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Stroke. 2003;34:1021-1026; originally published online March 20, 2003;
doi: 10.1161/01.STR.0000063374.89732.9F

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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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Constraint-Induced Movement Therapy and Rehabilitation Exercises Lessen Motor Deficits and Volume of Brain Injury After Striatal Hemorrhagic Stroke in Rats

Suzanne B. DeBow, BSc; Melissa L.A. Davies, BSc; Heather L. Clarke, BSc; Frederick Colbourne, PhD

Background and Purpose—Constraint-induced movement therapy (CIMT) promotes motor recovery after occlusive stroke in humans, but its efficacy after intracerebral hemorrhage (ICH) has not been investigated clinically or in the laboratory. In this study we tested whether CIMT and a rehabilitation exercise program would lessen motor deficits after ICH in rats.

Methods—Rats were subjected to striatal ICH (via infusion of collagenase) or sham stroke. Seven days later, treatment began with CIMT (8 h/d of ipsilateral forelimb restraint), rehabilitation exercises (eg, reaching, walking; 1 h/d), or both for 7 days. Some rats were not treated. Motor deficits were assessed up to the 60-day survival time, after which the volume of tissue lost was determined.

Results—Untreated ICH rats made more limb slips traversing a horizontal ladder and showed an asymmetry toward less use of the contralateral paw in the cylinder test of limb use asymmetry (day 28). These rats were also significantly less successful in the Montoya staircase test (days 55 to 59) of skilled reaching. Neither therapy alone provided much benefit. However, the combination of daily exercises and CIMT substantially and persistently improved recovery. Unexpectedly, this group had a statistically smaller volume of tissue lost than untreated ICH rats.

Conclusions—The combination of focused rehabilitation exercises and CIMT effectively promotes functional recovery after ICH, while either therapy alone is less effective. This therapy may work in part by reducing the volume of tissue lost, likely through reducing atrophy while promoting remodeling. (*Stroke*. 2003;34:1021-1026.)

Key Words: corpus striatum ■ intracerebral hemorrhage ■ motor activity ■ movement disorders ■ physical therapy techniques ■ rehabilitation ■ stroke ■ rats

Hemiparesis affects the majority of stroke survivors acutely. While some spontaneous recovery occurs, many are chronically impaired,¹ in part because of “learned non-use.”² This results from repeated failure with the impaired limb soon after injury combined with successful use of the normal limb. Restraint of the unimpaired limb (eg, via sling for 6 h/d for 2 weeks) to force the use of an impaired limb during normal daily activities and rehabilitation exercises counteracts learned non-use. This therapy, known as constraint-induced movement therapy (CIMT), persistently decreases motor deficits after stroke in humans.^{3–6}

Approximately 20% to 25% of ischemic stroke survivors could benefit from CIMT.⁷ Unfortunately, there are few clinical and no experimental data on the efficacy of CIMT after intracerebral hemorrhage (ICH). For instance, data from 2 hemorrhagic patients, discontinued from traditional therapy, showed that CIMT lessened hemiparesis.⁸ Further study is warranted especially because rodent studies show that early CIMT may worsen brain injury and depress recovery. In rats, CIMT initiated immediately after cortical injury produced by electrolytic lesions,^{9,10} middle cerebral artery occlusion,¹¹ and cortical devascularization (S. DeBow, BSc, et al,

unpublished data, 2000) results in enlarged lesions and poorer recovery. This is thought to result from heightened behavioral pressure (ie, neuronal overactivity) forced on vulnerable tissue.⁹ Thus, given the trend toward beginning rehabilitation soon after stroke,¹² early CIMT might worsen occlusive and hemorrhagic stroke outcome. Although preliminary data⁶ indicate that CIMT begun within 4 to 14 days after stroke in humans does not worsen outcome, hemorrhagic stroke patients were excluded.

We tested whether the combination of CIMT and rehabilitation exercises or each alone would improve motor recovery in rats after ICH.^{13,14} Therapies began 1 week after surgery and lasted for 7 days. Motor recovery on several tests was assessed over 2 months, and the volume of brain tissue destruction was quantified at 2 months.

Materials and Methods

Animals

Sixty-seven male Sprague-Dawley rats (weight, 250 to 350 g; obtained locally) were individually housed with food and water ad libitum. Procedures were approved by the Biosciences Animal Policy and Welfare Committee of the University of Alberta and in

Received August 23, 2002; final revision received September 30, 2002; accepted October 30, 2002.

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DOI: 10.1161/01.STR.0000063374.89732.9F

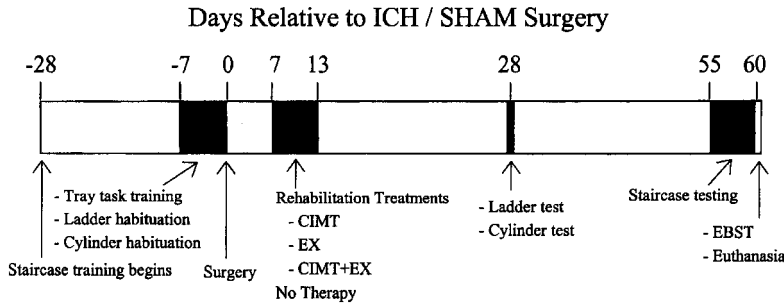


Figure 1. Group treatments (days relative to surgery). The Table shows group sizes.

accordance with the Canadian Council on Animal Care guidelines. Figure 1 summarizes procedures, and the Table indicates group sizes.

Behavior Training

Montoya Staircase Task

Rats were food deprived to 85% of their free-feeding weight 3 days before training.¹⁵ Training in the test, which measures independent forelimb reaching ability, consisted of two 15-minute trials per day separated by 4 to 5 hours for 5 d/wk over 3 weeks. Two rats were excluded because they failed to reach the criterion¹⁵ of 9 pellets (45 mg each) per forelimb on 3 consecutive days.

Tray Task

Rats were placed in the tray task for 30 min/d over 5 days before surgery. This apparatus is 19 cm wide×27 cm long×25 cm high and has 2-mm-wide vertical bars spaced 1 cm apart in the front through which rats reach for food (20- to 40-mg pellets) placed in a tray (4 cm wide and 5 mm deep).¹⁶ The rats' preferred paw was determined in this test.

Limb Use Asymmetry Test

Rats spontaneously explored (eg, by touching the wall) a transparent cylinder (45 cm in height by 33 cm in diameter) during one 5-minute session for each of 2 days before surgery.¹⁵

Horizontal Ladder Test

Rats walked across the ladder (1.0 m long) 3 times each day for 2 consecutive days before surgery.^{15,17} The ladder had randomly spaced rungs (1 to 3 cm).

Surgery

Rats were anesthetized with isoflurane (1.5% to 2% maintenance in 70% N₂O, 30% O₂), and a small hole was drilled in the skull (0.2 mm anterior and 3.0 mm lateral to bregma) contralateral to the preferred paw. A 28-gauge needle was inserted into the dorsal striatum (5.5 mm ventral to surface of the skull), and either 0.7 μL of sterile saline (SHAM group) or saline containing 0.14 U of bacterial collagenase (ICH group; type IV-S, Sigma Chemical Co) was infused.¹³⁻¹⁵ The needle was removed, and the craniotomy was sealed with a screw. Marcaine was infiltrated into the wound, which was then closed. Rectal temperature was maintained between 36.0°C and 37.0°C during surgery with the use of a heating pad. Two rats were excluded because of surgical error.

Group Sizes*

	CIMT		No CIMT	
	EX	No EX	EX	No EX
SHAM	6	6	5	6
ICH	11	9	9	11
	ICH+CIMT+EX	ICH+CIMT	ICH+EX	ICH

See Figure 1 for time line.

Rats were randomly assigned up to desired group size of at least 5 in SHAM groups and at least 8 in ICH groups.

Rehabilitation Therapies

One group each of SHAM and ICH rats received no therapy. Other groups received 7 days of rehabilitation beginning 1 week after surgery. Group assignment was random. Treated SHAM and ICH rats received either (1) CIMT alone (in standard home cage); (2) a daily exercise (EX) regimen; or (3) both (CIMT+EX). The CIMT involved restraining the forelimb ipsilateral to the lesion via a sleeveless jacket (model RJ02, Lomir Biomedical, Inc) that wrapped around the upper torso of the rat and attached to a metal wrist bracelet. The jacket was continuously worn for 7 days, while the bracelet restraint was used between 8 AM and 4 PM, mimicking human therapy.^{3,4} Rats had full range of motion when the bracelet was not used. The EX program lasted 1 h/d for 7 days. Exercises included 30 minutes in the tray task and 10 minutes in each of the cylinder, ladder, and running wheel tasks. Rats were required to walk across the ladder a minimum of 3 times and to run 10 m in the wheel. Rats were not exposed to the running wheel previously because it was not necessary.

Assessment of Recovery

Limb Use Asymmetry Test

Twenty-eight days after surgery, all rats were videotaped for 5 minutes in the cylinder. Forelimb use for initiating a rearing movement, for wall exploration, and for landing was analyzed.^{15,18} A "push-off" is the independent use of either forelimb or simultaneous use of both on rearing. "Wall exploration" is the initial placement of a forelimb on the wall and contact during lateral movements across it. Simultaneous use on the wall includes lateral walking movements, in which limbs are used alternatively. A "landing" is the use of either forelimb or simultaneous use of both to land after rearing. Testing at later times was not done to limit the already extensive amount of behavioral analyses.

Horizontal Ladder Test

Twenty-eight days after surgery, rats were videotaped crossing the ladder 3 times. The percentage of footfalls (slips through the bars were summed across 3 trials) with each paw while traversing the ladder was analyzed.

Staircase Testing

All rats were food deprived (to 85%) 2 days before testing, which began 55 days after surgery. Rats were tested twice per day over 5 days.¹⁵ The number of pellets consumed per side, expressed as a percentage of baseline (average of last 10 training trials, ie, an asymptotic performance), was analyzed.

The staircase test was, a priori, our primary end point because it was meant to provide an unbiased assessment of recovery. Accordingly, this test was not used during rehabilitation so that we could determine whether the effects of rehabilitation on other tasks transferred to the staircase and whether those effects were persistent.

Elevated Body Swing Test

Sixty days after surgery, rats were tested on the elevated body swing test (EBST),¹⁹ which involved recording the direction of rotation after rats were suspended by the tail (30 trials). Rats normally turn ipsilateral to the lesioned hemisphere. Experimenters were blind to treatment identity, as with all tests.

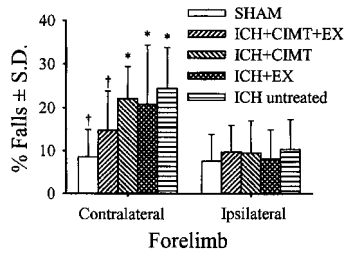


Figure 2. Number of falls (mean \pm SD) with ipsilateral and contralateral forelimbs while crossing the ladder on day 28 after surgery. * $P < 0.05$ vs SHAM; † $P < 0.05$ vs untreated ICH.

Histology

Rats were killed 60 days after surgery with sodium pentobarbital (80 mg/kg) and perfused with saline and then 10% formalin. Brains were processed, and 40- μ m (every 600 μ m) coronal sections were taken with a cryostat and stained with cresyl violet.¹⁵ The volume of tissue lost was determined with the use of Scion Image J 4.0 (Scion Corporation) and expressed as (1) Volume of Tissue Lost = Remaining Volume of Normal Hemisphere - Remaining Volume of Injured Hemisphere and (2) Volume of Hemisphere = Average Area of Complete Coronal Section of Hemisphere (Excluding Area of Ventricle and Area of Damage) \times Interval Between Sections \times Total Number of Sections.

Statistical Analysis

Data were analyzed with multiple factor (CIMT versus no CIMT, EX versus no EX, test trial) ANOVAs with subsequent simple effects and planned comparisons. In all cases the SHAM groups were combined (see Results), and thus comparisons with the combined SHAM group were made within a 1-way (eg, volume data) or 2-way (staircase test data; between/within design) ANOVA²⁰ or a *t* test that did not assume equality of error variances in cases of a significant Levene's test.

Results

Horizontal Ladder Test

Ipsilateral and contralateral fall rates were not different among SHAM groups ($P \geq 0.115$). The percentage of falls with the contralateral forelimb was significantly ($P \leq 0.001$) greater in ICH, ICH+EX, and ICH+CIMT groups than in the combined SHAM group (Figure 2). Whereas neither single therapy significantly lessened the ICH-induced error rate ($P \geq 0.430$), the combination treatment did ($P < 0.05$ versus untreated ICH; $P = 0.059$ versus SHAM). The ipsilateral forelimb fall rate in ICH groups did not differ statistically from the SHAM group ($P = 0.741$).

Cylinder Test

There were no significant group main effects in the percentage of simultaneous movements for push-off, landing, or wall contacts ($P \geq 0.082$). Therefore, the percent contralateral use was analyzed [(number of contacts with contralateral limb/ipsilateral+contralateral limb use) \times 100]. Rehabilitation treatments did not affect SHAM groups ($P \geq 0.380$), and data were combined. All ICH groups displayed an asymmetry favoring use of the ipsilateral limb (see Figure 3 for statistics). Because of high variability, only the ICH+CIMT+EX group was significantly better than the ICH group, but only for landing.

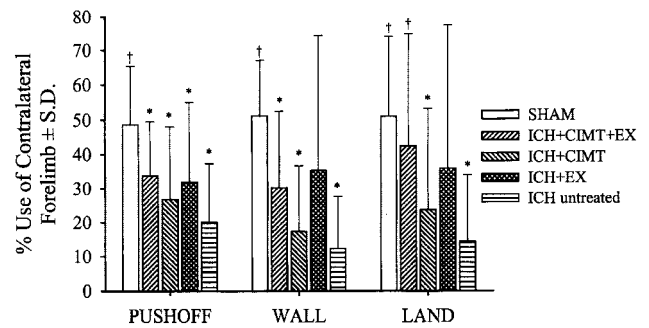


Figure 3. Spontaneous paw use (mean \pm SD percent use of contralateral forelimb) in the cylinder on day 28 after surgery. * $P < 0.05$ vs SHAM; † $P < 0.05$ vs untreated ICH.

Staircase Test

All groups performed similarly during training (data not shown). The SHAM groups did not differ significantly during testing (percent baseline success) with the ipsilateral or contralateral limbs ($P \geq 0.158$ for group main effects and group interaction). The untreated ICH group was impaired with the ipsilateral ($P = 0.041$) and contralateral ($P < 0.001$) forelimbs (Figure 4), although to a lesser extent with the ipsilateral limb. Neither the ICH+EX nor the ICH+CIMT group was significantly better than the untreated ICH group in terms of ipsilateral ($P \geq 0.599$) and contralateral reaching success rate ($P \geq 0.318$). The ICH+CIMT+EX group was significantly better (versus single or no therapies) with the contralateral ($P < 0.01$) but not ipsilateral limb ($P \geq 0.106$). This group was not significantly different than the SHAM group with either limb ($P \geq 0.547$).

The first 5 minutes of the second last staircase session was videotaped to determine the number of reaches and success rate for those reaches (contralateral limb data shown). Although the group effect was not significant ($P = 0.164$), there were trends toward less reaching in the ICH (mean \pm SD reaches in 5 minutes = 31.2 ± 22.9), ICH+EX (32.0 ± 32.2), and ICH+CIMT groups (33.9 ± 23.1) compared with SHAM (48.6 ± 25.9), whereas the ICH+CIMT+EX group (50.1 ± 18.2) was similar to the SHAM group. The ICH ($11.0 \pm 12.8\%$ [SD]), ICH+EX ($9.2 \pm 8.9\%$), and ICH+CIMT groups ($5.5 \pm 6.9\%$) had a lower success rate than SHAM ($25.1 \pm 14.9\%$; $P \leq 0.004$). The ICH+CIMT+EX group ($17.3 \pm 10.3\%$) performed better than ICH rats, but this was not significant ($P > 0.10$ versus ICH; $P = 0.091$ versus SHAM).

Elevated Body Swing Test

The turning bias (percent toward lesioned side) results during the EBST did not statistically differentiate among SHAM or ICH groups with or without EX or CIMT treatments and did not reveal significant differences among lesioned and SHAM groups ($P \geq 0.065$; SHAM averaged groups, -47.5% ; untreated ICH rats, -64.2% ; ICH+EX, -62.4% ; ICH+CIMT, -55.2% ; ICH+CIMT+EX, -49.1%).

Volume of Tissue Lost

Sham groups were not damaged (0.4 mm^3 of tissue lost ± 1.4 SD). There were significant differences among ICH groups (Figure 5), with a significant main effect for EX treatment

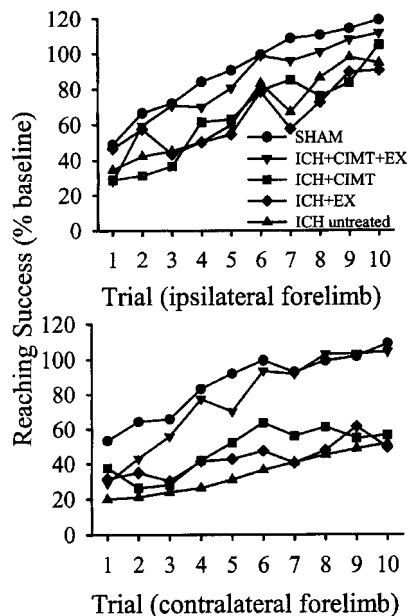


Figure 4. Average percent reaching success in the staircase with ipsilateral and contralateral forelimbs. Greater contralateral deficits were found than with the ipsilateral forelimb. The ICH, ICH+CIMT, and ICH+EX groups were significantly impaired compared with SHAM. The ICH+CIMT+EX group had near-total recovery (see Results for statistics).

($P=0.049$) and nonsignificant effects for CIMT treatment ($P=0.578$) and the interaction ($P=0.736$). Notably, the ICH+CIMT+EX and ICH+EX groups had a 28% and 18% smaller volume of tissue lost, respectively, than the untreated ICH group. A significant Levene's test for homogeneity of variances was found ($P=0.007$). Thus, additional t tests using separate or pooled error variances were also used depending on the outcome of each Levene's test. With this analysis, only the ICH+CIMT+EX group had a statistically smaller volume of tissue lost ($P=0.028$).

Each behavioral test (eg, contralateral limb in staircase test; $r=-0.609$, $P<0.001$) significantly predicted the volume of tissue lost (all groups entered in analysis). A forward multiple linear regression of the average performance in the cylinder (percent contralateral touches), ladder (percent contralateral falls), EBST (percent ipsilateral turning), and staircase (percent success with contralateral limb) yielded an $r=0.810$ ($P<0.001$) for predicting the volume of tissue lost.

The reduction in the volume of lost tissue in the ICH+CIMT+EX group does not easily account for improved recovery in that group because the behavioral tests did not significantly correlate with volume in that group (eg, average percent success with contralateral limb in staircase test; $r=0.388$, $P=0.238$). This is in contrast to the significant correlations within the ICH group (eg, staircase data versus lesion volume; $r=-0.674$, $P=0.023$), in which more direct relationships are expected.

Discussion

One week of combined CIMT and EX therapy significantly facilitated motor recovery on a number of behavioral tests after ICH in rats, whereas neither therapy alone was of as substantial benefit. This underscores the need for daily EX in combination with CIMT, as done clinically. Clinical studies show that CIMT, corresponding to our CIMT+EX treatment, successfully transfers improvement from the clinic to a real-life setting.^{2,3} Similarly, rats were substantially better on the staircase test even though they were not rehabilitated on it and testing started 6 weeks after the end of rehabilitation. Surprisingly, rats receiving combined therapy had significantly smaller volume of tissue lost, although this alone did not easily account for the improved recovery. While these results suggest that this therapy will benefit humans who suffer an ICH, further experimental studies examining the length, quality, and daily quantity of rehabilitation exercise and the insult severity are warranted given the findings that CIMT administered immediately after ischemic brain injury worsens outcome¹¹ (S. DeBow, BSc, et al, unpublished data, 2000).

While the absence of a detrimental effect in this study is likely due to the greater intervention delay,¹⁰ it might have also resulted from using a different type of brain lesion (hemorrhagic versus occlusive) and/or a less demanding rehabilitation regimen (eg, 8 h/d of limb restraint with bracelet and cotton jacket versus plaster of Paris cast). Thus, it is possible that immediate CIMT or the use of a 24-hour restraint method would aggravate an ICH. Our method of rehabilitation offers a definitive advantage over the casting method by allowing us to manipulate the duration of daily therapy. Another effective therapy is the combination of environmental enrichment and task-specific training, which has been shown to improve functional recovery when initiated 15 days after stroke in rats without affecting infarct

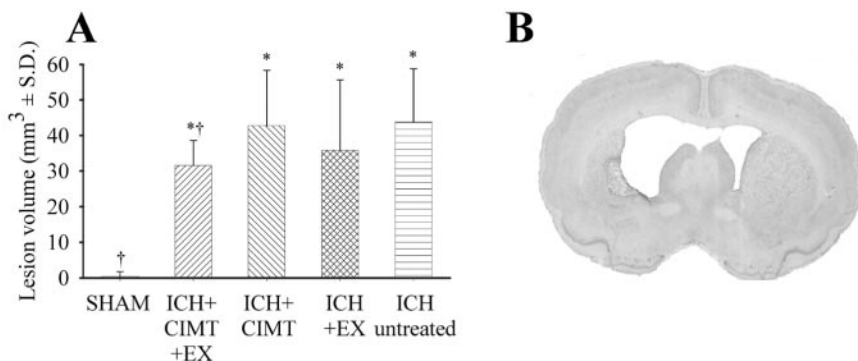


Figure 5. Volume of tissue lost at 60 days after ICH/SHAM surgery (A). The calculation includes the cavity and atrophy (eg, ventriculomegaly; photomicrograph represents a typical lesion [B]). Injury was less in the ICH+EX group and significantly less in the ICH+CIMT+EX group. * $P<0.05$ vs SHAM; † $P<0.05$ vs untreated ICH.

size.²¹ Enrichment combined with CIMT and EX therapies might prove maximally beneficial.

We did not anticipate that rehabilitation therapy would lessen the apparent volume of tissue lost. However, this is not surprising given that exercise diminishes cell death and functional deficits from multiple types of brain insults. For instance, treadmill running (1 km/d) for weeks before, or immediately after, neurotoxic (demoic acid, 3-acetylpyridine) and neurodegenerative insults (inherited Purkinje cell degeneration) is neuroprotective.²² Additionally, immediate forced use of the impaired forelimb ameliorates neurochemical injury and behavioral deficits in the 6-hydroxydopamine model.¹⁸ Currently, a cytoprotective effect was observed with rehabilitation delayed for 1 week after ICH. This is likely due to a reduction in atrophy and transneuronal degeneration because the rapid progression of the primary hemorrhagic injury in this model¹³ would make a direct neuroprotective effect unlikely. The reduction in injury may have occurred as a result of exercise-induced elevations in growth factors (eg, insulin-like growth factor²²) and exercise-induced angiogenesis.²³ Further studies are examining some of these possible mechanisms. While a reduction in lesion volume would be expected to result in fewer behavioral deficits, it is unlikely to account solely for the marked improvements in the ICH+CIMT+EX group (eg, poor correlation between volume and behavior within the ICH+CIMT+EX group). Functional recovery results from many factors including, but not limited to, overcoming learned non-use, cortical²⁴ and sub-cortical reorganization, synaptogenesis, and dendritic growth.^{21,25,26} The latter effects, if localized ipsilateral to the lesion, may contribute somewhat to the apparent reduction in the volume of lesion in the ICH+CIMT+EX group.

The superior performance of the ICH+CIMT+EX over the ICH+EX group in the staircase test is probably due to the fact that rats in the ICH+CIMT+EX group were forced to use their contralateral limb to obtain food in the tray task during rehabilitation, whereas the ICH+EX group likely used their ipsilateral limb even though it was not their originally preferred paw. We previously observed that rats with their preferred forelimbs impaired (via Botulinum A toxin injections) learned to use their nondominant limb until the toxin became inactive (J. McKenna, PhD, and F. Colbourne, PhD, unpublished data, 2000). Accordingly, the advantage of the CIMT therapy is that it forces the rats to use their contralateral limb during specific rehabilitation exercises and at other times (eg, grooming), although the contribution of each is unknown. The most impressive benefits were observed in the staircase and ladder tests probably because rats participated actively in the tray and ladder tests during rehabilitation. While the ICH+CIMT+EX group performed similar to the SHAM group with both limbs in the staircase test, only the contralateral limb results were significantly better than those in untreated ICH rats. This is likely due to a smaller ipsilateral deficit in untreated ICH rats because there was no residual deficit with the ipsilateral limb of the ICH+CIMT+EX rats. The less impressive recovery in the cylinder test may be due to rats becoming habituated and participating less. Additionally, the EBST and the cylinder test were less useful in detecting group differences because of substantial intragroup

variability. Nonetheless, all of the behavioral tests used significantly correlated with the volume of injury (with all animals included).

Of many putative neuroprotectants administered after striatal ICH in rats (eg, hypothermia,¹⁵ hematoma aspiration,²⁷ NXY-059,²⁸ anti-inflammatory therapies²⁹), none have provided benefit in the staircase test, in contrast to the substantial and persistent recovery presently observed with CIMT+EX therapy. Nonetheless, caution must be exercised in interpreting the observed recovery since rats may have used compensatory strategies.³⁰ For example, some groups had a lower percent reaching success rate in the staircase test. Further studies should examine whether CIMT+EX therapy promotes true recovery of function.

In summary, CIMT+EX therapy significantly and chronically improved motor deficits after ICH in rats. Clinical investigations are warranted in humans who suffer intrastriatal hemorrhagic stroke. Further experimental studies are also needed to elucidate the mechanisms underlying the facilitated recovery and reduction in the volume of tissue lost and the factors that promote maximal recovery (eg, duration of therapy).

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