Published in final edited form as:

Behav Brain Res. 2020 October 01; 395: 112879. doi:10.1016/j.bbr.2020.112879.

Choice-based assessments outperform traditional measures for chronic depressive-like behaviors in rats after brain injury

Michelle Frankot¹, Christopher O'Hearn¹, Cole Vonder Haar^{1,2}

¹Department of Psychology, West Virginia University, Morgantown, WV, USA

²Department of Neuroscience, West Virginia University, Morgantown, WV, USA

Abstract

Depression is the most common psychiatric comorbidity to be diagnosed following traumatic brain injury (TBI). In clinical populations, TBI-induced depression may be particularly difficult to treat due to both unique underlying causes and the propensity for treatment resistance. Preclinical assays are needed to characterize depressive-like behavior in models of TBI and evaluate treatments. In the current study, two traditionally-acute assays of depressive-like behaviors, the Forced Swim Task and Saccharin Preference, were extended longitudinally to evaluate chronic TBI-induced depressive-like behaviors in male rats. Two chronic measures of motivation, the Progressive Ratio (PR) task and Effort Discounting Task (EDT), were also tested. The PR measures motivation to exert effort, while the EDT parametrically evaluates choice between low- and high-effort requirements. The EDT was the only assay which captured chronic depressive-like behavior after TBI, albeit with a degree of recovery over time. We found that traditionally-acute measures (Forced Swim Task, Saccharin Preference), and even our other chronic measure (PR), failed to capture long-term deficits. We also challenged serotonin and dopamine systems (via fluoxetine and bupropion) to evaluate how TBI-induced changes to these systems might drive depressive-like behaviors. Although we found no effect of fluoxetine, high-dose bupropion differentially impaired TBI rats. These findings suggest that (1) TBI-induced depressive symptoms remain difficult to measure at the preclinical level, (2) treatment for TBI-induced depression requires further exploration, and (3) obstacles at the preclinical level may translate to treatment failure at the clinical level.

Corresponding author: Cole Vonder Haar, Department of Psychology, West Virginia University, PO Box 6040, 53 Campus Drive, Morgantown, WV 26505, Tel: 1-304-293-1787, Fax: 1-304-293-6606, cole.vonderhaar@mail.wvu.edu.

Author Credit Statement

Choice-based assessments outperform traditional measures for chronic depressive-like behaviors in rats after brain injury

Michelle Frankot

Conceptualization; Data curation; Formal analysis; Investigation; Visualization; Roles/Writing - original draft; Writing - review & editing

Christopher M. O'Hearn

Conceptualization; Data curation; Formal analysis; Investigation; Visualization; Roles/Writing - original draft;

Cole Vonder Haar

Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Project administration; Supervision; Validation; Roles/Writing - original draft; Writing - review & editing

Conflict of Interest Statement

The authors have no financial interests in the outcome of this research.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Depression; controlled cortical impact; emotion; anti-depressant

1. Introduction

Traumatic brain injury (TBI) is associated with long-term impairments that can elevate risk of psychiatric conditions [1, 2] including mood disorders, post-traumatic stress disorder, and anxiety [3–5]. Depression is the most common psychiatric disorder diagnosed following TBI [4], with a prevalence as high as 53% [6]. Because of the chronic nature of TBI-induced depression, these patients are at risk for quality of life impairments and functional disabilities that persist years after injury [3]. This condition is further complicated because TBI patients may represent a special population in their response to treatment [7, 8].

Even without the confounding factor of TBI, depression is particularly challenging to study. In clinical studies, barriers include recruitment [9], reluctance to seek treatment, and high attrition rates [10]. Preclinical studies are also complicated by methodological challenges, such as the inability to use self-report measures. Preclinical models of depression are also typically precipitated by a triggering event (e.g., stress). In these models, antidepressants may only remedy changes linked to the trigger (e.g., stress-induced hippocampal dysfunction) rather than additional neural mechanisms that underlie depression in humans [11, 12]. Clinical depression is a complex, multifaceted disorder, and preclinical models can typically only account for singular symptoms that approximate a depressive-like phenotype. As a result, the findings of preclinical studies may only apply to a narrow subgroup of patients with depression [13].

Unfortunately, the challenges of studying depression are amplified in a TBI population. In particular, disruptions to monoaminergic systems which occur after injury may affect the pharmacodynamics of traditional anti-depressive drugs [14]. While changes to dopaminergic systems have been well-established in clinical patients (e.g., reduced dopamine transporter levels, nigrostriatal tract damage) [15, 16], the study of serotonergic perturbations have been more limited to animal models. For example, TBI increased hippocampal serotonin receptor density in male rats, but behavioral deficits (i.e., motor, memory) were not recovered by treatment with fluoxetine, a common selective serotonin reuptake inhibitor (SSRI) [17]. Given that monoaminergic therapeutics (e.g., SSRIs) are a frontline treatment for depression, a better understanding of these neurotransmitter systems in TBI-induced depression is needed. In addition to these issues, TBI patients often struggle to maintain appropriate self-awareness, or the ability to objectively recognize and identify deficits caused by injury [18, 19], which can have effects on discerning treatment outcomes as well [20].

The study of depression in animal models of TBI has largely adopted the classic approach to measuring depressive-like symptoms. Typically, this involves brief, single measurements, such as the forced swim task (FST) and the saccharin/sucrose preference (SP) task. During the FST, rats are placed in a cylindrical tank of water and become immobile after an initial period of swimming and escape behaviors. Duration to immobility (“giving up” on escape)

is used to measure behavioral despair [21], however, it should also be noted that some have argued that immobility may be an adaptive (or “coping”) response to an inescapable stressor. Despite this, it is still one of the most widely used assays for preclinical depression and anti-depressive therapeutics [22]. Anhedonia, another characteristic symptom of depression, is measured using the SP task [23]. Rats exposed to stressors tend to decrease preference for sweetened water versus normal water [24]. Brain injury increased depressive behavior on the FST [25] and SP [26, 27], but it is difficult to identify how these symptoms evolve and/or resolve due to testing at single time points. Repeated FST testing gives mixed findings in healthy rats. In one study, experience with the task reduced struggling time and increased immobile time [28], but in another, repeat testing successfully demonstrated the acute and chronic effects of fluoxetine [29]. These limitations are large concerns when considering the chronic nature of TBI-induced depressive-like behaviors. Given the widespread use of FST, it is important to determine whether additional measures may be needed to fully assess depressive symptoms within the context of preclinical TBI.

Fortunately, several approaches have been developed using long-term operant behavioral methods to study preclinical depressive-like behaviors. These chronic measures, such as the progressive ratio (PR) task often focus on the motivational component of depression and can be tested daily as they measure “state-like”, or stable behaviors. During the PR task, the effort requirement increases every time a reinforcer is delivered, thus increasing the response cost as a function of earned reinforcers [30]. The primary measure is the “break point”, or effort requirement at which rats stop responding, and has been effective at dissecting motivational functions of the striatal dopaminergic system [31, 32]. While the PR task only has a single option, Salamone and colleagues [33] developed a PR task which provides a choice between lever-pressing for sucrose pellets or eating standard chow that is freely available in the chamber. This concurrent choice paradigm pits a high-effort/high-value reinforcer against a low-effort/low-value reinforcer and may more accurately reflect the variety of choices available in real life. However, the concurrent PR/chow task does not provide real-time data about changes in effort expenditure across multiple conditions within a session. This can be measured using a procedure called the effort discounting task (EDT) [34], which parametrically measures motivation at different effort requirements by presenting rats with an option between responding on a lever that delivers a low-magnitude reinforcer or a second lever that delivers a large-magnitude reinforcer but requires more effort (i.e., more presses). Disruptions in effort discounting have been associated with stress hormones [35] and dopamine modulation [34] but have not been studied after brain injury. The PR task has been used to measure motivation after injury but only in a very abbreviated fashion [36, 37]. Given these gaps in the literature, it is important to determine whether chronic operant measures such as the PR more appropriately capture chronic TBI-induced depressive-like symptoms and whether these measures can be augmented with choice-based procedures such as the EDT.

Thus, the goal of the current study was as follows: to (1) determine what type of behavioral assay is most effective in capturing chronic depressive-like symptoms after TBI. Because we have previously shown frontal TBI induces psychiatric symptoms [e.g., risky decision-making, impulsivity; 38], we used an identical procedure determine if these findings extended to depressive-like symptoms. Acute measures (FST and SP) were extended over

several weeks to determine if these traditional assays can be used chronically. Long-term measures (PR and EDT) were also used to assess the efficacy of operant procedures and characterize the role of choice in assaying depressive-like behaviors after TBI. We also aimed to (2) characterize the response to monoaminergic drug challenge after TBI. Fluoxetine and bupropion were used to challenge the serotonergic and dopaminergic systems because both are disrupted after TBI. Other neurotransmitter systems involved in depression, such as noradrenalin, were not assessed in the current study because they are not as well characterized in the context of TBI [14]. Specifically, we expected to see differential responses to drugs that increased the effects of serotonin and dopamine.

2. Materials and Methods

2.1 Subjects

Subjects were 44 male Long-Evans rats which began training at approximately 300 g body weight (Charles River, Wilmington MA). Rats were group-housed prior to surgery and single-housed after. All rats were maintained on a 12:12 reverse light-dark cycle with water available *ad libitum*. Two independent cohorts of rats were used to prevent the confound of concurrently learning two different operant tasks with different contingencies of reinforcement. A cohort of 21 rats (Experiment 1; Figure 1A) completed the FST, SP, and PR tasks; the other 23 rats (Experiment 2; Figure 1B) completed the EDT. Both cohorts of rats were food-restricted and maintained at 14–16 g of standard chow daily. The housing differed slightly between the two conditions due to a switchover in vivarium caging. In Experiment 1, rats were pair housed in standard cages prior to surgery and single housed after surgery. In Experiment 2, rats were triple housed in Optirat cages (Animal Care Systems, Centennial, CO) prior to surgery and then pair-housed with a divider separating the two rats after surgery. All animal procedures were approved by the institutional animal care and use committee under NIH guidelines.

2.2 Progressive Ratio (Motivation)

PR [30] testing was conducted in a bank of 16 operant chambers (Med Associates, St. Albans, VT) enclosed in individual sound-attenuating boxes with a white noise generator in the testing room. The floor of each chamber was composed of a grid of stainless-steel rods. The right side of each chamber was equipped with a food hopper to deliver sucrose pellets (BioServ F0021, 45 mg) and light. Retractable levers with cue lights were situated on each side of the food hopper. The left wall of the chamber was equipped with a 5-hole array. The chamber was also equipped with a houselight and tone generator. Only the levers, food hopper, hopper light, and houselight were used.

The day prior to training, 25 sucrose pellets (45 mg, BioServ, Fleming, NJ) were dropped into homecages to eliminate neophobia. Rats were then habituated to the chambers with 10 freely-available sucrose pellets in the hopper for 30 min. Lever-pressing was shaped using an autoshaping procedure [39]. During autoshaping sessions, a pellet was delivered every 35 s on average (VT-35), or could be produced by a lever press on the extended lever. Once pressing was acquired, rats began daily fixed ratio (FR) sessions (30 min). Rats progressed through escalating ratios (1, 5, 10, 20) if 30 or more reinforcers were earned in a session.

Upon completion of the FR-20, PR baseline was established (30 sessions). During the PR schedule, each subsequent reinforcer delivery increased the ratio requirement for subsequent reinforcers according to an exponential formula ($[5 * e^{0.2n} - 5]$, where n is the number of reinforcers; FR-1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 116, 143, 176, 216, 265, 324, 397, 485...)[40]. Each session continued for 90 minutes or until the rat abstained from responding for five minutes. The greatest ratio completed by the rat was considered the breakpoint. Once data were stable (no changes $\pm$ 10% the average of the last 5 sessions), rats moved onto surgery. PR sessions were conducted each week from Tuesday-Friday.

2.3 Saccharine Preference (Anhedonia)

The SP task [23] was conducted by placing two 400 mL bottles in the rat's home cage. One bottle contained tap water, the other contained a 0.05% sodium saccharine (Sigma-Aldrich) solution. Bottles were weighed before placement and after 72 hours exposure. Bottle position was alternated every 24 hours to reduce side bias. Saccharine preference was calculated as a percent of total intake. Trials occurred weekly after injury, and a pre-injury baseline assessment was taken. Sessions were conducted each week and initiated immediately after PR sessions on Friday and concluded immediately before FST on Monday.

2.4 Forced Swim Test (Behavioral Despair)

The FST [29] was conducted in three transparent cylindrical tanks (20 cm diameter). Tanks were filled to 19 cm height (6 L) with warm tap water (25° C). Testing was conducted in indirect red light and in the presence of a white noise generator. Upon conclusion of the session, the rat was removed from the cylinder, hand-dried with a towel, and placed in a heated cage for 10 minutes, before being returned to its home cage. Water was changed every third trial, and sessions were recorded using a video camera placed 70 cm lateral to the tank. ANY-maze behavioral tracking software (Stoelting Co., Wood Dale, IL) was used for data collection. The primary measure was immobility duration. FST testing occurred after injury beginning with a pretest conducted six days after injury. The pretest is conducted to induce behavioral despair and accentuate behavioral differences during subsequent testing sessions [41]. The pretest ran for 15 minutes; each subsequent weekly session ran for five minutes. These sessions were conducted each week on Monday. These three tasks (PR, SP, and FST) were all completed by one cohort of rats (Experiment 1).

2.5 Effort Discounting (Motivation)

The next cohort of rats (Experiment 2) trained in the same operant chambers and followed the same habituation and autoshaping parameters. Once lever pressing was acquired, they began effort discounting training according to published protocols [34]. First, rats were trained to press both levers on an FR-1 schedule of reinforcement until they pressed at least 50 times within a 30-minute session. Then, to reduce side bias, rats were presented with a single lever on an alternating basis on an FR-1 schedule of reinforcement. Once rats achieved fewer than 10 omissions per session on this alternating paradigm, they progressed to the full EDT.

The EDT consisted of 32-minute sessions of 48 trials divided into 4 blocks. Each block consisted of 2 forced-choice trials and 10 free-choice trials. One lever was designated as the low reward (LR) lever and the other as the high reward lever (HR). The LR lever delivered 1 pellet; the HR lever delivered 3 pellets. During forced-choice trials, a single lever was presented to ensure rats were exposed to the contingencies of each lever. During free-choice trials, both levers were extended. Responses on the LR lever were reinforced on an FR-1 schedule. Responses on the HR lever were reinforced on an FR-2, -5, -10, or -20 schedule (incremented each block). The first press caused the LR lever to retract, but the HR lever remained extended for 25 seconds or until the schedule requirement was met. Failure to respond or complete a schedule was scored as an omission. Baseline criteria were established when the HR lever was chosen on at least 75% of trials during the first block and when behavior was stable for 3 days, as assessed by a within-subjects analysis and visual confirmation. After 66 sessions, rats met baseline criteria and were separated into Sham or TBI groups for subsequent surgery.

2.6 Surgery

Surgical procedures were performed according to previous studies [42]. To mitigate potential infection, aseptic techniques were employed. Ketoprofen (5 mg/kg) was administered subcutaneously before surgery for analgesia. Bupivacaine (0.1 mL, 25% solution) was used as a local anesthetic. Rats were anesthetized using a combination of oxygen (0.5 L/min) and isoflurane (2–4%) and placed in a stereotaxic apparatus. A heating pad was used to regulate body temperature. Once the nociceptive flexion reflex could no longer be detected using the toe-pinch method, a 2.0 cm incision was made along the midline revealing the top of skull. Rats received a craniotomy, 6.0 mm in diameter, over the medial prefrontal cortex (AP +3.0, ML +0.0 from bregma) using a micro-drill. A stainless-steel circular impactor tip (5.0 mm diameter) was positioned over the craniotomy and an electromagnetic impactor device (Leica Biosystems, Buffalo Grove, IL) was used to drive the impactor tip into the cortex to a depth of 2.5 mm, at a rate of 3 m/s. The impactor tip remained in the cortex for 500 ms before it was retracted. Once post-injury bleeding was attenuated, the incision was sutured, and the rat was placed in a heated recovery chamber until it regained consciousness. Sham rats underwent an identical procedure, with the exception of brain injury. Rats were monitored daily for one-week post-surgery to assess pain signs, activity level, hydration, weight fluctuations, and infection.

2.7 Post-Surgical Behavioral Assessment

For Experiment 1, the FST was conducted, starting at post-surgery Week 2 once per week until Week 9. SP was conducted, started at post-surgery Week 2, once per week until Week 7 post-surgery. PR testing occurred four days per week, starting at Day 8 post-surgery, and continued until Week 9. Regular testing for PR was not conducted during Week 7. For Experiment 2, EDT testing was carried out 5 days per week and continued until Week 11 post-surgery.

2.8 Pharmacological Challenges

During post-surgery Week 8, rats in Experiment 1 received either 10 mg/kg fluoxetine (Sigma-Aldrich; in 5% dimethyl sulfoxide in PBS) or vehicle prior to FST and PR in a

counterbalanced design; rats received the other condition during Week 9. This dose was selected because it was efficacious in previous literature [43]. Fluoxetine administration occurred 24 h, 12 h, and 1 h before FST sessions, and 1 h before PR sessions.

During post-surgery Weeks 9–11, a bupropion challenge was conducted for the EDT cohort. Bupropion (Sigma-Aldrich, St Louis, MO) doses (0, 10, 20, and 40 mg/kg) Depression and TBI were selected from doses that have previously been effective in increasing motivation during effort-based operant tasks [44]. All four doses were counterbalanced using a Latin square design and administered 30-min prior to the task [44]. First, baseline performance was established during the 1–2 days prior to injections. After injections, there was a day of wash out followed by the weekend, during which no testing occurred. This follows previous protocols where rats received one dose per week [44].

2.9 Euthanasia

At 10 weeks post-surgery for the Experiment 1 cohort, and 12 weeks post-surgery for the Experiment 2 cohort, rats were transcardially perfused with 0.9% phosphate buffered saline followed by 3.7% phosphate buffered formaldehyde. Brains were then fixed in 3.7% phosphate buffered formaldehyde for 24 hr before being transferred to a 30% sucrose solution. Brains were embedded in a gelatin block (15% gelatin) with a maximum of four brains per block. The gelatin blocks were frozen and then sliced using a sliding microtome at a thickness of 30 μ m. Slices were then mounted on electrostatically-subbed slides for staining.

2.10 Nissl Stain & Lesion Analysis

Slides were rehydrated using a series of washes administered in the respective order: Citrisolv (1 $\times$ 10 min), 95% EtOH (1 $\times$ 3 min), 70%, EtOH (1 $\times$ 1 min), 50% EtOH (1 $\times$ 1 min), and dH₂O (1 $\times$ 2 min). They were then placed in 0.01% cresyl violet (3 min) or thionin (10 s) for staining and dehydrated by reversing the previous sequence of washes. After dehydration, slides were cover-slipped and allowed to dry overnight.

Digital images (600 dpi) of the brain slices, traversing the area of the lesion (+5.0, +4.0, +3.0, +2.0, +1.0 from bregma) [45], were measured using ImageJ (NIH, Bethesda, MD). The remaining brain volume was estimated by multiplying the thickness (30 μ m) by the average of the slices and the distance [42].

2.11 Data Analysis

Repeated measures data were analyzed using linear mixed-effects regression (LMER). For Experiment 1, this included the effect of TBI on time spent immobile in the FST, percent preference for saccharine, percent baseline PR breakpoint (breakpoint/baseline breakpoint*100), and the effects of Fluoxetine on PR and FST. For Experiment 2, this included the effects of TBI, effort requirement, and bupropion on percent choice of the HR lever over time on the EDT. Omissions, response latency, and reinforcer collection latency were assessed as well.

Arcsine-square root transformations were applied to the percent data (SP, FST, EDT), and log transformations were applied to omissions on the EDT, and progressive ratio breakpoints.

3. Results

3.1 Experiment 1

3.1.1 Progressive Ratio.—Percent of baseline breakpoint (Figure 2C, 2D) was analyzed using LMER (Injury [Sham, TBI] x Time [Week: 2–6]). There was a significant Injury x Time interaction such that TBI rats significantly improved across time from a low starting point ($\beta = 0.26$, $t = 7.15$, $p < 0.001$).

3.1.2 Saccharine Preference Test.—Percent preference (Figure 2B) was analyzed using LMER (Injury [Sham, TBI] x Time [Week: 2–7]). Preference for saccharine water escalated for both groups throughout the recovery period ($\beta = 0.25$, $t = 5.96$, $p < 0.001$), but did not differ as a function of injury or change differentially across time ($\beta = 0.06$, $t = 0.38$, $p = 0.740$; $\beta = -0.02$, $t = 0.34$, $p = 0.733$).

3.1.3 Forced Swim Test.—Percent of time spent immobile (Figure 2A) was analyzed using a t-test (pretest) or LMER (Injury [Sham, TBI] x Time [Week: 2–7]). Although Shams spent significantly more time immobile than TBI rats during the pretest ($t(18.12) = 3.08$, $p = 0.006$), both groups spent increasing amounts of time immobile following repeated testing ($\beta = 0.20$, $t = 2.92$, $p = 0.004$) and did not differ as a function of injury or change differentially across time ($\beta = -0.03$, $t = 0.15$, $p = 0.884$; $\beta = -0.15$, $t = 1.57$, $p = 0.119$).

3.1.4 Fluoxetine Challenge.—Breakpoint on the PR (Figure 3A) and percent immobile time on the FST (Figure 3B) were analyzed by LMER (Injury [Sham, TBI] x Drug [Fluoxetine, Vehicle]) to evaluate the effects of fluoxetine. Fluoxetine increased overall breakpoints ($\beta = 0.52$, $t = 3.02$, $p = 0.003$), but not differentially across the groups ($\beta = -0.01$, $t = 0.05$, $p = 0.961$). However, it had no effect on time spent immobile ($\beta = -0.46$, $t = 1.07$, $p = 0.295$) nor interaction with Group ($\beta = 0.54$, $t = 0.90$, $p = 0.374$).

3.2 Experiment 2

3.2.1 Effort Discounting Task.—HR lever choice (Figure 4) was analyzed using LMER (Injury [Sham, TBI] x Time [Week: 2–11] x Effort [FR: 2–20]). Choice of the HR lever significantly decreased after TBI at each effort requirement but slowly recovered over time ($\beta = 0.20$, $t = 11.29$, $p < 0.001$). TBI rats also did not show as much sensitivity to increasing effort requirements relative to shams ($\beta = 0.06$, $t = 3.89$, $p < 0.001$), presumably due generally-suppressed HR choice. Recovery over time from TBI on choice of the HR lever did not vary by effort requirement ($\beta = 0.00$, $t = 0.13$, $p = 0.895$).

Omissions were analyzed using LMER (Injury [Sham, TBI] x Time [Week: 2–11] x Effort [FR 2–20]). TBI rats had a slight increase in omissions (peaking at ~5% of trials omitted; data not shown) at higher effort requirements and quickly recovered to sham levels ($\beta = -0.10$, $t = 3.44$, $p = 0.001$). Further, there was no main effect of injury ($\beta = 0.08$, $t = 0.58$, p

= 0.568), indicating that the effects of TBI on lever choice were not due to changes in omissions.

3.2.2 Other Variables.—For a number of psychomotor variables (Figure 5), TBI rats experienced transient deficits during the initial one-three weeks of testing, which rapidly recovered to sham levels. These variables were analyzed using LMER (Injury [Sham, TBI] x Time [Week: 2–11] x Effort [FR 2–20]) and included trial initiation latency ($\beta = -0.08$, $t = 5.70$, $p < 0.001$; Figure 5A), choice latency ($\beta = -0.08$, $t = 5.48$, $p < 0.001$; Figure 5B), lever hold duration ($\beta = -0.09$, $t = 6.52$, $p < 0.001$; Figure 5C). These deficits were quickly recovered and consistent with expected transient post-injury psychomotor effects. The only variable which revealed extended deficits was the reinforcer collection latency, where TBI rats showed initial deficits which recovered over time ($\beta = -0.66$, $t = 8.14$, $p < 0.001$; Figure 5D), but did not reach sham levels.

3.2.3 Bupropion Challenge.—The effects of bupropion on choice (Figure 6A–D) were analyzed using LMER (Injury [Sham, TBI] x Bupropion Dose [0, 10, 20, 40 mg/kg] x Effort [FR 2–20]). There was an overall Injury by Dose interaction, $F(3, 323.19) = 5.08$, $p = 0.002$. Bupropion did not significantly reduce choice for the high-reward lever for Sham rats at any dose, although it approached significance at the highest one (10 mg/kg: $\beta = 0.15$, $t = 1.35$, $p = 0.177$; 20 mg/kg: $\beta = 0.14$, $t = 1.24$, $p = 0.214$; 40 mg/kg: $\beta = -0.21$, $t = 1.95$, $p = 0.052$). TBI rats showed a similar response, but had significantly reduced high-reward choice at the 40 mg/kg dose (10 mg/kg: $\beta = 0.10$, $t = 0.93$, $p = 0.355$; 20 mg/kg: $\beta = -0.11$, $t = 1.02$, $p = 0.308$; 40 mg/kg: $\beta = -0.75$, $t = 7.10$, $p < 0.001$). There was a significant effort by dose interaction ($F(3, 321.76) = 3.61$, $p = 0.013$); such that the highest dose decreased choice as effort requirement increased (10 mg/kg: $\beta = 0.06$, $t = 0.71$, $p = 0.478$; 20 mg/kg: $\beta = -0.05$, $t = 0.62$, $p = 0.535$; 40 mg/kg: $\beta = -0.31$, $t = 3.98$, $p < 0.001$).

The effects of bupropion on omissions (Figure 6E–H) were analyzed using LMER (Injury [Sham, TBI] x Bupropion [0, 10, 20, 40 mg/kg] x Effort [FR 2–20]). There was an overall Injury x Dose interaction ($F(3, 342) = 3.44$, $p = 0.017$). For the Sham rats, no dose affected omissions (10 mg/kg: $\beta = -0.32$, $t = 1.87$, $p = 0.061$; 20 mg/kg: $\beta = -0.31$, $t = 1.84$, $p = 0.066$; 40 mg/kg: $\beta = 0.08$, $t = 0.48$, $p = 0.629$). For the TBI rats, the highest dose increased omissions (10 mg/kg: $\beta = -0.03$, $t = 0.18$, $p = 0.861$; 20 mg/kg: $\beta = 0.22$, $t = 1.34$, $p = 0.181$; 40 mg/kg: $\beta = 0.79$, $t = 4.87$, $p < 0.001$).

3.3 Lesion Analysis (Experiment 1 & 2)

The effect of injury on brain volume (Figure 7) was assessed using an independent-sample t -test. In both Experiment 1 ($t(17.6) = 2.67$, $p = 0.016$) and Experiment 2 ($t(20.76) = 5.048$, $p < 0.001$) there were a significant difference between Sham and TBI groups, such that TBI rats had a lower brain volume than sham rats.

4. Discussion

Preclinical measures that assess chronic TBI-induced depressive-like behavior are necessary to develop effective treatments for patients. In the current study, there were no extended deficits on the FST, SP, and PR tasks, suggesting a lack of chronic depressive symptoms.

Despite this, we saw long-term TBI-induced deficits using the EDT, albeit with some recovery over time. While some transient psychomotor deficits accompanied motivational deficits in the EDT, they recovered more quickly, and were not present in other behaviors (e.g., swimming), suggesting that subtle long-term behavioral deficits were mediated by more than motoric impairments. This recovery curve mapped onto prior data from our lab on other tasks [46]. Together, these experiments suggest that a depressive phenotype does exist and that choice-based assays may be more effective for characterizing chronic deficits. However, they also highlight the overall difficulty of measuring TBI-induced depressive-like behavior in preclinical models.

Traditionally, the FST and SP have been used as assays of depressive-like behavior for single time points. However, it has been suggested that the FST is more useful for screening antidepressant drugs, rather than modeling behavior phenotypes [22]. Further, acute measures may not be sensitive enough to detect chronic TBI-induced emotional impairment. For example, the FST was successfully used to characterize helplessness following a focal TBI in mice 21 days post-injury but was unable to detect differences based on the severity of injury [47]. Acute TBI-induced anhedonia has been observed using the SP test [27] but this effect has not been tested in the chronic post-injury period. The SP test may be particularly difficult to implement in chronic testing given that rats substantially increased preference across time in the current data regardless of injury (Figure 2). This is also not the first time that these assays have failed to detect depressive phenotypes after TBI [48]; more work may be necessary to modify these assessments for chronic use. Given that we found a ceiling effect for saccharin preference, reducing concentration may increase the sensitivity of this measure for chronic testing. Modified versions of the FST, such as increased water depth [41] may also be more effective in capturing subtle, depressive phenotypes.

Although we expected the PR task to be useful in detecting long-term impairments, we found acute, but not chronic deficits in motivation following TBI. Prior studies using PR in experimental TBI have had mixed results, but also did not involve as thorough of measurements as the current data. One assessed PR performance only once every five days, but observed deficits to six weeks post-injury, which were rescued by a therapeutic drug [36]. However, in a separate study, when PR was briefly probed at closer to 10 weeks post-injury there were no motivational deficits [37]. Our current data represent the most thorough assessment to date of PR performance in experimental TBI. The PR task is traditionally regarded as providing relatively stable measurements of motivation [49], which suggests that motivation is changing across the acute post-injury period. Indeed, this reflects similar findings from our laboratory in which task engagement increases (e.g., more trials, fewer omissions) across the first few post-injury weeks on reinforcement-driven tasks [38]. An alternative explanation is that the PR was relatively insensitive to more subtle deficits and that choice-based variants on the PR, such as the concurrent chow procedure may increase the sensitivity.

Although the first three behavioral assays used were unsuccessful in measuring long-term depressive-like behaviors after brain injury, we were able to capture subtle, chronic deficits using the EDT. This may be reflective of the choice-based nature of the task, particularly when compared to the PR task, which, while similar, does not have a decision-making

element. We have observed robust deficits after brain injury on other choice paradigms to measure behaviors such as risky decision-making [38]. Thus, future assays of depressive-like behavior after TBI may benefit from assessing choice between low and high effort options, using tasks such as the EDT or the concurrent chow variant of the PR task [33]. Although the SP assessment involved a choice component, it seemed to lack the sensitivity for chronic assessment. The EDT may have been more sensitive than the SP because the differences between each choice (i.e., the effort requirement) were incrementally increased within each session. However, the EDT findings are also difficult to interpret due to increases in HR preference during the late post-injury period. The relatively short duration of TBI effects on the EDT could either reflect limitations of the task in capturing deficits or actual recovery of deficits over time. Moreover, if initial minor motoric deficits did reduce high-effort choice, this would suggest that post-TBI depression would recover alongside those improvements, which does not reflect the clinical case and would be problematic for translating animal models to human patients. Notably, depressive symptoms did not persist as long as other TBI-induced cognitive deficits that our lab has observed [38]. Further, it is possible that choice of the HR lever was affected by changes in discrimination after injury rather than motivational processes given the impairments often seen in discriminating contingencies after TBI [50, 51].

Another major concern after TBI is disruption to the monoaminergic systems. In the current study, we did not see any differential effects of fluoxetine, a serotonergic agonist, on behavior. However, when the fluoxetine challenge was conducted, there were no behavioral differences between TBI and sham rats, which limits the potential to observe injury by drug interactions. Further, while fluoxetine slightly increased motivation (Figure 3), the effects of fluoxetine may have been confounded by the stress of frequent injections. However, the current study provided no indications of serotonergic disruption after brain injury. This suggests that patients with TBI-induced depression may respond the same way to serotonergic drugs such as fluoxetine compared to individuals with traditional depression, although our conclusions are limited because we were unable to generate the expected therapeutic effects of fluoxetine even in control rats.

Similarly, acute administration of bupropion, a dopaminergic agonist, did not improve behavioral performance. However, a ceiling effect may have obscured potential drug by injury interactions. Preference for the HR lever was already between 80–90% when the bupropion challenge was conducted, and there was little room for improvement. The highest dose may also have been too potent to see appropriate effects; however, it has been used successfully to increase motivation in previous studies [44] and had no detrimental effect on sham rats. Bupropion has improved behavioral performance on other effort-based tasks [52], but it should be noted that the literature on effort discounting specifically is sparse and mainly consists of low doses (5–15 mg/kg) to reverse the effects of dopamine antagonists [52, 53]. Perhaps most relevant in the current study is that bupropion selectively impaired motivation and increased omissions for TBI rats at the highest dose used (40 mg/kg), suggesting sensitization of the dopamine system after brain injury. Our use of acute pharmacological challenge coupled with unexpected findings limits the conclusions that can be made about the treatment potential of dopaminergic drugs. Further research is still needed to assess the efficacy of dopaminergic drugs for TBI-induced depression, particularly given

the mounting evidence to suggest that TBI-induced depression may be “treatment resistant” [7, 8, 54].

In conclusion, it remains difficult to measure subtle, depressive-like behavior after TBI. A number of barriers, including monoaminergic disruptions and deficits in discrimination amplify the difficulties that already exist in modeling depression in rodents. However, we achieved moderate success using a chronic, choice-based measure. When assessing a TBI population, choice-based methods may provide the sensitivity needed to capture symptoms. Additionally, further work needs to be done to parse the contributions of various neurotransmitter systems to depressive symptoms after TBI. In the current study, increasing serotonin and dopamine activity did not mitigate depressive-like behavior; in fact, dopamine agonism resulted in unexpected detrimental outcomes for TBI rats. Thus, the behavioral and pharmacological profile of preclinical TBI-induced depressive symptoms require further resolution. These unanswered questions may be contributing to difficulties in treatment at the clinical level. Although further research will help bridge this gap, preclinical measures are still limited in that they often only capture one behavioral symptom of a complex disorder. The heterogeneity of emotional impairment after TBI contributes to the complexity of treatment; ultimately, it is difficult to treat a clinical disorder when the underlying causes and symptomology of the disorder are not well characterized.

Acknowledgements

Special thanks to Cassandra Modrak, Kristen Pechacek, Lauren Giesler, Virginia Milleon, Anastasios Lake, Cory Whitley, Taylor Scott and Caitlyn Cabral for their help in behavioral testing. This work was funded by the National Institutes of Health (NIGMS P20-GM109098), Southern Regional Education Board, and West Virginia University.

References

- [1]. Perry DC, Sturm VE, Peterson MJ, Pieper CF, Bullock T, Boeve BF, Miller BL, Guskiewicz KM, Berger MS, Kramer JH, Welsh-Bohmer KA, Association of traumatic brain injury with subsequent neurological and psychiatric disease: a meta-analysis, *J Neurosurg* 124(2) (2016) 511–26. [PubMed: 26315003]
- [2]. Reeves RR, Panguluri RL, Neuropsychiatric complications of traumatic brain injury, *Journal of psychosocial nursing and mental health services* 49(3) (2011) 42–50.
- [3]. Fann JR, Katon WJ, Uomoto JM, Esselman PC, Psychiatric disorders and functional disability in outpatients with traumatic brain injuries, *Am J Psychiatry* 152(10) (1995) 1493–9. [PubMed: 7573589]
- [4]. Hibbard MR, Uysal S, Kepler K, Bogdany J, Silver J, Axis I psychopathology in individuals with traumatic brain injury, *J Head Trauma Rehabil* 13(4) (1998) 24–39. [PubMed: 9651237]
- [5]. Zgaljardic DJ, Seale GS, Schaefer LA, Temple RO, Foreman J, Elliott TR, Psychiatric Disease and Post-Acute Traumatic Brain Injury, *J Neurotrauma* 32(23) (2015) 1911–25. [PubMed: 25629222]
- [6]. Bombardier CH, Fann JR, Temkin NR, Esselman PC, Barber J, Dikmen SS, Rates of major depressive disorder and clinical outcomes following traumatic brain injury, *Jama* 303(19) (2010) 1938–45. [PubMed: 20483970]
- [7]. Fann JR, Hart T, Schomer KG, Treatment for depression after traumatic brain injury: a systematic review, *J Neurotrauma* 26(12) (2009) 2383–402. [PubMed: 19698070]
- [8]. Dinan TG, Mobayed M, Treatment resistance of depression after head injury: a preliminary study of amitriptyline response, *Acta Psychiatr Scand* 85(4) (1992) 292–4. [PubMed: 1595364]
- [9]. Brown JSL, Murphy C, Kelly J, Goldsmith K, How can we successfully recruit depressed people? Lessons learned in recruiting depressed participants to a multi-site trial of a brief depression intervention (the ‘CLASSIC’ trial), *Trials* 20(1) (2019) 131–143. [PubMed: 30760305]

- [10]. Mojtabai R, Olfson M, Sampson NA, Jin R, Druss B, Wang PS, Wells KB, Pincus HA, Kessler RC, Barriers to mental health treatment: results from the National Comorbidity Survey Replication, *Psychol Med* 41(8) (2011) 1751–61. [PubMed: 21134315]
- [11]. Willner P, Belzung C, Treatment-resistant depression: are animal models of depression fit for purpose?, *Psychopharmacology (Berl)* 232(19) (2015) 3473–95. [PubMed: 26289353]
- [12]. Willner P, Scheel-Kruger J, Belzung C, The neurobiology of depression and antidepressant action, *Neurosci Biobehav Rev* 37(10 Pt 1) (2013) 2331–71. [PubMed: 23261405]
- [13]. Matthews K, Christmas D, Swan J, Sorrell E, Animal models of depression: navigating through the clinical fog, *Neurosci Biobehav Rev* 29(4–5) (2005) 503–13. [PubMed: 15925695]
- [14]. Ozga JE, Povroznik JM, Engler-Chiurazzi EB, Vonder Haar C, Executive (dys)function after traumatic brain injury: special considerations for behavioral pharmacology, *Behav Pharmacol* 29(7) (2018) 617–637. [PubMed: 30215621]
- [15]. Jenkins PO, De Simoni S, Bourke NJ, Fleminger J, Scott G, Towey DJ, Svensson W, Khan S, Patel M, Greenwood R, Cole JH, Sharp DJ, Dopaminergic abnormalities following traumatic brain injury, *Brain* 141(3) (2018) 797–810. [PubMed: 29360949]
- [16]. Jolly AE, Raymont V, Cole JH, Whittington A, Scott G, De Simoni S, Searle G, Gunn RN, Sharp DJ, Dopamine D2/D3 receptor abnormalities after traumatic brain injury and their relationship to post-traumatic depression, *Neuroimage Clin* 24 (2019) 101950–101960. [PubMed: 31352218]
- [17]. Wilson MS, Hamm RJ, Effects of fluoxetine on the 5-HT1A receptor and recovery of cognitive function after traumatic brain injury in rats, *Am J Phys Med Rehabil* 81(5) (2002) 364–72. [PubMed: 11964577]
- [18]. Bivona U, Ciarli P, Barba C, Onder G, Azicnuda E, Silvestro D, Mangano R, Rigon J, Formisano R, Executive function and metacognitive self-awareness after severe traumatic brain injury, *J Int Neuropsychol Soc* 14(5) (2008) 862–8. [PubMed: 18764981]
- [19]. Rigon J, Burro R, Guariglia C, Maini M, Marin D, Ciarli P, Bivona U, Formisano R, Self-awareness rehabilitation after Traumatic Brain Injury: A pilot study to compare two group therapies, *Restor Neurol Neurosci* 35(1) (2017) 115–127. [PubMed: 28059799]
- [20]. Lam CS, McMahon BT, Priddy DA, Gehred-Schultz A, Deficit awareness and treatment performance among traumatic head injury adults, *Brain Inj* 2(3) (1988) 235–42. [PubMed: 3251475]
- [21]. Porsolt RD, Anton G, Blavet N, Jalfre M, Behavioural despair in rats: a new model sensitive to antidepressant treatments, *Eur J Pharmacol* 47(4) (1978) 379–91. [PubMed: 204499]
- [22]. Petit-Demouliere B, Chenu F, Bourin M, Forced swimming test in mice: a review of antidepressant activity, *Psychopharmacology (Berl)* 177(3) (2005) 245–55. [PubMed: 15609067]
- [23]. Willner P, Towell A, Sampson D, Sophokleous S, Muscat R, Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant, *Psychopharmacology (Berl)* 93(3) (1987) 358–64. [PubMed: 3124165]
- [24]. Willner P, Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation, *Psychopharmacology (Berl)* 134(4) (1997) 319–29.
- [25]. Collins-Praino LE, Arulsamy A, Katharesan V, Corrigan F, The effect of an acute systemic inflammatory insult on the chronic effects of a single mild traumatic brain injury, *Behav Brain Res* 336 (2018) 22–31. [PubMed: 28855139]
- [26]. Cope EC, Morris DR, Scrimgeour AG, VanLandingham JW, Levenson CW, Zinc supplementation provides behavioral resiliency in a rat model of traumatic brain injury, *Physiol Behav* 104(5) (2011) 942–7. [PubMed: 21699908]
- [27]. Cope EC, Morris DR, Scrimgeour AG, Levenson CW, Use of zinc as a treatment for traumatic brain injury in the rat: effects on cognitive and behavioral outcomes, *Neurorehabil Neural Repair* 26(7) (2012) 907–13. [PubMed: 22331212]
- [28]. Dal-Zotto S, Marti O, Armario A, Influence of single or repeated experience of rats with forced swimming on behavioural and physiological responses to the stressor, *Behav Brain Res* 114(1–2) (2000) 175–81. [PubMed: 10996058]
- [29]. Mezadri TJ, Batista GM, Portes AC, Marino-Neto J, Lino-de-Oliveira C, Repeated rat-forced swim test: reducing the number of animals to evaluate gradual effects of antidepressants, *J Neurosci Methods* 195(2) (2011) 200–5. [PubMed: 21167866]

- [30]. Hodos W, Progressive ratio as a measure of reward strength, *Science* 134(3483) (1961) 943–4. [PubMed: 13714876]
- [31]. Randall PA, Pardo M, Nunes EJ, Lopez Cruz L, Vemuri VK, Makriyannis A, Baqi Y, Muller CE, Correa M, Salamone JD, Dopaminergic modulation of effort-related choice behavior as assessed by a progressive ratio chow feeding choice task: pharmacological studies and the role of individual differences, *PLoS One* 7(10) (2012) e47934–e47944. [PubMed: 23110135]
- [32]. Salamone JD, Correa M, Motivational views of reinforcement: implications for understanding the behavioral functions of nucleus accumbens dopamine, *Behav Brain Res* 137(1–2) (2002) 3–25. [PubMed: 12445713]
- [33]. Salamone JD, Steinpreis RE, McCullough LD, Smith P, Grebel D, Mahan K, Haloperidol and nucleus accumbens dopamine depletion suppress lever pressing for food but increase free food consumption in a novel food choice procedure, *Psychopharmacology (Berl)* 104(4) (1991) 515–21. [PubMed: 1780422]
- [34]. Floresco SB, Tse MT, Ghods-Sharifi S, Dopaminergic and glutamatergic regulation of effort- and delay-based decision making, *Neuropsychopharmacology* 33(8) (2008) 1966–79. [PubMed: 17805307]
- [35]. Bryce CA, Floresco SB, Perturbations in Effort-Related Decision-Making Driven by Acute Stress and Corticotropin-Releasing Factor, *Neuropsychopharmacology* 41(8) (2016) 2147–59. [PubMed: 26830960]
- [36]. Vonder Haar C, Maass WR, Jacobs EA, Hoane MR, Deficits in discrimination after experimental frontal brain injury are mediated by motivation and can be improved by nicotinamide administration, *J Neurotrauma* 31(20) (2014) 1711–20. [PubMed: 24934504]
- [37]. Vonder Haar C, Winstanley CA, Minor Functional Deficits in Basic Response Patterns for Reinforcement after Frontal Traumatic Brain Injury in Rats, *J Neurotrauma* 33(20) (2016) 1892–1900. [PubMed: 26756392]
- [38]. Shaver TK, Ozga JE, Zhu B, Anderson KG, Martens KM, Vonder Haar C, Long-term deficits in risky decision-making after traumatic brain injury on a rat analog of the Iowa gambling task, *Brain Res* 1704 (2019) 103–113. [PubMed: 30296430]
- [39]. Brown PL, Jenkins HM, Auto-shaping of the pigeon's key-peck, *J Exp Anal Behav* 11(1) (1968) 1–8. [PubMed: 5636851]
- [40]. Mobini S, Chiang TJ, Ho MY, Bradshaw CM, Szabadi E, Comparison of the effects of clozapine, haloperidol, chlorpromazine and d-amphetamine on performance on a time-constrained progressive ratio schedule and on locomotor behaviour in the rat, *Psychopharmacology (Berl)* 152(1) (2000) 47–54. [PubMed: 11041315]
- [41]. Slaterry DA, Cryan JF, Using the rat forced swim test to assess antidepressant-like activity in rodents, *Nat Protoc* 7(6) (2012) 1009–14. [PubMed: 22555240]
- [42]. Vonder Haar C, Anderson GD, Hoane MR, Continuous nicotinamide administration improves behavioral recovery and reduces lesion size following bilateral frontal controlled cortical impact injury, *Behav Brain Res* 224(2) (2011) 311–7. [PubMed: 21704653]
- [43]. Cryan JF, Valentino RJ, Lucki I, Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test, *Neurosci Biobehav Rev* 29(4–5) (2005) 547–69. [PubMed: 15893822]
- [44]. Randall PA, Lee CA, Podurgiel SJ, Hart E, Yohn SE, Jones M, Rowland M, Lopez-Cruz L, Correa M, Salamone JD, Bupropion increases selection of high effort activity in rats tested on a progressive ratio/chow feeding choice procedure: implications for treatment of effort-related motivational symptoms, *Int J Neuropsychopharmacol* 18(2) (2014).
- [45]. Paxinos G, Watson C, *The Rat Brain in Stereotaxic Coordinates*, Seventh ed., Elsevier Inc 1982.
- [46]. Vonder Haar C, Lam FC, Adams WK, Riparip LK, Kaur S, Muthukrishna M, Rosi S, Winstanley CA, Frontal Traumatic Brain Injury in Rats Causes Long-Lasting Impairments in Impulse Control That Are Differentially Sensitive to Pharmacotherapeutics and Associated with Chronic Neuroinflammation, *ACS Chem Neurosci* 7(11) (2016) 1531–1542. [PubMed: 27525447]
- [47]. Washington PM, Forcelli PA, Wilkins T, Zapple DN, Parsadanian M, Burns MP, 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) (2012) 2283–96. [PubMed: 22642287]
- [48]. Tucker LB, Burke JF, Fu AH, McCabe JT, Neuropsychiatric Symptom Modeling in Male and Female C57BL/6J Mice after Experimental Traumatic Brain Injury, *J Neurotrauma* 34(4) (2017) 890–905. [PubMed: 27149139]
- [49]. Stafford D, Branch MN, Effects of step size and break-point criterion on progressive-ratio performance, *J Exp Anal Behav* 70(2) (1998) 123–38. [PubMed: 9768504]
- [50]. Bauman RA, Widholm JJ, Petras JM, McBride K, Long JB, Secondary hypoxemia exacerbates the reduction of visual discrimination accuracy and neuronal cell density in the dorsal lateral geniculate nucleus resulting from fluid percussion injury, *J Neurotrauma* 17(8) (2000) 679–93. [PubMed: 10972244]
- [51]. Martens KM, Vonder Haar C, Hutsell BA, Hoane MR, A discrimination task used as a novel method of testing decision-making behavior following traumatic brain injury, *J Neurotrauma* 29(15) (2012) 2505–12. [PubMed: 22924664]
- [52]. Yohn SE, Thompson C, Randall PA, Lee CA, Muller CE, Baqi Y, Correa M, Salamone JD, The VMAT-2 inhibitor tetrabenazine alters effort-related decision making as measured by the T-maze barrier choice task: reversal with the adenosine A2A antagonist MSX-3 and the catecholamine uptake blocker bupropion, *Psychopharmacology (Berl)* 232(7) (2015) 1313–23. [PubMed: 25323625]
- [53]. Nunes EJ, Randall PA, Hart EE, Freeland C, Yohn SE, Baqi Y, Muller CE, Lopez-Cruz L, Correa M, Salamone JD, Effort-related motivational effects of the VMAT-2 inhibitor tetrabenazine: implications for animal models of the motivational symptoms of depression, *J Neurosci* 33(49) (2013) 19120–30. [PubMed: 24305809]
- [54]. Fann JR, Bombardier CH, Temkin N, Esselman P, Warms C, Barber J, Dikmen S, Sertraline for Major Depression During the Year Following Traumatic Brain Injury: A Randomized Controlled Trial, *J Head Trauma Rehabil* 32(5) (2017) 332–342. [PubMed: 28520672]

A. Experiment 1: Forced Swim Test (FST), Saccharin Preference (SP), and Progressive Ratio (PR)

B. Experiment 2: Effort Discounting Task (EDT)

Figure 1.

Experimental timeline for Experiment 1 (A) and Experiment 2 (B). In Experiment 1, PR testing was conducted 4 days per week (Tue-Fri) for 8 weeks post-injury. SP testing was conducted on the weekend (Fri-Mon) and FST (Mon) each week. The FST PT (pre-test; PT) was the first test conducted after injury and occurred one day prior to post-injury Week 2. In Experiment 2, training/testing on the EDT occurred 5 days per week (Mon-Fri).

Figure 2.

Sham/TBI results for (A) percent immobile on FST, (B) percent preference on SP, and (C) percent-baseline breakpoint on PR. There were no differences between TBI and Sham rats on the FST or SP (p 's > 0.740). On the PR, TBI rats were briefly impaired and quickly recovered to Sham levels (p < 0.001). Data are mean + SEM.

Figure 3.

Effects of fluoxetine/vehicle in Sham/TBI rats for (A) breakpoint on PR, and (B) percent immobile on FST. Fluoxetine increased break points for both groups ($p = 0.003$), but had no effect on time spent immobile ($p = 0.295$). Data are mean + SEM.

Figure 4. Percent choice of the high-reward (HR) lever in Sham/TBI rats for A) FR-2, B) FR-5, C) FR-10, and D) FR-20 schedules of reinforcement over time since injury. TBI rats had reduced preference for the HR lever compared to Shams but improved over time on all schedules ($p < 0.001$). Data are mean + SEM.

Figure 5.

TBI rats showed low-magnitude transient deficits but quickly recovered to sham levels. across the four effort requirements for (A) initiation latency ($p < 0.001$), (B) choice latency ($p < 0.001$), (C) lever hold duration ($p < 0.001$), and (D) reinforcer collection latency ($p < 0.001$), when compared to Sham rats. Data are mean + SEM.

Figure 6.

Change from baseline percent choice of the high-reward (HR) lever for (A) vehicle (0 mg/kg), (B) 10 mg/kg, (C) 20 mg/kg, and (D) 40 mg/kg bupropion, and change from baseline percent omissions at (E) vehicle (0 mg/kg), (F) 10 mg/kg, (G) 20 mg/kg, and (H) 40 mg/kg bupropion across effort requirements. Choice of the HR lever for the TBI group decreased ($p < 0.001$) and omissions increased ($p < 0.001$) following the 40 mg/kg dose only. Data are mean + SEM.

Figure 7.

Brain volume (A) and representative images (B) for Sham and TBI rats in Experiment 1 and Experiment 2. Average brain volume was significantly smaller for TBI rats relative to Shams in Experiment 1 ($p = 0.016$) and Experiment 2 ($p < 0.001$).