JOURNAL OF NEUROTRAUMA 33:1–9 (XXXX 00, 2016)

DOI: 10.1089/neu.2015.4276

Minor Functional Deficits in Basic Response Patterns for Reinforcement after Frontal Traumatic Brain Injury in Rats

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Abstract

Traumatic brain injury (TBI) is a major contributor to numerous psychiatric conditions and chronic behavioral dysfunction. Recent studies in experimental brain injury have begun to adopt operant methodologies to assess these deficits, all of which rely on the process of reinforcement. No studies have directly examined how reinforced behaviors are affected by TBI, however. The current study assessed performance under the four most common schedules of reinforcement (fixed ratio, variable ratio, fixed interval, variable interval) and one higher order schedule assessing motivation (progressive ratio) after bilateral, pre-frontal controlled cortical impact injury. TBI-induced differences on the basic schedules were minor, with the exception of the variable ratio, where increased efficacy (more reinforcers, higher response rates, lower interresponse times) at higher requirements was observed as a result of brain injury. Performance on the progressive ratio schedule showed some gross differences between the groups, in that sham rats became more efficient under this schedule while injured rats perseverated in lever pressing. Further, injured rats were specifically impaired at lower response requirements on the progressive ratio. Taken together, these findings indicate that simple reinforced behaviors are mostly unaffected after TBI, except in the case of variable ratio schedules, but the altered performance on the higher-order progressive ratio schedule suggests changes involving motivation or potentially perseveration. These findings validate operant measures of more complex behaviors for brain injury, all of which rely on reinforcement and can be taken into consideration when adapting and developing novel functional assessments.

Key words: behavior; cognition; controlled cortical impact; operant; schedules of reinforcement

Introduction

It is estimated that 1–2% of persons in the United States live with a chronic disability after sustaining a traumatic brain injury (TBI),[1,2] and an increasing number of studies are linking TBI to numerous psychiatric disorders and symptoms including depression, anxiety, poor impulse control, and addiction.[3,4] Despite this problem, there is a large gap in the experimental literature with regard to chronic behavioral disturbances in animals after TBI. Part of this may be explained by the heavy reliance on sensorimotor measures of functional outcome and the focus on treatments that act in the immediate post-injury phase.

Studies examining chronic outcomes have found relatively few enduring cognitive deficits. A study evaluating impairments up to 1 year post-injury found enduring deficits in Morris water maze (MWM) performance using lateral fluid percussion injury[5]; however, a similar study, using controlled cortical impact (CCI) observed only transient MWM deficits over the course of the year.[6] With sufficient training, brain-injured rats learn to perform the MWM at a high level and typically maintain this ability throughout continued testing.

Many studies have noted that MWM deficits resolve by 1–2 months post-injury across multiple models,[7–11] but some researchers have managed to show more extensive deficits by adjusting the testing schedule such that rats do not become overtrained.[12,13] Findings from these and many other studies using the MWM as the primary cognitive outcome measure highlight some of the inherent weaknesses in using this task for assessing long-term cognitive deficits.

Many researchers have begun to explore alternative behavioral assessments for experimental TBI to increase sensitivity to milder deficits and the subtler chronic impairments. Some researchers have begun using the sucrose preference task as a measure of anhedonia.[14,15] In this task, rats or mice in their home cage are offered an option between normal water and a water-sucrose solution. Researchers have found that frontal TBI reduces preference for the sweetened solution; however, it is unclear whether this depressive-like behavior is indicative of chronic dysfunction.

Recently, Bondi and colleagues[16] demonstrated the efficacy of using the attentional set-shifting test to detect sensitive deficits in the ability to attend to novel stimulus dimensions. In this task, rats dig in various media containing different olfactory cues to receive a

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cereal piece and must discriminate the correct scent or media. This test may have strong efficacy in assessing chronic outcomes if deficits persist through repeated testing, although this has yet to be tested in models of brain injury.

There is also an increased interest in the use of operant chambers to characterize cognitive deficits related to TBI. Operant chambers have been used in the fields of experimental analysis of behavior, behavioral pharmacology, and behavioral neuroscience to evaluate cognitive function for many years. Initially, very few experimental TBI studies used this methodology,[9,17,18] but recent studies have used them to evaluate a host of behaviors, including discrimination, inhibition, perseveration and conditioned fear.[19–24]

The common element among these assessments is the use of reinforcement to maintain responding on the behavioral task. Each assessment uses something that is typically rewarding (e.g. cereal, sucrose pellet, sucrose-water) to reinforce the action (e.g., correct discrimination, correct response, water bottle licking). The process of reinforcement was extensively studied and characterized during the early work of Ferster and Skinner,[25] and four basic schedules of reinforcement were identified: fixed ratio (FR), variable ratio (VR), fixed interval (FI), and variable interval (VI).

Given appropriate control over the experimental conditions, each of the four basic schedules produces specific patterns of behavior, which can then be studied under a multitude of conditions to determine how behavior is altered as a function of changes in reinforcement or physiological manipulations. These basic behaviors have been used recently in the field of experimental stroke, and deficits were highlighted under high response requirement FR schedules, modified VI schedules, and in switching between two FR schedules.[26–28]

Although previous work has identified deficits in motivation after frontal injury,[14,15,20] however, there have been no studies to date directly examining how TBI affects responding under different reinforcement schedules in animal models. It is therefore possible that fundamental alterations in the relationship between reinforcers and behavior may confound interpretation of TBI-induced deficits described in the above studies, all of which relied on the process of reinforcement.

To address this issue directly, we therefore examined whether a severe bilateral frontal CCI would alter responding under the four basic schedules of reinforcement. In addition, we examined performance under the progressive ratio (PR) schedule, a measure of motivation[29] that has previously been shown to be altered by TBI.[20] We hypothesized that a severe brain injury would cause considerable disruption in reinforcement processes, possibly shedding light on the varied deficits observed in reinforcement-based tasks that are more commonly being applied in the experimental TBI field.

Methods

Animals

Subjects were a mixture of Sprague-Dawley ($n = 8$) and Long-Evans ($n = 12$) rats, 3 months of age at the time of surgery, divided equally across surgical conditions. Rats were food restricted to 85% free-feeding weight (14–20 g maintenance chow daily); water was available *ad libitum*. Rats were housed singly in standard cages on a (12:12) reverse light cycle, with a plastic hut and shredded paper towel available as enrichment. Housing and testing were performed in accordance with the Canadian Council on Animal Care and all procedures were approved by the UBC Animal Care Committee.

Surgery

Rats were randomly assigned to TBI ($n = 12$) or sham ($n = 8$) groups. TBI rats were given a severe bilateral frontal CCI as described previously.[20,21,30] In brief, rats were anesthetized and placed in a stereotaxic frame. Buprenorphine (0.01 mg/kg, subcutaneously [SC]), lactated Ringer solution (8 mL, SC), and bupivacaine (0.1 mL of 0.5% solution, SC at incision site) were administered. Under aseptic conditions, a midline incision was made in the scalp and the fascia retracted. A 6.0 mm diameter circular craniotomy was performed centered at AP +3.0, ML 0.0 mm from bregma.

A TBI was then induced using an electromagnetic CCI device (Leica Biosystems, Buffalo Grove, IL) with a circular flat-faced, 5-mm diameter tip, at a rate of 3 m/sec to a depth of −2.5 mm for 0.5 sec. After injury, bleeding was stopped with sterile gauze and the incision sutured. Sham procedures included everything above with the exception of craniotomy and impact. Buprenorphine (0.01 mg/kg) was administered for pain management 10 and 24 h post-surgery.

Apparatus

Behavior was conducted in a bank of 12 standard operant chambers (Med Associates, St. Albans, VT) equipped on one side with a five-hole array and on the other with a tone generator, two retractable levers, two lights above the levers, a sucrose pellet dispenser (45 mg pellets, Bio-Serv, Flemington, NJ), and a houselight. Only the left lever, the pellet dispenser, and the houselight were used in this experiment.

Behavior assessment

Seven days after surgery, behavioral testing began. Each session was conducted with the houselight on and the left lever extended for the duration of the test session. Testing continued until approximately 3 months post-surgery. Rats' responses were assessed under five schedules of reinforcement: FR, VR, FI, VI, and PR.[25,29] The four basic schedules were assessed for 13–15 sessions each, with incrementing response requirements every 2–3 sessions, allowing for a rapid assessment of multiple response requirements within each schedule type. The PR schedule was assessed over five sessions. Successful completion of any schedule requirement resulted in delivery of a single sugar pellet.

Basic schedules

Fixed ratio schedule. Reinforcement under FR schedules engenders moderate rates of responding, with pauses between bouts of responses at higher schedule values. Rats were assessed at five incrementing fixed ratio levels for two-three sessions each (1, 3, 5, 10, or 20 responses required for reinforcement). A session ended when a rat reached a maximum of 50 reinforcers or 30 min had elapsed.

Variable ratio schedule. Reinforcement under VR schedules causes a high, constant rate of responding relative to the other basic schedules. Rats were assessed at five incrementing variable ratio levels for two-three sessions each (on average, 1, 3, 5, 10, or 20 responses required for reinforcement). A session ended when a rat reached a maximum of 50 reinforcers or 30 min had elapsed.

Fixed interval schedule. Reinforcement under FI schedules produces an accelerating rate of responding as time approaches the FI value. Rats were assessed at five incrementing interval levels for two-three sessions each (one response required after 5, 15, 30, 60, or 120 sec). A session ended when a rat reached a maximum of 50 reinforcers or 30 min had elapsed.

Variable interval schedule. Reinforcement under VI schedules generates a moderate, constant rate of responding relative to the other basic schedules. Rats were assessed at five incrementing variable interval levels for two-three sessions each (one response required after, on average, 5, 15, 30, 60, or 120 sec). A session ended when a rat reached a maximum of 50 reinforcers or 30 min had elapsed.

Progressive ratio schedule

Reinforcement under PR schedules is used to measure the motivation of the animal and primarily determines the point at which the number of responses is too costly for delivery of a single reinforcing event (referred to as the break point). Animals typically display initial high rates of responding. As the response requirement increases, responding decreases and is marked by more frequent and longer interresponse pauses until the rat stops responding completely. The break point is typically the primary measure of interest for motivation, but examination of response rates and interresponse times (IRTs) can yield additional information on how animals are motivated within a given component of the schedule.

Rats were assessed on a progressive ratio schedule for 5 days. The number of responses required increased every time a reinforcer was delivered by a given amount (sequence = FR2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 175, 200 … n). A session ended when the rat made no response for 5 min or 120 min had elapsed.

Outcome measures

Several levels of analysis are possible with the data collected in this experiment. For the basic schedules, we chose three levels of analysis from the coarse to the fine: reinforcers obtained (percent of maximum), press rate, and the IRT. For the progressive ratio, we analyzed the break point (maximum FR value obtained before giving up), the session length, and the efficiency at which rats reached the break point (Break/Time). In addition, we analyzed the response rates and IRTs across the different response requirements of the PR schedule.

Histology and lesion analysis

Rats were euthanized with a mixture of carbon dioxide and oxygen. Brains were then removed and post-fixed in a 3.7% formalin solution. After approximately 1 week, brains were placed in a 30% sucrose solution. After 3 days in sucrose, brains were sliced at –20° on a cryostat at 50 μm and mounted to gelatin-subbed slides. Slides were then stained with cresyl-violet and imaged on a Zeiss microscope. Six brain sections, evenly spaced through the injury location, were measured using ImageJ software (National Institutes of Health, Bethesda MD), and brain volumes were estimated using the Cavalieri method.

Data analysis

Statistical tests were conducted using R statistical software (http://www.r-project.org/). Transformations were applied to the data as appropriate to normalize distributions. A log transformation was used for data bound on the lower spectrum (Press Rate, IRT), and the arcsine-square root transformation was used for the percentage variable (Percent Maximum Reinforcers). Repeated measures data were analyzed using linear mixed effects regression in the lme4 library; individual baseline was used as the random effect. The p values were estimated using the lmerTest library. Lesion volume was compared in a one-way analysis of variance (ANOVA). A p value of less than 0.05 was considered significant. All reported R^2 statistics are adjusted for the number of predictors.

Results

Basic schedules

Strain effects. A linear mixed effects regression was performed to determine whether there were any strain differences in the number of reinforcers obtained (Reinforcers ~ Strain × Schedule). An ANOVA on the model showed no main effects or interactions of Strain ($F(1, 17.22) = 1.27$, $p = 0.276$; $F(3, 912.01) = 1.77$, $p = 0.151$, respectively; $R^2 = 0.39$). A regression examining the press rates (Press Rate ~ Strain × Schedule) showed no main effect of Strain ($F(1, 17.04) = 0.10$, $p = 0.753$) but did reveal a Strain × Schedule interaction ($F(3, 911.90) = 8.71$, $p < 0.001$; $R^2 = 0.65$). When the individual schedule was examined, however, there were no specific strain differences on the FR, VR, FI, or VI schedules ($\beta = 0.10$, $p = 0.718$; $\beta = 0.14$ $p = 0.619$; $\beta = -0.07$ $p = 0.078$; $\beta = -0.51$, $p = 0.811$, respectively).

A regression examining IRTs (IRT ~ Strain × Schedule), showed a similar Strain × Schedule interaction effect ($F(3, 898.8) = 16.72$, $p < 0.001$; $R^2 = 0.64$). When the individual schedule was examined, there were no specific strain differences on the FR, VR, or FI schedules ($\beta = -0.15$, $p = 0.567$; $\beta = 0.10$, $p = 0.698$; $\beta = 0.22$, $p = 0.400$, respectively); however, the Sprague-Dawley rats showed increased IRTs on the VI schedule ($\beta = 0.81$, $p = 0.004$, Fig. 1).

Fixed ratio. A linear mixed effects regression examining the effects of injury on the number of reinforcers obtained at different FR response requirements was performed (Reinforcers ~ Injury × Response Requirement). There was no main effect or interaction of Injury, ($\beta = -0.03$, $p = 0.856$; $\beta = 0.00$, $p = 0.943$, respectively; $R^2 = 0.52$), although the number of reinforcers obtained decreased as the response requirement increased ($\beta = -0.06$, $p < 0.001$, Fig. 2).

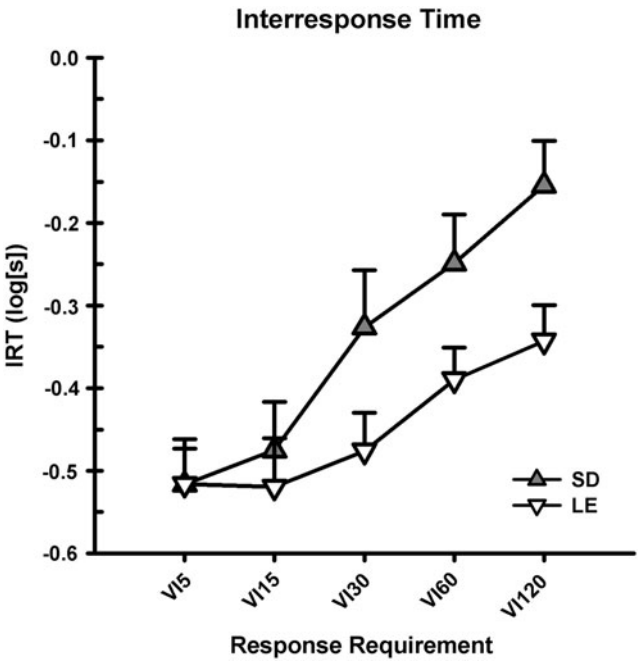

FIG. 1. Average interresponse times (IRTs) across variable interval schedule requirements. Sprague-Dawley (SD) rats had significantly increased IRTs as the interval requirement was increased compared with Long-Evans (LE) ($p = 0.004$). Data shown are mean ± standard error of the mean.

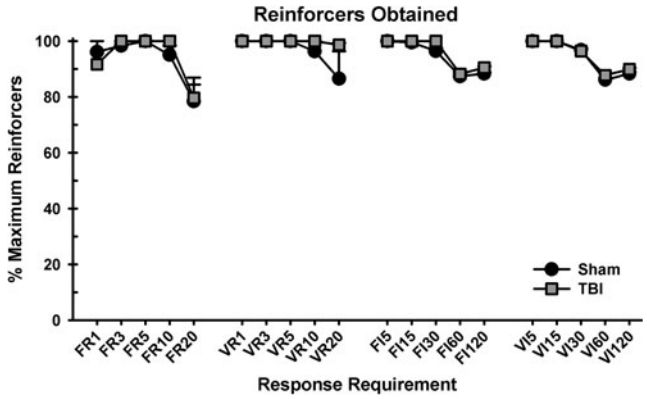

FIG. 2. Reinforcers obtained across various schedule requirements. Sham and TBI rats performed similarly across all schedules (p's > 0.463), except for the variable ratio (VR). On the VR schedule, sham rats obtained significantly less reinforcers at higher response requirements ($p = 0.001$). Data shown are mean ± standard error of the mean. FR, fixed ratio; FI, fixed interval; VI, variable interval.

A regression was also performed to examine effects of injury on the rate of pressing at different response requirements (Press Rate ~ Injury × Response Requirement). There was no main effect or interaction of Injury ($\beta = -0.22$, $p = 0.239$; $\beta = 0.01$, $p = 0.428$, respectively; $R^2 = 0.67$), although the press rate increased as the response requirement increased ($\beta = 0.06$, $p < 0.001$, Fig. 3). Another regression examined effects of injury on the average IRT at different response requirements (IRT ~ Injury × Response Requirement). The TBI group had significantly increased IRTs ($\beta = 0.58$, $p = 0.004$; $R^2 = 0.59$), and there was a main effect of Response Requirement with IRTs decreasing as the response requirement increased ($\beta = -0.05$, $p < 0.001$), but no interaction ($\beta = -0.02$, $p = 0.178$, Fig. 4).

Variable ratio. A linear mixed effects regression was performed to examine effects of injury on the number of reinforcers obtained at different VR response requirements (Reinforcers ~ Injury × Response Requirement). There was no main effect of Injury ($\beta = -0.28$, $p = 0.305$; $R^2 = 0.67$), but there was a main effect of Response Requirement ($\beta = -0.07$, $p < 0.001$) and an interaction between Injury and Response Requirement ($\beta = 0.06$, $p = p = 0.001$) such that sham rats obtained less reinforcers at higher response requirements than injured rats (Fig. 2).

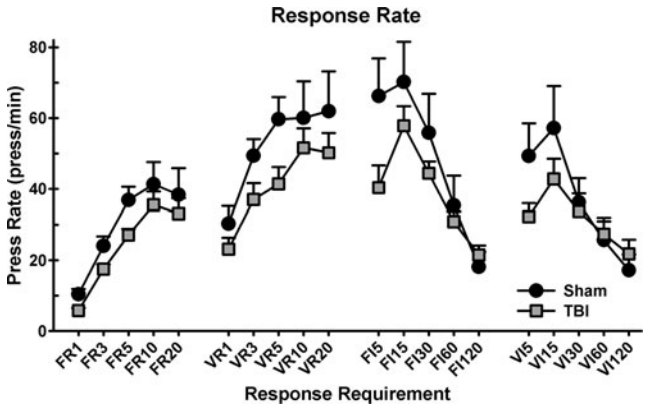

FIG. 3. Response rates across various schedule requirements. Sham and traumatic brain injury (TBI) rats were not significantly different on the fixed ratio (FR), fixed interval (FI), or variable interval (VI) schedules (p's > 0.239). On the variable ratio (VR) schedule, injured rats demonstrated an interaction such that they increased their rate of responding significantly more than sham rats as the response requirement increased ($p = 0.033$), despite having lower overall rates of responding. Data shown are mean ± standard error of the mean.

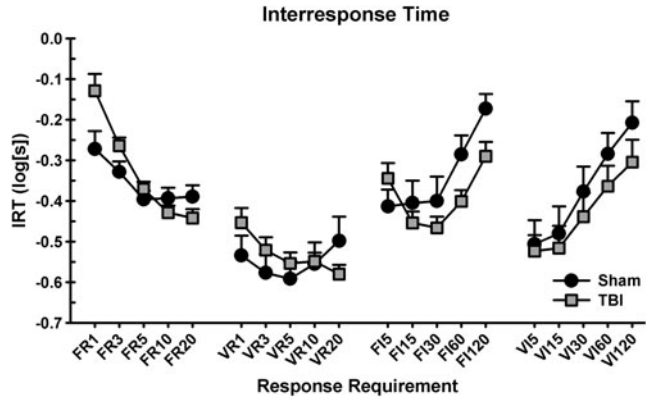

FIG. 4. Average interresponse times (IRTs) across various schedule requirements. Sham and traumatic brain injury (TBI) rats were not significantly different on the fixed interval (FI) or variable interval (VI) schedules (p's > 0.329). On the fixed ratio (FR) schedule, injured rats had significantly higher IRTs overall compared with sham rats ($p = 0.004$). On the variable ratio (VR) schedule, injured rats decreased their IRTs significantly more than sham rats as the response requirement increased ($p < 0.001$). Data shown are mean ± standard error of the mean.

A regression of the effects of injury on the rate of pressing at different response requirements (Press Rate ~ Injury × Response Requirement) revealed additional group differences. There was no main effect of Injury ($\beta = -0.21$, $p = 0.365$; $R^2 = 0.85$), but there was a main effect of Response Requirement ($\beta = 0.02$, $p = 0.016$) and an interaction between Injury and Response Requirement ($\beta = 0.03$, $p = 0.033$) such that TBI rats increased their response rates more than Sham rats across the response requirements (Fig. 3).

A regression was also performed to examine effects of injury on the average IRT at different response requirements (IRT ~ Injury × Response Requirement). There was a main effect of Injury ($\beta = 0.55$, $p = 0.044$; $R^2 = 0.74$), a main effect of Response Requirement ($\beta = 0.03$, $p = 0.007$), and an interaction between Injury and Response Requirement ($\beta = -0.06$, $p < 0.001$) such that the average IRT for TBI rats decreased more than sham rats as the response requirement was increased (Fig. 4).

Fixed interval. A linear mixed effects regression of the effects of injury on the number of reinforcers obtained at different FI response requirements (Reinforcers ~ Injury × Interval Requirement) showed no group differences or interaction ($\beta = 0.16$, $p = 0.463$; $\beta = 0.00$, $p = 0.833$, respectively; $R^2 = 0.76$), although the number of reinforcers obtained decreased as the interval increased ($\beta = -0.02$, $p < 0.001$, Fig. 2). A regression on the rate of pressing at different response requirements (Press Rate ~ Injury × Interval Requirement) showed a similar lack of Injury effects or interactions ($\beta = -0.03$, $p = 0.935$, $\beta = 0.00$; $p = 0.890$, respectively; $R^2 = 0.87$), although the press rate decreased as the interval increased ($\beta = -0.01$, $p < 0.001$, Fig. 3).

A regression was also performed to examine effects of injury on the average IRT at different response requirements (IRT ~ Injury × Interval Requirement). There was no main effect or interaction of

Injury ($\beta = 0.01$, $p = 0.976$, $\beta = 0.00$; $p = 0.329$, respectively; $R^2 = 0.82$), but again the average IRT increased as the interval was lengthened ($\beta = 0.01$, $p < 0.001$, Fig. 4).

Variable interval. A linear mixed effects regression was performed to examine effects of injury on the number of reinforcers obtained at different VI response requirements (Reinforcers ~ Injury × Interval Requirement). There was no main effect or interaction of Injury, ($\beta = -0.06$, $p = 0.790$; $\beta = 0.00$, $p = 0.809$, respectively; $R^2 = 0.67$), although the number of reinforcers obtained decreased as the interval increased ($\beta = -0.01$, $p < 0.001$, Fig. 2). A regression on the rate of pressing at different response requirements (Press Rate ~ Injury × Interval Requirement) revealed no main effect or interaction of Injury ($\beta = -0.11$, $p = 0.763$; $\beta = 0.00$, $p = 0.730$, respectively; $R^2 = 0.84$), yet the press rate decreased as the interval increased ($\beta = -0.01$, $p < 0.001$, Fig. 3).

A regression was also performed to examine effects of injury on the average IRT at different response requirements (IRT ~ Injury × Interval Requirement). There was no main effect or interaction of Injury ($\beta = -0.03$, $p = 0.932$; $\beta = 0.00$, $p = 0.767$, respectively; $R^2 = 0.85$), although again the average IRT increased as the interval was lengthened ($\beta = 0.01$, $p < 0.001$, Fig. 4).

Progressive ratio

Overall effects. A linear mixed effects regression was performed to determine whether there were any strain differences in the PR break point (Break Point ~ Strain × Session). There were no main effects or interactions of Strain ($\beta = -0.69$, $p = 0.089$; $\beta = 0.06$, $p = 0.392$, respectively; $R^2 = 0.88$). A regression was performed to assess the effects of injury on PR break point (Break Point ~ Injury × Session). There was no effect of Injury ($\beta = 0.19$, $p = 0.651$; $R^2 = 0.90$), but there was a significant effect of Session ($\beta = -0.29$, $p < 0.001$) and an Injury × Session interaction ($\beta = 0.18$, $p = 0.014$) such that sham rats decreased their break point more than injured rats across sessions (Fig. 5).

A regression on the latency to reach the break point (Time ~ Injury × Session) showed no effect of Injury ($\beta = 0.31$, $p = 0.303$; $R^2 = 0.83$), but there was a significant effect of Session ($\beta = -0.58$, $p < 0.001$) and an Injury × Session interaction ($\beta = 0.38$, $p < 0.001$) such that sham rats took less time than injured rats across sessions (Fig. 5). Another regression on the efficiency in reaching their break point (Time/Break Point) of rats on the PR schedule (Efficiency ~ Injury × Session) revealed no effect of Injury ($\beta = -0.18$, $p = 0.627$; $R^2 = 0.83$), but a significant effect of Session ($\beta = 0.39$, $p < 0.001$) and an Injury × Session interaction ($\beta = -0.25$, $p = 0.006$) such that sham rats reached their break point more efficiently across sessions (Fig. 5).

Within-session dynamics. A linear mixed effects regression was performed to examine how the press rates changed across increasing response requirements (Press Rate ~ Injury × Response Requirement). There was a significant effect of Injury ($\beta = -0.39$, $p < 0.001$; $R^2 = 0.52$) and Response Requirement ($\beta = -0.62$,

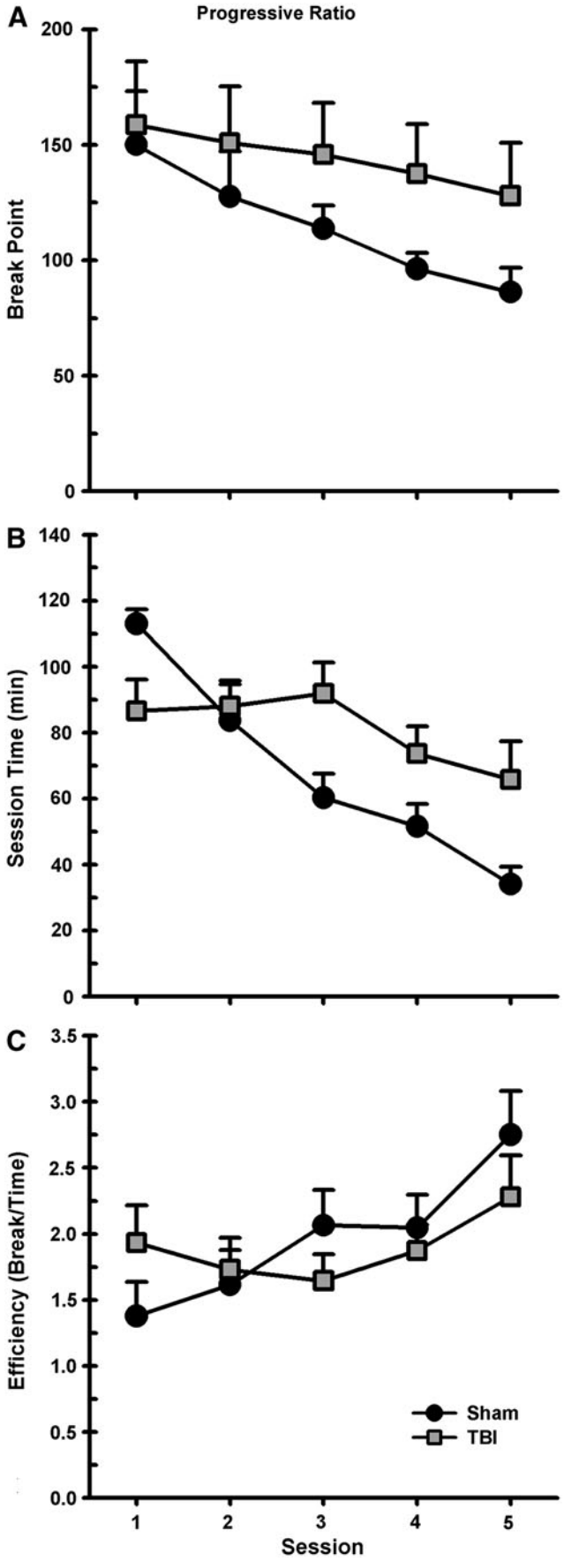

FIG. 5. Performance on the progressive ratio (PR) schedule across sessions. (**A**) Sham rats significantly decreased break points across sessions compared with injured rats ($p = 0.014$). (**B**) Sham rats significantly reduced their time spent on the PR across sessions compared with injured rats ($p < 0.001$). (**C**) Sham rats became significantly more efficient at reaching their break point across the sessions compared with injured rats ($p = 0.006$). Data shown are mean ± standard error of the mean. TBI, traumatic brain injury.

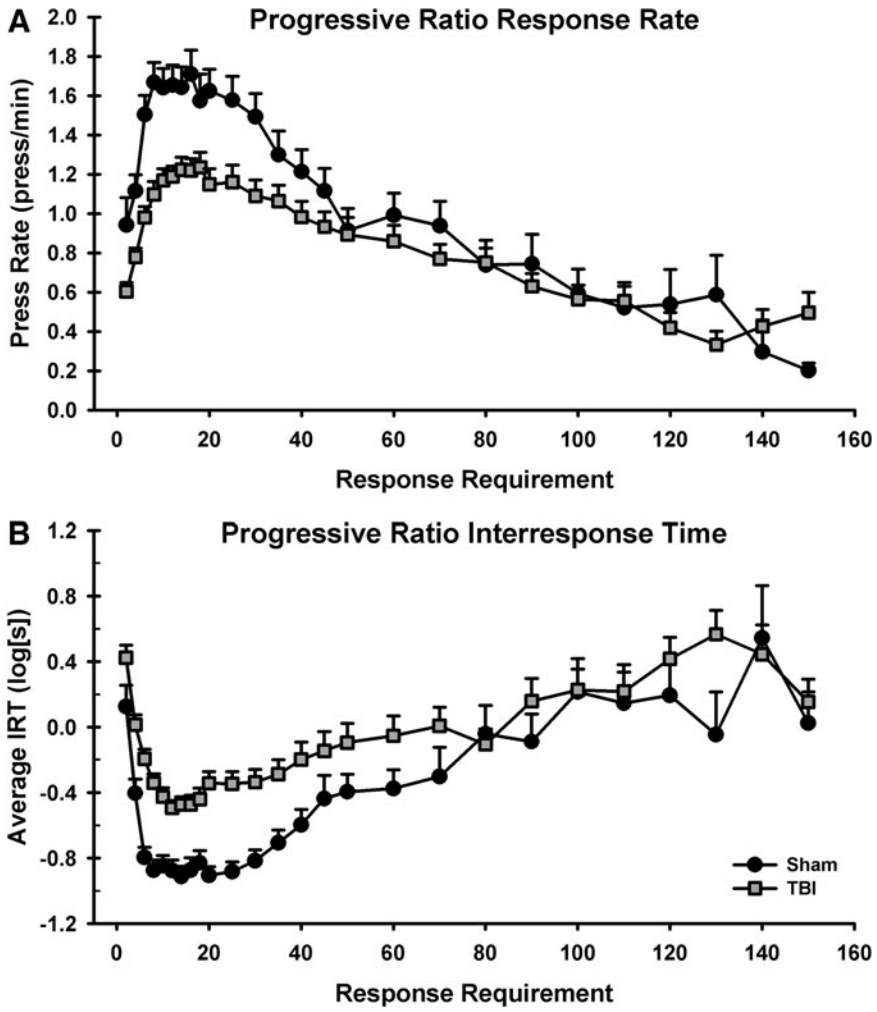

FIG. 6. Within-session dynamics on the progressive ratio (PR) schedule. (**A**) Injured rats had significantly lower press rates at lower response requirements compared with sham rats ($p<0.001$). (**B**) Injured rats had significantly higher interresponse times (IRTs) at lower response requirements compared with sham rats ($p=0.001$). Data shown are mean ± standard error of the mean. TBI, traumatic brain injury.

$p=0.006$) and an Injury × Response Requirement interaction ($\beta=0.15$, $p<0.001$) such that sham animals had a higher response rate on response requirements less than 50 (Fig. 6).

A regression was also performed to examine how the IRT distribution changed across increasing response requirements (IRT ~ Injury × Response Requirement). There was a significant effect of Injury ($\beta=0.18$, $p<0.001$; $R^2=0.44$) and Response Requirement ($\beta=0.62$, $p=0.001$) and an Injury × Response Requirement interaction ($\beta=-0.09$, $p=0.001$) such that sham animals had lower IRTs on response requirements less than 80 (Fig. 6).

Lesion analysis

Brain volumes were compared in a one-way ANOVA (Lesion Volume ~ Injury). The injured group had significantly reduced brain volumes compared with sham ($F(1,18)=71.58$, $p<0.001$) with mean brain loss (lesion size and ventricular enlargement) of 43.64 mm^3 ± 3.83 standard error of the mean (Fig. 7).

Discussion

This study marks an important step in the characterization of response patterns under a variety of schedules of reinforcement after experimental TBI. We examined responding at three levels, from coarse to fine: reinforcers obtained, response rate, and IRTs, across the various schedule requirements. Initially we hypothesized that there would be fundamental deficits in responding for reinforcement after a brain injury and that this could potentially explain multiple other impairments observed across a number of recent studies using reinforcement.[14–16,19–22]

Contrary to this hypothesis, however, there were minimal differences between injured and sham animals on all four of the basic schedules (FR, VR, FI, VI). On the FR schedule, the injured animals showed slight dysfunction in the form of higher IRTs despite similar response rates and nearly identical reinforcers obtained (Fig. 4). Given that this was the first schedule tested after injury, this may be indicative of minor transient motor deficits that subsequently resolved, or slower initial learning of the lever-pressing behavior.

The most interesting finding with regard to the basic schedules was the behavior on the VR schedule. A significant interaction on all three outcome variables demonstrated that the injured rats adapted to the changing schedule requirements in a considerably different fashion than sham rats (Fig. 2,3,4). Specifically, injured animals obtained more reinforcers than sham controls as the

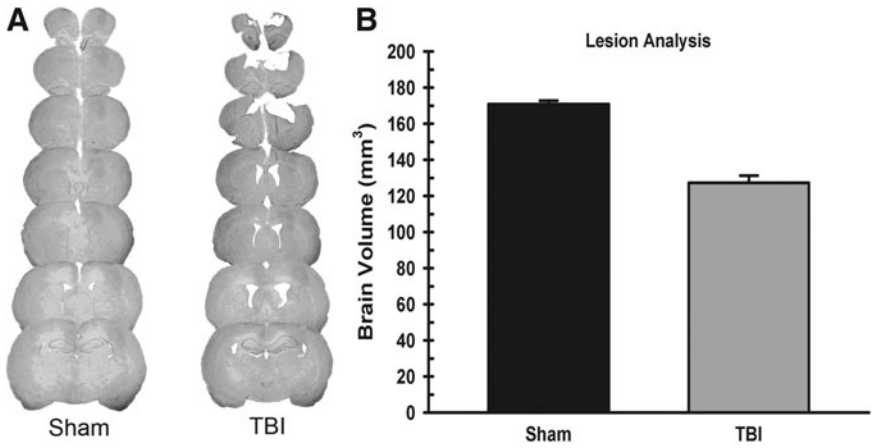

FIG. 7. Lesion quantification. (**A**) Histoplate demonstrating injury severity and location. Frontally injured rats had substantial cavitation, with lesions evident from the anterior of the brain to the striatum, as well as enlarged ventricles. (**B**) Injured rats had significantly reduced brain volumes relative to sham rats ($p < 0.001$). TBI, traumatic brain injury.

schedule requirement increased and failed to decrease their response rates as sham rats did while demonstrating a gradual decrease in IRTs across the response requirements.

These data are suggestive of a more constant rate of responding in the TBI rats despite a slightly lower overall response rate, with fewer long pauses. Because high-requirement VR schedules are the least predictable in how much effort is required for a single reinforcer (varying between 2–40 presses in the current study), it is possible that this may be reflective of perseverative behavior in the face of uncertain outcomes. In addition, this effect is particularly interesting because it is not observed on the other variable schedule, the VI schedule, which typically shows lower rates of responding and larger pauses between responses compared with the VR,[25] suggesting that a very high response rate is necessary to tease apart these differences.

Apart from the VR performance, the basic schedules showed primarily null effects. The PR schedule readily highlighted interesting group differences, however. First, the injured group did not show the same degree of decreasing break points across sessions compared with the sham group (Fig. 5). This finding stands in stark contrast to a previous study that used a nearly identical PR schedule[20] as well as other motivational studies after frontal TBI.[14,15] One possibility is that these differences may be because of the time frame of testing. In the current study, rats were tested at almost 3 months after injury for five sessions straight. In the previous PR study, rats were assessed once every 5 days and only until approximately 1.5 months post-injury. In the studies using sucrose preference, this was only assessed early after injury. It is possible that by two months, such motivational deficits largely recover in frontally injured rats, but this has yet to be tested.

Of note is that, despite the higher break points in the PR schedule suggesting increased motivation for food, injured rats still required the majority of the session to reach their break point, while sham rats became more efficient and took less time across sessions (Fig. 5). The performance of the sham rats is more consistent with traditional acquisition of PR schedules, which suggests aberrant behavior in injured animals despite the higher break points. This is further compounded by a finer examination of the press rates and IRTs of injured rats on the PR schedule. At lower values, they demonstrated lower response rates and higher IRTs, which are typically consistent with less motivated behavior. At higher response requirements, however, performance collapses into similar patterns observed in sham rats (Fig. 6).

These differences can potentially be attributed to several factors, including motor dysfunction (unlikely given other response rates in the current study), decreases in motivation (also unlikely given relative rates of responding at higher values), or perseveration in pressing behavior. Previous work has identified several variables that may affect motivational states, but these are commonly applied to understanding the higher PR values, and in intact animals.[31] The discrepancies in the current study, between increased break points, but blunted response rates at smaller schedule values highlight the importance of using multiple levels of analysis on data such as these and also suggest that more advanced behavioral assessments will be necessary to tease apart the complex behavioral changes that occur from brain injury.

One other finding that may require additional follow-up was the small difference identified between Sprague-Dawley and Long-Evans rats on IRTs during VI schedule performance (Fig. 1). Despite this, there was no significant strain difference in the overall response rate on the VI, and no significant differences were found on any other behaviors. Although this is a very small effect, it suggests a potential fundamental difference in how responses are allocated. Sprague-Dawley rats increased their IRTs more across the schedule values than Long-Evans rats, which represents an increase in either the amount, or the length, of long pauses between responses. Many studies have examined strain differences, but typically in the evaluation of more complex behaviors such as impulsivity or addiction.[32,33] The current study was likely underpowered to be able to fully investigate this phenomenon, but future studies may be justified in addressing this difference in behavior.

Most previous operant studies in TBI have made use of FR schedules (typically FR-1).[16–18,20–22,34] Here we confirmed that TBI leads to only minimal differences in reinforcers collected, response rates, and IRTs on such schedules. This validates several of the effects observed previously by showing that injured rats respond similarly to shams for FR reinforcement and paves the way for the use of a multitude of more sophisticated operant tasks that can assess complex cognitive behaviors.

It should be noted, though, that the findings from the VR schedule may be problematic when adapting some decision-making tasks for assessing TBI, specifically those that use probabilistic outcomes,

which have similarities to a VR schedule.[35–37] This finding, however, may also explain why neuropsychology assessments such as the Iowa Gambling Task show such robust deficits in persons with brain injury.[38–40] Further, the dissociation between PR break point and the within-session measures of motivation, combined with the increased reinforcers obtained on the VR schedule by injured animals, suggests some degree of perseveration, which has also been identified in human patients.[41,42]

Perseveration is a considerably understudied area in the experimental TBI literature with strong implications for many common assessments of learning. Perseverative behavior may belie cognitive inflexibility and rigidity of thought that can lead to difficulties in switching strategies based on changes in the environment and has long been associated with deficits in frontal functioning.[43] Perseverative deficits are a core symptom of a number of debilitating psychiatric conditions such as schizophrenia as well as compulsive disorders such as obsessive-compulsive disorder and trichotillomania. Whether such deficits arise through a failure to inhibit salient behaviors or an inability to engage in reappraisal of current response requirements deserves future study because impairment in such cognitive processes may indicate the need for dissociable therapeutic interventions.[44]

While this study was an important first step in addressing changes in reinforced behavior at the most basic level after TBI, more work is needed in the assessment of simple response requirements to ensure any effects of TBI on more complex cognitive measures are interpreted correctly. This study has characterized positive reinforcement, but it is unclear how injured rats respond under contingencies of negative reinforcement and punishment. This is especially important, because other groups have begun to characterize TBI-induced deficits in performance of avoidance tasks.[24,45,46] By using the information from these basic studies, the field can adapt more complex cognitive behaviors into the behavioral test battery for TBI and can more accurately model the real-world dysfunction that occurs in patients. Improving and refining the assessment of TBI can lead to better evaluation of therapeutic agents and hopefully yield treatments for those living with chronic problems because of TBI.

Acknowledgments

Funding for this project was provided by the Canadian Institutes for Health Research.

Author Disclosure Statement

No competing financial interests exist.

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