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Research Paper

Refining environmental enrichment to advance rehabilitation based research after experimental traumatic brain injury

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ABSTRACT

The typical environmental enrichment (EE) paradigm, which consists of continuous exposure after experimental traumatic brain injury (TBI), promotes behavioral and histological benefits. However, rehabilitation is often abbreviated in the clinic and administered in multiple daily sessions. While recent studies have demonstrated that a once daily 6-hr bout of EE confers benefits comparable to continuous EE, breaking the therapy into two shorter sessions may increase novelty and ultimately enhance recovery. Hence, the aim of the study was to test the hypothesis that functional and histological outcomes will be significantly improved by daily preclinical neurorehabilitation consisting of two 3-hr periods of EE vs. a single 6-hr session. Anesthetized adult male rats received a controlled cortical impact of moderate-to-severe injury (2.8 mm tissue deformation at 4 m/s) or sham surgery and were then randomly assigned to groups receiving standard (STD) housing, a single 6-hr session of EE, or two 3-hr sessions of EE daily for 3 weeks. Motor function (beam-balance/traversal) and acquisition of spatial learning/memory retention (Morris water maze) were assessed on post-operative days 1–5 and 14–19, respectively. Cortical lesion volume was quantified on day 21. Both EE conditions improved motor function and acquisition of spatial learning, and reduced cortical lesion volume relative to STD housing ($p < 0.05$), but did not differ from one another in any endpoint ($p > 0.05$). The findings replicate previous work showing that 6-hr of EE daily is sufficient to confer behavioral and histological benefits after TBI and extend the findings by demonstrating that the benefits are comparable regardless of how the 6-hrs of EE are accrued. The relevance of the finding is that it can be extrapolated to the clinic and may benefit patients who cannot endure a single extended period of neurorehabilitation.

1. Introduction

The World Health Organization predicts that traumatic brain injuries (TBI) will become the leading cause of death and disability by the year 2020 (Hyder et al., 2007). Through many convoluted mechanisms including both the injury caused by the primary initial impact and the damaging secondary and tertiary pathophysiological cascades that follow (Kline et al., 2016; Adelson et al., 1998; Bayir et al., 2009; Bramlett et al., 2016; Carlson et al., 2017), TBIs induce a wide variety of long-lasting symptoms including motor deficiencies (Kline et al., 1994; Blaya et al., 2014; Shear et al., 2015), disrupted cognitive function (Horneman and Emanuelson, 2009; Levin et al., 2010; Barry and Tomes, 2015; Richter et al., 2015), and fatigue (LaChapelle and Finlayson, 1998). While public awareness has increased regarding the dangers of TBI, the affective and economic costs of TBIs remain astronomical and effective treatment strategies are scarce (Doppenberg et al., 2004; Menon, 2009). However, promoting recovery of motor and cognition and managing fatigue continue to be primary concerns of health specialists as they strive to assimilate patients back into society.

Neurorehabilitation and its rodent analogue environmental enrichment (EE) have both been successful in conferring robust benefits after TBI (Chua et al., 2007; de Witt et al., 2011; Bondi et al., 2014;

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Radabaugh et al., 2016; Hart et al., 2016). Clinical rehabilitative treatments employ a transdisciplinary team of specialists to target multiple different aspects of each unique injury resulting in the patient being exposed to a wide variety of stimuli (Chua et al., 2007). However, with 29% to 47% of TBI patients reporting abnormal levels of fatigue within the first month after injury (Minderhoud et al., 1980), longer rehabilitation sessions may prove to be less productive as the fatigue becomes distracting or unbearable (Keshaven et al., 1981; LaChapelle and Finlayson, 1998). Hence, multiple shorter sessions of rehabilitation may ease fatigue and afford more productive rehabilitation efforts as well as increased interaction with the stimuli.

EE mimics many of the stimuli patients are exposed to in the clinic by encouraging voluntary exercise, increasing social interaction, and providing cognitive stimulation. While the stimuli are analogous to the clinic, the typically used paradigm, which consists of continuous exposure after TBI (Radabaugh et al., 2016; de Witt et al., 2011; Monaco et al., 2014; Sozda et al., 2010; Kline et al., 2007; Bondi et al., 2014, 2015) is quite dissimilar as patients do not receive rehabilitation on a continual basis. Previous studies have observed the effects of abbreviated EE after experimental TBI and reported that both male (de Witt et al., 2011) and female (Radabaugh et al., 2016) rats benefit from 6-hrs of EE exposure per day. Indeed, the benefits conferred by the 6-hr EE paradigm are not significantly different from the continuous approach. While patients in the clinic may spend up to 6-hrs participating in neurorehabilitation each day, it is often in multiple, shorter sessions that target different aspects of the recovery process (i.e., speech therapy, vocational therapy, exercise) (Chua et al., 2007). Therefore, progressive experimental manipulations to the typical EE paradigm could continue to refine EE and advance rehabilitation based research.

This study aimed to evaluate the effects generated by two 3-hr sessions of EE exposure each day compared to one 6-hr session. We hypothesized that the group receiving two shorter sessions of EE per day would recover more fully, as measured by well-established motor and cognitive tasks, than the one receiving one 6-hr session of EE. The rationale for the hypothesis is that rats placed into the EE cage twice per day and given a rest period will not be as negatively impacted by fatigue and may acclimate less to the environment thus promoting more interaction with the "novel" stimuli when reintroduced for the second session. This increased amount of engagement could provide further benefit from the rehabilitation and potentially lead to a greater degree of cognitive flexibility and motor ability. Support for this thesis comes from the work of Sozda et al. (2010) who showed that atypical EE groups, which had one of the critical components of EE (i.e., physical space, cognitive stimuli, or social interaction) excluded, did not recover to the same degree as those in the typical EE groups, indicating that exploration and engagement are important.

2. Materials and methods

2.1. Subjects and pre-surgical procedures

Fifty-one adult male Sprague-Dawley rats (Harlan, Indianapolis, Indiana) were paired 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 beam-walk task to ensure that they could traverse the entire length of the beam effortlessly, typically in under 5 s. On the morning of surgery, the rats were pre-assessed on the beam-balance and beam-walk tasks to determine baseline performance.

2.2. Surgery and acute neurological evaluation

Rats weighing 300–325 g on the day of surgery were subjected to a controlled cortical impact (CCI) injury as previously described (Dixon et al., 1991; Kline et al., 2000, 2007, 2010, 2012; Hoffman et al., 2008; Bondi et al., 2014; Leary et al., 2017). Briefly, a surgical level of anesthesia was induced with 4% isoflurane in 2:1 N_2O:O_2 and then the rats underwent an endotracheal intubation and secured in a stereotaxic frame and ventilated mechanically. The surgical plane of anesthesia was maintained with 2% isoflurane. Core temperature was maintained at 37 ± 0.5 °C with a heating pad. Utilizing aseptic procedures a midline scalp incision was made, the skull was exposed, 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 and removal from the stereotaxic apparatus 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. These neurological indices are used to determine the level of injury severity (Kline et al., 2007, 2010; Hoffman et al., 2008; Monaco et al., 2013, 2014). Following the acute neurological assessment the rats were randomly assigned to three TBI ($n = 12$ per condition) and three sham control ($n = 5$ per condition) groups.

2.4. Housing conditions: standard and environmental manipulation

After the effects of surgical anesthesia abated (as evidenced by spontaneous movement in the holding cage), all the rats were returned to the colony and placed in typical laboratory shoebox cages (37 × 25 × 18 cm, 2 rats per cage) with ad libitum food and water. Beginning at 9:00 a.m. the following morning, the rats randomized to a single 6-hr enrichment session were removed from the STD cages and placed in the EE cages until 3:00 p.m., at which time they were returned to STD housing. The rats assigned to two 3-hr sessions were also removed from STD conditions at 9:00 a.m. and returned to the STD cages at 12:00 p.m. where they remained until 3:00 p.m. and then placed back into enrichment until 6:00 p.m. These rehabilitative manipulations occurred each day for 21 days. Briefly, the EE is a specifically designed steel-wire cage (91 × 76 × 50 cm) that consists of three levels with ladders to ambulate from one level to another and contains 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). Ten to twelve rats, which included TBI and sham controls, were housed in the EE together to minimize variability among the groups.

2.5. Motor performance

Well-established beam-balance and beam-walk tasks were utilized to assess motor function (Kline et al., 2010, 2012; Cheng et al., 2008, 2012, 2016). Briefly, the beam-balance task consisted of placing the rat on an elevated narrow beam (1.5 cm wide) and recording the time it remained on for a maximum of 60 s. The beam-walk test consisted of recording the elapsed time to traverse the beam (2.5 cm wide × 100 cm long). Baseline performance was assessed 1 h prior to surgery. Following surgery, testing was conducted on days 1–5, and consisted of three trials (60 s allotted time with an inter-trial interval of 30 s) per day on each task. The average daily scores for each subject were used in the statistical analyses.