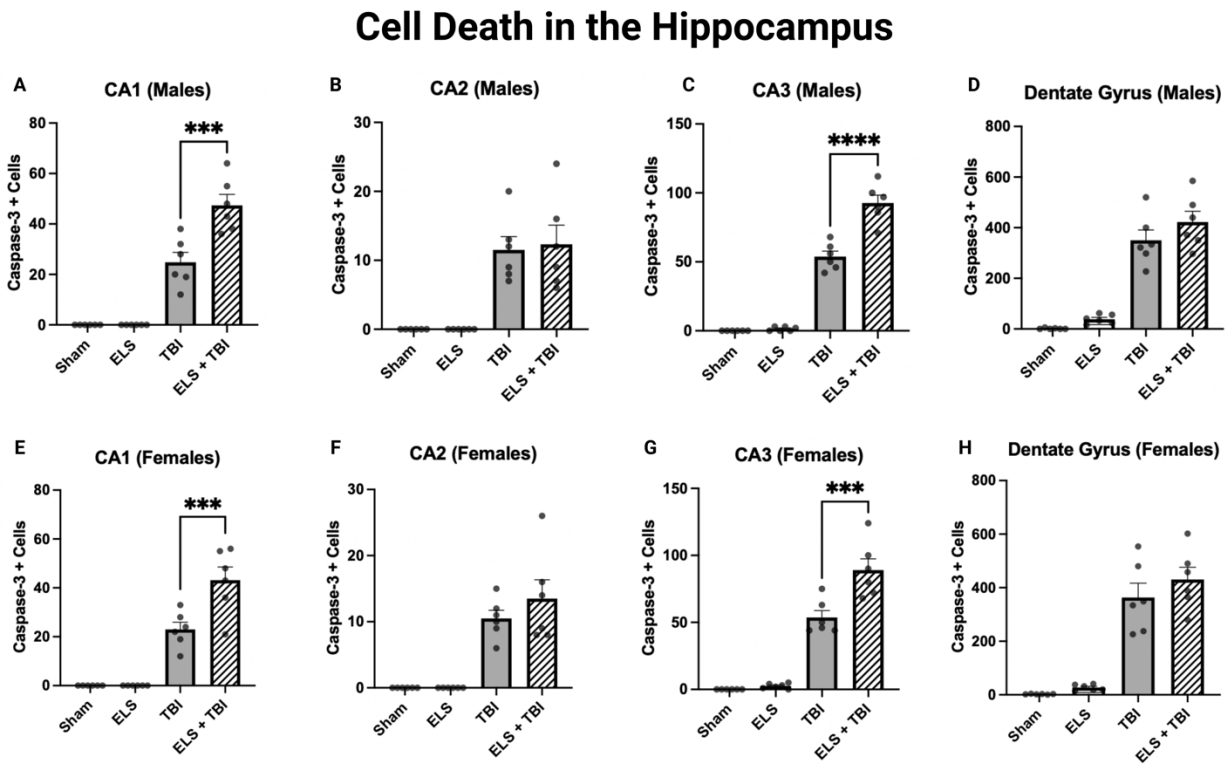


Figure 9. Caspase-3+ cells were quantified in hippocampal subfields. ELS + TBI was associated with increased numbers of caspase-3+ cells in both males (A-D) and females (E-H).

Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated.

In males, TBI resulted in an increase in caspase-3+ cells in A.) CA1 (****p<0.0001), B.) CA2 (***p<0.001), C.) CA3 (****p<0.0001), and D.) dentate gyrus (****p<0.0001). ELS resulted in a similar number of caspase-3 cells in A.) CA1 (n.s.), B.) CA2 (n.s.), C.) CA3 (n.s.), and D.) dentate gyrus (n.s.). ELS + TBI resulted in a greater number of caspase-3+ cells in A.) CA1 (****p<0.0001), B.) CA2 (***p<0.001), C.) CA3 (****p<0.0001), and D.) dentate gyrus (****p<0.0001). ELS + TBI resulted in the number of caspase-3 + cells in A.) CA1 (****p<0.0001) and C.) CA3 (****p<0.0001) compared to TBI.

In females, TBI yielded greater caspase-3+ cells in the E.) CA1 (***p<0.001), F.) CA2 (***p<0.001), G.) CA3 (****p<0.0001), and H.) dentate gyrus (****p<0.0001). ELS showed no differences in the number of caspase-3+ cells in E.) CA1 (n.s.), F.) CA2 (n.s.), G.) CA3 (n.s.), and H.) dentate gyrus (n.s.). ELS + TBI resulted in an increase in caspase-3+ cells in E.) CA1 (****p<0.0001), F.) CA2 (****p<0.0001), G.) CA3 (****p<0.0001), and H.) dentate gyrus (****p<0.0001). ELS + TBI, when compared to TBI, resulted in increased numbers of caspase-3+ cells in E.) CA1 (***p<0.001) and G.) CA3 (***p<0.001).

3.4 Anxiety, hyperactivity, learning and memory

Behavioral assessments were conducted at adulthood in mice exposed to ELS or TBI compared to sham controls. Additionally, ELS+TBI was compared to TBI, to determine if ELS altered recovery after brain injury.

3.4.1 The Open Field Test (OFT) and Elevated Zero Maze (EZM)

The OFT and EZM were selected to assess anxiety-like behavior, based upon thigmotaxis, greater time spent in the center versus time spent in the periphery (OFT, Fig. 10), and preference for the open versus closed arms (EZM, Fig.11). When exposed to ELS, males spent more time in the inner zone of the OFT (Fig. 10A) and open arms of the EZM (Fig. 11A). While TBI resulted in less time spent in the inner zone of the OFT (Fig. 10A), TBI resulted in more time spent in the EZM open arms (Fig. 11A). While males in the ELS + TBI group, compared to TBI, did not show a difference in time spent in the OFT (Fig. 10A), ELS + TBI spent more time in the open arms in the EZM compared to TBI (Fig 11A). In females, these outcomes were a bit more complex. In the OFT, mice in the ELS condition spent less time in the inner zone (Fig. 10B) but spent more time in the open arms in the EZM (Fig. 11B). Similarly, TBI females spent less time in the OFT inner zone (Fig.10B), but more time in the EZM open arms (Fig. 11B). ELS+TBI in the OFT (Fig. 10B) and EZM (Fig. 11B) did not show a difference in time spent in the measured areas compared to TBI alone.

We have previously shown that early age TBIs in mice result in hyperactivity at adulthood (Chen, Noble-Haeusslein et al. 2013). Here, the OFT and EZM were also used to assess distance moved in controls and experimental groups. (Fig. 10 and Fig. 11). In females, ELS resulted in an increase in distance moved when compared to shams, but a similar distinction was not noted in males. Conversely, TBI resulted in an increase in distance moved in the OFT

compared to shams that was limited to males. TBI in combination with ELS in females resulted in an increase in distance moved when compared to TBI alone, but this same comparison in males resulted in a decrease in thigmotaxis. These findings reveal that males and females show different response to measures of anxiety and hyperactivity after ELS and TBI are variable and may indicate that factors outside of ELS and incidence of head injury may influence these measures.

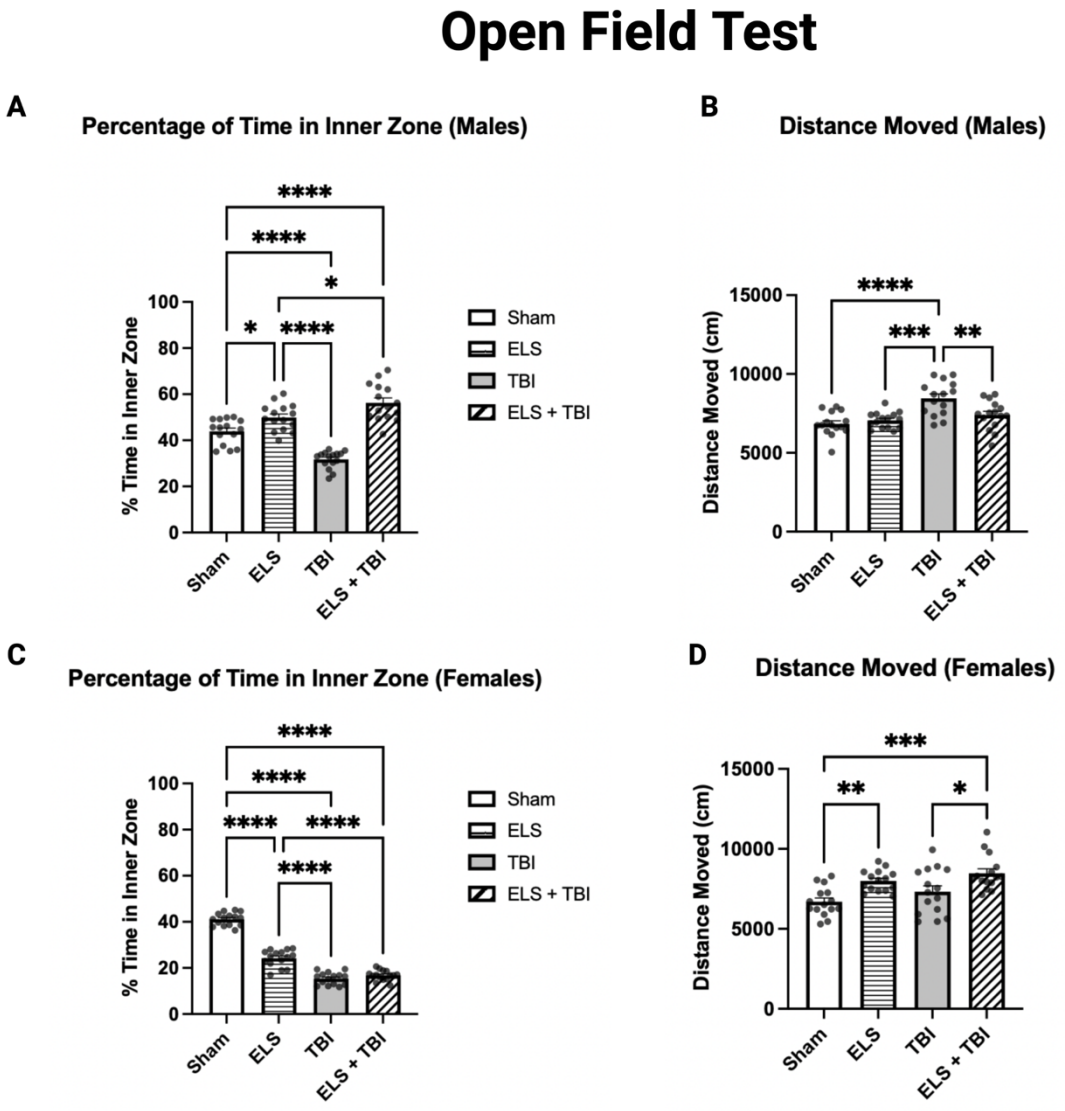


Figure 10. The open field test was used to assess anxiety-like behavior and hyperactivity. Mice were evaluated for their preference to the center of the field (inner zone) versus the perimeter (outer zone) and the distance traveled over a period of 10 minutes.

Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated.

A.) Brain-injured male mice spent less time in the outer zone (****p<0.0001). In contrast, ELS showed no differences in time spent in the outer zone. While ELS + TBI resulted in increased time spent in the outer zone (***p<0.001), ELS + TBI spent less time in the outer zone compared to TBI (****p<0.0001).
B.) In males, TBI resulted in greater distance moved (****p<0.0001). ELS and ELS +TBI did not show a difference in the distance moved. ELS + TBI resulted in reduced activity compared to TBI (**p<0.01).
C.) In females, TBI and ELS spent less time in the outer zone (****p<0.0001) and (****p<0.0001), respectively. ELS + TBI when compared to shams or TBI, resulted in reduced time spent time in the outer zone. (****p<0.0001, for both comparisons.)
D.) Female TBI mice spent a similar time in the outer zone (n.s.). ELS resulted in a reduction in time spent in the outer zone (**p<0.01). ELS + TBI resulted in an increase in the total distance moved compared to sham (**p<0.01) or TBI (*p<0.05).

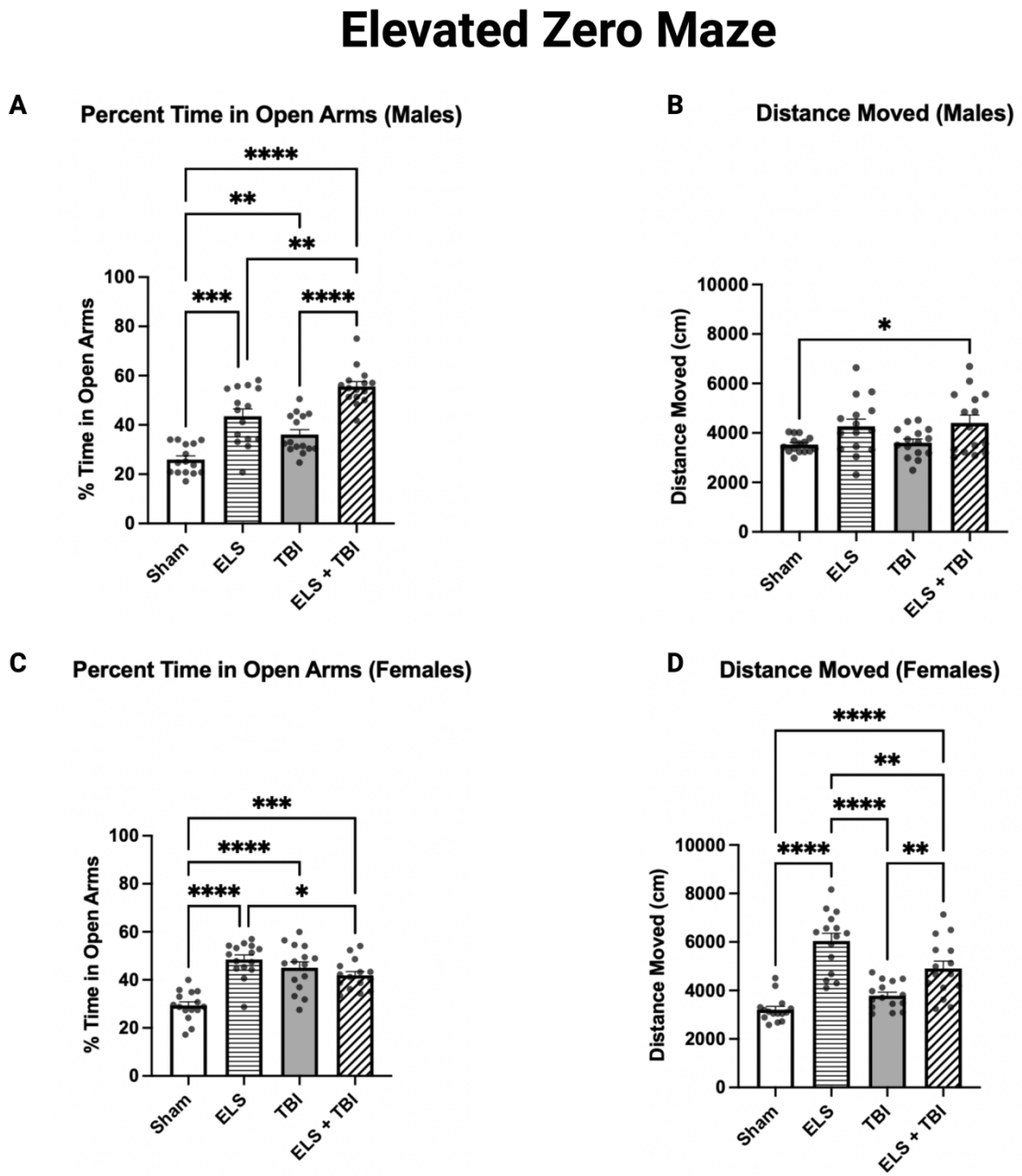


Figure 11. The elevated zero maze was used to assess anxiety-like behavior. ELS and TBI resulted in altered preference for open versus closed arms.
Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated.
A.) In males, TBI showed no difference in time spent in the open arms (**p<0.01). ELS resulted in more time spent in the open arms (***p<0.001). ELS + TBI showed more time spent in the open arms (****p<0.0001). Compared to TBI, ELS + TBI in males resulted in more time spent in the open arms (****p<0.0001).

B.) There was no difference in total distance moved between male TBI and sham animals (n.s.). ELS showed a similar distance moved (n.s.). ELS did not show a difference in distance traveled compared to sham (n.s.). ELS + TBI resulted in greater distance moved (*$p<0.05$). There was no difference in total distance traveled between ELS + TBI and TBI (n.s.).
C.) In females, TBI resulted in more time spent in the open arms (****$p<0.0001$). ELS showed more time spent in the open arms (****$p<0.0001$). ELS + TBI showed greater time spent in the open arms (***$p<0.001$). ELS + TBI spent a similar time in the open arms compared to TBI (n.s.).
D.) TBI females showed a similar distance moved. ELS resulted in greater distance moved (****$p<0.0001$). ELS + TBI showed a greater distance moved (****$p<0.0001$). ELS + TBI resulted in more total distance moved compared to TBI (**$p<0.01$).

3.4.2 Novel Object Recognition Test: Novelty Preference is impaired after TBI

Next, novelty processing and working memory was assessed using the novel object recognition test. A novelty preference index was used to determine the proportion of time each mouse spent with a novel object versus a familiar object (Fig. 12 A-B). TBI in males and females resulted in decreased preference for the novel object compared to sham controls (Fig. 12 C-D). Whereas female mice, exposed to ELS, showed less preference for the novel object compared to sham controls, there was no difference in novelty preference in males (Fig. 12 C-D). While, female mice, exposed to ELS+ TBI, showed less preference for novelty compared to TBI, a similar distinction was not evident in males (Fig. 12 C-D). These findings suggest that while TBI impairs novelty preference in both sexes, females are more vulnerable to deficits in novelty preference when ELS is introduced to the model.

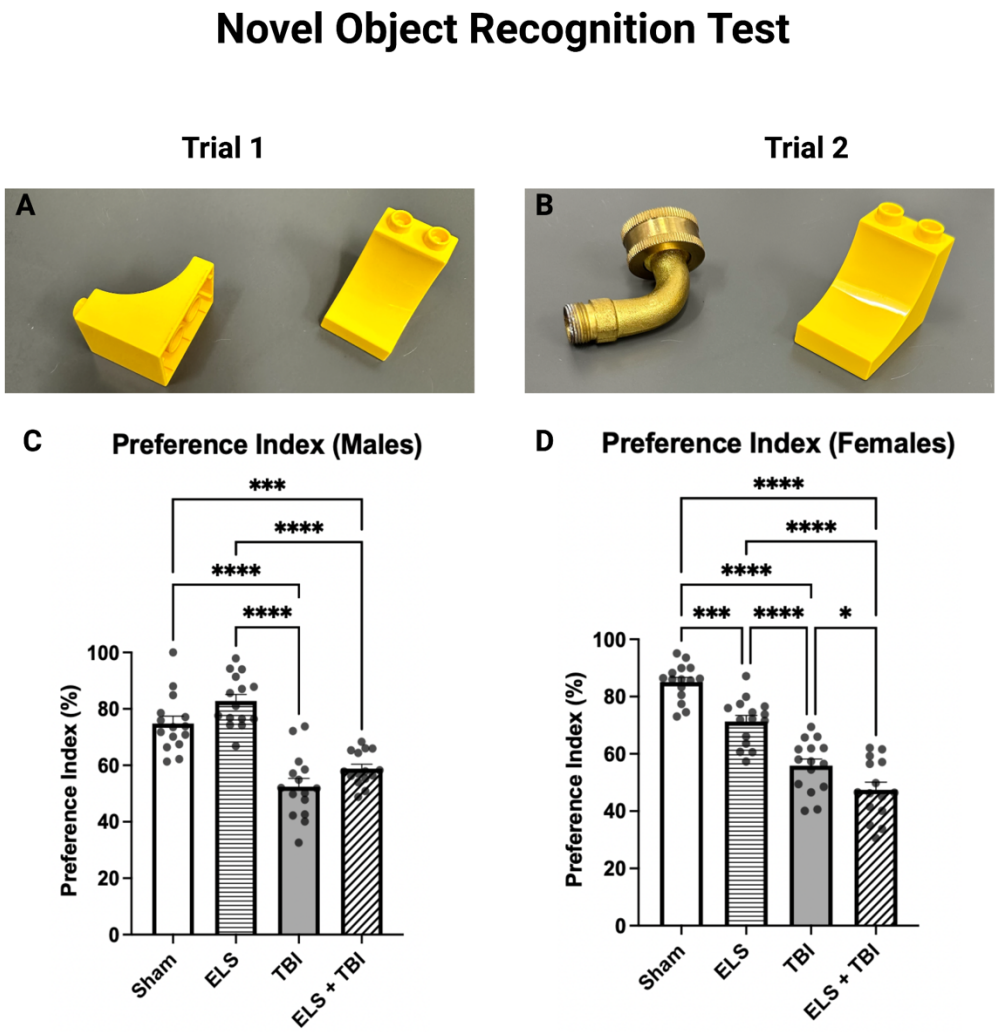


Figure 12. The novel object recognition test was used to evaluate learning and memory. A preference index, the time spent with a novel object versus a familiar object, was determined for each group.

A., B.) In trial 1, a mouse was exposed to 2 identical Duplo® blocks for 5 minutes (A). In trial 2, 1 block was replaced with a similar sized gold dishwasher elbow (B).

Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated.

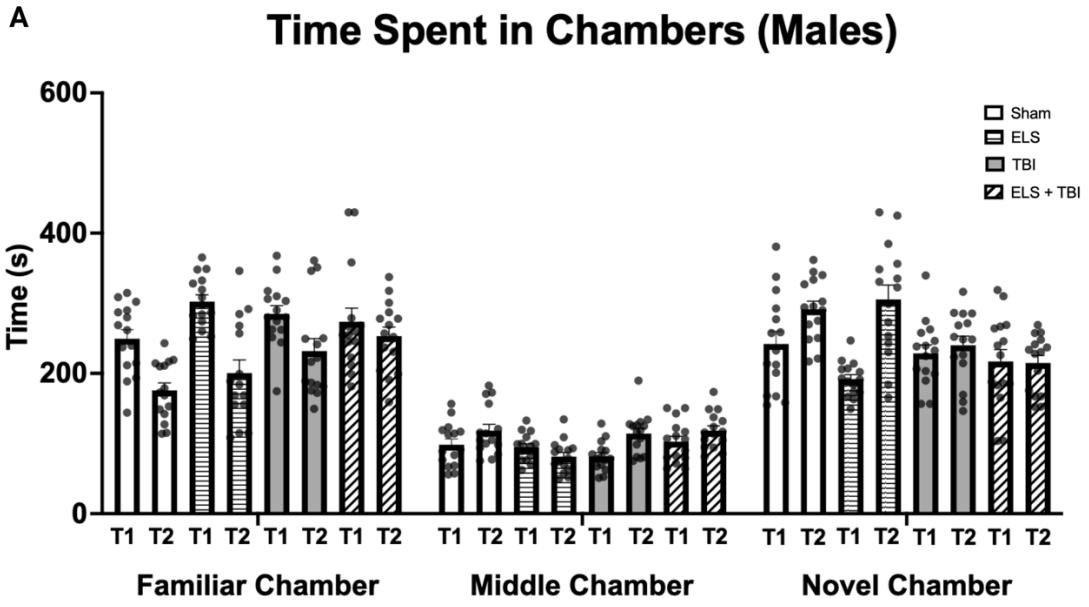
C.) In males, TBI resulted in a reduced time spent with the novel object (****p<0.0001). ELS did not show a difference in time spent with the novel object (n.s.). ELS + TBI resulted in decreased time spent with the novel object (***p<0.001). There was no difference in time spent with the novel object between ELS + TBI and TBI (n.s.).

D.) In females, TBI showed decreased time with the novel object (****$p<0.0001$). ELS resulted in less time spent with the novel object (***$p<0.001$). ELS + TBI decreased the time spent with the novel object (**** $p<0.0001$). ELS + TBI reduced time spent with the novel object compared to TBI (*$p<0.05$).

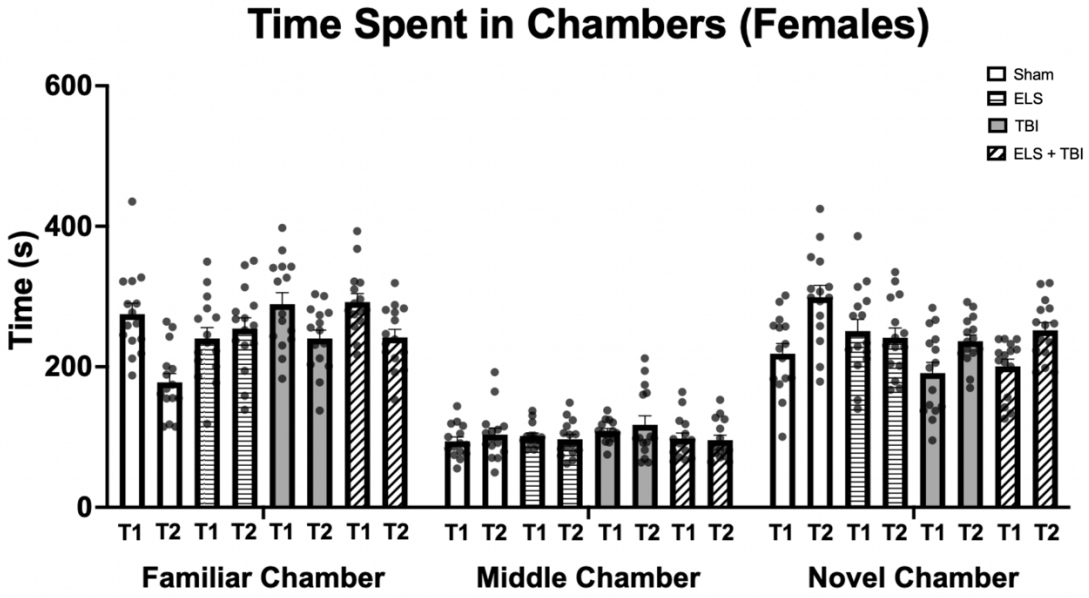
3.4.3 Three-Chamber Task: Social recognition is disrupted after TBI

The three-chamber task was used to test social recognition. We have previously shown decreased social novelty at long-term timepoints following a P21 TBI (Semple, Blomgren et al. 2013). The familiar chamber contained the familiar mouse (stimulus mouse 1), the novel chamber contained a new stimulus mouse, the middle chamber was the starting position for the assay and the mouse could freely cross the middle chamber during each trial. Raw data, based upon trials 1 and 2, summarize the amount of time spent in each chamber (Fig. 13A-B). Trial 2 determined if the test mouse can discriminate between the familiar mouse (familiar chamber) and the novel mouse (novel chamber). While TBI in males and females resulted in decreased social recognition compared to shams (Fig. 13 C-D), ELS did not impact social recognition in either males or females (Fig. 13 C-D). ELS prior to TBI in male and female mice did not show a difference in social recognition compared to TBI. Collectively, these findings suggest that social recognition is impaired by TBI but not ELS and when these insults are combined, the overall deficit is not further amplified.

A


Time Spent in Chambers (Males)
Time (s)
600
400
200
0
Sham
ELS
TBI
ELS + TBI
T1 T2 T1 T2 T1 T2 T1 T2 T1 T2 T1 T2 T1 T2 T1 T2 T1 T2 T1 T2 T1 T2 T1 T2
Familiar Chamber
Middle Chamber
Novel Chamber

B


Time Spent in Chambers (Females)
Time (s)
600
400
200
0
Sham
ELS
TBI
ELS + TBI
T1 T2 T1 T2 T1 T2 T1 T2 T1 T2 T1 T2 T1 T2 T1 T2 T1 T2 T1 T2 T1 T2 T1 T2
Familiar Chamber
Middle Chamber
Novel Chamber

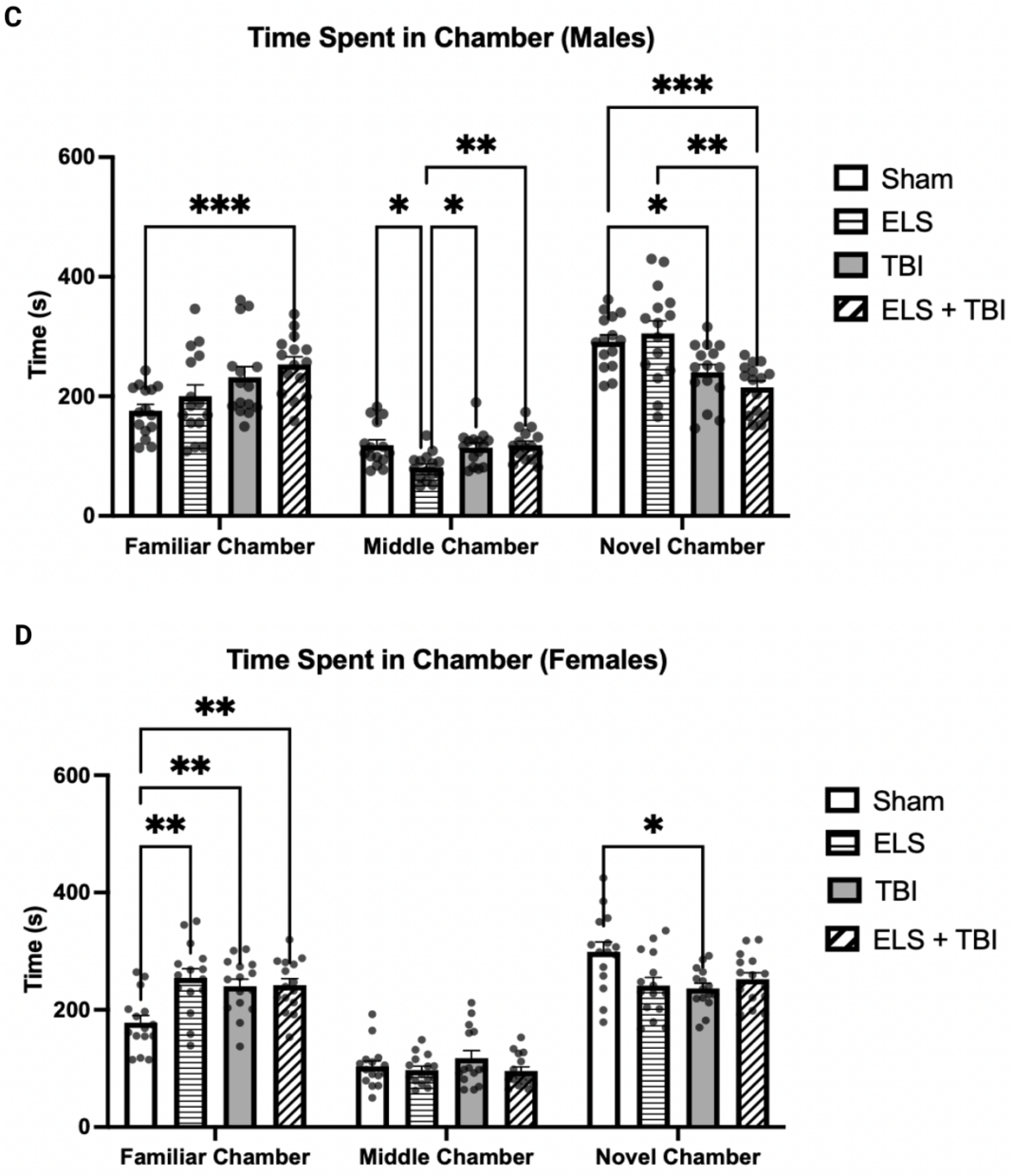


Figure 13. Social recognition was evaluated using the 3-chamber task
A-B.) A summary of the raw data from the 3-chamber task represents the time spent in each chamber during trial 1 (T1) and trial 2. (T2).

Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *$p<0.05$ significance threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated.

C.) In males, TBI reduced the amount of time spent in the novel chamber (*p<0.05). ELS showed similar time spent in the novel chamber (n.s.). ELS + TBI resulted in less time in the novel chamber (***p<0.001). There was no difference between time spent in the novel chamber between ELS + TBI and TBI (n.s.). D.) In females, TBI resulted in less time spent in the novel chamber (*p<0.05). ELS and ELS + TBI did not show a difference in time spent in the novel chamber (n.s.). There was no difference in time spent in the novel chamber between ELS + TBI and TBI (n.s.).

3.4.4 ELS and TBI impair spatial and contextual memory

Spatial and contextual learning and memory were assessed by the Barnes Maze. In each group, males and females learned the location of the target hole by the last acquisition day (Fig. 14 A, E). On the probe trial day, TBI in males and females resulted in a longer latency to locate the target hole compared to shams (Fig. 14 B, F). A similar finding was seen in females when exposed to ELS (Fig. 14 B, F). However, when ELS preceded TBI there were no differences, when compared to TBI in either sex (Fig. 13 B,F).

Time spent at the target hole was also used to measure spatial memory (Supplemental Figure 1). TBI alone and ELS alone resulted in a reduced time spent at the target hole in both sexes, compared to shams (Fig. S1A, B). Interestingly, only male mice, exposed to ELS + TBI spent more time at the target hole when compared to TBI alone (Fig. Supplemental 1B).

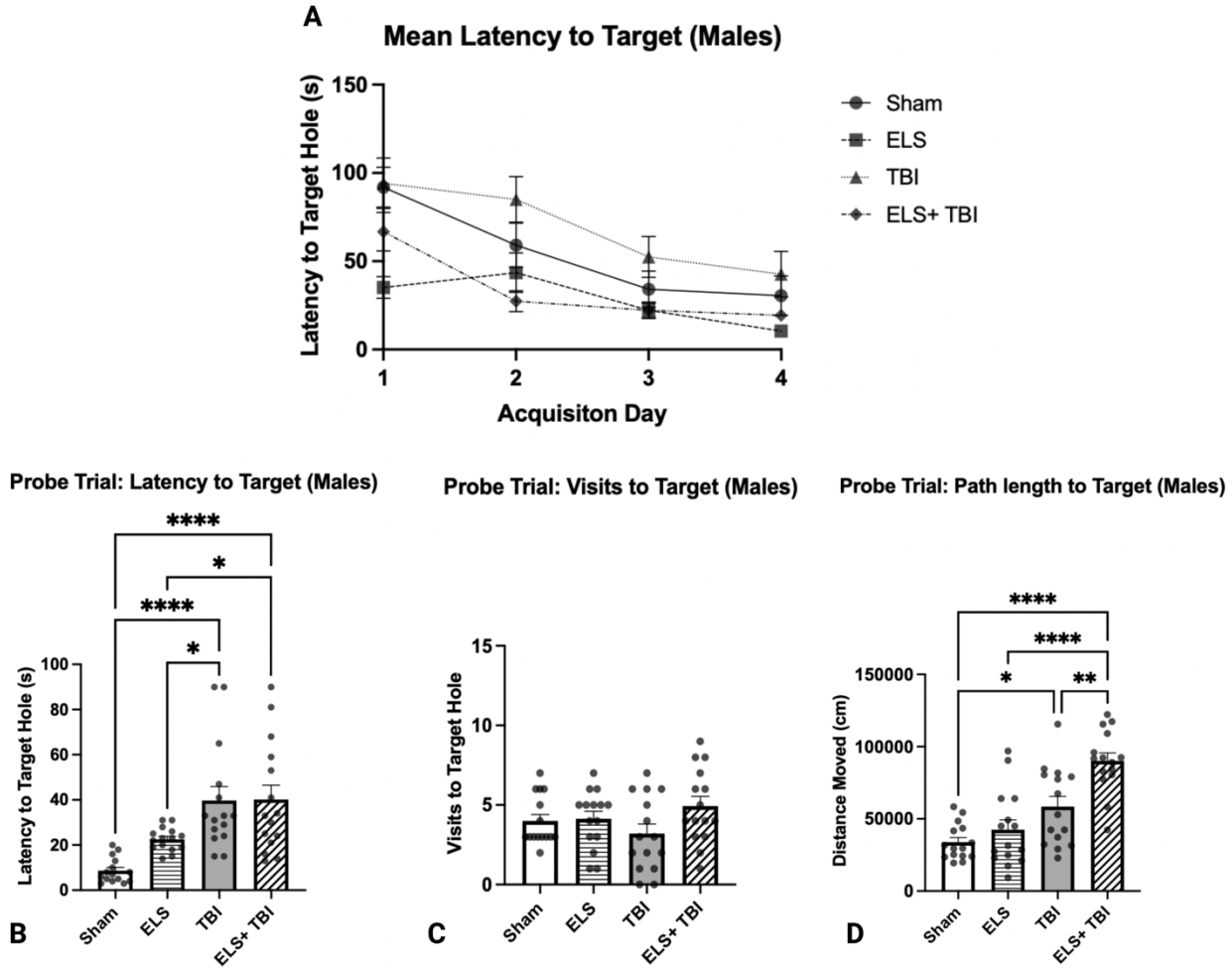
Visits to the target hole were also quantified on the probe trial day. While males did not show any differences in visits to the target hole between any condition, females, when exposed to ELS, visited the target hole significantly less than shams (Fig. 13 C, G).

Path length to the target hole was also quantified on the probe trial day. ELS showed no difference in the path length to the target hole compared to shams in males and females (Fig. 13 D, H). TBI in males resulted in a longer path length to the target hole compared to shams, but

females did not show this difference (Fig. 13 D, H). ELS prior to TBI in males and females took longer path lengths to the target hole compared to TBI (Fig. 13 D, H).

Path length to the target hole was complimented by the total distance traveled to assess overall activity on the probe trial day (Supplemental Figure 2). Exposure to ELS resulted in a greater total distance traveled compared to sham in both sexes (Fig. S2A, B). This same effect was seen in TBI, in which TBI males and females traveled around the maze more than sham (Fig. S1A, B). ELS prior to TBI did not contribute to the distance traveled in TBI males and females (Fig. S1A, B). Overall, these results indicate that ELS and TBI result in a memory but not learning deficit and ELS prior to TBI contributes to this impairment.

Barnes Maze Test



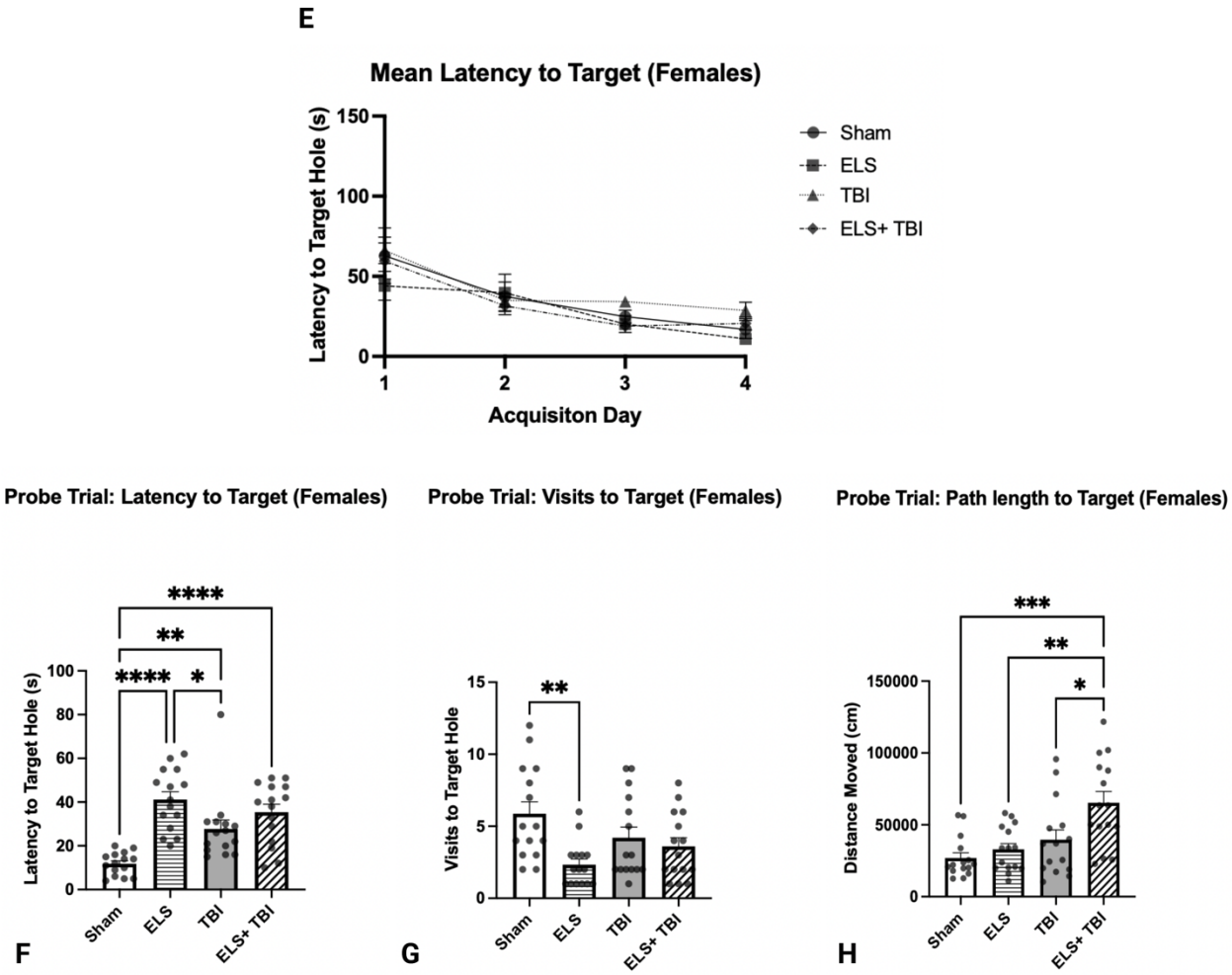


Figure 14. The Barne's maze was used to test spatial and contextual learning and memory.
A.) All male mice learned the location of the target hole by the last acquisition day (N=15/group, simple linear regression).
Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated.

B.) On probe trial day, TBI resulted in a greater latency to the target (****p<0.0001). ELS showed a similar latency to the target hole (n.s.). ELS + TBI showed a longer latency to find the target (****p<0.0001). ELS + TBI did not show a difference in latency to find the target hole compared to TBI (n.s.). C.) No differences in number of visits to the nest was seen in male mice. D.) In females, TBI resulted in greater path length (*p<0.05). ELS showed a similar path length to the target (n.s.). ELS + TBI showed a greater path length to the target hole (****p<0.0001). ELS + TBI resulted in greater path length compared to TBI (**p<0.01).
E.) Female mice from all groups learned the location of the target hole by the last acquisition day N=15 females/group, simple linear regression).

F.) On probe trial day, TBI in females resulted in greater latency to the target (**p<0.01). ELS showed longer latency to the target hole (****p<0.0001). ELS + TBI resulted in higher latency to the target hole (****p<0.0001). ELS + TBI in females did not contribute to a longer latency to target hole compared to TBI (n.s.).
G.) In females, TBI made a similar number of visits to the target hole. ELS made less visits to the target hole (**p<0.01). ELS + TBI did not show a difference in the number of visits made to the target hole (n.s.). ELS + TBI made a similar number of visits to the target hole compared to TBI.
H.) TBI and ELS in females showed no difference in path length to find the target hole compared to sham (n.s., for both comparisons). ELS + TBI showed a longer path length to the target hole (***p<0.001) and ELS + TBI resulted in longer path lengths to find the target hole compared to TBI (*p<0.05).

Behavior Summary

Behavior	Measure	Outcome Males	Outcome Females
Open Field Test	% Time in Inner Zone	**ELS:** ↑ **TBI:** ↓ **ELS+TBI:** No Change	**ELS:** ↓ **TBI:** ↓ **ELS+TBI:** No Change
	Distance Moved	**ELS:** No Change **TBI:** ↑ **ELS+TBI:** ↓	**ELS:** ↑ **TBI:** No Change **ELS+TBI:** ↑
Elevated Zero Maze	% Time in Open Arm	**ELS:** ↑ **TBI:** ↑ **ELS+TBI:** ↑	**ELS:** ↑ **TBI:** ↑ **ELS+TBI:** No Change
	Distance Moved	**ELS:** No Change **TBI:** No Change **ELS+TBI:** No Change	**ELS:** ↑ **TBI:** ↓ **ELS+TBI:** ↑
Novel Object Recognition	Novelty Preference	**ELS:** No Change **TBI:** ↓ **ELS+TBI:** No Change	**ELS:** ↓ **TBI:** ↓ **ELS+TBI:** ↓
Three-Chamber Task	Social Novelty Preference	**ELS:** No Change **TBI:** ↓ **ELS+TBI:** No Change	**ELS:** No Change **TBI:** ↓ **ELS+TBI:** No Change
Barnes Maze	Spatial Memory (Latency to Target)	**ELS:** No Change **TBI:** ↑ **ELS+TBI:** No change	**ELS:** ↑ **TBI:** ↑ **ELS+TBI:** No Change

Table 4. Summary of behaviors at adulthood. Behaviors measured at adulthood are summarized above. Outcome comparisons are the following: ELS = ELS vs sham; TBI = TBI vs sham; ELS + TBI = ELS + TBI vs TBI. The arrows in the OFT and EZM indicate an increase or decrease in the time spent or distance moved. For the NOR and 3-chamber task a down arrow indicates decreased performance in each task. In the Barnes Maze, an up arrow indicates decreased performance.

3.4.5 Groups of behavior that go together, stay together

Correlation Matrices were used to compare learning and memory outcomes (Fig. 14 A, C) and anxiety-like behaviors/overall activity (Fig. 14 B, D). In both males and females, the learning and memory-related outcomes/scores are related to the other. For example, in the male learning and memory matrix (Fig. 14 A), the lower the novel object preference index score, the greater the latency to find the target hole. Anxiety-like behaviors show an interesting correlation and it a bit more complex. For example, in the female anxiety-related behavior matrix (Fig. 14 D), the more time spent in the outer zone in the Open Field correlates with more time in the open arm of the Elevated Zero Maze. But more distance traveled in the Open Field was positively correlated with the distance traveled in the Elevated Zero Maze. Although complex, these particular matrices accurately describe the trends in males and females for each set of behaviors.

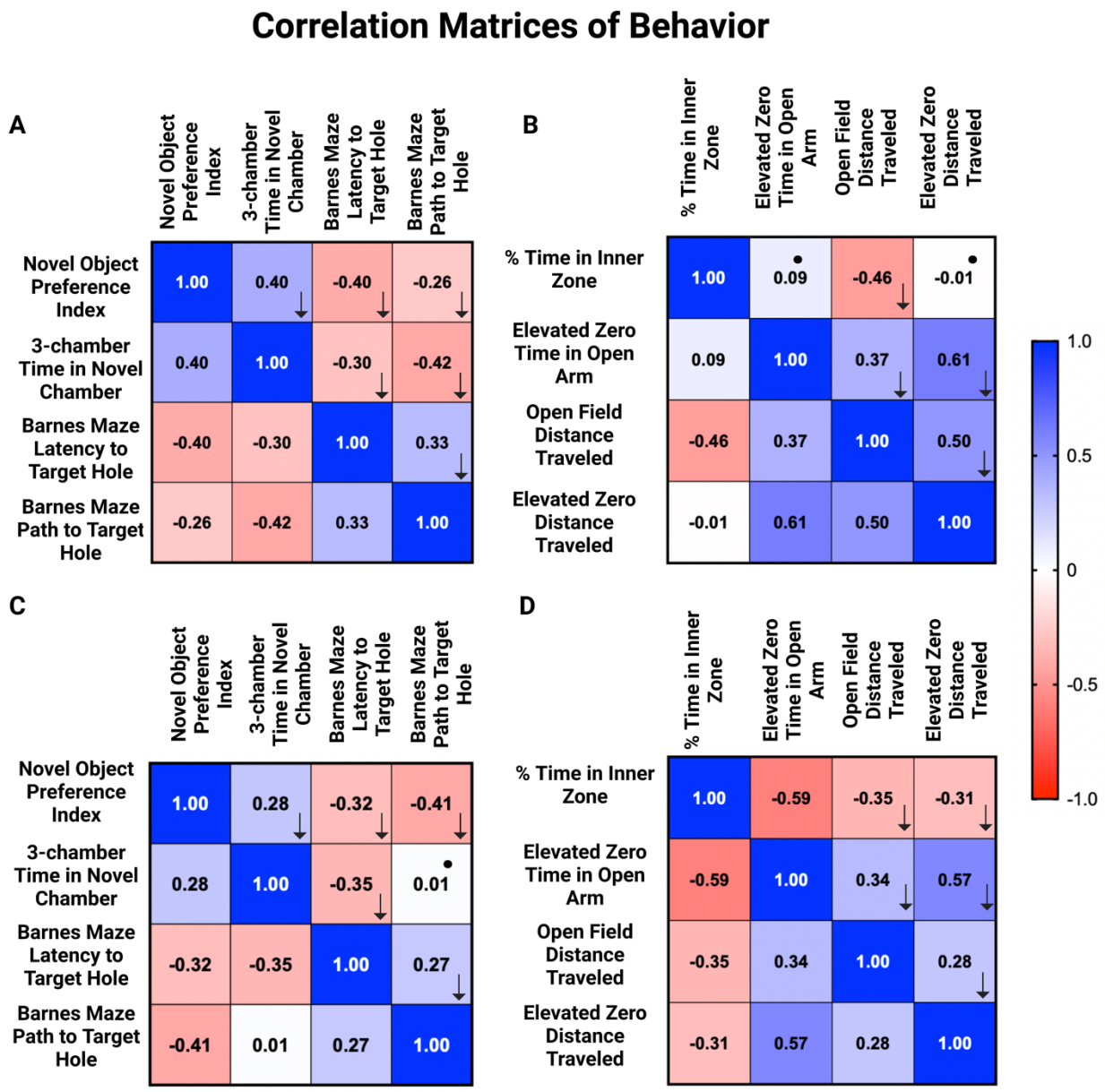


Figure 15. Learning and memory and anxiety-like behaviors were evaluated at adulthood.

Correlation matrices were used to summarize learning and memory related behaviors in A.) Males and C.) Females and measures of anxiety and overall activity in B.) Males and D.) Females. Outcomes from each category of behavior are correlated with one another. Pearson's r correlation coefficients are reported in each square (-1.0 to +1.0). The shades of red indicate a significant negative correlation (-1.0, $p<0.05$), shades of blue indicate a significant positive correlation (+1.0, $p<0.05$). A black dot in a square indicates non-significance and a down arrow indicates a decreased performance in both groups.

3.5 The hippocampus at adulthood

EPCP4, used to visualize CA2, and NeuN antibodies were used to quantify neuronal density in the subzones of the injured hippocampus. TBI resulted in a reduction of neurons in the CA1, CA2, and CA3, compared to the sham control for males and females (Fig. 15 A-F). ELS caused a loss of neurons in CA1, CA2, and CA3 compared to sham for females (Fig. 15 D-E), but only CA1 and CA3 in males (Fig. 15 A, C). Prior exposure of ELS to TBI caused a loss of neurons only in the CA1 in females compared to TBI (Fig. 15 D), and no additional loss of neurons in the other areas for males and females compared to TBI.

These results indicate that while TBI contributes to neuronal loss in the pyramidal cell subzones, ELS overall does not amplify this deficit.

Volumetric loss was evaluated in the ipsilateral cortex and hippocampus in mice exposed to ELS, TBI, and ELS in combination with TBI. TBI resulted in a volumetric loss in the cortex and hippocampus compared to sham controls in males and females (Fig. 16 A-D). While ELS resulted in hippocampal and cortical volumetric loss compared to shams, this was limited to in females. (Fig. 16 A-D). ELS in combination with TBI, compared to TBI, did not result in volume loss in the cortex and hippocampus for both males and females. (Fig. 16 A-D). These results indicate that TBI results in ipsilateral cortical and hippocampal atrophy but when combined with TBI, ELS does not worsen this effect.

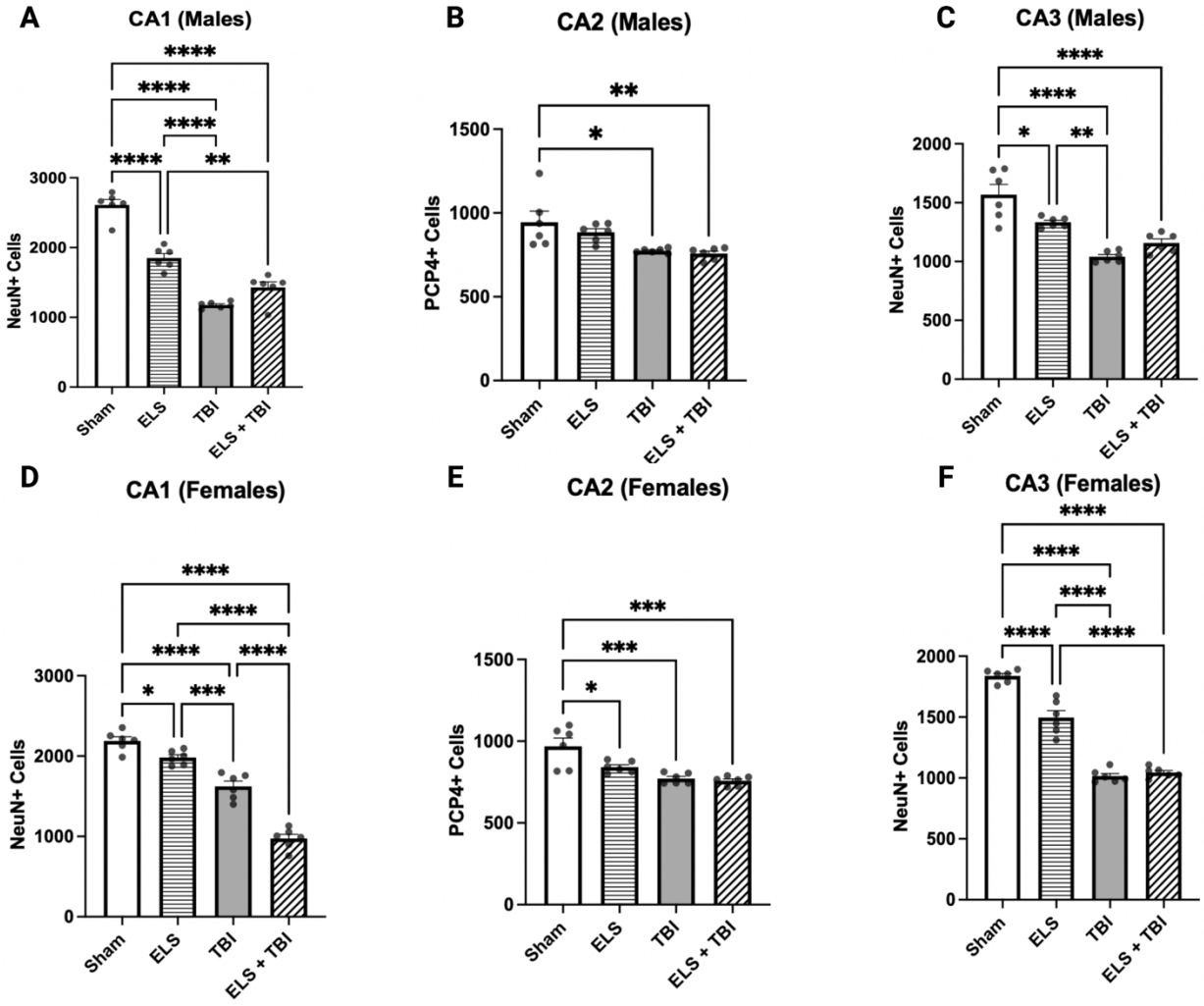


Figure 16. Stereological techniques were used to quantify neurons in CA1, CA2, and CA3.

Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated.

In males, TBI resulted in decreased labeling of NeuN+ in the A.) CA1 (****p<0.0001), decreased PCP4+ cells in the B.) CA2 (*p<0.05), and decreased NeuN+ cells in the C.) CA3 (****p<0.0001). ELS decreased labeling of NeuN+ cells in the A.) CA1 (****p<0.0001) and C.) CA3(*p<0.05). ELS + TBI showed less NeuN+ labeling in the A.) CA1 (****p<0.0001), PCP4+ cells in the B.) CA2 (**p<0.01), and NeuN+ cells in the C.) CA3 (****p<0.0001). ELS + TBI did not show differences in labeling in the A.) CA1 (n.s.), B.) CA2 (n.s.), and C.) CA3 (n.s.) compared to TBI. In females, TBI showed less NeuN+ labeling in the D.) CA1(****p<0.0001),

PCP4+ labeling in the E.) CA2 (***p<0.001), and NeuN+ cells in the F.) CA3 (****p<0.0001). ELS decreased NeuN+ cells in the D.) CA1 (*p<0.05), PCP4+ cells in the E.) CA2. (*p<0.05), and NeuN+ cells in the F.) CA3 (****p<0.0001). ELS + TBI resulted in decreased NeuN+ labeling in the D.) CA1 (****p<0.0001), PCP4+ labeling in the E.) CA2 (***p<0.001), and NeuN+ cells in the F.) CA3 (****p<0.0001). ELS + TBI decreased NeuN+ in the D.) CA1 (****p<0.0001), compared to TBI.

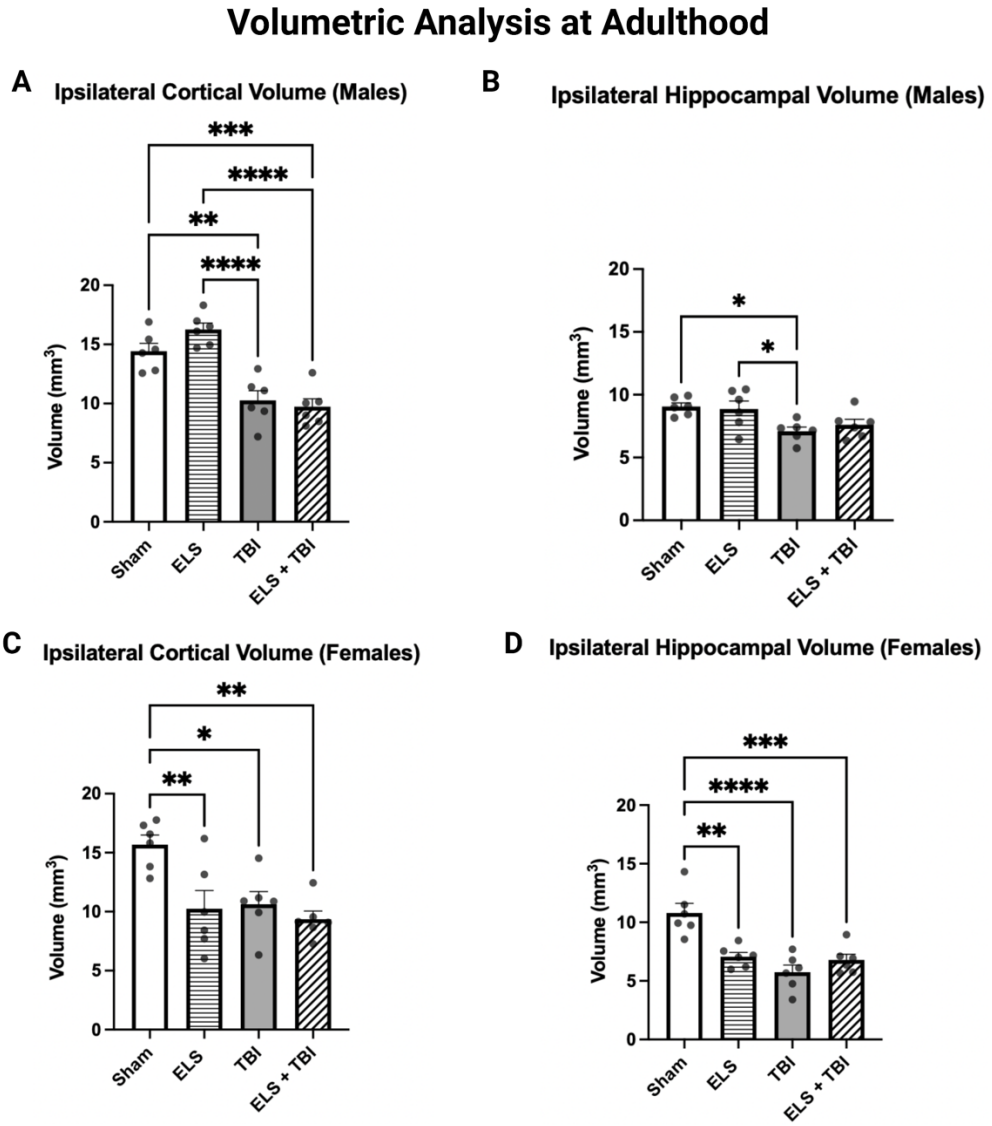


Figure 17. At adulthood, ELS+TBI resulted in similar cortical and hippocampal volumetric loss when compared to TBI alone.

Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance

threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated.

A.) In males, TBI resulted in a decrease in cortical volume (**p<0.01). ELS did not show a difference in cortical volume (n.s.). ELS + TBI showed cortical volume loss (***p<0.001). ELS + TBI did not show differences in cortical reduction compared to TBI. B.) TBI resulted in less hippocampal volume (*p<0.05). ELS did not show a difference in hippocampal volume (n.s.). ELS + TBI also did not show a difference in hippocampal volume (n.s.). C.) In females, TBI resulted in less cortical volume (**p<0.01). ELS reduced cortical volume (**p<0.01). ELS + TBI resulted in cortical loss (**p<0.01). There were no differences in cortical volume loss between ELS+ TBI and TBI. D.) TBI resulted in less hippocampal volume(****p<0.0001). ELS resulted in hippocampal (**p<0.01). ELS + TBI resulted in hippocampal loss (***p<0.001). No differences in hippocampal volume were seen between ELS + TBI and TBI (n.s.).

3.5.1 Long-term hippocampal structural deficits are correlated to hippocampal-dependent functional deficits after ELS and TBI

Pearson's r Correlation matrices were used to examine the degree to which neuronal cell loss and hippocampal volumetric loss at adulthood were related to hippocampal-dependent functional deficits (Figure 17). Most structural changes in the in the injured brain corresponded to volumetric loss. For example, in the matrix describing males, (Fig. 18 A), CA2 neurons were positively correlated with the time spent in the novel chamber Three-Chamber Task, suggesting a relationship between time spent with the novel mouse and number of CA2 neurons. In the matrix describing females, (Fig. 18 B), there is a negative correlation between CA1 neurons and Barne's Maze path length to the target hole. This suggests that with fewer neurons in less CA1, there is a longer path length to find the target hole. The matrix outputs indicate that the structural impairments in the hippocampal subzones are related to the deficits seen in the hippocampal-dependent behaviors and that each subregion neuron reduction corresponds to the deficit of its role in learning and memory.

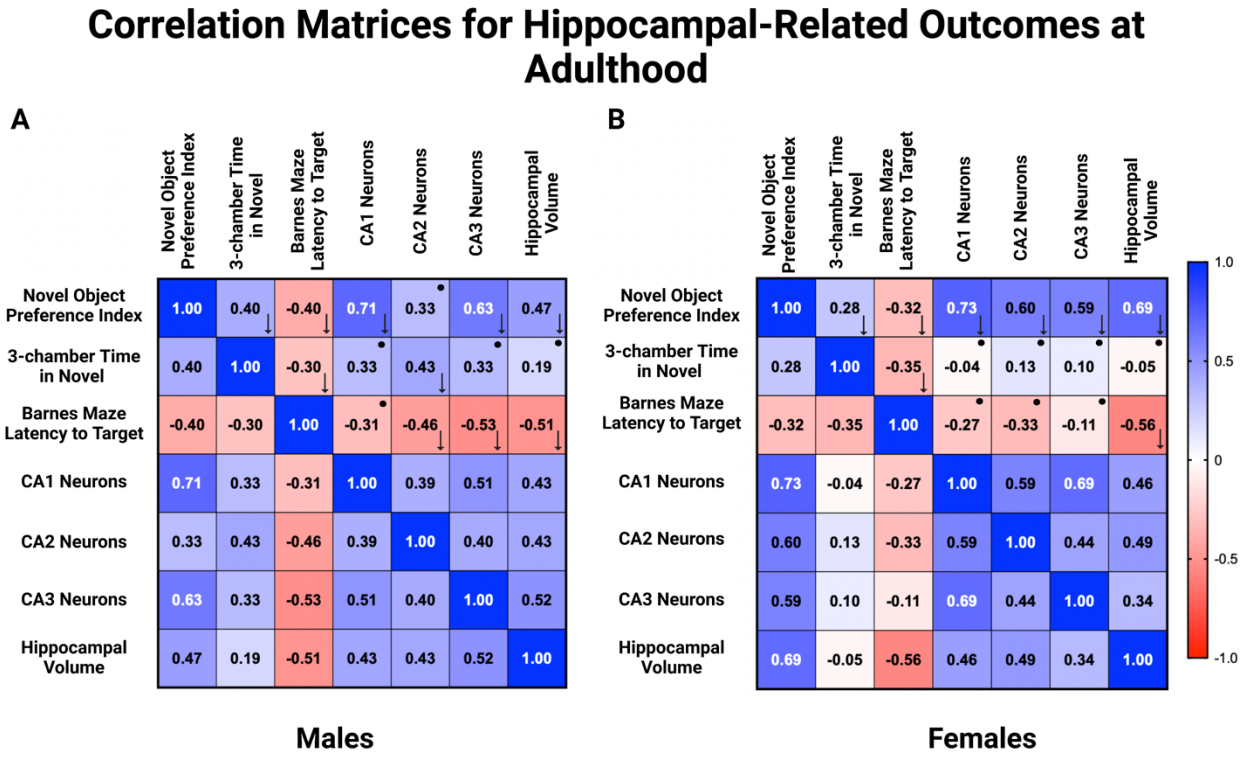


Figure 18. Hippocampal-dependent behaviors were correlated with neuronal loss in hippocampal CA1, CA2, and CA3 and cortical and hippocampal volumetric loss.
A, B.) Correlation matrices were used to summarize hippocampal dependent behaviors, assayed at adulthood, with ipsilateral cortical and hippocampal volumes and hippocampal subfields in males (A) and females (B). Pearson's r correlation coefficients are reported in each square (-1.0 to +1.0). Shades of red and blue indicate either a significant negative correlation (-1.0, $p<0.05$) or a significant positive correlation (+1.0, $p<0.05$), respectively. A black dot in a square indicates non-significance and a down arrow indicates a decrease in performance and a decrease in the number of neurons.

3.6 Acute pathogenesis after ELS and TBI is related to hippocampal subzone neuron loss at adulthood

Pearson's r correlation matrices were used to examine the relationship between the pathogenic response seen at P22 in the hippocampus and neuronal loss seen in hippocampal subregions at adulthood (Fig. 19). At P22, we examined microglial density and apoptosis via caspase-3. Using the correlation matrix, we assessed the relationship between acute microglial density and apoptosis in the subregions. The matrix indicates a strong positive correlation between microglial density and apoptosis. This suggests that the more microglia in the included subregions

correlates with greater apoptosis. The relationship between acute pathogenic outcomes and adulthood neuronal cell loss was also evaluated. There is a strong negative correlation between microglial density and cell death acutely and the number of neurons in the CA1, CA2, and CA3 subzones at adulthood. Such findings suggest that increased microglial density in each subregion at P22, correlates with fewer neurons at adulthood. Additionally, the acute pathogenic changes in hippocampal subregions may serve as early biomarkers of long-term neuronal loss in the hippocampus and may predict behavioral outcomes.

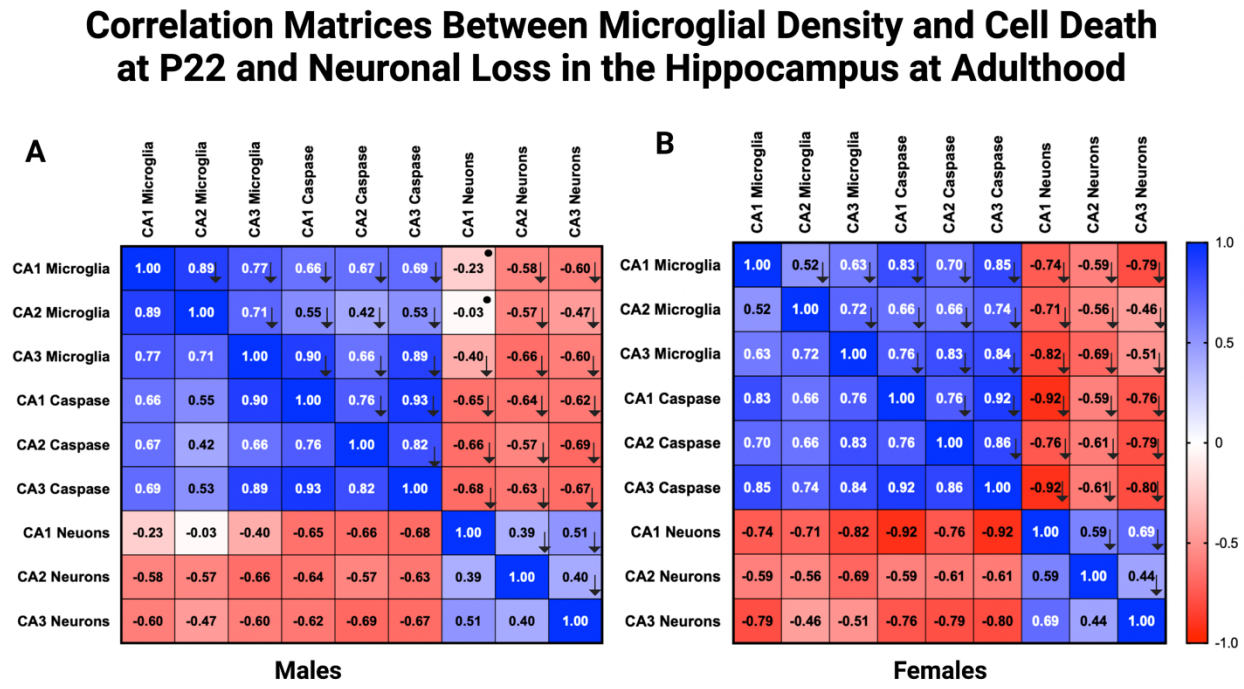


Figure 19. Indices of acute pathogenesis at P22 (microglial activation, increased caspase-3) were correlated with a reduction in neurons in hippocampal subfields at adulthood.
Correlation matrices were used to examine the temporal relationship between ELS and ELS+ TBI in A.) males and B.) females. Pearson's r correlation coefficients are reported in each square (-1.0 to +1.0) and a heat map was generated to visualize correlation strength between variables. shades of red indicate a significant negative correlation (-1.0, $p<0.05$), shades of blue indicate a significant positive correlation (+1.0, $p<0.05$). A black dot in a square indicates non-significance and a down arrow indicates an adverse relationship between the correlated outcomes.

Chapter 4: Discussion

Children who have sustained moderate to severe TBIs often present with deficits in learning, memory and sociality (Prins, Greco et al. 2013, Serpa, Ferguson et al. 2021). While advanced imaging has offered insights into volumetric changes in the cortex after a pediatric TBI (Lindsey, Wilde et al. 2019), few studies have considered how an early age TBI may lead to long-term, sustained deficits at adulthood (CDC reference). Recovery in this context is hampered by the interaction between injury-related processes that contribute to neurodegeneration which, in turn, are superimposed on brain development. Moreover, given the heterogeneity of these injuries, based upon imaging studies (Lindsey, Wilde et al. 2019), clinical trials are faced with the challenge of increasing size of recruitments.

To address this unmet need for longitudinal studies, an early age preclinical model of TBI was used to assess learning, memory and sociality, each of which are indices of quality of life in adults who have sustained an early age TBI. This study also considered ELS, defined in part by fragmental maternal care, that may alter the developmental trajectory of recovery after TBI and has been linked to later life psychiatric symptoms (Spadoni, Vinograd et al. 2022).

4.1 Cytokines after ELS and TBI

Cytokines in the developing brain play a role in neural development, including neuronal differentiation (Araujo and Cotman 1995, Schneider, Pitossi et al. 1998, Serpa, Ferguson et al. 2021). Importantly, proper neurodevelopment, relies, in part, on the balance of pro and anti-inflammatory cytokines. This is exemplified in studies that report disruption of these natural fluctuations in IL-1B, IL-1α, IL-6, IFNγ, and TNFα acutely after a TBI in both the young and adult brain (Sandhir, Puri et al. 2004, Perez-Polo, Rea et al. 2013, Chhor, Moretti et al. 2017, Taib, Leconte et al. 2017, Serpa, Ferguson et al. 2021). While a TBI results in a robust increase

in these cytokines in the cortex in both age groups during the acute phase post injury (Perez-Polo, Rea et al. 2013, Chhor, Moretti et al. 2017, Taib, Leconte et al. 2017, Serpa, Ferguson et al. 2021), this response is higher in the young brain (Sandhir, Puri et al. 2004, Semple, O'Brien et al. 2017, Serpa, Ferguson et al. 2021), namely in the cortex. This raises the possibility that the young brain may be particularly vulnerable to a TBI due to a more exaggerated inflammatory response.

In this study cytokines were differentially expressed in the cortex in response to TBI, ELS or ELS prior to TBI. IL-1B, IL-6, TNFα, IFNγ, IL-1α, and IL-10, were measured in homogenates of the cortical mantle at 1-day postinjury. Both ELS alone and TBI alone resulted in an increase in IL-1B in males and females at P22. These findings parallel with what is reported in clinical studies of ELS and TBI (Li and Liu 2013, Nwachuku, Puccio et al. 2016, Fogelman and Canli 2019, Sun, Bai et al. 2019, Doganyigit, Erbakan et al. 2022).

The present study showed a pronounced expression of IL-1B, compared to other cytokines, in the injured cortex. The clinical field has gained a better understanding of the underlying mechanisms behind neuroinflammation following a TBI and how to best utilize biomarkers in identifying this type of inflammation. Nucleotide oligomerization domain (NOD)-like receptor P3 (NLRP3) is part of the NLR family and plays an important role in regulating innate immunity (O'Brien, Pham et al. 2020). NLRP3 in particular is referred to as an "inflammasome" or a multiprotein complex that assembles in response to stress-associated stimuli (de Zoete, Palm et al. 2014) and specifically lead to the activation of IL-1B. In the context to TBI, NLRP3 in which it sends priming signals to signal transcriptional up-regulation of itself. Then, sends activation signals to activate the immune system to respond to the mechanical TBI (Bauernfeind, Horvath et al. 2009, Swanson, Deng et al. 2019, O'Brien, Pham et

al. 2020). This two-step priming and activation process is said to be the initial cascade of inflammatory events following at TBI and eventually leads to the conversion of inactive IL-1B to active IL-1B. Ultimately, active IL-1B is released in the tissue and serum and acts as a signal to microglia and promoters of cell death.

The concern of early neuroinflammation in the young brain, in which childhood injuries show elevated IL-1B (Giza and Prins 2006, Semple, Blomgren et al. 2013), and adulthood injuries show a strong correlation between acutely elevated IL-1B levels and long-term neuronal damage after injury and the functional deficits to follow (Nwachuku, Puccio et al. 2016, Liu and Quan 2018, Sun, Bai et al. 2019, Doganyigit, Erbakan et al. 2022). The elevation of IL-B has been reported in the cortex by others, in the context of adult TBIs in both humans and preclinical models. TBI patients evaluated within 24 hours of the injury show robust expression in IL-1B and can persist up to 5 days after the injury (Holmin and Hojeberg 2004). Preclinical models have expanded these with the utilization of inflammatory interventions and show that the neutralization of IL-1B can ameliorate cell loss and additional inflammation (Ozen, Ruscher et al. 2020), cell loss, microglial activation, and long-term impairment (Semple, O'Brien et al. 2017). Longitudinal clinical studies of ELS also show elevated IL-1B levels into adulthood and is the most prominent with patients with depression (Liu, Croft et al. 2013, Fogelman and Canli 2019), a disease state heavily studied in clinical ELS (Kessler and Magee 1994, Kaplow and Widom 2007, Saleh, Potter et al. 2017, Syed and Nemeroff 2017, Smith and Pollak 2020). Thus, while the present study finds support prior pre-clinical and clinical, they also suggest that IL-1B may be the most sensitive pro-inflammatory cytokine to TBI and ELS, and perhaps is an essential mediator in the cascade of inflammatory events to follow.

My studies addressed the pro-inflammatory environment in both males and females at P22 in the context of 3 major conditions, TBI, ELS and ELS+TBI. At P22, corresponding to 1-day after TBI, there was a broad expression of multiple cytokines, IL-1B, IL6, TNFα, IFNγ and IL-1α, in both males and females. In contrast, mice exposed to ELS only or ELS in combination with TBI resulted in a more limited expression of cytokines (IL-1B, TNFα, IL-10) that was restricted to males only. Such findings highlight the complexity of the acute, post-injury environment, characterized by a robust pro-inflammatory response in both sexes. While ELS alone resulted in a more modest response, it does suggest that ELS, limited to P2 to P9, has a sustained effect during subsequent brain development.

These findings raise an important question regarding the duration of the pro-inflammatory response after ELS. To the best of my knowledge, there is only one longitudinal study of ELS using the LBN model (Hoeijmakers, Ruigrok et al. 2017). The pro-inflammatory cytokines IL-1B, IL-6, and TNFα, as measured by mRNA, were evaluated at P9, when animals were returned to standard housing, and 4- and 10-months post LBN. While IL-1B mRNA was elevated at P9, there was no evidence of elevated cytokines at the later time points (Hoeijmakers, Ruigrok et al. 2017). In contrast, studies of other ELS models, such as maternal stress, report an elevation of pro-inflammatory cytokines in the hippocampus, serum, and prefrontal cortex, that remained elevated beyond the ELS exposure, extending into young adulthood (Wang, Jiao et al. 2011, Delpech, Wei et al. 2016, Reus, Fernandes et al. 2017, Amini-Khoei, Haghani-Samani et al. 2019, Nouri, Hashemzadeh et al. 2020, Lorigooini, Boroujeni et al. 2021). Together, these findings raise questions about the concept of immune priming (Danese, Pariante et al. 2007) that results in prolonged exposure to inflammatory mediators. The durability of early life stress, as

determined by indices of inflammation, may be related to the nature of this insult and based upon my findings, may differ between males and females.

4.2 Regional vulnerability of the hippocampus

Reactive microglia and apoptosis, commonly found in proximity to a primary cortical insult, are also evident within subcortical structures including the hippocampus, thalamus, corpus callosum, dorsal striatum, and cortex (Loane, Kumar et al. 2014, Loane and Kumar 2016) Tsuru (Tsuru-Aoyagi, Potts et al. 2009, Chen, Noble-Haeusslein et al. 2013, Hanlon, Raghupathi et al. 2019). Rodent CCI models (Tsuru-Aoyagi, Potts et al. 2009, Chen, Noble-Haeusslein et al. 2013) and closed head injury models (Hanlon, Raghupathi et al. 2019) address inflammation and cell death (apoptosis) in the immature brain (Tsuru-Aoyagi, Potts et al. 2009, Chen, Noble-Haeusslein et al. 2013, Hanlon, Raghupathi et al. 2019). Such findings indicate that the areas such as the dorsal striatum and corpus callosum (Hanlon, Raghupathi et al. 2019) and hippocampus (Tsuru-Aoyagi, Potts et al. 2009, Chen, Noble-Haeusslein et al. 2013) show greater levels of activated microglia following injury, and this has also been associated with greater cell death via apoptosis. This position has been supported by prior work from my lab, which studied the overexpression of the antioxidant glutathione peroxide (GPx). Overexpression of GPx resulted in a reduction in activated microglial density and apoptosis following an early-aged injury (Tsuru-Aoyagi, Potts et al. 2009) and was associated with better long-term behavioral recovery (Tsuru-Aoyagi, Potts et al. 2009).

The hippocampus in rodents is vulnerable to both ELS (Bath, Manzano-Nieves et al. 2016, Walker, Bath et al. 2017, Lumertz, Kestering-Ferreira et al. 2022) and focal cortical injuries (Shultz, Bao et al. 2012, Prins, Greco et al. 2013, Semple, Blomgren et al. 2013).

However, a clear delineation of this vulnerability has yet to be understood within the CA1, CA2 and CA3 subregions of the hippocampus. To address this challenge, microglial density and apoptosis in defined hippocampal CA subregions served as indicators of secondary pathogenesis. In my study, each subregion was first defined: CA2 was immunolabeled using an antibody directed against Purkinje cell protein 4 (PCP4), (Hitti and Siegelbaum 2014, San Antonio, Liban et al. 2014, Tzakis and Holahan 2019, Peng, Gu et al. 2023), and CA1 and CA3 were identified using NeuN, an antibody directed against neuronal nuclei. With these regional markers, Iba-1+ microglia and Caspase-3+ cells were then quantified within each region. As might be expected, while all experimental conditions were associated with an increase in microglial density and apoptosis in the hippocampus, there were differences within subregions. For example, TBI resulted in an increase in microglial density in all subregions for both males and females. While ELS alone also resulted in an increase in microglial density, this was limited to CA2 and CA3 in males and CA3 in females. Prompted by clinical relevance, I addressed the extent to which ELS, prior to a TBI would alter pathogenesis in the hippocampus. ELS+ TBI, when compared to TBI, resulted in a pronounced microglial response in CA3 in both males and females. The importance of this finding is reflected in a recent study in humans (Miller, Chong et al. 2020) who demonstrated a key role for CA3 in episodic memory. Techniques involving fMRI reveal that bilateral damage to CA3 resulted in the loss of functional integration across the medial temporal lobe that is part of the default network. This resulted in disruption in the retrieval of episodic memories which were established long after their initial acquisition. As brain injuries in children who have experienced ELS may present with this disorder, further studies are needed to address an effective remediation that is tailored to this condition.

4.3.1 Hippocampal-dependent behaviors

Given the reduction of neurons in the hippocampus after ELS and TBI, the specific learning and memory behaviors to the hippocampal subregions were assessed with the Novel Object Recognition Test (NOR), Three-Chamber Task, and the Barnes Maze.

Social memory and social development are significant determinants of adequate quality of life (Ryan, Catroppa et al. 2016). Interestingly, the evaluation of social deficits following early age and adulthood injury is limited. The three-chamber task was developed and validated in autism-spectrum rodent models and have effectively captured risk factors for social dysfunction (Silverman, Yang et al. 2010, Yang, Silverman et al. 2011). One model of a P21 TBI showed that TBI in the young brain elicits impairments to social recognition/novelty in at adulthood (Semple, Canchola et al. 2012) via the three-chamber task. My findings in this study validate these findings in both males and females, in which injured mice did not show a preference for the novel stimulus mouse over the familiar mouse. Early clinical studies of pediatric TBI indicate that the earlier incidence of injury, the greater vulnerability to poor neurobiological outcomes, especially in the learning and memory domains (Levin, Zhang et al. 2004, Anderson and Catroppa 2005, Catroppa, Anderson et al. 2008, Anderson, Spencer-Smith et al. 2009, Wells, Minnes et al. 2009, Semple, Blomgren et al. 2013). Importantly, patients with an incidence of a TBI report social dysfunction, in which the occurrence of a TBI reduced life satisfaction and ability to integrate into their community (Rosema, Crowe et al. 2012, Williams, Rapport et al. 2014, Shultz, McDonald et al. 2020). Clinical research suggests that social deficits may be related to the earlier incidence of injury, in which the earlier the injury occurs, the greater the risk for a social behavior deficit (Wells, Minnes et al. 2009, Ryan, Catroppa et al. 2016) and coincides with white matter pathology resulting from the injury, as indicated by neuroimaging techniques (Wells, Minnes et al. 2009, Rosema, Crowe et al. 2012).

To the best of my knowledge, there are few groups using the LBN model to assess adulthood social recognition with the three-chamber task or a social recognition test (Kohl, Riccio et al. 2013, Kohl, Wang et al. 2015, Wendel, Short et al. 2021). These findings indicate that ELS via the LBN model displays impairments in social recognition at adulthood (Kohl, Riccio et al. 2013, Kohl, Wang et al. 2015, Wendel, Short et al. 2021). and that this deficit is not seen in the young or adolescent timepoints (Kohl, Riccio et al. 2013, Kohl, Wang et al. 2015). This concept aligns with the TBI field, in which the later timepoints indicate a more pronounced deficit compared to other developmental timepoints; however, ELS males and females in the present study did not provoke a social deficit, as indicated by the three-chamber task. While the three-chamber task selected for the present study followed the protocol of the TBI field (Crawley 2008, Semple, Canchola et al. 2012), ELS mice did not show the same social impairments at adulthood of that in the literature. This finding suggests that assessment of social function via the selected version of the three-chamber task may not be the best measure of the social cognition following ELS and perhaps the terminology and measure of social recognition versus preference for social novelty are highly specific in the context of ELS.

Spatial, contextual, episodic, and working memory are key indicators of normal higher executive and cognitive function (Cowan, Rutherford et al. 2003, Cowan 2010, Cowan 2014). In developmental neuroscience, educational and developmental benchmarks of comprehension assess cognitive and executive function and incorporate elements of the ability to learn and retain information (Cowan, Rutherford et al. 2003, Cowan 2010, Cowan 2014). The Novel Object Recognition (NOR) test has been previously used in rodent models to assess object recall or preference (Ennaceur and Delacour 1988, Ennaceur 2010, Akkerman, Blokland et al. 2012, van Goethem, Rutten et al. 2012, Leger, Quiedeville et al. 2013, Lueptow 2017). The assessment of

object recall at adulthood via the NOR has not been well studied in rodent models assessing an early-aged injury; however, the few adult injury models show that TBI impairs novelty recall/preference (Zhao, Loane et al. 2012). NOR in my study suggest poor object recall in males and females following and early aged injury. The Barnes Maze has been used as an alternative to the Morris Water Maze, in which rodents working, spatial, and contextual memory are assessed without the use of an additional stressor (i.e. water) (Gee, Steffen et al. 2023). Working memory plays an important role in early childhood development and shapes the formation of long-term memories (Ericsson and Kintsch 1995, Cowan 2010, Cowan 2014). To date, only one TBI group has examined working and spatial learning and memory via the Barnes Maze in the adult injured brain (Gee, Steffen et al. 2023) and showed that TBI impaired spatial and contextual memory in all aspects of the maze. Injured mice in the present study showed significant spatial, contextual memory deficits at adulthood during the probe trial day of Barnes Maze. These findings support the implication seen in clinical studies of long-term memory following pediatric injury, in which the young brain presents a great vulnerability to spatial, contextual, and working memory impairments (Levin, Zhang et al. 2004, Anderson and Catroppa 2005, Catroppa, Anderson et al. 2008, Anderson, Spencer-Smith et al. 2009, Wells, Minnes et al. 2009, Semple, Blomgren et al. 2013, Shultz, McDonald et al. 2020)

ELS studies using the LBN model have assessed non-social learning and memory behaviors (Rice, Sandman et al. 2008, Oomen, Soeters et al. 2010, Gallo, Shleifer et al. 2019, Wendel, Short et al. 2021) using the NOR. The original LBN model (Rice, Sandman et al. 2008) indicated an impairment in preference for object novelty at adulthood (Rice, Sandman et al. 2008). While no LBN models have used the Barnes Maze to assess learning and spatial, working, and contextual memory impairment, the Morris Water Maze revealed that ELS produces

impairments in spatial and contextual memory at later timepoints (Oomen, Soeters et al. 2010). Human studies of stress resulting from childhood abuse, neglect, and poverty also reveal learning and memory dysfunction beyond the time of ELS exposure (Woon and Hedges 2008, De Brito, Mechelli et al. 2009, Chen, Hamilton et al. 2010, Gorka, Hanson et al. 2014, Hanson, van den Bos et al. 2017, Lawson, Camins et al. 2017, Teicher, Anderson et al. 2018, Smith and Pollak 2020) and these dysfunctions are associated with reduced hippocampal volume. It should be noted that the ELS studies use different versions of the NOR to assess learning and memory behavior and that no group has assessed spatial memory using the Barnes Maze test. While the results from the present study cannot be directly compared to the literature or the clinical outcomes, ELS in my study only prompted a spatial and contextual memory impairment in females but did not elicit deficits in non-social learning and memory in males and females.

While the ELS field suggests memory impairments, ELS does not impact learning and memory behavior to the same degree as TBI alone. Results are limited in measures of learning and memory after ELS prior to a TBI (Diaz-Chavez, Lajud et al. 2020). The findings indicate that ELS combined with a TBI amplifies the impairment in spatial and learning memory compared to TBI alone (Diaz-Chavez, Lajud et al. 2020). While this group selected a fear conditioning model to assess spatial working memory, ELS prior to TBI in the present study impaired spatial working memory as indicated by the Barnes Maze and this was most evident in females. Together, TBI elicits a deficit in learning and memory behaviors. Specifically, injury at the P21 timepoint suggests these deficits are worse compared to an injury at adulthood, especially social recognition. While I was surprised that ELS did not show memory deficits at extended timepoints, nor did ELS amplify memory deficits in all measures, learning and memory may be most impacted by the loss of neurons in the hippocampal subregions and the reduction of

hippocampal volume, produced most significantly by TBI rather than ELS. Additionally, my findings address longitudinal outcomes on hippocampal-driven behaviors when ELS precedes a pediatric TBI.

4.4 Anxiety and Hyperactivity after ELS and TBI

Anxiety-related behavior and hyperactivity assessments were included in the present study to provide a broad understanding of behaviors that are impaired by ELS and TBI. The Open Field Test (OFT) and Elevated Zero Maze (EZM) were selected to measure anxiety-related behavior, impulsivity, and hypo- or hyperactivity.

Psychiatric behaviors, like anxiety, have been historically difficult to assess in rodent models because it is difficult to assess emotion in animals (Shultz, Bao et al. 2012, Shultz, McDonald et al. 2020)). Rodent models present a difficult challenge in generating an anxiety-like state ((Shultz, Bao et al. 2012, Shultz, McDonald et al. 2020). Several rodent models examining the emergence of an anxiety-like response to injury have utilized the EZM due to simplicity and high sensitivity to animal manipulations (i.e. TBI or ELS) (Shultz, Bao et al. 2012, Popovitz, Mysore et al. 2019, Shultz, McDonald et al. 2020). Early-aged injuries have evaluated outcomes of the OFT and EZM into adulthood and show that TBI induces anxiety-like behavior (Popovitz, Mysore et al. 2019). Adult models of TBI have also observed increased anxiety using the OFT and EZM and persists for several months after the injury (Shultz, Bao et al. 2012, Shultz, McDonald et al. 2020) and this is consistent across rodent species. Longitudinal studies of pediatric head injuries show that younger children are at the greatest risk for developing anxiety disorders as early as 6 months after injury and persists well beyond the incidence of injury and is independent of anxiety prior to injury (Levi, Drotar et al. 1999, Luis

and Mittenberg 2002, Vasa, Grados et al. 2004, Max, Keatley et al. 2011, Babikian, Merkley et al. 2015). Neuroimaging studies suggest that the physical damage resulting from TBI may mediate dysregulation of the white matter systems in the injured cortex (Max, Keatley et al. 2011). Additionally, much like learning and memory, younger children continue to present the greatest risk for developing novel anxiety disorder following a TBI (Max, Keatley et al. 2011). It should be noted that in most assessments of anxiety following pediatric injury, emotional perception and hormonal irregularities are measured in conjunction, in which the anxiety is associated with impairment in emotional processing and a hormonal imbalance (Dunlop, Udvarhelyi et al. 1991, Blakemore 2008, Schmidt, Hanten et al. 2010, Ryan, Anderson et al. 2014, Serpa, Ferguson et al. 2021).

Interestingly, chronic stress is one of the most common ways to elicit an anxiety-like response in human and animal models (Shultz, Bao et al. 2012, Shultz, McDonald et al. 2020),Wilner). Few rodent models of ELS via the LBN model have assessed outcomes of the OFT and Elevated Plus Maze (similar to the EZM) at extended timepoints following ELS (Rice, Sandman et al. 2008) (Wang, Jiao et al. 2011, Dalle Molle, Portella et al. 2012, Walker, Bath et al. 2017, Gallo, Shleifer et al. 2019), all but one (Rice, Sandman et al. 2008)indicate anxiety-like behaviors at adulthood (Wang, Jiao et al. 2011, Dalle Molle, Portella et al. 2012, Walker, Bath et al. 2017, Gallo, Shleifer et al. 2019). Interestingly, the one group that did not show an anxiety-like response in the OFT or Elevates Plus Maze, showed hyperactivity at adulthood (Rice, Sandman et al. 2008). Clinical studies of adolescents and young adults indicate that ELS during childhood is a risk factor for anxiety and may persist into aged adulthood (Safren, Gershuny et al. 2002, Bandelow, Charimo Torrente et al. 2004, Norman, Byambaa et al. 2012, Hughes, Bellis et al. 2017, Syed and Nemeroff 2017, Lahdepuro, Savolainen et al. 2019, Smith and Pollak

2020); however these studies suggest that the anxiety that emerges is non-specific and may vary based on the type of ELS exposure (i.e. neglect, childhood abuse, malnutrition, institutional rearing, maltreatment, low socioeconomic status).

While the referenced studies and reviews indicate a straightforward anxiety-like response, the present findings show inconsistent results between the OFT and EZM with both males and females. I selected the OFT and EZM to determine anxiety-like and/or hyperactivity in chronically stressed and injured mice. While the OFT revealed anxiety-like behavior in TBI males and females, only males showed a hyperactive state. ELS resulted in anxiety-like behavior and hyperactivity in females; however, ELS in males resulted in impulsivity in both OFT and EZM but not hyperactivity in either test. When combined, ELS and TBI did not show anxiety or impulsivity in the OFT for males and females, but the EZM in males showed impulsivity and no hyperactivity. While these findings appear difficult to piece together, they indicate that there may be more complex factors beyond ELS and TBI that influence measures of anxiety, impulsivity, and hyperactivity. It may also be possible that these measures are not the best assays to assess these behaviors in tandem with one another in the context of ELS and TBI.

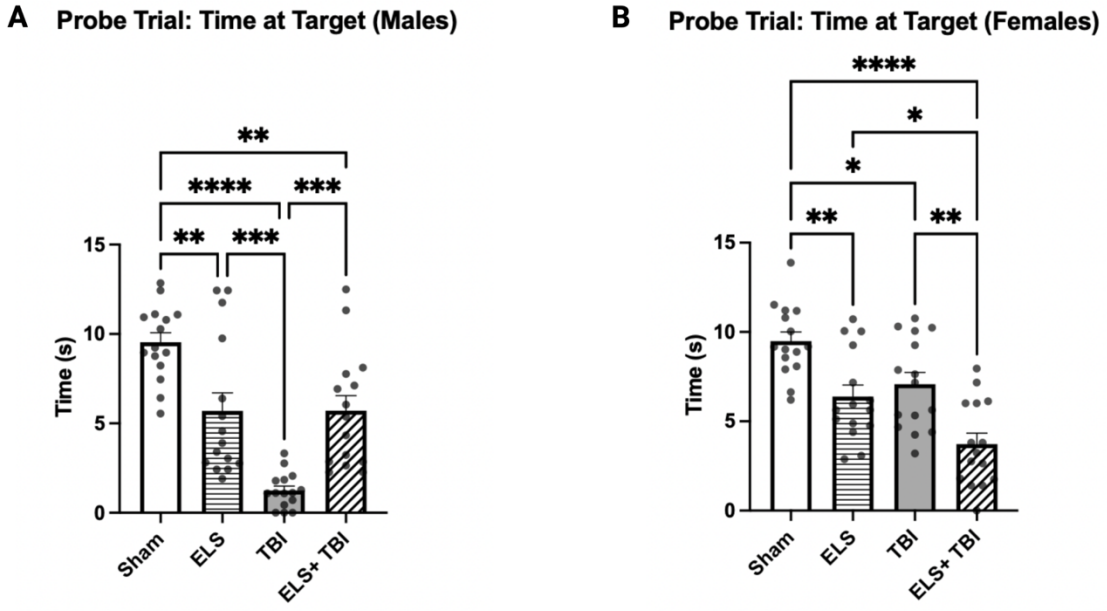
Conclusion

In conclusion these studies address the clinically relevant problem of heterogeneity of injury recovery, with the superimposition of early life stress. We show that the chronically stressed and injured brain amplifies elements of inflammation and pathogenesis and this in turn has significant implications on the long-term functional and structural consequences seen at adulthood. The findings provide an opportunity to potentially develop biomarkers in preclinical models to understand the short and long-term recovery following an injury. Translational studies

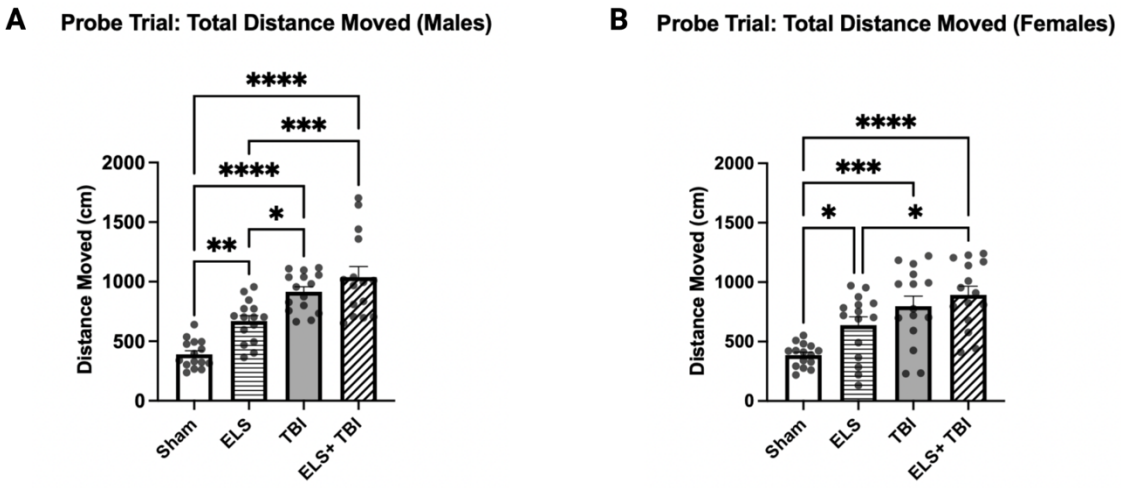
may be able to use this information to improve care management following a pediatric head injury.

Supplemental Data

Supplemental Figures



Supplemental Figure 1. The Barnes Maze was used to test spatial and contextual learning and Memory. Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 44-45; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated. On probe trial day, in males A.) TBI resulted in less time spent at the target hole (****p<0.0001). ELS showed less time spent at the hole (**p<0.01). ELS + TBI males spent more time at the target hole compared to TBI (***p<0.001). In females B.), TBI resulted in less time spent at the target hole (*p<0.05). ELS showed less time spent at the target hole (**p<0.01). ELS + TBI resulted in less time at the target hole compared to TBI (**p<0.01).



Supplemental Figure 2. The Barnes Maze was used to test spatial and contextual learning and Memory. Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 44-45; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated. Total distance was measured in male and female mice (A, B). In males A.), TBI showed more distance moved (****p<0.0001). ELS resulted in greater distance traveled (**p<0.01). ELS + TBI did not show differences in distance traveled compared to TBI (n.s.). In females, TBI mice showed greater distance moved (***p<0.001). ELS resulted in more distance moved (*p<0.05). ELS + TBI did not show differences in total distance moved compared to TBI (n.s.).

Supplemental Tables

S1. IL-1B (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	48	3	16.10	21.68	0.0000017
Residuals	15	20	0.74	NA	NA

Supplemental Table 1. ANOVA Table for cytokine IL-1B in males at P22.

S2. IL-6 (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	69	3	23.1	10.05	0.000301
Residuals	46	20	2.3	NA	NA

Supplemental Table 2. ANOVA Table for cytokine IL-16 in males at P22.

S3. TNFα (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	9.2	3	3.1	29.77	0.000000142
Residuals	2.1	20	0.1	NA	NA

Supplemental Table 3. ANOVA Table for cytokine TNFa in males at P22.

Supplemental Table 4. ANOVA Table for cytokine IFNg in males at P22.

S4. IFNγ (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	1.8	3	0.613	9.013	0.000559
Residuals	1.4	20	0.068	NA	NA

S5. IL-10 (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	0.57	3	0.190	4.18	0.0189
Residuals	0.91	20	0.045	NA	NA

Supplemental Table 5. ANOVA Table for cytokine IL-10 in males at P22.

S6. IL-1α (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	2.4	3	0.810	13.93	0.0000392
Residuals	1.2	20	0.058	NA	NA

Supplemental Table 6. ANOVA Table for cytokine IL-1a in males at P22.

S7. IL-1β (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	17.3	3	5.763	68.13	0.000000000112
Residuals	1.7	20	0.085	NA	NA

Supplemental Table 7. ANOVA Table for cytokine IL-1B in females at P22.

S8. IL-6 (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	226	3	75.4	64.51	0.000000000184
Residuals	23	20	1.2	NA	NA

Supplemental Table 8. ANOVA Table for cytokine IL-16 in females at P22.

S9. TNFα (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	7.3	3	2.44	10.34	0.000254
Residuals	4.7	20	0.24	NA	NA

Supplemental Table 9. ANOVA Table for cytokine TNFa in females at P22.

S10. IFNγ (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	0.59	3	0.198	5.882	0.00476
Residuals	0.67	20	0.034	NA	NA

Supplemental Table 10. ANOVA Table for cytokine IFNg in females at P22.

S11. IL-10 (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	0.17	3	0.055	1.858	0.169
Residuals	0.59	20	0.030	NA	NA

Supplemental Table 11. ANOVA Table for cytokine IL-10 in females at P22.

S12. IL-1α (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	0.52	3	0.174	4.613	0.0131
Residuals	0.75	20	0.038	NA	NA

Supplemental Table 12. ANOVA Table for cytokine IL-1a in females at P22.

S13. CA1 (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	298177	3	99392	22.07	0.00000148
Residuals	90050	20	4503	NA	NA

Supplemental Table 13. ANOVA Table for microglial density in CA1 in males at P22.

S14. CA2 (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	18278	3	6093	27.08	0.000000303
Residuals	4500	20	225	NA	NA

Supplemental Table 14. ANOVA Table for microglial density in CA2 in males at P22.

S15. CA3 (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	943370	3	314457	72.23	0.000000000066
Residuals	87073	20	4354	NA	NA

Supplemental Table 15. ANOVA Table for microglial density in CA2 in males at P22.

S16. Dentate Gyrus (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	248185	3	82728	16.22	0.000014
Residuals	102038	20	5102	NA	NA

Supplemental Table 16. ANOVA Table for microglial density in the dentate gyrus in males at P22.

S17. CA1 (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	1254047	3	418016	16.84	0.0000107
Residuals	496350	20	24817	NA	NA

Supplemental Table 17. ANOVA Table for microglial density in CA1 in females at P22.

S18. CA2 (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	37114	3	12371	9.175	0.000506
Residuals	26969	20	1348	NA	NA

Supplemental Table 18. ANOVA Table for microglial density in CA2 in females at P22.

S19. CA3 (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	519529	3	173176	31.71	0.000000085
Residuals	109234	20	5462	NA	NA

Supplemental Table 19. ANOVA Table for microglial density in CA3 in females at P22.

S20. Dentate Gyrus (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	519529	3	173176	31.71	0.000000085
Residuals	109234	20	5462	NA	NA

Supplemental Table 20. ANOVA Table for microglial density in the dentate gyrus in females at P22.

S21. CA1 (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	9331	3	3110	60.5	0.000000000327
Residuals	1028	20	51	NA	NA

Supplemental Table 21. ANOVA Table for cell death (apoptosis) in CA1 in males at P22.

S22. CA2 (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	854	3	285	16.61	0.0000118
Residuals	343	20	17	NA	NA

Supplemental Table 22. ANOVA Table for cell death (apoptosis) in CA2 in males at P22.

S23. CA3 (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	36139	3	12046	166	0.0000000000000268
Residuals	1452	20	73	NA	NA

Supplemental Table 23. ANOVA Table for cell death (apoptosis) in CA3 in males at P22.

S24. Dentate Gyrus (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	823798	3	274599	51.4	0.00000000139
Residuals	106843	20	5342	NA	NA

Supplemental Table 24. ANOVA Table for cell death (apoptosis) in the dentate gyrus in males at P22.

S25. CA1 (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	7787	3	2596	45.75	0.00000000386
Residuals	1135	20	57	NA	NA

Supplemental Table 25. ANOVA Table for cell death (apoptosis) in CA1 in females at P22.

S26. CA2 (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	891	3	297	20.55	0.00000253
Residuals	289	20	14	NA	NA

Supplemental Table 26. ANOVA Table for cell death (apoptosis) in CA2 in females at P22.

S27. CA3 (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	33234	3	11078	74.43	0.0000000000502
Residuals	2977	20	149	NA	NA

Supplemental Table 27. ANOVA Table for cell death (apoptosis) in CA3 in females at P22.

S28. Dentate Gyrus (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	891600	3	297200	39.97	0.0000000123
Residuals	148701	20	7435	NA	NA

Supplemental Table 28. ANOVA Table for cell death (apoptosis) in the dentate gyrus in females at P22.

S29. Time in Inner (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	4961	3	1654	46.73	0.00000000000000292
Residuals	1982	56	35	NA	NA

Supplemental Table 29. ANOVA Table for time in the inner area of the Open Field Test (OFT) in adult males

S30. Distance Moved (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	23174806	3	7724935	10.95	0.00000922
Residuals	39506507	56	705473	NA	NA

Supplemental Table 30. ANOVA Table for time in the distance moved in the OFT in adult males

S31. Time in Inner (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	6285	3	2095.0	255.7	0.000000000000000000000000000000121
Residuals	459	56	8.2	NA	NA

Supplemental Table 31. ANOVA Table for time in the inner area of the OFT in adult females

S32. Distance Moved (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	27298722	3	9099574	8.11	0.000142
Residuals	62832135	56	1122002	NA	NA

Supplemental Table 32. ANOVA Table for time in the distance moved in the OFT in adult females

S33. Time in Outer (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	7039	3	2346	32.85	0.0000000000022
Residuals	3999	56	71	NA	NA

Supplemental Table 33. ANOVA Table for time in the open arms of the EZM in adult males

S34. Distance Moved (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	9092325	3	3030775	3.738	0.0161
Residuals	45407058	56	810840	NA	NA

Supplemental Table 34. ANOVA Table for the distance moved in the EZM in adult males

S35. Time in Outer (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	3139	3	1046	18.9	0.0000000136
Residuals	3100	56	55	NA	NA

Supplemental Table 35. ANOVA Table for time in the open arms of the EZM in adult females

S36. Distance Moved (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	70763210	3	23587737	27.09	0.000000000059
Residuals	48765592	56	870814	NA	NA

Supplemental Table 36. ANOVA Table for distance moved in the EZM in adult females

S37. Time in Novel Chamber (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	81703	3	27234	8.606	0.0000864
Residuals	177218	56	3165	NA	NA

Supplemental Table 37. ANOVA Table for time in the novel chamber in the Three-chamber task in adult males

S38. Time in Novel Chamber (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	36785	3	12262	4.805	0.00477
Residuals	142907	56	2552	NA	NA

Supplemental Table 38. ANOVA Table for time in the novel chamber in the Three-chamber task in adult females

S39. Novel Preference Index (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	8811	3	2937	33.83	0.00000000000131
Residuals	4862	56	87	NA	NA

Supplemental Table 39. ANOVA Table for the Novel Preference index in Novel Object Recognition (NOR) in adult males

S40. Novel Preference Index (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	12494	3	4165	56.43	0.0000000000000000624
Residuals	4133	56	74	NA	NA

Supplemental Table 40. ANOVA Table for the Novel Preference index in NOR in adult females

S41. Latency to Target (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	10325	3	3442	11.14	0.00000775
Residuals	17302	56	309	NA	NA

Supplemental Table 41. ANOVA Table for the latency to target hole in the Barnes Maze for adult males

S42. Visits to Target (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	23	3	7.6	1.815	0.155
Residuals	233	56	4.2	NA	NA

Supplemental Table 42. ANOVA Table for the visits to target hole in the Barnes Maze for adult males

S43. Path Length to Target (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	27660951620	3	9220317207	17.96	0.0000000274
Residuals	28750840312	56	513407863	NA	NA

Supplemental Table 43. ANOVA Table for path length to target hole in the Barnes Maze for adult males

S44. Time at Target (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	518	3	172.6	22.08	0.00000000146
Residuals	438	56	7.8	NA	NA

Supplemental Table 44. ANOVA Table for time spent at the target hole in the Barnes Maze for adult males

S45. Total Distance (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	3692891	3	1230964	25.79	0.000000000131
Residuals	2673295	56	47737	NA	NA

Supplemental Table 45. ANOVA Table for total distance moved in the Barnes Maze for adult males

S46. Latency to Target (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	7284	3	2428	14.63	0.000000376
Residuals	9295	56	166	NA	NA

Supplemental Table 46. ANOVA Table for the latency to target hole in the Barnes Maze for adult females

S47. Visits to Target (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	97	3	32.3	4.876	0.00441
Residuals	371	56	6.6	NA	NA

Supplemental Table 47. ANOVA Table for visits to the target hole in the Barnes Maze for adult females

S48. Path Length to Target (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	12905513139	3	4301837713	8.323	0.000114
Residuals	28943782759	56	516853264	NA	NA

Supplemental Table 48. ANOVA Table for path length to the target hole in the Barnes Maze for adult females

S49. Time at Target (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	253	3	84.3	14.79	0.000000329
Residuals	319	56	5.7	NA	NA

Supplemental Table 49. ANOVA Table for time spent at the target hole in the Barnes Maze for adult females

S50. Total Distance (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	2211262	3	737087	11.05	0.00000838
Residuals	3734035	56	66679	NA	NA

Supplemental Table 50. ANOVA Table for total distance moved in the Barnes Maze for adult females

S51. CA1 (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	1254047	3	418016	16.84	0.0000107
Residuals	496350	20	24817	NA	NA

Supplemental Table 51. ANOVA Table for NeuN+ cells in CA1 in adult males

S52. CA2 (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	144992	3	48331	6.404	0.00323
Residuals	150950	20	7548	NA	NA

Supplemental Table 52. ANOVA Table for PCP4+ cells in CA2 in adult males

S53. CA3 (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	946298	3	315433	22.64	0.00000122
Residuals	278678	20	13934	NA	NA

Supplemental Table 53. ANOVA Table for NeuN+ cells in CA3 in adult males

S54. CA1 (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	5118342	3	1706114	104.1	0.00000000000224
Residuals	327705	20	16385	NA	NA

Supplemental Table 54. ANOVA Table for NeuN+ cells in CA1 in adult females

S55. CA2 (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	168234	3	56078	12.19	0.000093
Residuals	92017	20	4601	NA	NA

Supplemental Table 51. ANOVA Table for PCP4+ cells in CA2 in adult females

S56. CA3 (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	2785742	3	928581	138	0.000000000000156
Residuals	134574	20	6729	NA	NA

Supplemental Table 56. ANOVA Table for NeuN+ cells in CA3 in adult females

S57. Cortical Volume (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	182	3	60.7	22.01	0.00000152
Residuals	55	20	2.8	NA	NA

Supplemental Table 57. ANOVA Table for cortical volume in adult males

S58. Hippocampal Volume (Males)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	16	3	5.5	4.615	0.0131
Residuals	24	20	1.2	NA	NA

Supplemental Table 58. ANOVA Table for hippocampal volume in adult males

S59. Cortical Volume (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	147	3	49	6.969	0.00215
Residuals	140	20	7	NA	NA

Supplemental Table 59. ANOVA Table for cortical volume in adult females

S60. Hippocampal Volume (Females)

Source	Sum.of.Squares	Degrees.of.Freedom	Mean.Square	F.Value	P.Value
Treatment	88	3	29.3	13.71	0.0000436
Residuals	43	20	2.1	NA	NA

Supplemental Table 60. ANOVA Table for cortical volume in adult females

List of Figures

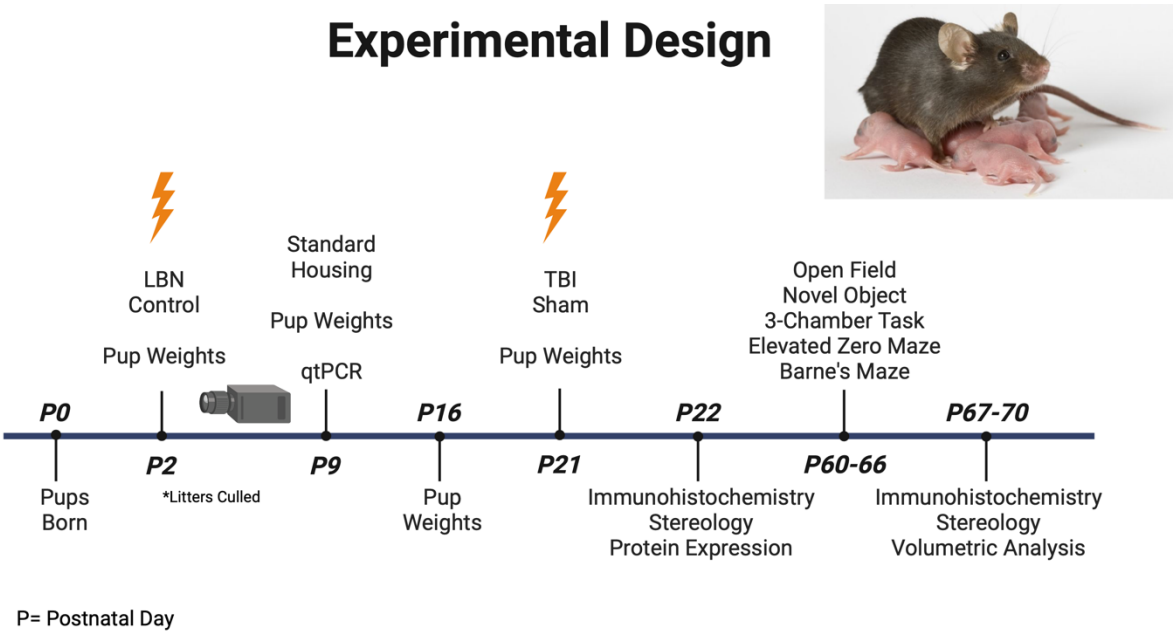


Figure 1. Experimental Design and Timeline. Mice were bred in house. On P2, litters were culled to 6-8 pups. Nursing dam and litters were randomized into LBN or Control cages until P9. Continuous home cage monitoring was used to track maternal behavior. A subset of animals was used for qTPCR to analyze collected for immunohistochemistry and protein expression. On P21, pups were weaned and randomized into TBI or Sham surgery. Brain tissue was collected for immunohistochemistry and protein expression. On 60-70, animals were analyzed for hippocampal structure and function.

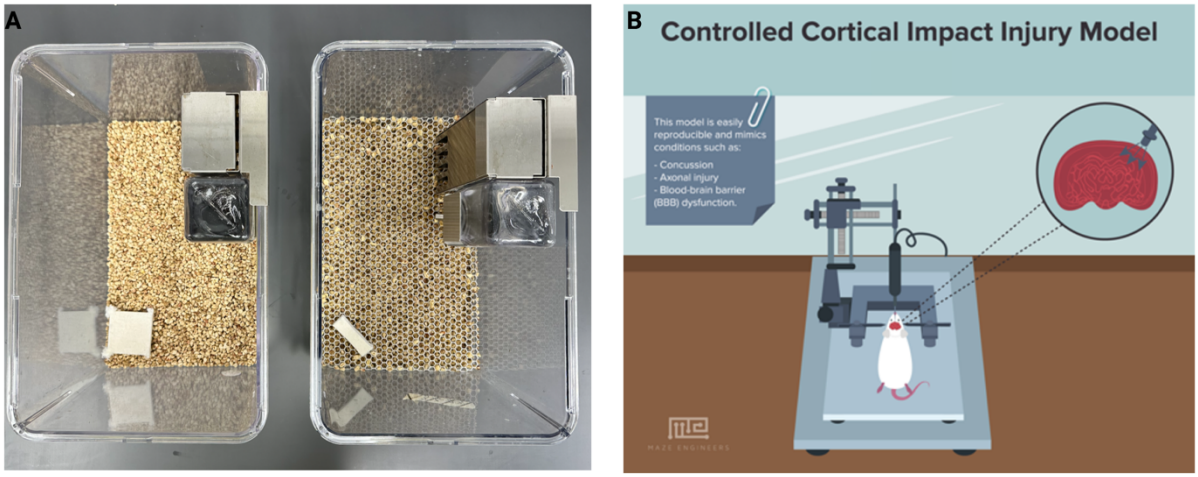


Figure 2. Standard murine models of ELS (limiting bedding nestlet, LBN) and TBI (controlled cortical impact) were used in this study.

A. The LBN model consisted of a metal, mesh bottom that was positioned on top of standard corncob bedding and a reduced nestlet square (right). The control cage contained corncob bedding with a full nestlet square (left).
B.) Schematic of a focal injury, produced by a controlled cortical impact, at P21.

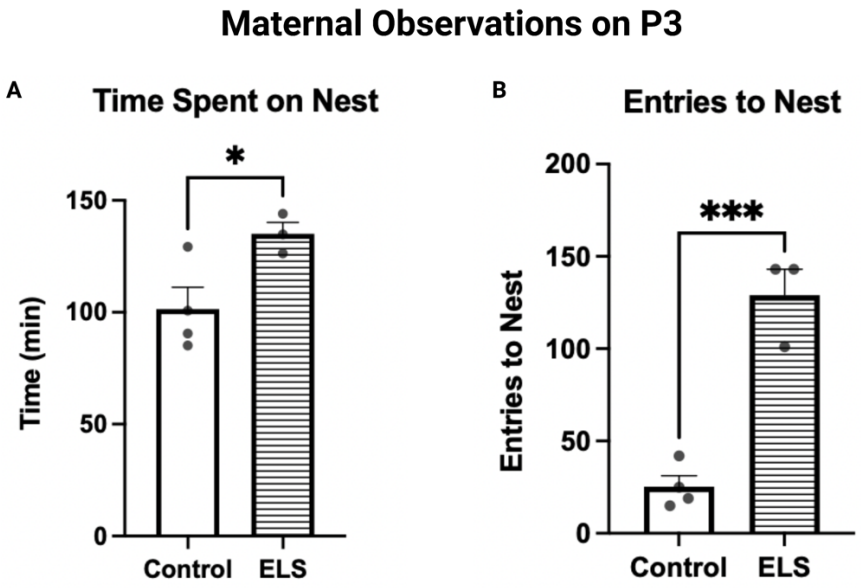


Figure 3. Maternal behaviors, measured at P3, revealed disrupted maternal behavior.
Time spent on the nest and entries of dams to the nest were scored and averaged across 0:00, 6:00, 11:00, 12:00, 18:00, and 23:00 Zeitgeber time hours. A.) ELS dams spent more time on their nests (unpaired t-test, $t=2.718$, $*p<0.05$) and B.) made more entries to the nest as compared to controls (unpaired t-test, $t=7.592$ $***p<0.0001$).

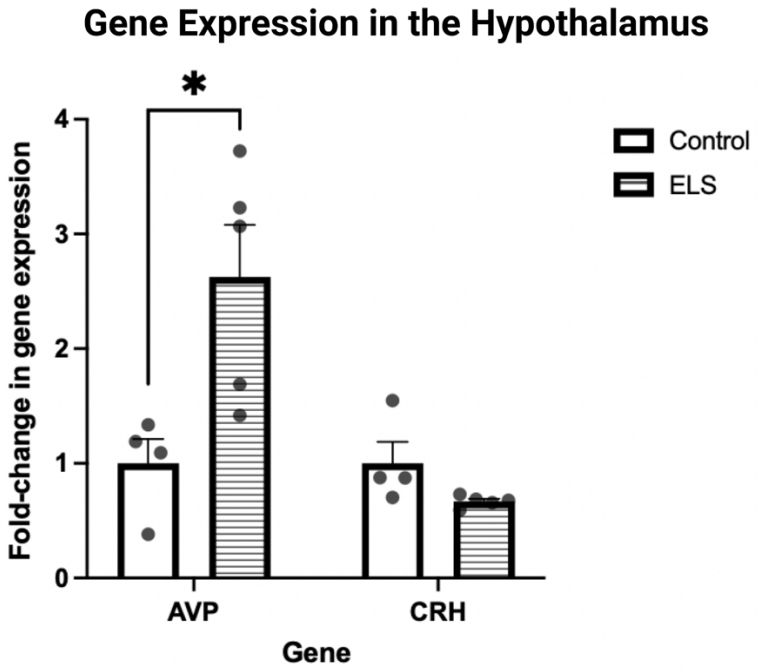


Figure 4. Arginine vasopressin (AVP) was increased in the hypothalamus after exposure to ELS. AVP and corticotropin-releasing hormone (CRH) were quantified in the hypothalamus at P9, a time point corresponding to the final day of exposure to ELS. While there was an increase in AVP (N=4-5, multiple t-test, Bonferroni Correction, t= 2.625, *p<0.05), there was no change in CRH (N=4-5, multiple t-test, Bonferroni Correction, t= 0.6694, p=0.08).

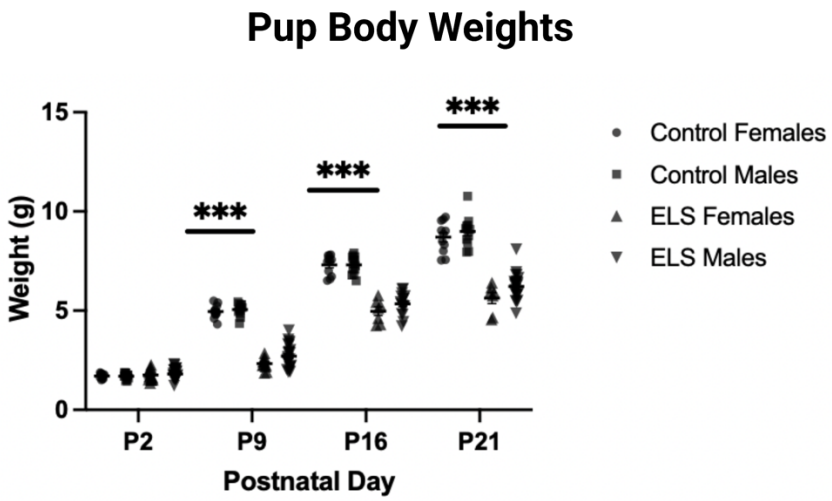
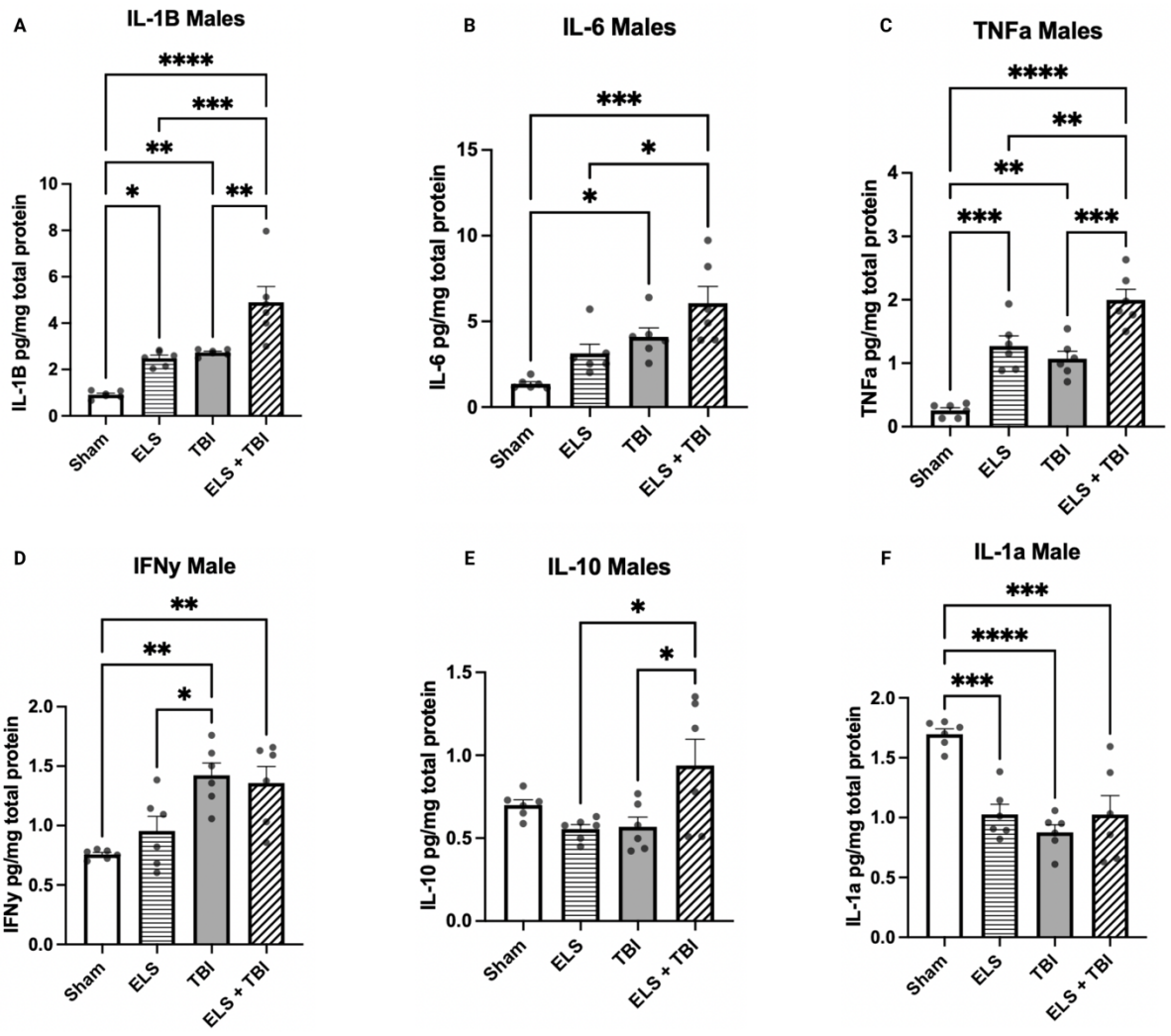


Figure 5. Body weights were tracked during and after exposure to ELS.
ELS was associated with a lower gain in weight than that of control pups (N=23-37, mixed effect analysis, $F_{3, 128}$=101.80, ***p<0.001). ELS females showed lower gains in body weights compared to ELS males (males, N=12-26, females, N=11-16; two-way ANOVA, $F_{1,129}$=10.30, **p<0.01).


Cytokine Expression in the Cortex at P22
A
IL-1B Males
IL-1B pg/mg total protein
B
IL-6 Males
IL-6 pg/mg total protein
C
TNFa Males
TNFa pg/mg total protein
D
IFNy Male
IFNy pg/mg total protein
E
IL-10 Males
IL-10 pg/mg total protein
F
IL-1a Male
IL-1a pg/mg total protein
Sham
ELS
TBI
ELS + TBI

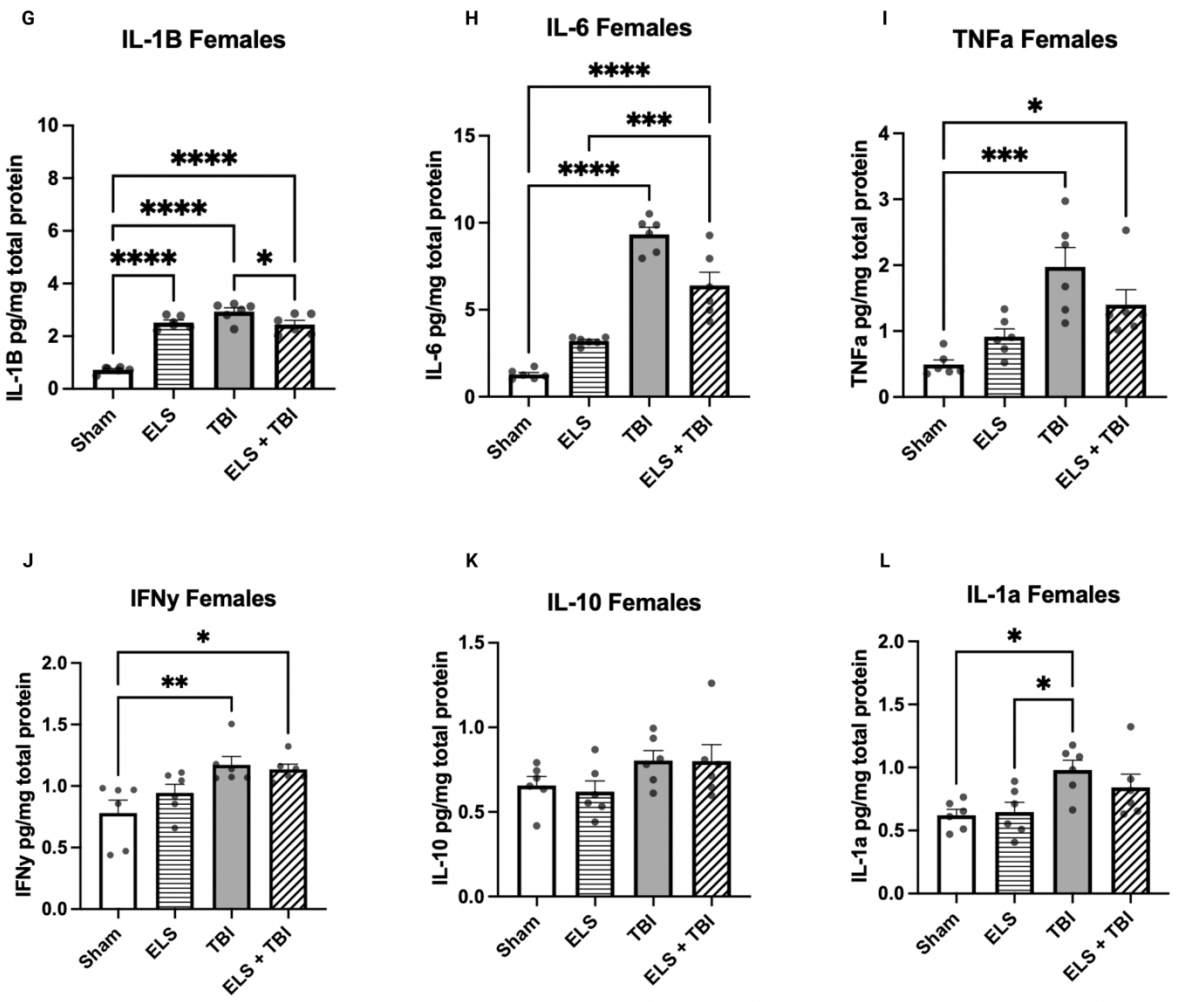


Figure 6. Cytokines, quantified in cortical homogenates at P22, revealed limited variation across experimental conditions.

Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance threshold, n.s.= not significant, one-way ANOVA). All reported stats were compared to the sham controls, unless otherwise stated.

(A-F) In males, TBI resulted in an increase in A.) IL-1B (**p<0.01), B.) IL-6 (*p<0.05), C.) TNFa (**p<0.01), and D.) IFNg (**p<0.01); there was a reduction in F.) IL-1a (****p<0.0001). Similarly, ELS resulted in increased A.) IL-1B (*p<0.05) and B.) TNFa (***p<0.001), but F.) reduced IL-1a (***p<0.001). ELS + TBI resulted in an increase in A.) IL-1B (****p<0.0001), B.) IL-6 (***p<0.001), C.) TNFa (****p<0.0001), D.) IFNg (**p<0.01), and F.) IL-1a (***p<0.001). When compared to TBI, ELS + TBI resulted in increased A.) IL-1B (**p<0.01) and E.) IL-10 (*p<0.05).

(E-F) In TBI resulted in G.) increased IL-1B (****p<0.0001), H.) IL-6 (****p<0.0001), I.) TNFa (***p<0.001), J) IFNg (**p<0.001), and L.) IL-1a (*p<0.05). ELS resulted in G). an increase that was limited to IL-1B (****p<0.0001). ELS+ TBI produced increased G.) IL-1B (****p<0.0001), H.) IL-6 (****p<0.0001), I.) TNFa (*p<0.05), and J.) IFNg (*p<0.05). When compared to TBI, ELS + TBI resulted in decreased G.) IL-1B (*p<0.05).

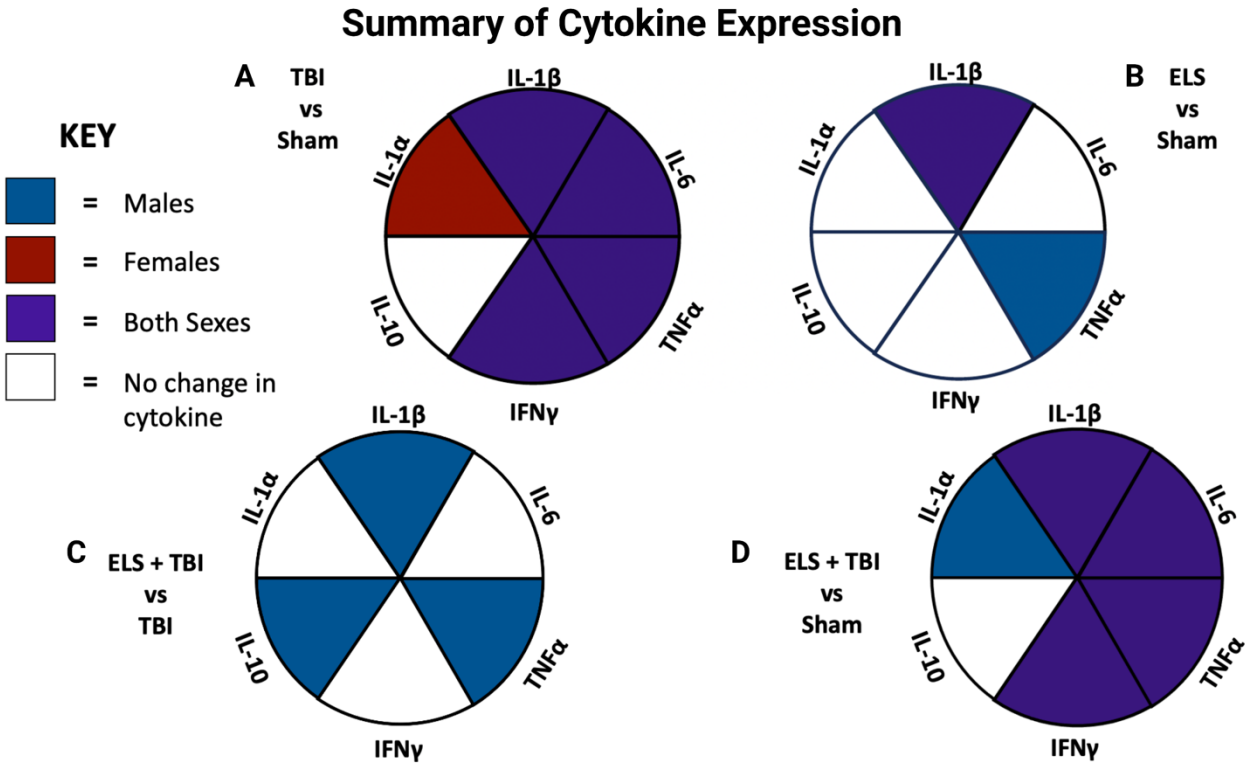


Figure 7. Summary of the differential response of cytokines in cortical homogenates at 1day post injury.
A.) With the exception of IL-10, cytokines were significantly elevated in the injured cortex, in both males and females, compared to shams.
B.) ELS resulted in a more limited increased expression of cytokines (IL-1B and TNFa only) compared to shams.
C.) Only male pups, when exposed to ELS+TBI, showed an elevation in cytokines, compared to TBI.
D.) With the exception of IL-10, cytokines were significantly elevated in the injured cortex for males and females, compared to shams.

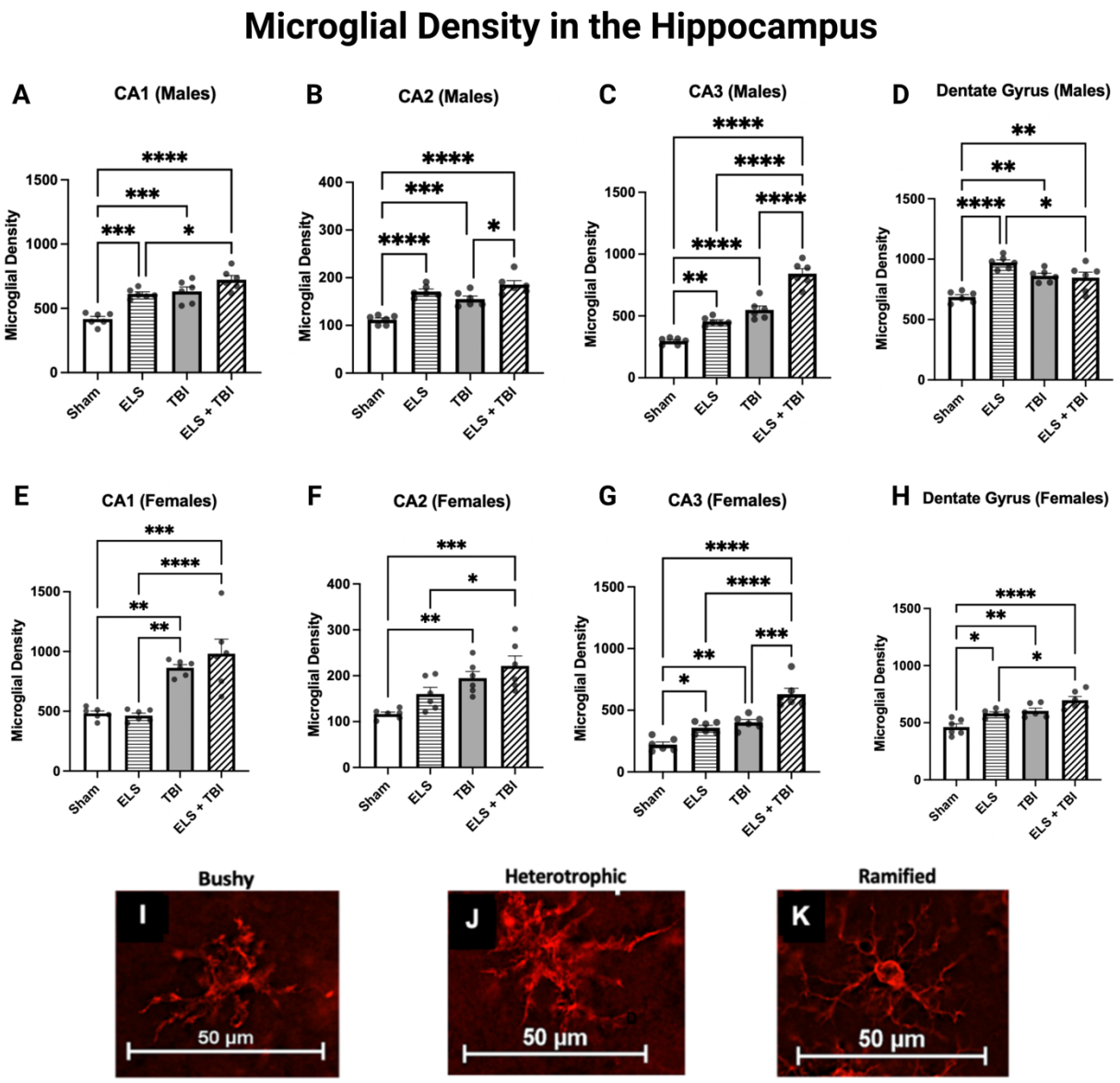


Figure 8. At 1 day post injury, there were regional differences in microglial density in the hippocampus in mice exposed to either ELS or TBI.

Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated.

(A-D) In males, there were significant changes in cytokines when compared across all groups (Supplemental tables 13-16; N= 6/group, One-Way ANOVA). TBI resulted in an increase in Iba-1+ cells in A) CA1 (***p<0.001), B) CA2 (***p<0.001), C), CA3 (****p<0.0001), and D) the dentate gyrus (**p<0.01). ELS resulted in an increase in Iba-1+ cells in A) CA1 (***p<0.001), B) CA2 (****p<0.0001), C) CA3 (**p<0.01), and D) the dentate gyrus (****p<0.0001). ELS+

TBI revealed similar findings to that of TBI alone. There was an increase in the number of Iba-1+ cells in the CA1 (****p<0.0001), CA2 (p****<0.0001), CA3 (****p<0.0001), and dentate gyrus (**p<0.01). ELS+ TBI in males, when compared to TBI, resulted in an increase in Iba-1+ cells in B.) CA2(*p<0.05) and C.) CA3(****p<0.0001) E-H.)

In females, TBI resulted in E.) an increase in Iba-1+ cells in CA1 (**p<0.01), F.) CA2 (**p<0.01), and G.) CA3 (**p<0.01). Exposure to ELS resulted in an increase in Iba-1+ cells in G.) CA3 (*p<0.05) and H.) dentate gyrus (*p<0.05). ELS + TBI resulted in an increase in Iba-1+ cells in E.) CA1 (***p<0.001), F.) CA2 (***p<0.001), G.) CA3 (****p<0.0001), and H.) dentate gyrus (****p<0.0001). ELS + TBI, when compared to TBI, resulted in an increase in Iba-1+ cells in G.) CA3 (****p<0.0001).

Distinct microglial phenotypes, randomly assessed throughout the hippocampus, were evident at 1-day post injury in the hippocampus. Representative images (I-K) were taken in the injured male hippocampus at P22.

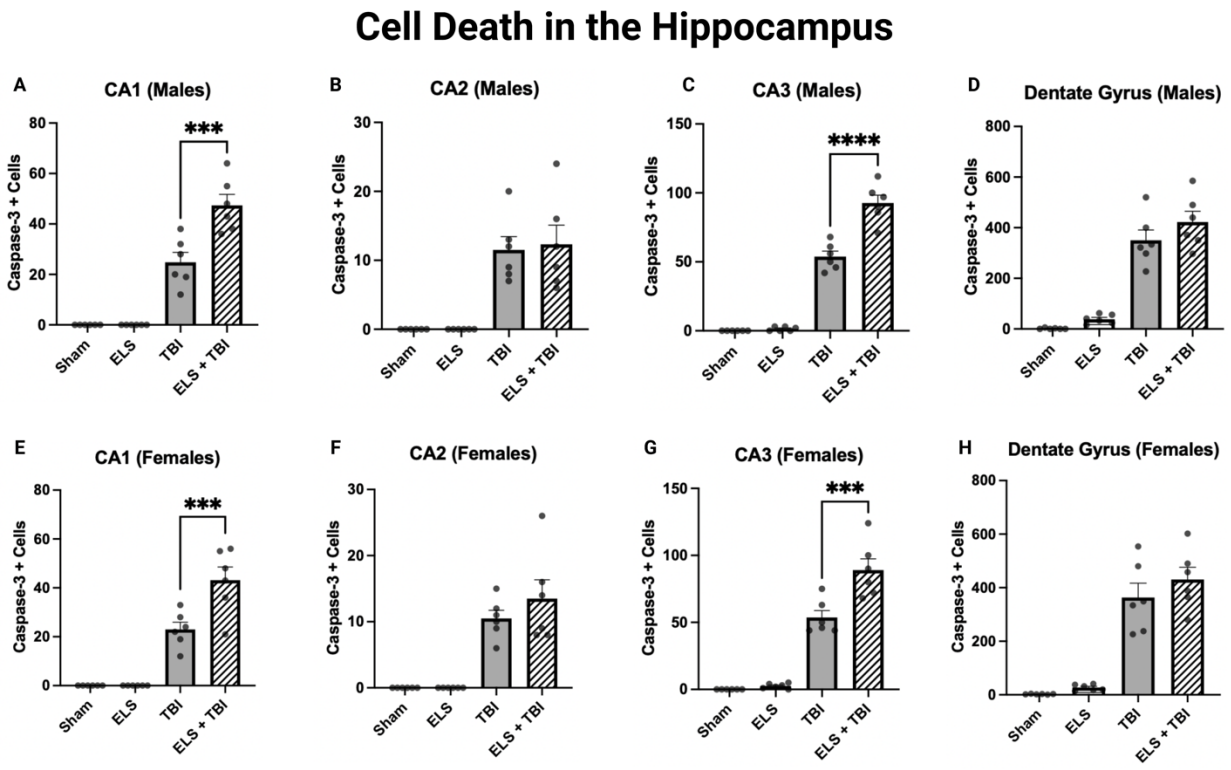


Figure 9. Caspase-3+ cells were quantified in hippocampal subfields. ELS + TBI was associated with increased numbers of caspase-3+ cells in both males (A-D) and females (E-H).

Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated.

In males, TBI resulted in an increase in caspase-3+ cells in A.) CA1 (****p<0.0001), B.) CA2 (***p<0.001), C.) CA3 (****p<0.0001), and D.) dentate gyrus (****p<0.0001). ELS resulted in a similar number of caspase-3 cells in A.) CA1 (n.s.), B.) CA2 (n.s.), C.) CA3 (n.s.), and D.) dentate gyrus (n.s.). ELS + TBI resulted in a greater number of caspase-3+ cells in A.) CA1 (****p<0.0001), B.) CA2 (***p<0.001), C.) CA3 (****p<0.0001), and D.) dentate gyrus (****p<0.0001). ELS + TBI resulted in the number of caspase-3 + cells in A.) CA1 (****p<0.0001) and C.) CA3 (****p<0.0001) compared to TBI.

In females, TBI yielded greater caspase-3+ cells in the E.) CA1 (***p<0.001), F.) CA2 (***p<0.001), G.) CA3 (****p<0.0001), and H.) dentate gyrus (****p<0.0001). ELS showed no differences in the number of caspase-3+ cells in E.) CA1 (n.s.), F.) CA2 (n.s.), G.) CA3 (n.s.), and H.) dentate gyrus (n.s.). ELS + TBI resulted in an increase in caspase-3+ cells in E.) CA1 (****p<0.0001), F.) CA2 (****p<0.0001), G.) CA3 (****p<0.0001), and H.) dentate gyrus (****p<0.0001). ELS + TBI, when compared to TBI, resulted in increased numbers of caspase-3+ cells in E.) CA1 (***p<0.001) and G.) CA3 (***p<0.001).

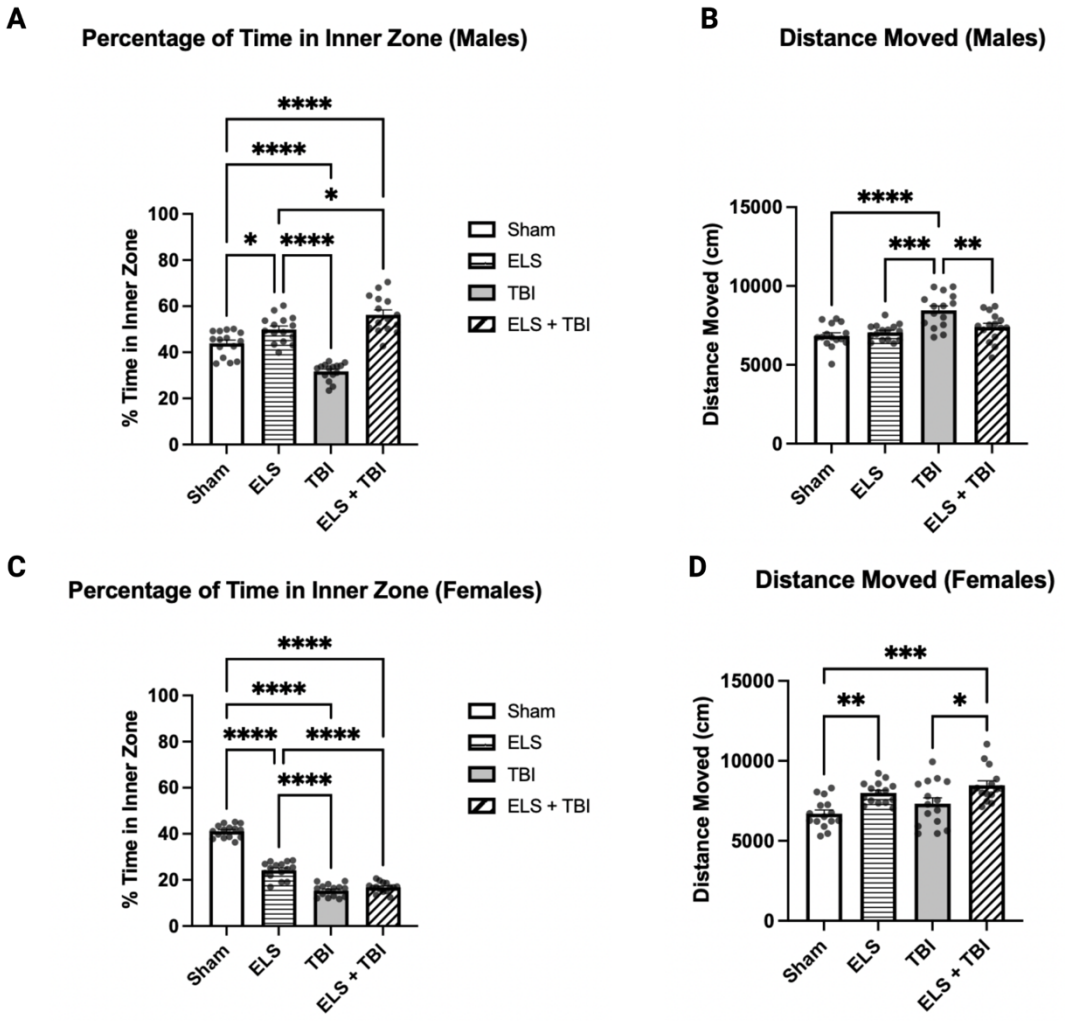


Figure 10. The open field test was used to assess anxiety-like behavior and hyperactivity. Mice were evaluated for their preference to the center of the field (inner zone) versus the perimeter (outer zone) and the distance traveled over a period of 10 minutes.

Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated.

A.) Brain-injured male mice spent less time in the outer zone (****p<0.0001). In contrast, ELS showed no differences in time spent in the outer zone. While ELS + TBI resulted in increased time spent in the outer zone (***p<0.001), ELS + TBI spent less time in the outer zone compared to TBI (****p<0.0001).

B.) In males, TBI resulted in greater distance moved (****p<0.0001). ELS and ELS +TBI did not show a difference in the distance moved. ELS + TBI resulted in reduced activity compared to TBI (**p<0.01).
C.) In females, TBI and ELS spent less time in the outer zone (****p<0.0001) and (****p<0.0001), respectively. ELS + TBI when compared to shams or TBI, resulted in reduced time spent time in the outer zone. (****p<0.0001, for both comparisons.)
D.) Female TBI mice spent a similar time in the outer zone (n.s.). ELS resulted in a reduction in time spent in the outer zone (**p<0.01). ELS + TBI resulted in an increase in the total distance moved compared to sham (**p<0.01) or TBI (*p<0.05).

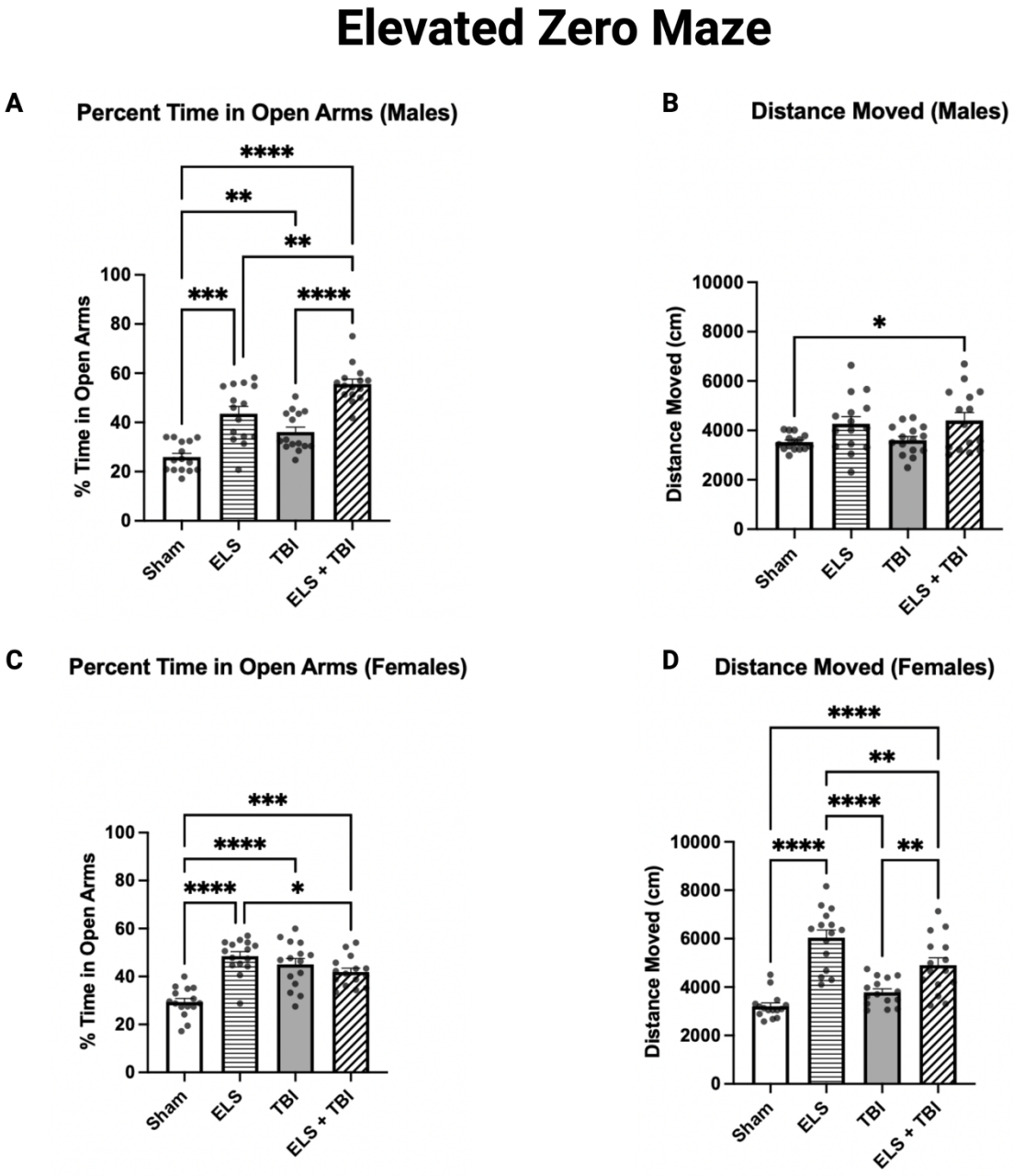


Figure 11. The elevated zero maze was used to assess anxiety-like behavior. ELS and TBI resulted in altered preference for open versus closed arms.
Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated.
A.) In males, TBI showed no difference in time spent in the open arms (**p<0.01). ELS resulted in more time spent in the open arms (***p<0.001). ELS + TBI showed more time spent in the open arms (****p<0.0001). Compared to TBI, ELS + TBI in males resulted in more time spent in the open arms (****p<0.0001).
B.) There was no difference in total distance moved between male TBI and sham animals (n.s.). ELS showed a similar distance moved (n.s.). ELS did not show a difference in distance traveled compared to sham (n.s.). ELS + TBI resulted in greater distance moved (*p<0.05). There was no difference in total distance traveled between ELS + TBI and TBI (n.s.).
C.) In females, TBI resulted in more time spent in the open arms (****p<0.0001). ELS showed more time spent in the open arms (****p<0.0001). ELS + TBI showed greater time spent in the open arms (***p<0.001). ELS + TBI spent a similar time in the open arms compared to TBI (n.s.).
D.) TBI females showed a similar distance moved. ELS resulted in greater distance moved (****p<0.0001). ELS + TBI showed a greater distance moved (****p<0.0001). ELS + TBI resulted in more total distance moved compared to TBI (**p<0.01).

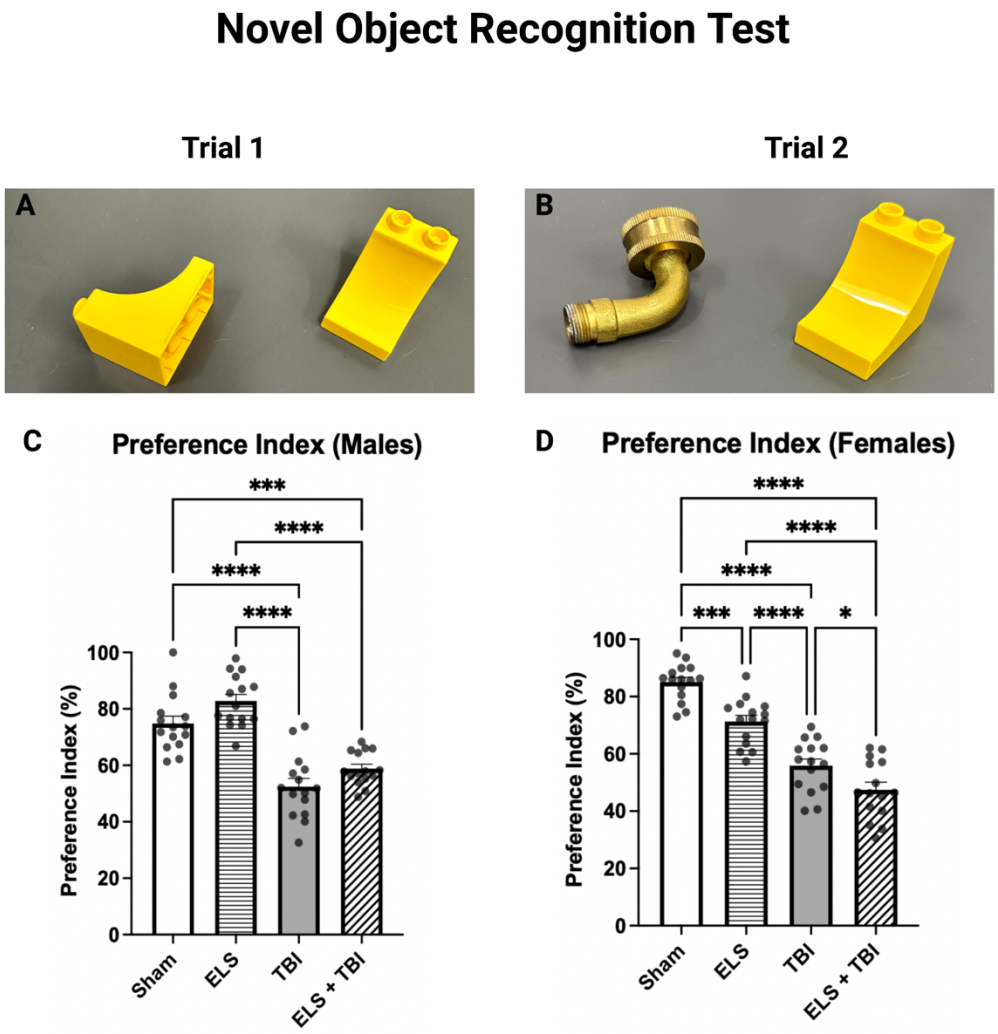
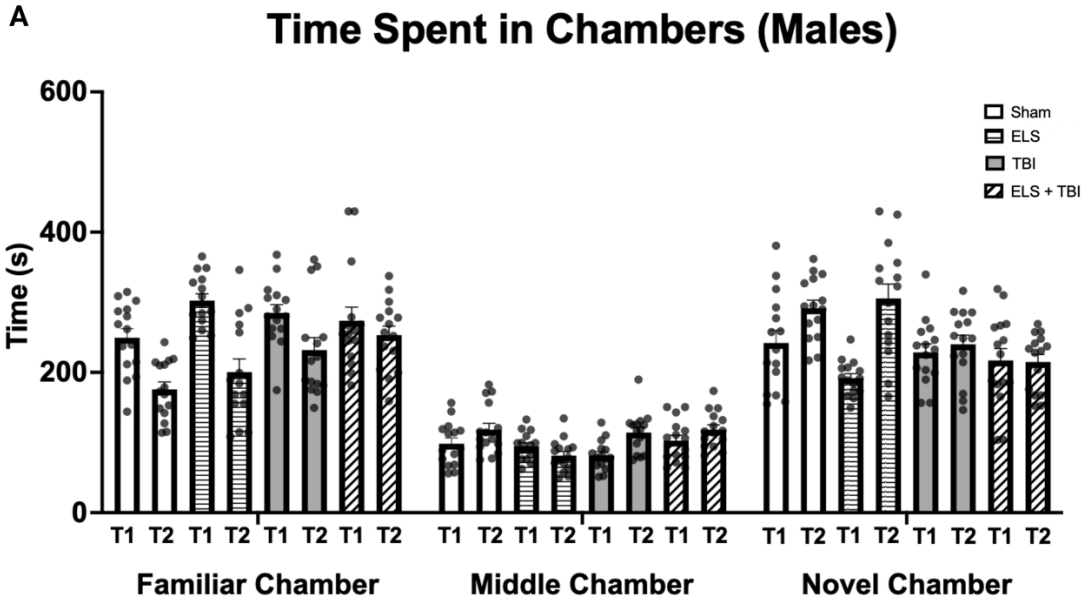


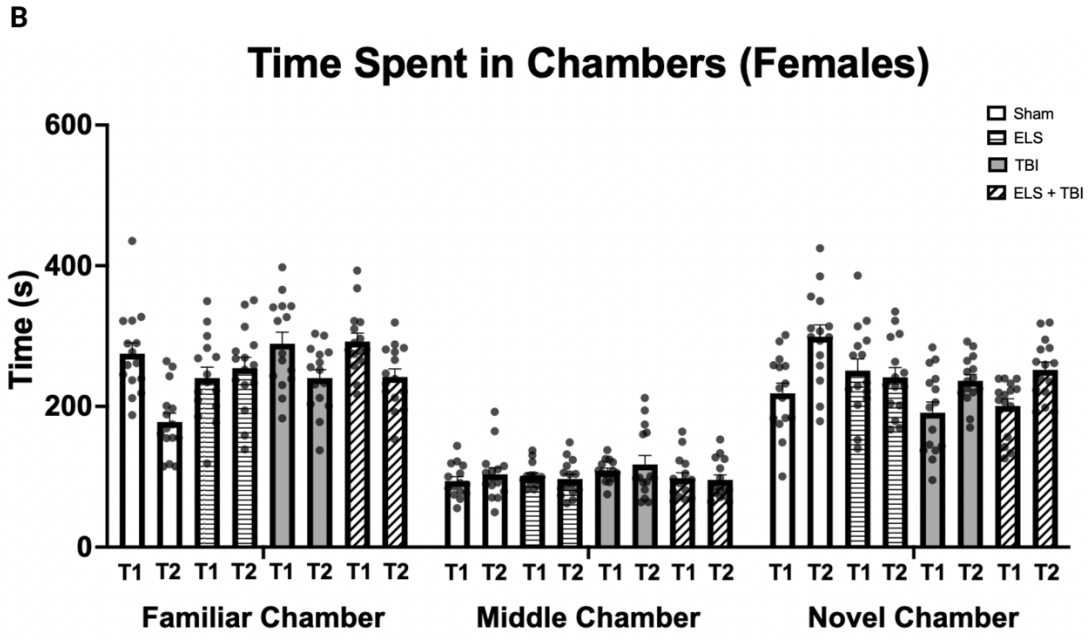
Figure 12. The novel object recognition test was used to evaluate learning and memory. A preference index, the time spent with a novel object versus a familiar object, was determined for each group.

A., B.) In trial 1, a mouse was exposed to 2 identical Duplo® blocks for 5 minutes (A). In trial 2, 1 block was replaced with a similar sized gold dishwasher elbow (B).

Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated.
C.) In males, TBI resulted in a reduced time spent with the novel object (****p<0.0001). ELS did not show a difference in time spent with the novel object (n.s.). ELS + TBI resulted in decreased time spent with the novel object (***p<0.001). There was no difference in time spent with the novel object between ELS + TBI and TBI (n.s.).
D.) In females, TBI showed decreased time with the novel object (****p<0.0001). ELS resulted in less time spent with the novel object (***p<0.001). ELS + TBI decreased the time spent with

the novel object (**** p<0.0001). ELS + TBI reduced time spent with the novel object compared to TBI (*p<0.05).





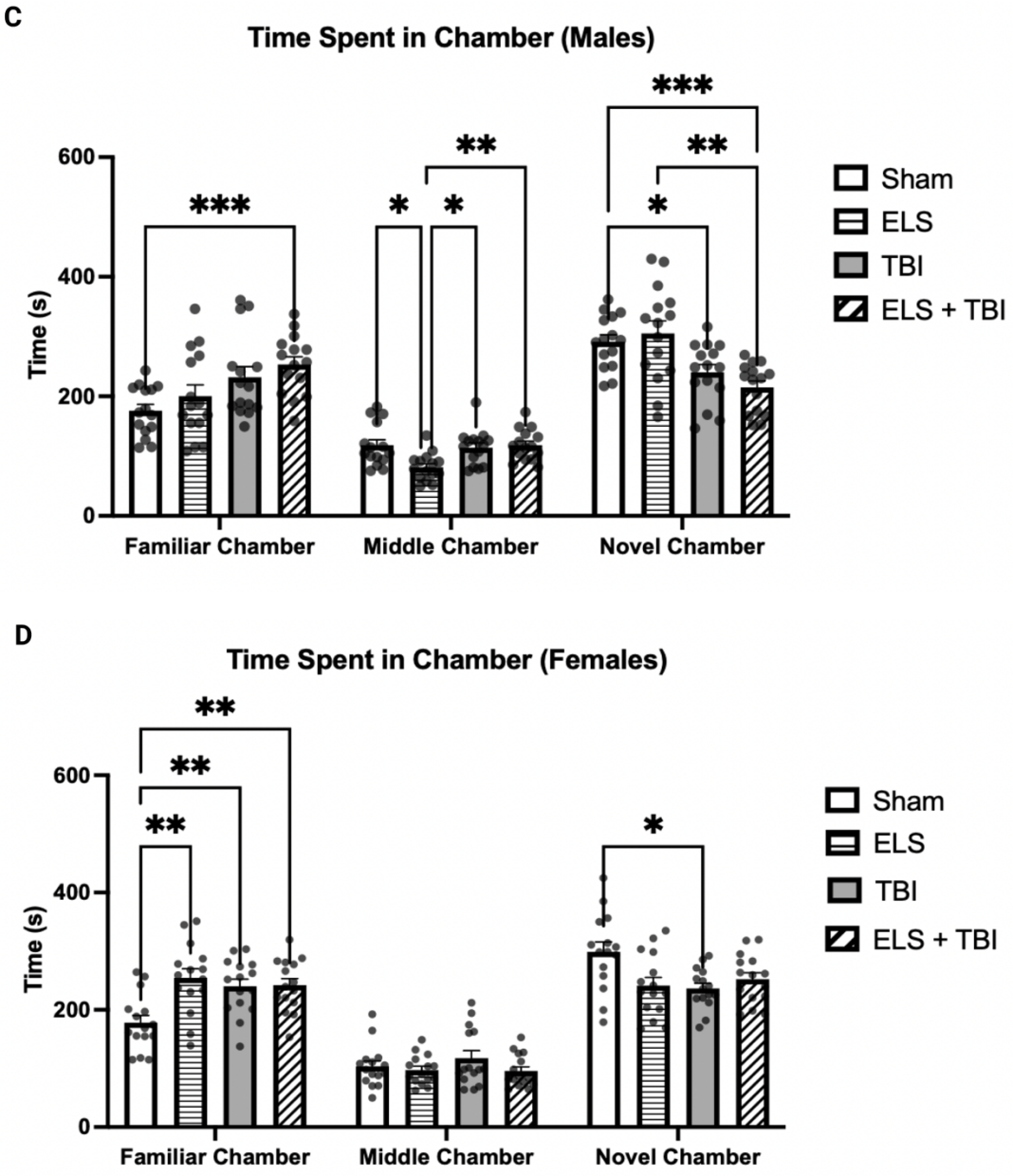
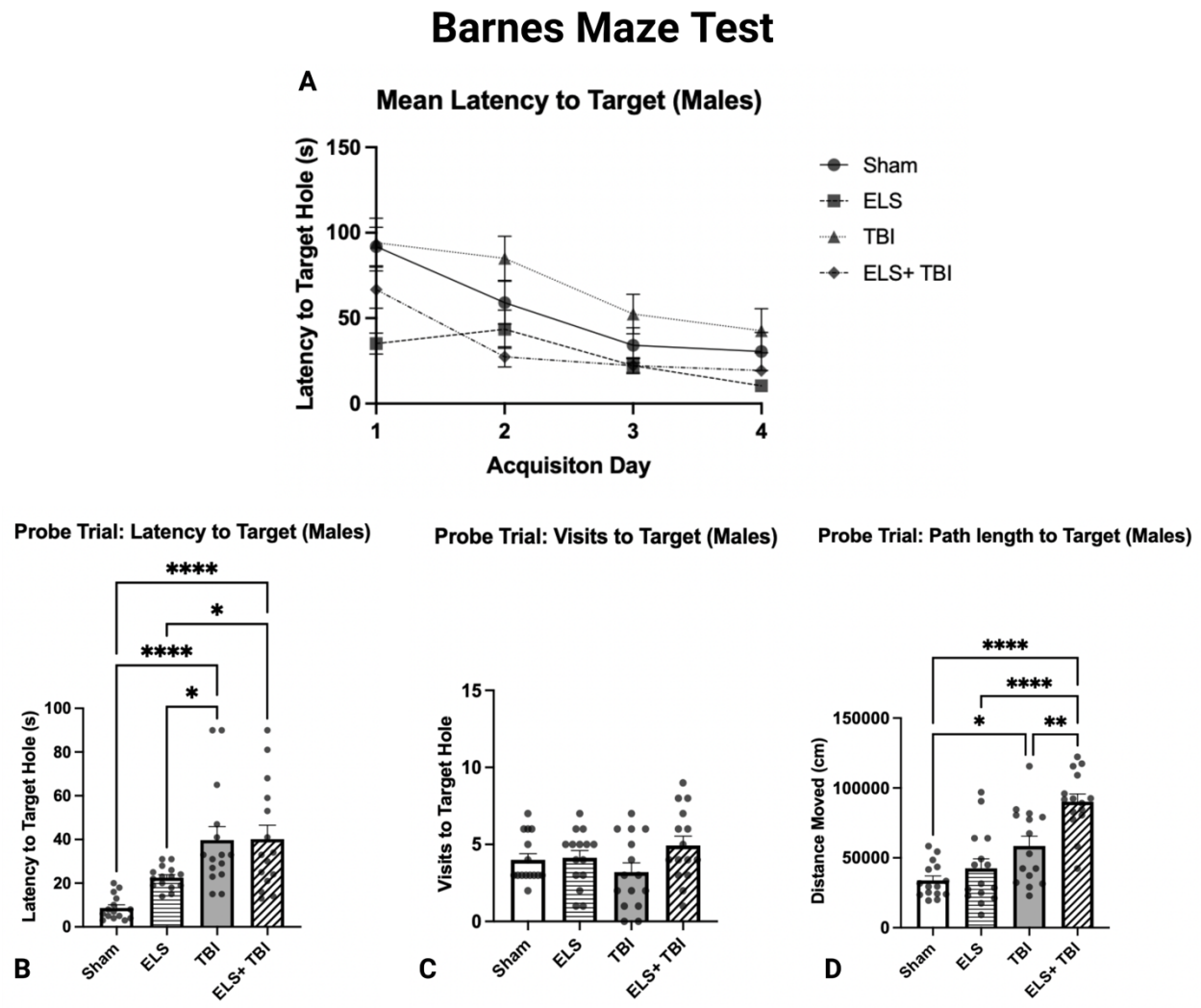


Figure 13. Social recognition was evaluated using the 3-chamber task
A-B.) A summary of the raw data from the 3-chamber task represents the time spent in each chamber during trial 1 (T1) and trial 2. (T2).

Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *$p<0.05$ significance threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated.

C.) In males, TBI reduced the amount of time spent in the novel chamber (*p<0.05). ELS showed similar time spent in the novel chamber (n.s.). ELS + TBI resulted in less time in the novel chamber (***p<0.001). There was no difference between time spent in the novel chamber between ELS + TBI and TBI (n.s.). D.) In females, TBI resulted in less time spent in the novel chamber (*p<0.05). ELS and ELS + TBI did not show a difference in time spent in the novel chamber (n.s.). There was no difference in time spent in the novel chamber between ELS + TBI and TBI (n.s.).



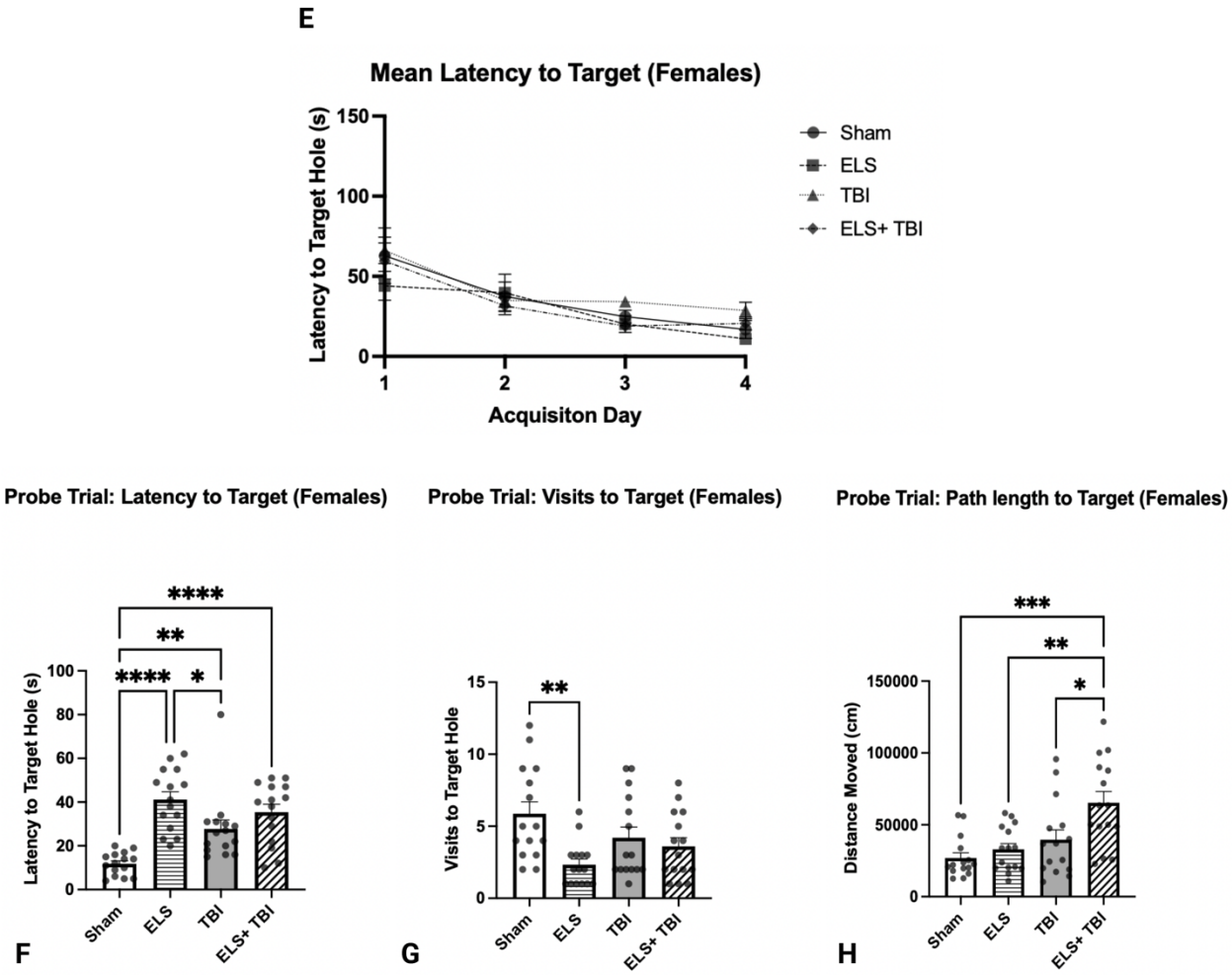


Figure 14. The Barnes maze was used to test spatial and contextual learning and memory.
A.) All male mice learned the location of the target hole by the last acquisition day (N=15/group, simple linear regression).
Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated.
B.) On probe trial day, TBI resulted in a greater latency to the target (****p<0.0001). ELS showed a similar latency to the target hole (n.s.). ELS + TBI showed a longer latency to find the target (****p<0.0001). ELS + TBI did not show a difference in latency to find the target hole compared to TBI (n.s.). C.) No differences in number of visits to the nest was seen in male mice.
D.) In females, TBI resulted in greater path length (*p<0.05). ELS showed a similar path length to the target (n.s.). ELS + TBI showed a greater path length to the target hole (****p<0.0001). ELS + TBI resulted in greater path length compared to TBI (**p<0.01).
E.) Female mice from all groups learned the location of the target hole by the last acquisition day N=15 females/group, simple linear regression).
F.) On probe trial day, TBI in females resulted in greater latency to the target (**p<0.01). ELS showed longer latency to the target hole (****p<0.0001). ELS + TBI resulted in higher latency

to the target hole (****p<0.0001). ELS + TBI in females did not contribute to a longer latency to target hole compared to TBI (n.s.).
G.) In females, TBI made a similar number of visits to the target hole. ELS made less visits to the target hole (**p<0.01). ELS + TBI did not show a difference in the number of visits made to the target hole (n.s.). ELS + TBI made a similar number of visits to the target hole compared to TBI.
H.) TBI and ELS in females showed no difference in path length to find the target hole compared to sham (n.s., for both comparisons). ELS + TBI showed a longer path length to the target hole (***p<0.001) and ELS + TBI resulted in longer path lengths to find the target hole compared to TBI (*p<0.05).

Behavior Summary

Behavior	Measure	Outcome Males	Outcome Females
Open Field Test	% Time in Inner Zone	**ELS:** ↑ **TBI:** ↓ **ELS+TBI:** No Change	**ELS:** ↓ **TBI:** ↓ **ELS+TBI:** No Change
	Distance Moved	**ELS:** No Change **TBI:** ↑ **ELS+TBI:** ↓	**ELS:** ↑ **TBI:** No Change **ELS+TBI:** ↑
Elevated Zero Maze	% Time in Open Arm	**ELS:** ↑ **TBI:** ↑ **ELS+TBI:** ↑	**ELS:** ↑ **TBI:** ↑ **ELS+TBI:** No Change
	Distance Moved	**ELS:** No Change **TBI:** No Change **ELS+TBI:** No Change	**ELS:** ↑ **TBI:** ↓ **ELS+TBI:** ↑
Novel Object Recognition	Novelty Preference	**ELS:** No Change **TBI:** ↓ **ELS+TBI:** No Change	**ELS:** ↓ **TBI:** ↓ **ELS+TBI:** ↓
Three-Chamber Task	Social Novelty Preference	**ELS:** No Change **TBI:** ↓ **ELS+TBI:** No Change	**ELS:** No Change **TBI:** ↓ **ELS+TBI:** No Change
Barnes Maze	Spatial Memory (Latency to Target)	**ELS:** No Change **TBI:** ↑ **ELS+TBI:** No change	**ELS:** ↑ **TBI:** ↑ **ELS+TBI:** No Change

Table 4. Summary of behaviors at adulthood. Behaviors measured at adulthood are summarized above. Outcome comparisons are the following: ELS = ELS vs sham; TBI = TBI vs sham; ELS + TBI = ELS + TBI vs TBI. The arrows in the OFT and EZM indicate an increase or decrease in the time spent or distance moved. For the NOR and 3-chamber task a down arrow indicates decreased performance in each task. In the Barnes Maze, an up arrow indicates decreased performance.

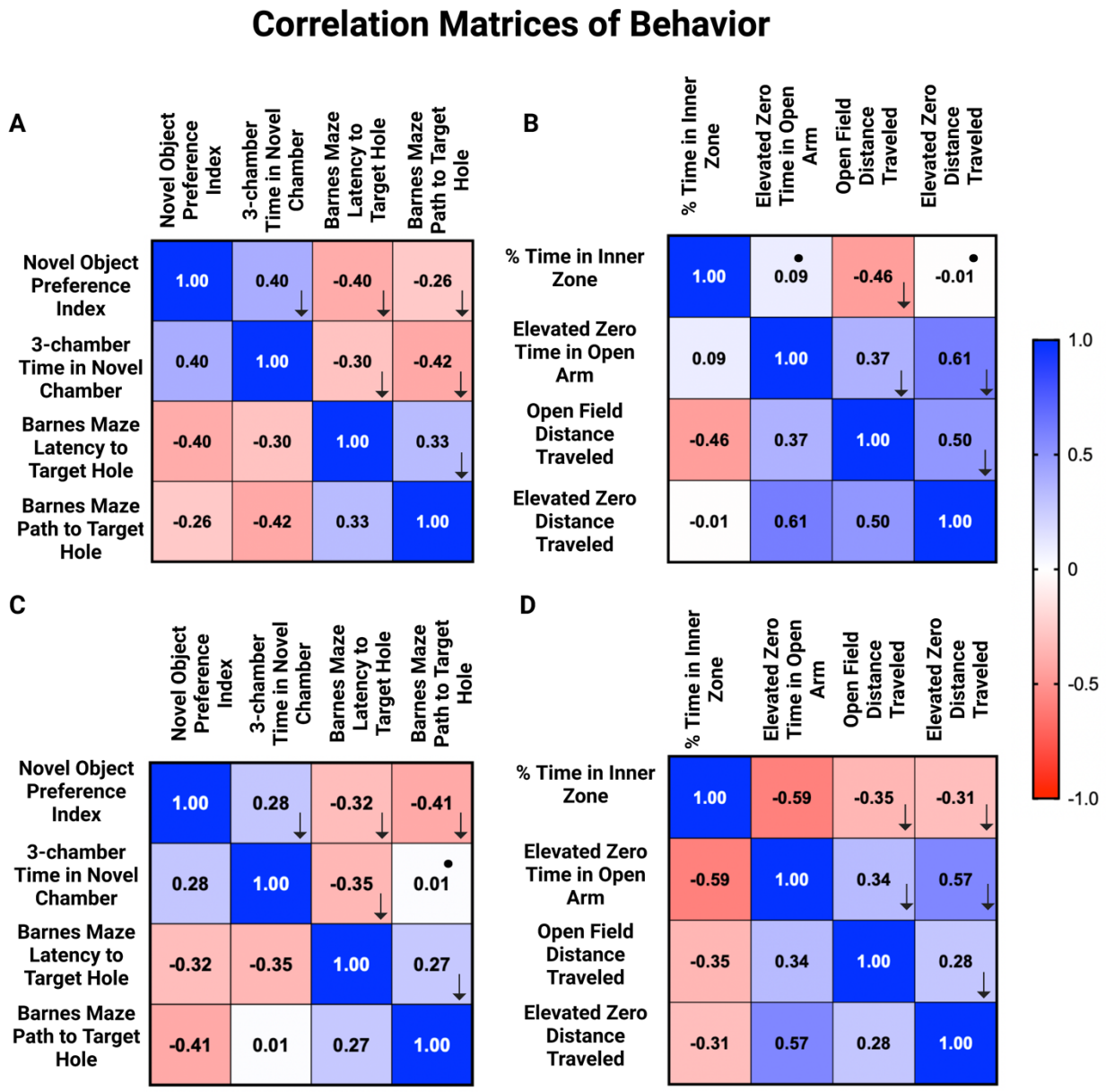


Figure 15. Learning and memory and anxiety-like behaviors were evaluated at adulthood.

Correlation matrices were used to summarize learning and memory related behaviors in A.) Males and C.) Females and measures of anxiety and overall activity in B.) Males and D.) Females. Outcomes from each category of behavior are correlated with one another. Pearson's r correlation coefficients are reported in each square (-1.0 to +1.0). The shades of red indicate a significant negative correlation (-1.0, $p<0.05$), shades of blue indicate a significant positive correlation (+1.0, $p<0.05$). A black dot in a square indicates non-significance and a down arrow indicates a decreased performance in both groups.

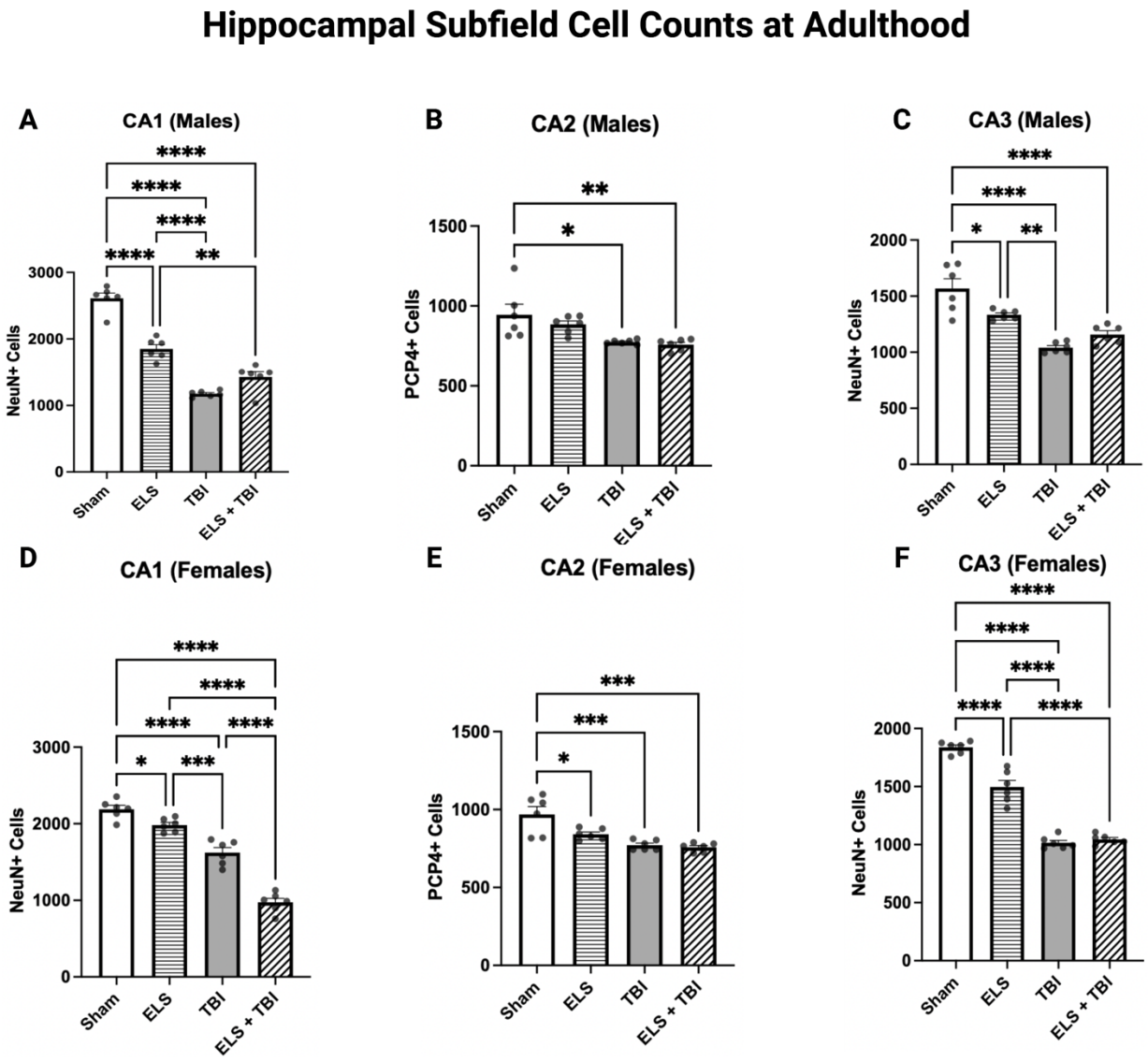


Figure 16. Stereological techniques were used to quantify neurons in CA1, CA2, and CA3.

Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated.

In males, TBI resulted in decreased labeling of NeuN+ in the A.) CA1 (****p<0.0001), decreased PCP4+ cells in the B.) CA2 (*p<0.05), and decreased NeuN+ cells in the C.) CA3 (****p<0.0001). ELS decreased labeling of NeuN+ cells in the A.) CA1 (****p<0.0001) and C.) CA3(*p<0.05). ELS + TBI showed less NeuN+ labeling in the A.) CA1 (****p<0.0001), PCP4+ cells in the B.) CA2 (**p<0.01), and NeuN+ cells in the C.) CA3 (****p<0.0001). ELS + TBI did not show differences in labeling in the A.) CA1 (n.s.), B.) CA2 (n.s.), and C.) CA3 (n.s.) compared to TBI. In females, TBI showed less NeuN+ labeling in the D.) CA1(****p<0.0001),

PCP4+ labeling in the E.) CA2 (***p<0.001), and NeuN+ cells in the F.) CA3 (****p<0.0001). ELS decreased NeuN+ cells in the D.) CA1 (*p<0.05), PCP4+ cells in the E.) CA2. (*p<0.05), and NeuN+ cells in the F.) CA3 (****p<0.0001). ELS + TBI resulted in decreased NeuN+ labeling in the D.) CA1 (****p<0.0001), PCP4+ labeling in the E.) CA2 (***p<0.001), and NeuN+ cells in the F.) CA3 (****p<0.0001). ELS + TBI decreased NeuN+ in the D.) CA1 (****p<0.0001), compared to TBI.

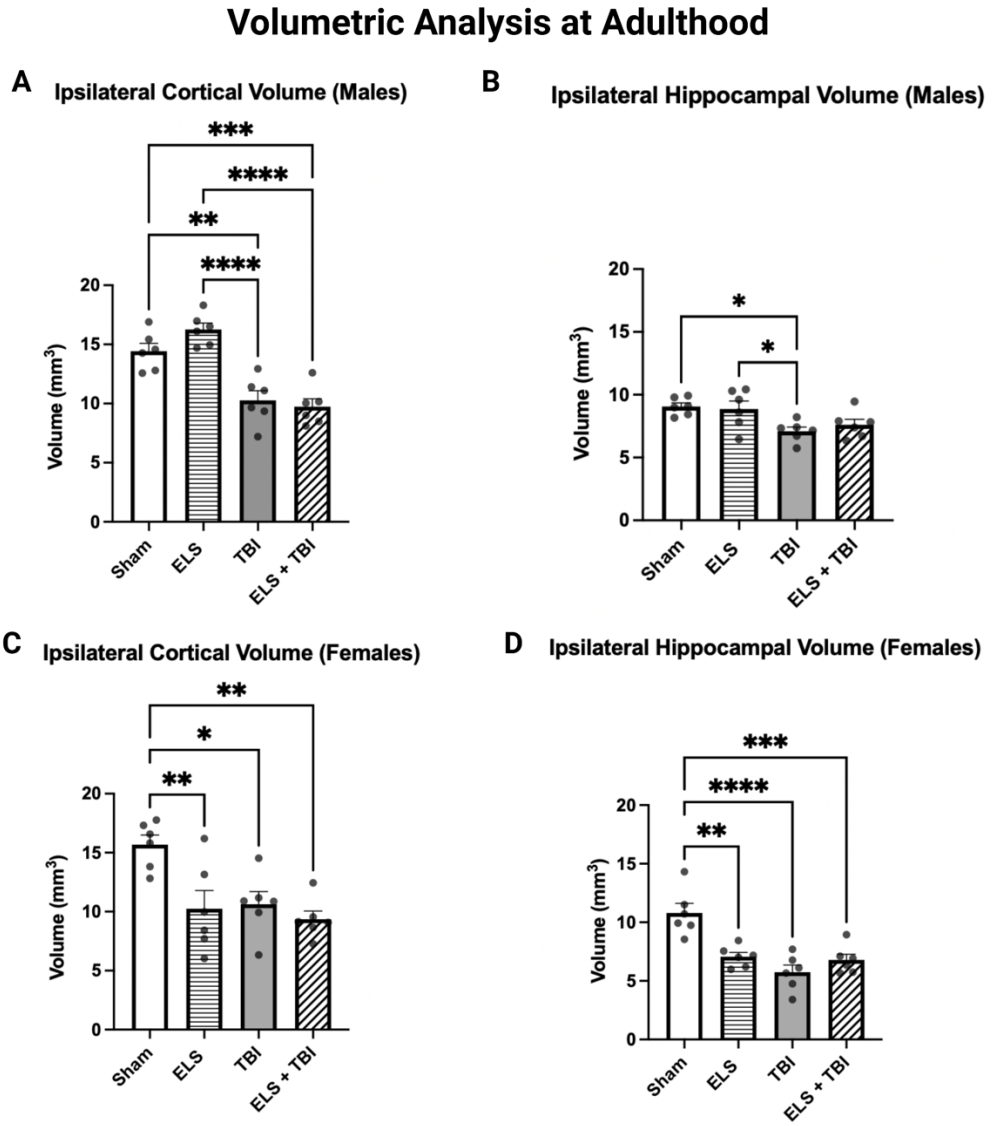


Figure 17. At adulthood, ELS+TBI resulted in similar cortical and hippocampal volumetric loss when compared to TBI alone.

Data in males and females were first separately evaluated across all groups and found to be significantly different (Supplemental tables, 45-50; N= 6/group, one-way ANOVA). Tukey's post hoc tests were then used for multiple comparisons (adjusted p, *p<0.05 significance

threshold, n.s.= not significant, one-way ANOVA). All reported stats are compared to the sham controls, unless otherwise stated.

A.) In males, TBI resulted in a decrease in cortical volume (**p<0.01). ELS did not show a difference in cortical volume (n.s.). ELS + TBI showed cortical volume loss (***p<0.001). ELS + TBI did not show differences in cortical reduction compared to TBI. B.) TBI resulted in less hippocampal volume (*p<0.05). ELS did not show a difference in hippocampal volume (n.s.). ELS + TBI also did not show a difference in hippocampal volume (n.s.). C.) In females, TBI resulted in less cortical volume (**p<0.01). ELS reduced cortical volume (**p<0.01). ELS + TBI resulted in cortical loss (**p<0.01). There were no differences in cortical volume loss between ELS+ TBI and TBI. D.) TBI resulted in less hippocampal volume(****p<0.0001). ELS resulted in hippocampal (**p<0.01). ELS + TBI resulted in hippocampal loss (***p<0.001). No differences in hippocampal volume were seen between ELS + TBI and TBI (n.s.).

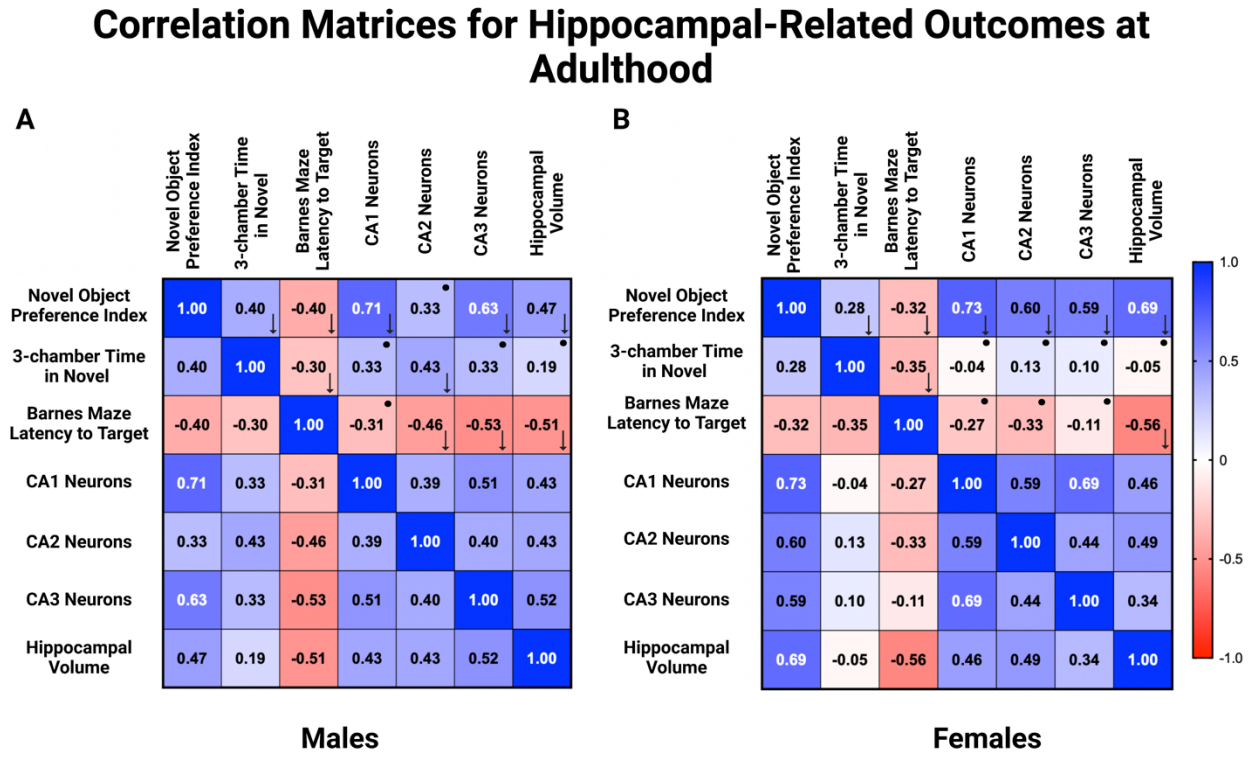


Figure 18. Hippocampal-dependent behaviors were correlated with neuronal loss in hippocampal CA1, CA2, and CA3 and cortical and hippocampal volumetric loss.
A, B.) Correlation matrices were used to summarize hippocampal dependent behaviors, assayed at adulthood, with ipsilateral cortical and hippocampal volumes and hippocampal subfields in males (A) and females (B). Pearson's r correlation coefficients are reported in each square (-1.0 to +1.0). Shades of red and blue indicate either a significant negative correlation (-1.0, p<0.05) or a significant positive correlation (+1.0, p<0.05), respectively. A black dot in a square indicates non-significance and a down arrow indicates a decrease in performance and a decrease in the number of neurons.

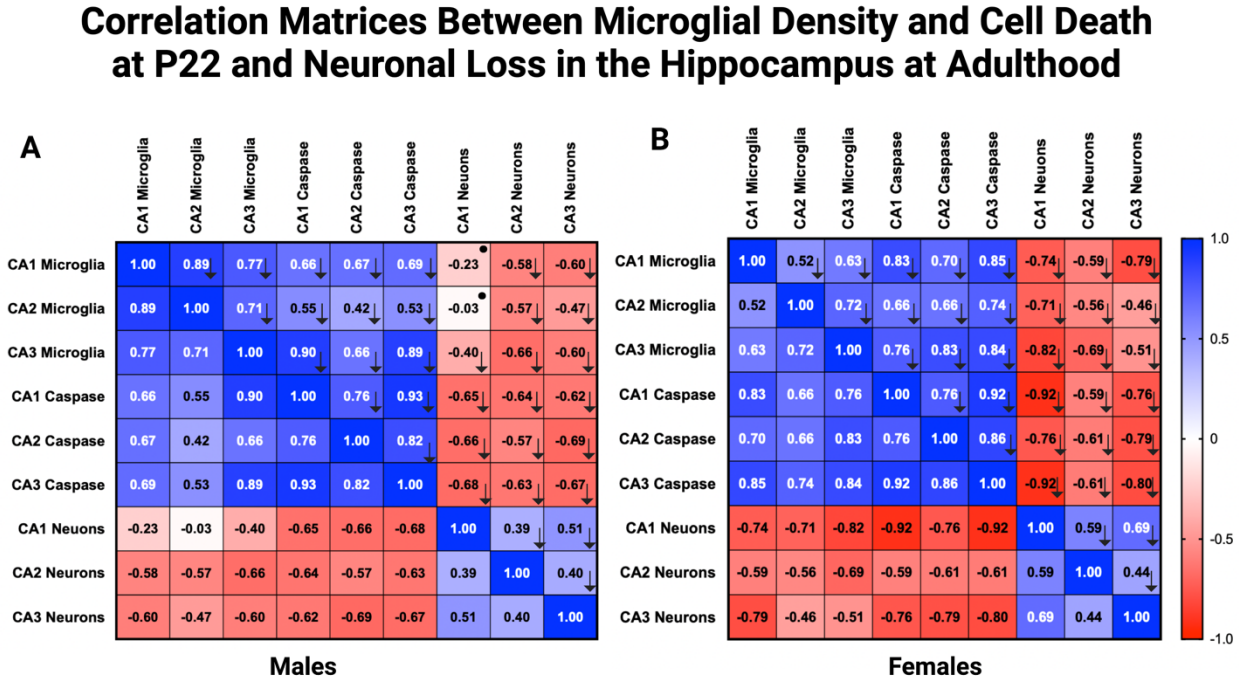


Figure 19. Indices of acute pathogenesis at P22 (microglial activation, increased caspase-3) were correlated with a reduction in neurons in hippocampal subfields at adulthood.
Correlation matrices were used to examine the temporal relationship between ELS and ELS+ TBI in A.) males and B.) females. Pearson's r correlation coefficients are reported in each square (-1.0 to +1.0) and a heat map was generated to visualize correlation strength between variables. shades of red indicate a significant negative correlation (-1.0, $p<0.05$), shades of blue indicate a significant positive correlation (+1.0, $p<0.05$). A black dot in a square indicates non-significance and a down arrow indicates an adverse relationship between the correlated outcomes.

References

Ader, R. (1970). "The effects of early experience on the adrenocortical response to different magnitudes of stimulation." Physiol Behav **5**(8): 837-839.

Agorastos, A., P. Pervanidou, G. P. Chrousos and D. G. Baker (2019). "Developmental Trajectories of Early Life Stress and Trauma: A Narrative Review on Neurobiological Aspects Beyond Stress System Dysregulation." Front Psychiatry **10**: 118.

Akkerman, S., A. Blokland, O. Reneerkens, N. P. van Goethem, E. Bollen, H. J. Gijselaers, C. K. Lieben, H. W. Steinbusch and J. Prickaerts (2012). "Object recognition testing: methodological considerations on exploration and discrimination measures." Behav Brain Res **232**(2): 335-347.

Amini-Khoei, H., E. Haghani-Samani, M. Beigi, A. Soltani, G. R. Mobini, S. Balali-Dehkordi, A. Haj-Mirzaian, M. Rafieian-Kopaei, A. Alizadeh, M. R. Hojjati and M. Validi (2019). "On the role of corticosterone in behavioral disorders, microbiota composition alteration and neuroimmune response in adult male mice subjected to maternal separation stress." Int Immunopharmacol **66**: 242-250.

Anderson, S. W., H. Damasio, D. Tranel and A. R. Damasio (2000). "Long-term sequelae of prefrontal cortex damage acquired in early childhood." Dev Neuropsychol **18**(3): 281-296.

Anderson, V., M. H. Beauchamp, K. O. Yeates, L. Crossley, S. J. Hearps and C. Catroppa (2013). "Social competence at 6 months following childhood traumatic brain injury." J Int Neuropsychol Soc **19**(5): 539-550.

Anderson, V. and C. Catroppa (2005). "Recovery of executive skills following paediatric traumatic brain injury (TBI): a 2 year follow-up." Brain Inj **19**(6): 459-470.

Anderson, V., C. Catroppa, S. Morse, F. Haritou and J. Rosenfeld (2005). "Functional plasticity or vulnerability after early brain injury?" Pediatrics **116**(6): 1374-1382.

Anderson, V., C. Godfrey, J. V. Rosenfeld and C. Catroppa (2012). "Predictors of cognitive function and recovery 10 years after traumatic brain injury in young children." Pediatrics **129**(2): e254-261.

Anderson, V., M. Spencer-Smith, R. Leventer, L. Coleman, P. Anderson, J. Williams, M. Greenham and R. Jacobs (2009). "Childhood brain insult: can age at insult help us predict outcome?" Brain **132**(Pt 1): 45-56.

Anderson, V. A., M. M. Spencer-Smith, L. Coleman, P. J. Anderson, M. Greenham, R. Jacobs, K. J. Lee and R. J. Leventer (2014). "Predicting neurocognitive and behavioural outcome after early brain insult." Dev Med Child Neurol **56**(4): 329-336.

Araujo, D. M. and C. W. Cotman (1995). "Differential effects of interleukin-1 beta and interleukin-2 on glia and hippocampal neurons in culture." Int J Dev Neurosci **13**(3-4): 201-212.

Arp, J. M., J. P. Ter Horst, M. Loi, J. den Blaauwen, E. Bangert, G. Fernandez, M. Joels, M. S. Oitzl and H. J. Krugers (2016). "Blocking glucocorticoid receptors at adolescent age prevents enhanced freezing between repeated cue-exposures after conditioned fear in adult mice raised under chronic early life stress." Neurobiol Learn Mem **133**: 30-38.

Avishai-Eliner, S., E. E. Gilles, M. Eghbal-Ahmadi, Y. Bar-El and T. Z. Baram (2001). "Altered regulation of gene and protein expression of hypothalamic-pituitary-adrenal axis components in an immature rat model of chronic stress." J Neuroendocrinol **13**(9): 799-807.

Babikian, T., T. Merkley, R. C. Savage, C. C. Giza and H. Levin (2015). "Chronic Aspects of Pediatric Traumatic Brain Injury: Review of the Literature." J Neurotrauma **32**(23): 1849-1860.

Bandelow, B., A. Charimo Torrente, D. Wedekind, A. Broocks, G. Hajak and E. Ruther (2004). "Early traumatic life events, parental rearing styles, family history of mental disorders, and birth risk factors in patients with social anxiety disorder." Eur Arch Psychiatry Clin Neurosci **254**(6): 397-405.
Banyard, V. L. (1999). "Childhood maltreatment and the mental health of low-income women." Am J Orthopsychiatry **69**(2): 161-171.
Barik, J., F. Marti, C. Morel, S. P. Fernandez, C. Lanteri, G. Godeheu, J. P. Tassin, C. Mombereau, P. Faure and F. Tronche (2013). "Chronic stress triggers social aversion via glucocorticoid receptor in dopaminoceptive neurons." Science **339**(6117): 332-335.
Bath, K. G., G. Manzano-Nieves and H. Goodwill (2016). "Early life stress accelerates behavioral and neural maturation of the hippocampus in male mice." Horm Behav **82**: 64-71.
Bauernfeind, F. G., G. Horvath, A. Stutz, E. S. Alnemri, K. MacDonald, D. Speert, T. Fernandes-Alnemri, J. Wu, B. G. Monks, K. A. Fitzgerald, V. Hornung and E. Latz (2009). "Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression." J Immunol **183**(2): 787-791.
Bayir, H., V. E. Kagan, Y. Y. Tyurina, V. Tyurin, R. A. Ruppel, P. D. Adelson, S. H. Graham, K. Janesko, R. S. Clark and P. M. Kochanek (2002). "Assessment of antioxidant reserves and oxidative stress in cerebrospinal fluid after severe traumatic brain injury in infants and children." Pediatr Res **51**(5): 571-578.
Bayir, H., P. M. Kochanek and V. E. Kagan (2006). "Oxidative stress in immature brain after traumatic brain injury." Dev Neurosci **28**(4-5): 420-431.
Bennett, E. R., K. Reuter-Rice and D. T. Laskowitz (2016). Genetic Influences in Traumatic Brain Injury. Translational Research in Traumatic Brain Injury. D. Laskowitz and G. Grant. Boca Raton (FL).
Bensley, L. S., J. Van Eenwyk, S. J. Spieker and J. Schoder (1999). "Self-reported abuse history and adolescent problem behaviors. I. Antisocial and suicidal behaviors." J Adolesc Health **24**(3): 163-172.
Bernhagen, J., T. Calandra, R. A. Mitchell, S. B. Martin, K. J. Tracey, W. Voelter, K. R. Manogue, A. Cerami and R. Bucala (1993). "MIF is a pituitary-derived cytokine that potentiates lethal endotoxaemia." Nature **365**(6448): 756-759.
Bhatnagar, S. and M. J. Meaney (1995). "Hypothalamic-pituitary-adrenal function in chronic intermittently cold-stressed neonatally handled and non handled rats." J Neuroendocrinol **7**(2): 97-108.
Biagini, G., E. M. Pich, C. Carani, P. Marrama and L. F. Agnati (1998). "Postnatal maternal separation during the stress hyporesponsive period enhances the adrenocortical response to novelty in adult rats by affecting feedback regulation in the CA1 hippocampal field." Int J Dev Neurosci **16**(3-4): 187-197.
Bilbo, S. D. (2010). "Early-life infection is a vulnerability factor for aging-related glial alterations and cognitive decline." Neurobiol Learn Mem **94**(1): 57-64.
Bilbo, S. D., J. C. Biedenkapp, A. Der-Avakian, L. R. Watkins, J. W. Rudy and S. F. Maier (2005). "Neonatal infection-induced memory impairment after lipopolysaccharide in adulthood is prevented via caspase-1 inhibition." J Neurosci **25**(35): 8000-8009.
Birrell, J. M. and V. J. Brown (2000). "Medial frontal cortex mediates perceptual attentional set shifting in the rat." J Neurosci **20**(11): 4320-4324.
Blakemore, S. J. (2008). "The social brain in adolescence." Nat Rev Neurosci **9**(4): 267-277.

Bodnar, C. N., K. N. Roberts, E. K. Higgins and A. D. Bachstetter (2019). "A Systematic Review of Closed Head Injury Models of Mild Traumatic Brain Injury in Mice and Rats." J Neurotrauma **36**(11): 1683-1706.
Bondar, N. P., A. A. Lepeshko and V. V. Reshetnikov (2018). "Effects of Early-Life Stress on Social and Anxiety-Like Behaviors in Adult Mice: Sex-Specific Effects." Behav Neurol **2018**: 1538931.
Bondi, C. O., J. D. Jett and D. A. Morilak (2010). "Beneficial effects of desipramine on cognitive function of chronically stressed rats are mediated by alpha1-adrenergic receptors in medial prefrontal cortex." Prog Neuropsychopharmacol Biol Psychiatry **34**(6): 913-923.
Briere, J. and M. Runtz (1988). "Multivariate correlates of childhood psychological and physical maltreatment among university women." Child Abuse Negl **12**(3): 331-341.
Brunson, K. L., E. Kramar, B. Lin, Y. Chen, L. L. Colgin, T. K. Yanagihara, G. Lynch and T. Z. Baram (2005). "Mechanisms of late-onset cognitive decline after early-life stress." J Neurosci **25**(41): 9328-9338.
Burnett, S., C. Sebastian, K. Cohen Kadosh and S. J. Blakemore (2011). "The social brain in adolescence: evidence from functional magnetic resonance imaging and behavioural studies." Neurosci Biobehav Rev **35**(8): 1654-1664.
Busillo, J. M., K. M. Azzam and J. A. Cidlowski (2011). "Glucocorticoids sensitize the innate immune system through regulation of the NLRP3 inflammasome." J Biol Chem **286**(44): 38703-38713.
Byrnes, K. R., D. J. Loane, B. A. Stoica, J. Zhang and A. I. Faden (2012). "Delayed mGluR5 activation limits neuroinflammation and neurodegeneration after traumatic brain injury." J Neuroinflammation **9**: 43.
Calandra, T., J. Bernhagen, C. N. Metz, L. A. Spiegel, M. Bacher, T. Donnelly, A. Cerami and R. Bucala (1995). "MIF as a glucocorticoid-induced modulator of cytokine production." Nature **377**(6544): 68-71.
Carpenter, L. L., C. E. Gawuga, A. R. Tyrka, J. K. Lee, G. M. Anderson and L. H. Price (2010). "Association between plasma IL-6 response to acute stress and early-life adversity in healthy adults." Neuropsychopharmacology **35**(13): 2617-2623.
Caspi, A., K. Sugden, T. E. Moffitt, A. Taylor, I. W. Craig, H. Harrington, J. McClay, J. Mill, J. Martin, A. Braithwaite and R. Poulton (2003). "Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene." Science **301**(5631): 386-389.
Catroppa, C., V. Anderson, M. Ditchfield and L. Coleman (2008). "Using magnetic resonance imaging to predict new learning outcome at 5 years after childhood traumatic brain injury." J Child Neurol **23**(5): 486-496.
Catroppa, C., V. A. Anderson, S. A. Morse, F. Haritou and J. V. Rosenfeld (2008). "Outcome and predictors of functional recovery 5 years following pediatric traumatic brain injury (TBI)." J Pediatr Psychol **33**(7): 707-718.
Cavaiola, A. A. and M. Schiff (1988). "Behavioral sequelae of physical and/or sexual abuse in adolescents." Child Abuse Negl **12**(2): 181-188.
Chapman, S. B. and L. McKinnon (2000). "Discussion of developmental plasticity: factors affecting cognitive outcome after pediatric traumatic brain injury." J Commun Disord **33**(4): 333-344.
Chastain, L. G., T. Franklin, O. Gangisetty, M. A. Cabrera, S. Mukherjee, P. Shrivastava, S. Jabbar and D. K. Sarkar (2019). "Early life alcohol exposure primes hypothalamic microglia to

later-life hypersensitivity to immune stress: possible epigenetic mechanism." Neuropsychopharmacology **44**(9): 1579-1588.
Chauhan, N. B. and R. Gatto (2011). "Restoration of cognitive deficits after statin feeding in TBI." Restor Neurol Neurosci **29**(1): 23-34.
Chen, C. Y., L. J. Noble-Haeusslein, D. Ferriero and B. D. Semple (2013). "Traumatic injury to the immature frontal lobe: a new murine model of long-term motor impairment in the absence of psychosocial or cognitive deficits." Dev Neurosci **35**(6): 474-490.
Chen, M. C., J. P. Hamilton and I. H. Gotlib (2010). "Decreased hippocampal volume in healthy girls at risk of depression." Arch Gen Psychiatry **67**(3): 270-276.
Chhor, V., R. Moretti, T. Le Charpentier, S. Sigaut, S. Lebon, L. Schwendimann, M. V. Ore, C. Zuiani, V. Milan, J. Josserand, R. Vontell, J. Pansiot, V. Degos, C. Ikonomidou, L. Titomanlio, H. Hagberg, P. Gressens and B. Fleiss (2017). "Role of microglia in a mouse model of paediatric traumatic brain injury." Brain Behav Immun **63**: 197-209.
Chu, J. A. and D. L. Dill (1990). "Dissociative symptoms in relation to childhood physical and sexual abuse." Am J Psychiatry **147**(7): 887-892.
Clarke, A. S. (1993). "Social rearing effects on HPA axis activity over early development and in response to stress in rhesus monkeys." Dev Psychobiol **26**(8): 433-446.
Cortes, D. and M. F. Pera (2021). "The genetic basis of inter-individual variation in recovery from traumatic brain injury." NPJ Regen Med **6**(1): 5.
Cory, C. Z., M. D. Jones, D. S. James, S. Leadbeatter and L. D. Nokes (2001). "The potential and limitations of utilising head impact injury models to assess the likelihood of significant head injury in infants after a fall." Forensic Sci Int **123**(2-3): 89-106.
Cowan, F., M. Rutherford, F. Groenendaal, P. Eken, E. Mercuri, G. M. Bydder, L. C. Meiners, L. M. Dubowitz and L. S. de Vries (2003). "Origin and timing of brain lesions in term infants with neonatal encephalopathy." Lancet **361**(9359): 736-742.
Cowan, N. (2010). "Multiple concurrent thoughts: The meaning and developmental neuropsychology of working memory." Dev Neuropsychol **35**(5): 447-474.
Cowan, N. (2014). "Working Memory Underpins Cognitive Development, Learning, and Education." Educ Psychol Rev **26**(2): 197-223.
Cox, G. (1995). "Glucocorticoid treatment inhibits apoptosis in human neutrophils. Separation of survival and activation outcomes." J Immunol **154**(9): 4719-4725.
Craft, T. K., N. Zhang, E. R. Glasper, P. D. Hurn and A. C. Devries (2006). "Neonatal factors influence adult stroke outcome." Psychoneuroendocrinology **31**(5): 601-613.
Crain, B., C. Cotman, D. Taylor and G. Lynch (1973). "A quantitative electron microscopic study of synaptogenesis in the dentate gyrus of the rat." Brain Res **63**: 195-204.
Crawley, J. N. (2008). "Behavioral phenotyping strategies for mutant mice." Neuron **57**(6): 809-818.
Dalle Molle, R., A. K. Portella, M. Z. Goldani, F. P. Kapczinski, S. Leistner-Segal, G. A. Salum, G. G. Manfro and P. P. Silveira (2012). "Associations between parenting behavior and anxiety in a rodent model and a clinical sample: relationship to peripheral BDNF levels." Transl Psychiatry **2**: e195.
Daneman, R., L. Zhou, A. A. Kebede and B. A. Barres (2010). "Pericytes are required for blood-brain barrier integrity during embryogenesis." Nature **468**(7323): 562-566.
Danese, A., T. E. Moffitt, H. Harrington, B. J. Milne, G. Polanczyk, C. M. Pariante, R. Poulton and A. Caspi (2009). "Adverse childhood experiences and adult risk factors for age-related

disease: depression, inflammation, and clustering of metabolic risk markers." Arch Pediatr Adolesc Med **163**(12): 1135-1143.
Danese, A., C. M. Pariante, A. Caspi, A. Taylor and R. Poulton (2007). "Childhood maltreatment predicts adult inflammation in a life-course study." Proc Natl Acad Sci U S A **104**(4): 1319-1324.
De Brito, S. A., A. Mechelli, M. Wilke, K. R. Laurens, A. P. Jones, G. J. Barker, S. Hodgins and E. Viding (2009). "Size matters: increased grey matter in boys with conduct problems and callous-unemotional traits." Brain **132**(Pt 4): 843-852.
de Kloet, E. R., R. M. Sibug, F. M. Helmerhorst and M. V. Schmidt (2005). "Stress, genes and the mechanism of programming the brain for later life." Neurosci Biobehav Rev **29**(2): 271-281.
De Miguel, C., I. E. Obi, D. H. Ho, A. S. Loria and J. S. Pollock (2018). "Early life stress induces immune priming in kidneys of adult male rats." Am J Physiol Renal Physiol **314**(3): F343-F355.
De Vries, G. J., E. F. Rissman, R. B. Simerly, L. Y. Yang, E. M. Scordalakes, C. J. Auger, A. Swain, R. Lovell-Badge, P. S. Burgoyne and A. P. Arnold (2002). "A model system for study of sex chromosome effects on sexually dimorphic neural and behavioral traits." J Neurosci **22**(20): 9005-9014.
de Zoete, M. R., N. W. Palm, S. Zhu and R. A. Flavell (2014). "Inflammasomes." Cold Spring Harb Perspect Biol **6**(12): a016287.
Delpech, J. C., L. Wei, J. Hao, X. Yu, C. Madore, O. Butovsky and A. Kaffman (2016). "Early life stress perturbs the maturation of microglia in the developing hippocampus." Brain Behav Immun **57**: 79-93.
Dent, G. W., M. A. Smith and S. Levine (2000). "Rapid induction of corticotropin-releasing hormone gene transcription in the paraventricular nucleus of the developing rat." Endocrinology **141**(5): 1593-1598.
Despins, E. H., L. S. Turkstra, M. A. Struchen and A. N. Clark (2016). "Sex-Based Differences in Perceived Pragmatic Communication Ability of Adults With Traumatic Brain Injury." Arch Phys Med Rehabil **97**(2 Suppl): S26-32.
Diaz-Chavez, A., N. Lajud, A. Roque, J. P. Cheng, E. Melendez-Herrera, J. J. Valdez-Alarcon, C. O. Bondi and A. E. Kline (2020). "Early life stress increases vulnerability to the sequelae of pediatric mild traumatic brain injury." Exp Neurol **329**: 113318.
Dickens, A. M., Y. R. L. B. Tovar, S. W. Yoo, A. L. Trout, M. Bae, M. Kanmogne, B. Megra, D. W. Williams, K. W. Witwer, M. Gacias, N. Tabatadze, R. N. Cole, P. Casaccia, J. W. Berman, D. C. Anthony and N. J. Haughey (2017). "Astrocyte-shed extracellular vesicles regulate the peripheral leukocyte response to inflammatory brain lesions." Sci Signal **10**(473).
Dixon, C. E., P. M. Kochanek, H. Q. Yan, J. K. Schiding, R. G. Griffith, E. Baum, D. W. Marion and S. T. DeKosky (1999). "One-year study of spatial memory performance, brain morphology, and cholinergic markers after moderate controlled cortical impact in rats." J Neurotrauma **16**(2): 109-122.
Doganyigit, Z., K. Erbakan, E. Akyuz, A. K. Polat, A. Arulsamy and M. F. Shaikh (2022). "The Role of Neuroinflammatory Mediators in the Pathogenesis of Traumatic Brain Injury: A Narrative Review." ACS Chem Neurosci **13**(13): 1835-1848.
Domowicz, M., N. L. Wadlington, J. G. Henry, K. Diaz, M. J. Munoz and N. B. Schwartz (2018). "Glial cell responses in a murine multifactorial perinatal brain injury model." Brain Res **1681**: 52-63.

Dunlop, T. W., G. B. Udvarhelyi, A. F. Stedem, J. M. O'Connor, M. L. Isaacs, J. G. Puig and J. H. Mather (1991). "Comparison of patients with and without emotional/behavioral deterioration during the first year after traumatic brain injury." J Neuropsychiatry Clin Neurosci **3**(2): 150-156.
Ehrlich, K. B., K. M. Ross, E. Chen and G. E. Miller (2016). "Testing the biological embedding hypothesis: Is early life adversity associated with a later proinflammatory phenotype?" Dev Psychopathol **28**(4pt2): 1273-1283.
Ennaceur, A. (2010). "One-trial object recognition in rats and mice: methodological and theoretical issues." Behav Brain Res **215**(2): 244-254.
Ennaceur, A. and J. Delacour (1988). "A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data." Behav Brain Res **31**(1): 47-59.
Ericsson, K. A. and W. Kintsch (1995). "Long-term working memory." Psychol Rev **102**(2): 211-245.
Eslinger, P. J. and K. R. Biddle (2000). "Adolescent neuropsychological development after early right prefrontal cortex damage." Dev Neuropsychol **18**(3): 297-329.
Fagundes, C. P., R. Glaser and J. K. Kiecolt-Glaser (2013). "Stressful early life experiences and immune dysregulation across the lifespan." Brain Behav Immun **27**(1): 8-12.
Fan, P., T. Yamauchi, L. J. Noble and D. M. Ferriero (2003). "Age-dependent differences in glutathione peroxidase activity after traumatic brain injury." J Neurotrauma **20**(5): 437-445.
Felitti, V. J., R. F. Anda, D. Nordenberg, D. F. Williamson, A. M. Spitz, V. Edwards, M. P. Koss and J. S. Marks (1998). "Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The Adverse Childhood Experiences (ACE) Study." Am J Prev Med **14**(4): 245-258.
Fine, I., A. R. Wade, A. A. Brewer, M. G. May, D. F. Goodman, G. M. Boynton, B. A. Wandell and D. I. MacLeod (2003). "Long-term deprivation affects visual perception and cortex." Nat Neurosci **6**(9): 915-916.
Fitzsimons, C. P., L. W. van Hooijdonk, M. Schouten, I. Zalachoras, V. Brinks, T. Zheng, T. G. Schouten, D. J. Saaltink, T. Dijkmans, D. A. Steindler, J. Verhaagen, F. J. Verbeek, P. J. Lucassen, E. R. de Kloet, O. C. Meijer, H. Karst, M. Joels, M. S. Oitzl and E. Vreugdenhil (2013). "Knockdown of the glucocorticoid receptor alters functional integration of newborn neurons in the adult hippocampus and impairs fear-motivated behavior." Mol Psychiatry **18**(9): 993-1005.
Flaherty, E. G., R. Thompson, H. Dubowitz, E. M. Harvey, D. J. English, L. J. Proctor and D. K. Runyan (2013). "Adverse childhood experiences and child health in early adolescence." JAMA Pediatr **167**(7): 622-629.
Fletcher, J. M., L. Ewing-Cobbs, M. E. Miner, H. S. Levin and H. M. Eisenberg (1990). "Behavioral changes after closed head injury in children." J Consult Clin Psychol **58**(1): 93-98.
Fogelman, N. and T. Canli (2019). "Early Life Stress, Physiology, and Genetics: A Review." Front Psychol **10**: 1668.
Fonken, L. K., A. D. Gaudet, K. R. Gaier, R. J. Nelson and P. G. Popovich (2016). "MicroRNA-155 deletion reduces anxiety- and depressive-like behaviors in mice." Psychoneuroendocrinology **63**: 362-369.
Fox, G. B., L. Fan, R. A. Levasseur and A. I. Faden (1998). "Sustained sensory/motor and cognitive deficits with neuronal apoptosis following controlled cortical impact brain injury in the mouse." J Neurotrauma **15**(8): 599-614.

Fox, G. B., R. A. LeVasseur and A. I. Faden (1999). "Behavioral responses of C57BL/6, FVB/N, and 129/SvEMS mouse strains to traumatic brain injury: implications for gene targeting approaches to neurotrauma." J Neurotrauma **16**(5): 377-389.
Frank, M. G., M. V. Baratta, D. B. Sprunger, L. R. Watkins and S. F. Maier (2007). "Microglia serve as a neuroimmune substrate for stress-induced potentiation of CNS pro-inflammatory cytokine responses." Brain Behav Immun **21**(1): 47-59.
Frank, M. G., L. K. Fonken, L. R. Watkins and S. F. Maier (2020). "Acute stress induces chronic neuroinflammatory, microglial and behavioral priming: A role for potentiated NLRP3 inflammasome activation." Brain Behav Immun **89**: 32-42.
Frank, M. G., M. D. Weber, L. K. Fonken, S. A. Hershman, L. R. Watkins and S. F. Maier (2016). "The redox state of the alarmin HMGB1 is a pivotal factor in neuroinflammatory and microglial priming: A role for the NLRP3 inflammasome." Brain Behav Immun **55**: 215-224.
Gallo, M., D. G. Shleifer, L. D. Godoy, D. Ofray, A. Olaniyan, T. Campbell and K. G. Bath (2019). "Limited Bedding and Nesting Induces Maternal Behavior Resembling Both Hypervigilance and Abuse." Front Behav Neurosci **13**: 167.
Galon, J., D. Franchimont, N. Hiroi, G. Frey, A. Boettner, M. Ehrhart-Bornstein, J. J. O'Shea, G. P. Chrousos and S. R. Bornstein (2002). "Gene profiling reveals unknown enhancing and suppressive actions of glucocorticoids on immune cells." FASEB J **16**(1): 61-71.
Gee, C. C., R. Steffen and F. M. Kievit (2023). "An updated Barnes maze protocol for assessing the outcome of controlled cortical impact mouse models of traumatic brain injury." J Neurosci Methods **392**: 109866.
Gerring, J. P., B. Slomine, R. A. Vasa, M. Grados, A. Chen, W. Rising, J. R. Christensen, M. B. Denckla and M. Ernst (2002). "Clinical predictors of posttraumatic stress disorder after closed head injury in children." J Am Acad Child Adolesc Psychiatry **41**(2): 157-165.
Giedd, J. N., J. Blumenthal, N. O. Jeffries, F. X. Castellanos, H. Liu, A. Zijdenbos, T. Paus, A. C. Evans and J. L. Rapoport (1999). "Brain development during childhood and adolescence: a longitudinal MRI study." Nat Neurosci **2**(10): 861-863.
Gilles, E. E., L. Schultz and T. Z. Baram (1996). "Abnormal corticosterone regulation in an immature rat model of continuous chronic stress." Pediatr Neurol **15**(2): 114-119.
Giovanelli, A., A. J. Reynolds, C. F. Mondi and S. R. Ou (2016). "Adverse Childhood Experiences and Adult Well-Being in a Low-income, Urban Cohort." Pediatrics **137**(4).
Giza, C. C. and M. L. Prins (2006). "Is being plastic fantastic? Mechanisms of altered plasticity after developmental traumatic brain injury." Dev Neurosci **28**(4-5): 364-379.
Gorka, A. X., J. L. Hanson, S. R. Radtke and A. R. Hariri (2014). "Reduced hippocampal and medial prefrontal gray matter mediate the association between reported childhood maltreatment and trait anxiety in adulthood and predict sensitivity to future life stress." Biol Mood Anxiety Disord **4**: 12.
Gould, D. A., N. G. Stevens, N. G. Ward, A. S. Carlin, H. E. Sowell and B. Gustafson (1994). "Self-reported childhood abuse in an adult population in a primary care setting. Prevalence, correlates, and associated suicide attempts." Arch Fam Med **3**(3): 252-256.
Grundy, P. L., M. S. Harbuz, D. S. Jessop, S. L. Lightman and P. M. Sharples (2001). "The hypothalamo-pituitary-adrenal axis response to experimental traumatic brain injury." J Neurotrauma **18**(12): 1373-1381.
Gunn, B. G., L. Cunningham, M. A. Cooper, N. L. Corteen, M. Seifi, J. D. Swinny, J. J. Lambert and D. Belelli (2013). "Dysfunctional astrocytic and synaptic regulation of hypothalamic

glutamatergic transmission in a mouse model of early-life adversity: relevance to neurosteroids and programming of the stress response." J Neurosci **33**(50): 19534-19554.
Gupte, R., W. Brooks, R. Vukas, J. Pierce and J. Harris (2019). "Sex Differences in Traumatic Brain Injury: What We Know and What We Should Know." J Neurotrauma **36**(22): 3063-3091.
Hagberg, H., A. David Edwards and F. Groenendaal (2016). "Perinatal brain damage: The term infant." Neurobiol Dis **92**(Pt A): 102-112.
Hall, F. S., L. S. Wilkinson, T. Humby and T. W. Robbins (1999). "Maternal deprivation of neonatal rats produces enduring changes in dopamine function." Synapse **32**(1): 37-43.
Hall, M. J., S. Levant and C. J. DeFrances (2012). "Hospitalization for stroke in U.S. hospitals, 1989-2009." NCHS Data Brief(95): 1-8.
Hamm, R. J., C. E. Dixon, D. M. Gbadebo, A. K. Singha, L. W. Jenkins, B. G. Lyeth and R. L. Hayes (1992). "Cognitive deficits following traumatic brain injury produced by controlled cortical impact." J Neurotrauma **9**(1): 11-20.
Hanlon, L. A., R. Raghupathi and J. W. Huh (2019). "Depletion of microglia immediately following traumatic brain injury in the pediatric rat: Implications for cellular and behavioral pathology." Exp Neurol **316**: 39-51.
Hanson, J. L., W. van den Bos, B. J. Roeber, K. D. Rudolph, R. J. Davidson and S. D. Pollak (2017). "Early adversity and learning: implications for typical and atypical behavioral development." J Child Psychol Psychiatry **58**(7): 770-778.
Hartmann, J., N. Dedic, M. L. Pohlmann, A. Hausl, H. Karst, C. Engelhardt, S. Westerholz, K. V. Wagner, C. Labermaier, L. Hoeijmakers, M. Kertokarijo, A. Chen, M. Joels, J. M. Deussing and M. V. Schmidt (2017). "Forebrain glutamatergic, but not GABAergic, neurons mediate anxiogenic effects of the glucocorticoid receptor." Mol Psychiatry **22**(3): 466-475.
Hirsch, C. and F. G. Evans (1965). "Studies on Some Physical Properties of Infant Compact Bone." Acta Orthop Scand **35**: 300-313.
Hitti, F. L. and S. A. Siegelbaum (2014). "The hippocampal CA2 region is essential for social memory." Nature **508**(7494): 88-92.
Hoeijmakers, L., S. R. Ruigrok, A. Amelianchik, D. Ivan, A. M. van Dam, P. J. Lucassen and A. Korosi (2017). "Early-life stress lastingly alters the neuroinflammatory response to amyloid pathology in an Alzheimer's disease mouse model." Brain Behav Immun **63**: 160-175.
Holmin, S. and B. Hojeberg (2004). "In situ detection of intracerebral cytokine expression after human brain contusion." Neurosci Lett **369**(2): 108-114.
Hu, B. R., C. L. Liu, Y. Ouyang, K. Blomgren and B. K. Siesjo (2000). "Involvement of caspase-3 in cell death after hypoxia-ischemia declines during brain maturation." J Cereb Blood Flow Metab **20**(9): 1294-1300.
Hubel, D. H. and T. N. Wiesel (1970). "The period of susceptibility to the physiological effects of unilateral eye closure in kittens." J Physiol **206**(2): 419-436.
Hughes, K., M. A. Bellis, K. A. Hardcastle, D. Sethi, A. Butchart, C. Mikton, L. Jones and M. P. Dunne (2017). "The effect of multiple adverse childhood experiences on health: a systematic review and meta-analysis." Lancet Public Health **2**(8): e356-e366.
Huttenlocher, P. R. (1979). "Synaptic density in human frontal cortex - developmental changes and effects of aging." Brain Res **163**(2): 195-205.
Ivy, A. S., K. L. Brunson, C. Sandman and T. Z. Baram (2008). "Dysfunctional nurturing behavior in rat dams with limited access to nesting material: a clinically relevant model for early-life stress." Neuroscience **154**(3): 1132-1142.

Jenkins, W. M. and M. M. Merzenich (1984). "Role of cat primary auditory cortex for sound-localization behavior." J Neurophysiol **52**(5): 819-847.
Johnson, F. K. and A. Kaffman (2018). "Early life stress perturbs the function of microglia in the developing rodent brain: New insights and future challenges." Brain Behav Immun **69**: 18-27.
Kalinichev, M., K. W. Easterling, P. M. Plotsky and S. G. Holtzman (2002). "Long-lasting changes in stress-induced corticosterone response and anxiety-like behaviors as a consequence of neonatal maternal separation in Long-Evans rats." Pharmacol Biochem Behav **73**(1): 131-140.
Kalsbeek, A., C. X. Yi, S. E. la Fleur, R. M. Buijs and E. Fliers (2010). "Suprachiasmatic nucleus and autonomic nervous system influences on awakening from sleep." Int Rev Neurobiol **93**: 91-107.
Kamper, J. E., V. Pop, A. M. Fukuda, D. O. Ajao, R. E. Hartman and J. Badaut (2013). "Juvenile traumatic brain injury evolves into a chronic brain disorder: behavioral and histological changes over 6months." Exp Neurol **250**: 8-19.
Kaplow, J. B. and C. S. Widom (2007). "Age of onset of child maltreatment predicts long-term mental health outcomes." J Abnorm Psychol **116**(1): 176-187.
Karver, C. L., B. Kurowski, E. A. Semple, T. Stancin, H. G. Taylor, K. O. Yeates, N. C. Walz and S. L. Wade (2014). "Utilization of behavioral therapy services long-term after traumatic brain injury in young children." Arch Phys Med Rehabil **95**(8): 1556-1563.
Karver, C. L., S. L. Wade, A. Cassedy, H. G. Taylor, T. Stancin, K. O. Yeates and N. C. Walz (2012). "Age at injury and long-term behavior problems after traumatic brain injury in young children." Rehabil Psychol **57**(3): 256-265.
Keller, J., R. Gomez, G. Williams, A. Lembke, L. Lazzeroni, G. M. Murphy, Jr. and A. F. Schatzberg (2017). "HPA axis in major depression: cortisol, clinical symptomatology and genetic variation predict cognition." Mol Psychiatry **22**(4): 527-536.
Kelly-Irving, M., B. Lepage, D. Dedieu, M. Bartley, D. Blane, P. Grosclaude, T. Lang and C. Delpierre (2013). "Adverse childhood experiences and premature all-cause mortality." Eur J Epidemiol **28**(9): 721-734.
Keshavan, M. S., V. A. Diwadkar, M. DeBellis, E. Dick, R. Kotwal, D. R. Rosenberg, J. A. Sweeney, N. Minshew and J. W. Pettegrew (2002). "Development of the corpus callosum in childhood, adolescence and early adulthood." Life Sci **70**(16): 1909-1922.
Kessler, R. C. and W. J. Magee (1994). "Childhood family violence and adult recurrent depression." J Health Soc Behav **35**(1): 13-27.
Kochanek, P. M., J. S. Wallisch, H. Bayir and R. S. B. Clark (2017). "Pre-clinical models in pediatric traumatic brain injury-challenges and lessons learned." Childs Nerv Syst **33**(10): 1693-1701.
Kohl, C., O. Riccio, J. Grosse, O. Zanoletti, C. Fournier, M. V. Schmidt and C. Sandi (2013). "Hippocampal neuroligin-2 overexpression leads to reduced aggression and inhibited novelty reactivity in rats." PLoS One **8**(2): e56871.
Kohl, C., X. D. Wang, J. Grosse, C. Fournier, D. Harbich, S. Westerholz, J. T. Li, A. Bacq, C. Sippel, F. Hausch, C. Sandi and M. V. Schmidt (2015). "Hippocampal neuroligin-2 links early-life stress with impaired social recognition and increased aggression in adult mice." Psychoneuroendocrinology **55**: 128-143.
Kolb, B., R. Gibb and D. van der Kooy (1994). "Neonatal frontal cortical lesions in rats alter cortical structure and connectivity." Brain Res **645**(1-2): 85-97.

Kolber, B. J., M. S. Roberts, M. P. Howell, D. F. Wozniak, M. S. Sands and L. J. Muglia (2008). "Central amygdala glucocorticoid receptor action promotes fear-associated CRH activation and conditioning." Proc Natl Acad Sci U S A **105**(33): 12004-12009.
Kozera, B. and M. Rapacz (2013). "Reference genes in real-time PCR." J Appl Genet **54**(4): 391-406.
Kozlowski, D. A. and T. Schallert (1998). "Relationship between dendritic pruning and behavioral recovery following sensorimotor cortex lesions." Behav Brain Res **97**(1-2): 89-98.
Kraus, M. F., T. Susmaras, B. P. Caughlin, C. J. Walker, J. A. Sweeney and D. M. Little (2007). "White matter integrity and cognition in chronic traumatic brain injury: a diffusion tensor imaging study." Brain **130**(Pt 10): 2508-2519.
Krugers, H. J., J. M. Arp, H. Xiong, S. Kanatsou, S. L. Lesuis, A. Korosi, M. Joels and P. J. Lucassen (2017). "Early life adversity: Lasting consequences for emotional learning." Neurobiol Stress **6**: 14-21.
Kurowski, B. G., A. Treble-Barna, V. Pilipenko, S. L. Wade, K. O. Yeates, H. G. Taylor, L. J. Martin and A. G. Jegga (2019). "Genetic Influences on Behavioral Outcomes After Childhood TBI: A Novel Systems Biology-Informed Approach." Front Genet **10**: 481.
L.J. Grota, R. A. (1969). "Continuous recording of maternal behavior in Rattus norvegicus." Animal Behavior **17**(4): 772, IN779, 723-729.
Lahdepuro, A., K. Savolainen, M. Lahti-Pulkkinen, J. G. Eriksson, J. Lahti, S. Tuovinen, E. Kajantie, A. K. Pesonen, K. Heinonen and K. Raikkonen (2019). "The Impact of Early Life Stress on Anxiety Symptoms in Late Adulthood." Sci Rep **9**(1): 4395.
Lajud, N., A. Roque, J. P. Cheng, C. O. Bondi and A. E. Kline (2020). "Early Life Stress Preceding Mild Pediatric Traumatic Brain Injury Increases Neuroinflammation but Does Not Exacerbate Impairment of Cognitive Flexibility during Adolescence." J Neurotrauma.
Lakens, D. (2013). "Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs." Front Psychol **4**: 863.
Lawson, G. M., J. S. Camins, L. Wisse, J. Wu, J. T. Duda, P. A. Cook, J. C. Gee and M. J. Farah (2017). "Childhood socioeconomic status and childhood maltreatment: Distinct associations with brain structure." PLoS One **12**(4): e0175690.
Leger, M., A. Quiedeville, V. Bouet, B. Haelewyn, M. Boulouard, P. Schumann-Bard and T. Freret (2013). "Object recognition test in mice." Nat Protoc **8**(12): 2531-2537.
Lenroot, R. K. and J. N. Giedd (2006). "Brain development in children and adolescents: insights from anatomical magnetic resonance imaging." Neurosci Biobehav Rev **30**(6): 718-729.
Levi, R. B., D. Drotar, K. O. Yeates and H. G. Taylor (1999). "Posttraumatic stress symptoms in children following orthopedic or traumatic brain injury." J Clin Child Psychol **28**(2): 232-243.
Levin, H. S., G. Hanten, C. C. Chang, L. Zhang, R. Schachar, L. Ewing-Cobbs and J. E. Max (2002). "Working memory after traumatic brain injury in children." Ann Neurol **52**(1): 82-88.
Levin, H. S., L. Zhang, M. Dennis, L. Ewing-Cobbs, R. Schachar, J. Max, J. A. Landis, G. Roberson, R. S. Scheibel, D. L. Miller and J. V. Hunter (2004). "Psychosocial outcome of TBI in children with unilateral frontal lesions." J Int Neuropsychol Soc **10**(3): 305-316.
Levine, S. (1957). "Infantile experience and resistance to physiological stress." Science **126**(3270): 405.
Levine, S. (1962). "Plasma-free corticosteroid response to electric shock in rats stimulated in infancy." Science **135**(3506): 795-796.
Levine, S. (2000). "Influence of psychological variables on the activity of the hypothalamic-pituitary-adrenal axis." Eur J Pharmacol **405**(1-3): 149-160.

Levine, S., D. M. Huchton, S. G. Wiener and P. Rosenfeld (1991). "Time course of the effect of maternal deprivation on the hypothalamic-pituitary-adrenal axis in the infant rat." Dev Psychobiol **24**(8): 547-558.
Lewis, T. L. and D. Maurer (2005). "Multiple sensitive periods in human visual development: evidence from visually deprived children." Dev Psychobiol **46**(3): 163-183.
Li, L. and J. Liu (2013). "The effect of pediatric traumatic brain injury on behavioral outcomes: a systematic review." Dev Med Child Neurol **55**(1): 37-45.
Lidow, M. S., P. S. Goldman-Rakic and P. Rakic (1991). "Synchronized overproduction of neurotransmitter receptors in diverse regions of the primate cerebral cortex." Proc Natl Acad Sci U S A **88**(22): 10218-10221.
Lindsey, H. M., E. A. Wilde, K. Caeyenberghs and E. L. Dennis (2019). "Longitudinal Neuroimaging in Pediatric Traumatic Brain Injury: Current State and Consideration of Factors That Influence Recovery." Front Neurol **10**: 1296.
Liu, X. and N. Quan (2018). "Microglia and CNS Interleukin-1: Beyond Immunological Concepts." Front Neurol **9**: 8.
Liu, Y., J. M. Cousin, J. Hughes, J. Van Damme, J. R. Seckl, C. Haslett, I. Dransfield, J. Savill and A. G. Rossi (1999). "Glucocorticoids promote nonphlogistic phagocytosis of apoptotic leukocytes." J Immunol **162**(6): 3639-3646.
Liu, Y., J. B. Croft, D. P. Chapman, G. S. Perry, K. J. Greenlund, G. Zhao and V. J. Edwards (2013). "Relationship between adverse childhood experiences and unemployment among adults from five U.S. states." Soc Psychiatry Psychiatr Epidemiol **48**(3): 357-369.
Loane, D. J. and A. Kumar (2016). "Microglia in the TBI brain: The good, the bad, and the dysregulated." Exp Neurol **275 Pt 3**: 316-327.
Loane, D. J., A. Kumar, B. A. Stoica, R. Cabatbat and A. I. Faden (2014). "Progressive neurodegeneration after experimental brain trauma: association with chronic microglial activation." J Neuropathol Exp Neurol **73**(1): 14-29.
Lorigooini, Z., S. N. Boroujeni, M. Sayyadi-Shahraki, M. Rahimi-Madiseh, E. Bijad and H. Amini-Khoei (2021). "Limonene through Attenuation of Neuroinflammation and Nitrite Level Exerts Antidepressant-Like Effect on Mouse Model of Maternal Separation Stress." Behav Neurol **2021**: 8817309.
Lucassen, P. J., E. F. Naninck, J. B. van Goudoever, C. Fitzsimons, M. Joels and A. Korosi (2013). "Perinatal programming of adult hippocampal structure and function; emerging roles of stress, nutrition and epigenetics." Trends Neurosci **36**(11): 621-631.
Lueptow, L. M. (2017). "Novel Object Recognition Test for the Investigation of Learning and Memory in Mice." J Vis Exp(126).
Luis, C. A. and W. Mittenberg (2002). "Mood and anxiety disorders following pediatric traumatic brain injury: a prospective study." J Clin Exp Neuropsychol **24**(3): 270-279.
Lumertz, F. S., E. Kestering-Ferreira, R. Orso, K. C. Creutzberg, S. G. Tractenberg, B. A. Stocchero, T. W. Viola and R. Grassi-Oliveira (2022). "Effects of early life stress on brain cytokines: A systematic review and meta-analysis of rodent studies." Neurosci Biobehav Rev **139**: 104746.
Magno, L. A. V., M. Collodetti, H. Tenza-Ferrer and M. A. Romano-Silva (2019). "Cylinder Test to Assess Sensory-motor Function in a Mouse Model of Parkinson's Disease." Bio Protoc **9**(16): e3337.

Makinde, H. M., C. M. Cuda, T. B. Just, H. R. Perlman and S. J. Schwulst (2017). "Nonclassical Monocytes Mediate Secondary Injury, Neurocognitive Outcome, and Neutrophil Infiltration after Traumatic Brain Injury." J Immunol **199**(10): 3583-3591.
Markostamou, I., A. Ioannidis, E. Dandi, M. A. Mandyla, E. Nousiopoulou, C. Simeonidou, E. Spandou and D. A. Tata (2016). "Maternal separation prior to neonatal hypoxia-ischemia: Impact on emotional aspects of behavior and markers of synaptic plasticity in hippocampus." Int J Dev Neurosci **52**: 1-12.
Mattei, D., A. Ivanov, C. Ferrai, P. Jordan, D. Guneykaya, A. Buonfiglioli, W. Schaafsma, P. Przanowski, W. Deuther-Conrad, P. Brust, S. Hesse, M. Patt, O. Sabri, T. L. Ross, B. J. L. Eggen, E. Boddeke, B. Kaminska, D. Beule, A. Pombo, H. Kettenmann and S. A. Wolf (2017). "Maternal immune activation results in complex microglial transcriptome signature in the adult offspring that is reversed by minocycline treatment." Transl Psychiatry **7**(5): e1120.
Max, J. E., E. Keatley, E. A. Wilde, E. D. Bigler, H. S. Levin, R. J. Schachar, A. Saunders, L. Ewing-Cobbs, S. B. Chapman, M. Dennis and T. T. Yang (2011). "Anxiety disorders in children and adolescents in the first six months after traumatic brain injury." J Neuropsychiatry Clin Neurosci **23**(1): 29-39.
McCauley, J., D. E. Kern, K. Kolodner, L. Dill, A. F. Schroeder, H. K. DeChant, J. Ryden, L. R. Derogatis and E. B. Bass (1997). "Clinical characteristics of women with a history of childhood abuse: unhealed wounds." JAMA **277**(17): 1362-1368.
McIntosh, J., H. Anisman and Z. Merali (1999). "Short- and long-periods of neonatal maternal separation differentially affect anxiety and feeding in adult rats: gender-dependent effects." Brain Res Dev Brain Res **113**(1-2): 97-106.
McLaughlin, R. J., S. Verlezza, J. M. Gray, M. N. Hill and C. D. Walker (2016). "Inhibition of anandamide hydrolysis dampens the neuroendocrine response to stress in neonatal rats subjected to suboptimal rearing conditions." Stress **19**(1): 114-124.
McNally, K. A., B. Bangert, A. Dietrich, K. Nuss, J. Rusin, M. Wright, H. G. Taylor and K. O. Yeates (2013). "Injury versus noninjury factors as predictors of postconcussive symptoms following mild traumatic brain injury in children." Neuropsychology **27**(1): 1-12.
McPherson, R. J., M. Mascher-Denen and S. E. Juul (2009). "Postnatal stress produces hyperglycemia in adult rats exposed to hypoxia-ischemia." Pediatr Res **66**(3): 278-282.
Meaney, D. F., D. H. Smith, D. I. Shreiber, A. C. Bain, R. T. Miller, D. T. Ross and T. A. Gennarelli (1995). "Biomechanical analysis of experimental diffuse axonal injury." J Neurotrauma **12**(4): 689-694.
Mehta, M. and C. Schmauss (2011). "Strain-specific cognitive deficits in adult mice exposed to early life stress." Behav Neurosci **125**(1): 29-36.
Merrill, L. L., C. E. Newell, C. J. Thomsen, S. R. Gold, J. S. Milner, M. P. Koss and S. G. Rosswork (1999). "Childhood abuse and sexual revictimization in a female Navy recruit sample." J Trauma Stress **12**(2): 211-225.
Milaniak, I. and S. R. Jaffee (2019). "Childhood socioeconomic status and inflammation: A systematic review and meta-analysis." Brain Behav Immun **78**: 161-176.
Miller, T. D., T. T. Chong, A. M. Aimola Davies, M. R. Johnson, S. R. Irani, M. Husain, T. W. Ng, S. Jacob, P. Maddison, C. Kennard, P. A. Gowland and C. R. Rosenthal (2020). "Human hippocampal CA3 damage disrupts both recent and remote episodic memories." Elife **9**.
Mills, K. L., F. Lalonde, L. S. Clasen, J. N. Giedd and S. J. Blakemore (2014). "Developmental changes in the structure of the social brain in late childhood and adolescence." Soc Cogn Affect Neurosci **9**(1): 123-131.

Moeller, T. P., G. A. Bachmann and J. R. Moeller (1993). "The combined effects of physical, sexual, and emotional abuse during childhood: long-term health consequences for women." Child Abuse Negl **17**(5): 623-640.
Molet, J., K. Heins, X. Zhuo, Y. T. Mei, L. Regev, T. Z. Baram and H. Stern (2016). "Fragmentation and high entropy of neonatal experience predict adolescent emotional outcome." Transl Psychiatry **6**: e702.
Moreno, J. A. and M. McKerral (2015). "Differences according to Sex in Sociosexuality and Infidelity after Traumatic Brain Injury." Behav Neurol **2015**: 914134.
Moussaoui, N., M. Larauche, M. Biraud, J. Molet, M. Million, E. Mayer and Y. Tache (2016). "Limited Nesting Stress Alters Maternal Behavior and In Vivo Intestinal Permeability in Male Wistar Pup Rats." PLoS One **11**(5): e0155037.
Murphy, F. C., A. Michael, T. W. Robbins and B. J. Sahakian (2003). "Neuropsychological impairment in patients with major depressive disorder: the effects of feedback on task performance." Psychol Med **33**(3): 455-467.
Naninck, E. F., L. Hoeijmakers, N. Kakava-Georgiadou, A. Meesters, S. E. Lazic, P. J. Lucassen and A. Korosi (2015). "Chronic early life stress alters developmental and adult neurogenesis and impairs cognitive function in mice." Hippocampus **25**(3): 309-328.
Neher, J. J. and C. Cunningham (2019). "Priming Microglia for Innate Immune Memory in the Brain." Trends Immunol **40**(4): 358-374.
Niu, L., S. S. Luo, Y. Xu, Z. Wang, D. Luo, H. Yang, W. Li, J. He, X. L. Zhong, Z. H. Liu, J. Y. Zeng, W. Y. Cao and W. Wan (2020). "The critical role of the hippocampal NLRP3 inflammasome in social isolation-induced cognitive impairment in male mice." Neurobiol Learn Mem **175**: 107301.
Norman, R. E., M. Byambaa, R. De, A. Butchart, J. Scott and T. Vos (2012). "The long-term health consequences of child physical abuse, emotional abuse, and neglect: a systematic review and meta-analysis." PLoS Med **9**(11): e1001349.
Nouri, A., F. Hashemzadeh, A. Soltani, E. Saghaei and H. Amini-Khoei (2020). "Progesterone exerts antidepressant-like effect in a mouse model of maternal separation stress through mitigation of neuroinflammatory response and oxidative stress." Pharm Biol **58**(1): 64-71.
Nugent, B. M., C. L. Wright, A. C. Shetty, G. E. Hodes, K. M. Lenz, A. Mahurkar, S. J. Russo, S. E. Devine and M. M. McCarthy (2015). "Brain feminization requires active repression of masculinization via DNA methylation." Nat Neurosci **18**(5): 690-697.
Nwachuku, E. L., A. M. Puccio, A. Adeboye, Y. F. Chang, J. Kim and D. O. Okonkwo (2016). "Time course of cerebrospinal fluid inflammatory biomarkers and relationship to 6-month neurologic outcome in adult severe traumatic brain injury." Clin Neurol Neurosurg **149**: 1-5.
O'Brien, W. T., L. Pham, G. F. Symons, M. Monif, S. R. Shultz and S. J. McDonald (2020). "The NLRP3 inflammasome in traumatic brain injury: potential as a biomarker and therapeutic target." J Neuroinflammation **17**(1): 104.
Ogawa, T., M. Mikuni, Y. Kuroda, K. Muneoka, K. J. Mori and K. Takahashi (1994). "Periodic maternal deprivation alters stress response in adult offspring: potentiates the negative feedback regulation of restraint stress-induced adrenocortical response and reduces the frequencies of open field-induced behaviors." Pharmacol Biochem Behav **49**(4): 961-967.
Oomen, C. A., H. Soeters, N. Audureau, L. Vermunt, F. N. van Hasselt, E. M. Manders, M. Joels, P. J. Lucassen and H. Krugers (2010). "Severe early life stress hampers spatial learning and neurogenesis, but improves hippocampal synaptic plasticity and emotional learning under high-stress conditions in adulthood." J Neurosci **30**(19): 6635-6645.

Osier, N. D. and C. E. Dixon (2016). "The Controlled Cortical Impact Model: Applications, Considerations for Researchers, and Future Directions." Front Neurol **7**: 134.
Ozen, I., K. Ruscher, R. Nilsson, J. Flygt, F. Clausen and N. Marklund (2020). "Interleukin-1 Beta Neutralization Attenuates Traumatic Brain Injury-Induced Microglia Activation and Neuronal Changes in the Globus Pallidus." Int J Mol Sci **21**(2).
Pace, T. W., T. C. Mletzko, O. Alagbe, D. L. Musselman, C. B. Nemeroff, A. H. Miller and C. M. Heim (2006). "Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress." Am J Psychiatry **163**(9): 1630-1633.
Parker, K. N., M. H. Donovan, K. Smith and L. J. Noble-Haeusslein (2021). "Traumatic Injury to the Developing Brain: Emerging Relationship to Early Life Stress." Front Neurol **12**: 708800.
Paul, M. S. and F. Limaiem (2023). Histology, Purkinje Cells. StatPearls. Treasure Island (FL).
Pena, C. J., M. Smith, A. Ramakrishnan, H. M. Cates, R. C. Bagot, H. G. Kronman, B. Patel, A. B. Chang, I. Purushothaman, J. Dudley, H. Morishita, L. Shen and E. J. Nestler (2019). "Early life stress alters transcriptomic patterning across reward circuitry in male and female mice." Nat Commun **10**(1): 5098.
Peng, S., W. Gu, W. Zhu, Y. Zhuang, X. Yang, Y. Lv, S. Meng, W. Xie and M. Li (2023). "A new AAV tool for highly preferentially targeting hippocampal CA2." Mol Brain **16**(1): 50.
Penke, Z., K. Felszeghy, B. Fernette, D. Sage, C. Nyakas and A. Burlet (2001). "Postnatal maternal deprivation produces long-lasting modifications of the stress response, feeding and stress-related behaviour in the rat." Eur J Neurosci **14**(4): 747-755.
Perez-Polo, J. R., H. C. Rea, K. M. Johnson, M. A. Parsley, G. C. Unabia, G. Xu, S. K. Infante, D. S. Dewitt and C. E. Hulsebosch (2013). "Inflammatory consequences in a rodent model of mild traumatic brain injury." J Neurotrauma **30**(9): 727-740.
Piemonti, L., P. Monti, P. Allavena, M. Sironi, L. Soldini, B. E. Leone, C. Socci and V. Di Carlo (1999). "Glucocorticoids affect human dendritic cell differentiation and maturation." J Immunol **162**(11): 6473-6481.
Plotsky, P. M. and M. J. Meaney (1993). "Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats." Brain Res Mol Brain Res **18**(3): 195-200.
Popovitz, J., S. P. Mysore and H. Adwanikar (2019). "Long-Term Effects of Traumatic Brain Injury on Anxiety-Like Behaviors in Mice: Behavioral and Neural Correlates." Front Behav Neurosci **13**: 6.
Potts, M. B., S. E. Koh, W. D. Whetstone, B. A. Walker, T. Yoneyama, C. P. Claus, H. M. Manvelyan and L. J. Noble-Haeusslein (2006). "Traumatic injury to the immature brain: inflammation, oxidative injury, and iron-mediated damage as potential therapeutic targets." NeuroRx **3**(2): 143-153.
Prins, M., T. Greco, D. Alexander and C. C. Giza (2013). "The pathophysiology of traumatic brain injury at a glance." Dis Model Mech **6**(6): 1307-1315.
Prins, M. L., S. M. Lee, C. L. Cheng, D. P. Becker and D. A. Hovda (1996). "Fluid percussion brain injury in the developing and adult rat: a comparative study of mortality, morphology, intracranial pressure and mean arterial blood pressure." Brain Res Dev Brain Res **95**(2): 272-282.
Pryce, C. R. and J. Feldon (2003). "Long-term neurobehavioural impact of the postnatal environment in rats: manipulations, effects and mediating mechanisms." Neurosci Biobehav Rev **27**(1-2): 57-71.
Pullela, R., J. Raber, T. Pfankuch, D. M. Ferriero, C. P. Claus, S. E. Koh, T. Yamauchi, R. Rola, J. R. Fike and L. J. Noble-Haeusslein (2006). "Traumatic injury to the immature brain results in

progressive neuronal loss, hyperactivity and delayed cognitive impairments." Dev Neurosci **28**(4-5): 396-409.
Raineki, C., M. R. Cortes, L. Belnoue and R. M. Sullivan (2012). "Effects of early-life abuse differ across development: infant social behavior deficits are followed by adolescent depressive-like behaviors mediated by the amygdala." J Neurosci **32**(22): 7758-7765.
Raineki, C., E. Sarro, M. Rincon-Cortes, R. Perry, J. Boggs, C. J. Holman, D. A. Wilson and R. M. Sullivan (2015). "Paradoxical neurobehavioral rescue by memories of early-life abuse: the safety signal value of odors learned during abusive attachment." Neuropsychopharmacology **40**(4): 906-914.
Reus, G. Z., G. C. Fernandes, A. B. de Moura, R. H. Silva, A. C. Darabas, T. G. de Souza, H. M. Abelaira, C. Carneiro, D. Wendhausen, M. Michels, B. Pescador, F. Dal-Pizzol, D. S. Macedo and J. Quevedo (2017). "Early life experience contributes to the developmental programming of depressive-like behaviour, neuroinflammation and oxidative stress." J Psychiatr Res **95**: 196-207.
Rice, C. J., C. A. Sandman, M. R. Lenjavi and T. Z. Baram (2008). "A novel mouse model for acute and long-lasting consequences of early life stress." Endocrinology **149**(10): 4892-4900.
Richards, M. and M. E. Wadsworth (2004). "Long term effects of early adversity on cognitive function." Arch Dis Child **89**(10): 922-927.
Riggs, S., A. J. Alario and C. McHorney (1990). "Health risk behaviors and attempted suicide in adolescents who report prior maltreatment." J Pediatr **116**(5): 815-821.
Rincon-Cortes, M. and R. M. Sullivan (2016). "Emergence of social behavior deficit, blunted corticolimbic activity and adult depression-like behavior in a rodent model of maternal maltreatment." Transl Psychiatry **6**(10): e930.
Roe, S. Y., E. M. McGowan and N. J. Rothwell (1998). "Evidence for the involvement of corticotrophin-releasing hormone in the pathogenesis of traumatic brain injury." Eur J Neurosci **10**(2): 553-559.
Romeo, R. D., A. Mueller, H. M. Sisti, S. Ogawa, B. S. McEwen and W. G. Brake (2003). "Anxiety and fear behaviors in adult male and female C57BL/6 mice are modulated by maternal separation." Horm Behav **43**(5): 561-567.
Rorty, M., J. Yager and E. Rossotto (1994). "Childhood sexual, physical, and psychological abuse in bulimia nervosa." Am J Psychiatry **151**(8): 1122-1126.
Rosema, S., L. Crowe and V. Anderson (2012). "Social function in children and adolescents after traumatic brain injury: a systematic review 1989-2011." J Neurotrauma **29**(7): 1277-1291.
Roth, T. L., D. Nayak, T. Atanasijevic, A. P. Koretsky, L. L. Latour and D. B. McGavern (2014). "Transcranial amelioration of inflammation and cell death after brain injury." Nature **505**(7482): 223-228.
Rowe, R. K., B. M. Rumney, H. G. May, P. Permana, P. D. Adelson, S. M. Harman, J. Lifshitz and T. C. Thomas (2016). "Diffuse traumatic brain injury affects chronic corticosterone function in the rat." Endocr Connect **5**(4): 152-166.
Russo, M. V., L. L. Latour and D. B. McGavern (2018). "Distinct myeloid cell subsets promote meningeal remodeling and vascular repair after mild traumatic brain injury." Nat Immunol **19**(5): 442-452.
Ryan, N. P., V. Anderson, C. Godfrey, M. H. Beauchamp, L. Coleman, S. Eren, S. Rosema, K. Taylor and C. Catroppa (2014). "Predictors of very-long-term sociocognitive function after pediatric traumatic brain injury: evidence for the vulnerability of the immature "social brain"." J Neurotrauma **31**(7): 649-657.

Ryan, N. P., C. Catroppa, C. Godfrey, L. J. Noble-Haeusslein, S. R. Shultz, T. J. O'Brien, V. Anderson and B. D. Semple (2016). "Social dysfunction after pediatric traumatic brain injury: A translational perspective." Neurosci Biobehav Rev **64**: 196-214.
Saavedra, L. M., M. G. Hernandez-Velazquez, S. Madrigal, A. Ochoa-Zarzosa and L. Torner (2021). "Long-term activation of hippocampal glial cells and altered emotional behavior in male and female adult rats after different neonatal stressors." Psychoneuroendocrinology **126**: 105164.
Safren, S. A., B. S. Gershuny, P. Marzol, M. W. Otto and M. H. Pollack (2002). "History of childhood abuse in panic disorder, social phobia, and generalized anxiety disorder." J Nerv Ment Dis **190**(7): 453-456.
Sagae, S. C., B. Zanardini, E. D. Ribeiro-Paz, A. C. Amaral, G. A. Bronczek, C. Lubaczeuski, S. Grassiolli, P. Koehler-Santos, J. R. de Oliveira, M. V. F. Donadio and C. Raineki (2018). "Metabolic dysfunction in a rat model of early-life scarcity-adversity: Modulatory role of cafeteria diet." Exp Physiol **103**(11): 1481-1493.
Saleh, A., G. G. Potter, D. R. McQuoid, B. Boyd, R. Turner, J. R. MacFall and W. D. Taylor (2017). "Effects of early life stress on depression, cognitive performance and brain morphology." Psychol Med **47**(1): 171-181.
San Antonio, A., K. Liban, T. Ikrar, E. Tsyganovskiy and X. Xu (2014). "Distinct physiological and developmental properties of hippocampal CA2 subfield revealed by using anti-Purkinje cell protein 4 (PCP4) immunostaining." J Comp Neurol **522**(6): 1333-1354.
Sanchez, C. M., D. J. Titus, N. M. Wilson, J. E. Freund and C. M. Atkins (2020). "Early Life Stress Exacerbates Outcome after Traumatic Brain Injury." J Neurotrauma.
Sandhir, R., V. Puri, R. M. Klein and N. E. Berman (2004). "Differential expression of cytokines and chemokines during secondary neuron death following brain injury in old and young mice." Neurosci Lett **369**(1): 28-32.
Schallert, T., S. M. Fleming, J. L. Leasure, J. L. Tillerson and S. T. Bland (2000). "CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, parkinsonism and spinal cord injury." Neuropharmacology **39**(5): 777-787.
Scheff, S. W., D. A. Price, R. R. Hicks, S. A. Baldwin, S. Robinson and C. Brackney (2005). "Synaptogenesis in the hippocampal CA1 field following traumatic brain injury." J Neurotrauma **22**(7): 719-732.
Schmidt, A. T., G. R. Hanten, X. Li, K. D. Orsten and H. S. Levin (2010). "Emotion recognition following pediatric traumatic brain injury: longitudinal analysis of emotional prosody and facial emotion recognition." Neuropsychologia **48**(10): 2869-2877.
Schmidt, M. V. (2010). "Molecular mechanisms of early life stress--lessons from mouse models." Neurosci Biobehav Rev **34**(6): 845-852.
Schmidt, M. V., M. S. Oitzl, S. Levine and E. R. de Kloet (2002). "The HPA system during the postnatal development of CD1 mice and the effects of maternal deprivation." Brain Res Dev Brain Res **139**(1): 39-49.
Schneider, H., F. Pitossi, D. Balschun, A. Wagner, A. del Rey and H. O. Besedovsky (1998). "A neuromodulatory role of interleukin-1beta in the hippocampus." Proc Natl Acad Sci U S A **95**(13): 7778-7783.
Schwartz, L., H. G. Taylor, D. Drotar, K. O. Yeates, S. L. Wade and T. Stancin (2003). "Long-term behavior problems following pediatric traumatic brain injury: prevalence, predictors, and correlates." J Pediatr Psychol **28**(4): 251-263.

Scott, C., A. McKinlay, T. McLellan, E. Britt, R. Grace and M. MacFarlane (2015). "A comparison of adult outcomes for males compared to females following pediatric traumatic brain injury." Neuropsychology **29**(4): 501-508.
Semple, B. D., K. Blomgren, K. Gimlin, D. M. Ferriero and L. J. Noble-Haeusslein (2013). "Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species." Prog Neurobiol **106-107**: 1-16.
Semple, B. D., S. A. Canchola and L. J. Noble-Haeusslein (2012). "Deficits in social behavior emerge during development after pediatric traumatic brain injury in mice." J Neurotrauma **29**(17): 2672-2683.
Semple, B. D., L. J. Noble-Haeusslein, M. Gooyit, K. G. Tercovich, Z. Peng, T. T. Nguyen, V. A. Schroeder, M. A. Suckow, M. Chang, J. Raber and A. Trivedi (2015). "Early Gelatinase Activity Is Not a Determinant of Long-Term Recovery after Traumatic Brain Injury in the Immature Mouse." PLoS One **10**(11): e0143386.
Semple, B. D., T. J. O'Brien, K. Gimlin, D. K. Wright, S. E. Kim, P. M. Casillas-Espinosa, K. M. Webster, S. Petrou and L. J. Noble-Haeusslein (2017). "Interleukin-1 Receptor in Seizure Susceptibility after Traumatic Injury to the Pediatric Brain." J Neurosci **37**(33): 7864-7877.
Serpa, R. O., L. Ferguson, C. Larson, J. Bailard, S. Cooke, T. Greco and M. L. Prins (2021). "Pathophysiology of Pediatric Traumatic Brain Injury." Front Neurol **12**: 696510.
Shultz, S. R., F. Bao, V. Omana, C. Chiu, A. Brown and D. P. Cain (2012). "Repeated mild lateral fluid percussion brain injury in the rat causes cumulative long-term behavioral impairments, neuroinflammation, and cortical loss in an animal model of repeated concussion." J Neurotrauma **29**(2): 281-294.
Shultz, S. R., S. J. McDonald, F. Corrigan, B. D. Semple, S. Salberg, A. Zamani, N. C. Jones and R. Mychasiuk (2020). "Clinical Relevance of Behavior Testing in Animal Models of Traumatic Brain Injury." J Neurotrauma **37**(22): 2381-2400.
Silverman, J. L., M. Yang, C. Lord and J. N. Crawley (2010). "Behavioural phenotyping assays for mouse models of autism." Nat Rev Neurosci **11**(7): 490-502.
Simon, D. W., M. J. McGeachy, H. Bayir, R. S. B. Clark, D. J. Loane and P. M. Kochanek (2017). "The far-reaching scope of neuroinflammation after traumatic brain injury." Nat Rev Neurol **13**(9): 572.
Smith, K. E. and S. D. Pollak (2020). "Early life stress and development: potential mechanisms for adverse outcomes." J Neurodev Disord **12**(1): 34.
Somera-Molina, K. C., S. Nair, L. J. Van Eldik, D. M. Watterson and M. S. Wainwright (2009). "Enhanced microglial activation and proinflammatory cytokine upregulation are linked to increased susceptibility to seizures and neurologic injury in a 'two-hit' seizure model." Brain Res **1282**: 162-172.
Spadoni, A. D., M. Vinograd, B. Cuccurazzu, K. Torres, L. M. Glynn, E. P. Davis, T. Z. Baram, D. G. Baker, C. M. Nievergelt and V. B. Risbrough (2022). "Contribution of early-life unpredictability to neuropsychiatric symptom patterns in adulthood." Depress Anxiety **39**(10-11): 706-717.
Spencer-Booth, Y. and R. A. Hinde (1971). "The effects of 13 days maternal separation on infant rhesus monkeys compared with those of shorter and repeated separations." Anim Behav **19**(3): 595-605.
Suarez, M. M., M. A. Rivarola, S. M. Molina, N. I. Perassi, G. M. Levin and R. Cabrera (2001). "Periodic maternal deprivation and lesion of anterodorsal thalami nuclei induce alteration on hypophyso adrenal system activity in adult rats." Life Sci **69**(7): 803-813.

Sun, Y., L. Bai, X. Niu, Z. Wang, B. Yin, G. Bai, D. Zhang, S. Gan, C. Sun, S. Wang, F. Zhu and M. Zhang (2019). "Elevated Serum Levels of Inflammation-Related Cytokines in Mild Traumatic Brain Injury Are Associated With Cognitive Performance." Front Neurol **10**: 1120.
Swanson, K. V., M. Deng and J. P. Ting (2019). "The NLRP3 inflammasome: molecular activation and regulation to therapeutics." Nat Rev Immunol **19**(8): 477-489.
Syed, S. A. and C. B. Nemeroff (2017). "Early Life Stress, Mood, and Anxiety Disorders." Chronic Stress (Thousand Oaks) **1**.
Taib, T., C. Leconte, J. Van Steenwinckel, A. H. Cho, B. Palmier, E. Torsello, R. Lai Kuen, S. Onyeomah, K. Ecomard, C. Benedetto, B. Coqueran, A. C. Novak, E. Deou, M. Plotkine, P. Gressens, C. Marchand-Leroux and V. C. Besson (2017). "Neuroinflammation, myelin and behavior: Temporal patterns following mild traumatic brain injury in mice." PLoS One **12**(9): e0184811.
Tapp, Z. M., J. P. Godbout and O. N. Kokiko-Cochran (2019). "A Tilted Axis: Maladaptive Inflammation and HPA Axis Dysfunction Contribute to Consequences of TBI." Front Neurol **10**: 345.
Tasker, R. C. (2006). "Changes in white matter late after severe traumatic brain injury in childhood." Dev Neurosci **28**(4-5): 302-308.
Tata, D. A., I. Markostamou, A. Ioannidis, M. Gkioka, C. Simeonidou, G. Anogianakis and E. Spandou (2015). "Effects of maternal separation on behavior and brain damage in adult rats exposed to neonatal hypoxia-ischemia." Behav Brain Res **280**: 51-61.
Taylor, A. N., S. U. Rahman, N. C. Sanders, D. L. Tio, P. Prolo and R. L. Sutton (2008). "Injury severity differentially affects short- and long-term neuroendocrine outcomes of traumatic brain injury." J Neurotrauma **25**(4): 311-323.
Taylor, A. N., S. U. Rahman, D. L. Tio, M. J. Sanders, J. K. Bando, A. H. Truong and P. Prolo (2006). "Lasting neuroendocrine-immune effects of traumatic brain injury in rats." J Neurotrauma **23**(12): 1802-1813.
Teicher, M. H., C. M. Anderson, K. Ohashi, A. Khan, C. E. McGreenery, E. A. Bolger, M. L. Rohan and G. D. Vitaliano (2018). "Differential effects of childhood neglect and abuse during sensitive exposure periods on male and female hippocampus." Neuroimage **169**: 443-452.
Terranova, M. L. and G. Laviola (2005). "Scoring of social interactions and play in mice during adolescence." Curr Protoc Toxicol **Chapter 13**: Unit13 10.
Tertil, M., U. Skupio, J. Barut, V. Dubovyk, A. Wawrzczak-Bargiela, Z. Soltys, S. Golda, L. Kudla, L. Wiktorowska, K. Szklarczyk, M. Korostynski, R. Przewlocki and M. Slezak (2018). "Glucocorticoid receptor signaling in astrocytes is required for aversive memory formation." Transl Psychiatry **8**(1): 255.
Thompson, H. J., J. Lifshitz, N. Marklund, M. S. Grady, D. I. Graham, D. A. Hovda and T. K. McIntosh (2005). "Lateral fluid percussion brain injury: a 15-year review and evaluation." J Neurotrauma **22**(1): 42-75.
Threlkeld, S. W., G. D. Rosen and R. H. Fitch (2007). "Age at developmental cortical injury differentially alters corpus callosum volume in the rat." BMC Neurosci **8**: 94.
Tong, K. A., S. Ashwal, B. A. Holshouser, J. P. Nickerson, C. J. Wall, L. A. Shutter, R. J. Osterdock, E. M. Haacke and D. Kido (2004). "Diffuse axonal injury in children: clinical correlation with hemorrhagic lesions." Ann Neurol **56**(1): 36-50.
Tong, K. A., S. Ashwal, B. A. Holshouser, L. A. Shutter, G. Herigault, E. M. Haacke and D. K. Kido (2003). "Hemorrhagic shearing lesions in children and adolescents with posttraumatic diffuse axonal injury: improved detection and initial results." Radiology **227**(2): 332-339.

Tottenham, N., T. A. Hare, B. T. Quinn, T. W. McCarry, M. Nurse, T. Gilhooly, A. Millner, A. Galvan, M. C. Davidson, I. M. Eigsti, K. M. Thomas, P. J. Freed, E. S. Booma, M. R. Gunnar, M. Altemus, J. Aronson and B. J. Casey (2010). "Prolonged institutional rearing is associated with atypically large amygdala volume and difficulties in emotion regulation." Dev Sci **13**(1): 46-61.
Treble-Barna, A., J. Patronick, S. Uchani, N. C. Marousis, C. K. Zigler, E. L. Fink, P. M. Kochanek, Y. P. Conley and K. O. Yeates (2020). "Epigenetic Effects on Pediatric Traumatic Brain Injury Recovery (EETR): An Observational, Prospective, Longitudinal Concurrent Cohort Study Protocol." Front Neurol **11**: 460.
Trojan, E., K. Chamera, N. Bryniarska, K. Kotarska, M. Leskiewicz, M. Regulska and A. Basta-Kaim (2019). "Role of Chronic Administration of Antidepressant Drugs in the Prenatal Stress-Evoked Inflammatory Response in the Brain of Adult Offspring Rats: Involvement of the NLRP3 Inflammasome-Related Pathway." Mol Neurobiol **56**(8): 5365-5380.
Tsuda, M. C. and S. Ogawa (2012). "Long-lasting consequences of neonatal maternal separation on social behaviors in ovariectomized female mice." PLoS One **7**(3): e33028.
Tsuru-Aoyagi, K., M. B. Potts, A. Trivedi, T. Pfankuch, J. Raber, M. Wendland, C. P. Claus, S. E. Koh, D. Ferriero and L. J. Noble-Haeusslein (2009). "Glutathione peroxidase activity modulates recovery in the injured immature brain." Ann Neurol **65**(5): 540-549.
Tyzio, R., G. L. Holmes, Y. Ben-Ari and R. Khazipov (2007). "Timing of the developmental switch in GABA(A) mediated signaling from excitation to inhibition in CA3 rat hippocampus using gramicidin perforated patch and extracellular recordings." Epilepsia **48 Suppl 5**: 96-105.
Tzakis, N. and M. R. Holahan (2019). "Social Memory and the Role of the Hippocampal CA2 Region." Front Behav Neurosci **13**: 233.
van der Goes, A., K. Hoekstra, T. K. van den Berg and C. D. Dijkstra (2000). "Dexamethasone promotes phagocytosis and bacterial killing by human monocytes/macrophages in vitro." J Leukoc Biol **67**(6): 801-807.
van Goethem, N. P., K. Rutten, F. J. van der Staay, L. A. Jans, S. Akkerman, H. W. Steinbusch, A. Blokland, J. van't Klooster and J. Prickaerts (2012). "Object recognition testing: rodent species, strains, housing conditions, and estrous cycle." Behav Brain Res **232**(2): 323-334.
van Reedt Dortland, A. K., E. J. Giltay, T. van Veen, F. G. Zitman and B. W. Penninx (2010). "Metabolic syndrome abnormalities are associated with severity of anxiety and depression and with tricyclic antidepressant use." Acta Psychiatr Scand **122**(1): 30-39.
van Reedt Dortland, A. K., E. J. Giltay, T. van Veen, F. G. Zitman and B. W. Penninx (2012). "Personality traits and childhood trauma as correlates of metabolic risk factors: the Netherlands Study of Depression and Anxiety (NESDA)." Prog Neuropsychopharmacol Biol Psychiatry **36**(1): 85-91.
Vanbesien-Mailliot, C. C., I. Wolowczuk, J. Mairesse, O. Viltart, M. Delacre, J. Khalife, M. C. Chartier-Harlin and S. Maccari (2007). "Prenatal stress has pro-inflammatory consequences on the immune system in adult rats." Psychoneuroendocrinology **32**(2): 114-124.
Vandewalle, G., B. Middleton, S. M. Rajaratnam, B. M. Stone, B. Thorleifsdottir, J. Arendt and D. J. Dijk (2007). "Robust circadian rhythm in heart rate and its variability: influence of exogenous melatonin and photoperiod." J Sleep Res **16**(2): 148-155.
Vasa, R. A., M. Grados, B. Slomine, E. H. Herskovits, R. E. Thompson, C. Salorio, J. Christensen, C. Wursta, M. A. Riddle and J. P. Gerring (2004). "Neuroimaging correlates of anxiety after pediatric traumatic brain injury." Biol Psychiatry **55**(3): 208-216.

Vazquez, V., J. Penit-Soria, C. Durand, M. J. Besson, B. Giros and V. Dauge (2005). "Maternal deprivation increases vulnerability to morphine dependence and disturbs the enkephalinergic system in adulthood." J Neurosci **25**(18): 4453-4462.
Veenema, A. H., R. Bredewold and I. D. Neumann (2007). "Opposite effects of maternal separation on intermale and maternal aggression in C57BL/6 mice: link to hypothalamic vasopressin and oxytocin immunoreactivity." Psychoneuroendocrinology **32**(5): 437-450.
Viau, V., S. Sharma, P. M. Plotsky and M. J. Meaney (1993). "Increased plasma ACTH responses to stress in nonhandled compared with handled rats require basal levels of corticosterone and are associated with increased levels of ACTH secretagogues in the median eminence." J Neurosci **13**(3): 1097-1105.
von Leden, R. E., K. N. Parker, A. A. Bates, L. J. Noble-Haeusslein and M. H. Donovan (2019). "The emerging role of neutrophils as modifiers of recovery after traumatic injury to the developing brain." Exp Neurol **317**: 144-154.
Walker, C. D., K. G. Bath, M. Joels, A. Korosi, M. Larauche, P. J. Lucassen, M. J. Morris, C. Raineki, T. L. Roth, R. M. Sullivan, Y. Tache and T. Z. Baram (2017). "Chronic early life stress induced by limited bedding and nesting (LBN) material in rodents: critical considerations of methodology, outcomes and translational potential." Stress **20**(5): 421-448.
Walker, C. D., K. A. Scribner, C. S. Cascio and M. F. Dallman (1991). "The pituitary-adrenocortical system of neonatal rats is responsive to stress throughout development in a time-dependent and stressor-specific fashion." Endocrinology **128**(3): 1385-1395.
Wang, D., J. L. S. Levine, V. Avila-Quintero, M. Bloch and A. Kaffman (2020). "Systematic review and meta-analysis: effects of maternal separation on anxiety-like behavior in rodents." Transl Psychiatry **10**(1): 174.
Wang, L., J. Jiao and S. C. Dulawa (2011). "Infant maternal separation impairs adult cognitive performance in BALB/cJ mice." Psychopharmacology (Berl) **216**(2): 207-218.
Wang, X. D., Y. A. Su, K. V. Wagner, C. Avrabos, S. H. Scharf, J. Hartmann, M. Wolf, C. Liebl, C. Kuhne, W. Wurst, F. Holsboer, M. Eder, J. M. Deussing, M. B. Muller and M. V. Schmidt (2013). "Nectin-3 links CRHR1 signaling to stress-induced memory deficits and spine loss." Nat Neurosci **16**(6): 706-713.
Washington, P. M., P. A. Forcelli, T. Wilkins, D. N. Zapple, M. Parsadanian and M. P. Burns (2012). "The effect of injury severity on behavior: a phenotypic study of cognitive and emotional deficits after mild, moderate, and severe controlled cortical impact injury in mice." J Neurotrauma **29**(13): 2283-2296.
Weinberg, J. (1987). "Effects of early experience on responsiveness to ethanol: a preliminary report." Physiol Behav **40**(3): 401-406.
Wells, R., P. Minnes and M. Phillips (2009). "Predicting social and functional outcomes for individuals sustaining paediatric traumatic brain injury." Dev Neurorehabil **12**(1): 12-23.
Wendel, K. M., A. K. Short, B. P. Noarbe, E. Haddad, A. M. Palma, M. A. Yassa, T. Z. Baram and A. Obenaus (2021). "Early life adversity in male mice sculpts reward circuits." Neurobiol Stress **15**: 100409.
Wigger, A. and I. D. Neumann (1999). "Periodic maternal deprivation induces gender-dependent alterations in behavioral and neuroendocrine responses to emotional stress in adult rats." Physiol Behav **66**(2): 293-302.
Wilde, E. A., Z. Chu, E. D. Bigler, J. V. Hunter, M. A. Fearing, G. Hanten, M. R. Newsome, R. S. Scheibel, X. Li and H. S. Levin (2006). "Diffusion tensor imaging in the corpus callosum in children after moderate to severe traumatic brain injury." J Neurotrauma **23**(10): 1412-1426.

Wilde, E. A., Hunter and E. D. Bigler (2012). "Pediatric traumatic brain injury: neuroimaging and neurorehabilitation outcome." NeuroRehabilitation **31**(3): 245-260.
Williams, M. W., L. J. Rapport, S. R. Millis and R. A. Hanks (2014). "Psychosocial outcomes after traumatic brain injury: life satisfaction, community integration, and distress." Rehabil Psychol **59**(3): 298-305.
Williamson, L. L., P. W. Sholar, R. S. Mistry, S. H. Smith and S. D. Bilbo (2011). "Microglia and memory: modulation by early-life infection." J Neurosci **31**(43): 15511-15521.
Wilson, R. S., P. A. Boyle, S. R. Levine, L. Yu, S. E. Anagnos, A. S. Buchman, J. A. Schneider and D. A. Bennett (2012). "Emotional neglect in childhood and cerebral infarction in older age." Neurology **79**(15): 1534-1539.
Woon, F. L. and D. W. Hedges (2008). "Hippocampal and amygdala volumes in children and adults with childhood maltreatment-related posttraumatic stress disorder: a meta-analysis." Hippocampus **18**(8): 729-736.
Yamazaki, A., Y. Ohtsuki, T. Yoshihara, S. Honma and K. Honma (2005). "Maternal deprivation in neonatal rats of different conditions affects growth rate, circadian clock, and stress responsiveness differentially." Physiol Behav **86**(1-2): 136-144.
Yang, M., J. L. Silverman and J. N. Crawley (2011). "Automated three-chambered social approach task for mice." Curr Protoc Neurosci **Chapter 8**: Unit 8 26.
Yeates, K. O., E. D. Bigler, M. Dennis, C. A. Gerhardt, K. H. Rubin, T. Stancin, H. G. Taylor and K. Vannatta (2007). "Social outcomes in childhood brain disorder: a heuristic integration of social neuroscience and developmental psychology." Psychol Bull **133**(3): 535-556.
Young, J. W. (1966). "Selected facial measurements of children for oxygen-mask design. AM 66-9." AM Rep: 1-11.
Zhao, S., Z. Yu, Y. Liu, Y. Bai, Y. Jiang, K. van Leyen, Y. G. Yang, J. M. Lok, M. J. Whalen, E. H. Lo and X. Wang (2017). "CD47 deficiency improves neurological outcomes of traumatic brain injury in mice." Neurosci Lett **643**: 125-130.
Zhao, Z., D. J. Loane, M. G. Murray, 2nd, B. A. Stoica and A. I. Faden (2012). "Comparing the predictive value of multiple cognitive, affective, and motor tasks after rodent traumatic brain injury." J Neurotrauma **29**(15): 2475-2489.