Research report

Influence of amphetamine on recovery after intracerebral hemorrhage in rats

Angela M. Auriat a,∗, Frederick Colbourne a, b

a Department of Psychology, University of Alberta, Edmonton, Alta., Canada
b Centre for Neuroscience, University of Alberta, Edmonton, Alta., Canada

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Abstract

d-amphetamine (AMP) paired with physical activity (e.g., beam walking) improves recovery after ischemic injury to the cortical motor system of rodents. We tested whether AMP promotes recovery after intracerebral hemorrhage (ICH) in rats. A moderate-sized ICH was produced by stereotaxically injecting collagenase into the striatum. Five days later rats were placed into either environmental enrichment cages (EE) or a control condition (group housing in standard cages) until euthanasia at 4 weeks post-ICH. Animals were injected with either AMP (2 mg/kg i.p.) or sterile saline on days 7, 9 and 11 after ICH. Rats in EE also received training on beam (walking) and tray (skilled reaching) tasks 30 min after each injection. Walking (beam and ladder task), skilled reaching (tray and staircase tasks) and neurological deficits (NDS) were repeatedly assessed. We predicted that EE would improve recovery and that AMP would further enhance it. Results showed that EE, but not AMP, significantly and consistently improved recovery on the beam and ladder task. Neither treatment significantly affected skilled reaching. Lesion volume was not significantly different among groups (overall average: 44.6 mm³ of tissue lost ± 15.3 S.D.). In conclusion, EE provides modest benefit for striatal ICH whereas AMP does not. This suggests that AMP will not provide substantial benefit to those patients with severe ICH affecting the basal ganglia.

Keywords: Recovery; Striatum; Environmental enrichment; Rehabilitation; Stroke

1. Introduction

d-amphetamine (AMP) coupled with physical activity promotes sensorimotor recovery after brain injury in rodents [1,5,7,30], whereas AMP alone is not considered an effective treatment. Feeney and colleagues have shown that AMP treatment paired with training for traversing a narrow elevated beam significantly improves beam-walking skill after a mechanical insult to the motor cortex. Conversely, a single AMP injection without concomitant training on the beam provided no benefit [5]. Furthermore, recent studies using clinically relevant ischemic insults to the motor cortex show improvements with AMP treatment [1,7]. Nonetheless, clinical trials evaluating AMP have yielded mixed results; some report significant improvements [8,32], whereas others find none [29,31]. Perhaps the discrepancies are due to patient selection and the fact that clinical studies have been small. Accordingly, further animal experimentation should identify those factors that influence AMP’s efficacy (e.g., type and location of stroke) to increase the likelihood of finding benefit in defined clinical stroke populations.

Approximately 15% of all strokes are hemorrhagic and of these significant basal ganglia damage commonly occurs [16]. Survivors are often left with long-term disabilities due to permanent functional impairment (e.g., hemiplegia) [15]. It is possible that AMP might facilitate recovery in these patients. Given that the pathophysiology of intracerebral hemorrhage (ICH) differs substantially from ischemic and traumatic brain injury [33], it is likely that the mechanisms of recovery and the response to rehabilitative treatments, such as AMP, will also differ. To date, most animal and clinical studies have focused on ischemic stroke. Studies that included hemorrhagic stroke had very few hemorrhage patients, making it difficult to determine the efficacy of treatment for these patients [8]. Thus, neither pre-clinical nor clinical studies have determined whether AMP might promote recovery after ICH.
In this study we assessed whether AMP with or without rehabilitation improves performance on several behavioral tests after a striatal ICH in rats. The collagenase model of ICH, developed by Rosenzweig et al. [27], was used because it results in consistent hemorrhaging, and a well-characterized pattern of behavioral deficits [20]. Environmental enrichment (EE; multilevel cages with ramps, toys, and a running wheel) with additional training on reaching and walking (tray and beam tests, respectively) was used as our rehabilitation intervention. Enrichment was used because it enhances behavioral recovery in many models of brain injury [9,12], although the effects of EE on striatal ICH are not known. We compared four groups: rats housed in EE with AMP injections (EE + AMP), those housed in EE with saline injections (EE + SAL), group housed in standard cages with AMP injections (GH + AMP), and group housed in standard cages with saline injections (GH + SAL). Rats in EE also received experience for both walking and skilled reaching (beam and tray tasks, respectively) 30 min after each injection of SAL or AMP. We predicted that EE with additional rehabilitation training, henceforth simply referred to as EE, would improve recovery and that AMP treatment would provide additional benefit. Amphetamine alone (GH + AMP) was not expected to improve recovery.

2. Methods

2.1. Animals

Sixty, 7-week-old, male, Sprague–Dawley rats, obtained locally (Biological Sciences Animal Services; University of Alberta, Edmonton, Alta., Canada), weighing approximately 250 g were entered into this study. Rats were group housed, four per cage, in standard plastic cages (width: 38 cm; length: 49 cm; height: 20 cm) with wood chip bedding. After being acclimatized to our animal room for 3 days they were handled daily for 4 days (5–10 min/day) before starting the experiment. To minimize stress that is likely to result from changing cage mates each group of four rats were randomly assigned to one of two housing groups (GH or EE). This ensured that the same rats remained housed together for the duration of the study. Rats were then randomly assigned to one of two injection groups (SAL or AMP), resulting in four treatment groups: GH + SAL (n = 17), GH + AMP (n = 15), EE + SAL (n = 14), EE + AMP (n = 14). Animals were given free access to food and water, except when food deprivation was needed for behavioral training and testing. All procedures were approved by the Biological Sciences Animal Policy and Welfare Committee of the University of Alberta and were in accordance with the Canadian Council on Animal Care guidelines.

2.2. Behavior training

2.2.1. Montoya staircase test

Three days before training rats were food-deprived to 90% of their free feeding weight adjusted for natural gains in body weight over this period [20]. Prior to surgery, rats were trained to reach for food reward pellets (45 mg each; Bio-Serv, Frenchtown, NJ, USA) in the staircase test (length: 30 cm; width: 6.8 cm; height: 12 cm) twice daily (15 min trials separated by 3–4 h) for 5 days a week over 3 weeks (Fig. 1). The test was baited with 3 pellets per stair for a maximum of forelimbs to 2 for full wrist flexion and shoulder adduction when the rat was laterally; (4) contralateral forelimb flexion rated from 0 for uniform extension to grasp at all on a elevated bar (diameter: 3 mm); (3) hind limb retraction rated as a 0 (rat fell off the beam within 10 s), 1 (rat remained on the beam for more than 10 s but could not cross), 2 (rat could not cross but was able to place affected limb on beam), 3 (rat crossed but was unable to place affected limb on beam), 4 (rat crossed beam and placed affected limb on beam at least once), 5 (rat crossed with more than 50% foot slips with the affected limb), 6 (rat crossed with fewer than 50% foot slips with affected limb), or 7 (rat crossed with two or less foot slips). This test is sensitive to striatal ICH [20].

2.2.2. Tray task

Rats were trained in the tray task over 14 consecutive days (30 min/day) beginning the final week of staircase training. In the tray task (Plexiglas box; width: 19 cm; height: 25 cm; length: 27 cm) the rats must reach through vertical bars (1 cm separation) to obtain food pellets (17% Layer Prostock feed; Masterfeeds, Edmonton, Alberta) placed in a shallow tray located just outside of the box [6]. We recorded the first 10 min of the final training day for subsequent video analysis. The numbers of successful and unsuccessful reaches were determined and the % success was calculated as: (successful/reach attempts) × 100. In order for the rat to score a reach, the paw had to be inserted through the bars of the cage, and in order for the reach to be considered successful the animal had to consume some food from its paw. Rats were given free access to their regular food after completing this training.

2.2.3. Horizontal ladder walking test

Rats were given 1 day of training on the ladder (four crosses) prior to recording baseline performance. On the last day of tray task training rats were videotaped crossing the middle 0.5 m section of a 1 m long horizontal ladder with variably spaced rungs (1–3 cm). Several variations of rung spacing are sensitive to brain injury, including ICH [20,22]. Thus, this test, with the parameters used in this study, can detect significant error rates at up to 6 weeks following a moderate-sized ICH [2]. The number of errors (complete limb slips through the rungs) made for each limb was determined during four trials. The performance on this test is expressed as the % success = # successful footsteps/# successful footsteps + # foot slips)×100.

2.2.4. Beam walking test

On the final day of tray task training the rats were also trained to cross an elevated beam (length: 1.1 m; width: 3.2 cm) by placing them on the beam at increasing distances from the goal box until they successfully crossed the entire beam. An additional three crosses were then videotaped and analyzed on an eight-point scale [2,5]. Briefly, each cross was scored as a 0 (rat fell off the beam within 10 s), 1 (rat remained on the beam for more than 10 s but could not cross), 2 (rat could not cross but was able to place affected limb on beam), 3 (rat crossed but was unable to place affected limb on beam), 4 (rat crossed beam and placed affected limb on beam at least once), 5 (rat crossed with more than 50% foot slips with the affected limb), 6 (rat crossed with fewer than 50% foot slips with affected limb), or 7 (rat crossed with two or less foot slips). This test is sensitive to striatal ICH [20].

2.2.5. Neurological deficit scale (NDS)

Neurological deficit scores were assessed at multiple times, once prior to surgery, and 3, 5, 14, 21 and 28 days following ICH [20,25]. The score was compiled from five components: (1) spontaneous circling rated from 0 for no circling to 3 for continuous circling when placed in a plexi glass cylinder (diameter: 33 cm); (2) bilateral forelimb grasp rated from 0 for normal to 3 for unable to grasp at all on an elevated bar (diameter: 3 mm); (3) hind limb retraction rated from 0 for immediate replacement to 3 for no retraction after limb was displaced laterally; (4) contralateral forelimb flexion rated from 0 for uniform extension of forelimbs to 2 for full wrist flexion and shoulder adduction when the rat was lifted by the base of the tail; (5) beam walking ability rated from 0 for a rat that crosses easily to a 3 for a rat unable to stay on the beam for more than 10 s (length: 1.1 m; width: 3.2 cm). Scores for each test were added for a maximum score of 14 indicating greatest impairment. This NDS is sensitive to striatal ICH [20].
2.3. Surgery

Rats were food deprived for 12 h prior to surgery, which was performed aseptically under isoflurane anesthesia (4% induction; 2% maintenance in 70% N2O and 30% O2). Rectal temperature was maintained at ~37.0 °C during surgery with a heating pad. Rats were anesthetized and a catheter placed in the tail artery to measure mean arterial blood pressure (MABP) and to take blood samples for measurement of blood gases, pH, glucose, and hemoglobin concentrations. Two blood samples were taken—one at the start of surgery and one following the infusion of collagenase. Measurements of MABP were recorded every 5 min. Heparinized saline was used to prevent clotting in the tail artery and catheter. An identical amount of heparinized saline (1 mL; 10.0 U) was infused into all rats. Rats were placed in a stereotaxic frame. A midline incision was made and a hole was drilled at 3.5 mm lateral at the level of the bregma contralateral to the preferred paw (limb with highest average number of pellets retrieved over the last week of staircase training). A 26-gauge needle (Hamilton syringe; Hamilton, Reno, NV, USA) was lowered 5.5 mm below the surface of the skull. After waiting 5 min with the needle in place 0.5 μL of sterile saline containing 0.10 U of bacterial collagenase (Type IV-S; Sigma, Oakville, Ont., Canada) was manually infused over 5 min into the striatum [24]. Previous studies indicate that this dose of collagenase produces moderate lesion sizes [20,27] whereas saline injections cause only minimal injury (needle tract) [20,19]. The needle remained undisturbed for 5 min after the injection. A metal screw (model MX-080-2; Small Parts, Miami Lakes, FL, USA) sealed the hole and Marcaine (Sanofi Canada, Markham, Ont., Canada) was placed into the wound, which was then closed with staples.

We opted to affect the preferred paw, by lesioning the contralateral hemisphere, to allow for a more consistent level of performance and to avoid floor effects (i.e., not being able to worsen the performance of the non-preferred forelimb). Furthermore, the preferred limb in the staircase test is usually the limb that performs better on other tasks such as the ladder and tray tasks (C. MacLellan, F. Colbourne, unpublished data).

2.4. Amphetamine and rehabilitation

All rats were food-deprived to 90% of their free feeding weight adjusted for normal increases in body weight over days 3–14 post-ICH. Starting 5 days after ICH, and lasting until euthanasia, rats in the EE conditions were placed into a cage (width: 35 cm; length: 75 cm; height: 75 cm) with 3 levels, tunnels, ramps, various toys, and a running wheel. Cages were cleaned and changed weekly, with new objects introduced. Animals in the GH conditions remained in standard group housing (see Section 2.1). Injections of 2 mg/kg d-amphetamine sulphate (i.p.; 2 mg/mL; US Pharmacopedia, Rockville, MD, USA) or an equivalent volume of sterile saline were given on days 7, 9 and 11 following ICH. Thirty minutes after the injections of AMP or SAL rats in the EE conditions were given rehabilitation training consisting of five consecutive crosses on the elevated beam and NDS scores. The rats were then returned to ad lib feeding. Animals were only tested on skilled reaching tasks for a limited number of days in order to reduce the need for food deprivation. Additional testing was also not thought necessary in order to accurately gauge recovery. NDS testing was conducted on days 3, 5, 14, 21 and 28.

2.5. Behavioral testing

Rats were videotaped on the beam (three crosses) and ladder (four crosses) tasks on days 5, 14, 21 and 28. Tray testing (10 min) was conducted on days 5, 14 and 24. Two days before staircase testing animals were food deprived to 90% of their free feeding weight. Rats were tested on days 21–23 in staircase, two times a day for 15 min each. On day 24 rats were tested on tray task and then returned to ad lib feeding. Animals were only tested on skilled reaching tasks for a limited number of days in order to reduce the need for food deprivation. Additional testing was also not thought necessary in order to accurately gauge recovery. NDS testing was conducted on days 3, 5, 14, 21 and 28.

2.6. Histology

Rats were euthanized 4 weeks following ICH surgery with an overdose of sodium pentobarbital (Somnotol; MTC Pharmaceuticals, Cambridge, Ont., Canada; 80 mg/kg i.p.). They were perfused with saline followed by 10% formalin. Brains were cut into 40 μm coronal sections using a cryostat. Every fifth section was saved starting anterior to and extending to well after the end of the lesion ensuring that we analyzed locations where atrophy may occur. The tissue was stained with cresyl violet and every 600 μm a section was assessed for the extent of lesion. Volume of tissue lost was assessed using Scion Image J.4.0 (Scion Corporation, Frederick, MD, USA) and was calculated by subtracting the remaining volume of injured hemisphere from the remaining volume of normal hemisphere. The volume of each hemisphere was calculated as: (area of complete coronal section of the hemisphere − area of damage − area of ventricle) × interval between sections × number of sections [20].

2.7. Statistical analysis

Results are presented as mean ± standard deviation (S.D.). The behavioral scores and lesion volume were analyzed as multiple factor ANOVA (SPSS 11.0; SPSS Inc., Chicago, IL, USA). In cases of a significant Levene’s test for equality of error variances we used the Kruskal–Wallis test. For nonparametric data (beam and NDS scores) we used Wilcoxon signed ranks, Kruskal–Wallis and Mann–Whitney tests. The sum of the scores for the three daily trials was analyzed for the beam data. The $x^2$-test was used to assess dropout rate (number of rats failing to reach with contralateral limb) in tray task. The level of significance was set at $p < 0.05$.

3. Results

One rat (GH + SAL) died during surgery, presumably due to anesthesia. Three other animals, one from each of the GH + SAL, GH + AMP, and EE + AMP groups, were excluded at the time of histological analysis due to surgical error. The remaining

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Physiological variables measured before (top) and after ICH (bottom)</th>
<th>GH + SAL</th>
<th>GH + AMP</th>
<th>EE + SAL</th>
<th>EE + AMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.41 ± 0.04</td>
<td>7.41 ± 0.02</td>
<td>7.42 ± 0.04</td>
<td>7.41 ± 0.03</td>
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<tr>
<td>$pCO_2$ (mm Hg)</td>
<td>41.6 ± 4.6</td>
<td>40.3 ± 3.2</td>
<td>39.7 ± 5.7</td>
<td>40.3 ± 5.4</td>
<td></td>
</tr>
<tr>
<td>$pO_2$ (mm Hg)</td>
<td>122.4 ± 18.9</td>
<td>114.9 ± 13.5</td>
<td>115.3 ± 9.49</td>
<td>115.2 ± 15.6</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>15.4 ± 1.5</td>
<td>15.9 ± 0.65</td>
<td>15.1 ± 0.82</td>
<td>15.5 ± 0.72</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>8.8 ± 2.0</td>
<td>8.3 ± 1.4</td>
<td>8.7 ± 2.9</td>
<td>8.9 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>105.3 ± 7.7</td>
<td>100.7 ± 9.4</td>
<td>96.8 ± 7.5</td>
<td>100.3 ± 9.1</td>
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</tr>
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</table>

The hemoglobin and glucose values are from the first reading, whereas the mean arterial blood pressure (MABP) is averaged throughout surgery. The values are within normal ranges and are similar among groups and across time points.
group sizes were 15, 14, 14 and 13 in GH + SAL, GH + AMP, EE + SAL, and EE + AMP groups, respectively.

3.1. Physiological parameters

Weight data was recorded for all groups during food deprivation and the period following surgery to ensure the health of all animals. There were no significant differences in weight among the groups at any time (data not shown).

Physiological parameters (e.g., pCO₂) were not significantly different among groups either prior to or following collagenase infusion (e.g., pCO₂: drug main effect—\( F_{1,50} = 0.197, p = 0.659 \); housing main effect—\( F_{1,50} = 0.037, p = 0.849 \); interaction—\( F_{1,50} = 0.402, p = 0.529 \)). These parameters did not change significantly over time (e.g., pCO₂: \( F_{1,50} = 0.254, p = 0.617; \) Table 1).

3.2. Lesion volume

Inspection of coronal sections at a 30-day survival showed that ICH-induced brain injury primarily occurred within the striatum but occasionally affected other structures (e.g., globus pallidus) along with usually causing marked enlargement of the ipsilateral ventricle. This pattern was similar among group as was the lesion volume (\( \chi^2(3) = 2.508, p = 0.474; \) Fig. 2a), which was analyzed by the Kruskal–Wallis test owing to a significant Levene’s test (\( F_{1,25} = 5.650, p = 0.002 \)). A representative lesion is illustrated in Fig. 2b.

3.3. Behavioral testing

All animals received a normal score (i.e., 7 on each of the three trials) on the elevated beam task prior to ICH. All groups were significantly impaired on day 5 post-ICH (\( Z = -6.342, p < 0.001, \) Fig. 3) and the groups were not significantly different at this time (\( \chi^2(3) = 0.896, p = 0.826 \)), which was prior to rehabilitation treatment. However, significant group differences emerged on day 14 (\( \chi^2(3) = 18.076, p < 0.001 \), day 21 (\( \chi^2(3) = 20.646, p < 0.001 \)) and day 28 (\( \chi^2(3) = 9.785, p = 0.020 \)). Pairwise comparisons indicate that EE + SAL rats performed significantly better than GH + SAL rats on day 14 (\( U = 46.500, p = 0.003 \), day 21 (\( U = 37.000, p = 0.001 \)) and day 28 (\( U = 60.500, p = 0.014 \)). EE + SAL rats were also significantly better than GH + AMP rats on day 14 (\( U = 32.000, p = 0.001 \), day 21 (\( U = 31.000, p = 0.001 \)), and day 28 (\( U = 46.500, p = 0.004 \)). EE + AMP rats were significantly better than the GH + SAL on day 14 (\( U = 53.000, p = 0.020 \)) and day 21 (\( U = 36.500, p = 0.002 \)). EE + AMP rats were also significantly better than GH + AMP rats on day 14 (\( U = 36.000, p = 0.003 \)) and day 21 (\( U = 33.000, p = 0.002 \)). However, there was no significant effect of AMP as there were no significant difference between the GH + SAL versus GH + AMP on day 14 (\( U = 92.000, p = 0.561 \), day 21 (\( U = 92.000, p = 0.565 \)) or day 28 (\( U = 96.500, p = 0.693 \)) or between EE + AMP versus EE + SAL on day 14 (\( U = 82.500, p = 0.450 \), day 21 (\( U = 89.500, p = 0.906 \)) or day 28 (\( U = 68.500, p = 0.107 \)). Therefore, EE treatment improved beam walking ability, but AMP did not.

The NDS scores were not significantly different among groups at baseline (prior to ICH) as all rats received the minimum score of 0 (i.e., no impairment). Three days following ICH all groups were significantly impaired compared to baseline (\( Z = -6.559, p < 0.001 \)). However, there were no significant group differences prior to rehabilitation treatment on day 3 (\( \chi^2(3) = 3.661, p = 0.300 \)) or day 5 (\( \chi^2(3) = 7.464, p = 0.058 \)) post-ICH. Significant group differences emerged on day 14 (\( \chi^2(3) = 15.937, p = 0.001 \)) and day 21 (\( \chi^2(3) = 9.614, p = 0.022 \)), but not on day 28 (\( \chi^2(3) = 5.861, p = 0.119 \)) post-ICH (Fig. 4). On day 14, each EE-treated group was significantly less impaired than their respective GH control group: EE + SAL versus GH + SAL (\( U = 47.000, p = 0.009 \)), and EE + AMP versus GH + AMP (\( U = 32.500, p = 0.003 \)). On day 21, the EE + SAL (\( U = 53.000, p = 0.019 \)) and EE + AMP (\( U = 46.000, p = 0.011 \)) groups were significantly better than GH + SAL rats. As it is possible that the beam sub-scale of the NDS contributes considerably to these effects, we also analyzed the NDS without the beam sub-scale. There were no significant differences between groups on day 14 (\( \chi^2(3) = 1.78, p = 0.619 \)), day 21 (\( \chi^2(3) = 7.049, p = 0.070 \)), and day 28 (\( \chi^2(3) = 1.685, p = 0.640 \)) indicating that EE only improved NDS scores through enhancing beam performance.
Fig. 3. Each symbol represents the sum of three scored trials for each rat in the beam-walking test on days 5, 14, 21 and 28 ((†) indicates a significant difference from GH + AMP and (*) indicates a significant difference from GH + SAL; see results for statistics).

Fig. 4. Neurologic impairments for days 3, 5, 14, 21 and 28. Each symbol represents the score for one rat ((†) indicates a significant difference from GH + AMP and (*) indicates a significant difference from GH + SAL).
Owing to significant Levene’s test ($F_{3.52} = 6.203, p = 0.001$) for baseline ladder data, indicating heterogeneity, we used a nonparametric test and found no significant baseline differences among groups ($U = 66.500, p = 0.144$). The overall baseline performance for all groups was $95.2 \pm 5.3\%$ success. Analysis of day 5 data with ANOVA revealed a significant drug main effect ($F_{1,49} = 7.036, p = 0.010$). Given the presence of a main effect prior to treatment, the following test days were expressed as % improvement (% success on treatment day − % success on day 5). This analysis of days 14, 21 and 24 data revealed a significant main effect of Drug ($F_{1,48} = 7.880, p = 0.007$), but neither the Housing ($F_{1,48} = 0.501, p = 0.483$) nor the Drug × Housing interaction ($F_{1,48} = 0.301, p = 0.591$) were significant. Therefore, EE, but not AMP, improved performance on the ladder test.

Rats failing to reach baseline staircase exclusion criteria were excluded from the analysis, leaving group sizes of: 10, 10, 12 and 10 in the GH + SAL, GH + AMP, EE + SAL, and EE + AMP groups, respectively. Performance improved over the test days for all groups (day effect: $F_{2,76} = 5.529, p = 0.006$; Fig. 6), but there were no significant drug ($F_{1,38} = 0.076, p = 0.784$) or housing ($F_{1,38} = 0.117, p = 0.735$) main effects and no interaction ($F_{1,38} = 3.138, p = 0.085$).

Analysis of the tray task data indicated that there were no baseline differences among the groups (drug: $F_{1,41} = 0.107, p = 0.745$; housing: $F_{1,41} = 0.537, p = 0.468$; interaction, $F_{1,41} = 0.337, p = 0.565$). Following the ICH several animals would not reach with their impaired arm so they were excluded from the tray task analysis. The dropout rates for each group were compared for each test day. The day 5 dropout rates were 20, 36, 43 and 31% in the GH + SAL, GH + AMP, EE + SAL and EE + AMP groups, respectively; which is not significantly different ($\chi^2_{(1)} = 0.573, p = 0.449$). However, on day 14 ($\chi^2_{(1)} = 5.916, p = 0.015$) and day 24 ($\chi^2_{(1)} = 6.103, p = 0.013$) significantly more rats in the EE groups stopped reaching with their contralateral limbs. The dropout rates were 40, 36, 71 and 69% on days 14 and 33, 50, 71 and 77% on day 24 in the GH + SAL, GH + AMP, EE + SAL and EE + AMP groups, respectively. Given the differences in dropout rates tray data was not analyzed further.

4. Discussion

This is the first study to test the separate and combined effects of EE and AMP after ICH. Environmental enrichment, which included rehabilitation exercises, improved recovery after ICH, and this was not facilitated by AMP. Furthermore, EE-facilitated recovery was incomplete and did not occur on all behavioral tests. Notably, EE enhanced walking ability on beam and ladder tests. The improvement found with the NDS was apparently due to the beam sub-scale. Neither treatment facilitated skilled reaching in the staircase test. These results indicate that AMP does not provide significant functional benefit following striatal ICH. Accordingly, further research is required before AMP should be considered for evaluation in striatal ICH patients.

Beam training during AMP exposure was no better than training (EE) alone. However, the EE + SAL and EE + AMP groups approached a full recovery on the beam test suggesting that it may not have been sensitive enough to detect any further benefit of AMP beyond that provided by EE (i.e., ceiling effect). Accordingly, our beam data are not necessarily at odds with reports that the combination of AMP and beam training improves performance [5,28]. Interestingly, Goldstein and Davis [10] found that if non-AMP treated rats were forced to cross a beam they recovered at a rate similar to AMP treated rats. It follows then that our EE cage, which contained a beam that we frequently observed our rats using, both with and without AMP, was a sufficient treatment to allow recovery on subsequent beam tests. Thus, when sufficient training and recovery time is given the beam test will likely be insensitive to treatment effects [20]. This highlights a limitation of studies using these tests as the sole indicator of functional outcome following AMP treatment [5]. Although, it is possible that the beam test may be more sensitive under other conditions, such as cases where there is limited exposure to the test, or in other models of brain injury. In contrast to
the beam test, persistent deficits occurred in the ladder test indicating that this is the more sensitive measure of recovery in this study. We found that significant improvement occurred in the ladder test when rats were housed in EE, but AMP provided no benefit either alone or combined with rehabilitation. Recovery on this test may have generalized from training on other tasks, such as beam, or from the experiences the rats had in the EE cages (e.g., climbing and walking).

Amphetamine treatment for skilled reaching has produced equivocal results perhaps owing to study differences such as variations in lesion size, location and insult type, along with the dose of AMP. Two studies found benefit with AMP (<2 mg/kg) and skilled reach training following cortical ischemic injury [1,7]. In contrast, Rasmussen et al. [26] using an embolic stroke model found that reach training improves performance in the staircase test, but this effect disappears with AMP treatment (3.5 mg/kg). This effect was attributed to AMP suppressing reaching indicating the importance of proper dosing and training following AMP treatment. We selected 2 mg/kg as a dose because it did not block reaching and is a commonly used dose in rehabilitation studies in rats. Presently, neither EE nor AMP improved recovery after ICH in the staircase test. We counted the number of pellets retrieved in this test; thus, there may have been performance differences among groups (e.g., % successful reaches). Unfortunately, rats often stopped reaching with their impaired limb in tray task following ICH and thus we were unable to accurately judge reaching success. Furthermore, the switch in limb preference in tray task was significantly different among groups thereby confounding this test data.

Enrichment has been found to improve recovery in several brain injury models [9,12,17], including ICH as presently shown (i.e., walking). We used EE as a way to enhance rehabilitation during AMP exposure, providing abundant opportunity for exploration and exercise. Rats without AMP were repeatedly observed exploring the multiple objects in the cage. This behavior increased with AMP treatment and some stereotypical behaviors (e.g., head bobbing) emerged that did not appear to notably impact mobility nor did they block performance on the other rehabilitation exercises. Unfortunately, behavior was not formally quantified in the EE cages or other exercises, and thus it is not possible to determine whether the stereotypical behaviors somehow impeded rehabilitation. Additionally, it is possible that the altered activity of AMP treated rats influenced co-housed animals. However, we did not observe any abnormal interactions between animals following drug treatment. Johansson et al. [13] combined AMP treatment, at the same dose we used (2 mg/kg), with EE following middle cerebral artery occlusion in rats and found that AMP did not provide any additional benefit to EE. Our findings suggest that the inability of AMP to provide benefit to animals housed in EE may be related to the demands of the function being assessed. Finally, AMP was unable to provide additional benefit on beam because EE animals achieved a near maximum score. However, this was not a limitation of the ladder test where AMP also failed to improve recovery.

Beneficial effects of EE are usually limited to tasks that involve body coordination and balance, such as beam, ladder and rotarod [12,14,17] whereas improvements on skilled reaching come from additional forced reach training of the impaired limb [3]. Perhaps this is because EE naturally encourages rats to use their non-injured limb for reaching, whereas all limbs must be used for effective locomotion. This learning may be most effective when the impaired limb is used early after injury as during EE exposure. Indeed, more animals in the EE than in the GH groups stopped reaching with their contralateral limb in the tray task on test days 14 and 24. Future studies should consider whether AMP combined with more intensive and forced reach training would facilitate recovery of skilled reaching ability. For instance, constraint-induced movement therapy (CIMT), which involves restraint of the unimpaired limb to force the use of the impaired limb during exercises (e.g., tray training), improved staircase performance following striatal ICH [4]. Further improvement, especially needed for severe ICH [21], may be achieved with the addition of AMP to CIMT.

The present findings do not exclude the possible use of AMP for striatal ICH; however, neither do they support the use of AMP for this type of stroke, as there was no benefit of AMP. Accordingly, alternative AMP treatments should be tested as they may better promote recovery. First, AMP treatment was not started until 7 days after ICH and an earlier start may have improved outcome to a greater extent. However, one must question whether very early treatments have clinical relevance and there is concern about early behavioral interventions aggravating injury as found following ischemia [18]. Second, the dosage of AMP may need to be varied to suit the particular rehabilitation goal (e.g., walking versus skilled reaching). Third, more frequent treatment may provide greater benefit. Unfortunately, the number of treatment days in clinical and experimental AMP studies varies considerably with several studies reporting benefit from a single dose of AMP [1,5,26]. Only one study, using a model of cortical injury in cats, directly compared single and multiple dosing. They found that multiple doses increase the rate of recovery. However, the single dose eventually reaches the same level of improvement [11]. We chose three treatment days because this would likely provide an adequate indication of AMP’s effectiveness while avoiding the problem of rats becoming habituated and refusing to complete testing trials. Finally, it is possible that additional behavioral tests might have revealed further group effects. However, not all aspects of recovery can be assessed in a single study and we used several tests thought to be sensitive to a striatal ICH lesion.

Location, size and type of injury critically modulate the extent of recovery following stroke thereby necessitating a thorough evaluation of putative treatment in multiple stroke types. Here we report that with our selected treatment protocol AMP did not improve function after ICH in rats. Additionally, our results suggest that although EE with additional training improves performance on some tasks, while its use early after injury may further inhibit limb use for other tasks such as reaching. Robust functional benefits in animal models are likely needed if treatments are to be effective clinically. Therefore, our current findings indicate that AMP treatment may not provide substantial benefit to ICH patients.
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References