Environmental Enrichment as a Viable Neurorehabilitation Strategy for Experimental Traumatic Brain Injury

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Abstract

Environmental enrichment (EE) emerged as a robust independent variable capable of influencing behavioral outcome in experimental studies after the fortuitous observation by renowned neuropsychologist Donald O. Hebb that rats raised as pets in his home performed markedly better on problem-solving tasks than those kept in the laboratory. In the subsequent years, numerous studies ensued demonstrating that EE was also capable of inducing neuroplasticity in normal (i.e., noninjured) rats. These behavioral and neural alterations provided the impetus for investigating EE as a potential therapy for traumatic brain injury (TBI), which, over the past two decades, has resulted in several reports. Hence, the aim of this review is to integrate the findings and present the current state of EE as a viable neurorehabilitation strategy for TBI. Using the specific key term searches “traumatic brain injury” and “environmental enrichment” or “enriched environment,” 30 and 30 experimental TBI articles were identified by PubMed and Scopus, respectively. Of these, 27 articles were common to both search engines. An additional article was found on PubMed using the key terms “enriched environment” and “fluid percussion.” A review of the bibliographies in the 34 articles did not yield additional citations. The overwhelming consensus of the 34 publications is that EE benefits behavioral and histological outcome after brain injury produced by various models. Further, the enhancements are observed in male and female as well as adult and pediatric rats and mice. Taken together, these cumulative findings provide strong support for EE as a generalized and robust preclinical model of neurorehabilitation. However, to further enhance the model and to more accurately mimic the clinic, future studies should continue to evaluate EE during more rehabilitation-relevant conditions, such as delayed and shorter time periods, as well as in combination with other therapeutic approaches, as we have been doing for the past few years.

Key words: behavior; brain injury; cognition, environmental enrichment; functional recovery; rehabilitation; water maze

What Is Environmental Enrichment?

ENVIRONMENTAL ENRICHMENT (EE) can be operationally defined as an experimental strategy in which several research subjects, typically rodents, are exposed to multiple objects of various shapes and sizes in a vast living space (Fig. 1). The environment provides the opportunity for enrichment and integration of exploratory, physical, and social elements.^{1–9} This unique milieu is in marked contrast to standard (STD) housing, where single or paired subjects live in traditional laboratory-sized cages and receive only basic sustenance (i.e., food and water). EE produces numerous plasticity-associated alterations and/or significant behavioral improvements in normal (i.e., noninjured) rodents^{10–12} as well as in models of various central nervous system (CNS) syndromes, such as Alzheimer’s disease,^{13,14} Parkinson’s disease,^{15–17}

spinal cord injury (SCI),^{18,19} chronic hypoxic injury,²⁰ stroke,^{21–25} and cortical lesions.^{26–28} EE similarly promotes cognitive and motor improvement and attenuates histopathology after traumatic brain injury (TBI) in rodents^{1–3,6,29} and thus has been likened to physiotherapeutic rehabilitation.^{1–3,6–9,30}

The Beginning: Environmental Enrichment and Neuroplasticity

Donald O. Hebb’s serendipitous discovery in the mid-1940s that rats raised as pets in his home outperformed those kept in standard laboratory cages in problem solving, specifically being able to negotiate their way through a maze in a shorter amount of time,³¹ answered an important scientific question regarding whether the environment plays a role in how an organism behaves. A plethora

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FIG. 1. Photograph of our environmental enrichment (EE) cage with multiple levels and wide array of sensory stimuli (e.g., balls, ramps, tubes, and nesting materials). In our laboratory, 10–12 rats, which include both traumatic brain injury and sham controls, are housed together to ensure equal conditions. For studies investigating continuous EE, rats were only removed briefly for behavioral assessments, weighing, and, if applicable, drug administrations. Together, these components make up the “typical” EE. However, it should be noted that EE cage setups differ from laboratory to laboratory in terms of the number of rats housed together, the design of the cages, and the complexity of the cage contents.

of subsequent research confirmed these findings and elaborated further on how environmental manipulations have a significant role in shaping the brain and affecting behavior.

EE originated as an empirical variable formally in the 1960s with the pioneering efforts of Bennett, Diamond, and Rosenzweig, who examined environmental influences on chemical and anatomical brain plasticity in normal rats. Specifically, in 1964, Bennett and colleagues³² demonstrated that *adult* rats exposed to EE developed heavier and thicker cerebral cortices, relative to STD-housed controls. These results were in marked contrast to the zeitgeist that neurodevelopment halted in childhood, and ultimately demonstrated that brains are capable of reshaping and reconfiguring into adulthood—a premise that is well accepted today. Diamond and colleagues, in 1966, provided further EE-induced neuroplastic evidence by showing that cortical thickening was directly related to a significant increase in the number and length of

glia.³³ In 1968, La Torre and colleagues compared rats raised in an impoverished environment, consisting of single housing in small cages with covered sides and often in separate rooms, to those in EE and showed that the latter condition increased brain weight and levels of acetylcholinesterase (AChE), which replicated the work of Bennett and colleagues.³⁴ An increase in the size of the hippocampus was also demonstrated by Walsh and colleagues.³⁵ In subsequent studies, researchers were able to better estimate depth differences³³ and identify particular regions of cortical thickening,³⁶ confirming EE-induced increases in cortical depth.^{33,36–39} It was then also established that direct interaction with the environment is necessary to confer benefits,³⁹ a finding that was recently confirmed by researchers in the laboratory of Kline and colleagues,^{4,8} and that a threshold of EE is necessary to promote cortical depth differences, relative to an impoverished environment.³⁸ A certain threshold of EE was also recently shown to be

necessary for improved cognitive benefits and histological protection after experimental brain trauma.^{4,7,8}

The Progression: Environmental Enrichment and Neuroplasticity

EE progressed with what can be considered a second wave of empirical research during the mid-1990s and for the past two decades has expanded on the seminal anatomical and behavioral studies and is currently emerging into the investigation of molecular changes in various experimental manipulations. Specifically, it has been shown that rats raised in EE exhibit larger synapses⁴⁰ and higher densities of synaptic vesicles.⁴¹ EE also positively affects neurogenesis, even in the adult brain, as determined by increased newborn neuronal cell survival in rats⁴² and mice,^{43–45} an effect that may be mediated by neuron-selective survival-promoting factors.³⁰ Moreover, EE exposure has been shown to increase the pre- and postsynaptic plasticity markers synaptophysin and postsynaptic density protein-95, respectively, in a variety of brain regions.⁴⁶ Taken together, these neural alterations may explain, at least in part, potential mechanisms for EE-mediated neurobehavioral and cognitive benefits.

The Impetus for Environmental Enrichment as a Therapeutic Approach

Numerous TBI studies have demonstrated changes in brain morphology, loss of cortical and hippocampal cells, alterations in neurotransmitter and neuromodulatory systems, and down-regulation of neurotrophin expression, which correlate with behavioral dysfunction.^{47–49} Given the plethora of findings described above showing that exposing normal rodents to EE results in a variety of neuroanatomical and neurochemical changes,^{30,33,38,50} it seemed reasonable to hypothesize that these same neuroplastic priming factors may influence the brain's response to injury and thus serve as a potential reparative therapy.

The significance of determining a potentially efficacious therapeutic strategy, such as EE, after TBI is that over 10 million people worldwide are afflicted each year, with close to 2 million of them occurring in the United States. The severity of TBI ranges from mild concussions to severe trauma or even death. In the United States alone, there are over 500,000 emergency room visits for TBI and nearly 50,000 fatalities.^{49,51–56} In addition, more than 120,000 individuals suffer long-term and/or permanent neurological dysfunction in motor and cognitive ability, for which there are limited treatments. Whereas the emotional toll of TBI on an afflicted individual's interpersonal relationships with family, friends, and coworkers is immeasurable, the economic cost to society accounts for billions of dollars each year.^{53,57} For these reasons, TBI is an important area of health and rehabilitation research and the importance of continued development and improvement of therapeutic strategies for TBI-induced deficits is paramount. Hence, the aim of this review is to integrate the findings from the literature and briefly present historical and current perspectives that have served as the impetus and guide for EE as an applicable, potentially efficacious therapeutic strategy for experimental TBI. Using the specific key term searches "traumatic brain injury" and "environmental enrichment" or "enriched environment," 30 and 30 experimental TBI articles were identified by PubMed and Scopus, respectively. Of these, 27 articles were common to both search engines. An additional article was found on PubMed using the key terms "enriched environment" and "fluid percussion." In addition to the key terms, further inclusion criteria were the use of a none-

nriched comparison group, randomization, and blinding. A total of 79.4% of the included studies specifically reported randomization and blinding. However, though not all studies saliently reported these manipulations, it cannot be assumed that they were not performed. We relied on the fact that all studies were peer-reviewed publications. Whereas blinding is important, several studies may not have reported it because it is difficult to blind the enrichment group. All studies are described in the text and summarized in Table 1.

Multi-Faceted Benefits of Environmental Enrichment after Brain Injury

The first reported confirmations that EE is protective and/or produces benefits after brain injury were published in the mid-1970s by Will and colleagues, who showed that after suction ablation of the occipital cortex (with inadvertent underlying hippocampal and subcortical damage occurring occasionally) in neonatal and young adult rats, EE improved behavior on the standard 12 Hebb-Williams problems task.^{58,59} Subsequent confirmations came from the studies of Held and colleagues,²⁸ Gentile and colleagues,⁶⁰ and Rose and colleagues.⁶¹ Specifically, Held and colleagues²⁸ delved into the variable of timing exposure to EE and subjected rats to both pre- and postoperative EE versus STD housing after bilateral sensorimotor cortex lesions or sham surgery. The results revealed that providing enrichment before a cortical lesion did not result in motor deficits and that the rats did not exhibit any aberrant movement of the hindlimbs during testing. Moreover, impoverishment before the cortical lesion followed by postoperative EE decreased the initial deficit and improved recovery time, relative to the continuous impoverished condition, where rats exhibited significantly worse initial deficit and delayed recovery time.²⁸ In a study comparing the effects of EE to those of exercise, Gentile and colleagues also subjected rats to bilateral sensorimotor cortex ablations, randomized them to groups receiving EE or exercise versus STD controls, and found that whereas exercise was effective, it did not yield the same protective effects as EE.⁶⁰ Exposure to EE has also been shown to improve spatial learning and attenuate hippocampal neurodegeneration after chemical lesions of the ventral subiculum.⁶²

Behavioral and histological benefits of EE have also been shown using models of brain injury that are more traumatic by design (e.g., controlled cortical impact [CCI] and fluid percussion [FP]) and, by extension, have greater clinical validity.^{63–66} Both CCI and FP injuries produce significant cognitive and histological dysfunction that persist up to 1 year after trauma.^{67–71}

Several studies, which are the main focus of this review, have shown that EE after CCI or FP injury confers significant improvement in motor performance, acquisition of spatial learning, and/or histopathology, when compared with animals placed in STD housing conditions.^{1,4–6,28,29,59,72} One of the first studies evaluating EE after TBI was conducted by Hamm and colleagues, who sought to determine whether exposure to EE would promote the recovery of cognitive function. Rats were subjected to a moderate level of FP or sham injury and then were immediately placed in either EE (i.e., complex environment) or STD conditions. Spatial learning performance was assessed in a Morris water maze (MWM)⁷³ on postoperative days 11–15. The data showed that TBI rats recuperating in the EE located the escape platform significantly quicker than those housed in STD conditions. Moreover, the enriched rats performed as well as the sham controls. These data demonstrate a marked EE-induced improvement in cognitive function after TBI.⁵

TABLE 1. SUMMARY OF NEUROBEHAVIORAL, HISTOLOGICAL, AND MOLECULAR CHANGES INDUCED BY EE IN EXPERIMENTAL MODELS OF TBI

<i>Injury model</i>	<i>Animal age and gender</i>	<i>Environmental enrichment</i>	<i>Behavioral and physiological effects</i>	<i>Brain alterations</i>	<i>Concomitant drug treatment</i>	<i>Reference</i>
Fluid percussion	Adult, male rats (300–350 g)	Immediately after TBI for 15 days	<ul style="list-style-type: none">• ↑ acquisition of MWM spatial learning in injured EE vs. STD rats• TBI rats exposed to EE performed comparable to sham controls	<ul style="list-style-type: none">• No differences among groups in brain weight	N/A	Hamm et al. (1996) ⁵
	Pups, male rats (PND 17–20)	17 days after weaning (PND 21), then 2 weeks in STD	<ul style="list-style-type: none">• TBI + EE did not differ from TBI + STD controls	<ul style="list-style-type: none">• ↑ thickness in sham + EE group in frontal and occipital cortices; effect abolished by TBI, even after EE	N/A	Fineman et al. (2000) ⁹⁷
	Adult, male rats (275–325 g)	2 weeks postinjury	<ul style="list-style-type: none">• ↓ TBI-induced spatial learning deficits vs. STD• EE housed rats displayed ↓ learning acquisition, compared to shams	<ul style="list-style-type: none">• ↓ cortical lesion volume• Microglial proliferation and inflammatory processes observed in the injured cortex, even after EE	N/A	Passineau et al. (2001) ²⁹
	Adult, male rats (325–375 g)	EE + daily handling for 2 weeks postinjury	<ul style="list-style-type: none">• ↑ acquisition of spatial learning in injured EE vs. STD rats, as assessed in the MWM	<ul style="list-style-type: none">• No changes in neurotrophin/receptor mRNA levels (i.e., BDNF, trkB, and NT-3) after either EE or STD housing	N/A	Hicks et al. (2002) ⁷²
	Pups, male rats (PND 19–20)	17 days after weaning (PND 20), then up to 2 weeks in STD housing	N/A	<ul style="list-style-type: none">• Golgi-Cox dendritic density and branching analyses in frontal, parietal, and occipital cortices (PNDs 47–51)• EE ↑ dendritic density in occipital cortex, TBI ↑ dendritic density in regions distant from injury (contralateral parietal cortex, ipsi- and contralateral occipital cortex)• TBI attenuated EE-induced dendritic density increases, except in ipsilateral occipital cortex, where TBI + EE had additive effects.	N/A	Ip et al. (2002) ⁹⁸
	Adult, male rats (325–375 g)	1 h/day, from 1 to 20 days after TBI	<ul style="list-style-type: none">• ↑ acquisition of the MWM task in injured rats, when tested over two trial blocks per day for 2 days after cessation of EE	<ul style="list-style-type: none">• ↑ presence of progenitor cells (BrdU⁺) in the dentate (granule cell layer and hilus) on the ipsilateral side of injury	N/A	Gaulke et al. (2005) ¹¹³
	Pups, male rats (PND 19)	Early EE: 17 days after weaning + 2 weeks in STD; Delayed EE: 2 weeks in STD after weaning, then 17 days of EE	<ul style="list-style-type: none">• Early EE ↑ MWM spatial learning and probe trial performance for both sham and injured animals, albeit less for the latter• Delayed EE had no effects on TBI rats	N/A	N/A	Giza et al. (2005) ¹⁰²
	Adult, male rats (300–350 g)	EE vs. EE + MEOS for 2 weeks postinjury	<ul style="list-style-type: none">• Neuromotor deficits assessed by a composite neuroscore were comparable in all TBI rats at 1 day postinjury, but EE + MEOS ↑ outcome at 7 and 15 days postinjury, compared to TBI + STD and TBI + EE groups• EE + MEOS ↑ spatial reference memory in the Barnes maze test, compared to TBI + STD and TBI + EE groups	N/A	N/A	Maegele et al. (2005) ⁷⁷
	Adult, male rats (300–350 g)	EE vs. EE + MEOS for 2 weeks postinjury	<ul style="list-style-type: none">• Neuromotor deficits were comparable in all TBI animals at 1 day postinjury, but EE + MEOS ↑ outcome at 15 days postinjury, compared to TBI + STD and TBI + EE groups	<ul style="list-style-type: none">• ↓ lesion volumes in EE-only and EE + MEOS groups, with the EE + MEOS condition displaying greater protection	N/A	Maegele et al. (2005) ⁷⁸
	Adult, male rats (300–350 g)	EE + MEOS + rotarod motor training for 5 days postinjury	<ul style="list-style-type: none">• ↑ neurofunctional recovery on postinjury days 7 and 15, compared to STD group	<ul style="list-style-type: none">• ↓ Fluoro-Jade⁺ degenerating neurons in the early rehabilitation group	N/A	Lippert-Gruner et al. (2007) ⁷⁹

(continued)

TABLE 1. (CONTINUED)

<i>Injury model</i>	<i>Animal age and gender</i>	<i>Environmental enrichment</i>	<i>Behavioral and physiological effects</i>	<i>Brain alterations</i>	<i>Concomitant drug treatment</i>	<i>Reference</i>
Controlled cortical impact	Adult, male rats (300–350 g)	EE vs. EE + MEOS for 30 days postinjury	<ul style="list-style-type: none"> • EE + MEOS performed better than EE-only and STD at 15 days post-TBI • ↑ recovery in EE-only and EE + MEOS, but not STD, at 30 days vs. 15 days postinjury 	<ul style="list-style-type: none"> • Lesion volumes not significantly different in the EE-only and EE + MEOS rats at 30 days postinjury vs. STD 	N/A	Lippert-Gruener et al. (2007) ⁸²
	Adult, male rats (350–375 g)	3 × 45 min/week for 3 weeks, beginning 1 week postinjury (nine total sessions)	<ul style="list-style-type: none"> • Sensitivity to whisker stimulation up to 8 weeks after moderate TBI, whereas shams were soothed by the stimulation (i.e., low scores) • ↑ total free serum corticosterone • No significant improvement in behavioral outcome after limited EE 	N/A	N/A	McNamara et al. (2010) ¹⁰⁷
	Adult, male rats (350–450 g)	Early multi-sensory rehabilitation (EMR; 24 to 15 days postinjury)	<ul style="list-style-type: none"> • ↑ recovery of neuromotor function (neuroscore) in EMR group at 15 days postinjury • No differences on Barnes maze in latency to reach protective box and path length improvement on days 11–15 postinjury, but ↓ random search strategies in EMR group 	N/A	N/A	Lippert-Gruener et al. (2011) ⁸³
	Adult, C57BL/6J male mice (25–30 g)	IntelliCage exposure with neurobehavioral monitoring for 9 days pre- and up to 5 days postinjury	<ul style="list-style-type: none"> • ↑ recovery of locomotive activity (i.e., number of visits and nose pokes) at 24 h postinjury; complete restoration in locomotive activity at 72 h post-TBI 	<ul style="list-style-type: none"> • ↓ number of dead cells in the striatum at 72 h postinjury, compared to STD housing 	N/A	Muthuraju et al. (2012) ⁷⁶
	Adult male (310 g) and normally cycling female (266 g) rats	3 weeks postinjury	<ul style="list-style-type: none"> • EE ↑ spatial memory performance (latency to reach platform and path length) in male, but not female, rats 	N/A	N/A	Wagner et al. (2002) ⁸⁹
	Pups, male rats (PND 21)	15 days after weaning, then STD housing until injury at 3 months of age, followed by 41 days of recovery	<ul style="list-style-type: none"> • ↑ initial deficit in forelimb use, but rescued motor coordination in the foot fault test 	<ul style="list-style-type: none"> • ↑ cortical contusion cavity formation through the forelimb area of the sensorimotor cortex 	N/A	Kozlowski et al. (2004) ¹⁰⁶
	Adult male (310 g) and normally cycling female (266 g) rats	4 weeks postinjury	N/A	<ul style="list-style-type: none"> • BDNF protein levels ↑ in frontal cortex ipsilateral to injury in males, with no effects of EE; no effects of injury or EE on BDNF in females • BDNF expression ↓ in hippocampus ipsilateral to injury in both males and females; EE ↑ postinjury BDNF protein expression in females, but not in males • BDNF expression ↑ in hippocampus contralateral to injury in females, with no effects of EE 	N/A	Chen et al. (2005) ²⁹
	Adult male (310 g) and normally cycling female (266 g) rats	Began immediately after injury and continued for 4 weeks	N/A	<ul style="list-style-type: none"> • DAT expression analyzed in ipsi- and contralateral frontal cortex and striatum in males and females • ↓ DAT expression in analyzed regions of TBI + STD, but no additional EE effects • EE ↓ DAT expression postinjury in striatum and ipsilateral frontal cortex of females, compared to sham/STD rats, but no TBI-alone effects • Larger DAT decreases in males after injury, but EE had larger effects on postinjury DAT expression for females 	N/A	Wagner et al. (2005) ¹³⁷

(continued)

TABLE 1. (CONTINUED)

Injury model	Animal age and gender	Environmental enrichment	Behavioral and physiological effects	Brain alterations	Concomitant drug treatment	Reference
	Adult, male rats (300–325 g)	Began immediately after injury and continued for 3 weeks	<ul style="list-style-type: none"> • 8-OH-DPAT ↑ spatial learning • EE ↑ motor and cognitive recovery • EE enhanced the effect of 8-OH-DPAT on spatial learning, but no additive effects were observed with the combined treatments. 	<ul style="list-style-type: none"> • Acute 8-OH-DPAT and EE significantly ↓ CA₃ cell loss, but no additive effects with combined treatments 	8-OH-DPAT (0.5 mg/kg), i.p., 15 min post-surgery	Kline et al. (2007) ¹
	Young adult, male rats (200–300 g)	EE + running wheel for 4 weeks postinjury beginning immediately after injury	<ul style="list-style-type: none"> • EE ↑ recovery of function in the skilled forepaw reaching, ladder, and beam walking tasks, although control animals attained similar improvements by 28 days 	N/A	N/A	Smith et al. (2007) ⁷⁴
	Adult, male rats (300–325 g)	Continuous (3 weeks), early (1 week EE + 2 weeks STD), or delayed (1 week STD + 2 weeks EE)	<ul style="list-style-type: none"> • ↑ motor beam walk performance (days 1–5 post-TBI) in continuous and early EE groups, compared to delayed and no EE groups • ↑ spatial learning acquisition (days 14–18 post-TBI) in continuous and delayed EE groups vs. early and no EE groups 	<ul style="list-style-type: none"> • Continuous EE significantly rescued hippocampal CA₃ cells vs. STD housing. 	N/A	Hoffman et al. (2008) ⁴
	Adult, male rats (300–325 g)	Began immediately after injury and continued for 3 weeks	<ul style="list-style-type: none"> • 8-OH-DPAT and EE ↑ motor and cognitive recovery post-TBI • No additive beneficial effects observed with combined treatments 	<ul style="list-style-type: none"> • Chronic 8-OH-DPAT and EE ↓ hippocampal CA₃ and ChAT + medial septal cell loss • EE + 8-OH-DPAT effects on cholinergic cells were additive (i.e., combined treatment group ↑ sparing of ChAT⁺ cells vs. either the EE-alone or 8-OH-DPAT-alone groups) 	8-OH-DPAT (0.1 mg/kg), i.p., beginning 24 h postinjury, once-daily for 3 weeks	Kline et al. (2010) ²
	Adult, male rats (300–325 g)	Early and continuous vs. three atypical EEs (EE minus social, EE minus sensory stimuli) and STD plus sensory stimuli	<ul style="list-style-type: none"> • Continuous EE ↑ motor and cognitive recovery • Each the three atypical EE conditions provided limited and nonsignificant benefits when EE components were presented individually. 	<ul style="list-style-type: none"> • Continuous EE ↓ CA₃ cell loss and cortical lesion volume • No significant reductions observed in histopathology in any of the three atypical EE conditions, relative to STD 	N/A	Sozda et al. (2010) ⁶
	Adult, male rats (300–325 g)	Early and continuous vs. abbreviated (i.e., rehabilitation-relevant): 2, 4, or 6 h/day	<ul style="list-style-type: none"> • Continuous and 6-h EE groups were comparable on all behavioral assessments after TBI; ↑ motor and cognitive performance vs. injured rats housed in STD and 2-h and 4-h EE. • Two- and 4-h EE groups not different from STD 	N/A	N/A	de Witt et al. (2011) ⁷
	Adult, male rats (300–350 g)	2 h/day, beginning 1 day after injury and lasting for 14 days	<ul style="list-style-type: none"> • ↓ TBI-related impairments of spontaneous object recognition in a Y-maze arena 	N/A	N/A	Darwish et al. (2014) ¹¹⁴
	Adult, male rats (300–325 g)	Early and continuous (3 weeks), early (1 or 2 weeks postinjury), delayed (beginning at 1 or 2 weeks postinjury), early, and late EE (1 week EE + 1 week STD + 1 week EE) or early and late STD (1 week STD + 1 week EE + 1 week STD)	<ul style="list-style-type: none"> • Continuous, early (7 days), and early and late (7-day EE, 7-day STD, 7-day EE) exposure ↑ motor recovery • Continuous and delayed (starting at 7 days post-TBI) exposure ↑ cognitive recovery 	N/A	N/A	Matter et al. (2011) ⁸
	Adult, male rats (300–325 g)	Phase I: continuous (3 weeks) postinjury; phase II: half of EE groups transferred to STD housing (TBI + EE + STD and sham + EE + STD); neurobehavior reassessed once per month for 6 months	<ul style="list-style-type: none"> • ↑ beam balance and spatial learning acquisition after TBI • EE benefits are long-lasting, as motor and cognitive recovery was maintained for up to 6 months after EE cessation • TBI-induced cognitive deficits in untreated rats are long term and remained relatively unchanged from day 18 up to 6 months postinjury. 	N/A	N/A	Cheng et al. (2012) ⁹

(continued)

TABLE 1. (CONTINUED)

Injury model	Animal age and gender	Environmental enrichment	Behavioral and physiological effects	Brain alterations	Concomitant drug treatment	Reference
	Adult, male rats (300–325 g)	Began immediately after injury and continued for 3 weeks	<ul style="list-style-type: none"> • Buspirone and EE ↑ motor and cognitive recovery post-TBI • EE enhanced buspirone effect on spatial learning, but there were no additive effects with combined treatments. 	<ul style="list-style-type: none"> • TBI + EE, regardless of whether buspirone or vehicle-treated, displayed ↓ CA₃ cell loss 	Buspirone (0.3 mg/kg), i.p., beginning 24 h postsurgery, once-daily for 3 weeks	Kline et al. (2012) ³
	Adult, male rats (300–325 g)	Began immediately after injury and continued for 4 weeks	N/A	<ul style="list-style-type: none"> • mRNA expression profile in SN and VTA by microarray analysis to assess differential gene expression • Alterations in genes involved in inflammation, cellular plasticity, calcium, and DA signaling pathways in TBI + EE rats • EE induced alterations in neurotrophins (BDNF, nerve growth factor, and NT-3) in other brain regions, but not SN or VTA 	N/A	Shin et al. (2013) ¹³⁸
	Adult, male rats (375–400 g)	Continuous EE for 38 days postinjury	<ul style="list-style-type: none"> • Four weeks of EE ↓ mTBI-induced increases in swim latency and the number of errors in the non-matching-to-sample task • EE normalized mTBI-related errors made in the delayed non-matching-to-sample task. 	<ul style="list-style-type: none"> • EE ↓ IL-1β and TNF-α and ↑ IL-10 levels in prefrontal cortex and hippocampus of mTBI rats • EE ↓ injury-induced reductions in pAMPK/AMPK ratio and uMtCK protein levels 	N/A	Briones et al. (2013) ⁷⁵
	Adult, male rats (400–500 g)	EE + running wheel, individual motor skills training (2 × day) and olfactory stimulation (1 × day) for 15 days preceding injury	<ul style="list-style-type: none"> • EE ↓ TBI-induced sensory neglect at days 8 and 25 postinjury • EE ↓ TBI-induced deficits in spatial memory at 18–21 days postinjury 	<ul style="list-style-type: none"> • ↓ Nissl neuronal profiles in dorsal prefrontal cortex (anterior cingulate cortex and, occasionally, prelimbic cortex) • Extent of cortical damage similar between the two groups, but quantitative mapping not performed 	N/A	Johnson et al. (2013) ⁸⁴
	Adult, normal cycling female (250–270 g) rats	Began immediately after injury and continued for 3 weeks	<ul style="list-style-type: none"> • No effects of estrous stage at the time of TBI were detected with regard to behavioral performance. • EE ↑ beam balance, beam walk, rotarod fine motor ability, and spatial learning in injured rats, compared to STD-housed females 	<ul style="list-style-type: none"> • ↑ histological protection (i.e., increased CA_{1/3} cell survival and decreased cortical lesion volume) 	N/A	Monaco et al. (2013) ⁹⁶
	Adult, male rats (250–300 g)	36 days postinjury	<ul style="list-style-type: none"> • TBI + EE + eSC no different from control groups in spatial learning (days 14–23 post-injury), whereas TBI + EE or TBI + eSC did not reduce TBI-induced deficits • TBI + EE and TBI + EE + eSC similar to sham group on Barnes maze (days 24–29 postinjury) • TBI + EE + eSC no different than sham group at end of rotarod motor training (days 30–34 postinjury) 	<ul style="list-style-type: none"> • EE and/or eSC therapy ↓ neuronal loss near lesion site and hippocampus 	Murine cortical eSCs implanted in injured cortex 1 week post-TBI	Peruzzaro et al. (2013) ¹²²
Blast	Adult, male rats (245–265 g)	71 days postinjury	<ul style="list-style-type: none"> • EPM and Barnes maze tests starting at 15, 44, and 66 days postinjury • Normalization of anxiety > 2 months, independent of housing condition • ↑ spatial memory postinjury 	<ul style="list-style-type: none"> • VEGF and tau proteins, but not IL-6, normalized in dorsal hippocampus • ↓ IL-6 expression in ventral hippocampus of bTBI rats 	N/A	Kovesdi et al. (2011) ⁸⁵

Only effects that reached statistical significance are reported, whether demonstrating EE-induced benefits or lack thereof

EE, environmental enrichment; TBI, traumatic brain injury; PND, postnatal day; STD, standard; MEOS, multimodal early onset stimulation; EMR, early multisensory rehabilitation; MW, Morris water maze; N/A, not available; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino) tetralin; eSC, embryonic stem cell; EPM, elevated plus-maze; mRNA, messenger RNA; BDNF, brain-derived neurotrophic factor; trkB, tropomyosin related kinase B; NT-3, neurotrophin 3; BrdU, bromodeoxyuridine; VEGF, vascular endothelial growth factor; IL, interleukin; bTBI, blast TBI; DAT, dopamine transporter; ChAT, choline acetyltransferase; SN, substantia nigra; VTA, ventral tegmental area; TNF- α , tumor necrosis factor alpha; mTBI, mild TBI; AMPK, adenosine-monophosphate-activated protein kinase; pAMPK, phosphorylated AMPK; uMtCK, ubiquitous mitochondrial creatine kinase; i.p., intraperitoneal.

Using a similar injury device, but producing a more severe injury, Passineau and colleagues showed that rats recovering in an enriched environment displayed faster learning rates in the MWM spatial acquisition task, relative to the injured STD-housed group. Additionally, at 2 weeks postinjury, cortical lesion volume was significantly smaller in rats exposed to EE. This study provided evidence that EE can be effective in reducing cognitive deficits and histopathology in a TBI model that exhibits severe cerebral contusion and hemodynamic consequences.²⁹ Hicks and colleagues proceeded to further evaluate the relationship between EE and behavioral recovery after TBI and found that 2 weeks of EE exposure improved the acquisition of spatial learning in the MWM.⁷² After CCI injury to the forelimb region of the rat sensorimotor cortex, Smith and colleagues reported faster recovery of skilled forelimb function in rats chronically exposed to EE postinjury.⁷⁴ In a recent study by Briones and colleagues,⁷⁵ 4 weeks of continuous EE exposure significantly normalized CCI-induced increases in mean swim latency and errors made in a non-matching-to-sample task conducted in a water maze. Also, in a delayed non-matching-to-sample task, TBI rats displayed greater performance errors as the time delay between the sample and test trials increased, whereas EE attenuated the injury-related behavioral deficits.⁷⁵

In addition to rats, beneficial effects of EE exposure have also been reported in adult mice. Specifically, C57BL/6 male mice were maintained in an IntelliCage enclosure (i.e., a fully automated and computer-operated system) with neurobehavioral monitoring for 9 days pre- and up to 5 days post-FP injury.⁷⁶ Locomotive activity (i.e., number of visits and nose pokes) at 24 h postinjury was enhanced in IntelliCage-exposed mice and there was a complete restoration at 72 h post-TBI. Moreover, the number of dead cells in the striatum at 72 h postinjury was significantly reduced, compared to STD housing. Hence, it appears that IntelliCage exposure enhanced the neurorehabilitation process post-TBI because it provided three important factors for locomotive recovery (EE, motivated voluntary movement, and social interaction) and accelerated striatum-derived neurogenesis.⁷⁶

As an adjunct to typical EE, Maegele and colleagues added auditory and olfactory stimuli to further enhance the paradigm. Specifically, they devised a clinically relevant combined paradigm of enriched housing and complex multi-modal early onset stimulation (MEOS) by exposing TBI rats to a specific protocol of auditory, motor, olfactory, and visual stimuli.⁷⁷ Post-traumatic neuromotor deficits were assessed at 1, 7, and 15 days after injury using a standardized composite neuroscore, whereas recovery of spatial reference function was measured on days 11–15 postinjury using the Barnes circular maze task. Injured rats performed significantly better in both tasks on postinjury day 15 when targeted multi-modal external stimuli were added to the EE paradigm, compared to injured animals housed in EE alone or STD conditions. This study was the first to indicate that the rescue of neuromotor and cognitive function is greater when MEOS is added to EE, thus conferring an enhanced potential for functional recovery resulting from greater stimulation and, perhaps, neuronal plasticity.⁷⁷ The researchers followed up with a subsequent study aimed at investigating morphological correlates of neurological recovery in a combined EE plus MEOS paradigm after lateral FP injury.⁷⁸ Both EE TBI groups displayed improvements 7 days postinjury, with the EE plus MEOS group performing significantly better on day 15 postinjury, compared to all other groups, whereas neuromotor dysfunction in injured STD-housed rats remained unchanged throughout the study. Analyses of lesion volume measured in brain sections stained for neuron-specific enolase, the apoptosis marker,

caspase 3-active/C3A, and the astrocyte-specific marker, glial fibrillary acidic protein (GFAP), revealed that rats in the EE plus MEOS group had the least damage. It is possible that the combined exposure attenuated secondary tissue injury, as well as enhanced plasticity responses and/or recruited compensatory mechanisms from intact, adjacent cortical regions.⁷⁸

Follow-up studies from the same laboratory⁷⁹ proceeded to further refine the combined EE plus MEOS paradigm with a 5-day rotarod motor training procedure applied early after injury in order to better mimic multi-sensory rehabilitation used to enhance neural plasticity and regain functional capacities in TBI patients.^{80,81} After injury, the composite neuroscore test revealed reduced neuromotor function in both injured groups, housed in either the rehabilitation setting or STD conditions, though rats in the rehabilitation model group performed significantly better at 7 and 15 days postinjury. Identification of injury-induced structural alterations, such as degenerating neural cell bodies and processes, revealed a significantly lower number of positive cells in the rehabilitation combined paradigm group, compared to STD housing. Although the effects of individual components of the rehabilitation model have not been disentangled, these findings suggested that the combination of early EE and multi-sensory stimulation along with motor training conferred a definite improvement of neuromotor functions and attenuated neural loss, compared to STD housing.⁷⁹

A subsequent study sought to determine whether the EE plus MEOS rehabilitation model produced beneficial effects that persist for longer postoperative periods (i.e., 30 days).⁸² After injury, rats were distributed among EE plus MEOS, EE only, or STD housing conditions and then assessed for neurological function using a composite neuroscore, whereas post-traumatic motor deficits were measured by computerized analysis of vibrissal (i.e., whisker) motor performance. When compared with rehabilitation exposure for 15 days post-TBI,^{77,78} the neuroscores of injured rats receiving 30 days of EE plus MEOS exposure were significantly better. Also, cortical lesion volumes were measured after immunostaining for neuron-specific enolase, C3A, and GFAP, whereas cortical lesion volumes containing regeneration-associated proteins (RAPs) were determined in sections stained for GAP-43, a marker for axonal growth and sprouting, microtubule-associated protein 2, a dendritic marker, and neuronal class III β -tubulin, a microtubule marker for apical dendrites and axons in the cortex. Overall, no significant differences were detected in motor performance of vibrissae musculature, cortical lesion volumes, and cortical lesion volumes plus RAP between enriched and nonenriched animals. The results indicated that experience-dependent plasticity after EE plus MEOS exposure may be sufficient to rescue neurological function and may act by recruiting adjacent intact cortical areas to complement necessary functional pathways.⁸²

In order to further quantify the effect of multi-sensory rehabilitation on rodent cognitive function postinjury, injured rats were exposed to early multi-sensory rehabilitation (EMR; from 24 h to 15 days postinjury), which consisted of acoustic, olfactory, and visual stimulation. Animals in the EMR group exhibited potentiated recovery of neuromotor function (neuroscore) at 15 days postinjury.⁸³ There were no differences on the Barnes maze test in latencies to reach the protective box and path length improvement on days 11–15 postinjury, but EMR exposure significantly reduced the random search strategy chosen by injured rats, suggesting that this experimental model conferred some benefit on functional recovery.

Although EE exposure is overwhelmingly provided post-TBI, Johnson and colleagues assessed the potential benefits of a 15-day

course of EE pre-exposure in preventing neurobehavioral deficits after an impact to the prefrontal cortex.⁸⁴ The data showed that TBI-induced deficits in sensory neglect and spatial learning were significantly reduced in enriched versus nonenriched rats, prompting the researchers to conclude that the presentation of EE before TBI does indeed confer neuroprotection.⁸⁴ As indicated earlier, Held and colleagues²⁸ also provided EE preceding cortical injury and reported that the paradigm protected against motor deficits. Neuroprotective effects of EE exposure before ischemia/reperfusion injury have also been reported.²⁵ However, despite the behavioral protection, such an approach has limited clinical relevance.

In addition to CCI and FP brain injury, EE has also been shown to provide benefit after blast TBI (bTBI). In a recent study, Kovessdi and colleagues⁸⁵ investigated the beneficial effects of EE on behavioral, cellular, and molecular alterations induced by bTBI, a leading cause of disability among soldiers that is associated with memory impairments and increased anxiety.⁸⁶ Briefly, the researchers used a rodent model of bTBI through a compression-driven shock tube and performed behavioral assessments on the elevated plus-maze (EPM) and Barnes maze tests on separate days starting at 15, 44, and 66 days postinjury in order to provide a more longitudinal behavioral study relevant for the clinical setting. They first found that normalization of anxiety postinjury on EPM may take more than 2 months and is independent of housing conditions; however, long-term EE significantly improved spatial memory in the Barnes maze, compared to normal housing (NH; i.e., STD).⁸⁵ Further, the vascular endothelial growth factor (VEGF), a signaling protein acting as a positive regulator of adult *de novo* hippocampal neurogenesis,⁸⁷ and tau protein, a marker of axonal degeneration, but not the proinflammatory molecule known to inhibit hippocampal neurogenesis, interleukin-6 (IL-6),⁸⁸ were normalized in the dorsal hippocampus after long-term EE exposure, compared to NH rats. Enriched housing also reduced IL-6 expression in the ventral hippocampus of bTBI rats.⁸⁵ Overall, functional recovery after bTBI may involve complex neurogenesis-dependent and -independent processes, some of which are seemingly attributable to EE exposure.

The Benefits of Environmental Enrichment Extend Beyond Adult Male Rats

Although the studies just described have reliably shown that EE confers recovery of motor performance and enhances spatial learning and memory after CCI, FP, and bTBI, the studies have been conducted exclusively in male rats. Clearly, this is a limitation of the current model because it largely ignores half of the TBI population. Thus, in an attempt to address this limitation, male and normal cycling female rats were subjected to a CCI injury, placed in either STD or enriched housing for 3 weeks, and tested for motor and cognitive ability. TBI females, regardless of whether housed in EE or STD conditions, performed significantly better than their male counterparts on beam walking, although there were no differential effects attributed to EE. In contrast, males, but not females, exhibited EE-enhanced spatial memory performance.⁸⁹

Given that EE produces many changes in the brain, it seemed unlikely that the EE benefits were confined only to males, particularly because several studies in other CNS injury models,^{90–92} as well as non-CNS injury paradigms,^{93–95} have consistently reported that EE induces cognitive benefits in females. Hence, to further address this disparity, Monaco and colleagues⁹⁶ set out to reassess the effect of EE on functional and histological outcome in female

rats after brain trauma, using similar experimental procedures to the previous study by Wagner and colleagues.⁸⁹ Briefly, females were injured at various phases of the estrous stage to mimic real-world events. No effects of estrous stage at the time of TBI were detected with regard to behavioral performance. In the TBI groups, EE improved beam balance, beam walk, fine motor ability as assessed on a rotarod, and MWM spatial learning performance, compared to STD-housed females. EE also provided significant histological protection, as determined by increased hippocampal CA_{1/3} cell survival and decreased cortical lesion size.⁹⁶ This study is the first to demonstrate that EE confers robust benefits in female rats on a variety of tasks post-TBI, which parallels numerous studies in males and provides further support for EE as a pre-clinical model of neurorehabilitation.^{1–9,29} The marked behavioral differences between the two CCI studies may be a result of the inclusion of the rotarod task in the Monaco and colleagues study, which provided additional rehabilitation that manifest in previously undetected improvements in spatial learning.

EE has also been shown to exert benefits in pediatric rats. Specifically, the effects of EE during development were assessed in rats subjected to a FP injury on postnatal days (PNDs) 17–20 and placed in EE for 17 days.⁹⁷ EE significantly increased cortical thickness in the frontal and occipital cortices of sham controls, relative to the STD shams, but did not increase cortical thickness after FP injury. EE also enhanced MWM performance in shams versus FP-injured rats in STD conditions, as would be expected, but did not improve behavior in the injured group. These findings suggest that moderate concussive brain injuries in juveniles, albeit eliciting limited neuronal degeneration, attenuate outcomes of experience-dependent neural plasticity.⁹⁷ To further determine whether these anatomical and behavioral alterations during development in response to FP injury and EE, alone or in combination, correlate with changes in dendritic arborization, Ip and colleagues reported an ample description of dendritic analyses.⁹⁸ Briefly, PND 19 injured or sham rats were placed in either EE or STD housing on PND 20 for 17 days, followed by rehousing in STD environment until sacrifice on PNDs 47–51 for Golgi-Cox dendritic density and branching analysis in frontal, parietal, and occipital cortices. Using the Golgi method, it has been previously shown that animals exposed to EE display increased dendritic branching at higher orders,⁹⁹ enriched dendritic tree size,¹⁰⁰ and increased number of spines per dendrite.¹⁰¹ The results indicated that enriched housing increased dendritic density, predominantly within the occipital cortex, whereas receiving a TBI led to dendritic density increases in regions distant from the site of injury, such as the contralateral parietal cortex and the ipsi- and contralateral occipital cortex.⁹⁸ Moreover, in animals subjected to TBI followed by EE housing, there were injury-induced attenuations in EE-dependent dendritic density increases, except in the ipsilateral occipital cortex, where FP injury and EE showed additive effects with regard to dendritic density increases. Overall, it appeared that dendritic cortical changes correlate with the compromised behavioral function and developmental plasticity after brain injury, although these changes may be region dependent.⁹⁸

To further characterize the time-dependent behavioral responses to EE exposure after moderate TBI during development, PND 19 rats underwent lateral FP injury followed by rearing in EE or STD housing.¹⁰² Experience-dependent plasticity was assessed by two different EE treatments consisting of an early EE group exposed to enriched housing for 17 days from PNDs 20–37 and a delayed EE group undergoing enrichment exposure from PNDs 34–50. All rats were trained on the MWM from PNDs 50–57 with a probe trial test

on PND 63. Early EE rearing conferred memory benefits in task acquisition and probe trial performance for both sham and injured animals, albeit the magnitude of the improvement was stronger for uninjured rats. When EE treatment was applied 2 weeks postinjury, it did not affect performance in TBI rats. Thus, EE rehabilitation-induced benefits after experimental TBI during development differentially arise as a function of timing postinjury.¹⁰²

Further, the benefits of EE are not limited to young subjects because several non-TBI studies have shown that rats up to 24 months of age show an EE-induced attenuation of age-related dendritic regression and synaptic loss,¹⁰³ a reduction in spatial memory decline,¹⁰⁴ and increased cognitive function in complex blind-alley maze tasks.¹⁰⁵ EE also confers benefits in aged rats after middle cerebral artery electrocoagulation, as evidenced by improved rate and extent of neurobehavioral recovery, which correlated with smaller infarcts and a reduction in the number of proliferating astrocytes.²¹ Clearly lacking, but necessary, are studies evaluating the potential efficacy of EE after TBI in aged rodents.

Caveats to the Environmental Enrichment Effect

Although the overwhelming consensus of all the published studies is that EE confers benefits after TBI and can therefore be considered a viable neurorehabilitation strategy for experimental TBI, a few studies showed that there are caveats to the EE effect. In one such study, rats exposed to enriched housing for 15 days during postweaning, and who subsequently received a TBI at 3 months of age, displayed larger contusion cavities. These same rats also exhibited a greater initial deficit in forelimb use, albeit they subsequently recovered motor coordination in the foot fault test 1 week earlier.¹⁰⁶ In a paradigm where rats were placed in EE 1 week after a mid-line FP injury for 45 min per day (three times per week for 3 weeks for a total of nine sessions), no significant improvements in behavioral outcome were observed.¹⁰⁷ Moreover, EE did not restore MWM spatial learning,⁹⁷ or the latency to reach protective box and path length improvement on the Barnes maze.⁸³ A lack of EE-induced benefits was also noted for brain alterations, such as lesion volume⁸² and neurotrophin levels.⁷² Some of these limitations may have been the result of differential timing parameters. Thus, taken together, these findings elucidate the importance of timing, such as when is the latest time that EE can be initiated to promote optimal outcomes and what is the least amount of EE that is capable of conferring benefits. Such factors are the focus of the subsequent section.

Environmental Enrichment as a Pre-Clinical Model of Neurorehabilitation: Moving Beyond the Typical Paradigm

Long-term efficacy of environmental enrichment after brain trauma

A clinically relevant, but unanswered, question regarding the EE paradigm is whether the benefits can be maintained after EE (i.e., rehabilitation) is discontinued. Demonstrating longevity is critical because it would strengthen the validity of EE as a pre-clinical model of neurorehabilitation, considering that clinical rehabilitative therapy typically lasts for a finite period of time with little or no opportunity for follow-up care. Thus, in a recent study designed to study the possible long-term benefits of EE, Cheng and colleagues⁹ subjected rats to a CCI or sham injury and then, during phase I of

the experiment, they were randomly assigned to 3 weeks of EE or STD housing. Assessment of motor (days 1–5) and cognitive (days 14–19) ability was performed while the rats were in their respective environments. The findings showed that behavior was significantly improved in the EE group, relative to STD controls, which replicated previous studies.^{1–4} Moreover, in phase II, which was designed to mimic a real-world rehabilitative strategy, half of the improved rats in the EE condition were transferred back to STD housing conditions—effectively withdrawing rehabilitation—and then retested for motor and cognition at 1-month intervals for 6 months. The EE-continuous and EE-withdrawal groups performed at the same level and were both significantly better than the continuous STD-housed group. These data indicate that EE-induced motor and cognitive benefits are maintained for up to 6 months after the cessation of EE. Moreover, the study also confirmed that TBI-induced cognitive deficits in untreated rats are long term, because the spatial learning curve for the STD-housed group remained relatively unchanged from day 18 up to 6 months postinjury. These findings are in agreement with long-term TBI-induced cognitive impairments previously described both in the clinical literature¹⁰⁸ and in pre-clinical studies employing CCI^{68,70} and FP⁶⁹ injury models.

Therapeutic window of environmental enrichment after brain trauma

Another significant step in the continued progression of EE as a clinically relevant experimental model of rehabilitation was achieved by Hoffman and colleagues, who sought to determine whether the EE-mediated beneficial effects on behavioral recovery after TBI are dependent on early and constant exposure.⁴ This is important for model development because TBI patients will not engage in rehabilitation until after critical care. The findings revealed that rats provided early (immediately after surgery) and continuous EE postinjury recovered from the motor deficits and acquired spatial learning significantly quicker than STD-housed rats, which replicated previous studies from our laboratory.^{1–3} The data further showed that delaying EE by 1 week after TBI still enhanced cognition, but did not benefit motor function. In contrast, providing EE immediately after TBI and then withdrawing it after 1 week facilitated the recovery of the motor deficits, which are present during the first week after TBI, but did not benefit spatial learning, which takes place during the second week. The interpretation of the findings was that EE-induced benefits are contingent on task-specific neurobehavioral experience and not necessarily on the duration of EE.⁴ However, whereas benefits were observed when EE was provided concurrently with specific neurobehavioral training, only half of the possible temporal permutations for EE exposure were evaluated in the initial study. Hence, to further characterize this timing issue, Matter and colleagues extended the original study by Hoffman and colleagues by including additional temporal permutations.⁸ Briefly, rats were randomly assigned to eight groups receiving continuous, early, delayed, or intermittent EE. Again, early EE enhanced motor recovery, whereas late EE facilitated cognitive recovery. Further, the TBI group receiving enrichment for a total of 14 days (1 week before and during maze training) performed significantly better than both the TBI groups receiving 1 week of EE postinjury, followed by 1 week of STD housing then another 1 week of EE exposure during the cognitive task, suggesting that there may be a threshold of approximately 2 weeks of contiguous EE required to promote cognitive benefits after TBI.⁸

In addition to TBI patients not engaging in rehabilitation until after critical care has ended, another important variable to consider for successful EE model development is that, once rehabilitation is initiated, the duration of the therapy will be significantly limited. In the clinical setting, rehabilitation therapy is typically provided for a finite period of time, typically 3–8 h, and is dependent on patients being able to withstand and participate in rehabilitative programs.^{109–112} To reduce discrepancies between the experimental EE model and the clinical setting, a recent study by de Witt and colleagues focused on evaluating the potential efficacy of an abbreviated enrichment (i.e., rehabilitation-relevant) paradigm consisting of 2, 4, or 6 h of EE per day, compared to either continuous EE or STD housing.⁷ No significant differences were observed between the continuous and 6-h dose of EE, but both exhibited significant improvements in motor and cognitive performance, when compared to groups receiving 2 or 4 h of EE or STD housing.⁷ Additionally, no differences were revealed between the 2- or 4-h EE groups versus the STD group. These novel findings suggest that abbreviated EE is sufficient for promoting optimal functional benefits after moderate TBI in rats, but also indicate that a minimum threshold of daily enrichment exposure may be required to elicit neurobehavioral recovery after TBI, as previously reported by Matter and colleagues.⁸

Although a small number of studies have reported that lesser amounts of EE can be effective, the experimental designs (i.e., behavioral assessments in models of TBI) for those studies were different from the ones conducted by Kline and colleagues. Specifically, 1 h of EE per day appeared sufficient to enhance cognitive recovery after FP injury.¹¹³ Further, TBI-related impairments of spontaneous object recognition in a Y-maze were reversed when EE exposure began 24 h postinjury and consisted of 2 h per day for 14 days.¹¹⁴ Moreover, 2 h of EE after bilateral occipital cortex lesions was as effective as continuous EE in reducing spatial navigation errors in a Hebb-Williams maze.⁵⁹ Last, in non-TBI models, 3 h of EE per day for 2 weeks improved long-term novel object recognition memory and enhanced adult hippocampal neurogenesis,¹¹ whereas 1 h of object exposure (i.e., EE) was sufficient to enhance memory consolidation.¹¹⁵ Taken together, these studies suggest the EE can provide benefits after CNS injury, but, as with other therapies, there may be a limit to its abilities that is dependent on timing, dosing, and injury.

Combinational therapies: Environmental enrichment and pharmaceuticals or stem cell implantation

The limited translational success of pre-clinical findings employing experimental TBI models is, according to current beliefs, at least partially attributed to the use of monotherapies, as opposed to a more translatable approach of combined or polytherapies.^{2,116} In an attempt to initiate beneficial effects in TBI models from multiple angles concomitantly, another group of studies focused on combining treatment with EE and administration of pharmacologic agents targeting neurotransmitter systems likely altered postinjury. First, it has been shown that acute treatment with the serotonin_{1A} (5-HT_{1A}) receptor agonist, 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), promotes behavioral recovery and restores neuropathological alterations post-TBI.^{117,118} Therefore, Kline and colleagues sought to investigate whether combining 8-OH-DPAT with EE would confer additional benefit, relative to either treatment alone.¹ As per the acute pharmacotherapy regimen, rats received a single administration of 8-OH-DPAT 15 min after TBI or sham injury and then were provided early and continuous EE. The results

indicated that both 8-OH-DPAT and EE exposure were sufficient to individually enhance behavioral recovery and rescue hippocampal CA₃ cell loss after TBI, as observed in previous studies.^{117,118} Specifically, both EE and 8-OH-DPAT improved motor function, as demonstrated by a significant reduction in time to traverse an elevated narrow beam in both groups versus the STD-housed vehicle group. Moreover, both EE and 8-OH-DPAT enhanced spatial learning, relative to the STD-housed group receiving vehicle. Cognitive differences were also observed after the combination of EE and 8-OH-DPAT versus the STD plus 8-OH-DPAT group, with the former being significantly better, suggesting that EE enhanced the effect of 8-OH-DPAT. However, there were no significant differences in behavioral recovery between the combination treatment group and the EE-alone group, indicating that there was no additional benefit when overlapping the two therapeutic strategies.¹

Studies from Kline and colleagues have also shown that chronic 8-OH-DPAT reduces histopathology (i.e., decreases cortical lesion volume and enhances hippocampal neuron survival) and improves neurobehavioral recovery after CCI injury.¹¹⁹ Thus, to enhance the relevance for physiotherapeutic intervention when patients may also be given drugs chronically, a subsequent study combined EE with chronic 8-OH-DPAT given daily for 3 weeks postinjury.² The researchers reported cognitive and motor recovery, as well as attenuated hippocampal CA₃ and choline acetyltransferase (ChAT) medial septal cell loss when each therapy was applied individually. Subsequently, the combined therapy reduced TBI-induced ChAT cell loss, but did not enhance hippocampal cell survival or neurobehavioral performance beyond that of either treatment alone.² A possible explanation for the lack of an additive behavioral effect in some of the reported measures from the above studies could be that the typical EE paradigm (i.e., early and continuous) is a very effective treatment and thus there may be a ceiling effect that precludes 8-OH-DPAT from conferring additional statistically significant improvement.

The 5-HT_{1A} receptor agonist, buspirone, has been previously shown to facilitate spatial learning on MWM and reduce histopathology after TBI¹²⁰ and it is already used in the clinical setting to treat anxiety and depression; therefore, safety and tolerability parameters are well characterized.¹²¹ To enhance the clinical translation of single-therapy studies, Kline and colleagues placed injured or sham rats into EE or STD housing and administered either chronic buspirone or vehicle.³ TBI animals in EE, regardless of whether buspirone or vehicle treated, displayed diminished CA₃ hippocampal cell loss as well as facilitated motor recovery and enhanced spatial learning and memory retention, compared to STD housing. Buspirone alone also provided significant neurobehavioral recovery in injured rats. Overall, these results demonstrated significant benefits by EE and buspirone, although the expected outcome of larger beneficial effects, when provided in combination, did not materialize.³ The lack of an additive effect may again be attributable to a ceiling effect produced by an early-and-continuous EE paradigm, similar to findings by Kline and colleagues,² indicating therefore that future studies aimed at unveiling synergistic effects of drug plus EE may require various doses and/or dosing schedules in clinically relevant delayed-and-abbreviated EE paradigms. As discussed in the previous section, recent studies embracing this strategy have recently been conducted by Kline and colleagues and continue to be explored.

Another combinational treatment approach was recently presented by Peruzzaro and colleagues, who evaluated the efficiency of EE plus murine cortical embryonic stem cell (eSCs) as an adjunct therapy in reversing TBI-induced behavioral deficits. Briefly,

after a bilateral prefrontal CCI injury, rats were placed in EE for 36 days postinjury, which overlapped with the eSCs that were implanted in the injured cortex 1 week post-TBI.¹²² Though the combination of eSC implants plus EE exposure was, for the most part, not effective at facilitating recovery in the rotarod and Barnes maze tests, the combined treatment group performed similarly to control groups, suggesting some degree of neuroprotection was achieved. Stringent quantitative analyses for eSC survival, migration, and differentiation into neural or glial cells were not performed in this study; therefore, the direct functional relevance of eSC implantation combined with EE remains to be determined.

Optimal Benefits from Typical versus Atypical Enrichment Paradigms

The robust changes induced by EE and described throughout the literature have prompted questions asking what it is about the paradigm that mediates the effects. As mentioned earlier, typical EE consists of three basic components, which include increased space for exploration and exercise, sensory experience, and socialization.⁶ Rosenzweig and colleagues reported that socialization alone is insufficient to account for cerebral effects of EE and speculated that the inanimate stimulus conditions might also play a synergistic role.¹²³ In a model of inflammatory pain, Gabriel and colleagues showed that physical enrichment had a bigger effect.¹²⁴ Lambert and colleagues reported that exercise, but not cognitive stimulation or acrobat training, improved spatial learning, relative to controls.⁹⁵ However, both social and EE improved sensory and motor function after SCI in the rat.¹⁸ Taken together, these studies suggest that different elements of the EE paradigm may contribute to the observed benefits.

To address this question in greater detail, Sozda and colleagues designed a study to further determine which of these aspects, or combination of them, was necessary to confer benefits after TBI.⁶ Rats were distributed among various EE conditions, namely, typical EE (i.e., all components included), typical STD (i.e., no components), and atypical EE conditions that consisted of EE minus social (i.e., toys in large cage, but pair housing), EE minus stimuli (i.e., group housing, but no toys), and STD plus stimuli (a few toys added to the STD housing condition). The data showed that, albeit not statistically significant, when compared to the STD group, the atypical EE groups exhibited slightly more improvement in the MWM.⁶ However, the typical EE paradigm was most beneficial in providing motor and cognitive recovery, as well as histological protection versus the typical STD group and each of the three atypical EE conditions, which indicates that concomitant presentation of all the components is necessary to provide an optimal EE effect, thus supporting Rosenzweig's speculation of an additive or synergistic effect. This finding has significant clinical relevance because it suggests that rehabilitation programs that focus on multi-modal rehabilitative approaches may provide benefits to TBI patients above and beyond those focusing on specific symptomatology.

Potential Mechanisms for Environmental Enrichment-Induced Benefits after Traumatic Brain Injury

A number of studies^{72,125–129} have reported significant changes in the expression of neurotrophins after TBI. Briefly, after a moderate FP injury, Hicks and colleagues¹²⁵ reported increased expression of neurotrophin brain-derived neurotrophic factor (BDNF) messenger RNA (mRNA) in the CA₃ and dentate gyrus at

1, 3, and 6 h postinjury, with BDNF in the dentate gyrus remaining significantly elevated up to 72 h. Further, neurotrophin 3 (NT-3) mRNA was significantly reduced in the dentate at 6 and 24 h postinjury, supporting the hypothesis that secondary events after TBI are multi-faceted and may include neurotrophin plasticity. Further, because long-term exposure to EE housing in non-TBI animals has been shown to modulate mRNA brain expression for these neurotrophins,^{130–132} Hicks and colleagues proceeded to further evaluate the relationship between EE and neurotrophin gene expression after TBI.⁷² This study found that 2 weeks of EE exposure improved acquisition of the MWM task in FP-injured rats, but did not produce changes in BDNF, tropomyosin related kinase B (trkB), and NT-3 mRNA levels in hippocampal subregions.⁷² In contrast, Chen and colleagues¹²⁹ reported that BDNF protein levels were significantly elevated in the frontal cortex ipsilateral to CCI injury in males, but were not further affected by EE exposure. Moreover, cortical BDNF expression was not affected by either injury or EE in females. In the hippocampus ipsilateral to injury, qualitative decreases in BDNF expression were found for both sexes exposed to STD housing, whereas significant increases in BDNF protein expression was observed in enriched females. Moreover, injured females also displayed a significant increase in BDNF expression in the hippocampus contralateral to the injury, which was not further augmented by EE.¹²⁹

Given that dopamine (DA) system alterations have been reported in rodents subsequent to EE exposure^{15,133} or CCI injury,^{134–136} Wagner and colleagues investigated gender-dependent responsiveness to EE and DA regulation postinjury. Briefly, male and cycling female rats were subjected to TBI and placed into EE or STD housing for 4 weeks followed by quantification of frontal cortex and striatum dopamine transporter (DAT) protein expression. The researchers concluded that DAT expression in males undergoes larger decreases after injury, compared to females, but EE exposure has larger effects on postinjury DAT expression in females versus males.¹³⁷ Shin and colleagues¹³⁸ characterized the mRNA expression profile in substantia nigra (SN) and ventral tegmental area (VTA) at 4 weeks postinjury alone and in combination with enriched housing by using efficiency analysis of microarray data¹³⁹ to assess differential gene expression. Aside from alterations in genes involved in inflammation and cellular plasticity, there were also chronic alterations in genes important for calcium and DA signaling pathways in TBI animals exposed to EE, such as down-regulation of tyrosine hydroxylase and up-regulation of alpha-synuclein, which may represent compensatory mechanisms to counteract the detrimental effects of TBI.¹³⁸

In addition to potential neurotrophin and dopaminergic mechanisms mediating the benefits conferred by EE, Briones and colleagues provided additional possibilities.⁷⁵ Specifically, continuous EE reversed the injury-induced increases of the pro-inflammatory cytokines, IL-1 β and tumor necrosis factor alpha (TNF- α), as well as decreases of the anti-inflammatory cytokine, IL-10, as measured in the prefrontal cortex and hippocampus. Markers of brain energy homeostasis, such as the ratio of phosphorylated adenosine-monophosphate-activated protein kinase (pAMPK)/AMPK and the ubiquitous mitochondrial creatine kinase (uMtCK) were decreased postinjury in the same brain regions, an effect significantly normalized by EE exposure.

Taken together, these studies demonstrate complex, region-specific, and gender differences in various potential molecular mechanisms mediating EE-induced recovery. Importantly, the elucidation of such varied alterations may provide new avenues for pharmaceutical targets and rehabilitative approaches after TBI.

Conclusion

The conclusion from the majority of the 34 currently published TBI studies using EE as a therapy is that enrichment benefits behavioral and histological outcome in male and female, as well as adult and pediatric rats and mice, after brain injury produced by various models. Though there are cases where EE was ineffective, those instances are in the minority. Taken together, these cumulative findings provide strong support for EE as a generalized, robust pre-clinical model of neurorehabilitation. However, to further enhance the model and more accurately mimic the clinic, additional experimental manipulations should be considered. For example, future studies should continue to evaluate EE during more rehabilitation-relevant conditions, such as delayed and shorter time periods as well as in combination with other therapeutic approaches, as our laboratory has been doing for the past few years. This review has highlighted possible therapies, such as pharmaceuticals, that affect dopaminergic and inflammatory pathways that can be coupled with EE. Focusing on developing and refining these applicable variables will advance a clinically relevant experimental model of rehabilitation that can be applied to the TBI setting to facilitate translatable rehabilitation research. The translatability will be strengthened further by continuing to expand the model in males and females, as well as developing it further in pediatric and aged populations.

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References

- Kline, A.E., Wagner, A.K., Westergom, B.P., Malena, R.R., Zafonte, R.D., Olsen, A.S., Sozda, C.N., Luthra, P., Panda, M., Cheng, J.P., and Aslam, H.A. (2007). Acute treatment with the 5-HT_{1A} receptor agonist 8-OH-DPAT and chronic environmental enrichment confer neurobehavioral benefit after experimental brain trauma. *Behav. Brain Res.* 177, 186–194.
- Kline, A.E., McAloon, R.L., Henderson, K.A., Bansal, U.K., Ganti, B.M., Ahmed, R.H., Gibbs, R.B., and Sozda, C.N. (2010). Evaluation of a combined therapeutic regimen of 8-OH-DPAT and environmental enrichment after experimental traumatic brain injury. *J. Neurotrauma* 27, 2021–2032.
- Kline, A.E., Olsen, A.S., Sozda, C.N., Hoffman, A.N., and Cheng, J.P. (2012). Evaluation of a combined treatment paradigm consisting of environmental enrichment and the 5-HT_{1A} receptor agonist buspirone after experimental traumatic brain injury. *J. Neurotrauma* 29, 1960–1969.
- Hoffman, A.N., Malena, R.R., Westergom, B.P., Luthra, P., Cheng, J.P., Aslam, H.A., Zafonte, R.D., and Kline, A.E. (2008). Environmental enrichment-mediated functional improvement after experimental traumatic brain injury is contingent on task-specific neurobehavioral experience. *Neurosci. Lett.* 431, 226–230.
- Hamm, R.J., Temple, M.D., O'Dell, D.M., Pike, B.R., and Lyeth, B.G. (1996). Exposure to environmental complexity promotes recovery of cognitive function after traumatic brain injury. *J. Neurotrauma* 13, 41–47.
- Sozda, C.N., Hoffman, A.N., Olsen, A.S., Cheng, J.P., Zafonte, R.D., and Kline, A.E. (2010). Empirical comparison of typical and atypical environmental enrichment paradigms on functional and histological outcome after experimental traumatic brain injury. *J. Neurotrauma* 27, 1047–1057.
- de Witt, B.W., Ehrenberg, K.M., McAloon, R.L., Panos, A.H., Shaw, K.E., Raghavan, P.V., Skidmore, E.R., and Kline, A.E. (2011). Abbreviated environmental enrichment enhances neurobehavioral recovery comparably to continuous exposure after traumatic brain injury. *Neurorehabil. Neural Repair* 25, 343–350.
- Matter, A.M., Folweiler, K.A., Curatolo, L.M., and Kline, A.E. (2011). Temporal effects of environmental enrichment-mediated functional improvement after experimental traumatic brain injury in rats. *Neurorehabil. Neural Repair* 25, 558–564.
- Cheng, J.P., Shaw, K.E., Monaco, C.M., Hoffman, A.N., Sozda, C.N., Olsen, A.S., and Kline, A.E. (2012). A relatively brief exposure to environmental enrichment after experimental traumatic brain injury confers long-term cognitive benefits. *J. Neurotrauma* 29, 2684–2688.
- Veena, J., Srikumar, B.N., Mahati, K., Bhagya, V., Raju, T.R., and Shankaranarayana Rao, B.S. (2009). Enriched environment restores hippocampal cell proliferation and ameliorates cognitive deficits in chronically stressed rats. *J. Neurosci. Res.* 87, 831–843.
- Brüel-Jungerman, E., Larocque, S., and Rampon, C. (2005). New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. *Eur. J. Neurosci.* 21, 513–521.
- Doulames, V., Lee, S., and Shea, T.B. (2013). Environmental enrichment and social interaction improve cognitive function and decrease reactive oxidative species in normal adult mice. *Int. J. Neurosci.* Oct 31. doi:10.3109/00207454.2013.848441. [Epub ahead of print]
- Jankowsky, J.L., Melnikova, T., Fadale, D.J., Xu, G.M., Slunt, H.H., Gonzales, V., Younkin, L.H., Younkin, S.G., Borchelt, D.R., and Savonenko, A.V. (2005). Environmental enrichment mitigates cognitive deficits in a mouse model of Alzheimer's disease. *J. Neurosci.* 25, 5217–5224.
- Cracchiolo, J.R., Mori, T., Nazian, S.J., Tan, J., Potter, H., and Arendash, G.W. (2007). Enhanced cognitive activity-over and above social or physical activity-is required to protect Alzheimer's mice against cognitive impairment, reduce A β deposition, and increase synaptic immunoreactivity. *Neurobiol. Learn. Mem.* 88, 277–294.
- Bezard, E., Dovero, S., Belin, D., Duconger, S., Jackson-Lewis, V., Przedborski, S., Piazza, P.V., Gross, C.E., and Jaber, M. (2003). Enriched environment confers resistance to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and cocaine: involvement of dopamine transporter and trophic factors. *J. Neurosci.* 23, 10999–11007.
- Faherty, C.J., Raviie Shepherd, K., Herasimtschuk, A., and Smye, R.J. (2005). Environmental enrichment in adulthood eliminates neuronal death in experimental Parkinsonism. *Brain Res. Mol. Brain Res.* 134, 170–179.
- Jadavji, N.M., Kolb, B., and Metz, G.A. (2006). Enriched environment improves motor function in intact and unilateral dopamine-depleted rats. *Neuroscience* 140, 1127–1138.
- Berrolcal, Y., Pearse, D.D., Singh, A., Andrade, C.M., McBroom, J.S., Puentes, R., and Eaton, M.J. (2007). Social and environmental enrichment improves sensory and motor recovery after severe contusive spinal cord injury in the rat. *J. Neurotrauma* 24, 1761–1772.
- Fischer, F.R., and Peduzzi, J.D. (2007). Functional recovery in rats with chronic spinal cord injuries after exposure to an enriched environment. *J. Spinal Cord Med.* 30, 147–155.
- Salmaso, N., Silbereis, J., Komitova, M., Mitchell, P., Chapman, K., Ment, L.R., Schwartz, M.L., and Vaccaro, F.M. (2012). Environmental enrichment increases the GFAP+stem cell pool and reverses hypoxia-induced cognitive deficits in juvenile mice. *J. Neurosci.* 32, 8930–8939.
- Buchhold, B., Mogoanta, L., Suofu, Y., Hamm, A., Walker, L., Kessler, C., and Popa-Wagner, A. (2007). Environmental enrichment improves functional and neuropathological indices following stroke in young and aged rats. *Restor. Neurol. Neurosci.* 25, 467–484.
- Zai, L., Ferrari, C., Dice, C., Subbaiah, S., Havton, L.A., Coppola, G., Geschwind, D., Irwin, N., Huebner, E., Strittmatter, S.M., and Benowitz, L.I. (2011). Inosine augments the effects of a Nogo receptor blocker and of environmental enrichment to restore skilled forelimb use after stroke. *J. Neurosci.* 31, 5977–5988.
- Nygren, J., and Wieloch, T. (2005). Enriched environment enhances recovery of motor function after focal ischemia in mice, and downregulates the transcription factor NGF1-A. *J. Cereb. Blood Flow Metab.* 25, 1625–1633.
- Zhao, L.R., Mattsson, B., and Johansson, B.B. (2000). Environmental influence on brain-derived neurotrophic factor messenger RNA expression after middle cerebral artery occlusion in spontaneously hypertensive rats. *Neuroscience* 97, 177–184.

25. Yu, K., Wu, Y., Hu, Y., Zhang, Q., Xie, H., Liu, G., Chen, Y., Guo, Z., and Jia, J. (2013). Neuroprotective effects of prior exposure to enriched environment on cerebral ischemia/reperfusion injury in rats: the possible molecular mechanism. *Brain Res.* 1538, 93–103.
26. Rema, V., and Ebner, F.F. (2003). Lesions of mature barrel field cortex interfere with sensory processing and plasticity in connected areas of the contralateral hemisphere. *J. Neurosci.* 23, 10378–10387.
27. Goldstein, L.B. (2003). Model of recovery of locomotor ability after sensorimotor cortex injury in rats. *ILAR J.* 44, 125–129.
28. Held, J.M., Gordon, J., and Gentile, A.M. (1985). Environmental influences on locomotor recovery following cortical lesions in rats. *Behav. Neurosci.* 99, 678–690.
29. Passineau, M.J., Green, E.J., and Dietrich, W.D. (2001). Therapeutic effects of environmental enrichment on cognitive function and tissue integrity following severe traumatic brain injury in rats. *Exp. Neurol.* 168, 373–384.
30. Will, B., Galani, R., Kelche, C., and Rosenzweig, M.R. (2004). Recovery from brain injury in animals: relative efficacy of environmental enrichment, physical exercise or formal training (1990–2002). *Prog. Neurobiol.* 72, 167–182.
31. Hebb, D.O. (1947). The effects of early experience on problem solving at maturity. *Am. Psychol.* 2, 306–307.
32. Bennett, E.L., Diamond, M.C., Krech, D., and Rosenzweig, M.R. (1964). Chemical and anatomical plasticity brain. *Science* 146, 610–619.
33. Diamond, M.C., Law, F., Rhodes, H., Lindner, B., Rosenzweig, M.R., Krech, D., and Bennett, E.L. (1966). Increases in cortical depth and glia numbers in rats subjected to enriched environment. *J. Comp. Neurol.* 128, 117–126.
34. La Torre, J.C. (1968). Effect of differential environmental enrichment on brain weight and on acetylcholinesterase and cholinesterase activities in mice. *Exp. Neurol.* 22, 493–503.
35. Walsh, R.N., Budtz-Olsen, O.E., Penny, J.E., and Cummins, R.A. (1969). The effects of environmental complexity on the histology of the rat hippocampus. *J. Comp. Neurol.* 137, 361–366.
36. Mollgaard, K., Diamond, M.C., Bennett, E.L., Rosenzweig, M.R., and Lindner, B. (1971). Quantitative synaptic changes with differential experience in rat brain. *Int. J. Neurosci.* 2, 113–127.
37. Diamond, M.C., Rosenzweig, M.R., Bennett, E.L., Lindner, B., and Lyon, L. (1972). Effects of environmental enrichment and impoverishment on rat cerebral cortex. *J. Neurobiol.* 3, 47–64.
38. Diamond, M.C., Ingham, C.A., Johnson, R.E., Bennett, E.L., and Rosenzweig, M.R. (1976). Effects of environment on morphology of rat cerebral cortex and hippocampus. *J. Neurobiol.* 7, 75–85.
39. Ferchmin, P.A., and Bennett, E.L. (1975). Direct contact with enriched environment is required to alter cerebral weights in rats. *J. Comp. Physiol. Psychol.* 88, 360–367.
40. Rosenzweig, M.R., and Bennett, E.L. (1996). Psychobiology of plasticity: effects of training and experience on brain and behavior. *Behav. Brain Res.* 78, 57–65.
41. Nakamura, H., Kobayashi, S., Ohashi, Y., and Ando, S. (1999). Age-changes of brain synapses and synaptic plasticity in response to an enriched environment. *J. Neurosci. Res.* 56, 307–315.
42. Nilsson, M., Perfilieva, E., Johansson, U., Orwar, O., and Eriksson, P.S. (1999). Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. *J. Neurobiol.* 39, 569–578.
43. Kempermann, G., Kuhn, H.G., and Gage, F.H. (1997). More hippocampal neurons in adult mice living in an enriched environment. *Nature* 386, 493–495.
44. Kempermann, G., Brandon, E.P., and Gage, F.H. (1998). Environmental stimulation of 129/SvJ mice causes increased cell proliferation and neurogenesis in the adult dentate gyrus. *Curr. Biol.* 8, 939–942.
45. Kempermann, G., and Gage, F.H. (1999). Experience-dependent regulation of adult hippocampal neurogenesis: effects of long-term stimulation and stimulus withdrawal. *Hippocampus* 9, 321–332.
46. Nithianantharajah, J., Levis, H., and Murphy, M. (2004). Environmental enrichment results in cortical and subcortical changes in levels of synaptophysin and PSD-95 proteins. *Neurobiol. Learn. Mem.* 81, 200–210.
47. McIntosh, T.K. (1994). Neurochemical sequelae of traumatic brain injury: therapeutic implications. *Cerebrovasc. Brain Metab. Rev.* 6, 109–162.
48. Thompson, H.J., Lifshitz, J., Marklund, N., Grady, M.S., Graham, D.I., Hovda, D.A., and McIntosh, T.K. (2005). Lateral fluid percussion brain injury: a 15-year review and evaluation. *J. Neurotrauma* 22, 42–75.
49. Bales, J.W., Wagner, A.K., Kline, A.E., and Dixon, C.E. (2009). Persistent cognitive dysfunction after traumatic brain injury: a dopamine hypothesis. *Neurosci. Biobehav. Rev.* 33, 981–1003.
50. Diamond, M.C., Krech, D., and Rosenzweig, M.R. (1964). The effects of an enriched environment on the histology of the rat cerebral cortex. *J. Comp. Neurol.* 123, 111–120.
51. Centers for Disease Control and Prevention (CDC) National Center for Injury Prevention and Control. (2003). *Report to Congress on Mild Traumatic Brain Injury in the United States: Steps to Prevent a Serious Public Health Problem*. Centers for Disease Control and Prevention: Atlanta, GA.
52. Moore, E.L., Terryberry-Spohr, L., and Hope, D.A. (2006). Mild traumatic brain injury and anxiety sequelae: a review of the literature. *Brain Inj.* 20, 117–132.
53. Selassie, A.W., Zaloshnja, E., Langlois, J.A., Miller, T., Jones, P., and Steiner, C. (2008). Incidence of long-term disability following traumatic brain injury hospitalization, United States, 2003. *J. Head Trauma Rehabil.* 23, 123–131.
54. Summers, C.R., Ivins, B., and Schwab, K.A. (2009). Traumatic brain injury in the United States: an epidemiologic overview. *Mt. Sinai J. Med.* 76, 105–110.
55. Faul, M., Xu, L., Wald, M.M., and Coronado, V.G. (2010). *Traumatic Brain Injury in the United States: Emergency Department Visits, Hospitalizations and Deaths 2002–2006*. Centers for Disease Control and Prevention, National Center for Injury Prevention and Control: Atlanta, GA.
56. Garcia, A.N., Shah, M.A., Dixon, C.E., Wagner, A.K., and Kline, A.E. (2011). Biologic and plastic effects of experimental traumatic brain injury treatment paradigms and their relevance to clinical rehabilitation. *PM R* 3, S18–S27.
57. Max, W., MacKenzie, E.J., and Rice, C.P. (1991). Head injuries: costs and consequences. *J. Head Trauma Rehabil.* 6, 76–91.
58. Will, B.E., Rosenzweig, M.R., and Bennett, E.L. (1976). Effects of differential environments on recovery from neonatal brain lesions, measured by problem-solving scores and brain dimensions. *Physiol. Behav.* 16, 603–611.
59. Will, B.E., Rosenzweig, M.R., Bennett, E.L., Hebert, M., and Morimoto, H. (1977). Relatively brief environmental enrichment aids recovery of learning capacity and alters brain measures after post-weaning brain lesions in rats. *J. Comp. Physiol. Psychol.* 91, 33–50.
60. Gentile, A.M., Beheshti, Z., and Held, J.M. (1987). Enrichment versus exercise effects on motor impairments following cortical removals in rats. *Behav. Neural Biol.* 47, 321–332.
61. Rose, F.D., Davey, M.J., Love, S., and Dell, P.A. (1987). Environmental enrichment and recovery from contralateral sensory neglect in rats with large unilateral neocortical lesions. *Behav. Brain Res.* 24, 195–202.
62. Dhanushkodi, A., Bindu, B., Raju, T.R., and Kutty, B.M. (2007). Exposure to enriched environment improves spatial learning performances and enhances cell density but not choline acetyltransferase activity in the hippocampus of ventral subicular-lesioned rats. *Behav. Neurosci.* 121, 491–500.
63. Statler, K.D., Jenkins, L.W., Dixon, C.E., Clark, R.S., Marion, D.W., and Kochanek, P.M. (2001). The simple model versus the super model: translating experimental traumatic brain injury research to the bedside. *J. Neurotrauma* 18, 1195–1206.
64. Dixon, C.E., Clifton, G.L., Lighthall, J.W., Yaghmai, A.A., and Hayes, R.L. (1991). A controlled cortical impact model of traumatic brain injury in the rat. *J. Neurosci. Methods* 39, 253–262.
65. Dixon, C.E., Lyeth, B.G., Povlishock, J.T., Findling, R.L., Hamm, R.J., Marmarou, A., Young, H.F., and Hayes, R.L. (1987). A fluid percussion model of experimental brain injury in the rat. *J. Neurosurg.* 67, 110–119.
66. Kline, A.E., and Dixon, C.E. (2001). Contemporary in vivo models of brain trauma and a comparison of injury responses, in: *Head Trauma: Basic, Preclinical, and Clinical Directions*. Miller, L.P., and Hayes, R.L. (eds). John Wiley & Sons, Inc.: New York, pps. 65–84.
67. Smith, D.H., Chen, X.H., Pierce, J.E., Wolf, J.A., Trojanowski, J.Q., Graham, D.I., and McIntosh, T.K. (1997). Progressive atrophy and neuron death for one year following brain trauma in the rat. *J. Neurotrauma* 14, 715–727.
68. Lindner, M.D., Plone, M.A., Cain, C.K., Frydel, B., Francis, J.M., Emerich, D.F., and Sutton, R.L. (1998). Dissociable long-term cog-

- nitive deficits after frontal versus sensorimotor cortical contusions. *J. Neurotrauma* 15, 199–216.
69. Pierce, J.E., Smith, D.H., Trojanowski, J.Q., and McIntosh, T.K. (1998). Enduring cognitive, neurobehavioral and histopathological changes persist for up to one year following severe experimental brain injury in rats. *Neuroscience* 87, 359–369.
 70. Dixon, C.E., Kochanek, P.M., Yan, H.Q., Schiding, J.K., Griffith, R.G., Baum, E., Marion, D.W., and DeKosky, S.T. (1999). One-year study of spatial memory performance, brain morphology, and cholinergic markers after moderate controlled cortical impact in rats. *J. Neurotrauma* 16, 109–122.
 71. Bramlett, H.M., and Dietrich, W.D. (2002). Quantitative structural changes in white and gray matter 1 year following traumatic brain injury in rats. *Acta Neuropathol.* 103, 607–614.
 72. Hicks, R.R., Zhang, L., Atkinson, A., Stevenon, M., Veneracion, M., and Seroogy, K.B. (2002). Environmental enrichment attenuates cognitive deficits, but does not alter neurotrophin gene expression in the hippocampus following lateral fluid percussion brain injury. *Neuroscience* 112, 631–637.
 73. Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *J. Neurosci. Methods* 11, 47–60.
 74. Smith, J.M., Lunga, P., Story, D., Harris, N., Le Belle, J., James, M.F., Pickard, J.D., and Fawcett, J.W. (2007). Inosine promotes recovery of skilled motor function in a model of focal brain injury. *Brain* 130, 915–925.
 75. Briones, T.L., Woods, J., and Rogozinska, M. (2013). Decreased neuroinflammation and increased brain energy homeostasis following environmental enrichment after mild traumatic brain injury is associated with improvement in cognitive function. *Acta Neuropathol. Commun.* 1, 57.
 76. Muthuraju, S., Pati, S., Rafiqul, M., Abdullah, J.M., and Jaafar, H. (2012). IntelliCage provides voluntary exercise and an enriched environment, improving locomotive activity in mice following fluid percussion injury. *Basal Ganglia* 2, 143–151.
 77. Maegele, M., Lippert-Gruener, M., Ester-Bode, T., Sauerland, S., Schafer, U., Molcanyi, M., Lefering, R., Bouillon, B., Neiss, W.F., Angelov, D.N., Klug, N., McIntosh, T.K., and Neugebauer, E.A. (2005). Reversal of neuromotor and cognitive dysfunction in an enriched environment combined with multimodal early onset stimulation after traumatic brain injury in rats. *J. Neurotrauma* 22, 772–782.
 78. Maegele, M., Lippert-Gruener, M., Ester-Bode, T., Garbe, J., Bouillon, B., Neugebauer, E., Klug, N., Lefering, R., Neiss, W.F., and Angelov, D.N. (2005). Multimodal early onset stimulation combined with enriched environment is associated with reduced CNS lesion volume and enhanced reversal of neuromotor dysfunction after traumatic brain injury in rats. *Eur. J. Neurosci.* 21, 2406–2418.
 79. Lippert-Gruener, M., Maegele, M., Pokorny, J., Angelov, D.N., Svestkova, O., Wittner, M., and Trojan, S. (2007). Early rehabilitation model shows positive effects on neural degeneration and recovery from neuromotor deficits following traumatic brain injury. *Physiol. Res.* 56, 359–368.
 80. Gruener, M.L., and Terhaag, D. (2000). Multimodal early onset stimulation (MEOS) in rehabilitation after brain injury. *Brain Inj.* 14, 585–594.
 81. Lippert-Gruener, M., Wedekind, C., Ernestus, R.I., and Klug, N. (2002). Early rehabilitative concepts in therapy of the comatose brain injured patients. *Acta Neurochir. Suppl.* 79, 21–23.
 82. Lippert-Gruener, M., Maegele, M., Garbe, J., and Angelov, D.N. (2007). Late effects of enriched environment (EE) plus multimodal early onset stimulation (MEOS) after traumatic brain injury in rats: ongoing improvement of neuromotor function despite sustained volume of the CNS lesion. *Exp. Neurol.* 203, 82–94.
 83. Lippert-Gruener, M., Maegele, M., Svestkova, O., Angerova, Y., Ester-Bode, T., and Angelov, D.N. (2011). Rehabilitation intervention in animal model can improve neuromotor and cognitive functions after traumatic brain injury: pilot study. *Physiol. Res.* 60, 367–375.
 84. Johnson, E.M., Traver, K.L., Hoffman, S.W., Harrison, C.R., and Herman, J.P. (2013). Environmental enrichment protects against functional deficits caused by traumatic brain injury. *Front. Behav. Neurosci.* 7, 44.
 85. Kovesdi, E., Gyorgy, A.B., Kwon, S.K., Wingo, D.L., Kamnakh, A., Long, J.B., Kasper, C.E., and Agoston, D.V. (2011). The effect of enriched environment on the outcome of traumatic brain injury: a behavioral, proteomics, and histological study. *Front. Neurosci.* 5, 42.
 86. Taber, K.H., Warden, D.L., and Hurley, R.A. (2006). Blast-related traumatic brain injury: what is known? *J. Neuropsychiatry Clin. Neurosci.* 18, 141–145.
 87. Rosenstein, J.M., and Krum, J.M. (2004). New roles for VEGF in nervous tissue-beyond blood vessels. *Exp. Neurol.* 87, 246–253.
 88. Vallieres, L., Campbell, I.L., Gage, F.H., and Sawchenko, P.E. (2002). Reduced hippocampal neurogenesis in adult transgenic mice with chronic astrocytic production of interleukin-6. *J. Neurosci.* 22, 486–492.
 89. Wagner, A.K., Kline, A.E., Sokoloski, J., Zafonte, R.D., Capulong, E., and Dixon, C.E. (2002). Intervention with environmental enrichment after experimental brain trauma enhances cognitive recovery in male but not female rats. *Neurosci. Lett.* 334, 165–168.
 90. Pereira, L.O., Strapasson, A.C., Nabinger, P.M., Achaval, M., and Netto, C.A. (2008). Early enriched housing results in partial recovery of memory deficits in female, but not in male, rats after neonatal hypoxia-ischemia. *Brain Res.* 1218, 257–266.
 91. Pereira, L.O., Nabinger, P.M., Strapasson, A.C., Nardin, P., Gonçalves, C.A., Siqueira, I.R., and Netto, C.A. (2009). Long-term effects of environmental stimulation following hypoxia-ischemia on the oxidative state and BDNF levels in rat hippocampus and frontal cortex. *Brain Res.* 1247, 188–195.
 92. Martinez-Cué, C., Baamonde, C., Lumbrales, M., Paz, J., Davisson, M.T., Schmidt, C., Dierssen, M., and Florez, J. (2002). Differential effects of environmental enrichment on behavior and learning of male and female Ts65Dn mice, a model for Down syndrome. *Behav. Brain Res.* 134, 185–200.
 93. Frick, K.M., and Fernandez, S.M. (2003). Enrichment enhances spatial memory and increases synaptophysin levels in aged female mice. *Neurobiol. Aging* 24, 615–626.
 94. Frick, K.M., Stearns, N.A., Pan, J.Y., and Berger-Sweeney, J. (2003). Effects of environmental enrichment on spatial memory and neurochemistry in middle-aged mice. *Learn. Mem.* 10, 187–198.
 95. Lambert, T.J., Fernandez, S.M., and Frick, K.M. (2005). Different types of environmental enrichment have discrepant effects on spatial memory and synaptophysin levels in female mice. *Neurobiol. Learn. Mem.* 83, 206–216.
 96. Monaco, C.M., Mattioli, V.V., Folweiler, K.A., Tay, J.K., Yellswarup, N.K., Curatolo, L.M., Matter, A.M., Cheng, J.P., and Kline, A.E. (2013). Environmental enrichment promotes robust functional and histological benefits in female rats after controlled cortical impact injury. *Exp. Neurol.* 247, 410–418.
 97. Fineman, I., Giza, C.C., Nahed, B.V., Lee, S.M., and Hovda, D.A. (2000). Inhibition of neocortical plasticity during development by a moderate concussive brain injury. *J. Neurotrauma* 17, 739–749.
 98. Ip, E.Y., Giza, C.C., Griesbach, G.S., and Hovda, D.A. (2002). Effects of enriched environment and fluid percussion injury on dendritic arborization within the cerebral cortex of the developing rat. *J. Neurotrauma* 19, 573–585.
 99. Volkmar, F.R., and Greenough, W.T. (1972). Rearing complexity affects branching of dendrites in the visual cortex of the rat. *Science* 176, 1445–1447.
 100. Holloway, R.L., Jr. (1966). Dendritic branching: some preliminary results of training and complexity in rat visual cortex. *Brain Res.* 2, 393–396.
 101. Globus, A., Rosenzweig, M.R., Bennett, E.L., and Diamond, M.C. (1973). Effects of differential experience on dendritic spine counts in rat cerebral cortex. *J. Comp. Physiol. Psychol.* 82, 175–181.
 102. Giza, C.C., Griesbach, G.S., and Hovda, D.A. (2005). Experience-dependent behavioral plasticity is disturbed following traumatic injury to the immature brain. *Behav. Brain Res.* 157, 11–22.
 103. Darmopil, S., Petanjek, Z., Mohammed, A.H., and Bogdanovic, N. (2009). Environmental enrichment alters dentate granule cell morphology in oldest-old rat. *J. Cell. Mol. Med.* 13, 1845–1856.
 104. Bennett, J.C., McRae, P.A., Levy, L.J., and Frick, K.M. (2006). Long-term continuous, but not daily, environmental enrichment reduces spatial memory decline in aged male mice. *Neurobiol. Learn. Mem.* 85, 139–152.
 105. Winocur, G. (1998). Environmental influences on cognitive decline in aged rats. *Neurobiol. Aging* 19, 589–597.
 106. Kozlowski, D.A., Nahed, B.V., Hovda, D.A., and Lee, S.M. (2004). Paradoxical effects of cortical impact injury on environmentally enriched rats. *J. Neurotrauma* 21, 513–519.
 107. McNamara, K.C., Lisembee, A.M., and Lifshitz, J. (2010). The whisker nuisance task identifies a late-onset, persistent sensory sensitivity in diffuse brain-injured rats. *J. Neurotrauma* 27, 695–706.

108. Binder, L.M. (1986). Persisting symptoms after mild head injury: a review of the postconcussive syndrome. *J. Clin. Exp. Neuropsychol.* 8, 323–346.
109. Blackerby, W.F. (1990). Intensity of rehabilitation and length of stay. *Brain Inj.* 4, 167–173.
110. Shiel, A., Burn, J.P., Henry, D., Clark, J., Wilson, B.A., Burnett, M.E., and McLellan, D.L. (2001). The effects of increased rehabilitation therapy after brain injury: results of a prospective controlled trial. *Clin. Rehabil.* 15, 501–514.
111. Zhu, X.L., Poon, W.S., Chan, C.C., and Chan, S.S. (2007). Does intensive rehabilitation improve the functional outcome of patients with traumatic brain injury (TBI)? A randomized controlled trial. *Brain Inj.* 21, 681–690.
112. Vanderploeg, R.D., Schwab, K., Walker, W.C., Fraser, J.A., Sigford, B.J., Date, E.S., Scott, S.G., Curtiss, G., Salazar, A.M., and Warden, D.L.; Defense and Veterans Brain Injury Center Study Group. (2008). Rehabilitation of traumatic brain injury in active duty military personnel and veterans: Defense and Veterans Brain Injury Center randomized controlled trial of two rehabilitation approaches. *Arch. Phys. Med. Rehabil.* 89, 2227–2238.
113. Gaulke, L.J., Horner, P.J., Fink, A.J., McNamara, C.L., and Hicks, R.R. (2005). Environmental enrichment increases progenitor cell survival in the dentate gyrus following lateral fluid percussion injury. *Brain Res. Mol. Brain Res.* 141, 138–150.
114. Darwish, H., Mahmood, A., Schallert, T., Chopp, M., and Therrien, B. (2014). Simvastatin and environmental enrichment effect on recognition and temporal order memory after mild-to-moderate traumatic brain injury. *Brain Inj.* 28, 211–226.
115. Degroot, A., Wolff, M.C., and Nomikos, G.G. (2005). Acute exposure to a novel object during consolidation enhances cognition. *Neuroreport* 16, 63–67.
116. Margulies, S., and Hicks, R.; Combination Therapies for Traumatic Brain Injury Workshop Leader. (2009). Combination therapies for traumatic brain injury: prospective considerations. *J. Neurotrauma* 26, 925–939.
117. Kline, A.E., Yu, J., Massucci, J.L., Zafonte, R.D., and Dixon, C.E. (2002). Protective effects of the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino)tetrinalin against traumatic brain injury-induced cognitive deficits and neuropathology in adult male rats. *Neurosci. Lett.* 333, 179–182.
118. Kline, A.E., Massucci, J.L., Dixon, C.E., Zafonte, R.D., and Bolinger, B.D. (2004). The therapeutic efficacy conferred by the 5-HT_{1A} receptor agonist 8-Hydroxy-2-(di-n-propylamino)tetrinalin (8-OH-DPAT) after experimental traumatic brain injury is not mediated by concomitant hypothermia. *J. Neurotrauma* 21, 175–185.
119. Cheng, J.P., Hoffman, A.N., Zafonte, R.D., and Kline, A.E. (2008). A delayed and chronic treatment regimen with the 5-HT_{1A} receptor agonist 8-OH-DPAT after cortical impact injury facilitates motor recovery and acquisition of spatial learning. *Behav. Brain Res.* 194, 79–85.
120. Olsen, A.S., Sozda, C.N., Cheng, J.P., Hoffman, A.N., and Kline, A.E. (2012). Traumatic brain injury-induced cognitive and histological deficits are attenuated by delayed and chronic treatment with the 5-HT_{1A}-receptor agonist buspirone. *J. Neurotrauma* 29, 1898–1907.
121. Chew, E., and Zafonte, R.D. (2009). Pharmacological management of neurobehavioral disorders following traumatic brain injury—a state-of-the-art review. *J. Rehabil. Res. Dev.* 46, 851–879.
122. Peruzzaro, S.T., Gallagher, J., Dunkerson, J., Fluharty, S., Mudd, D., Hoane, M.R., and Smith, J.S. (2013). The impact of enriched environment and transplantation of murine cortical embryonic stem cells on recovery from controlled cortical contusion injury. *Restor. Neurol. Neurosci.* 31, 431–450.
123. Rosenzweig, M.R., Bennett, E.L., Hebert, M., and Morimoto, H. (1978). Social grouping cannot account for cerebral effects of enriched environments. *Brain Res.* 153, 563–576.
124. Gabriel, A.F., Paoletti, G., Della Seta, D., Panelli, R., Marcus, M.A., Farabollini, F., Carli, G., and Joosten, E.A. (2010). Enriched environment and the recovery from inflammatory pain: Social versus physical aspects and their interaction. *Behav. Brain Res.* 208, 90–95.
125. Hicks, R.R., Numan, S., Dhillon, H.S., Prasad, M.R., and Seroogy, K.B. (1997). Alterations in BDNF and NT-3 mRNAs in rat hippocampus after experimental brain trauma. *Brain Res. Mol. Brain Res.* 48, 401–406.
126. Hicks, R.R., Zhang, L., Dhillon, H.S., Prasad, M.R., and Seroogy, K.B. (1998). Expression of trkB mRNA is altered in rat hippocampus after experimental brain trauma. *Brain Res. Mol. Brain Res.* 59, 264–268.
127. Hicks, R.R., Martin, V.B., Zhang, L., and Seroogy, K.B. (1999). Mild experimental brain injury differentially alters the expression of neurotrophin and neurotrophin receptor mRNAs in the hippocampus. *Exp. Neurol.* 160, 469–478.
128. Yang, K., Perez-Polo, J.R., Mu, X.S., Yan, H.Q., Xue, J.J., Iwamoto, Y., Liu, S.J., Dixon, C.E., and Hayes, R.L. (1996). Increased expression of brain-derived neurotrophic factor but not neurotrophin-3 mRNA in rat brain after cortical impact injury. *J. Neurosci. Res.* 44, 157–164.
129. Chen, X., Li, Y., Kline, A.E., Dixon, C.E., Zafonte, R.D., and Wagner, A.K. (2005). Gender and environmental effects on regional brain-derived neurotrophic factor expression after experimental traumatic brain injury. *Neuroscience* 135, 11–17.
130. Falkenberg, T., Mohammed, A.K., Henriksson, B., Persson, H., Winblad, B., and Lindefors, N. (1992). Increased expression of brain-derived neurotrophic factor mRNA in rat hippocampus is associated with improved spatial memory and enriched environment. *Neurosci. Lett.* 138, 153–156.
131. Ickes, B.R., Pham, T.M., Sanders, L.A., Albeck, D.S., Mohammed, A.H., and Granholm, A.C. (2000). Long-term environmental enrichment leads to regional increases in neurotrophin levels in rat brain. *Exp. Neurol.* 164, 45–52.
132. Torasdotter, M., Metsis, M., Henriksson, B.G., Winblad, B., and Mohammed, A.H. (1998). Environmental enrichment results in higher levels of nerve growth factor mRNA in the rat visual cortex and hippocampus. *Behav. Brain Res.* 93, 83–90.
133. Zhu, J., Green, T., Bardo, M.T., and Dwoskin, L.P. (2004). Environmental enrichment enhances sensitization to GBR 12935-induced activity and decreases dopamine transporter function in the medial prefrontal cortex. *Behav. Brain Res.* 148, 107–117.
134. Yan, H.Q., Kline, A.E., Ma, X., Hooghe-Peters, E.L., Marion, D.W., and Dixon, C.E. (2001). Tyrosine hydroxylase, but not dopamine beta-hydroxylase, is increased in rat frontal cortex after traumatic brain injury. *Neuroreport* 12, 2323–2327.
135. Yan, H.Q., Kline, A.E., Ma, X., Li, Y., and Dixon, C.E. (2002). Traumatic brain injury reduces dopamine transporter protein expression in the rat frontal cortex. *Neuroreport* 13, 1899–1901.
136. Wilson, M.S., Chen, X., Ma, X., Ren, D., Wagner, A.K., Reynolds, I.J., and Dixon, C.E. (2005). Synaptosomal dopamine uptake in rat striatum following controlled cortical impact. *J. Neurosci. Res.* 80, 85–91.
137. Wagner, A.K., Chen, X., Kline, A.E., Li, Y., Zafonte, R.D., and Dixon, C.E. (2005). Gender and environmental enrichment impact dopamine transporter expression after experimental traumatic brain injury. *Exp. Neurol.* 195, 475–483.
138. Shin, S.S., Bales, J.W., Yan, H.Q., Kline, A.E., Wagner, A.K., Lyons-Weiler, J., and Dixon, C.E. (2013). The effect of environmental enrichment on substantia nigra gene expression after traumatic brain injury in rats. *J. Neurotrauma* 30, 259–270.
139. Jordan, R., Patel, S., Hu, H., and Lyons-Weiler, J. (2008). Efficiency analysis of competing tests for finding differentially expressed genes in lung adenocarcinoma. *Cancer Inform.* 6, 389–421.

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