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Research Paper

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-to-severe injury (2.8 mm tissue deformation at 4 m/s) or sham surgery and then were randomly assigned to groups receiving standard (STD) housing or 4 h, 6 h, or 24 h of EE daily. Motor function (beam-balance/walk and rotarod) was assessed on post-operative days 1–5 and every other day from 1 to 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 h and 24 h enhanced 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.

1. 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 Tomes, 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).

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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.

2. Materials and methods

2.1. 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 ± 1 °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 h); n = 10, TBI + EE (4 h); 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.

2.2. 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 N_2O:O_2. After endotracheal intubation the rats (250–270 g) were secured in a stereotaxic frame and ventilated mechanically. Core temperature was maintained at 37 ± 0.5 °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/s). 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.

2.3. Acute neurological evaluation

Hind limb reflexive ability was assessed immediately following the cessation of anesthesia by gently squeezing the rats' paw every 5 s 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.

2.4. 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 h or 6 h every day and then transferred back to the STD cage until the next enrichment session.

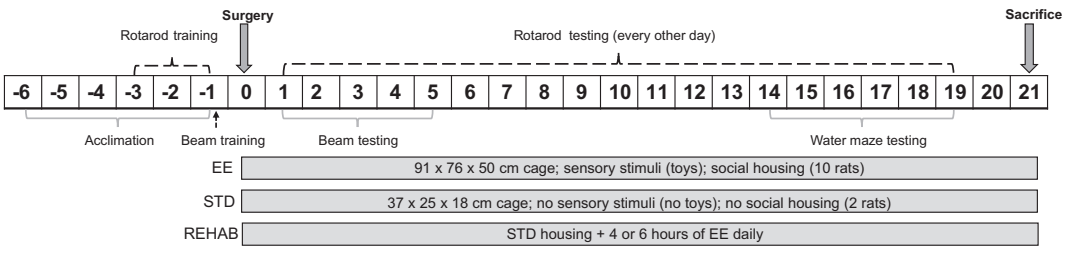

Fig. 1. Flow chart of the experimental paradigm depicting pre-and-post injury manipulations. Note that the beam tests consisted of two separate evaluations (i.e., beam-balance and beam-walk). Also, the rotarod test was performed every other day during post-operative days 1–19. Lastly, the water maze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