TRAUMATIC BRAIN INJURY AND GENDER: IMPLICATIONS FOR REHABILITATION

by

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Traumatic brain injury (TBI) is often thought to be a disease of young men. However, women comprise approximately 40% of the TBI population. In the era of personalized medicine, it is critical that we examine questions related to gender and outcomes after TBI, not only from the perspective of natural recovery, but also from the response to treatment. Many pharmacological agents are administered post-TBI, and given biological differences in males and females, the beneficial or deleterious effects of these pharmacological agents may or may not be equivalent between the sexes.

Haloperidol (HAL) is often prescribed in the clinic to manage TBI-induced agitation and aggression. HAL has consistently been shown to hinder functional recovery in male rats after experimental TBI, but whether these effects extend to females is not well established. Therefore, the objective of this study is to conduct an experiment examining differences between male and female rats in the response to HAL. To further understand this clinically relevant issue male and female rats received either a SHAM injury or a controlled cortical impact and were treated with either a vehicle solution (1mL/kg) or HAL (0.5mg/kg) starting 24 hours after injury and continuing once a day for 19 consecutive days. In regards to motor function TBI females performed better than TBI males. Furthermore, TBI females that received HAL performed better than TBI male that received HAL. With respect to cognitive function, which consisted of acquisition of spatial learning and memory retention, HAL was found to be deleterious to spatial learning acquisition in

the male TBI group, but did not appear to affect retention, as measured in the probe trial. In conclusion, the results of my experiment demonstrate that chronic administration of the antipsychotic drug HAL produces differences in recovery between males and females, as illustrated through tests of motor and cognitive function. Specifically, males performed worse than females, which replicate previous work from our laboratory. This finding is clinically significant because it can allow medical professionals to individualize medicine and to determine the most efficient treatment plan.

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1.0 TRAUMATIC BRAIN INJURY AND ITS CONSEQUENCES

In the United States alone approximately 1.7 million people suffer a TBI annually. Moreover, TBI is a contributing factor to nearly one-third of all injury related deaths (Center for Disease Control, 2010). These alarmingly high rates cost the United States over $60 billion in indirect and direct medical costs (Center for Disease Control, 2010). There are numerous ways in which individuals can obtain a TBI. According to the *Center for Disease Control* falls made up over 40% of all TBIs between 2006 and 2010, followed by unknown causes, and then motor vehicle accidents (Center for Disease Control, 2004). The risk of different causes of TBI may vary based on many factors, including age and gender (Center for Disease Control, 2004).

Once a TBI has occurred, mechanisms of change in the brain can be categorized into primary, secondary, and repair-regeneration stages (Marion, 1999). The primary injury stage is the result of the mechanical disruption of the brain that occurs as a direct response to trauma of the head. Primary injuries include penetrating damage, cortical contusions, damage to the vascular supply, and axonal shearing. Secondary injury results from the neurochemical and physiological effects that are initiated due to the primary trauma (Marion, 1999). The results of a secondary injury can include, but are not limited to, vascular changes, hypotension, cerebral edema, excitotoxicity and increased intracranial pressure. The repair-regeneration, is the third stage, and is the least understood stage of TBI, which is why current research needs to focus on this stage.

There is little time for intervention in the primary and secondary stages of injury making the repair-regeneration stage the most logical to study.

There are multiple functional and behavioral consequences of TBI, including changes in sensation, cognition, motor function, and emotion. An overview of these changes is provided in the following paragraphs.

1.1 SENSATION

The manner in which an individual with a TBI senses the environment can be severely altered. Impairments within the primary senses, such as somatosensation, vision, hearing, vestibular function, smell and taste are all potential consequences of TBI (Bach & David, 2006). In addition, individual with TBI may also experience altered sensation. Tingling and painful sensations are common. Scotoma and diplopia may cause blind spots in the visual field, blindness, and poor visiomotor integration. A consistent ringing in the ear, tinnitus, may add to a person's inability to concentrate. Vertigo and balance problems are also common after TBI. Hypogleusia alters the way a person perceives taste after TBI. Agnosia, the inability to discriminate one sensation from another is another possible deficit after TBI; this may cause a person to have difficulties recognizing objects (Bach & David, 2006). These TBI induced alterations in sensation make it difficult for a person to successfully reintegrate into society.

1.2 COGNITIVE FUNCTION

Following a TBI a plethora of cognitive deficits may be present depending on the severity and location of the injury. Attention and memory problems are a common complaint after a TBI, potentially affecting short-term and long-term memory (DiPaola et al., 2014). Often a person may have trouble concentrating after a TBI and may be easily distracted, and have difficulty staying on task or sitting for long periods of time. When a person's attention and memory both acutely and in the long term are affected by a TBI, understanding and processing information may become difficult. Attention and memory problems are not only an inconvenience but can lead to dangerous situations such as leaving a stove unattended or forgetting to take medication.

Besides attention and memory deficits, a person with TBI may endure may other kinds of cognitive deficits. The ability to processes and form language is a highly complex process that is often affected by TBI not only due to cognitive deficits but motor deficits can impact one's ability to communicate (Center for Disease Control, 2004). Furthermore, executive functioning such as working memory, reasoning, task flexibility and problem solving can be severely impacted (Hamm et al., 1992; Ratcliff et al., 2007). Sequencing abilities may be disrupted as a result of a TBI, which makes activities such as following directions or participating in multistep tasks extremely challenging (Liossi et al., 2009).

1.3 MOTOR FUNCTION

While cognitive dysfunction can be the result of injuries throughout the brain, motor dysfunction is associated with focal injuries to selected regions of the brain (Fujimoto et al., 2004). Spastic weakness, defined by resistance to passive stretch in the muscles and lack of voluntary motor control, can cause difficulties when performing even basic motor tasks. As a result, persons with spasticity may have difficulty with even the simplest activities of daily living. A person with a TBI may also have difficulties with balance and coordination (Murphy & Carmine, 2012). Slowness in movement and responses, bradykinesia, is often a common complaint after injury (Murphy & Carmine, 2012). Speech problems due to motor dysfunction such as dysarthria and oral apraxia may make communication post-injury difficult. These are only some of the most common motor dysfunctions after TBI. However it is possible to have a wide range of other symptoms depending on individual circumstances.

1.4 EMOTIONAL/BEHAVIORAL FUNCTION

After TBI, a person's behavior and emotional well-being may be altered due to neurochemical changes and situational factors. Agitation is often one of the earliest changes in behavior, particularly among individuals with moderate to severe TBI. Treating agitation is challenging because most of the pharmacological agents have sedative effects hindering the patient's ability to participate in a rehabilitation program and some agents have been known to cause long-term cognitive deficits, even after discontinuing the treatment (Phelps et al., 2014).

However, long after the acute period of recovery, changes in behavior and emotional well- being may persist. Emotional dysregulation may make it difficult for a person to deal with stressors of everyday life. A person with emotional dysregulation may have unchecked emotional responses or exhibit inappropriate emotions in situations (Paradiso et al., 1999). Depression is a very common outcome of a TBI due to changes in brain physiology and the situational circumstances (Fedio et al., 2014). Depression makes it difficult for a person to participate in therapy hindering the rehabilitation process. Likewise, anxiety is a common consequence, which can make the individual difficult to treat in the clinical setting. Aggression is a frequent product of a TBI, which can make the person anxious, and, in some circumstances, dangerous to treat or even dangerous to themselves.

In summary, there are multiple functional and behavioral consequences of TBI. The particular pattern of changes may vary based on the cause of the injury, the location and severity of the injury, and the specific mechanisms of change in the brain associated with these factors. Additional factors may also influence functional and behavioral changes. Among these factors, gender is of particular interest.

2.0 RATIONALE FOR EXAMINING GENDER AS A FACTOR INFLUENCING TBI OUTCOMES

There are a number of reasons why gender is of particular interest when examining TBI outcomes. Although TBI is generally more common among men, women comprise 40% of individuals with TBI (Center for Disease Control, 2010). Furthermore, meta-analyses and systematic reviews suggest that gender may be critical factor influencing functional outcomes after TBI (Farace & Wayne, 2000). Differences in outcomes may be due to differences in causes of injury, psychological and social factors, and physiological factors.

In general, men are at a greater risk of acquiring a TBI, particularly during late adolescence and young adulthood (up to the age of 30) (Center for Disease Control, 2010). During these high-risk years, often christened the "testosterone years," men are at a higher risk of motor vehicle accidents and interpersonal violence. Specifically men are six times more likely to receive a TBI due to a gunshot wound and about three times more likely to suffer a TBI as a result of a motor vehicle accident (Marion, 1999). However, among older adults (ages 65 and above), women are at higher risk for TBI (typically due to falls) (Bruns & Hauser, 2003). The higher risk for women in this age group could be explained by the fact that, in general, women have a longer life expectancy, and therefore are more prevalent in this age group.

There are many psychological and social differences between males and females that have the potential to impact functional outcomes after TBI. Depression is a disorder that causes a depressed mood and loss of interest in activities that normally bring pleasure for at least two weeks. Depression is nearly twice as prevalent in females as compared to males (Ven de Velde, Piet &Leveque, 2010). Depression has been linked with poor outcomes after TBI because it can

interfere with a person's ability to participate in activities of daily living and instrumental activities of daily living. Therefore, women may be more likely to experience poor outcomes (Richardson et al., 2014).

Societal expectations may also influence outcomes for males and females after TBI. For example, in current American society, there is a higher expectation for males to be employed outside of the home than females. If this pressure is present prior to injury it is likely going to be present after injury. Thus, males may be more likely to return to work following a TBI thus achieving a better outcome, according to selected measures, than females. This difference in outcomes may not be based purely on differences in neurological recovery, but also due to differences in the societal pressure to be employed outside of the home.

Another example of differences in societal expectations between males and females relates to expectations for the role of the caregiver. Approximately 78% of caregivers of individuals with TBI are females (Sander et al., 2007). This could be explained not only by the higher incidence of TBI in males leaving females to be the primary caregiver but also by societal expectations for females to assume roles as caregivers. This, added to the fact that females with TBI tend to be older, and outlive their partners, leads to the implication that females are less likely to return home and instead remain in institutions (thus an unfavorable outcome). These are just a few of the psychological and societal differences that can influence functional outcomes after TBI however.

Males and females are not only psychologically and socially different, they are also physiologically different. Among the more obvious differences between males and females are testosterone and estrogen levels. Due to hormonal differences, male and female brains do not respond to neurotransmitters and drugs the same way (McEwen, 1991). Females are more likely to express higher levels of Interleukin-1, which is pro-inflammatory after stress, such as trauma

(Arakawa et al., 2014). Conversely, estrogen has been shown to have neuroprotective properties by the activation of cell survival mechanisms (Chakrabarti et al., 2014; Djebaili et al., 2004; Roof & Hall, 2000). These differences could influence not only severity of secondary injuries but also the path of recovery for both sexes.

3.0 GENDER AND ITS INFLUENCE ON FUNTCIONAL OUTCOMES AFTER TBI

To better understand differences in functional outcomes between men and women, I conducted a search of the recent literature examining differences in functional outcomes based on gender. Specifically, a PubMed search for data-based research articles was initiated using the search terms “traumatic brain injury”, “gender” and “outcomes”. Articles were excluded if they: focused on pediatrics, did not examine gender as an independent variable, and those published before 1998 (when the last systematic review examining gender and its influences on functional outcomes after TBI was published). The bibliographies of the included articles were searched for additional relevant articles that may have not been found in the PubMed search. This process was continued until saturation was achieved. From the selected articles, information related to the setting (acute, subacute or chronic), experimental comparison, and male and female outcomes was extracted. A summary of the included studies and extracted information is presented in Figure 1.

The results of the review suggest that females fared better on the Glasgow Coma Scale (Slewa-Younan et al., 2004) and the Glasgow Outcome Scale (Brazinova et al., 2010). The Glasgow Coma Scale is a basic scale that measures responsiveness acutely while the Glasgow Outcome Scale measures disability chronically. These two studies suggest that females have less severe injuries initially and continue to have lower levels of disability chronically.

On the other hand, males fared better on outcome measures such as the Functional Independence Measure (FIM) (Graham et al., 2010 & Lecours et al., 2012), as well as discharge location and levels of scheduled home health care (Graham et al.,2010). The FIM is an assessment of basic self-care and functional mobility, and is strongly influenced by motor function. Given that males had higher FIM scores at discharge, it is not surprising that they were more likely to be

discharged home (rather than to an institution) and had less scheduled home health care. Discharge disposition and home health follow-up might also be influenced by the fact that males are more likely to have a primary caregiver, as previously discussed. Females could possibly be more likely to go to assisted living or have higher levels of home health care simply because they were the primary caregiver of their household so there is no one to take care of them post-injury due to practical reasons.

The results of this review suggest that there are no differences in post-injury complication such as pneumonia, acute respiratory distress syndrome, and bacteremia. Males and females also exhibited comparable levels of agitation post injury (Kayden et al., 2004; Magnotti et al., 2008). It is important to note that the Agitated Behavior Scale was used to measure agitation, and it has poor inter-rater reliability. Also, the behaviors on the scale are highly reflective of severe agitation such as: violence, explosive anger and repetitive behaviors. These indices may be comparable between males and females, whereas other indices of agitation such as shortness of temper may be different between males and females.

The findings are mixed with respect to differences in mortality, cognitive and affective outcomes, and employment outcomes after TBI. The three articles within this review that examined mortality differences reported three different conclusions. Davis et al., (2006) suggested that females were less susceptible to mortality than males; however, the groups were extremely unequal, with over seven thousand more male participants in the study. Ottochain et al., (2009) suggested that males fared better, but their sample contained female participants who were significantly older and more severely injured than their male counterparts. Magnotti et al., (2008) found that males and females were equivalent in terms of mortality; however, once again the comparison groups were highly unequal with nearly ten thousand more male participants than

female participants; furthermore, male participants had significantly lower Glasgow Coma scale scores upon admission on average. Thus, age and severity may be stronger predictors of mortality after TBI than gender. However, it remains unclear.

Furthermore, studies that examined cognitive and affective outcomes after injury also presented mixed findings. Liossi et al., (2009) examined cognitive and affective outcomes after TBI and found that females were significantly more impaired in verbal and visual memory and these deficits increased in severity with increasing age. Females also had higher levels of anxiety and depression. The correlation between age and degree of cognitive impairment in females suggest that estrogen, which decreases with age, is connected to these measures. Unfortunately, this study contained no control participants. Given that females in general are more prone to anxiety in general, it is unclear whether these differences can be attributed to the TBI. According to Ratcliff et al., (2007) females after TBI perform worse than males in tests of visual analytic skills, which is reflective of the general population and not limited to the TBI population alone. This study also found that males perform worse in tests of language, which is incongruent with the findings of Liossi et al., (2009). These two studies do a good job describing the affective and cognitive differences after brain injury. However, little is spoken about differences prior to injury making it unclear if the differences after injury are due to preexisting variances or the results of injury.

Differences in employment and work capacity after TBI are also difficult to interpret. Pre-existing gender differences and societal expectations with respect to employment and work capacity confound post-TBI outcomes. Bounds et al., (2003) found significant differences in successful employment after TBI in their sample which contained people with a lower than average socioeconomic status (SES). Groswasser et al., (1998) looked at work capacity rather than actual

successful employment, which eliminated some of the societal biases. All participants were at an inpatient rehabilitation center and the method for measuring work capacity was not clearly stated in the study. However, the study suggests that females fared better than their male counterparts in this specific study. The disagreement between actual employment and work capacity suggests that societal pressures may be a factor in this measure.

Figure 3-1: Scoping Review

	Year	Outcome measure	Setting	Comparison Populations	Findings	Who Fared Better?
Groswasser	*1998*	*Work Capacity*	*Acute/ Subacute*	*Females: n=34 & Males: n=79,Age 5-65years, Mechanism of injury not reported*	*Females had significantly better functional outcome (p<0.016)*	*Females*
Kraus	*2000*	*Mortality*	*Acute/ Subacute*	*Females: 16-29 yrs (n=49), 30-49yrs (n=47),≥50 yrs(n=47), Blunt injury (n=129), Penetrating Injury (n=14) Males: 16-29 yrs (n=285), 30-49 yrs (n=240), ≥50 yrs (n=127), Blunt Injury (n=556), Penetrating Injury (n=96)*	*Females had 1.28 times higher mortality than males*	*Males*
Bounds	*2003*	*Successful employment through DVR*	*Chronic*	*Females (n=23) and Males (n=55), 18-57 yrs*	*4.3% of females successfully employed as compared to 23.6% of males*	*Males*
Slewa-Younan	*2004*	*GCS,PTA and multiple outcome measures*	*Acute*	*Females (n=54) and Males(n=54) in high speed MVA, 16-37 years of age*	*Females had lesser severity of injury and length of PTA*	*Females*
Kadyan	*2004*	*Agitation (ABS)*	*Acute*	*Females(n=38) and Males (n=120) between 18-86 yrs. Mechanism of injury: MVA (n=99), Violent assault n=24), Falls (n=18), Other (n=17)*	*Females are equally likely to exhibit signs of agitation as males in peak intensity and average intensity*	*Equal*
Davis	*2006*	*Mortality*	*Acute*	*Females (15-49 years n=1881, ≥50 n=1297) and Males (15-49 years n=7891, ≥50 n=2368). Mechanism of injury not reported.*	*Post-menopausal females had greater survival rate was significantly better than males of the same age.*	*Females*

Corrigan	*2007*	*Employment 1 year after TBI*	*Chronic*	*Females (n=957) and Males (n=2487) 18-65 years of age.*	*Females were more likely to decrease work hours or stop working. Except in the oldest age group (55-64yrs) where males were more likely to stop working. Female employment outcomes got better as the age increased.*	*Mixed*
Ratcliff	*2007*	*1 year after injury- attention/workin g memory, visual memory, verbal memory, language, visual analytic skilled, problem solving and motor functioning*	*Chronic*	*Females (n=100) and Males (n=225) 16-45 yrs. Mechanism of injury was not reported.*	*Females performed better in tests of attention/working memory and language however did worse in tests of visual analytic skills.*	*Mixed*
Magnotti	*2008*	*Ventilator associated pneumonia, ARDS, bacteremia, ventilator days, ICU days, length of hospital stay and mortality*	*Acute*	*Females (≤40yrs n=6,476, >50 yrs n=3,007) and Males (≤40yrs n=14,934, >50 yrs n=5,194) who suffered a blunt impact injury.*	*Females had equal survival Gender alone offers no survival advantage.*	*Equal*

Liossi	*2009*	*Cognitive and Affective outcomes*	*Subacute /Chronic*	*Females (n=162) and Males (n=358) between 20-60 yrs. Mechanism of injury: MVA (70%), Falls (15%), Assault (8%), Pedestrian Injury (7%)*	*Females were significantly more impaired in verbal and **visual memory** and cognitive deficits increased with age in Females (role of estrogen on hippocampus?). Females had higher levels of anxiety and depression post TBI*	*Males*
Ottochain	*2009*	*Mortality*	*Acute*	*Females (<14y n=44, 14-44 n=123, 45-54 n=55, ≥55 n=184) and Males (<14y n=27, 14-44 n=172, 45-54 n=67, ≥55 n=147). Mechanism of injury not reported.*	*Females over the age of 55 (post menopause?) had worse outcomes*	*Males*
Brazinova	*2010*	*GCS score greater than or equal to 4*	*Chronic*	*Females (n=29) and Males (n=71) ≥65 with initial GCS ≤8. Mechanism of injury not clearly stated.*	*Females had more favorable outcomes.*	*Females*
Graham	*2010*	*Rehab length of stay, discharge FIM motor and cognitive, discharge setting and scheduled home health*	*Subacute*	*Females (n=8,650) and Males ≥65 (n=18,413). Mechanism of injury not clearly reported.*	*Females had lower length of stay, lower cognitive and motor FIM and greater scheduled home healthcare*	*Mixed*
Brown	*2012*	*Discharge location*	*Acute*	*Females (n=1,899, MVA n=222, , Other n=1677) and Males (n=1,581, MVA n=242, , Other n=133) ≥65yrs.*	*Females 1.3 times more likely to be sent to a care facility instead of home*	*Males*
Lecours	*2012*	*13 motor factors of FIM*	*Chronic*	*Females (n=63) and Males (n=73) ≥ 55 years, mechanism of injury not reported.*	*Males fared better*	*Males*

One can conclude from this review that it is reasonable to consider gender when examining TBI outcomes and the effects of intervention after TBI. Nonetheless, methodological issues limit conclusions that can be drawn from this review. Unfortunately, studies in the review varied greatly in recruitment and assessment methods, limiting the conclusions to be drawn from the review. Sampling methods yielded highly variable samples with respect to cause, location and severity of injury, let alone respective numbers of male and female participants. Furthermore, many of the assessment methods have questionable validity. Finally, variability in pharmacological and rehabilitation interventions was not measured or reported, raising questions about the consistency of treatment exposure that TBI participants had across studies. It is impossible for clinical researchers to control for factors such as age and gender.

Some of the methodological issues that are apparent in clinical studies may be addressed through pre-clinical studies. Laboratory experiments, using rat models, allow for the scientists to control factors that might influence outcomes, and isolate the effects of gender. For example, variability due to genetic make-up, pre-injury and post-injury environment, and cause, location, and severity of injury can be better controlled in rat models. Furthermore, pre- injury function and post-injury pharmacotherapy and rehabilitation can also be delivered in a more controlled fashion than what can be done in clinical studies. Thus, laboratory experiments with rat models offer advantages for isolating the effects of gender on TBI outcomes.

4.0 DIFFERENTIAL RESPONSE TO PHARMACOLOGICAL AGENTS: IMPLICATIONS BASED ON A SINGLE EXPERIMENT

Given the reasons to suspect gender differences in outcomes, and the mixed results in the literature, we designed a single controlled experiment examining gender differences in response to a pharmacological agent often used to treat agitation after TBI. For the purposes of this experiment, we chose to focus on a treatment for agitation is a common symptom after TBI, and our review suggested that males and females experience comparable levels of agitation after TBI (Kadyan et al., 2004). We chose to focus on haloperidol (HAL) because it is frequently used to treat agitation after TBI, and the differences in HAL response attributed to gender are unclear. Previous studies have examined the effects of HAL in male rats alone but the results have not been compared to those of female rats (Kline et al., 2008). Our intent was to clarify the impact of HAL as a preface to future studies examining alternative treatments.

HAL is highly effective in producing sedative effects to treat TBI-induced agitation and aggression. However, HAL is known to produce Tardive dyskinesia in ten to twenty percent of patients receiving this treatment long term (Horn & Zasler, 1996). It is important to note the possible motor deficits caused by HAL. With the neurological burden that HAL can place on patients with a TBI it is important to weigh the risks with the benefits. The risk to benefit ratio may however be different depending on the gender of the patient receiving this pharmacological treatment.

4.1 MECHANISM OF ACTION

HAL is a high potency typical antipsychotic drug that acts by antagonizing D2 receptors. Due to the D2 receptor blockade, extrapyramidal symptoms are very common. These pyramidal symptoms include: tremor, spasticity, akinesia and bradykinesia. Furthermore, HAL has effects on 5-$HT_{1 \text{ and } 2}$, D1 and α_1 and α_2 receptors however this activity is low. Also, HAL has minimal affinity to H1 and 5-HT1A receptors (Karl et al., 2006).

4.2 GENDER AND MEDICATION RESPONSE

In general, female patients are more likely to report side effects due to a medication. However, male patients may be more sensitive. Morag, Oved and Gurwitz (2012) compared immortalized human lymphoblastoid cell lines (LCLs) from unrelated females and males to examine sensitivity to common drugs, and demonstrated that males were more responsive and sensitive, to most medications. This finding suggests that there may be differences between how females and males respond to HAL but limited research in the differences in sensitivity to HAL between the two genders has been completed.

4.3 HALOPERIDOL AND BRAIN INJURY

HAL is commonly used after TBI to alleviate symptoms of agitation and aggression. Based on a survey by Fugate et al., (1997) of rehabilitation physicians with expertise in TBI, HAL was listed as physicians' drug of choice for the treatment of agitation 11% of the time. However, 25% of physicians reported using HAL when other treatments were ineffective. Furthermore, non-experts were significantly more likely to use HAL than experts (those who spent 70% of their time treating TBI or published at least two evaluation papers focusing on pharmacologic intervention after TBI in the last five years)(Fugate et al., 1997).

Even though HAL is frequently prescribed, it has been associated with poor cognitive and motor outcomes in experimental models of TBI. In a study by Kline et al., (2008) the effects of HAL on behavioral outcome were examined in male rats after a controlled cortical impact (CCI). The study and others found that HAL impaired the acquisition of spatial learning in the Morris water maze (Hoffman et al., 2008; Kline et a., 2008). This preclinical study suggests that HAL is detrimental to functional recovery. Unpublished studies from this same laboratory show that HAL reduces the efficacy of environmental enrichment, which has been shown to robustly improve function recovery after CCI (Monaco et al., 2013; Kline et al., 2008; Phelps et al., 2014).

Furthermore, although HAL is highly effective as a sedative agent it has the potential to produce serious side effects in patients (such as TBI patients) including neuroleptic malignant syndrome (NMS) (Bellamy et al., 2009). Symptoms of NMS include rigidity, fever, autonomic instability and cognitive changes that are thought to be result of the dopamine receptor blockade caused by HAL (Wilkinson et al., 1999). Interestingly, NMS is more common in males as compared to females suggesting that males are more sensitive to the D_2 receptor antagonistic activity of HAL.

In order to further explore the clinically relevant issue of gender differences in functional outcome after the chronic administration of HAL after TBI a single experiment was conducted. The goal of this study was to determine if gender differences in neurologic functional outcomes are different due to the effects of HAL. By using a rat model more variables could be held constant than in a clinical setting allowing any difference between the groups to be associated with gender and HAL.

4.4 METHODS

4.4.1 SUBJECTS

Thirty-two normal adult cycling female and thirty male Sprague-Dawley rats were subject to the experimental protocol. Rats were placed into the following conditions illustrated in figure 4-1.

Figure 4-1: Experimental Groups

	Male		Female	
	CCI	SHAM	CCI	SHAM
VEH	n=10	n=5	n=6	n=6
HAL	n=10	n=5	n=10	n=10

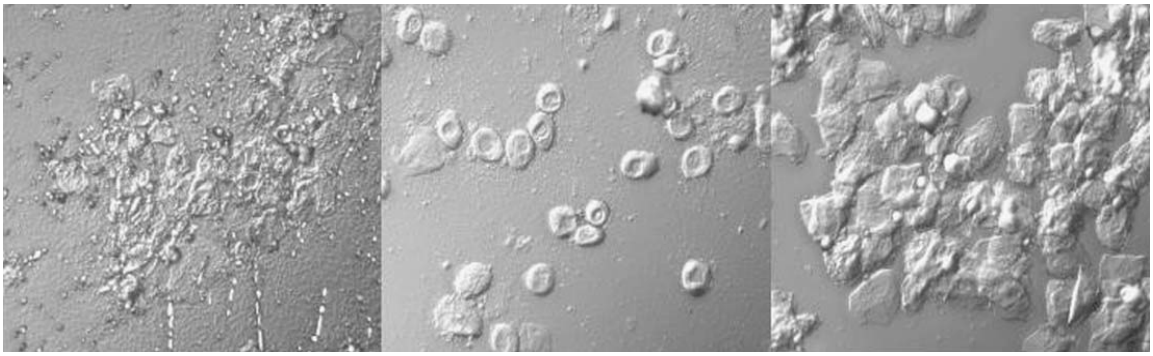
4.4.2 HOUSING CONDITIONS

Ten days prior to surgery rats arrived to the laboratory to become acclimated to conditions. All rats were housed in the same colony room, which was kept at a constant temperature (21±1°C). A light cycle was also kept constant for all rats with light being on between 7:00am-7:00pm. Food and water was available continuously throughout the experiment. Rats were housed two per cage. Female and male rats were kept separate throughout the experiment.

4.4.3 ESTROUS CYCLE MONITORING

Prior to surgery and the first day of Morris water maze testing, all female rats were evaluated for estrous stage. Following vaginal smear epithelial cells were examined by a light microscope. Rats were categorized as: proestrous, estrous, or diestrous based on highly distinguishable cellular characteristics (Monaco et al., 2013).

Figure 4-2: Estrous Stages



Differential interference contract micrographs of primary stages of the rat estrous cycle. (A) Typical cell pattern for diestrous. Smears include large numbers of leukocytes with scattered nucleated epithelial and cornified cells. (B) Typical cell pattern for proestrous. Smears include

primary epithelial cells with large nuclei. (C) Typical cell pattern for estrous. Smears include primarily cornified epithelial cells. (Wagner et al., 2004).

4.4.4 SURGERY

All rats received either a controlled cortical impact (CCI) or a SHAM injury (Dixon et al., 1991; Kline et al., 2008). Surgical anesthesia was induced using 4% isoflurane then maintained at 2% isoflurane. Rats were ventilated mechanically and maintained at a temperature of 37±0.5°C by use of a heating pad. Before the procedure started, each rat was secured using a stereotaxic frame. All rats received a midline scalp incision and the fascia was moved to expose the skull. A craniotomy was made over the right hemisphere of the brain by use of a hand held trephine; the bone flap was then removed.

The impacting rod (6mm, flat) was carefully positioned, centered in the craniotomy until it lightly touched the Dura mater. The impact tip was advanced 2.8mm further to produce a brain injury of moderate severity (2.8 mm tissue deformation at 4 m/sec). Immediately after impact anesthesia was discontinued. Sham rats were subjected to the same procedure, excluding impact.

4.4.5 ACUTE NEUROLOGICAL TESTING

Following the discontinuation of anesthesia acute neurological testing was administered. Hindlimb withdraw reflex was tested bilaterally by pinching the rat's paw with forceps every 5 seconds. The time it took for the withdraw reflex to return was recorded. The return of the righting

reflex was monitored by placing the rat supine and recording the time it took to return to a prone position (Monaco et al., 2013). These acute measures are sensitive indicators of injury severity and anesthetic effects.

4.4.6 DRUG ADMINISTRATION

HAL and VEH were made fresh daily by dissolving in 1:1 dimethyl sulfoxide and saline, which also served as the vehicle. Drug administration started 24 hours after surgery once daily following behavioral testing at a dose of 0.5mg/kg HAL or 1mL/kg vehicle. Drugs were administered intraperitoneally. The dose of HAL was chosen due to the concentrations being comparable to that used in the clinical setting (Zou et al., 2010).

4.4.7 MOTOR ASSESMENT: BEAM TASKS

Motor function was assessed using the beam-balance and beam walk tasks (Kline et al., 2008, Phelps et al., 2014, Manaco et al., 2013). Prior to injury, all rats received equal training on motor tasks to ensure an equal baseline. For the beam-balance task, the rat was placed on a beam that is 90 cm above ground level and 1.5 cm wide and the time is recorded that the rat balances for a maximum for 60 s. Prior to injury, all rats were trained to be able to balance for 60 seconds.

The beam walk task used the negative reinforcement of pink noise and bright light to encourage the rat traverse across a raised narrow beam of 100 centimeters. At the end of the beam

there was a darkened goal box, once the rat reached the box the negative stimuli were discontinued. Distance traversed and times to traverse were both recorded. A rating scale of 0- 5 was used to rate distance traversed with, 0 being failure to move from the starting box and a 5 being entrance to goal box. A maximum of 60 seconds was allotted for each trial of this task.

Motor function was assessed prior to surgery and post-operatively for five days and at the same time daily. For all motor testing the average of three trials was subjected to statistical analysis.

4.4.8 COGNITIVE ASSESSMENT: MORRIS WATER MAZE

The well-established *Morris Water Maze* was used to measure acquisition of spatial learning and memory (Morris, 1984). The maze consists of a blue plastic pool (160cm diameter, 60cm high) that was filled with tap water to 28cm. A clear platform made of Plexiglas was placed in the southwest quadrant of the pool 26 cm from the wall of the pool and 2 cm below the water level. Water temperature was held constant throughout testing at 26±1°C. Around the room which held the pool are constant visual cues. The position of the platform was held constant throughout all trials and rats. Spatial learning acquisition started on day 14 post- operative and continued for five consecutive days.

Cognitive testing was conducted at the same time each day. Each rat underwent four trials with a maximum allotted time of 120 seconds with a four-minute rest period between trials during which the rat was placed in a heated incubator. The rat was placed in the pool from one of four starting positions (North, East, South, and West) in a randomized order. If the rat failed to locate the platform in the 120 seconds allotted time it was manually guided to the platform. Once the rat

found the platform it was given a 30 s learning period on the platform. The average of the four daily trials were averaged and subjected to statistical analysis.

On post-operative day 19, the platform was raised 2 centimeters out of the water and outlined with white tape therefore, making the platform visible to the rat. Other than the raised visible platform all other water maze procedures were kept constant for this trial. These trials were used for a control procedure to ensure that the rats' visual acuity was intact. The state of the rats' visual acuity was important to check to ensure that rat was able to see the visual cues around the room.

4.4.9 PROBE TRAIL: MEMORY RETANTION

On day 19 prior to the visual trials, a single probe trial was conducted to assess memory retention. For this trial the platform was removed from the pool and the percent time the rat spent in the target quadrant was recorded. It was expected that a rat that learned where the platform was located would spend significantly more time in the target quadrant. The rat was placed in the pool and allowed to freely explore for 30 seconds. Swim speeds were also gathered during this trial. Swim speeds were collected to ensure that there were no differences in swim speeds between the groups because this would impact the amount of time it took the rat to find the platform.

4.5 STATISTICAL ANALYSIS

All statistical analyses were performed using *Statview* by observers who were blinded to the experimental conditions. The behavioral data were analyzed with repeated-measures analysis of variance (ANOVA). If the ANOVA revealed statically significant results the data were then further analyzed with the Fisher's PLSD post-hoc test to reveal specific differences between the experimental groups. The data were considered to be significant when the *p* value was $\leqslant 0.05$.

4.6 RESULTS

Rats were excluded from statistical analysis if they were unsuccessful in finding the platform during the visible platform trial because this suggested that their visual acuity was not intact. There were no differences in any of the SHAM injury conditions between the drug conditions therefore; their data were pooled together for simplicity.

4.6.1 ACUTE NEUROLOGICAL FUNCTION

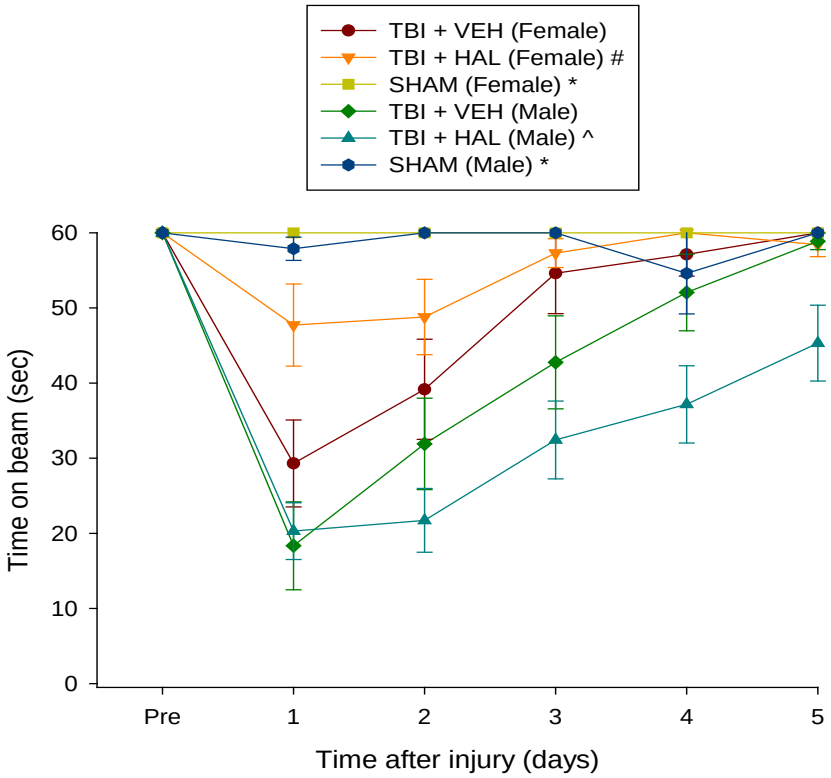
Significant differences in the left hind limb withdraw reflex were detected between TBI+ VEH females and the TBI +HAL males (p=0.0027), between both TBI + HAL (p=0.0010), and between the TBI and SHAM injury groups (p <0.0001). Significant differences occurred in right hind limb withdraw between the TBI + VEH females and TBI + HAL males (p=0.0015). No other

significant differences existed between the TBI groups. No significant differences were observed between any of the TBI groups in the time to exhibit the righting reflex (p <0.0001), which is a robust measure of injury severity.

4.6.2 MOTOR FUNCTION: BEAM-BALANCE

There were no pre-surgical differences between any of the rats, as they were all able to balance on the beam for the maximum allotted time of 60 seconds. Following CCI the ANOVA revealed significant Group [$F_{5.62}$ = 14.093, p <0.0001] and Day [$F_{5,310}$ = 45.197, p < 0.0001] differences, as well as a significant Group × Day interaction [$F_{25,310}$ = 7.430, p <0.0001]. Post-hoc analysis revealed that the TBI+ VEH female group recovered significantly faster than the TBI + HAL male group (p <0.0001, Fig 1.). In addition the TBI + HAL females recovered significantly faster than the TBI + VEH males (p<0.0001). Lastly, the TBI + VEH male group receiving vehicle recovered significantly faster than their counterparts receiving TBI+ HAL (p=0.002).

Figure 4-3: Beam Balance



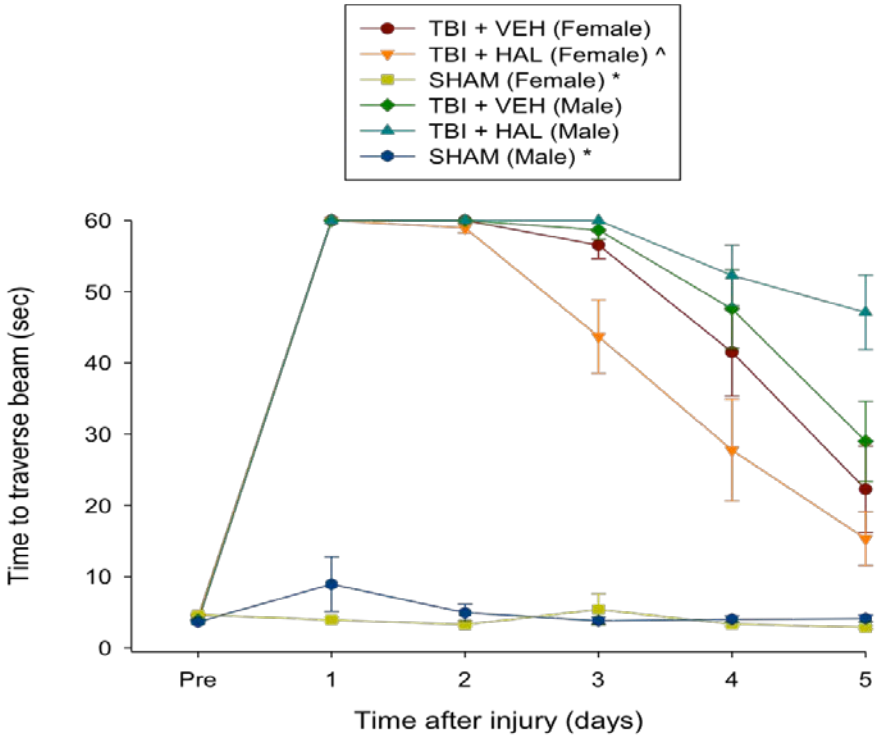
Mean (±S.E.) beam balance ability prior to, and after, TBI or SHAM injury. The * indicates SHAM $p<0.001$ vs. all TBI groups regardless of treatment. The # symbol indicates that the female TBI + VEH group was significantly different than TBI + HAL males ($p<0.001$). The ^ symbol indicates that the TBI + VEH males were significantly different than the TBI+ HAL females ($p<0.001$) and TBI + HAL males ($p=0.0020$).

4.6.3 MOTOR FUNCTION: BEAM- WALK (TIME)

No pre-surgical differences in the ability to traverse the beam were revealed among the groups. Following CCI the ANOVA revealed significant Group [$F_{5,62} = 145.040$, $p < 0.0001$] and Day [$F_{5,310} = 195.137$ $p < 0.0001$] differences, as well as a significant Group × Day interaction [$F_{25,310} = 21.966$ $p < 0.0001$]. Post-hoc analysis revealed that the only significant differences

among TBI groups existed between the TBI + HAL females and the TBI + HAL males (p= 0.0003). Females recovered significantly faster than their male counterparts receiving the same treatment condition.

Figure 4-4: Beam Walk Time



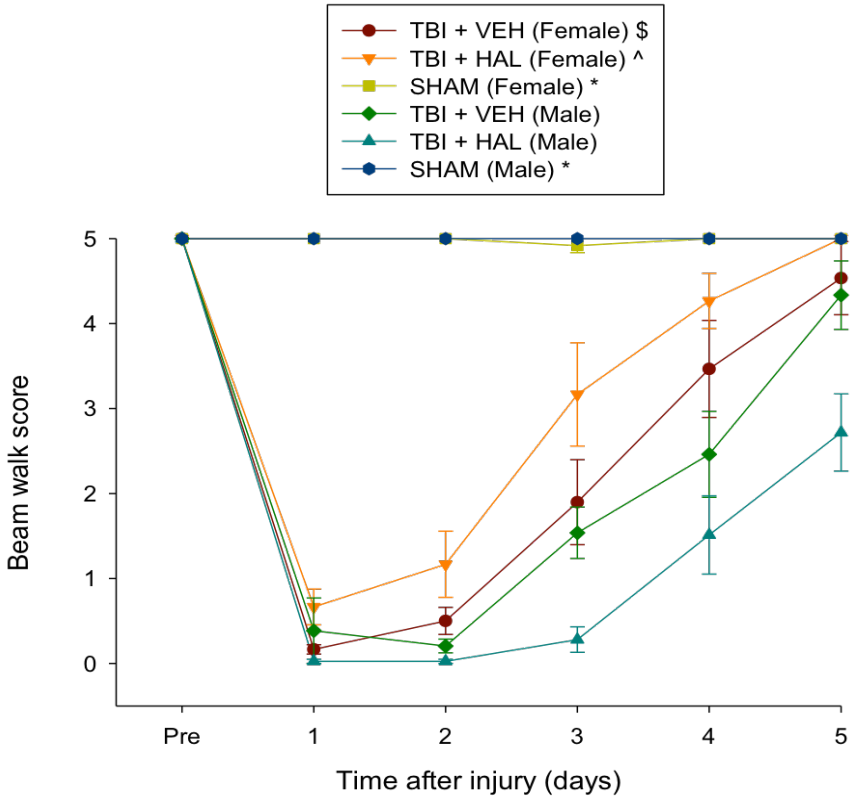
Mean (±S.E.) beam-walking ability (time) prior to, and after TBI or SHAM injury. * All SHAM injury groups were significantly different than all TBI groups (p<0.001). ^ The TBI + HAL female group was significantly different than the TBI + HAL male group (p=0.003).

4.6.4 MOTOR FUNCTION: BEAM-WALK (DISTANCE)

No pre-surgical differences were revealed. All rats were capable of traversing the entire length of the beam. Following CCI the ANOVA revealed significant Group [$F_{5,62}$ = 91.509,p <0.0001] and Day [$F_{5,310}$ = 144.066, p < 0.0001] differences, as well as a significant Group ×

Day interaction [$F_{25,310}$ = 16.076, p <0.0001]. Post-hoc analysis revealed three significant differences observed among the TBI groups. The TBI + VEH female group recovered significantly faster than the TBI + HAL male group (p=0.0004). In addition the TBI + HAL female group recovered faster than the TBI + VEH male (p=0.0030) group and the TBI + HAL male group (p<0.0001).

Figure 4-5: Beam Walk Distance

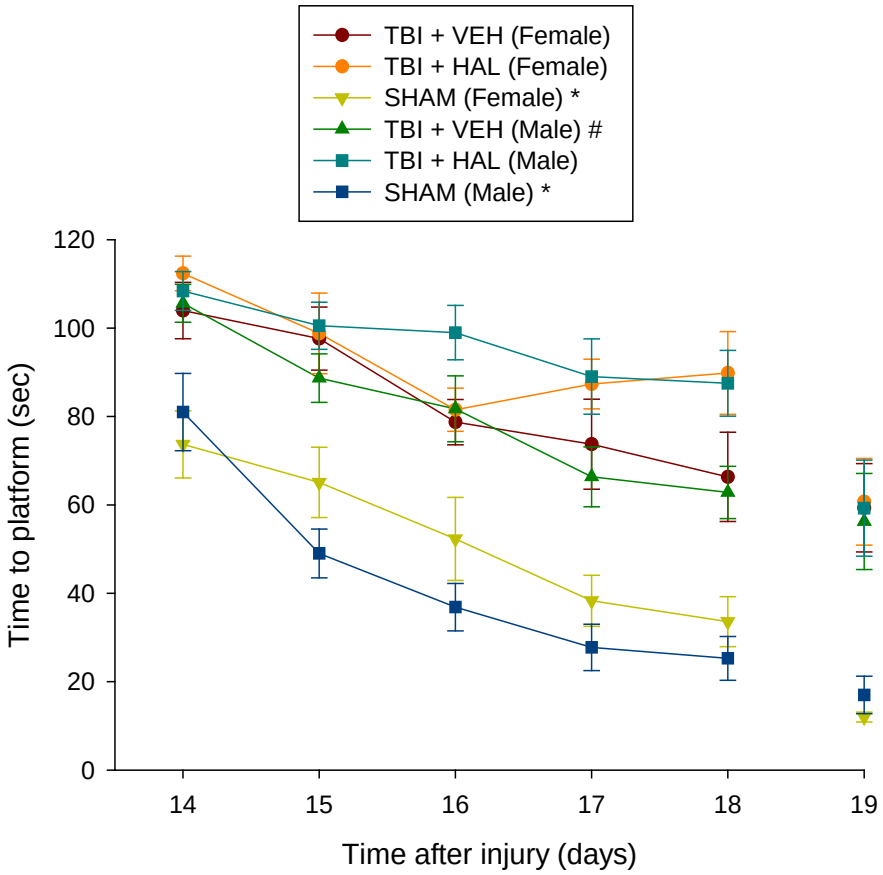


Mean (±S.E.) beam-walking ability (distance) prior to, and after TBI or SHAM injury. * All SHAM injury groups were significantly different than all TBI groups (p<0.001). $ TBI + VEH females preformed significantly different than the TBI + HAL males (p=0.0004). ^ TBI + HAL females traversed the beam significantly different better than both male TBI groups: VEH (p=0.0030) and HAL (p<0.0001).

4.6.5 COGNIIVE FUNCTION: ACQUISITION OF SPATIAL LEARNING (TIME TO PLATFORM)

The ANOVA revealed significant Group [$F_{5, 62}$ = 22.465, p <0.0001] and Day [$F_{4,248}$= 40.929, p < 0.0001] differences. Post-hoc analysis of the Morris water maze data revealed that the TBI + VEH male group was able to locate the platform significantly faster than the TBI + HAL male group (p=0.0008). No other significant differences were observed among the TBI groups. There were no significant differences in any groups during the visible trials (p<0.0001).

Figure 4-6 Morris Water Maze

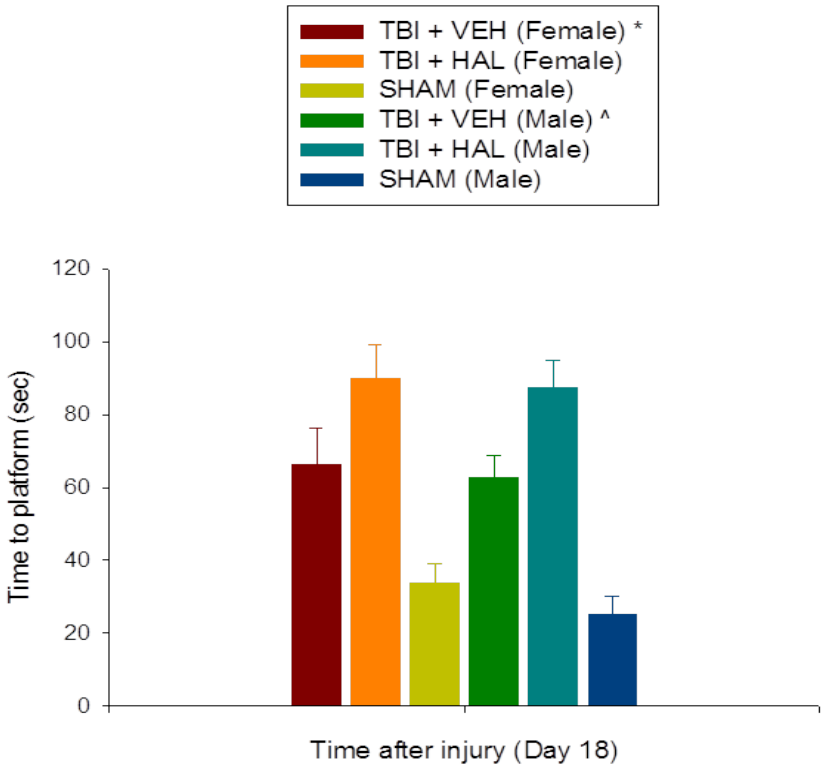


Mean (±S.E.) to find platform (sec). *SHAM groups preformed significantly better than all TBI groups (p<0.0001). # The only other statistically significant difference is the male TBI + VEH preformed significantly better than the male TBI+ HAL group (p=0.0008). Day 19 shows that with a visible platform all animals were able to find the platform in fewer than 120 sec.

4.6.6 COGNTIVE FUNCTION: AQUISTITION OF SPATIAL LEARNING (TIME TO PLATFORM ON DAY 18)

Following CCI the ANOVA revealed significant Group [$F_{5,\ 62}$ = 13.053, p <0.0001] differences. Post-hoc analysis of time to platform on day 18 revealed the female TBI + VEH group was significantly better than either group receiving HAL regardless of gender. Additionally, the female TBI + VEH group was significantly better on day 18 than the male TBI + VEH group. Furthermore, the male TBI + VEH group performed better than the male TBI +HAL group, which replicated previous studies from my laboratory.

Figure 4-7: Morris Water Maze Day 18

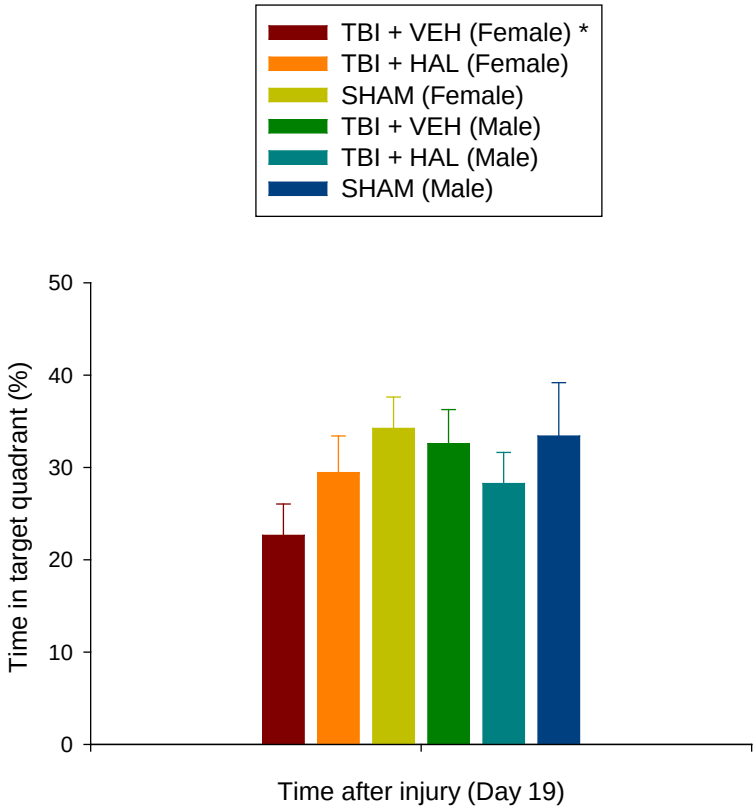


Mean (±S.E.) time to find platform on day 18 post injury. SHAM injury groups performed significantly better than all TBI groups (p<0.0001). * Female TBI + VEH group was significantly different than female TBI + HAL (p=0.0367) and male TBI + VEH (p=0.0450). ^ Male TBI + VEH performed significantly different than female TBI + HAL (p=0.0114) and male TBI + HAL (p=0.0130).

4.6.7 COGNTIVE FUNCTION: PROBE TRIAL (MEMORY RETENTION)

Following CCI the ANOVA revealed no significant Group [$F_{5,62}$ = 1.134, p = 0.3522] differences. Post-hoc analysis showed the only difference the existed among the groups during the probe trial was between the female TBI+VEH group and the SHAM female group (p=0.0455).

Figure 4-8: Probe Trial

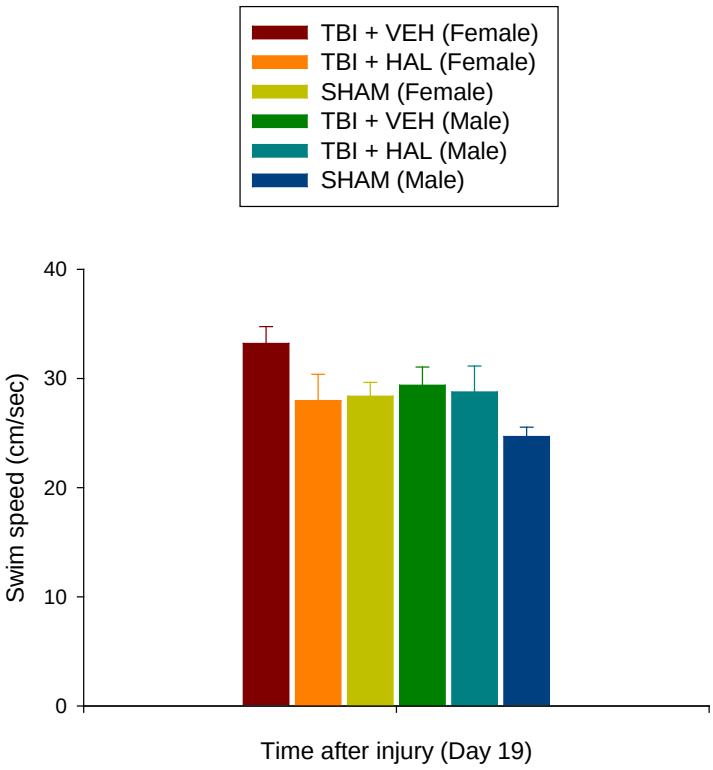


Mean (±S.E.) percent time in the target quadrant during the probe trial. * Female TBI+VEH group and the SHAM female group (p=0.0455).

4.6.8 COGNTIVE FUNCTION: SWIM SPEED

Following CCI the ANOVA revealed no significant Group [$F_{5,62}$ = 2.008, p =0.0898] differences.

Figure 4-9: Swim Speed



Mean (±S.E.) swim speed in cm/sec on day 19 post injury. No significant difference between any of the groups.

4.7 DISCUSSION

Previous studies have shown that the antipsychotics drugs (APDs) HAL and risperidone prevent recovery of cognitive function in male rats after TBI (Kline et al., 2008; Hoffman et al.,

2008; Phelps et al., 2014). Unfortunately, there has been very little research on the drug's effects on recovery in females, leading to a lack of understanding of whether these deleterious effects are similar between males and females. The goal of this experiment was to determine if HAL produces the same detrimental effects in females as it does in males. The results suggest that females do respond differently to HAL after TBI, in most measures HAL does not impede recovery to the same degree as it does in males.

Specifically regarding motor function, there were no pre-injury differences between male and female rats. However after TBI, the SHAM injury groups performed better than the TBI groups. Additionally, in general, female TBI rats performed better than male TBI rats, but the effects of HAL varied. Furthermore, female TBI + HAL performed better than male TBI +HAL. These findings suggests that with respect to motor function, TBI may not impair females as severely as males, and that HAL may not have the same deleterious effects on female's with TBI. Support for males rats faring worse after TBI + HAL treatment comes from a study by Feeney et al. (1982) who showed that HAL produced negative motor recovery outcomes in males after TBI. This preclinical finding also supports clinical data that human males are more sensitive to HAL than females (Oved & Gurwitz, 2012).

With respect to cognitive function, which consisted of acquisition of spatial learning and memory retention, HAL was found to be deleterious to spatial learning acquisition in the male TBI group, but did not appear to affect retention, as measured in the probe trial. The swim speed measure obtained during the probe trial may be influenced by both motor and cognitive performance.

It has been demonstrated that TBI produces significant disruptions of normal dopaminergic neurotransmitter physiology (Massucci et al, 2004; McIntosh et al, 1994). As previously indicted,

HAL demonstrates a high affinity for dopamine D_2 receptors, where it acts as an antagonist. Additionally, D_2 receptor agonists, such as bromocriptine and amantadine, produce more favorable outcomes after TBI in males (Dixon et al, 1999; Kline et al., 2004; Phelps et al., 2014). This could lead one to believe that there may be differences between genders in D_2 receptor affinity to HAL and/or gender differences in the metabolism of HAL.

Hormones are a variable that differ between male and females and hence it is reasonable to believe that testosterone and estrogen may play a part in the differences observed when HAL as a treatment after TBI. Previously McEwen (1991) found that hormonal differences cause the brain to respond differently to neurotransmitters and pharmacological treatments. Additionally, estrogen has been found to have neuroprotective properties because of its role in activation of cell survival mechanism (Chakrabarti et al., 2014). The neuroprotective properties of estrogen may lessen the deleterious effects of HAL after TBI.

This study serves as a stepping-stone for future studies to explore gender differences after TBI. Another strategy that may lessen the negative effects of HAL is environmental enrichment (EE) has been shown to provide robust neurological benefits after TBI in both males and females (Bondi et al., 2014; Cheng et al., 2012; de Witt et al., 2011; Monaco et al., 2013). Unpublished studies from our laboratory have demonstrated that EE lessens the appallingly negative outcomes associated with HAL. Future studies should examine females exposed to environmental enrichment and HAL in combination and compare the results to that of males.

Another interesting approach that could be taken is to study estrogen therapy in combination with HAL in male rats after TBI. By studying this combination of treatments experimenters would be able to speculate further if hormones are responsible for the differences between males and females after TBI when HAL is used as a treatment.

These findings are important because of the potential implications for rehabilitation. This study suggests that HAL inhibits motor recovery more significantly in males, than in females. This could diminish the benefits of occupational and physical therapy for males who sustain TBI. Furthermore, this study suggests that HAL causes significant cognitive deficits after TBI, which could lead to further decline in a patient's cognitive status after TBI. For example, if D_2 receptor antagonists are used more therapy may be required for the patient to reach the same milestones as if they were not being treated with a D_2 receptor antagonist.

Despite the data showing that antipsychotic drugs such as HAL is detrimental to the recovery process, such agents are still required for patient management. Fortunately, our laboratory has shown that antipsychotic drugs, which have different mechanisms of action, do not impair recovery after TBI and in fact may actually provide some cognitive benefit. Unpublished data from our laboratory suggests that aripiprazole does not negatively impact motor function and actually positively impacted spatial learning acquisition. The different outcomes may be attributed to the mechanism of action in that aripiprazole is a D_2 agonist and a 5HT1A partial agonist. Studies from our lab have shown that drugs with this mechanism of action may be beneficial to functional recovery after TBI (Kline et al., 2004; Phelps et al., 2014). Moreover, the sedative drug lorazepam also does not appear to produce negative effects on outcome after TBI. Data from our laboratory suggests that lorazepam does not negatively impact motor or cognitive functional outcomes after TBI and therefore should be considered a valid alternative to HAL (unpublished).

Taken together, the two studies just described suggest that patients may be treated efficiently for TBI-induced agitation and aggression without restricting their ability to recover spontaneously after TBI. Whether these drugs produce the same effects in females is not known, but is the focus of future studies in our laboratory.

5.0 CONCLUSION

In conclusion, the literature suggests, that although the findings are yet unclear, there are reasons to suspect that males and females may respond differently to treatment after TBI. The results of my experiment support this premise, demonstrating that chronic administration of the antipsychotic drug HAL produces differences in recovery between males and females, as illustrated through tests of motor and cognitive function. Specifically, males performed worse than females, which replicate previous work from our laboratory. The novelty of this experiment is that the data show that females were not as impaired. These findings may help rehabilitation practitioners design more effective and personalized interventions to meet the needs of all TBI patients exhibiting agitation and aggression. In addition, these findings provide support for additional controlled experiments examining differences in treatment response between males and females who have sustained TBI.

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