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Abbreviated environmental enrichment confers neurobehavioral, cognitive, and histological benefits in brain-injured female rats

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Abstract

Environmental enrichment (EE) promotes behavioral recovery after experimental traumatic brain injury (TBI). However, the chronic rehabilitation provided in the laboratory is not analogous to the clinic where physiotherapy is typically limited. Moreover, females make up approximately 40% of the clinical TBI population, yet they are seldom studied in brain trauma. Hence, the goal of this study was to test the hypothesis that abbreviated EE would confer neurobehavioral, cognitive, and histological benefits in brain injured female rats. Anesthetized rats received a cortical impact of moderate severity (2.8 mm tissue deformation at 4 m/s) or sham injury and then were randomly assigned to groups receiving standard (STD) housing or 4-hr, 6-hr, or 24-hr of EE daily. Motor function (beam-balance/walk and rotarod) was assessed on post-operative days 1-5 and every other day from 1-19, respectively. Spatial learning/memory (Morris water maze) was evaluated on days 14-19, and cortical lesion volume was quantified on day 21. No statistical differences were appreciated among the sham controls in any assessment and thus the data were pooled. All EE conditions improved motor function and memory retention, but only 6-hr and 24-hr enhanced

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spatial learning relative to STD ($p < 0.05$). Moreover, EE, regardless of duration reduced cortical lesion volume ($p < 0.05$). These data confirm that abbreviated EE confers robust neurobehavioral, cognitive, and histological benefits in TBI female rats, which supports the hypothesis and strengthens the utility of EE as a pre-clinical model of neurorehabilitation.

Keywords

behavioral outcome; controlled cortical impact; environmental enrichment; functional recovery; learning and memory; Morris water maze; traumatic brain injury

Introduction

From wars around the world to major sports like football and hockey, recent media buzz reporting the dangers of traumatic brain injury (TBI) has brought this once silent epidemic (Goldstein, 1990) out of the shadows and into the eyes of the general public. Despite the enormity of the problem, which encompasses 10 million TBIs worldwide each year (Hyder et al., 2007), effective translatable treatments are scarce (Doppenberg et al., 2004; Menon, 2009). According to the World Health Organization, the incidence of TBI has been consistently increasing and is expected to surpass many diseases and disorders as a leading cause of death and disability by the year 2020 (Hyder et al., 2007). While many patients exhibit deficits in motor performances, the most prolonged symptoms tend to be cognitive impairments such as learning deficits and memory loss (Horneman and Emanuelson, 2009; Levin et al., 2010; Barry and Tones, 2015; Richter et al., 2015). A patient's quality of life is certainly impaired by these dysfunctions and the costs of managing these symptoms account for billions of dollars each year (Max et al., 1991; Selassie et al., 2008; Faul et al., 2010). Hence, it is urgent that potential preclinical treatments that can easily be translated to the clinic, such as rehabilitative approaches, are further evaluated and refined.

Environmental enrichment (EE) is a paradigm that provides rodents living in an expansive space a plethora of novel stimuli, which serves as cognitive stimulation. EE also promotes social interaction and encourages exploration and exercise (Sozda et al., 2010; Kline et al., 2007). EE is considered a preclinical model of neurorehabilitation because of its effectiveness in conferring motor, cognitive, and histological benefits after TBI (Hamm et al., 1996; Passineau et al., 2001; Hicks et al., 2002; Kline et al., 2007; Hoffman et al., 2008; Sozda et al., 2010; Matter et al., 2011; Monaco et al., 2013; Bondi et al., 2014b, 2015). Importantly, the EE-induced beneficial effects are enduring, lasting up to at least 6 months (Cheng et al., 2012) and can be achieved even when initiation of exposure is delayed (Hoffman et al., 2008; Matter et al., 2011).

However, despite its ability to confer robust benefits and maintain the efficacy, the typical EE paradigm does currently have limitations. Specifically, the continuous exposure of enrichment may not be applicable at the clinical level where duration of therapy is generally limited (Blackerby, 1990; Shiel et al., 2001; Zhu et al., 2007; Vanderploeg et al., 2008). Female rats subjected to a controlled cortical impact (CCI) injury have previously been shown to recover motor and cognitive function after continuous EE (Monaco et al., 2013). Here we test the hypothesis that abbreviated EE will confer greater motor recovery,

acquisition of spatial learning/memory, and histological benefits versus STD housing after TBI. Moreover, the abbreviated EE groups will perform comparably to the continuous EE group.

Determining the effects of a preclinical model of neurorehabilitation in females is necessary as they make up approximately 41% of the TBI population and like males will receive rehabilitation as part of their prescribed therapeutic strategy. Moreover, females are vastly understudied in experimental models of TBI (Kline et al., 2016), and even more so in pre-clinical rehabilitation paradigms, thus it is paramount to determine the potential efficacy of this approach to further bolster the utility of EE as a therapeutic paradigm with clinical translatability.

Materials and methods

Subjects and pre-surgical procedures

Sixty adult (3 months old) normal cycling female Sprague-Dawley rats (Harlan, Indianapolis, IN) were used as this paradigm more closely mimics clinical TBI. Moreover, we have previously shown that estrous stage at the time of injury does not impact subsequent recovery (Wagner et al., 2004; Monaco et al., 2013). Rats were paired housed in ventilated polycarbonate rat cages and maintained in a temperature ($21 \pm 1^\circ\text{C}$) and light (on 0700-1900 h) controlled environment with food and water available *ad libitum*. During the week of acclimatization the rats were pre-trained on the rotarod and beam-walk tasks (see Fig. 1 for schematic of experimental paradigm) and then were randomly assigned to the following groups: TBI + STD; n=10, TBI + EE (continuous); n=10, TBI + EE (6 hours); n=10, TBI + EE (4 hours); n=10 and their respective Sham controls (n=20). All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh. Every attempt was made to limit the number of rats used and to minimize suffering.

Surgery

A controlled cortical impact (CCI) injury was produced as previously described (Kline et al., 2007, 2010, 2012; Bondi et al., 2014a). Briefly, surgical anesthesia was induced and maintained with 4% and 2% concentrations of isoflurane, respectively, in 2:1 N₂O:O₂. After endotracheal intubation the rats (250-270 g) were secured in a stereotaxic frame and ventilated mechanically. Core temperature was maintained at $37 \pm 0.5^\circ\text{C}$ with a heating pad. Employing aseptic procedures a midline scalp incision was made, the skin and fascia were reflected to expose the skull, and a craniectomy (6-mm in diameter) was made in the right hemisphere with a hand held trephine. The bone flap was removed and the craniectomy was enlarged further to accommodate the impact tip (6 mm, flat), which was centered and lowered through the craniectomy until it touched the dura mater, then the rod was retracted and the impact tip was advanced 2.8 mm farther to produce a moderate-to-severe brain injury (2.8 mm tissue deformation at 4 m/sec). Anesthesia was discontinued immediately after the impact and the incision was promptly closed. Once sutured, the rats were extubated and assessed for acute neurological outcome. Sham rats underwent all surgical procedures, except the impact.

Acute neurological evaluation

Hind limb reflexive ability was assessed immediately following the cessation of anesthesia by gently squeezing the rats' paw every 5 sec and recording the time to elicit a withdrawal response. Return of the righting reflex was determined by the time required to turn from the supine to prone position on three consecutive trials.

Housing conditions: environmental manipulation

After the effects of surgical anesthesia abated and the rats were able to ambulate spontaneously, they were returned to the colony where those designated for continuous enrichment were placed in specifically designed steel-wire cages (91×76×50 cm). The EE cages consisted of three levels with ladders to ambulate from one level to another and contained various toys (e.g., balls, blocks, and tubes), nesting materials (e.g., paper towels), and *ad libitum* food and water (Kline et al., 2007; Sozda et al., 2010; Bondi et al., 2014b, 2015). To maintain novelty, the objects were rearranged every day and changed each time the cage was cleaned, which was twice per week. Ten rats, including TBI and sham controls, were housed together to minimize variability between groups. Rats in the STD and abbreviated EE conditions were placed back into the standard ventilated polycarbonate rat cages (37×25×18 cm, 2 rats per cage) with only food and water. The abbreviated EE rats were removed from the STD cage and placed in the EE for their prescribed time of 4 hr or 6 hr every day and then transferred back to the STD cage until the next enrichment session.

Motor performance: beam-balance and beam-walk

Motor function was assessed using well-established beam-balance and beam-walk tasks (Kline et al., 2010, 2012; Cheng 2008, 2012, 2016). Performance on the beam-balance is assessed by recording the time (maximum of 60 sec per trial) that the rats can maintain their balance on an elevated narrow wooden beam (90 cm above floor level, 1.5 cm wide, and 34 cm in length). The beam-walk task, modified from that originally devised by Feeney and colleagues (Feeney et al., 1982), and used extensively in our laboratory (Yelleswarapu et al., 2012; Cheng et al., 2008, 2012, 2016), consists of training/assessing rats using a negative-reinforcement paradigm to escape bright light and white noise by traversing an elevated narrow beam (90 cm above floor level, 2.5 cm wide, and 100 cm in length) and entering a darkened goal box at the opposite end. Performance on the beam-walk consists of recording time to traverse the beam as well as the distance traveled. The scoring criteria for distance traveled is based on a rating scale from 0 to 5, where 0 indicates an inability to ambulate beyond the start point, 1-4 corresponds to distal segments of 20, 40, 60, or 80 cm from the starting point, respectively, and 5 indicates traversal of the entire length of the beam (100 cm) and entrance into the goal box. Rats were trained prior to TBI or sham injury to perform the tasks without errors (i.e., maintain their balance for 60 sec and traverse the beam in under 5 sec). A baseline performance assessment was taken on the day of surgery. Performance was assessed on post-operative days 1-5 and consisted of three trials (60 sec allotted time per trial) per day on each task. The average daily scores for each subject were used in the statistical analyses.

Motor performance: rotarod

A well-established rotarod task was utilized to evaluate fine locomotor and balance function (Hamm et al., 1994; Hamm, 2001; Monaco et al., 2013). Briefly, training began three days prior to surgery and consisted of both fixed rate and accelerating protocols until the rats were proficient. On the day of surgery the rats received three trials according to the accelerating protocol (i.e., 15-80 rotations per minute [rpm] for a maximum of 90 sec) to establish baseline performance. The testing procedure consisted of three trials (inter-trial interval of 5 min) where duration (maximum 90 sec) and speed (15-80 rpm), before losing balance and falling off the accelerating rotating rod (or completing two full revolutions, whichever occurred first), were recorded. Assessments were conducted every other day beginning on post-operative days 1 and continuing until day 19. The average daily scores for each subject were used in the statistical analyses.

Cognitive performance: spatial learning

Spatial learning was assessed using a well-established Morris water maze (MWM) task (Morris, 1984; Kline et al., 2002a,b, 2007, 2010, 2012; Olsen et al., 2012). The maze consisted of a plastic pool (180 cm diameter; 60 cm high) filled with tap water ($26 \pm 1^\circ\text{C}$) to a depth of 28 cm and was positioned in a room with prominent extra-maze cues. The platform was a clear Plexiglas stand (10 cm diameter, 26 cm high) that was positioned 26 cm from the maze wall in the southwest quadrant and held constant for each rat. Acquisition of spatial learning began on post-operative day 14 and entailed providing a block of four daily trials for five consecutive days (14-18) to locate the escape platform when it was submerged 2 cm below the water surface. On day 19 the platform was raised 2 cm above the water surface as a control procedure to determine the contributions of non-spatial factors (e.g., sensory-motor function, motivation, and visual acuity) on cognitive performance. For each daily block of trials the rats were placed in the pool facing the wall at each of the four possible start locations (north, east, south, and west) in a quasi-randomized manner. Each trial lasted until the rat climbed onto the platform or until 120 sec had elapsed, whichever occurred first. The rats that failed to locate the escape platform within the allotted time were manually guided to it. All rats remained on the platform for 30 sec before being placed in a heated incubator between trials (4-min inter-trial interval). The times of the 4 daily trials for each rat were averaged and used in the statistical analyses.

Cognitive performance: memory retention

One day after the final acquisition training session (day 19), all rats were given a probe trial to measure retention. Briefly, the platform was removed from the pool and the rats were placed in the pool from the location point most distal to the quadrant where the platform was previously located (i.e., "target quadrant") and allowed to freely explore the pool for 30 sec. The time spent searching in the target quadrant is recorded and used in the statistical analyses. The data were obtained using ANY-maze video tracking software.

Histology: quantification of cortical lesion volume

Three weeks after surgery the rats were anesthetized with Fatal-Plus® (0.3 mL, i.p.) and perfused transcardially with 200 mL 0.1 M phosphate buffered saline (pH 7.4) followed by

300 mL 4% paraformaldehyde. The heads were placed in the perfusate and one week later the brains were extracted, post-fixed further, dehydrated with alcohols, and embedded in paraffin. Coronal sections (7 μ m thick) were cut at 1-mm intervals through the lesion on a rotary microtome and mounted on Superfrost®/Plus glass microscope slides. After drying at room temperature, the sections were deparaffinized in xylenes, rehydrated, and stained with Cresyl violet. An observer blinded to experimental conditions analyzed cortical lesion volumes (mm³) by calculating the area of the lesion (mm²), which was done by outlining the inferred area of missing cortical tissue for each section (typically 5-7) taken at 1-mm intervals as previously reported (Olsen et al., 2012; Monaco et al., 2013, 2014).

Statistical analyses

All analyses were performed using Statview 5.0.1 software (Abacus Concepts, Inc., Berkeley, CA) on data collected by blinded experimenters. The motor and cognitive analyses were conducted using repeated-measures analysis of variance (ANOVA). The acute neurological data (i.e., hind limb withdrawal reflex and righting reflex) as well as the data for the visible platform, probe trial, swim speed, and cortical lesion volume were analyzed using one-factor ANOVAs. When the overall ANOVA revealed significant effects, the Newman-Keuls post-hoc test was used to determine specific group differences. The results are expressed as the mean $\pm$ standard error of the mean (S.E.M.) and were considered significant when $p < 0.05$.

Results

There were no exclusions and thus the statistical analyses were performed on the data from all 60 rats. No significant differences ($p > 0.05$) were observed in any behavioral measure between the sham controls regardless of housing condition so their data were pooled into one group designated as SHAM.

Acute neurological function

No differences were observed among the TBI groups in hind limb withdrawal reflex after a brief paw pinch [left range = 154.4 ± 2.9 sec to 177.7 ± 5.6 sec, $p > 0.05$; right range = 150.3 ± 3.1 sec to 173.6 ± 5.5 sec, $p > 0.05$] or for righting reflex [range 290.4 ± 11.2 sec to 333.9 ± 17.8 sec, $p > 0.05$] following the termination of anesthesia. The lack of significant differences with these acute neurological indices suggests that all groups experienced an equivalent level of injury and anesthesia.

Motor function: beam-balance

Prior to surgery, each rat was able to balance on the beam for the allotted 60 sec (Fig. 2). After surgery, the repeated measures ANOVA revealed significant Group [$F_{4,55} = 16.411$, $p < 0.0001$] and Day [$F_{5,275} = 51.387$, $p < 0.0001$] differences, as well as a significant Group $\times$ Day interaction [$F_{20,275} = 7.133$, $p < 0.0001$]. The post-hoc analysis revealed that the TBI + EE groups, regardless of duration (i.e., continuous, 6 hr, and 4 hr) performed significantly better than the TBI + STD group [$p < 0.05$]. Moreover, the TBI + EE (continuous) and TB + EE (6 hr) performed better than the TBI + EE (4 hr) [$p < 0.05$], but did not differ from one

another [$p > 0.05$]. Lastly, only the TBI + STD and TBI + EE (4 hr) groups were impaired relative to the SHAM controls [$p < 0.05$].

Motor function: beam-walk (time)

Prior to surgery, each rat consistently traversed the 100 cm beam to the reward box in under 5 sec (Fig. 3). After surgery, the statistical analysis revealed significant Group [$F_{4,55} = 48.453$, $p < 0.0001$] and Day [$F_{5,275} = 205.318$, $p < 0.0001$] differences, as well as a significant Group $\times$ Day interaction [$F_{20,275} = 21.649$, $p < 0.0001$]. The post-hoc analysis revealed that the TBI + EE groups, regardless of duration of exposure (i.e., continuous, 6 hr, and 4 hr) performed significantly better than the TBI + STD group [$p < 0.05$], with no differences among each other [$p > 0.05$]. Additionally, all TBI groups, regardless of housing or duration of EE were impaired relative to the SHAM controls [$p < 0.05$].

Motor function: beam-walk (distance)

Prior to surgery, each rat traversed the entire length of the beam to reach the goal box (Fig. 4). After surgery, the statistical analysis revealed significant Group [$F_{4,55} = 25.426$, $p < 0.0001$] and Day [$F_{5,275} = 156.424$, $p < 0.0001$] differences, as well as a significant Group $\times$ Day interaction [$F_{20,275} = 17.1451$, $p < 0.0001$]. The post-hoc analysis revealed that the TBI + EE groups, regardless of duration (i.e., continuous, 6 hr, and 4 hr) performed significantly better than the TBI + STD group [$p < 0.05$], with no differences among one another [$p > 0.05$]. Additionally, while locomotor function improved over time, all TBI groups, regardless of housing or duration of EE were impaired relative to the SHAM controls [$p < 0.05$].

Motor function: rotarod (duration and speed)

No pre-surgical differences were observed among groups for time on the rotarod [$p > 0.05$] or speed of locomotion [$p > 0.05$]. Following CCI injury, analysis of duration revealed significant Group [$F_{4,55} = 3.040$, $p = 0.0248$] and Day [$F_{10,550} = 12.240$, $p < 0.0001$] differences, as well as a significant Group $\times$ Day interaction [$F_{40,550} = 1.543$, $p = 0.0198$]. The post-hoc analysis revealed that all TBI + EE groups were able to maintain their balance on the rotarod longer than the TBI + STD [$p < 0.05$]. The TBI + EE (continuous) group did not differ from the TBI + EE (6 hr) group [$p > 0.05$], but was better than the TBI + EE (4 hr) group [$p < 0.05$]. There was no difference between the TBI + EE (6 hr) and the TBI + EE (4 hr) groups [$p > 0.05$; Fig 5]. Regarding speed, the analysis revealed significant Group [$F_{4,55} = 3.910$, $p = 0.0073$] and Day [$F_{10,550} = 15.935$, $p < 0.0001$] differences, as well as a significant Group $\times$ Day interaction [$F_{40,550} = 2.072$, $p = 0.0002$]. The post-hoc analysis revealed that all TBI + EE groups were able to maintain their balance on the rotarod longer despite accelerating speeds (i.e., increased rpms) vs. the TBI + STD group [$p < 0.05$]. TBI + EE (continuous) was better than TBI + EE (4 hr) [$p < 0.05$], but not TBI + EE (6 hr) [$p > 0.05$]. The SHAM control group was significantly better than all TBI groups, regardless of housing and duration of EE exposure [$p < 0.05$; Fig 6].

Cognitive function: acquisition of spatial learning

Analysis of the water maze data for locating the hidden platform revealed significant Group [$F_{4,55} = 23.097$, $p < 0.0001$] and Day [$F_{4,220} = 37.367$, $p < 0.0001$] differences, as well as a

significant Group $\times$ Day interaction [$F_{16,220} = 2.095$, $p = 0.0095$]. The post-hoc analysis indicated that the SHAM controls were better than all TBI groups [$p < 0.05$; Fig. 7]. The TBI + EE (continuous) and TBI + EE (6 hr) groups were significantly better than the TBI + STD and TBI + EE (4hr) groups were [$p < 0.05$], but did not differ from each other [$p > 0.05$]. Furthermore, no differences were revealed between the TBI + EE (4 hr) and TBI + STD groups [$p > 0.05$]. Swim speed did not differ among the groups (range = 32.2 ± 0.9 cm/sec to 36.7 ± 2.1 cm/sec; $p > 0.05$). Time to locate the visible platform was reduced in the SHAM controls relative to the TBI + STD, TBI + EE (6 hr) and TBI + EE (4 hr) groups [$p < 0.05$]. No differences were revealed among the TBI groups [$p > 0.05$].

Cognitive function: probe trial

Analysis of the probe data revealed a significant difference among the groups [$F_{4,55} = 7.741$, $p < 0.0001$]. The post-hoc analysis revealed that enhanced memory retention, as demonstrated by a greater percentage of the 30 sec allotted time spent in the target quadrant, was observed in the SHAM and TBI groups receiving EE compared to the TBI + STD group [$p < 0.05$]. No differences were observed among the TBI + EE groups, regardless of EE duration. (Fig. 8).

Histology: cortical lesion volume

Analysis of the lesion data revealed a significant group effect [$F_{3,16} = 3.579$, $p = 0.037$]. The mean cortical lesion volumes of the TBI + EE (continuous), TBI + EE (6 hr), and TBI + EE (4 hr) groups were 35.2 ± 3.9 mm³, 34.5 ± 1.3 mm³, and 38.4 ± 5.1 mm³, which did not differ from one another [$p > 0.05$], but were significantly smaller than the 49.3 ± 3.0 mm³ of the TBI + STD group [$p < 0.05$; Fig. 9].

Discussion

Numerous studies have reported EE-mediated motor and cognitive benefits after TBI produced by various experimental models (for excellent reviews see Bondi et al., 2014b, 2015). Because of these positive effects, EE has been touted as a preclinical model of neurorehabilitation (de Witt et al., 2011; Matter et al., 2011; Bondi et al., 2014b, 2015; Garcia et al., 2011). However, the chronic rehabilitation typically provided in the laboratory is not analogous to the clinic where physiotherapy is limited to less than 6-8 hr per day (Blackerby, 1990; Shiel et al., 2001; Zhu et al., 2007; Vanderploeg et al., 2008). Hence, the temporal logistics of EE (e.g., when is it given and for how long) need to be further clarified to solidify it as a preclinical model of neurorehabilitation.

Advances in these lines of research are ongoing. For example, Hoffman and colleagues (2008) first reported that significant benefits in cognitive performance are achieved even when exposure to EE is delayed by a week after TBI. Similar findings were subsequently replicated by Matter and colleagues (2011), who also demonstrated that the duration of EE is important for functional recovery. Refining the model further, de Witt and colleagues (2011) showed that when the duration of EE after CCI injury is abbreviated in males, such that it parallels more closely with the times provided in the clinic (e.g., 6 hr), it is still sufficiently robust to confer significant cognitive benefits in a 5-day water maze task. Gaulke and

colleagues (2005) also showed that an abridged EE paradigm in males after a fluid percussion brain injury increased progenitor cells in the ipsilateral dentate gyrus of the hippocampus and improved cognitive function in a 2-day water maze task.

Despite the abundance of evidence suggesting that there are inherent biological differences between females and males that play key roles in central nervous system functions, studies using exclusively males still significantly outnumber those using females 5.5 to 1 in the field of neuroscience (Beery and Zucker, 2011). Females are thought to be better protected from TBI than males mainly due to female sex steroid hormones (Berry et al., 2009; Groswasser et al., 1998; Roof and Hall, 2000). Increased levels of estrogen and progesterone have been reported to confer beneficial effects after TBI (Khaksari et al., 2011; Maghool et al., 2013; Naderi et al., 2015). Furthermore, EE restores brain derived neurotrophic factor expression in the hippocampus ipsilateral to injury more robustly in females than males (Chen et al., 2005). However, previous results from our group showed that females living for 3 weeks in continuous EE exhibited improved motor and cognitive ability as well as increased hippocampal cell survival and decreased cortical lesion size compared to STD-housed females, but do not show differences in behavioral performance regardless of the estrous phase at the time of CCI injury (Monaco et al., 2013), indicating that normal cycling females are equally sensitive to the beneficial effects of continuous EE as males.

The question remained whether abbreviated EE could also instill benefit in female rats and therefore addressing this limitation was the goal of the current study. An abbreviated EE paradigm consisting of 4 hr and 6 hr was chosen because these times fall within the range of those typically provided to clinical rehabilitation patients (Blackerby, 1990; Shiel et al., 2001; Zhu et al., 2007; Vanderploeg et al., 2008) and also because this protocol has previously been assessed in male rats (de Witt et al., 2011); comparing the efficacy of EE in both sexes is critical for validation of the rehabilitation paradigm (Kline et al., 2016). Analogous to previous studies (Wagner et al., 2004; Monaco et al., 2013), female rats were injured at various phases of the estrous stage to mimic real-world experience (i.e., clinical applicability).

Motor performance on the beam tasks was significantly enhanced in the EE groups, regardless of whether they received continuous or abbreviated exposure, relative to the STD-housed controls. However, performance in the rotarod task was facilitated only in the continually enriched rats. The differences may be attributable to the complexity of the task as success on this more sensitive measure is dependent on both balance and locomotion while the rotating rod accelerates. The additional symptom relevant experience afforded to the rats while in the EE cage for a longer duration may also account for the differences.

Regarding cognitive ability, the continuous and 6 hr EE groups were superior to the 4 hr EE and STD-housed controls, but did not differ from one another. A similar pattern where 4 hr of EE was inefficient in enhancing cognition was observed in male rats after CCI injury (de Witt et al., 2011). These findings indicate that the cognitive benefits mediated by EE do not require continuous exposure, but do suggest that there is a threshold requirement for acquiring spatial learning performance. Despite not exhibiting a significant benefit in the acquisition of spatial learning, the 4 hr EE group was equally effective as the continuous EE

and 6 hr EE groups in memory retention as indicated by significantly more time spent in the target quadrant. Indeed, none of the TBI + EE groups, regardless of duration of EE exposure differed from the SHAM controls. The differences in the acquisition of spatial learning among the EE groups, particularly the 4- hr vs. both the 6-hr and the continuous were not contingent on cortical lesion volume as all EE groups exhibited significantly smaller lesions relative to the STD controls, but did not differ from each other. This finding underscores the disparity reported when correlating histological outcomes with behavioral performance (Lyeth et al., 1990; Kline et al., 2016).

It is interesting that additional enrichment did not yield greater benefits. One explanation is that the benefits achieved in all endpoints reached a ceiling effect and despite more therapy there was little more that could be gained with rehabilitation alone. Alternatively, it is possible that the novelty of the EE paradigm wanes over time, and as such the rehabilitative efficacy is also diminished. Indeed, it has been shown that for optimal rehabilitative benefit, the rats must interact with all facets of the environment (Sozda et al., 2010). This interaction may become less desirable when the rats habituate to the daily environment after a few hours and regardless of how much longer they remain in there they do not benefit further. If this theory is correct, changing the milieu may provide renewed novelty and subsequently afford additional neurorehabilitation as the rats engage further with the environment. To test, this idea, we are currently providing EE twice per day with a change of the stimuli between sessions. This approach is also more clinically relevant as inpatients often receive dual therapy sessions.

In conclusion, the findings revealed that motor performance and spatial learning were facilitated by continuous EE vs. STD housing, which replicates previous findings from our laboratory and others (Bondi et al., 2014b, 2015). Additionally, the data demonstrate that abbreviated EE confers behavioral and histological benefit to the same level as continuous EE, which supports the hypothesis. These findings in females parallel those in males (de Witt et al., 2011) and validate the model as a relevant rehabilitation paradigm. That the 4 hr abbreviated EE group did not differ from the STD-housed controls suggest that there are still opportunities for improving the rehabilitative paradigm. To this end, we are currently combining sub-therapeutic doses of EE with pharmacotherapies that are routinely provided in the clinical setting. Overall, the findings have the potential to significantly impact, and advance, rehabilitation based research.

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Highlights

- Abbreviated EE produces robust cognitive benefits after experimental TBI in female rats
- Abbreviated EE significantly enhances motor performance after TBI in female rats
- Abbreviated EE significantly reduces cortical lesion volume
- These findings parallel those seen in males and provide further support for EE as a pre-clinical model of rehabilitation

Fig. 1.

Flow chart of the experimental paradigm depicting pre-and-post injury manipulations. Note that the beam tests consisted of two separate evaluations (i.e., beam-balance and beam-walk). Also, the rotarod test was performed every other day during post-operative days 1-19. Lastly, the water maze paradigm consisted of hidden platform assessments that occurred on days 14-18, a single 30-sec probe trial that was conducted on post-operative day 19 prior to a single visible platform assessment. Rats were sacrificed at 3 weeks.

Fig. 2.

Mean ($\pm$ S.E.M.) time (sec) balancing on an elevated narrow beam prior to, and after, TBI or sham injury. There were no significant differences among the three EE (i.e., continuous, 6 hr, and 4 hr) and STD sham groups and thus the data were pooled (SHAM). * $p < 0.05$ vs. TBI + STD. group [$p < 0.05$]. ^ $p < 0.05$ vs. TBI + EE (4 hr). ** $p < 0.05$ vs. TBI + STD and TBI + EE (4 hr). No other comparisons were significant.

Fig. 3.

Mean ($\pm$ S.E.M.) time (sec) to traverse an elevated narrow beam after TBI or sham injury.

There were no significant differences among the three EE (i.e., continuous, 6 hr, and 4 hr)

and STD sham groups and thus the data were pooled (SHAM). * $p < 0.05$ vs. TBI + STD. **

$p < 0.05$ vs. all TBI groups. No other comparisons were significant.

Fig. 4.

Mean ($\pm$ S.E.M.) distance traveled score along an elevated narrow beam before and after TBI or sham injury. There were no significant differences among the three EE (i.e., continuous, 6 hr, and 4 hr) and STD sham groups and thus the data were pooled (SHAM). * $p < 0.05$ vs. TBI + STD. ** $p < 0.05$ vs. all TBI groups. No other comparisons were significant.

Fig. 5.

Mean ($\pm$ S.E.M.) time (sec) on an accelerating rotarod prior to, and after, TBI or sham injury. There were no significant differences among the three EE (i.e., continuous, 6 hr, and 4 hr) and STD sham groups and thus the data were pooled (SHAM). * $p < 0.05$ vs. TBI + STD. ^ $p < 0.05$ vs. TBI + EE (4 hr). ** $p < 0.05$ vs. all TBI groups, except TBI + EE (continuous). No other comparisons were significant.

Fig. 6.

Mean ($\pm$ S.E.M.) speed (rpm) on an accelerating rotarod prior to, and after, TBI or sham injury. There were no significant differences among the three EE (i.e., continuous, 6 hr, and 4 hr) and STD sham groups and thus the data were pooled (SHAM). * $p < 0.05$ vs. TBI + STD. ^ $p < 0.05$ vs. TBI + EE (4 hr). ** $p < 0.05$ vs. all TBI groups, except TBI + EE (continuous). No other comparisons were significant.

Fig. 7.

Mean ($\pm$ S.E.M.) time (sec) to locate a hidden and visible platform in the water maze. There were no significant differences among the three EE (i.e., continuous, 6 hr, and 4 hr) and STD sham groups and thus the data were pooled (SHAM). For the hidden platform assessments, $*p < 0.05$ vs. TBI + STD and TBI + EE (4 hr). $**p < 0.05$ vs. all TBI groups. For the visible platform assessment, $**p < 0.05$ vs. all TBI groups, except TBI + EE (continuous). No other comparisons were significant.

Fig. 8.

Mean ($\pm$ S.E.M.) percent time spent in the target quadrant. The lined area shows the % time that each group spent in the target quadrant and the black area represents the % time spent in the non-target quadrants. The smaller circle in the middle of each chart illustrates a representative swim path of the respective group with the top right quadrant denoting the target. * $p < 0.05$ vs. TBI + STD. ** $p < 0.05$ vs. TBI + STD. No other comparisons were significant.

Fig. 9A.

Mean ($\pm$ S.E.M.) cortical lesion volume (mm³) 3 weeks after cortical impact injury. Figs. 9B-E depict average size lesions, at the level of the dorsal hippocampus, for the TBI + STD, TBI + EE (continuous), TBI + EE (6 hours), and TBI + EE (4 hours) groups, respectively. **p* < 0.05 vs. TBI + STD. No other comparisons were significant.