Experience-dependent behavioral plasticity is disturbed following traumatic injury to the immature brain

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Abstract

Traumatic brain injury (TBI) is most prevalent in children and young adults. The long-term effects of pediatric TBI include cognitive and behavioral impairments; however, over time, it is difficult to distinguish individual variability in intellect and behavior from sequelae of early injury. Postnatal day (PND) 19 rats underwent lateral fluid percussion (FP) injury, followed by rearing in either standard (STD) or enriched environment (EE) conditions. The hypothesis was that the traditional enhancement of cognitive functioning following EE rearing would be attenuated when this rearing is preceded by TBI at PND19. Thirty days after injury, Morris water maze (MWM) acquisition and subsequent probe trial retention were used to assess the behavioral effects of injury on experience-dependent plasticity induced by housing in EE at two different time windows. MWM acquisition demonstrated improvements following early EE rearing in both sham and injured animals; however, the degree of improvement was greater for uninjured animals. When EE rearing was delayed for 2 weeks after injury, the injury effect was absent and the effect of rearing even stronger. Memory testing in the early EE groups using a delayed probe trial showed an effect of injury and housing, with the sham EE animals benefiting the most. After the delayed EE, sham EE animals again showed more probe target hits, while FP EE animals did not, demonstrating an enduring memory deficit. These data confirm that early TBI has effects on experience-dependent plasticity resulting in long-term neurobehavioral deficits. In addition, the ability to benefit from environmental stimulation following TBI is dependent upon time after injury.

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1. Introduction

Traumatic brain injury (TBI) is a leading cause of death and disability in children [1]. Injuries to the brain (ischemia, seizures, surgical resection) that occur during development are distinct from similar types of injuries sustained in adulthood [2–4]. While clinical experience suggests that biomechanical injury to the immature brain can result in abnormal development and long-standing neurobehavioral impairment [5–8], these deficits are difficult to characterize and their underlying mechanisms are uncertain. The current study was designed to further characterize the disruption of experience-dependent plasticity following TBI to the immature brain.

Fluid percussion (FP) is a well-established model of diffuse TBI that elicits a widespread depolarization of neurons, resulting in the indiscriminate release of glutamate and
potassium efflux into the extracellular space [9,10]. This triggers a pathophysiologic cascade that includes an acute increase of cerebral glucose utilization, followed by a period of several days of metabolic depression and corresponding reduced cerebral blood flow. Furthermore, a relatively prolonged (up to 4 days) accumulation of intracellular calcium also occurs [11,12]. In preweanling rat pups, FP has been shown to cause a similar acute increase in cerebral glucose uptake as seen in adults. However, the subsequent period of reduced glucose metabolism, which can last up to 7–10 days in adults, is scarcely detectable in injured pups [13]. Another important age-dependent difference is that within the same level of severity, lateral FP injury in rat pups does not result in the same morphological damage reported in adults. In fact, in PND17–19 pups, there is little histological evidence of acute cell death after lateral FP, nor is there a significant reduction in the number of cortical or hippocampal neurons 14 days after injury when measured using rigorous stereological techniques [14]. However, even though these animals exhibit little in the way of injury-induced cell death, the capacity of neuronal plasticity is compromised, as revealed by the fact that injured animals cannot respond to rearing in an enriched environment (EE) [5,6].

Rearing in an EE is one of the earliest experimental models of experience-dependent plasticity. In this paradigm, animals are communally housed in a large cage with multiple “toys” that are changed in type, location and number on a daily basis. Over time, these novel stimuli result in anatomical changes such as increased cortical thickness [5,15], increased dendritic branching [6,16], and even increased hippocampal neurogenesis [17]. In addition to sustained morphological changes, animals reared in EE manifest enhanced neurocognitive performance on radial arm [18] and Morris water maze (MWM) [5,19,20] tasks. Although adult animals benefit from being reared in an EE, the effects of EE rearing appear to be the most robust when it occurs early during development [18,21]. A paradoxical interaction between TBI and EE exposure has recently surfaced when comparing animals at different ages. It is now quite well accepted that adult rats benefit from housing in EE, the effects of EE rearing being targeted to the appropriate temporal window. However, even though these animals exhibit little in the way of injury-induced cell death, the capacity of neuronal plasticity is compromised, as revealed by the fact that injured animals cannot respond to rearing in an enriched environment (EE) [5,6].

2. Materials and methods

2.1. Subjects and experimental design

Male Sprague–Dawley rat pups (see Table 1) underwent sham or FP injury on PND19. All pups were housed as littermates with access to mothers until weaning on PND20, 1 day following surgery. In the early EE study, sham and injured pups 1 day post-surgery were weaned and housed in differential environments for 17 days. They were then returned to standard conditions until behavioral testing commenced at PND50. For the early EE study, two separate experiments of 24 rat pups each were run and the results were pooled. A total of 48 pups were randomly divided into 4 separate groups as follows: (A) sham standard housing (SSTD) n = 12 (13-1 excluded; see below); (B) sham enriched environment (SEE) n = 11; (C) fluid percussion standard (FPSTD) n = 10 (11–1 excluded); and (D) fluid percussion enriched environment (FPPEE) n = 13. Two animals (one SSTD and one FPSTD) were unable to swim adequately and were excluded. For the delayed EE study, 2 experiments, each using 24 rats, were performed. Animals were housed in standard conditions for 14 days, followed by differential housing for 17 days. The 48 animals in this arm were randomly divided into the following 4 groups: (A) SSTD, n = 12; (B) SEE, n = 12; (C) FPSTD, n = 11 (12–1 excluded); and (D) FPPEE, n = 12. One FPSTD rat developed a presumed inner ear infection with a head tilt, and was also excluded from the study. Behavioral testing was initiated on PND51. These two experimental designs are illustrated in Fig. 1. The UCLA Chancellor’s Committee for Animal Research approved all animal studies.
<table>
<thead>
<tr>
<th>Group</th>
<th>Weight start (g)</th>
<th>Drop (°)</th>
<th>Apnea (s)</th>
<th>LOC (s)</th>
<th>Weight end (g)</th>
<th>n</th>
</tr>
</thead>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>SSTD</td>
<td>46.0 ± 1.1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>360 ± 19.2</td>
<td>11</td>
</tr>
<tr>
<td>SEE</td>
<td>42.8 ± 1.0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>335 ± 4.6</td>
<td>12</td>
</tr>
<tr>
<td>FPSTD</td>
<td>42.8 ± 1.5</td>
<td>15.5 ± 0.2</td>
<td>242.0 ± 32.8</td>
<td>276.0 ± 31.4</td>
<td>367 ± 8.1</td>
<td>10</td>
</tr>
<tr>
<td>FPEE</td>
<td>44.2 ± 1.4</td>
<td>15.5 ± 0.2</td>
<td>236.8 ± 30.2</td>
<td>285.2 ± 32.8</td>
<td>366 ± 16.4</td>
<td>13</td>
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<tr>
<td>Delayed</td>
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<tr>
<td>SSTD</td>
<td>45.3 ± 1.4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>343.9 ± 9.7</td>
<td>12</td>
</tr>
<tr>
<td>SEE</td>
<td>44.0 ± 1.3</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>320.9 ± 14.0</td>
<td>12</td>
</tr>
<tr>
<td>FPSTD</td>
<td>46.2 ± 1.4</td>
<td>15.6 ± 0.1</td>
<td>160.5 ± 31.6</td>
<td>219 ± 24.3</td>
<td>337.0 ± 8.9</td>
<td>11</td>
</tr>
<tr>
<td>FPEE</td>
<td>42.4 ± 1.2</td>
<td>15.7 ± 0.1</td>
<td>240.7 ± 48.7</td>
<td>262.5 ± 45.5</td>
<td>317.8 ± 10.7</td>
<td>12</td>
</tr>
</tbody>
</table>

Group abbreviations are as follows: sham injury standard housing (SSTD), sham injury enriched environment housing (SEE), FP injury standard housing (FPSTD), and FP injury enriched environment housing (FPEE). The final column (n) represents the number of subjects in a particular group that completed the study. No significant differences were detected between groups in any of these measures, suggesting a similar level of injury.

2.2. Fluid percussion injury

PND19 pups were anesthetized with isoflurane (1.5–2.0 ml/min in 100% O₂) and placed into a stereotaxic frame. Body temperature was maintained between 37–38 °C with a thermostatically controlled heating pad. 

FP animals underwent lateral FP injury as previously described [23]. A 3 mm diameter craniotomy was placed 2 mm posterior to bregma and 6 mm lateral (left) of the midline, using a high speed drill (Dremel). A plastic injury cap was then fixed in place over the craniotomy using silicone adhesive, cyanoacrylate and finally dental cement.

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**Fig. 3.** Timeline representing the experimental design of the study. All animals underwent sham or FP injury on PND19. The early EE group underwent 17 days of differential housing from PND20–37; the delayed EE group underwent 17 days of differential housing from PND34–50. Behavioral assessment was identical for all animals.
O₂ was administered until spontaneous respirations returned by 45 s, positive pressure 100% of the hind paw withdrawal reflex. If no spontaneous respirations and the unconsciousness time by the return of the fluid pulse was administered. After the injury, apnea was determined by pinching the middle toe of the left rear paw every 15 s and noting whether the animal retracted the limb. At the first sign of the hind paw withdrawal reflex, a fluid pulse was administered. After the injury, apnea time was determined by the resumption of spontaneous respiration and the unconsciousness time by the return of the hind paw withdrawal reflex. If no spontaneous respirations were evident by 45 s, positive pressure 100% O₂ was administered until spontaneous respirations returned. Upon return of the hind paw withdrawal reflex, the animal was placed back under anesthesia for removal of the injury cap and closure of the surgical wound. Sham animals were subjected to anesthesia and incision but no craniotomy was made and injury was not induced. Previous experience in our group demonstrated no significant differences between naive (no craniotomy) and sham (with craniotomy) animals with regards to histology [5,14,15].

2.3. Enriched environment

For the early EE study, on PND20, rat pups were weaned from their mothers and transferred to EE or STD housing. Animals in the late EE study were initially placed in STD and after 14 days, the differential rearing assignments were made randomly. For all animals, food and water were freely available, and they were maintained on a 12-h light/dark cycle.

The EE chamber, similar to that previously described [5,15,19,30], consisted of various toys, tunnels, and ladders placed in a two-level cage measuring 78 cm × 36 cm × 48 cm. Sixteen to eighteen animals were housed together for 17 days, followed by housing in standard vivarium conditions until behavioral testing began on PND50–51. All animals were manually removed from the cage daily, objects/toys were rearranged and moved, and animals were then manually placed back into the cage. Animals were alternated between the top and bottom levels of the cage on a daily basis.

2.4. Morris water maze training

On PND50 (30 days post-injury), all animals began acquisition training in the MWM. In summary, the MWM is a 1.5 m in diameter, 0.6 m in height circular tank, filled with water opacified using organic white paint (Stechler, Albuquerque, NM) and maintained near 20 °C. A 15 cm × 15 cm platform, 2 cm below the water surface, was fixed in position in the NW quadrant of the tank. Each animal underwent two blocks of training per day. Each block consisted of four consecutive trials where the subject was released from one of the four cardinal directions (N, S, E, W) selected at random, such that each direction was used once per block. For each trial, the animals had 45 s to locate the hidden platform, after which time it was guided to the platform. The time required to find the platform was recorded as the latency. The swim paths, velocity, and timing for each trial were recorded using a digital tracking system (SMART, San Diego Instruments). Between trials, each animal remained on the platform for a 1 min period. Animals were run in groups of four, so the interval between blocks on a particular day for a given subject was kept constant at 28 min. Animals underwent this training at the same time each day for 7 consecutive days. The use of two blocks of four trials per day was originally utilized to permit analysis of block averages after a short time delay (14 min) or a longer time delay (overnight). Furthermore, this regimen permits over 95% of animals to reach the criterion level of performance at the end of 7 days of training. This training paradigm has been used before in this experimental setting and follows our previously published methods [5,14].

2.5. Probe trial

After the 7 days of acquisition training, all animals were returned to standard vivarium housing for an additional 7 days. On the day of the probe trial, the hidden platform was removed. Each animal was then released in the center of the tank facing S for a single 30 s trial. The swim paths, velocity and timing were recorded using the same digital tracking system described above.

2.6. Morphology

At the end of all behavioral studies animals were sacrificed and gross inspection revealed that these animals were similar to previous published work [5,14,23]. No residual hematomata were evident on the brain’s surface. No cavitation or overt atrophy was noted. The brains were then stored for future studies.

2.7. Analysis

All data are expressed as a group mean ± standard error of the mean. Serial body weights and escape latency in the MWM were analyzed using a repeated measures analysis of variance (ANOVA) by injury condition (sham versus FP) and housing (STD versus EE). Trials to criterion, slope of learning, and probe trial target hits were subjected to ANOVA by injury condition and housing. Between the FPSTD and FPEE groups, apnea and unresponsiveness times were compared using one-way ANOVA. For STD versus EE comparisons in trials to criterion and probe trial target hits, t-tests were calculated for specific comparisons. Analysis of proportions of “fast learners,” as defined by slope of learning, was done with a rate-ratio comparison. All analyses were performed using SPSS v11.0 software.
3. Results

3.1. Physiological response to injury and housing

3.1.1. Early EE

The average apnea times and duration of unresponsiveness to toe pinch did not differ between FPSTD and FPEE (Table 1). The average starting weights of the animals (Table 1) showed no significant differences by injury (ANOVA $F_{1,44} = 0.65, P = 0.40$) or housing ($F_{1,44} = 0.40, P = 0.53$). During differential housing, from post-injury day 1–17, animals were weighed 6 times. There was a significant effect of injury (repeated measures ANOVA, $F_{1,39} = 14.79; P < 0.001$) and no significant effect of housing ($F_{1,39} = 2.61; P = 0.11$); however, there was evidence of an injury by housing interaction ($F_{1,39} = 4.85; P < 0.05$). Injured animals weighed less than shams, and EE animals weighed less than STD animals. At the end of the study (post-injury days 45–50), there were no significant differences in weight between groups (Table 1; injury $F_{1,29} = 2.73; P = 0.11$, housing $F_{1,29} = 1.29; P = 0.27$).

3.1.2. Delayed EE

FPSTD rats tended to have slightly shorter apnea and LOC times than FPEE rats (ANOVA $P = 0.17$ and 0.40, respectively, not significant; see Table 1). There were no significant

Fig. 2. Average latency (±S.E.M.) to find the hidden platform in the Morris water maze over time. Each block consists of four trials, and two blocks were run each day (see methods for details). Early EE (A) shows greater improvement in SEE vs. SSTD than FPEE vs. FPSTD. Delayed EE (B) shows SEE and FPEE perform similarly, and are both better than SSTD and FPSTD groups (see text for statistics).
There was a significant effect of injury on weight ($F_{1,41} = 10.8, P < 0.005$), an effect of housing ($F_{1,41} = 4.2, P < 0.05$), and no injury by housing interaction. During EE rearing (post-injury days 14–31), rats were weighed 5 times and there was a strong effect of housing on weight ($F_{1,41} = 10.8, P < 0.005$), but not of injury or injury by housing interaction. This effect of housing was almost significant at the end of the study ($F_{1,41} = 3.9, P = 0.06$). There was no effect of injury or injury by housing interaction. In general, therefore, EE reared animals weighed less than STD-housed ones.

3.2. Morris water maze acquisition: latency

3.2.1. Early EE

Injury or housing had no effect on latency to find the hidden platform during the first block (4 trials) in the MWM. However, repeated measures ANOVA over 14 blocks of training showed a significant effect of both injury ($F_{1,41} = 11.20; P < 0.005$) and housing ($F_{1,41} = 4.11; P < 0.05$, Fig. 2A). In general, the injured groups took longer to locate the hidden platform than the shams. Also, the EE animals performed better than those animals housed in standard cages (STD). However, there was no difference between FPEE and SSTD animals ($F_{1,41} = 1.00; P = 0.33$).

3.2.2. Delayed EE

Again, there were no significant effects of injury or housing on block 1 in the MWM. Repeated measures ANOVA over the entire 14 blocks of training showed a significant effect of housing ($F_{1,41} = 38.52; P < 0.001$) but the effect of injury seen after early EE was absent ($F_{1,41} = 1.86; P = 0.18$). Both EE groups were able to find the hidden platform more rapidly than the STD-housed animals (Fig. 2B).

3.3. Morris water maze acquisition: trials to criterion

3.3.1. Early EE

Criterion performance represents a standard against which each individual animal can be measured [5,31]. In our previous studies, we have determined that in our MWM, an animal swimming directly from the release point to the platform at normal speed should reach the platform in ≤4 s. Criterion was thus operationally defined as achieved for a given individual when the average latency of 4 consecutive trials (1 block) from 4 different release points was ≤4 s. ANOVA showed a significant effect of injury ($F_{1,43} = 7.58; P < 0.01$) and housing ($F_{1,43} = 5.39; P < 0.05$) on the number of trials to criterion. However, there was no significant interaction between injury and housing.

The SSTD group achieved the 4 s criterion with a mean of $45.1 ± 4.0$ trials. The SEE animals learned faster, requiring only $32.7 ± 4.3$ trials to achieve criterion level performance (SSTD versus SEE, $t$-test, Bonferroni correction, $P < 0.05$). FPSTD and FPEE rats reached the 4 s criterion in 52.4 ± 3.3 trials and 46.8 ± 3.4 trials respectively, (FPSTD versus FPEE, $t$-test, Bonferroni correction, $P = 0.26$, Fig. 3A).

3.3.2. Delayed EE

As with the early EE study, these data revealed a significant effect of housing in terms of the number of trials to criterion ($≤4 s$) ($F_{1,45} = 25.01; P < 0.001$); however, in contrast, in this study arm there was no effect of injury ($F_{1,47} = 0.62; P = 0.44$, Fig. 3B). Again, there was no specific interaction between injury and housing. Using $t$-tests, there was a significant difference between SSTD (49.0 ± 4.5 trials) and SEE (26.7 ± 3.4 trials; $t$-test, Bonferroni, $P < 0.001$). The difference between FPSTD (49.5 ± 4.5 trials) and FPEE (32.5 ± 3.2 trials) was also significant ($t$-test, Bonferroni, $P < 0.005$).

3.4. Morris water maze acquisition: slope of learning

3.4.1. Early EE

Animals began learning on the 1st block of Morris water maze training, and plateaued after reaching criterion ≤4 s. For each animal, between block 1 and the block when that animal reached criterion, latencies were plotted and the slope of the linear regression was obtained. ANOVA of the slope of learning showed an effect of injury ($F_{1,45} = 4.1; P = 0.05$); housing approached significance ($F_{1,45} = 3.6; P = 0.06$), and the interaction between injury and housing ($F_{1,45} = 2.1; P = 0.16$) was not significant (Fig. 4A). Animals with a learning slope of less than −1.0 learned the task particularly rapidly. The group breakdown of these “fast learners” was...
Fig. 4. Slope of learning plotted by group. Learning slope was calculated as the slope of the linear regression for an individual animal’s latency curve between block 1 and the block in which the animal reached criterion performance. An animal with a learning slope of less than \(-1.0\) was designated as a “fast learner”.

When early (A) and delayed (B) EE experiments are combined, rate–ratio comparison showed a significantly higher likelihood (6.2-fold, 95% confidence interval: 1.9–20.3) of a “fast learner” being in the SEE group. Interestingly, in the delayed EE study arm (B), FPEE animals tended to demonstrate a greater proportion of “fast learners” (4/12) than in the early EE (A, 1/13).

In this study, the majority of the fast learners were in the SEE group (5/12), as compared with the other 3 groups (3/34). Using a rate–ratio comparison, this demonstrates a 7.4-fold (95% confidence interval: 1.2–53.7) increased likelihood of an SEE animal being a fast learner compared to the other 3 groups.

3.4.2. Delayed EE

Using the same calculations, learning slopes were calculated for the individuals in the delayed EE study. In distinction to the animals reared in EE early after injury, learning slopes of the delayed EE animals showed no effect of injury ($F_{1,47} = 2.81; P < 0.10$) but a strong effect of housing ($F_{4,47} = 11.98; P < 0.001$; Fig. 4B). The interaction between injury and housing was not significant ($F_{4,47} = 0.60; P = 0.44$). Furthermore, the pattern of fast learners suggested a slight improvement in the delayed FPEE group: SSTD (1/12), SEE (7/13), FPSTD (1/10), and FPEE (4/12). Using the same rate–ratio comparison described above reveals a 5.6-fold chance (95% confidence interval: 1.2–29.6) of a fast learner being in the SEE group. Thus, in both arms of the study, the SEE individuals exhibited the greatest likelihood of being fast learners.

3.5. Morris water maze retention: probe trial

3.5.1. Early EE

At the completion of 1 week of MWM training, latency times were not significantly different between groups. This suggests that each group had learned the location of the platform adequately. After a 1 week hiatus, a probe trial was administered where the platform was removed and each animal’s swimming path traced. When the paths were scored for visits to the location of the recently removed platform, ANOVA showed significant effects of both injury ($F_{3,45} = 4.13; P < 0.05$) and housing ($F_{4,45} = 4.05; P = 0.05$). The
interaction between injury and housing was not significant ($F_{1,45} = 1.76, P = 0.19$). This mirrors the results of the trials to criterion: the SEE animals had significantly more target visits ($2.7 \pm 0.4$) than SSTD ($1.5 \pm 0.4$, 1-tailed $t$-test, $P < 0.05$), but FPEE ($1.5 \pm 0.2$) did not differ from FPSTD ($1.3 \pm 0.4$, 1-tailed $t$-test, $P = 0.23$; Fig. 5A). Thus, using MWM retention as measured by probe trial performance, again SEE animals demonstrated improvement over their STD controls while FPEE rats did not improve relative to their STD-housed controls.

### 3.5.2 Delayed EE

The probe trial results for the delayed EE study revealed no significant effects of either injury ($F_{1,45} = 0.39, P = 0.54$) or housing ($F_{1,45} < 1.36, P = 0.25$). This lack of an effect, unlike what was seen in the early EE study, may be due to increased variability in the FPEE animals. However, even using $t$-tests, differences between FPSTD and FPEE were not seen (1-tailed $t$-test, $P = 0.47$). Interestingly, SEE rats still had more target hits than SSTD rats, and this result did approach but not reach statistical significance (1-tailed $t$-test, $P = 0.06$, Fig. 5B).

The pattern of probe trial target entries was similar for both the early and delayed EE arms of the study. The SEE group alone had more target entries than the other three groups. When the data from the early and delayed EE arms was pooled, there was a significant effect of housing ($F_{1,95} = 4.54, P < 0.05$) and a trend toward an effect of injury ($F_{1,95} = 2.91, P = 0.09$). Analysis of the pooled probe trial data showed SEE had a significantly greater number of target entries than SSTD ($SSTD: 1.52 \pm 0.26$, SEE: $2.56 \pm 0.31$, 1-tailed $t$-test, $P < 0.01$). In contrast, no differences were detected between the pooled FPSTD ($1.48 \pm 0.25$) and FPEE ($1.64 \pm 0.28$) data (1-tailed $t$-test, $P = 0.34$).

### 3.6 Morris water maze: swim speeds

Swim speeds were measured at the conclusion of Morris water maze training in both studies and there were no significant differences between groups. For the early EE study, SSTD rats averaged $22.6 \pm 1.4$ cm/s in their probe trial. SEE rats’ average swim speed was $24.1 \pm 1.5$ cm/s, FPSTD $25.1 \pm 1.6$, and FPEE $22.6 \pm 1.7$. In the delayed EE experiment, the average swim speeds for each group were as follows (in cm/s): SSTD $(25.1 \pm 1.9)$, SEE $(26.6 \pm 1.2)$, FPSTD $(26.3 \pm 2.1)$ and FPEE $(25.3 \pm 1.3)$.

### 4 Discussion

This study further characterizes the change in responsiveness to EE that is seen following a moderate traumatic injury to the immature brain, revealing persistent post-TBI deficits in both learning and memory. Using a model of moderate TBI, one that results in little overt morphological injury in developing animals, deficits in experience-dependent plasticity are manifested by impaired behavioral outcomes following EE rearing. In this case, impairment of Morris water maze acquisition and retention are present over 1 month after the initial injury. These deficits were also influenced by the timing of differential experience. Animals reared in EE shortly after TBI showed less benefit in Morris water acquisition and probe trial performance than SEE rats; however, if EE housing was delayed 14 days after injury, there was a greater effect of housing on Morris water maze acquisition. Interestingly, some impairment in slope of learning and probe trial performance was detectable regardless of the timing of EE.
These differences suggest that experience-dependent cognitive enhancements have distinct subtypes, perhaps being mediated by different mechanisms. For example, different measures of spatial learning task acquisition showed a similar pattern of effects, improving when EE housing was delayed. Timing of EE after TBI appears to have a significant effect on the eventual behavioral outcome, and responsiveness to experience-dependent plasticity can, to some extent, recover. However, when using a probe trial paradigm to assess memory function, the FPEE rats showed a more lasting impairment, even when EE housing was delayed until 14 days post-injury. Thus, mechanisms mediating experience-dependent plasticity may show incomplete recovery, and persistent deficits, or more appropriately, loss of potential in particular domains, can occur.

Our findings are distinct from studies using EE housing as a therapeutic intervention to aid recovery following TBI in adult animals [22,32]. In the immature model, the injury alone does not appear to induce a deficit. Only by using EE rearing to raise the pups’ performance to a superior level (that may actually more closely mimic wild-type conditions) does the injury-induced behavioral dysfunction become evident.

4.1. Development and brain injury

Other models of brain injury, including ischemia [2,33,34], surgical ablation [35–37] or prolonged seizures [3,38,39] have demonstrated differences between injury sustained during development from that occurring in the mature brain, with the younger brain being either more vulnerable or more resilient. Studies investigating TBI have demonstrated the resiliency of the immature brain. Lateral FP injury in adult rats results in significant cell death [40] and prolonged Morris water maze latencies [31,41]. Moderate FP in preweaning rats results in little anatomical injury [5,14,23] and minimal behavioral impairment [31].

In weight drop model of diffuse developmental TBI, Adelson and co-workers demonstrated evidence of glial reactivity and diffuse edema. No neuronal loss and no Morris water maze deficits were detected, except at the highest levels of injury severity [42,43]. At this severity prolonged MWM impairments were noted, although there was still no overt evidence of neuron loss [24,44].

4.2. Development and plasticity

The developmental literature has long supported the concept of critical windows for plasticity in response to environmental manipulation. Rearing in EE also exhibits a developmental “window”. Although EE rearing has been shown to benefit both immature and mature animals [30,45], the effects of EE are more robust in younger animals [18,21,46]. Both behavioral enhancements [18,21] and morphological changes [47] are greater in magnitude in younger animals. In addition, younger animals can generally demonstrate these beneficial effects with a shorter duration of environmental stimulation [5,6,30].

Environmental effects on recovery from injury also show age-specific features. Using bifrontal ablations, Kolb et al. [4] demonstrated worse anatomical and behavioral outcomes when injury occurred during the first postnatal week or adulthood compared to PND10. Rearing in EE was capable of facilitating recovery, but interestingly, the benefits of EE were more robust in the perinatally-injured animals (that were more severely affected by the ablations) than in those injured later (that had better spontaneous recovery) [48-50]. One interpretation of this apparent paradox put forth by Kolb and his colleagues is that developmental plasticity can manifest either as enhanced spontaneous recovery or as experience-dependent functional improvement, but not both.

This concept may have some support in TBI studies showing that despite the apparent lack of anatomical damage after developmental TBI, there is also evidence of altered neuroplasticity and later neurocognitive dysfunction. Preweanling-injured rats reared in EE failed to develop enhancements in MWM acquisition seen in their sham-injured, EE-reared counterparts [5]. Additionally, the EE-induced expansion of cortical dendritic arbors was also abnormal in animals subjected to early TBI [6]. These findings are in agreement with those reported in the current study.

The early EE component in the current study again demonstrates that developmental TBI reduces the beneficial effects of rearing in a stimulating environment. Two separate cognitive impairments were seen: a “learning” deficit with difficulty in latency, learning slope and trials to criterion performance, and a “retention” or “memory” deficit evident in the probe trial. If enriched housing was delayed, the latency and trials to criterion measures improved, yet the probe trial impairment remained. This implies that the persistent behavioral impairment seen here is not simply an acute injury-induced deficit that recovers with time, but also a loss of potential that may result in enduring problems. This post-FP unresponsiveness to EE at either time point appears to be mediated by ongoing neuronal dysfunction, rather than cell death.

4.3. Mechanisms of injury-induced impaired neuroplasticity

Studies of brain injury have repeatedly focused on cell death as a mediator of physiological and behavioral dysfunctions; however, particularly in sublethal injuries such as diffuse brain trauma, it is likely that the effects of dysfunctional neurons outweigh those of dead neurons. Given proper conditions, injured dysfunctional neurons could conceivably recover. Nonetheless, there are at least two circumstances in which impaired neurons would take on greater significance. The first would be in a setting of repeated or combined injuries and the second would be when injury occurs in an immature brain.
In both animal and human TBI, it is known that within a critical post-injury period a second injury can exacerbate the damage of the primary one, presumably pushing physiologically stressed cells to the point of energy failure and cell death [51–54]. This has been demonstrated both experimentally and clinically in situations where an initial traumatic injury is combined with hypoxia-ischemia [55–56], hypotension [57] or seizure [58]. Another scenario in which transiently dysfunctional cells could result in long-lasting problems is during development, where a critical window for developmental plasticity may be missed and an individual’s full developmental potential not realized. The current study provides a strong example of the latter.

A number of potential mechanisms can underlie this post-injury dysfunction. The well-known post-TBI period of impaired glucose metabolism lasting 7–10 days in adults [59] lasts only 1–2 days in preweaning rats [13]. Furthermore, metabolic activation during Morris water maze appears to be intact following developmental FP injury [25]. Thus, this mechanism appears unlikely to underlie the loss of plasticity described here.

Electrophysiological studies have demonstrated reduced long-term potentiation (LTP) in traumatically injured adult [60–62] and juvenile PND24–37 [63] rats. In fact, impaired maintenance of LTP can persist up to 8 weeks after moderate FP injury [64], a time frame consistent with the lasting deficits in probe trial performance and in the likelihood to be a “fast learner” shown in the current study.

Reduced excitatory neurotransmission and/or diminished activation of molecular signal transduction pathways is yet another means whereby plasticity may be impaired in the absence of appreciable neuronal loss. In adults, receptor binding studies have shown injury-induced reductions in glutamate binding [26,27], NMDA receptor subunit levels [65–67], and cholinergic neurotransmission [68]. In a setting of impaired excitatory neurotransmission, activity-dependent molecular pathways such as those mediated by brain derived neurotrophic factor (BDNF) may also be affected. Acute reductions in BDNF are seen following lateral FP injury in both adult [69] and developing rats [70].

4.4. Methodological issues

In this study and our previous studies [5,31], several distinct components of the Morris water maze assessment deserve mention. The use of a 45 s cut-off for latency is helpful to prevent hypothermia and exhaustion in younger animals undergoing extensive (8 trials/day) testing. However, this also creates a ceiling effect that may reduce injury-induced differences in latency on early days of training. At the other end of the curve, 8 trials/day and 7 consecutive days of training ensure that over 95% of animals achieve the fairly strict criterion level of performance by the end of training, but may blunt group differences in latency by creating a floor effect. The benefits of this training paradigm are that it permits analysis of trials to criterion without excluding animals, and also that all groups have the same relative performance at the start of their 1 week hiatus prior to the probe trial.

5. Conclusion

Using careful behavioral analysis and an established model of experience-dependent developmental plasticity, our study reveals both acute and chronic effects of traumatic injury to the developing brain in the absence of overt morphological damage. When EE housing occurs early after developmental TBI, measures of Morris water maze acquisition are reduced. If differential housing is delayed until 14 days after injury, the detrimental effects recover and the housing effects persist. Thus, the timing of environmental change has important implications for post-injury functional outcome. However, particularly with a measure of memory, injury-induced loss of potential may not resolve. These problems are not immediately evident between sham and injured STD housed animals, marking an important distinction of this study from most earlier investigations, where direct effects of injury are compared. However, STD laboratory environmental conditions are generally a poor correlate of the “wild-type” situation for either animals or humans, and it has been argued that STD conditions are actually relatively impoverished compared to normal [71]. By utilizing the EE to better simulate real-world experience, we have demonstrated and characterized post-injury impairment of developmental potential. These neurobehavioral sequelae of early TBI are representative of some of the common problems seen in children after head injury and clearly show the importance of environment in assessing outcome and recovery. This research marks an early milestone to understanding the mechanisms of cerebral dysfunction following TBI during development. The next step lies in uncovering the underlying physiological and molecular processes involved, thereby providing the potential for meaningful therapeutic intervention in head-injured children.

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