Experimental Neurology 247 (2013) 410–418

Contents lists available at SciVerse ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Environmental enrichment promotes robust functional and histological benefits in female rats after controlled cortical impact injury

Christina M. Monaco [a,b], Vincent V. Mattiola [a,b], Kaitlin A. Folweiler [a,b], Justin K. Tay [a,b], Narayana K. Yelleswarapu [a,b], Lauren M. Curatolo [a,b], Ashley M. Matter [a,b], Jeffrey P. Cheng [a,b], Anthony E. Kline [a,b,c,d,e,f,*]

[a] Physical Medicine & Rehabilitation, University of Pittsburgh, Pittsburgh, PA 15213, USA
[b] Safar Center for Resuscitation Research, University of Pittsburgh, Pittsburgh, PA 15213, USA
[c] Psychology, University of Pittsburgh, Pittsburgh, PA 15213, USA
[d] Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15213, USA
[e] Center for the Neural Basis of Cognition, University of Pittsburgh, Pittsburgh, PA 15213, USA
[f] Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA 15213, USA

ARTICLE INFO

Article history:
Received 15 November 2012
Revised 2 January 2013
Accepted 9 January 2013
Available online 16 January 2013

Keywords:
Behavior
Controlled cortical impact
Environmental enrichment
Functional recovery
Learning and memory
Morris water maze
Traumatic brain injury

ABSTRACT

Environmental enrichment (EE) consistently induces marked benefits in male rats after traumatic brain injury (TBI), but whether similar efficacy extends to females is not well established. Hence, the aim of this study was to reassess the effect of EE on functional and histological outcome in female rats after brain trauma. Twenty-four normal cycling adult female rats underwent verification of estrous stage prior to controlled cortical impact (CCI) or sham injury and then were assigned to EE or standard (STD) housing. Motor function was assessed with beam-balance/beam-walk and rotarod tasks on post-operative days 1–5 and every other day from 1–19, respectively. Spatial learning/memory was evaluated in a Morris water maze on days 14–19. Morphologically intact hippocampal $CA_{1/3}$ cells and cortical lesion volume were quantified 3 weeks after injury. No differences were observed between the EE and STD sham groups in any endpoint measure and thus the data were pooled. In the TBI groups, EE improved beam-balance, beam-walk, rotarod, and spatial learning performance vs. STD (p's<0.05). EE also provided significant histological protection as confirmed by increased $CA_{1/3}$ cell survival and decreased cortical lesion size vs. STD. These data demonstrate that EE confers robust benefits in female rats after CCI injury, which parallels numerous studies in males and lends further credence for EE as a preclinical model of neurorehabilitation.

Introduction

Traumatic brain injury (TBI) is a significant health care issue that affects approximately ten million individuals worldwide each year (Hyder et al., 2007); of these TBI occurrences, the United States accounts for approximately 1.7 million cases (Faul et al., 2010; Sosin et al., 1995; Summers et al., 2009) that result in neurobehavioral dysfunction for 90,000 patients (Goldstein, 1990; Selassie et al., 2008). Although motor deficits are common, cognitive impairments are often more pronounced and prolonged (Horneman and Emanuelson, 2009) which adversely affects quality of life (Binder, 1986; Millis et al., 2001). While the affective toll of TBI on interpersonal relationships with family, friends, and coworkers is incalculable, the economic cost to society accounts for billions of dollars each year (Max et al., 1991; Selassie et al., 2008).

Given the devastating consequences of TBI, the development and initiation of treatment paradigms capable of promoting neurobehavioral and cognitive recovery is vital. To this end, numerous therapeutic strategies have been implemented (Garcia et al., 2011; Kokiko and Hamm, 2007; Parton et al., 2005; Wheaton et al., 2009). Although these approaches have provided significant functional and histological benefits in the laboratory, successful translation to the clinic has been inconsistent (Doppenberg et al., 2004; Menon, 2009). The relative lack of translational success with these fairly invasive approaches has prompted the investigation of non-invasive manipulations as alternative therapies for TBI (de Witt et al., 2011; Gomez-Pinilla et al., 2011; Griesbach et al., 2008, 2009; Hamm et al., 1996; Hoffman et al., 2008; Kline et al., 2007; Matter et al., 2011; Sozda et al., 2010; Will et al., 2004).

One approach that has steadily gained momentum as a potentially advantageous therapy for experimental TBI is environmental enrichment (EE). EE consists of providing rats a milieu that is conducive for engaging in sensory stimulation and physical exercise in an expansive social environment (Kline et al., 2007; Sozda et al., 2010) that may be akin to multi-modal clinical rehabilitation. The EE paradigm has

* Corresponding author at: Physical Medicine & Rehabilitation, Safar Center for Resuscitation Research, University of Pittsburgh, 3471 Fifth Ave., Suite 201, Pittsburgh, PA 15213, USA. Fax: +1 412 624 0943.
E-mail address: klineae@upmc.edu (A.E. Kline).

0014-4886/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.expneurol.2013.01.007

consistently demonstrated significant improvement in motor function, spatial learning, and memory retention (de Witt et al., 2011; Hoffman et al., 2008; Kline et al., 2007; Matter et al., 2011; Sozda et al., 2010). Furthermore, EE induces significant histological protection as demonstrated by smaller cortical lesions and decreased hippocampal $CA_{1/3}$ cell loss after TBI. Importantly, the benefits of EE are observed across several models of brain trauma (de Witt et al., 2011; Hamm et al., 1996; Hicks et al., 2002; Hoffman et al., 2008; Kline et al., 2007; Matter et al., 2011; Passineau et al., 2001; Rose et al., 1987; Sozda et al., 2010) and consequently may be considered a preclinical model of neurorehabilitation.

However, an important limitation of the current EE paradigm is that the benefits observed after TBI have been, with the exception of a single study (Wagner et al., 2002), exclusively evaluated in male rats (de Witt et al., 2011; Hamm et al., 1996; Hicks et al., 2002; Hoffman et al., 2008; Kline et al., 2007; Matter et al., 2011; Passineau et al., 2001; Rose et al., 1987; Sozda et al., 2010). Thus, whether similar efficacy extends to females is not established. The relevance of this line of investigation is that females account for a large percentage of the clinical TBI population, yet this group is grossly understudied in experimental models of TBI, and even more so in pre-clinical rehabilitation models. Hence, this study was conducted to determine whether three weeks of EE after cortical impact injury (CCI) promotes motor, cognitive, and histological benefits in female rats just as it has consistently been shown in parallel male studies (de Witt et al., 2011; Hoffman et al., 2008; Kline et al., 2007; Matter et al., 2011; Sozda et al., 2010).

Materials and methods

Subjects and pre-surgical procedures

Twenty-four adult normal cycling female Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing 250–270 g on the day of surgery were initially housed in standard steel-wire mesh cages and maintained in a temperature (21 ± 1 °C) and light (on 7:00 a.m. to 7:00 p.m.) 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 (Fig. 1) and then were randomly assigned to either TBI + STD (n = 8), TBI + EE (n = 8), Sham + STD (n = 4), or Sham + EE (n = 4) groups. Additionally, on the morning of surgery the rats were evaluated for estrous stage using classic cytology. Briefly, following a vaginal smear the epithelial cells were examined by light microscopy and based on traditional distinguishable characteristics of each stage (proestrous = predominantly nucleated epithelial cells with few cornified epithelial cells and leukocytes; estrous = predominantly cornified epithelial cells; diestrous = predominantly leukocytes with some nucleated epithelial cells) the rats were classified accordingly. 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

TBI was produced using the well established CCI injury model as previously described (Cheng et al., 2007, 2008; Dixon et al., 1999; Kline et al., 2004). Briefly, surgical anesthesia was induced and maintained with inspired concentrations of 4% and 2% isoflurane, respectively, in 2:1 N_2O:O_2. After endotracheal intubation the rats were secured in a stereotaxic frame, ventilated mechanically, and maintained at 37 ± 0.5 °C with a heating blanket. Utilizing aseptic procedures a midline scalp incision was made, the skin and fascia were reflected to expose the skull, and a craniectomy was made in the right hemisphere (between bregma/lambda and the sagittal suture/coronal ridge) with a hand held trephine. The bone flap was removed and the craniectomy was enlarged further with cranial rongeurs. Subsequently, the impacting rod was extended and the impact tip (6 mm, flat) 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 brain injury of moderate severity (2.8 mm tissue deformation at 4 m/s). Immediately after surgery the anesthesia was discontinued, the incision was sutured, the rats were extubated, and acute neurologic evaluations were performed. Sham rats underwent similar surgical procedures, but were not subjected to the impact.

Acute neurological evaluation

Immediately following the cessation of anesthesia hindlimb reflexive ability was assessed by gently squeezing the rat's paw with forceps every 5 s and recording the latency to elicit a withdrawal response. Return of the righting reflex was determined by placing the rat on its back and recording the time required to turn from the supine to prone position. These natural reflexive responses are sensitive indicators of injury severity and anesthetic effects (Cheng et al., 2007, 2008; Dixon et al., 1999; Kline et al., 2004).

Housing conditions: environmental manipulation

After the acute effects of surgical anesthesia abated, which was verified by the return of spontaneous movement in the holding cage, the rats were returned to the colony where those designated for enrichment were immediately placed in specifically designed $36 \times 30 \times 20$ inch stainless steel-wire cages consisting of three levels and ladders to ambulate from one level to another such that interaction with the various toys (e.g., balls, blocks, and tubes), nesting materials (e.g., paper towels), cage mates, and *ad libitum* food and water would be achievable (for depiction of EE cage, see Kline et al., 2007; Sozda et al., 2010). To maintain novelty, the objects were rearranged every day and changed each time the cage was cleaned, which was twice per week. Twelve rats, which included eight TBIs and four shams, were housed together to minimize variability. Rats in the STD conditions were placed in standard steel-wire mesh cages (2 rats per cage) with only food and water available.

Motor performance: beam tasks

Motor function was assessed with well established beam-balance (BB) and beam-walk (BW) tasks. Briefly, the BB test consists of placing the rat on an elevated (90 cm) narrow (1.5 cm wide) wooden beam

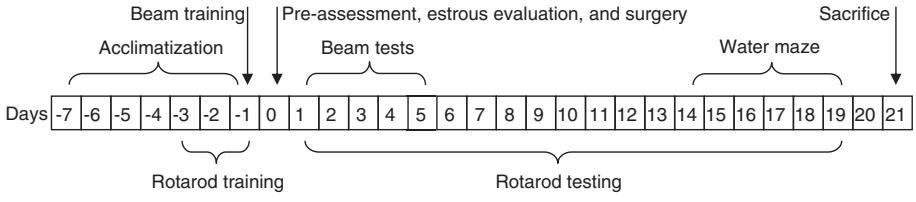

Fig. 1. Time line of the experimental paradigm depicting pre-and-post injury manipulations. Note that the beam tests consisted of two separate evaluations (i.e., beam-balance and beam-walk). Also, the rotarod test was performed every other day during post-operative days 1–19. Lastly, the water maze paradigm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