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Environmental enrichment-mediated functional improvement after experimental traumatic brain injury is contingent on task-specific neurobehavioral experience

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Abstract

Environmental enrichment (EE) is superior to standard (STD) housing in promoting functional recovery after traumatic brain injury (TBI). However, whether the EE-mediated benefits after TBI are dependent on exposure to enrichment during neurobehavioral training has not been elucidated. To address this issue, isoflurane-anesthetized adult male rats received either a cortical impact or sham injury and were then randomly assigned to early EE, delayed EE, continuous EE, or no EE (i.e., STD conditions). Continuous EE or no EE was initiated immediately after surgery and continued for the duration of the study. Early EE began directly after surgery, continued for one week, and was then followed by STD living (2 rats per cage) for the remainder of the study, while delayed EE commenced one week after early STD housing. Functional outcome was assessed with established motor and cognitive tests on post-injury days 1–5 and 14–18, respectively. CA_1/CA_3 neurons were quantified at 3 weeks. CA_3 cell loss was significantly attenuated in the TBI + continuous EE group vs. the TBI + no EE group. Beam-walking was facilitated in the TBI groups that received either early or continuous EE vs. those receiving delayed or no EE. Cognitive training was enhanced in the TBI groups that received continuous or delayed EE vs. the early EE or no EE groups. These data suggest that EE-mediated functional improvement after TBI is contingent on task-specific neurobehavioral experience.

Keywords

beam-walking; controlled cortical impact; functional recovery; learning and memory; Morris water maze; neurobehavior; traumatic brain injury

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Introduction

The administration of various pharmacotherapies is a widely used preclinical strategy to enhance motor and/or cognitive outcome after experimental TBI. Examples include acute and chronic treatment with the dopamine receptor agonists bromocriptine [14] and methylphenidate [12], as well as the 5-HT_{1A} receptor agonist 8-OH-DPAT [13,15,16]. Alternative therapeutic strategies such as neurotrophic support [3,20] or hypothermia [1,2] have also been successfully utilized. Non-invasive therapies such as voluntary exercise are also reported to influence functional outcome [6,11,22]. Another non-invasive and rehabilitation-relevant intervention that enhances functional outcome after TBI entails enriching the animals' living environment.

Environmental enrichment (EE) involves the integration of social interaction, increased living space, and various novel stimuli to create an environment that promotes motor and cognitive stimulation [22]. EE, which may be considered a rodent correlate of physiotherapeutic intervention has been extensively studied in numerous experimental conditions and has been reported to produce a plethora of anatomical responses, such as cortical thickening, dendritic arborization, hippocampal CA_3 cell survival, and growth factor expression [5,10,16–19,21,22]. Furthermore, early implementation and continuous presentation of EE enhances motor performance and spatial learning vs. standard (STD) housing after controlled cortical impact injury [16], fluid percussion TBI [8,19], or cortical aspiration lesions [9]. However, it has not been elucidated whether the EE-mediated benefits after TBI are contingent on continuous enrichment or if abbreviated exposure prior to or during neurobehavioral training is sufficient. It is plausible that an interaction between EE and neurobehavioral training is necessary for optimal recovery just as it has been shown for certain pharmacotherapeutic regimens combined with symptom relevant experience (SRE) [4,12]. Hence, the aim of the current study was to assess continuous, early, or delayed EE on functional and histological outcome after experimental TBI where the timing of the EE corresponded with specific behavioral training. The relevance of finding an optimal time to provide enrichment after TBI is based on the premise that EE is a relevant experimental analogue of rehabilitation, and may provide clinical utility alone or as an adjunct to pharmacotherapy if provided at the appropriate time.

Fifty adult male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 300–325 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 controlled (on 7:00 a.m. to 7:00 p.m.) environment with food and water available *ad libitum*. After one week of acclimatization the rats underwent a single day of beam-walk training, which consisted of five trials to traverse the beam in order to escape aversive stimuli (i.e., light and white noise), and then were prepared for surgery. Surgical procedures have been reported in detail elsewhere [13–16]. Briefly, isoflurane (4% in 2:1 N_2O/O_2) anesthetized rats were intubated, mechanically ventilated, and then subjected to either a right hemisphere controlled cortical impact (2.8 mm tissue deformation at 4 m/sec) or sham injury. Core temperature was maintained at 37 ± 0.5°C during surgery. Following the cessation of anesthesia, the rats underwent acute neurological assessments. Briefly, hind limb reflexive ability was assessed 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. Following surgery, the rats were randomly assigned to TBI + early EE (n=10), TBI + delayed EE (n=10), TBI + continuous EE (n=10), TBI + no EE (n=10), Sham + continuous EE (n=5), and Sham + no EE (n=5). The groups designated for early or continuous EE were immediately placed in a 92×76×51 cm steel-wire cage consisting of three levels (and ladders to ambulate from one level to another) with various toys (e.g., balls and blocks of various sizes and colors), tubes for tunneling, nesting materials (e.g., cloth and paper towels), and *ad*

libitum food and water [16]. To maintain novelty, the objects were rearranged every day and changed each time the cage was cleaned, which was approximately every 3 days. Ten to twelve rats, which included both TBI and sham controls, were housed in the EE at any given time. Rats in the STD conditions (i.e., no EE) were placed in standard sized steel-wire mesh cages (2 rats per cage) with only food and water. See Fig. 1 for a schematic of the experimental housing paradigm. 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* (National Academy Press, 1996). Every attempt was made to limit the number of animals used and to minimize suffering.

Motor function was assessed by established beam-balance (BB) and beam-walk (BW) tasks. The BB task consists of placing the rat on an elevated (90 cm) narrow wooden beam (1.5 cm wide) and recording the time it remains on for a maximum of 60 sec. The BW task consists of recording the elapsed time to traverse the beam. Rats were tested for BB and BW performance immediately before surgery (to establish a baseline measure), as well as on post-operative days 1–5, and consisted of three trials (60 sec allotted time with an intertrial interval of 30 sec) per day on each task. The average daily scores for each subject were used in the statistical analyses.

Spatial learning was assessed in a Morris water maze (MWM) task demonstrated to be sensitive to cognitive function/dysfunction after TBI [7,14]. Briefly, the maze consisted of a plastic pool (180 cm diameter; 60 cm high) filled with tap water (26 ± 1°C) to a depth of 28 cm and was situated in a room with salient visual cues. The platform was a clear Plexiglas stand that was positioned in the southwest quadrant and held constant for each rat. Spatial learning acquisition began on post-operative day 14 and consisted of providing a block of four daily trials (4-min inter-trial interval) for five consecutive days (14–18) to locate the platform when it was submerged 2 cm below the water surface (i.e., invisible to the rat). On post-operative day 19 the platform was raised 2 cm above the water surface (i.e., visible to the rat) as a control procedure to determine the contributions of non-spatial factors (e.g., sensory-motor performance, motivation, and visual acuity) on MWM performance. Swim speed was also assessed on this day. 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 goal 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. The times of the 4 daily trials for each rat were averaged and used in the statistical analyses. The data were obtained using a spontaneous motor activity recording & tracking (SMART) system (San Diego Instruments, San Diego, CA).

Three weeks after CCI or sham injury the rats were prepared for histological assessment as previously reported [13,14]. Briefly, the rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and then perfused transcardially with heparinized saline (pH 7.4) followed by 10% buffered formalin. The brains were extracted, post-fixed in formalin, dehydrated with alcohols, and embedded in paraffin. 7-μm thick coronal sections were cut at 1-mm intervals through the lesion and mounted on gelatin-coated glass microscope slides. After drying, the sections were deparaffinized in xylenes, rehydrated, and stained with Cresyl violet. An observer blinded to experimental conditions analyzed one coronal section underlying the area of contusion (~ 3.5 mm posterior to bregma) from all rats in each group for determination of treatment efficacy on selectively vulnerable hippocampal CA_1 and CA_3 neurons. To reduce counting errors associated with false positive identification of dying neurons, the total number of CA_1 and CA_3 morphologically intact neurons were counted using a Nikon Eclipse E600 microscope (Nikon Corporation, Tokyo, Japan) with a 40× objective. Morphologically intact neurons were characterized by a clearly defined cell body

and nucleus. The data are reported as the percent of total neurons in the ipsilateral (injured) CA_1 and CA_3 regions relative to the contralateral hippocampus.

Statistical analyses were performed on data collected by observers blinded to treatment 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 acute neurological assessments, histological, and swim speed data were analyzed by one-factor ANOVAs. When the overall ANOVAs revealed a significant effect, the Bonferroni/Dunn post-hoc test was used to determine specific group differences. The data are presented as the mean ± standard error (SE) and are considered significant when corresponding *p* values are < 0.05 or as determined by the Bonferroni/Dunn statistic after correcting for multiple comparisons.

No significant differences were observed among the TBI groups in hind limb withdrawal response latencies after a brief paw pinch [range 179.5 ± 3.2 sec to 192.3 ± 5.1 sec, $p > 0.05$] or for return of the righting reflex [range 331.4 ± 14.35 sec to 366.8 ± 10.8 sec, $p > 0.05$] after the cessation of anesthesia. The lack of significant differences with these acute neurological indices suggests that all TBI groups experienced an equivalent level of injury and anesthesia. Despite similar injury severity, one rat from the TBI + early EE group was unable to locate the visible platform, which may be indicative of visual deficits, and was therefore excluded from the study. Thus, the statistical analyses are based on forty-nine rats. Furthermore, because there were no significant differences in any outcome measure among the sham-injured groups [*p*'s > 0.05], the data were pooled and analyzed as one group (designated as SHAM).

All rats were capable of balancing on the beam and as such no pre-surgical differences were observed among groups. Similarly, there was no significant difference among groups in time to traverse the beam prior to injury [$p > 0.05$]. However, after the cortical impact significant impairments were detected in both BB and BW tasks for all TBI groups vs. SHAM controls [$p < 0.05$]. No significant difference in BB ability was observed among the TBI groups post-injury regardless of housing condition [$p > 0.05$, data not shown]. However, as depicted in Fig. 2, being in the enriched environment during locomotor testing improved BW ability as demonstrated by both the TBI + early EE and TBI + continuous EE groups recovering significantly faster than the TBI + delayed EE and TBI + no EE groups [*p*'s < 0.05]. No other group comparisons were significant [$p > 0.05$].

Analysis of spatial learning acquisition revealed significant group [$F_{4, 44} = 19.631$, $p < 0.0001$] and day [$F_{4, 176} = 19.902$, $p < 0.0001$] effects. All TBI groups were significantly impaired relative to SHAM controls, which were quite proficient in performing the task over the testing period. As depicted in Fig. 3, exposure to the enriched environment during training facilitated spatial learning and memory as evidenced by shorter times to locate the escape platform in the TBI + continuous EE and TBI + delayed EE groups vs. the TBI + early EE and TBI + no EE groups [*p's* < 0.0001]. No significant differences were revealed between the delayed and continuous EE groups [$p = 0.48$] or the early EE vs. no EE groups [$p = 0.30$].

No significant difference in swim speed (range = 23.1 ± 2.8 cm/sec to 33.6 ± 0.9 cm/sec) or visible platform acquisition (Fig. 3) was observed among the TBI groups vs. SHAM controls, suggesting that the accurate assessment of place learning was not precluded by extraneous factors.

TBI produced significant reductions in normal (i.e., morphologically intact) CA_1 and CA_3 neurons in the hippocampus ipsilateral to the impact. All TBI groups differed from the SHAM group, but not from one another in the percentage of normal appearing CA_1 neurons

(Table 1). The mean ratio of morphologically intact CA_3 neurons was significantly reduced in all TBI groups, regardless of condition, vs. SHAM [$p < 0.05$]. Furthermore, as detailed in Table 1, EE conferred selective neuroprotection as revealed by greater CA_3 intact cells in the TBI + continuous EE group vs. the TBI + no EE group [$p = 0.0337$]. No other comparisons were significant.

Several laboratories have reported positive effects with EE as a therapeutic approach after experimental brain trauma [8,9,16,19,22]. The current study is the first to use an adult rat model of controlled cortical impact injury to determine whether the EE-mediated beneficial effects on recovery are contingent on continuous exposure to enrichment or if abbreviated exposure prior to or during task-specific training is sufficient. The study showed that beam-walking performance was facilitated in both the continuous EE and early EE groups compared to the delayed EE and no EE groups. Acquisition of spatial learning was expedited in the continuous and delayed EE groups versus the early EE and no EE groups. Lastly, continuous EE conferred significant protection of hippocampal CA_3 cells versus all other TBI conditions. Collectively, these findings suggest that the EE-mediated functional benefits are contingent on task-specific neurobehavioral experience, but not necessarily on duration of exposure.

Support for the experience versus exposure dichotomy stems, in part, from the cognitive data in the current study revealing that both the continuous EE and delayed EE groups - which did not differ significantly from one another despite the former having received an additional week of enrichment - performed better than the early EE and no EE groups. Moreover, unpublished data from our laboratory suggest that exposing rats to EE for two weeks after surgery and then transferring them to STD conditions while undergoing training in the water maze is ineffective in acquiring spatial learning and is similar to that observed in the early EE group in the present study, which received only one week of enrichment. Lastly, a study by Gaulke and colleagues showed that exposure to EE for as little as one hour on the days of testing improved functional outcome [5]. The data suggest that EE exposure is necessary during the training, but the length of exposure is not crucial. However, both timing and duration of EE does appear to be important for histological protection as only the continuous EE group exhibited a greater percentage of surviving CA_3 neurons. This histological finding suggests that EE-mediated protection of CA_3 cells is a continuous process.

The overall findings suggest that an interaction between EE and neurobehavioral training is important for recovery just as it has been shown for certain pharmacotherapies combined with SRE [4,12]. For example, d-amphetamine enhances BW performance in TBI rats when provided during the period of drug action [4], but if a similar dose of amphetamine is provided to rats without BW experience (i.e., no SRE), no recovery is observed. A similar finding is observed after a single dose of methylphenidate coupled with several rehabilitation trials (i.e., BW) [12]. In the current study, motor function was assessed on post-operative days 1–5 and thus both the early EE and continuous EE groups were in the EE cage during testing. It is plausible that performance was enhanced due to the increased area of the cage and the availability of ladders both of which allow for greater locomotion potentially mimicking physical rehabilitation. In marked contrast, both the delayed and no EE groups were in the small STD sized cages during the motor testing, which significantly restricts physical activity. The cognitive abilities may have been enhanced by the increased social behavior and constructive mental stimulation provided by the association with interchangeable objects (e.g., colorful toys, tunnels and nesting materials) that provided a novel and enriching environment.

In conclusion, EE is a robust, non-invasive therapeutic strategy that enhances neurobehavioral recovery and confers histological protection after experimental TBI. Moreover, the current data indicate that EE may not have to be initiated immediately after TBI for enhanced cognitive recovery, which contrasts with pharmacologic strategies targeting various neurotransmitter systems [12–16] or non-pharmacologic approaches such as hypothermia [1,2] that necessitate relatively early initiation for therapeutic efficacy. This study provides important clinical implications regarding the positive effects of noninvasive therapies for human TBI such as EE, which may benefit rehabilitation alone or as an adjunct to pharmacotherapies.

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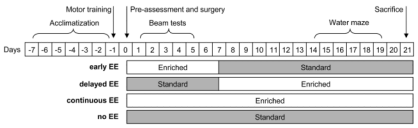

Fig. 1.

Flow chart of the experimental paradigm for TBI rats exposed to early EE, delayed EE, continuous EE, or no EE. Only TBI groups are depicted in the chart for simplicity.

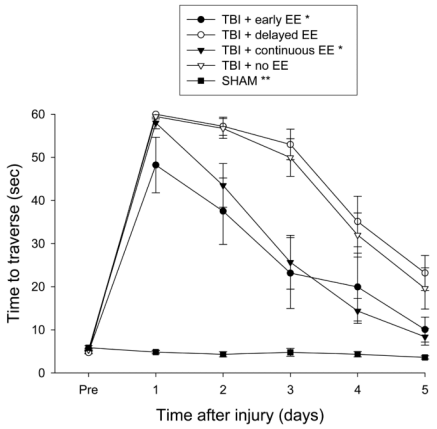

Fig. 2.

Mean (± SE) walking ability as measured by time (sec) to traverse an elevated wooden beam prior to, and after, TBI or SHAM injury. All TBI groups exhibited significant impairment vs. SHAM controls [$^{**}p < 0.05$]. Both the TBI + early and TBI + continuous groups, which were exposed to enriched living conditions during motor assessment exhibited an accelerated rate of recovery vs. the TBI + delayed EE and TBI + no EE groups that were housed in STD conditions during motor testing [$^{*}p < 0.05$].

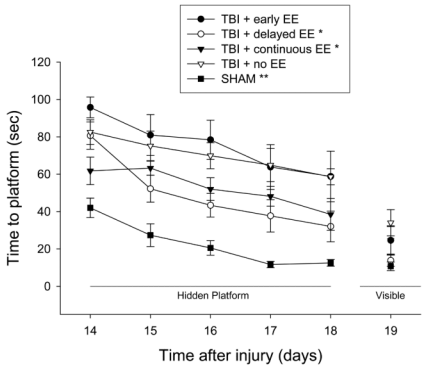

Fig. 3.

Mean (± SE) time (sec) to locate either a hidden (submerged) or visible (raised) platform in a water maze. All TBI groups were significantly different vs. SHAM controls [$^{**}p < 0.05$]. However, over the subsequent 5 days of training, the TBI + delayed EE and TBI + continuous EE, which were exposed to enriched living conditions during water maze training, learned the location of the escape platform significantly quicker than the TBI + early EE and TBI + no EE groups that were housed in STD conditions during cognitive training [$^{*}p < 0.05$]. No differences were observed among groups in locating the visible platform.

Table 1

Effect of early, delayed, continuous, or no EE on hippocampal cell survival quantified three weeks after TBI or sham injury.

Groups	*CA1*	*CA3*
TBI + early EE	39.8 ± 13.8	58.5 ± 15.9
TBI + delayed EE	35.9 ± 7.5	49.9 ± 6.7
TBI + continuous EE	50.2 ± 10.0	65.1 ± 5.6*
TBI + no EE	28.7 ± 12.8	35.9 ± 10.2
SHAM	100.1 ± 1.3**	99.9 ± 1.4**

Mean (± SE) normal appearing neurons expressed as a percentage of the contralateral hippocampus.

* $P < 0.05$ vs. TBI + no EE.

** $P < 0.05$ vs. all TBI groups.