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Research report

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

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ABSTRACT

An early (i.e., 15 min) single systemic administration of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT enhances behavioral recovery after experimental traumatic brain injury (TBI). However, acute administration of pharmacotherapies after TBI may be clinically challenging and thus the present study sought to investigate the potential efficacy of a *delayed and chronic* 8-OH-DPAT treatment regimen. Forty-eight isoflurane-anesthetized adult male rats received either a controlled cortical impact or sham injury and beginning 24 h later were administered 8-OH-DPAT (0.1 or 0.5 mg/kg) or saline vehicle (1.0 mL/kg) intraperitoneally once daily until all behavioral assessments were completed. Neurobehavior was assessed by motor and cognitive tests on post-operative days 1–5 and 14–19, respectively. The lower dose of 8-OH-DPAT (0.1 mg/kg) enhanced motor performance, acquisition of spatial learning, and memory retention vs. both the higher dose (0.5 mg/kg) and vehicle treatment ($p < 0.05$). These data replicate previous findings from our laboratory showing that 8-OH-DPAT improves neurobehavior after TBI, and extend those results by demonstrating that the benefits can be achieved even when treatment is withheld for 24 h. A *delayed and chronic* treatment regimen may be more clinically feasible.

1. Introduction

Traumatic brain injury (TBI) affects 1.5–2 million individuals in the United States each year. Approximately 100,000 severe-TBI survivors endure long-term memory and/or physical impairments that require rigorous and costly rehabilitative therapy [18,41,58]. Treatment options for brain injury are limited and typically consist of augmenting or restoring dysfunctional neurotransmitter systems. Preclinical evaluation of various pharmacological agents has yielded several potential treatment candidates. For example, both agonists and antagonists affecting acetylcholine (ACh), dopamine (DA), and glutamate neurotransmission have shown marked benefits in the laboratory [10,12,14,15,29,30,33,39]. Unfortunately, with the exception of a few small clinical studies [42,47,49,61], translating from bench to bedside has not yielded the same beneficial effects observed in the laboratory. This realization suggests that additional therapies or other neurotransmitter systems should be evaluated.

In part because of its widespread modulation of the major neurotransmitters ACh, DA, and glutamate, the serotonin (5-HT) system, and in particular the 5-HT$_{1A}$ receptor, is considered a significant pharmacological target for the treatment of various central nervous system (CNS) diseases [63]. While a plethora of reports exist showing benefits of 5-HT$_{1A}$ receptor stimulation in the treatment of anxiety and depression [2,44,45], there is a paucity of studies evaluating the role of this 5-HT receptor subtype on CNS trauma.

The few studies that do exist indicate that 5-HT$_{1A}$ receptor agonists exert beneficial effects. Administration of 5-HT$_{1A}$ receptor agonists before or after focal cerebral ischemia provides neuroprotection as evidenced by decreased histopathology [8,55,59]. A significant reduction in cortical lesion volume has also been

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reported following treatment with the 5-HT_{1A} receptor agonist BAY × 3702 after subdural hematoma [1]. Studies from our laboratory using the controlled cortical impact (CCI) injury model, which produces many of the characteristics of human TBI [27], have demonstrated that an early and continuous infusion of the 5-HT_{1A} receptor agonist repinotan HCL or a single administration of 8-OH-DPAT enhances cognitive recovery in a water maze task, decreases cortical lesion volume, and confers hippocampal neuron survival [5,28,32,34,35]. Taken together, these findings suggest that 5-HT_{1A} receptor agonists are beneficial in a variety of brain injury models. However, while the benefits of this early therapeutic paradigm are compelling, the potential efficacy of *delayed and chronic* 8-OH-DPAT treatments after TBI is unknown. This issue is paramount given the secondary sequelae that are prevalent hours to days after TBI that affect the recovery process.

2. Materials and methods

2.1. Subjects

Forty-eight adult male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 300–325 g on the day of surgery were housed in standard steel-wire mesh cages and maintained in a temperature (21 ± 1 °C) and light (on 7:00 a.m. to 7:00 p.m.) controlled environment with free access to food and water. After one week of acclimatization the rats underwent beam-walk training and then were randomly assigned to one of the following group conditions: TBI + 8-OH-DPAT (0.1 mg/kg; $n = 12$), TBI + 8-OH-DPAT (0.5 mg/kg; $n = 12$), TBI + Vehicle (1 mL/kg; $n = 12$), Sham + 8-OH-DPAT (0.1 mg/kg; $n = 4$), Sham + 8-OH-DPAT (0.5 mg/kg; $n = 4$), or Sham + Vehicle (1 mL/kg; $n = 4$).

2.2. Surgery

A surgical level of anesthesia was induced and maintained with inspired concentrations of 4% and 2% isoflurane, respectively, in 2:1 N_2O:O_2 in a vented anesthesia chamber. Following endotracheal intubation the rats were secured in a stereotaxic frame and mechanically ventilated. Utilizing aseptic techniques a 6-mm craniectomy was made in the right hemisphere between bregma and lambda and from midline to the coronal ridge. A CCI of 2.8 mm tissue deformation at 4 m/s produced an injury of moderate severity as previously described [9,26,32,34]. Sham control rats underwent all anesthetic and surgical manipulations except the impact. Anesthesia was discontinued immediately after CCI or sham injury and the incision was promptly closed with nylon sutures. The rats were subsequently extubated and acute neurological evaluations were performed. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh and were conducted in accordance with the recommendations provided in the *Guide for the Care and Use of Laboratory Animals* (National Academy Press, 1996). Every attempt was made to limit the number of subjects used and to minimize suffering.

2.3. Acute neurological evaluation

Hind limb reflexive ability was assessed following the cessation of anesthesia by gently squeezing the rats' paw every 5 s and recording the time to elicit a withdrawal response. Return of the righting reflex was determined by the time required to turn from the supine to prone position.

2.4. Drug administration

8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) was purchased from Sigma–Aldrich (St. Louis, MO) and was prepared daily by dissolving in sterile physiological saline. 8-OH-DPAT (0.1 or 0.5 mg/kg) or a comparable volume of vehicle (1 mL/kg saline) was administered intraperitoneally beginning 24 h after CCI or sham injury and once daily until all behavioral evaluations were completed (i.e., post-operative day 19). On the days when behavioral assessments were conducted, treatments were administered 1 h prior to testing by an experimenter unaware of group conditions. The doses of 8-OH-DPAT and route of administration were selected based on previous studies from our laboratory [5,28,35] showing this regimen to confer neuroprotection and promote behavioral recovery after TBI.

2.5. Motor performance

Beam-balance and beam-walking performance was assessed with well-established tests [5,10,26,28–35]. The beam-balance task consists of placing the rat on an elevated (90 cm) narrow wood beam (1.5 cm wide) and recording the time it remains on for a maximum of 60 s. The beam-walk task, originally devised by Feeney et al. [13], consists of training/assessing rats using a negative-reinforcement paradigm to escape ambient light and white noise by traversing an elevated narrow wood beam (2.5 cm × 100 cm) and entering a darkened goal box situated at the opposite end. When the rat enters the goal box the adverse stimuli (i.e., light and noise) are terminated and thus serve as reinforcement for completing the task. Performance was assessed by recording both the elapsed time to traverse the beam as well as the 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 location, 1–4 corresponds to distal segments of 20, 40, 60, or 80 cm from the start point, respectively, and 5 corresponds with traversing the entire length of the beam (100 cm) and entering the goal box. Rats were tested for beam-balance and beam-walk performance on post-operative days 1–5 and were provided three trials (60 s allotted time) per day on each task. The average daily scores for each subject were used in the statistical analyses.

2.6. Cognitive function

2.6.1. Acquisition of spatial learning

Spatial learning was assessed in a water maze task demonstrated to be sensitive to cognitive function/dysfunction after TBI [20,32,51,57] The maze was a plastic pool (180 cm diameter, 60 cm high) filled with tap water (26 ± 1 °C) to a depth of 28 cm and was situated in a room with salient visual cues. The platform was a clear Plexiglas stand (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. Acquisition of spatial learning was initiated on post-operative day 14 and continued until day 18. The paradigm consisted of providing a block of four daily trials for five consecutive days to locate the platform when it was submerged 2 cm below the water surface (i.e., invisible to the rat). For each daily block of trials the rats were placed in the pool facing the wall at each of the four possible start locations (north, east, south, and west) in a randomized manner. Each trial lasted until the rat climbed onto the platform or until 120 s had elapsed, whichever occurred first. Rats that failed to locate the goal within the allotted time were manually guided to it. All rats remained on the platform for 30 s before being placed in a heated incubator between trials (4-min inter-trial interval). The times of the four daily trials for each rat were averaged and used in the statistical analyses.

One day after the final acquisition training session (day 19), all rats were given a single probe trial to measure retention. Briefly, the platform was removed from the pool and the rats were placed in the maze from the location point most distal to the quadrant where the platform was previously situated (i.e., "target quadrant") and allowed to freely explore the pool for 30 s. Typically, rats that have learned the specific location of the escape platform exhibit a spatial bias and spend significantly more time in the target quadrant. The percent time spent in the target quadrant was used in the statistical analysis. Following the probe assessment, the rats were provided four additional trials to locate the platform when it was raised 2 cm above the water surface (i.e., visible to the rat). While this task has been used to test for non-hippocampal damage [3], its use in the present study was as a control procedure to determine the contributions of non-spatial factors (e.g., sensory-motor performance, motivation, and visual acuity) on water maze outcome.

The data were obtained using a spontaneous motor activity recording and tracking (SMART) system (San Diego Instruments, San Diego, CA).

2.7. Data analyses

Statistical analyses were performed on data collected by observers blinded to treatment conditions using Statview 5.0.1 software (Abacus Concepts, Inc., Berkeley, CA). The motor and cognitive data were analyzed by repeated-measures analysis of variance (ANOVA). The acute neurological, probe trial, and swim speed data were analyzed by one-factor ANOVAs. When the overall ANOVA revealed a significant effect, the data were further analyzed with the Bonferroni/Dunn post-hoc test to determine specific group differences. The data are presented as the mean ± standard error (S.E.) and are considered significant when corresponding p values are ≤ 0.05 or as determined by the Bonferroni/Dunn statistic after adjusting for multiple comparisons.

3. Results

One sham control rat received an inadvertent dura mater tear during the craniectomy and was omitted from the study. Hence, the statistical analyses are based on 47 rats.

3.1. Acute neurological function

No significant differences were observed among the TBI groups in hind limb withdrawal latency in response to a brief paw pinch (range 163.0 ± 6.3 s–177.2 ± 4.9 s, $p > 0.05$) or for return of righting ability (range 369.1 ± 14.6 s–403.5 ± 14.8 s, $p > 0.05$) after the cessation of anesthesia. The lack of significant group differences in