Intermittent Administration of Haloperidol after Cortical Impact Injury Neither Impedes Spontaneous Recovery Nor Attenuates the Efficacy of Environmental Enrichment

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Abstract

The administration of haloperidol (HAL) once-daily for 19 days after experimental traumatic brain injury (TBI) impedes recovery and attenuates the efficacy of environmental enrichment (EE). However, it is unknown how intermittent administration of HAL affects the recovery process when paired with EE. Addressing the uncertainty is relevant because daily HAL is not always warranted to manage TBI-induced agitation in the clinic, and indeed intermittent therapy may be a more common approach. Hence, the aim of the study was to test the hypothesis that intermittent HAL would neither impair recovery in standard (STD)-housed controls nor attenuate the efficacy of EE. Anesthetized adult male rats received a cortical impact or sham injury and then were housed in STD or EE conditions. Beginning 24 h later, HAL (0.5 mg/kg; intraperitoneally [i.p.]) was administered either once-daily for 19 days or once every other day, whereas vehicle (VEH; 1 mL/kg; i.p.) was administered once daily. Motor performance and cognition were assessed on post-injury days 1–5 and 14–19, respectively. Cortical lesion volume was quantified on day 21. SHAM controls performed better than all TBI groups on motor and spatial learning [$p < 0.05$], but did not differ from the TBI + EE + daily VEH group on memory retention [$p > 0.05$]. The TBI + EE + daily VEH and TBI + EE + intermittent HAL groups did not differ from one another on beam-walk or spatial learning [$p > 0.05$], and both performed better than all other TBI groups [$p < 0.05$]. In contrast, the TBI + STD + daily HAL group performed worse than all TBI groups on spatial learning [$p < 0.05$]. No difference in any endpoint was revealed between the TBI + STD + intermittent HAL and TBI + STD + daily VEH groups [$p > 0.05$]. The results support the hypothesis that HAL is not detrimental when provided intermittently. If translatable to the clinic, intermittent HAL may be used to control TBI-induced agitation without negatively affecting spontaneous recovery or rehabilitative efficacy.

Keywords: antipsychotic drugs; behavioral outcome; controlled cortical impact; environmental enrichment; functional recovery; learning and memory; Morris water maze; traumatic brain injury

Introduction

TRAUMATIC BRAIN INJURY (TBI) affects 10 million people worldwide and induces a plethora of pathophysiological and behavioral disturbances.^{1–4} In addition to the debilitating motor and cognitive dysfunction,^{5–7} other salient maladaptive behaviors resulting from TBI include agitation and aggression.^{8–12} These complications are not rare given that studies report that up to 96% of patients surviving TBI exhibit agitation^{11–14} whereas 11% demonstrate aggression.¹⁵ Moreover, 86.3% of TBI patients exhibited agitation during the acute admission phase.¹³ The percentage dropped slightly to 70% during rehabilitation.¹³ Agitation

may be caused by the TBI itself or other hospital-related factors that can lead to discomfort in patients, such as interrupted sleep attributed to routine medical care, poorly managed pain, extraneous noises, lighting conditions, or a host of other events. The impact of agitated behavior is significant because it disrupts patient care and subsequent rehabilitation because of uncooperativeness resulting from impulsivity and distractibility.^{9,16}

Antipsychotic drugs (APDs) have been the principal treatment approach to manage TBI-induced agitation.^{17,18} Indeed, their use is supported by expert guidelines, which suggest that they are a vital part of the pharmacological armamentarium to be used post-TBI in the management of agitation and aggression.^{19,20} However, though

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effective in controlling outbursts, the administration of daily APDs can result in other significant problems for the patient.^{9,18} Several pre-clinical studies have shown that daily administration of the APD, haloperidol (HAL), after various types of brain trauma produces deleterious effects on functional recovery.^{21–25} After unilateral ablation of the sensorimotor cortex, Feeney and colleagues showed that HAL impaired beam-walking ability relative to vehicle-treated controls.²¹ After fluid percussion brain injury, Wilson and colleagues showed that daily HAL impaired cognitive performance in a sensitive Morris water maze (MWM) task.²³ Similar negative effects on cognitive recovery were reported when HAL was administered once-daily after controlled cortical impact (CCI) injury in male and female rats.^{24–28} Moreover, the negative effect on cognitive recovery by HAL after TBI is long-lasting.²⁹ It has recently been demonstrated that daily administration of HAL after TBI attenuates the beneficial effects of environmental enrichment (EE), a pre-clinical model of neurorehabilitation.^{30–32}

However, TBI-induced agitation may not manifest daily in the clinic and therefore continuous treatment is not warranted. Indeed, intermittent treatment paradigms may be a more common approach, particularly during outpatient rehabilitation when the patient is no longer restricted to bed and receiving a combination of prescribed drugs. Hence, the aim of the study is to determine the effect of intermittent (i.e., once every other day) administration of HAL alone or in combination with EE. The working hypothesis is that intermittent HAL will neither impair recovery in standard (STD)-housed controls nor attenuate the efficacy of EE.

Methods

Subjects

Eighty-four adult male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 300–325 g on the day of surgery were pair-housed in ventilated polycarbonate cages and maintained in a temperature- ($21 \pm 1^\circ\text{C}$) and light-controlled (on 7:00 AM to 7:00 PM) controlled environment with *ad libitum* food and water. After 1 week of acclimatization all rats underwent a single day of beam-walk training, which consisted of 3–5 trials to reach the criterion of traversing the length of the beam in under 5 sec. After training, the rats were randomly assigned to TBI + STD + daily VEH (1.0 mL/kg; $n = 10$), TBI + EE + daily VEH ($n = 10$), TBI + STD + daily HAL (0.5 mg/kg; $n = 10$), TBI + EE + daily HAL ($n = 10$), TBI + STD + intermittent HAL ($n = 10$), TBI + EE + intermittent HAL ($n = 10$), and respective sham controls ($n = 4$ per condition; $N = 24$). All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh (Pittsburgh, PA). Every attempt was made to limit the number of rats used and to minimize suffering.

Surgery

A CCI injury was produced as previously described.^{26–30,33} Briefly, surgical anesthesia was induced and maintained with 4% and 2% isoflurane, respectively, in 2:1 $\text{N}_2\text{O}:\text{O}_2$. Rats were intubated and mechanically ventilated before being secured in a stereotaxic frame. Utilizing aseptic procedures, a midline scalp incision was made, the skin and fascia were reflected to expose the skull, and a craniectomy was made with a dental drill in the right hemisphere over the parietal cortex. The impacting rod was extended and the impact tip (6 mm, flat) was centered and lowered through the craniectomy until it contacted the dura mater, then the rod was retracted and the impact tip was advanced 2.8 mm farther to produce an injury of moderate severity (2.8 mm brain deformation at 4 m/s). Except for the cortical impact, sham controls experienced all surgical

procedures. Body temperature was maintained at $37 \pm 0.5^\circ\text{C}$ with a heating blanket and recorded with a rectal thermistor. Immediately after the CCI or sham injury, anesthesia was discontinued, the incision was sutured, rats were extubated, and acute neurological evaluations were conducted.

Acute neurological assessments

Immediately after the termination of anesthesia, hindlimb reflexive ability was assessed by gently squeezing the rats' paw every 5 sec and recording the time to elicit a withdrawal response. The time required to turn from the supine to prone position on three consecutive attempts was the criterion for return of the righting reflex. These tests are sensitive indicators of injury severity and duration of anesthesia.^{26–30}

Housing conditions: environmental manipulation

After surgery, and after the effects of anesthesia abated (as evidenced by spontaneous movement in the holding cage), rats were returned to the colony where those designated for enrichment were immediately placed in specifically designed stainless steel-wire cages ($91 \times 76 \times 50$ cm). EE cages consisted of three levels with ladders to ambulate from one level to another and contained various toys and objects with which to engage (e.g., balls, blocks, and tubes) and *ad libitum* food and water.^{31,32,34,35} To maintain novelty, objects were rearranged every day and changed each time the cage was cleaned, which took place 2× per week. Ten to 12 rats, which included HAL and vehicle-treated TBI and sham controls, were housed in the EE together to minimize variability. Rats in the STD conditions were placed in typical ventilated polycarbonate cages ($37 \times 25 \times 18$ cm, 2 rats per cage) with only food and water.

Drug administration

HAL (Sigma-Aldrich, St. Louis, MO) was prepared daily by dissolving in 20% dimethyl sulfoxide, which also served as the VEH. The dose of HAL was chosen because it has been reported to be comparable to that used clinically to control psychosis³⁶ and has been used in several TBI studies investigating functional outcome.^{24,26–30} Treatments began 24 h after CCI or sham surgery and were provided intraperitoneally (i.p.) either once-daily for 19 days (daily treatment TBI and sham groups) or once every other day (intermittent treatment TBI and sham groups). The half-life of HAL in rats using this dose and route is reported to be 2.6 h,³⁷ and thus it was provided *after* the daily behavioral assessments to circumvent sedative effects, which may confound the results. However, the biological effect of HAL was active during the exposure to EE.

Motor performance

Motor function was assessed using the well-established beam-balance and beam-walk tasks.^{26–30} Briefly, the beam-balance task consisted of placing the rat on an elevated (90 cm) narrow beam (1.5 cm wide) and recording the time it remained on for a maximum of 60 sec. The beam-walk task, modified from Feeney and colleagues²¹ and used extensively in our laboratory,^{26–30} consists of training/assessing rats using a negative-reinforcement paradigm to escape bright light and white noise by traversing an elevated narrow beam (2.5 cm wide $\times$ 100 cm long) and entering a darkened goal box at the opposite end. Beam-walk performance was assessed by recording the elapsed time to traverse the beam as well as distance traveled. The scoring criteria for distance traveled is based on a rating scale from 0 to 5, where 0 indicates an inability to ambulate beyond the start point, 1–4 corresponds to distal segments of 20, 40, 60, or 80 cm from the starting point, respectively, and 5 indicates walking down the entire length of the beam (100 cm) and entering the goal box. Rats were trained before CCI or sham injury to perform the tasks without errors (i.e., maintain their balance for 60 sec

and traverse the beam in under 5 sec). A baseline performance assessment was taken on the day of surgery. Performance on the beam tasks was assessed on post-operative days 1–5 and consisted of three trials (60 sec allotted time per trial) per day on each task. Average daily scores for each subject were used in the statistical analyses.

Cognitive function: acquisition of spatial learning

Spatial learning was assessed in a MWM task that has consistently been shown to be sensitive to cognitive performance after TBI.^{26–30,38,39} The maze was a plastic pool (180 cm diameter; 60 cm high) filled with tap water ($26 \pm 1^\circ\text{C}$) to a depth of 28 cm and was situated in a room with salient visual cues that remained constant throughout the study. The escape platform was a clear Plexiglas stand (10 cm diameter, 26 cm high) that was positioned 26 cm from the maze wall in the southwest quadrant and held constant for each rat. Spatial learning began on post-operative day 14 and consisted of providing a block of four daily trials (4-min intertrial interval) for 5 consecutive days (14–18) to locate the platform when it was submerged 2 cm below the water surface. On day 19, the platform was made visible to the rats by raising it 2 cm above the water surface as a control procedure to determine the contributions of non-spatial factors (e.g., sensory-motor performance, motivation, and visual acuity) on cognitive performance. For each daily block of trials, rats were placed in the pool facing the wall at each of the four possible start locations (north, south, west, and east) in a randomized manner. Each trial lasted until the rat climbed onto the platform or until 120 sec had elapsed, whichever occurred first. Rats that failed to escape the water within the allotted time were manually guided to the platform. All rats remained on the platform for 30 sec before being placed in a heated incubator between trials. The times of the four daily trials for each rat were averaged and used in the statistical analyses. All data were obtained using ANY-maze video tracking software (Stoelting Co., Wood Dale, IL).

Cognitive function: probe trial (memory retention)

One day after the final acquisition training session (i.e., day 19), but before the visible platform test, all rats were given a single probe trial to assess memory retention. Briefly, the platform was removed from the pool and the rats were placed in the maze from the quadrant opposite of where the platform was previously situated (i.e., “target quadrant”) and allowed to freely explore the pool for 30 sec. The percent time spent in the target quadrant was used in the statistical analysis as a measure of intact memory.

Histology: quantification of cortical lesion volume

Three weeks after CCI or sham injury, the rats were anesthetized with Fatal-Plus® (0.3 mL, i.p.) and perfused transcardially with 200 mL of 0.1 M of phosphate-buffered saline (pH 7.4) followed by 300 mL of 4% paraformaldehyde. Brains were extracted, post-fixed in the perfusate for 1 week, dehydrated with alcohols, and embedded in paraffin. Coronal sections (7 μm thick) were cut at 1-mm intervals through the lesion on a rotary microtome and mounted on Superfrost®/Plus glass microscope slides. After drying at room temperature, sections were deparaffinized in xylenes, rehydrated, and stained with cresyl violet. Cortical lesion volumes (mm^3) were determined by calculating the area of the lesion (mm^2), which was done by outlining the inferred area of missing cortical tissue for each section (typically 5–7) taken at 1-mm intervals (MCID; Imaging Research, Inc., St. Catharines, Ontario, Canada), and then by summing the lesions obtained from each section as previously reported.^{40–44}

Statistical analysis

All data were collected by experimenters blinded to treatment conditions and statistical analyses were performed using Statview software (version 5.0.1; SAS Institute Inc., Cary NC). Motor and cognitive data were analyzed by repeated-measures analysis of variance (ANOVA). Acute neurological assessments, swim speed, and cortical lesion volume were analyzed by one-factor ANOVAs. When the overall ANOVAs revealed a significant effect, the Newman-Keuls post-hoc test was used to determine group differences. Data are presented as the mean $\pm$ standard error of the mean (SEM) and are considered significant when corresponding p values are ≤ 0.05 .

Results

Three rats (1 from each of the three groups; TBI + EE + daily VEH, TBI + STD + daily HAL, and TBI + EE + intermittent HAL) were excluded from the study because of an inability to locate the visible platform in under 120 sec, which may be indicative of visual deficits and therefore would be a potential confound given the necessity to visualize the distal cues on the walls to acquire spatial learning. Hence, statistical analyses are based on 81 rats. There were no significant differences in any outcome measure among the sham control groups, regardless of treatment (daily VEH, daily HAL, intermittent HAL, or housing condition), and therefore the data were pooled into a single SHAM group.

Acute neurological assessments

No significant differences were observed among the TBI groups for return of hindlimb reflexive ability (range for right, 146.1 ± 2.8 to 161.9 ± 6.6 sec; range for left, 151.3 ± 2.8 to 166.2 ± 6.5 sec) or righting reflex time (range = 322.3 ± 18.8 to 350.2 ± 18.6 sec). The lack of significant differences in these post-surgical neurological assessments among the TBI groups indicates that all rats received similar levels of injury severity and anesthesia.

Motor function: beam-balance

No pre-surgical differences were observed among groups given that all rats were capable of balancing on the beam for the allotted 60 sec (Fig. 1). After TBI, the repeated-measures ANOVA revealed significant Group [$F_{6,74} = 16.569$; $p < 0.0001$] and Day [$F_{5,370} = 139.151$; $p < 0.0001$] differences, as well as a significant Group $\times$ Day interaction [$F_{30,370} = 8.992$; $p < 0.0001$]. The post-hoc analysis revealed that all TBI groups were significantly impaired relative to the SHAM group, which maintained balance for the allotted 60 sec [$ps < 0.05$]. Among the TBI comparisons, the TBI + EE + daily VEH group performed better than the TBI + STD + daily VEH and TBI + STD + daily HAL groups [$p < 0.05$]. The TBI + EE + intermittent HAL group was better than TBI + STD + daily HAL [$p < 0.05$], but did not differ from any other TBI group [$p > 0.05$]. No difference was revealed between the TBI + STD + intermittent HAL and the TBI + STD + daily VEH groups [$p > 0.05$].

Motor function: beam-walk

No pre-surgical differences in time to traverse the beam were revealed among groups given that all rats walked the full length and entered the goal box in approximately 5 sec (Fig. 2). After TBI, the repeated-measures ANOVA revealed significant Group [$F_{6,74} = 31.508$; $p < 0.0001$] and Day [$F_{5,370} = 204.898$; $p < 0.0001$] differences, as well as a significant Group $\times$ Day interaction [$F_{30,370} = 10.281$; $p < 0.0001$]. The post-hoc analysis revealed that

FIG. 1. Mean ($\pm$ SEM) time (seconds) balancing on an elevated narrow beam before, and after, TBI or sham injury. There were no significant differences among the sham groups and thus they were pooled (SHAM). # $p < 0.05$ versus all TBI groups. * $p < 0.05$ versus TBI + STD + daily VEH and TBI + STD + daily HAL. § $p < 0.05$ versus TBI + STD + daily HAL. No difference was revealed between the TBI + STD + intermittent HAL and the TBI + STD + daily VEH groups [$p > 0.05$]. EE, environmental enrichment; HAL, haloperidol; SEM, standard error of the mean; STD, standard; TBI, traumatic brain injury; VEH, vehicle.

all TBI groups were significantly impaired relative to the SHAM group [$ps < 0.05$]. Among the TBI comparisons, the TBI + EE + daily VEH group performed better than the TBI + STD + daily VEH, TBI + STD + daily HAL, and TBI + STD + intermittent HAL groups [$p < 0.05$]. TBI + EE + intermittent HAL was better than TBI + STD + daily HAL and TBI + STD + daily VEH [$p < 0.05$], but did not differ from any other TBI group [$p > 0.05$]. No difference was revealed between the TBI + STD + intermittent HAL and the TBI + STD + daily VEH groups [$p > 0.05$].

Motor function: beam-walk score

No pre-surgical differences in beam-walk score were revealed among groups because all rats walked the full length of the beam and entered the goal box for a maximal score of 5 (Fig. 3). After TBI, the repeated-measures ANOVA revealed significant Group [$F_{6,74} = 31.549$; $p < 0.0001$] and Day [$F_{5,370} = 261.6$; $p < 0.0001$] differences, as well as a significant Group $\times$ Day interaction [$F_{30,370} = 16.965$; $p < 0.0001$]. All TBI groups were significantly impaired relative to the SHAM group [$p < 0.05$]. The TBI + EE + daily VEH performed better than the TBI + STD + daily VEH, TBI + STD + daily HAL, and TBI + STD + intermittent HAL groups [$p < 0.05$]. TBI + EE + intermittent HAL was better than TBI + STD + daily VEH and TBI + STD + daily HAL [$p < 0.05$], but did not differ from the other EE groups, regardless of treatment [$p > 0.05$]. No differences were revealed between the TBI + EE + daily VEH group and the TBI + EE + intermittent HAL and TBI + EE + daily HAL groups [$p > 0.05$].

FIG. 2. Mean ($\pm$ SEM) time (seconds) to traverse an elevated narrow beam before, and after, TBI or sham injury. There were no significant differences among the sham groups and thus they were pooled (SHAM). # $p < 0.05$ versus all TBI groups. * $p < 0.05$ versus TBI + STD + daily VEH, TBI + STD + daily HAL, and TBI + STD + intermittent HAL. § $p < 0.05$ versus TBI + STD + daily HAL and TBI + STD + daily VEH. No difference was revealed between the TBI + STD + intermittent HAL and the TBI + STD + daily VEH groups [$p > 0.05$]. EE, environmental enrichment; HAL, haloperidol; SEM, standard error of the mean; STD, standard; TBI, traumatic brain injury; VEH, vehicle.

Cognitive function: acquisition of spatial learning

Repeated-measures ANOVA of the MWM data revealed significant Group [$F_{6,74} = 38.18$; $p < 0.0001$] and Day [$F_{4,296} = 80.899$; $p < 0.0001$] differences, as well as a significant Group $\times$ Day interaction [$F_{24,296} = 2.508$; $p = 0.0002$]. The SHAM group located the escape platform significantly faster than all TBI groups [$p < 0.05$; Fig. 4]. Additional post-hoc analyses revealed that the TBI + EE + daily VEH group performed better relative to all other TBI groups [$p < 0.05$], except the TBI + EE + intermittent HAL group [$p > 0.05$]. Moreover, the TBI + STD + daily HAL group performed worse than all other groups [$p < 0.05$]. In contrast, the TBI + EE + intermittent HAL group was better than TBI + STD + intermittent HAL, TBI + STD + daily VEH, and TBI + EE + daily HAL groups [$p < 0.05$]. No difference was revealed between the TBI + STD + intermittent HAL and the TBI + STD + daily VEH groups [$p > 0.05$]. Additionally, no significant differences in swim speed (range = 26.9 ± 0.8 to 33.1 ± 1.5 cm/s) were observed among any of the TBI or SHAM groups [$p > 0.05$]. There was a difference in the visible platform task because the SHAM controls performed better than all TBI groups [$p < 0.05$], except the TBI + EE + daily VEH and TBI + EE + daily HAL [$p > 0.05$].

Cognitive function: probe trial

Analysis of the probe data revealed a greater percentage of the allotted time spent in the target quadrant for the SHAM and TBI + EE + daily VEH groups ($42.2 \pm 2.3\%$ and $39.7 \pm 1.8\%$, respectively)

FIG. 3. Mean ($\pm$ SEM) score for distance traveled along an elevated narrow beam before, and after, TBI or sham injury. There was no significant difference among the sham groups and thus they were pooled (SHAM). $^{\#}p < 0.05$ versus all TBI groups and $^*p < 0.05$ versus TBI + STD + daily VEH, TBI + STD + daily HAL, and TBI + STD + intermittent HAL. $^{\S}p < 0.05$ versus TBI + STD + daily VEH and TBI + STD + daily HAL. No differences were revealed between the TBI + EE + daily VEH group and the TBI + EE + intermittent HAL and TBI + EE + daily HAL groups [$p > 0.05$]. EE, environmental enrichment; HAL, haloperidol; SEM, standard error of the mean; STD, standard; TBI, traumatic brain injury; VEH, vehicle.

versus the TBI + STD + daily VEH ($25.8 \pm 2.1\%$), TBI + STD + daily HAL ($27.4 \pm 3.1\%$), TBI + STD + intermittent HAL ($28.4 \pm 2.9\%$), and TBI + EE + daily HAL ($28.9 \pm 2.5\%$) groups [$p < 0.05$; Fig. 5]. No significant differences were revealed between the SHAM controls and the TBI + EE + daily VEH group [$p > 0.05$] or between the TBI + EE + daily VEH and TBI + EE + intermittent HAL groups [$p > 0.05$].

Histology: quantification of cortical lesion volume

Quantification of the cortical lesion volume data revealed a significant difference among the groups [$F_{5,24} = 2.970$; $p = 0.03$]. Specifically, the post-hoc analysis revealed that the difference was attributed to the TBI + EE + daily VEH group having a smaller lesion volume ($32.9 \pm 2.4 \text{ mm}^3$) relative to the TBI + STD + daily VEH ($44.4 \pm 3.2 \text{ mm}^3$), TBI + STD + daily HAL ($45.5 \pm 2.0 \text{ mm}^3$), TBI + STD + intermittent HAL ($45.8 \pm 3.5 \text{ mm}^3$), and TBI + EE + daily HAL ($43.8 \pm 3.9 \text{ mm}^3$) groups [$p < 0.05$; Fig. 6]. No statistical difference was revealed between the TBI + EE + daily VEH and TBI + EE + intermittent HAL ($39.1 \pm 1.9 \text{ mm}^3$) groups [$p > 0.05$].

Discussion

Although non-pharmacological strategies, such as aromatherapy, exercise, massage, and music therapy, are beneficial in managing maladaptive behavior (e.g., agitation) over time,^{45,46} the use of pharmacotherapies, such as APDs, is often the first choice be-

FIG. 4. Mean ($\pm$ SEM) time (seconds) to locate a hidden platform in the Morris water maze. There was no significant difference among the sham groups and thus they were pooled (SHAM). $^{\#}p < 0.05$ versus all TBI groups. $^*p < 0.05$ versus all TBI groups except the TBI + EE + intermittent HAL group. $^{\S}p < 0.05$ versus TBI + STD + intermittent HAL, TBI + STD + daily VEH, and TBI + EE + daily HAL. $^{\wedge}p < 0.05$ versus all groups. No difference was revealed between TBI + STD + intermittent HAL and the TBI + STD + daily VEH [$p > 0.05$]. No difference in swim speed was observed among the TBI groups versus SHAM controls [$p > 0.05$], but the SHAM group did locate the visible platform quicker than all TBI groups [$p < 0.05$], except the TBI + EE + daily VEH and TBI + EE + daily HAL [$p > 0.05$]. EE, environmental enrichment; HAL, haloperidol; SEM, standard error of the mean; STD, standard; TBI, traumatic brain injury; VEH, vehicle.

cause they work relatively quick and are available in multiple formulations, meaning that they can be delivered orally, intravenously, or intramuscularly, with the latter providing an advantage in a crisis when a patient may refuse to take the medication orally. Because APDs are frequently provided after TBI, and according to published data markedly interfere with the recovery process when provided daily,^{23–30} the aim of this study was to assess motor and cognitive recovery after TBI in adult male rats administered HAL or VEH once every other day (i.e., intermittently) and compared to groups receiving HAL once-daily while housed in STD or enriched conditions. The hypothesis was that that intermittent HAL would not impede recovery in STD-housed controls and would also not attenuate the efficacy of EE, which alone would enhance functional outcome beyond that of STD controls as has been demonstrated repeatedly.^{31,32,34,35}

EE facilitated beam-balance and beam-walking recovery and significantly improved spatial learning relative to STD-housed VEH-treated controls, which replicates previous studies^{31,32,34,35,41,43,44} and affirms its viability as a robust preclinical model of neurorehabilitation. Moreover, EE reduced the negative effects of daily HAL on cognitive performance because the combination group did not statistically differ from the VEH-treated STD group.³⁰ Additionally, daily HAL attenuated the effectiveness

FIG. 5. Mean ($\pm$ SEM) percent of time spent in the target quadrant (i.e., where platform was previously located) after a single probe trial provided on day 19 after TBI or sham injury. There was no significant difference among the sham groups and thus they were pooled (SHAM). # $p < 0.05$ versus all TBI groups, except TBI + EE + daily VEH [$p > 0.05$]. * $p < 0.05$ versus all TBI groups except TBI + EE + intermittent HAL. The dotted line represents chance (25%) performance. EE, environmental enrichment; HAL, haloperidol; SEM, standard error of the mean; STD, standard; TBI, traumatic brain injury; VEH, vehicle.

of EE in the cognitive task because the combined treatment group did not perform as well as the VEH-treated EE group.³⁰ In contrast, intermittent HAL did not impair cognitive recovery as demonstrated by a lack of statistical difference between the STD-housed HAL and the STD VEH-treated groups. Moreover, intermittent HAL did not reduce the effectiveness of EE because there was no difference between the EE groups receiving intermittent HAL versus VEH. Indeed, showing relatively equal effectiveness with EE alone on spatial learning, the EE + intermittent HAL group performed better than the EE + daily HAL group, the STD + daily VEH group, and the STD + daily HAL group. Unlike EE alone, intermittent HAL combined with EE did not reduce cortical lesion size, which suggests that HAL, albeit used infrequently, can still impair recovery in some endpoints. That no spatial learning difference was observed between the EE + VEH and EE + intermittent HAL groups, despite a significant difference in histological outcome, supports studies affirming that behavioral benefits and histological findings do not always correlate.^{47,48}

As an antipsychotic, HAL exerts its effects as a high-affinity D₂ postsynaptic receptor antagonist. Though effective in controlling symptoms of schizophrenia, such as psychosis, D₂ receptor antagonism impairs cognition in non-TBI patients^{49,50} and impedes the recovery of cognitive performance after TBI.^{17,18} Potential mechanisms for the deleterious effects of daily administration of HAL may include cellular dysfunction by the loss of antioxidant enzymes and increased lipid peroxidation,⁵¹ as well as oxidative stress and mitochondrial dysfunction.^{52,53} The depletion of tyrosine hydroxylase (TH)⁵⁴ may also play a role in cognitive deficits given that studies from our laboratory have shown that increased TH after

FIG. 6. Mean ($\pm$ SEM) cortical lesion volume (mm³) 3 weeks after cortical impact injury. * $p < 0.05$ versus all TBI groups, except TBI + EE + intermittent HAL. No other comparisons were significant. EE, environmental enrichment; HAL, haloperidol; SEM, standard error of the mean; STD, standard; TBI, traumatic brain injury; VEH, vehicle.

TBI correlates with the cognitive recovery time frame observed in our model.⁵⁵ In contrast, the D₂ receptor agonist, bromocriptine, has been reported to reduce markers of oxidative stress after cortical impact injury and improve spatial learning and memory.^{29,56,57} Moreover, methylphenidate and amantadine, also D₂ receptor agonists, enhance recovery after pre-clinical and clinical TBI.^{58–65} The neutral effects observed with intermittent HAL treatments on the recovery process may result from the lack of full receptor blockade and the absence of the deleterious mechanisms observed with daily HAL treatment. Alternatively, the neutral effects may be attributed to reducing the total amount of HAL by half over the course of the study. To determine whether this speculation has merit, future studies will need to reduce the daily dose of HAL from 0.5 to 0.25 mg/kg and assess the rats using the same surgical and behavioral paradigms. Given the robust detrimental behavioral effects observed when the current dose of HAL is provided chronically, it is likely that negative effects would still be observed with a lower dose. Lowering the APD dose too much in the clinic may lead to therapeutic failure,⁶⁶ but an every other day treatment approach with a standard dose may be effective given that the half-life of HAL in humans ranges from 14.5 to 36.7 h.⁶⁷

EE, on the other hand, has been reported to confer its beneficial effects by several potential mechanisms,^{31,32} particularly neuroplastic changes, such as synaptic strengthening and increased expression of neurotrophic factors.^{68–70} Although increased neurogenesis is typically cited as an EE-mediated event that benefits outcome,^{71,72} a recent report from our laboratory demonstrated that although continuous EE increased hippocampal neurogenesis beyond that of an abbreviated EE paradigm, there was no difference in behavioral outcomes between the two groups, suggesting that increasing neurogenesis after TBI beyond that of STD-housed controls is not necessary for EE-mediated benefits.⁷³ EE may also provide benefits after TBI by reducing proinflammatory and/or increasing anti-inflammatory responses.⁷⁴ That EE reduced the deleterious effects

of HAL on functional outcome is not surprising given that it has consistently been shown to robustly enhance motor and cognitive behavior after TBI produced by various models.^{30–32,34,35,44,75–77}

Moreover, non-aggressive interactions among rats group-housed, as in an enriched environment that may be a mild stressor, protect rats against consequent stressors and/or drugs of abuse.⁷⁸ This inoculation stress theory may explain why EE attenuates negative or disruptive behaviors, such as dependence and voluntary consumption of morphine,⁷⁹ heroin seeking,⁸⁰ and methamphetamine-induced withdrawal symptoms,⁸¹ in non-TBI rats. Extrapolating this theory to the current research design could explain the EE-mediated attenuation of HAL-induced detrimental effects.

Overall, these results support the hypothesis and suggest that intermittent HAL may be used to control TBI-induced agitation without negatively affecting spontaneous recovery or rehabilitative efficacy. However, if daily HAL treatment is necessary because of chronic agitation and/or aggression, the finding that this approach reduces the efficacy of neurorehabilitation (i.e., EE) would indicate that rehabilitation may need to be intensified or augmented such that the greatest benefit is achieved with the combination of daily HAL and EE.

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