JOURNAL OF NEUROTRAUMA 27:1047–1057 (June 2010)
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DOI: 10.1089/neu.2010.1313

Empirical Comparison of Typical and Atypical Environmental Enrichment Paradigms on Functional and Histological Outcome after Experimental Traumatic Brain Injury

Christopher N. Sozda,[1,2,a] Ann N. Hoffman,[1,2,b] Adam S. Olsen,[1,2,c] Jeffrey P. Cheng,[1,2] Ross D. Zafonte,[6–8] and Anthony E. Kline[1–5]

Abstract

Several studies have shown that housing rats in an enriched environment (EE) after traumatic brain injury (TBI) improves functional and histological outcome. The typical EE includes exploratory, sensory, and social components in cages that are often vastly larger than standard (STD) housing. It is uncertain, however, whether a single or specific component is sufficient to confer these benefits after TBI, or if all, perhaps in an additive or synergistic manner, are necessary. To clarify this ambiguity, anesthetized adult male rats were subjected to either a controlled cortical impact or sham injury, and then were randomly assigned to five different housing paradigms: (1) EE (typical), (2) EE (−social), (3) EE (−stimuli), (4) STD (typical), and (5) STD (+stimuli). Motor and cognitive function were assessed using conventional motor (beam-balance/traversal) and cognitive (spatial learning in a Morris water maze) tests on postoperative days 1–5 and 14–19, respectively, and cortical lesion volume and CA1/CA3 cell loss were quantified at 3 weeks. No significant differences were observed among the sham groups in any comparison and thus their data were pooled (i.e., SHAM). In the TBI groups, typical EE improved beam-balance versus both STD (+stimuli) and EE (−social), it facilitated the acquisition of spatial learning and memory retention versus all other housing conditions ($p < 0.003$), and it reduced lesion volume and CA3 cell loss versus STD (typical) housing. While rats in the three atypical EE conditions exhibited slightly better cognitive performance and histological protection versus the typical STD group, the overall effects were not significant. These data suggest that exposing TBI rats to any of the three components individually may be more advantageous than no enrichment, but only exposure to typical EE yields optimal benefits.

Key words: behavior; controlled cortical impact; enrichment; recovery; spatial learning; water maze

Introduction

Traumatic brain injury (TBI) is a significant health care issue for which there are limited treatment options. As such, numerous preclinical therapeutic approaches have been attempted. While the vast majority of studies have focused on pharmacological agents (Bales et al., 2009; Feeney and Sutton, 1987; Kokiko and Hamm, 2007; McIntosh, 1993; Parton et al., 2005), physiotherapeutic paradigms such as exercise and enriched environment (EE) have also been, and continue to be, evaluated (Gaulke et al., 2005; Griesbach et al., 2009; Hamm et al., 1996; Hicks et al., 2002; Hoffman et al., 2008; Kleim et al., 2003; Kline et al., 2007; Passineau et al., 2001; Wagner et al., 2002; Will et al., 2004). Regarding the latter, typical EEs consist of expansive accommodations filled with several rats and novel objects, which afford exploration and physical exercise, as well as sensory and social integration that stimulate and enrich the senses. These unique environments are in marked

[1]Physical Medicine and Rehabilitation, [2]Safar Center for Resuscitation Research, [3]Department of Psychology, [4]Center for Neuroscience, and [5]Center for the Neural Basis of Cognition, University of Pittsburgh, Pittsburgh, Pennsylvania.
[6]Physical Medicine and Rehabilitation, Harvard Medical School, [7]Spaulding Rehabilitation Hospital, and [8]Physical Medicine and Rehabilitation, Massachusetts General Hospital, Boston, Massachusetts.
Current address: [a]Department of Clinical and Health Psychology, University of Florida, Ganesville, Florida.
[b]Department of Psychology, Arizona State University, Tempe, Arizona.
[c]College of Medicine, University of Toledo, Toledo, Ohio.

contrast to standard (STD) housing, where rats, typically two per standard steel-wire mesh cage, are provided with only basic sustenance (i.e., food and water).

In addition to the plasticity-associated adaptations observed in non-injured animal models (Bennett et al., 1969; Bruel-Jungerman et al., 2005; Falkenberg et al., 1992; Frick and Fernandez, 2003; Ickes et al., 2000; Kempermann et al., 1997; Leggio et al., 2005; Nilsson et al., 1999; Nithianantharajah et al., 2004; Olson et al., 2006; Torasdotter et al., 1998; van Praag et al., 2000; Zhao et al., 2000, 2001), EE has also been studied in a number of brain-injury paradigms. In the developing rat pup, EE has been reported to improve behavioral performance after fluid percussion brain injury (Giza et al., 2005), and to enhance dendritic density in the occipital cortex ipsilateral to the injury (Ip et al., 2002). In the adult rat, EE has been shown to enhance motor performance on a beam-walk task after sensorimotor cortex lesions (Gentile et al., 1987; Held et al., 1985; Rose et al., 1987). Enrichment has also been shown to enhance the acquisition of spatial learning in a water maze task (Morris, 1984) after moderate (Hamm et al., 1996) or severe (Passineau et al., 2001) fluid percussion brain injury. EE also significantly benefits cognitive and motor function after controlled cortical impact (CCI) injury (Hoffman et al., 2008; Kline et al., 2007), and attenuates hippocampal CA3 cell loss (Kline et al., 2007).

Given the aforementioned benefits in behavioral and histological outcomes after TBI, EE can be considered a reasonable animal correlate of the clinical rehabilitation process. The parallel can be appreciated in that often after severe injuries, patients may receive therapeutic intervention in a rehabilitation setting, or they may simply receive care at home or at a nursing facility. Although not well validated through rigorous clinical trials, the cognitive and physical stimulation associated with acute rehabilitation is believed to provide an enriching environment for patients, which enhances recovery compared to a convalescent setting. Further, during the rehabilitation phase of recovery, pharmacotherapies may also be utilized, which may act in concert with the rehabilitation program in promoting recovery. A recent study from our laboratory suggests that combining EE and chronic treatment with the 5-HT_{1A} receptor agonist 8-OH-DPAT may provide an additive effect on some aspects of the recovery process (Kline et al., in review).

Hence, understanding what aspects of EE are important for mediating the observed beneficial effects seen after TBI is critical for optimizing the rehabilitation experience. However, as indicated earlier, the typical EE consists of three basic components (increased space for exploration and exercise, sensory experience, and socialization), and it is still unknown which of these, or combination of them, is most important for promoting functional recovery after TBI. It is possible that only a single component is needed to confer benefits, or it could also be that each aspect is necessary by contributing in an additive or synergistic fashion. Determining the component of EE that facilitates the recovery process after experimental brain trauma in rodents could potentially help to tailor more effective rehabilitation strategies in human TBI survivors, as the elements deemed to be more important could be implemented more robustly. Importantly, determining which enrichment component most effectively mediates recovery is not limited to TBI, as exposure to EE has been shown to produce benefit across a range of neurodegenerative disorders, including experimental parkinsonism (Jankowsky et al., 2005), and Alzheimer's disease (Cracchiolo et al., 2007; Faherty et al., 2005).

Given the potential neuroprotective advantages of customizing human physiotherapy, the current study was designed to elucidate what component of the EE paradigm is most important for instilling functional recovery after experimental brain trauma. The general hypothesis is that the typical EE paradigm (i.e., one combining exploratory opportunities, sensory stimulation, and socialization) would confer greater behavioral recovery than any other housing condition (i.e., atypical EE or STD).

Methods

Subjects

Seventy-two adult male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 300–325 g on the day of surgery were initially housed (two per cage) in standard steel-wire mesh cages and maintained in a temperature- and light-controlled environment ($21 \pm 1^{\circ}$C; 12-h light/dark cycle), with food and water available *ad libitum*. After 1 week of acclimatization the rats were prepared for surgery.

Surgical procedure

Surgical anesthesia was induced and maintained with inspired concentrations of 4% and 2% isoflurane (IsoFlo®; Halocarbon Products Corp., North Augusta, SC), respectively, in 2:1 N_2O:O_2. After endotracheal intubation the rats were secured in a stereotaxic frame, ventilated mechanically (Harvard Rodent Ventilator Model 683, Harvard Apparatus Inc., Holliston, MA), and temperature maintained at $37 \pm 0.5^{\circ}$C with a heating blanket. Utilizing aseptic procedures a midline scalp incision was made, the skin and fascia were reflected, and a craniectomy (6 mm in diameter) was made in the right hemisphere (between the bregma/lambda and the sagittal suture and coronal ridge) with a hand-held trephine (Miltex Instrument Company Inc., Bethpage, NY). The bone flap was removed and the craniectomy was enlarged further with cranial rongeurs. CCI injury was produced as previously described (Cheng et al., 2008; Dixon et al., 1991; Hoffman et al., 2008; Kline et al., 2004a,b). Briefly, the impacting rod was extended and the impact tip (6 mm, flat) was centered and lowered over the craniectomy until it contacted 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/sec). Immediately after the CCI, anesthesia was discontinued, the incision was promptly sutured, and the rats were extubated. Sham rats underwent analogous surgical preparations, but were not subjected to the impact. All experimental procedures were approved by the Animal Care and Use Committee at the University of Pittsburgh, and were conducted in accordance with the recommendations provided in the *Guide for the Care and Use of Laboratory Animals* (1996). Every attempt was made to limit the number of subjects used and to minimize discomfort.

Acute neurological evaluation

Immediately following the cessation of anesthesia, hindlimb reflexive ability was assessed by gently squeezing the

rat's paw every 5 sec 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 to turn from the supine to prone position. These evaluations have been reported to be sensitive indicators of injury severity and anesthetic effects (Cheng et al., 2008; Dixon et al., 1999, 2003; Hoffman et al., 2008; Kline et al., 2004a,b)

Group randomization

After completion of the acute neurological evaluations, five TBI ($n = 10$ per group) and five sham ($n = 4$–5 per group) groups were randomly assigned to five housing conditions, which consisted of typical EE, typical STD, and three atypical EEs [i.e., EE (−social), EE (−stimuli), and STD (+stimuli)]. See Figure 1 and the following section for details on what components made up the atypical environments.

Housing conditions: Environmental manipulation

Following surgery the EE rats were returned to the colony designated for typical EE. They were placed in a 36″ × 30″ × 20″ steel-wire cage with three levels (and ladders to ambulate from one level to another), containing various toys (e.g., balls, blocks, and tubes for tunneling), and materials for nesting (e.g., cloth and paper towels), as well as *ad libitum* food and water (Fig. 2). To maintain novelty, the objects were rearranged daily and changed each time the cage was cleaned, which was approximately every 3 days. Ten rats, which included both TBI and sham controls, were housed in the EE at any given time. For those animals that were assigned to the atypical EE conditions, the EE cage was altered according to group assignment (Fig. 1). For example, rats in the EE (−social) condition received the toys and large cage, but were housed two per cage. Rats in the EE (−stimuli) condition were housed in the large cage ($n = 10$), but were not exposed to the toys. Rats in the typical STD conditions were placed in standard steel-wire mesh cages (two rats per cage) with only food and water, while those assigned to the STD (+stimuli) condition were provided with a few toys in the STD cage.

Motor performance

Established beam-balance and beam-walk tasks were used to assess motor function. Briefly, the beam-balance task consists of placing the rat on an elevated (90 cm) narrow beam (1.5 cm wide) and recording the time it remains on up to a maximum of 60 sec. The beam-walk task, originally devised by Feeney and colleagues (1982), consists of training/assessing rats using a negative-reinforcement paradigm to escape a bright light and white noise by traversing a narrow elevated beam (2.5 × 100 cm), and entering a darkened goal box situated at the opposite end. When the rat entered the goal box the adverse stimuli (light and noise) were terminated, thus serving as negative reinforcement (reward) for completing the task. Performance was assessed by recording the time it took for the rat to traverse the beam. The rats were trained on both motor tasks 1 day prior to surgery, and baseline performance was assessed on the day of surgery. Post-surgery motor function was assessed on postoperative days 1–5. Balance and traversal times were recorded. Each rat was given three trials (60 sec allotted time with an inter-trial interval of 30 sec) per day on each task. The average daily scores for each subject were used in the statistical analyses.

Cognitive function: Acquisition of spatial learning and memory retention

Spatial learning was assessed for five consecutive days (postoperative days 14–18) in a Morris water maze (MWM) task established as a sensitive measure of cognitive function after TBI (Hamm et al., 1992; Hoffman et al., 2008; Kline et al., 2001, 2002a,b; Scheff et al., 1997). Briefly, the maze consisted of a plastic pool (180 cm diameter and 60 cm high) filled with tap water (26 ± 1°C) to a depth of 28 cm that was situated in a room with salient visual cues that remained constant throughout the study. The platform was a clear acrylic glass stand (10 cm diameter and 26 cm high) that was positioned 26 cm from the maze wall in the southwest quadrant and held constant for each rat. Each rat was given a block of four daily trials (120 sec allotted time with an inter-trial interval of 4 min) to locate the platform when it was submerged 2 cm below the water's surface (i.e., invisible to the rat). To control for the contributions of non-spatial factors (e.g., sensorimotor performance, motivation, and visual acuity) on maze performance, each rat was provided with an additional day (postoperative day 19) of testing to locate the platform when it was raised 2 cm above the water's surface (i.e., visible to the rat). 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 randomized manner. Each trial lasted until the rat climbed onto the platform or until 120 sec had elapsed, whichever occurred first. Rats that failed to locate the platform within the allotted time were manually guided to it by the experimenter. All rats remained on the platform for 30 sec before being placed in a heated incubator between trials. The average

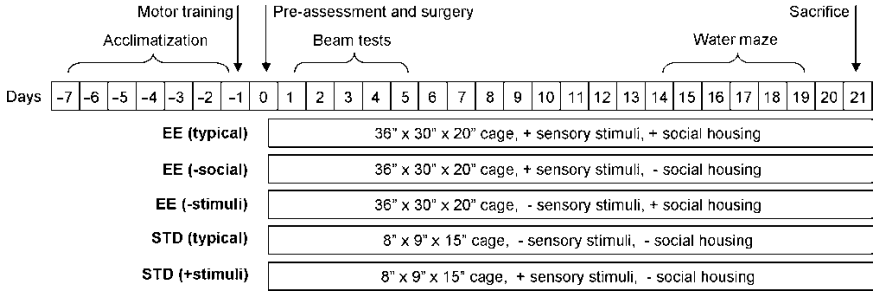

FIG. 1. Flow chart of the experimental paradigm and enrichment manipulations. EE (typical) refers to typical enrichment housing (see Fig. 2), and STD (typical) refers to typical standard housing (i.e., two rats in a standard size steel-wire cage with only food and water). EE (−social), EE (−stimuli), and STD (+stimuli) each refer to atypical EE paradigms.

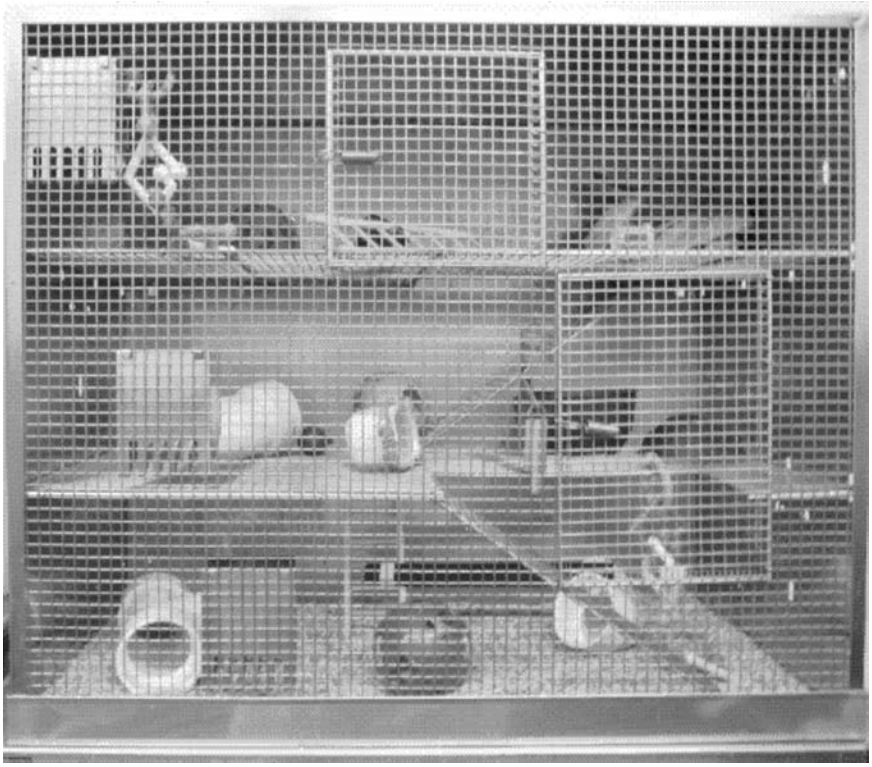

FIG. 2. Photograph of the EE cage with multiple levels and a wide array of sensory stimuli (e.g., balls, ramps, tubes, and nesting materials). Ten rats, which included both TBI animals and sham controls, were continuously housed here with the exception of brief removal for behavioral assessments. Together these components make up the "typical" EE.

time of the four daily trials for each rat were used in the statistical analyses. To measure retention of spatial learning all rats were given a single probe trial on postoperative day 19, which was 1 day after the final acquisition training session. Briefly, the platform was removed from the pool and the rats were placed in the maze at a location most distant from the quadrant where the platform was previously situated (i.e., the target quadrant) and allowed to freely explore the pool for 30 sec. In principle, rats that have learned the specific location of the hidden escape platform will exhibit a spatial bias and thus spend significantly more time in the target quadrant. All the data, which included time to locate the platform, distance to the platform, time in the target quadrant, and swim speed (assessed during the visible platform test), were obtained using a spontaneous motor activity recording and tracking (SMART) system (San Diego Instruments, San Diego, CA).

Histological assessments

Tissue sectioning and hippocampal cell quantification. Three weeks after CCI injury or sham injury, the rats were anesthetized with pentobarbital (50 mg/kg IP) and perfused transcardially with 200 mL heparinized 0.1 M phosphate-buffered saline (pH 7.4), followed by 300 mL 10% buffered formalin. The brains were extracted, post-fixed in 10% buffered formalin for 1 week, dehydrated with alcohol, and embedded in paraffin. Coronal sections 7 μm thick were cut at 1-mm intervals through the lesion on a rotary microtome and mounted on gelatin-coated glass microscope slides. After drying at room temperature, the sections were deparaffinized in xylene, rehydrated, and stained with cresyl violet. An observer blinded to experimental conditions analyzed coronal sections underlying the area of contusion ($\sim$3.5 mm posterior to bregma) of all rats in each group for determination of treatment efficacy (i.e., housing conditions) on selectively vulnerable hippocampal CA1/CA3 neurons. To reduce counting errors associated with false-positive identification of dying neurons, the total number of CA1 and CA3 morphologically intact neurons (i.e., those with a clearly defined cell body and nucleus) were counted using a Nikon Eclipse E600 microscope (Nikon Corp., Tokyo, Japan) with a 40× objective. To maintain consistency in quantification with previously published findings, the data are reported as the percent of total neurons in the ipsilateral (injured) CA1/CA3 regions relative to the contralateral hippocampus (Cheng et al., 2008; Dixon et al., 1999, 2003; Hoffman et al., 2008; Kline et al., 2007).

Cortical lesion volume

The area of the lesion (mm^2) was calculated in a subset ($n = 5$) of TBI animals from each housing condition by outlining the cortical lesion for each section taken at 1-mm intervals through the extent of the lesion (MCID; Imaging Research, Ontario, Canada). The volume (mm^3) of the lesion was determined by summing the areas of the lesion obtained from each section, as previously reported (Cheng et al., 2008; Hoffman et al., 2008; Kline et al., 2004a,b).

Statistical analysis

Statistical analyses were performed on data collected by observers blinded to housing conditions using StatView 5.0.1

software (Abacus Concepts, Inc., Berkeley, CA). The motor and cognitive data were analyzed by repeated-measures analysis of variance (ANOVA). The data for acute neurological assessments, histology, probe trial, and swim speed were analyzed by one-factor ANOVAs. When the overall ANOVA revealed a significant effect, the data were further analyzed with the Bonferroni/Dunn *post-hoc* test to determine specific group differences. The data are presented as the mean ± standard error of the mean (SEM), and are considered significant when corresponding p values are ≤ 0.05, or as determined by the Bonferroni/Dunn statistic after adjusting for multiple comparisons.

Results

No significant differences in acute neurological function, histological outcome, or in any of the behavioral assessments were observed among the sham groups, regardless of housing condition, and thus the data were pooled and analyzed as one inclusive group (denoted as "SHAM").

Acute neurological functioning

No significant differences were observed among the TBI groups in latency to elicit a withdrawal reflex in response to a brief paw pinch of the left and right limbs (range 182.3 ± 3.6 sec to 202.3 ± 7.7 sec, and 174.8 ± 3.5 sec to 197.1 ± 7.4 sec; $p > 0.05$, respectively), or for return of righting ability (range 362.4 ± 14.7 sec to 404.9 ± 22.3 sec; $p > 0.05$) after the cessation of anesthesia. The lack of significant group differences in these acute neurological indices suggests that all rats experienced an equivalent level of brain injury and anesthesia.

Motor function

No pre-surgical beam-balance differences were observed among groups, as all rats were capable of balancing for the allotted 60 sec (Fig. 3). However, a repeated-measures ANOVA on post-TBI data revealed significant group ($F_{5,66} = 7.724$; $p < 0.0001$) and day ($F_{5,330} = 72.955$; $p < 0.0001$) differences, as well as a significant group×day interaction ($F_{25,330} = 6.656$; $p < 0.0001$). The *post-hoc* test revealed that the typical STD and atypical EE groups were significantly different from the SHAM controls ($p < 0.0001$), while the typical EE was not ($p = 0.028$; $p = 0.003$ required for statistical significance after post-hoc adjustment). The *post-hoc* test further showed that the TBI + EE (typical) group recovered significantly quicker than the TBI + STD (+stimuli) and TBI + EE (−social) groups ($p = 0.003$ and $p = 0.0009$, respectively). No other group comparisons were significant. Similar to the beam-balance test, there were no pre-surgical differences in beam-walking among groups, as all rats were proficient and reached the goal box in approximately 5 sec. However, following TBI the ANOVA revealed significant group ($F_{5,66} = 14.831$; $p < 0.0001$) and day ($F_{5,330} = 168.702$; $p < 0.0001$) differences, as well as a significant group×day interaction ($F_{25,330} = 11.758$; $p < 0.0001$). The *post-hoc* analyses revealed that all TBI groups, regardless of housing condition, were significantly different from the SHAM controls ($p < 0.0001$), but were no different from one another, as they all reached baseline performance by the last day of testing (data not presented graphically due to a lack of statistical significance).

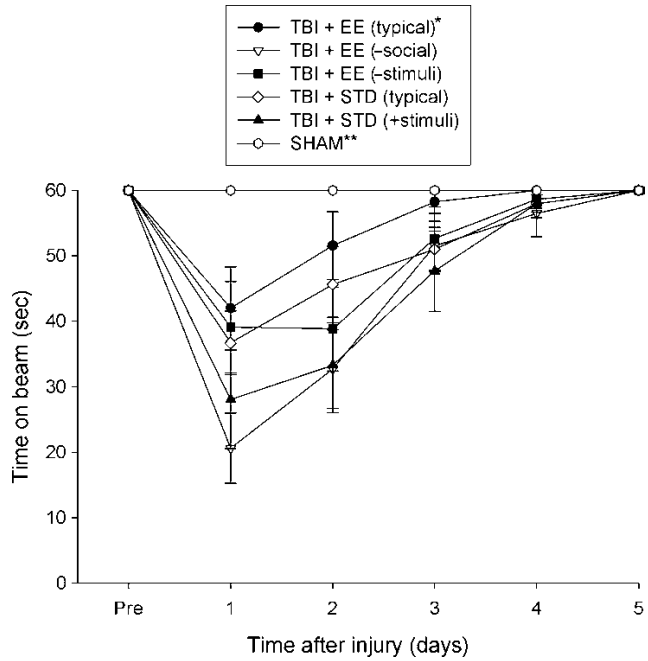

FIG. 3. Mean (±SEM) time (sec) of maintaining balance on an elevated narrow beam before and after TBI or SHAM injury [*$p = 0.003$ versus TBI + STD (+stimuli); *$p = 0.0009$ versus TBI + EE (−social); **$p < 0.0001$ versus the typical STD and atypical EE groups, but not the typical EE group].

Cognitive function: Acquisition of spatial learning

Analysis of spatial learning revealed significant group ($F_{5,66} = 20.152$; $p < 0.0001$) and day ($F_{4,264} = 36.267$; $p < 0.0001$) effects. While the TBI + EE (typical) group appears to be protected against TBI-induced cognitive deficits based on its performance on the first day of training (i.e., day 14) versus all other TBI groups, the *post-hoc* analysis for this single day was not significant. However, the *post-hoc* test did reveal that the TBI + EE (typical) group located the escape platform significantly quicker over time than all other TBI groups ($p < 0.001$). Moreover, the typical EE group was not statistically different from the SHAM controls after adjusting for multiple comparisons ($p = 0.0054$ versus 0.003). As depicted in Figure 4, all other TBI groups were significantly impaired relative to SHAM controls ($p < 0.0001$). Moreover, although the three atypical groups required less time to find the platform on the last day of training than the typical STD group, they were not statistically significantly different from one another. Analysis of path length, as a function of spatial learning, revealed significant differences between the SHAM and TBI groups. Specifically, all TBI groups swam farther distances to find the platform, which suggests significant impairments in the acquisition of spatial learning versus SHAM controls ($p < 0.0001$). However, the distance required to find the platform was significantly shorter for the TBI + EE (typical) group than for the TBI + EE (−social), TBI + STD (+stimuli), and TBI + STD (typical) groups ($p < 0.0029$; see Figs. 5 and 6 for representative swimming patterns and path lengths on the first day and last day of spatial learning). No differences were revealed among the other TBI groups, regardless of housing condition. A one-factor ANOVA performed on the visible

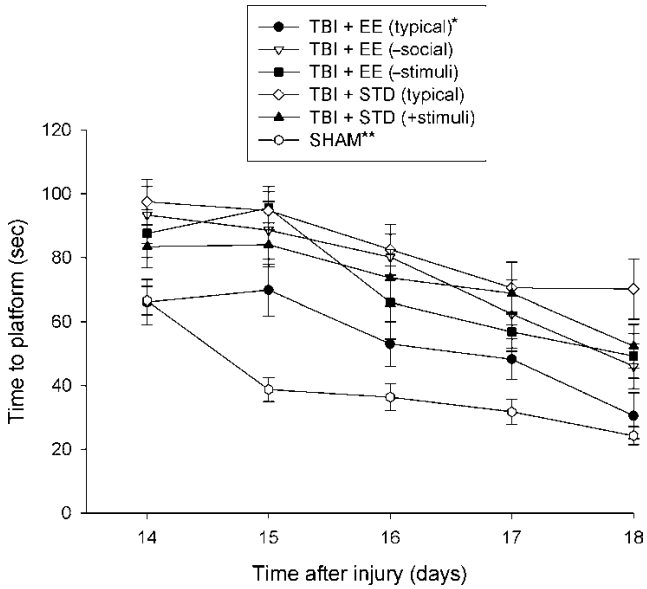

FIG. 4. Mean (±SEM) time (sec) to locate a hidden (submerged) platform in a water maze [*$p < 0.0013$ versus all other TBI groups; **$p < 0.0001$ versus all TBI groups, except TBI + EE (typical)].

platform data revealed a significant group [$F_{5,66} = 6.463$; $p < 0.0001$) effect, which was attributed to the SHAM group locating the platform quicker than the TBI + STD (typical) and TBI + EE (−stimuli) groups, which did not differ from the other TBI groups. No significant differences in swim speed (range = 26.7 ± 1.6 cm/sec to 28.3 ± 1.1 cm/sec) were observed among the groups.

Probe trial assessment

Analysis of probe data revealed a significant group ($F_{5,66} = 6.268$; $p < 0.0001$) effect, which was attributed to both the TBI + EE (typical) and SHAM groups performing better than all other TBI groups. Specifically, as depicted in Figure 7, typical EE enhanced memory retention, as evidenced by a significant spatial bias for the target quadrant in the TBI + EE (typical) group (39.3 ± 5.6%) versus all other TBI groups (all $p < 0.0012$). Moreover, the TBI + EE (typical) group did not differ from the SHAM controls (40.0 ± 1.6%; $p = 0.823$). Lastly, no differences were revealed among the TBI + EE (−social), TBI + EE (−stimuli), TBI + STD (+stimuli), and TBI + STD (typical) groups, which spent 29.4 ± 1.7%, 28.9 ± 1.9%, 28.3 ± 1.8%, and 25.6 ± 2.1%, respectively, of the 30-sec allotted time in the target quadrant.

Histology

Quantification of hippocampal neurons. CCI produced significant reductions in normal-appearing (i.e., morphologically intact) CA1 and CA3 neurons in the hippocampus ipsilateral to the impact. Regarding the CA1 region, all TBI groups differed from the SHAM control group ($p < 0.0001$). Specifically, the percentage of normal-appearing neurons in the SHAM group was 99.8 ± 1.8%, while in the TBI + EE (typical), TBI + EE (−social), TBI + EE (−stimuli), TBI + STD (typical), and TBI + STD (+stimuli) groups the percentages were 49.6 ± 6.0%, 43.9 ± 7.8%, 44.7 ± 6.4%, 39.6 ± 4.7%, and 42.2 ± 5.0%, respectively (the data are not presented graphically due to the lack of statistical significance). Regarding the CA3 region, all TBI groups differed from the SHAM group ($p < 0.0001$). Specifically, the percentage of normal-appearing neurons in the SHAM group was 99.7 ± 2.3%, while in the TBI + EE (typical), TBI + EE (−social), TBI + EE (−stimuli), TBI + STD (typical), and TBI + STD (+stimuli) groups the percentages were 63.2 ± 5.4%, 55.5 ± 6.7%, 52.3 ± 4.5%, 38.9 ± 4.2%, and 46.9 ± 5.2%, respectively. Furthermore, as depicted in Figure 8, typical EE conferred greater neuroprotection, as revealed by significantly more intact CA3 cells in the TBI + EE (typical) group than in the TBI + STD (typical) group ($p = 0.0026$). While all the atypical EE groups had slightly more viable CA3 neurons than the typical STD group, the differences were not statistically significant.

Cortical lesion volume

Cortical lesion volumes after CCI injury ranged from 33.4 ± 1.7 mm^3 to 42.1 ± 2.5 mm^3. The only significant difference was between the TBI + EE (typical) and TBI + STD (typical) groups ($p = 0.002$), which had the smallest and the largest volumes, respectively (Fig. 9). No other group comparisons were significant [TBI + EE (−social) = 40.6 ± 1.9 mm^3, TBI + EE (−stimuli) = 37.6 ± 1.5 mm^3, and TBI + STD (+stimuli) = 36.3 ± 1.1 mm^3].

Discussion

Typical EE includes the combination of exploratory, sensory, and social components, often in cages that are considerably larger than those used in most animal facilities, and exposing rats to such an environment confers neurobehavioral and histological benefits after brain trauma (Hamm et al., 1996; Hicks et al., 2002; Hoffman et al., 2008; Kline et al., 2007; Passineau et al., 2001), spinal cord injury (Berrocal et al.,

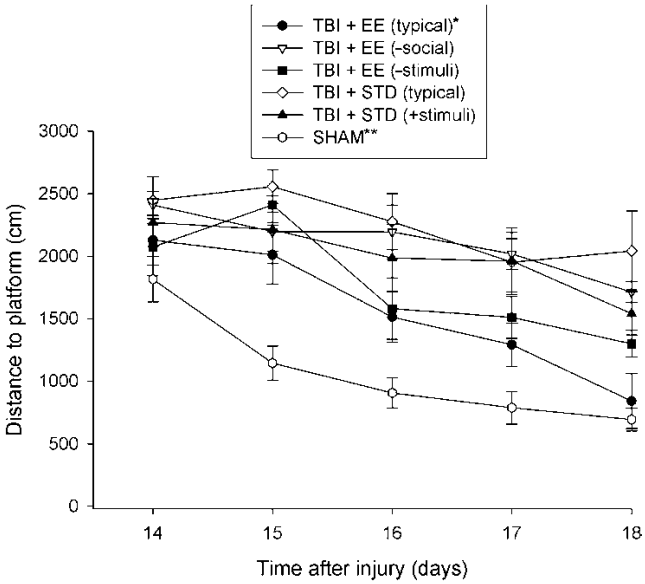

FIG. 5. Mean (±SEM) path length (cm) during acquisition of spatial learning in the water maze [*$p < 0.0029$ versus TBI + EE (−social), TBI + STD (+stimuli), and TBI + STD (typical); **$p < 0.0001$ versus all TBI groups].

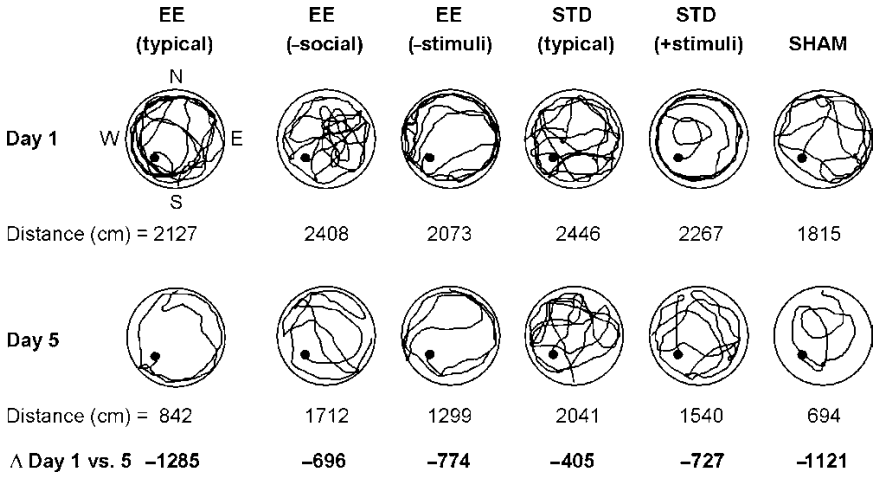

FIG. 6. Representative swim paths showing the mean distance traveled on training days 1 and 5 (i.e., postoperative days 14 and 18) in the water maze (• = location of platform in the southwest quadrant; Δ = mean change in swimming distance from training day 1 to day 5). Note that the typical EE and atypical EE groups had larger decreases in distance traveled than the typical STD group.

2007; Fischer and Peduzzi, 2006), and ischemia (Buchhold et al., 2007; Briones et al., 2009; Dahlqvist et al., 2004; Johansson and Ohlsson, 1996). Given the effectiveness of this therapy and the potential relevance for clinical TBI, the current study sought to determine whether a single or specific component is sufficient to confer these benefits, or if all (i.e., in an additive or synergistic manner) are necessary. In this study, the environments were manipulated such that each component of the typical EE could be evaluated individually. The rationale and significance of elucidating which component is more valuable for mediating recovery are that this aspect could be applied more robustly in clinical rehabilitation.

The data showed that typical EE accelerated the recovery of beam-balance performance more than the STD and atypical

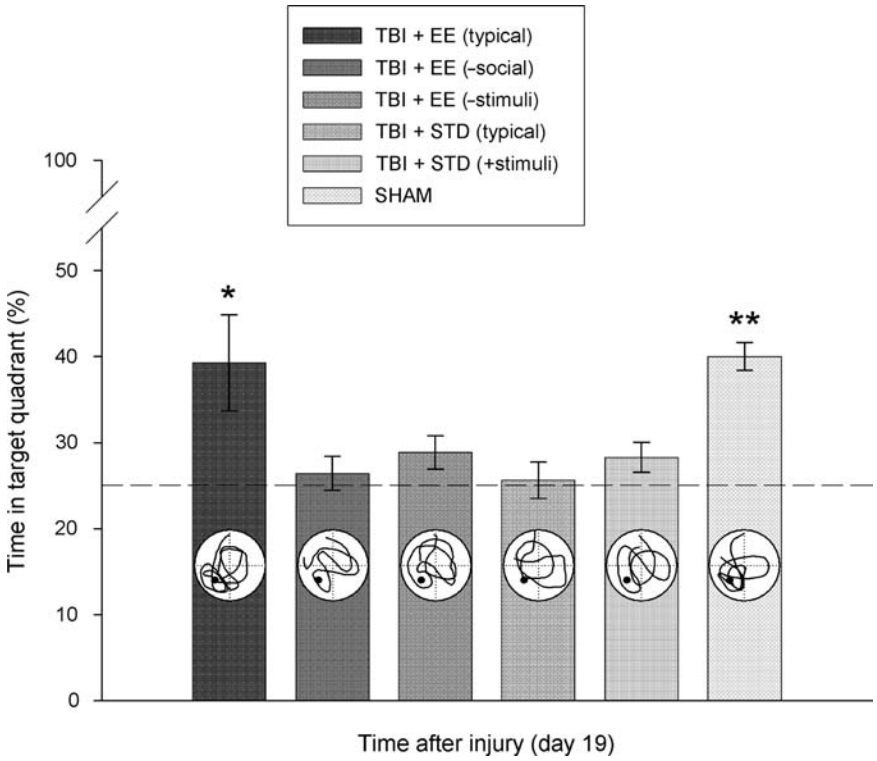

FIG. 7. Mean (±SEM) percentage of time spent in the target quadrant (i.e., where the platform had been previously located) following a single probe trial 19 days after TBI or sham injury [*$p < 0.0012$ versus all TBI groups; **$p < 0.003$ versus all TBI groups, except TBI + EE (typical), $p = 0.823$]. The dotted line represents performance at the chance level (25%), and the image of the maze inserted into each data bar depicts representative swim paths showing the percent of time in each quadrant (• = target quadrant).

EE conditions. This finding is consistent with previous reports from our laboratory, in which continuous EE was compared with STD housing (Hoffman et al., 2008; Kline et al., 2007). Unlike in previous reports, beam-walking ability did not differ overall among any of the TBI groups (Hoffman et al., 2008; Kline et al., 2007). Surprisingly, the STD-housed rats recovered to baseline levels at the same rate as the enriched rats. In previous studies from our laboratory, animals housed in the larger EE cage showed a more marked recovery of motor function than STD-housed rats. This has always been thought to result from the increased space available (i.e., the exploratory/exercise component) to the rats in the larger cage, which in turn afforded ample opportunity for locomotion that was manifested as improved beam-walking. Support for this notion is provided by a study by Hoffman and colleagues (2008), who showed that beam-walking ability was facilitated only in rats that were in the early, but not the delayed, EE group. This finding underscores the potential variability in using a gross motor function test that simply evaluates the time to traverse the beam, versus the more sensitive assessment of looking at how an animal traverses it (i.e., the quality of locomotion), such as the well-established hindlimb stepping paradigm of Feeney and associates (1982). Future studies evaluating motor recovery after EE warrant more sensitive evaluations.

In addition, the typical EE significantly improved the acquisition of spatial learning more than standard housing, which is also consistent with previous reports from our laboratory (Hoffman et al., 2008; Kline et al., 2007), and those of others (Hamm et al., 1996; Hicks et al., 2002; Passineau et al., 2001; Wagner et al., 2002). Moreover, the typical EE paradigm was also more effective in improving spatial acquisition than any of the atypical EE conditions, which though no different from one another were slightly better, albeit not significantly, than typical STD housing. Typical EE also improved memory retention more than all other housing conditions, as evidenced by a greater percentage of time spent in the target quadrant during a single-day probe trial. Lastly, typical EE significantly attenuated CA3 cell loss and cortical lesion volume more than typical STD. Again, this finding replicates previous data from our laboratory (Kline et al., 2007), and reinforces the significant neuroprotective benefit afforded by typical EE. Notably, all of the atypical groups also exhibited reductions in histopathology compared to the typical STD group, but these effects were not statistically significant.

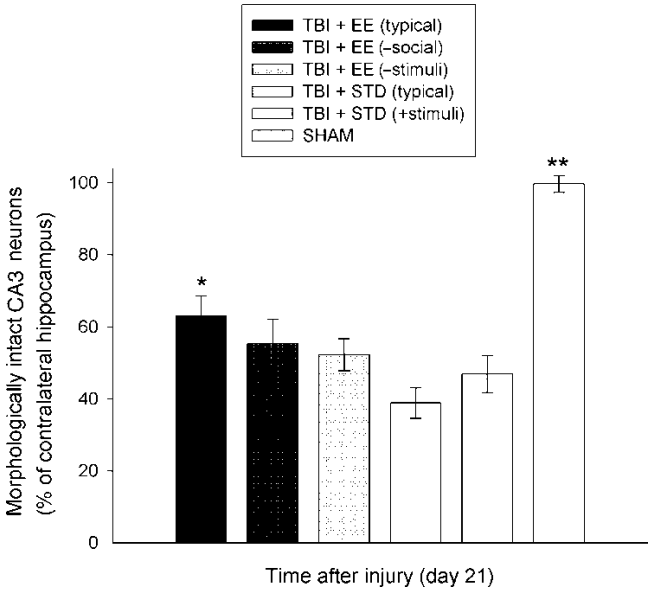

FIG. 8. Mean (±SEM) morphologically intact CA3 neurons (as % of those in the contralateral hippocampus) 3 weeks after TBI or sham injury [*$p = 0.0026$ versus TBI + STD (typical); **$p < 0.0001$ versus all TBI groups].

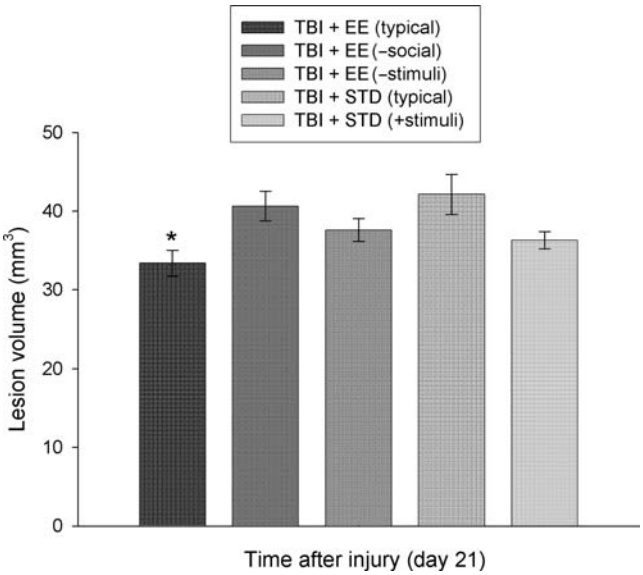

FIG. 9. Mean (±SEM) cortical lesion volume (mm^3) 3 weeks after cortical impact injury [*$p = 0.002$ versus TBI + STD (typical).

Taken together, these findings suggest that each individual component of EE adds some benefit to the overall effect seen after TBI, but the typical EE paradigm is optimal, which supports our hypothesis. While our findings of an additive effect with each individual EE component after TBI are novel, similar additive effects have been reported in models of ischemia (Johansson and Ohlsson, 1996), and Alzheimer's disease (Cracchiolo et al., 2007). Specifically, Johansson and Ohlsson showed that after ischemia, social interaction, accomplished by placing animals in a large cage, was superior to wheel-running in a standard cage, but an environment allowing free physical activity combined with social interaction resulted in the best performance on a series of motor tasks. In an Alzheimer's disease model, Cracchiolo and colleagues (2007) sought to determine which component of EE would protect mutant APP transgenic mice against cognitive impairment. The data showed that transgenic mice reared in impoverished conditions were not more impaired cognitively than transgenic mice reared in social or physical conditions, suggesting that individually neither is a viable form of behavioral enrichment. Indeed, the conclusion stated by the authors was that enhanced activity over and above that produced by social and physical activity was required to protect against cognitive impairment. In a seminal study evaluating the effects of enriched components in normal (i.e., non-injured) animals, Rosenzweig and colleagues (1978) included the typical exploratory, sensory, and social conditions in rodent cages, but also utilized a semi-natural environment,

which consisted of an outdoor pit filled with dirt, branches, stones, and growing weeds. Not surprisingly, the semi-natural environment produced the greatest increase in brain weight, it also decreased acetylcholinesterase (AChE) activity more than all other conditions. Moreover, the typical EE and social conditions showed lower levels of AChE activity than the impoverished condition, but the EE condition was better than the social component. Overall, these classic data demonstrate an additive effect of EE.

While it is generally acknowledged that the "typical" EE paradigm affords the greatest benefit, and our current data suggest that there is no real distinction between any of the components when provided individually, there is support for a more prominent role of social housing. From a theoretical perspective, it could be argued that the social component of the EE paradigm increases exploration and locomotor activity, as the rats "play" in an interactive milieu as suggested by Will and associates (2004). In terms of experimental data, as noted in the study by Johansson and Ohlsson (1996), the social group was much better in motor functioning than the exercise group. In a study assessing open field activity, social enrichment, but not physical enrichment, exerted the greatest benefit for both male and female rats (Elliott and Grunberg, 2005). In contrast, Schrijver and colleagues (2004) reported that the absence of socialization affected neither the acquisition of simple or complex discriminations, nor water maze performance, suggesting that it was not necessary. Furthermore, in a model of chronic inflammatory pain, Gabriel and co-workers (2009) reported that while both the physical and social aspects of the environment reduced the duration of mechanical allodynia versus the restricted or standard condition, the physical component had a greater effect than the social setting.

Despite the plethora of studies detailing the neurobehavioral benefits attributable to EEs, there is still much inconsistency in the literature regarding how each component is defined. For example, while there is agreement that social interaction is important, although some exceptions exist as noted above, there are usually significant differences in the number of animals that constitute a social environment. Studies such as ours (Hoffman et al., 2008; Kline et al., 2007) typically house 10–12 rats together, which encompasses a subset of all investigative groups. However, other investigators house only 3–5 rodents in a cage and consider that to be sufficient for EE or social grouping (Cracchiolo et al., 2007; Lambert et al., 2005; Schrijver et al., 2004). Further, there are vast differences in the size of cages and the materials that are placed in them. Some EE cages can be quite elaborate in size and detail (see Fig. 2 and Gabriel et al., 2009), and others are not much bigger than standard laboratory acrylic glass containers containing few, if any, stimuli. Thus, while we have shown that each individual component of the EE paradigm is important, as together they provide an additive effect on the recovery process after TBI, and others using various CNS injury models have come to the same conclusion, there is still a significant need for standardization of exactly what constitutes enrichment (Burke et al., 2007). Identification and standardization of EE components, cages, and stimuli is needed if this paradigm can someday be successfully translated into clinically-relevant rehabilitation approaches.

In conclusion, the findings of this study suggest that exposing TBI rats to any of the three EE components may be more advantageous than no enrichment, but only exposure to a typical EE yields optimal benefits. These findings may help tailor clinical rehabilitation so that maximal benefits can be achieved after brain trauma.

Acknowledgments

This work was supported, in part, by National Institutes of Health grants HD046700 and NS060005, awarded to A.E.K.

Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Anthony E. Kline, Ph.D.
Department of Physical Medicine & Rehabilitation
Department of Rehabilitation Research
Safar Center for Resuscitation Research
University of Pittsburgh
3471 Fifth Avenue, Suite 201
Pittsburgh, PA 15213

E-mail: klineae@upmc.edu