J. Phys. Ther. Sci.
27: 131–133, 2015

Original Article

The effect of a skilled reaching task on hippocampal plasticity after intracerebral hemorrhage in adult rats

Song-Hee Cheon, PT, PhD[1)]

[1)] *Department of Physical Therapy, Youngsan University: San 150 Joonam-dong, Yangsan, Gyeongsangnam-do, Republic of Korea*

Abstract. [Purpose] The primary objective of this study was to assess the effects of a skilled reaching task on cognition, as indexed by the pattern of GAP-43 expression in the hippocampus, following intracerebral hemorrhage (ICH) in rats (when the hippocampus plays a critical role in spatial memory and learning). [Subjects and Methods] The model of ICH used in the present study involved intrastriatal injection of collagenase. Sixty male Sprague-Dawley rats (aged 12 weeks) were randomly assigned to either a control (n = 30; CON) or skilled reaching training group (n = 30; SRT). The SRT group were trained 5 days per week for 4 weeks following ICH. Animals were sacrificed 1, 2, or 4 weeks after ICH. Western blot analysis was used to evaluate GAP-43 expression. [Results] GAP-43 expression was increased in the SRT group, in accordance with greater elapsed time, but decreased in the CON group. At 1 week post injury, there were no significant differences between the CON and SRT groups. However, there were significant differences at both 2 and 4 weeks. [Conclusion] The present findings suggest that increased GAP-43 expression in the hippocampus following skilled reaching training may result in enhanced cognition and neural plasticity following ICH.

Key words: Stroke, Skilled reaching training, Hippocampus

(This article was submitted Jun. 16, 2014, and was accepted Jul. 24, 2014)

INTRODUCTION

Stroke is defined as a sudden disturbance in the blood supply to the brain caused by hemorrhage or ischemia that provokes an acute inflammatory response and secondary cascade of neurodegenerative processes leading to cell death in the weeks and months following the injury[1)]. The symptoms, which vary according to the site and extent of brain damage, can be severely disabling and may result in impaired motor and sensorimotor domains and cognitive task performance. Physical rehabilitation therapy remains the first-line intervention strategy for attenuating chronic impairments in sensory-motor function[2)].

During skilled reaching, an individual reaches for a food item that has been placed in the mouth for eating. This natural human behavior —the first complex behavior displayed by human infants— is engaged in daily by adults and requires no special training[3)]. Among the many motor impairments that follow neurological damage in humans, the loss of manual dexterity, i.e., skilled use of the hands, is one of the most debilitating. Impairments in skilled hand use are seen in many neurological conditions including Huntington's disease[4)] and stroke[5)].

The adult brain responds to physical exercise and environmental stimulation with increased neurogenesis in the dentate gyrus[6)]. These new cells mature locally into granule neurons, form dendritic arbors and axonal projections, and functionally integrate into the existing hippocampal network[7)]. Several studies have demonstrated an association between increased dentate neurogenesis and improved performance in hippocampal learning tasks[8)].

GAP-43, a growth- and plasticity-associated protein, is developmentally regulated, concentrated in growth cones, excluded from dendrites, and selectively transported to axons and their terminals. Protein kinase C (PKC) regulates GAP-43 function via phosphorylation at ser-41 in rats, mice, and humans and at ser-42 in chicks[9)]. Phosphorylation of the PKC site is highly correlated with memory formation and the synaptic plasticity of long-term potentiation (LTP)[10)].

Animal studies have furthered our understanding of stroke pathology and mechanisms of recovery[11)]. For example, enriched rehabilitation (ER), which is the coupling of environmental enrichment and daily reach training, improves forelimb use following middle cerebral artery occlusion (MCAo) in rats[12)]. Therefore, the principal objective of the present study was to assess the effects of a skilled reaching task on cognition, as indexed by the pattern of GAP-43 expression in the hippocampus, following intracerebral hemorrhage (ICH) in rats.

Corresponding author. Song-Hee Cheon (E-mail: 1000sh@ysu.ac.kr)

SUBJECTS AND METHODS

All procedures were performed according to the protocols established by the Animal Experiment Committee of Daegu University, based on the NIH Guidelines for the Care and Use of Laboratory Animals (NIH, 1996). Sixty male Sprague-Dawley rats (aged 12 weeks), weighing 250–270 g, were maintained on a 12-hour on/12-hour off light/dark cycle with *ad libitum* access to food and water.

Rats were anesthetized with 2 mL/kg of a 50% Zoletil/50% Xylazine hydrochloride mixture and placed in a stereotactic frame. A needle (Hamilton instrument syringe, 75 N, 5 ul; Hamilton Company USA, Reno, NV, USA) was implanted into the left striatum (3.5 mm lateral and 7 mm deep relative to the bregma), according to the atlas of Paxinos and Watson[13]. Five minutes after insertion of the needle, collagenase (type VII; Sigma-Aldrich Coporation, St. Louis, MO, USA; 0.12 U in 1 μL NaCl, 0.9%) was injected over the course of the following 5 min using a pump (Precidor®, Infors HT, Basel, Switzerland). The needle was left in place for another 5 min and then removed slowly. The wound was sutured. Body temperature was monitored throughout surgery using a rectal probe and maintained at 37.5 ± 0.5 °C using a homoeothermic blanket control unit (Harvard Apparatus, Edenbridge, UK). Animals were randomly assigned to either the control (n = 30; CON) or skilled reaching training (n = 30; SRT) group.

Following surgery, animals were returned to their home cage, which was maintained at 26–28 °C to allow recovery from the anesthesia.

The animals in the SRT group were trained on a single pellet-reaching task modified from that of Whishaw and Pellis[14]. Boxes were made of clear Plexiglas (45 × 14 × 35 cm). A 1-cm-wide vertical slit was placed in the center of the front wall. On the exterior of the wall in front of the slit, a 2-cm-wide shelf was mounted 3 cm above the floor. Two indentations on the surface of the shelf, located 1 cm from the inside of the wall and aligned with the edges of the slit (1 cm apart), ensured that the rats could reach the pellet with either paw. A pellet was placed in the indentation contralateral to the limb with which the rat preferred to use for reaching. The lateral placement of the food pellet prevented the rats from using their tongue to obtain the pellet and also prevented use of the non-preferred paw to grasp the food[14]. A metal bar was positioned in front of the slit and approximately 1 cm from the horizontal shelf, thereby preventing the animal from dragging the food after reaching and preventing performance of a full and correct grasping movement.

The first 5 days comprised a shaping phase during which a number of food pellets were placed on the shelf to attract the animals. At the end of this period, rats could reliably retrieve the food from the shelf using the preferred limb in each trial. Subsequently, a single pellet was placed in the food indentation contralateral to the rat's preferred paw, and the training phase, consisting of 25 trials, was repeated three times during each daily session on the 7 days prior to surgery[15].

Animals were sacrificed 1, 2, and 4 weeks after ICH via anesthesia using 2 mL/kg of a 50% Zoletil/50% Xylazine hydrochloride mixture. Brains were removed for Western blot analysis.

The results were expressed as means ± standard errors (SE). All experiments were analyzed via analysis of variance. In some cases, comparisons between treatment and control means were performed via the Bonferroni-Dunn test. Differences were regarded as statistically significant at $p < 0.05$.

Table 1. GAP-43 expression in the hippocampus

Group	Relative optical density (percentage of control)		
	1 week	2 weeks	4 weeks
CON	100±14.0	58.8±8.5	49.7±7.6*
SRT	100±25.8	156.9±23.1#	197.7±23.5*,#

The amounts of GAP-43 were detected via Western blotting with anti-GAP-43 as described in the Materials and Methods section. Each example shown is representative of three experiments. The values represent the means±SE of three independent experiments conducted in triplicate dishes. *p<0.05 vs. 1 week groups. #p<0.05 vs. 2 weeks and 4 weeks groups, respectively, in CON.

RESULTS

A pattern of GAP-43 expression in the hippocampus was observed for each group following extraction of the brains. Optical density values determined at 1, 2, and 4 weeks post intracranial hemorrhage are presented in Table 1. GAP-43 expression was shown to increase in the SRT group in accordance with greater time elapsed; however, it decreased in the CON group ($p < 0.05$). Statistical significance was only reached after 4 weeks; additionally, a significant difference was observed between the 1- and 4-week groups (Table 1). At 1 week post injury, there was no significant difference between the CON and SRT groups ($p > 0.05$), but at 2 and 4 weeks, significant differences were observed ($p > 0.05$) (Table 1).

DISCUSSION

This study demonstrated that specific motor training via SRT and following experimental ICH promotes a histological change in the hippocampus associated with the time course of GAP-43 expression.

The model of ICH used in the present study consisted of an intrastriatal injection of collagenase, a proteolytic enzyme that interrupts the basal lamina of cerebral blood vessels, thus causing intraparenchymal bleeding[16]. In addition to its quick and easy execution, another feature of this model is that hemorrhage is spontaneous in nature, in contrast to the alternative model of ICH, which involves autologous blood injection. Furthermore, a recent study comparing collagenase to blood infusion models of ICH in rats demonstrated that neurological deficits resolve more rapidly and completely in the blood. Despite similar initial hematoma volumes, rats infused with collagenase did indeed exhibit greater neurological impairment at every time

point evaluated following striatal injection and remained significantly impaired at 28 days (the endpoint of the study). Consequently, the collagenase model should be preferred in studies assessing long-term functional outcomes[17].

Several recent studies in mice and rats clearly demonstrated that dentate neurogenesis is strongly stimulated by voluntary exercise in a running wheel[18, 19] and that the new neurons are functionally integrated into the hippocampal circuitry[7]. Another study showed that this training increased the physical fitness of the animals, stimulated the release of local hippocampal and circulating neurogenic growth factors, and enhanced angiogenesis in the hippocampal vasculature[8].

Wurm et al.[20] observed that daily training of a single forelimb greatly improved dentate neurogenesis in the healthy brain. Reaching training significantly increased the survival rate of newly developed neurons, whereas environmental enrichment did not influence dentate neurogenesis. In addition, following cortical infarcts in the forelimb sensorimotor cortex, both rehabilitative training of the impaired forelimb and environmental stimulation increased dentate neurogenesis.

The present study indicated that GAP-43 expression in the hippocampus was significantly reduced by ICH at 4 weeks in the CON group. In contrast, the SRT group exhibited a robust increase in GAP-43 expression, but the difference in expression between the two groups was statistically significant only at 2 and 4 weeks. Mirescu et al.[21] reported that animals with cortical infarcts in the forelimb sensorimotor cortex exhibited reduced reaching success during the first 5–10 days following formation of the lesion, which might serve to stress the animals and thereby impair the neurogenic response. Moreover, Wurm et al.[20] observed that skilled forelimb training effectively stimulated dentate neurogenesis and spatial learning in both infarcted and healthy brains; however, this training-induced increase in neurogenesis was reduced following cortical infarcts. No significant difference was noted between the CON and SRT groups at 1 week in this study.

Auriat et al.[22] reported that rehabilitation comprising an enriched environment and skilled reach training improved recovery after ICH in rats via a plasticity response (e.g., increased dendritic growth) that did not involve neurogenesis. The growth-associated protein GAP-43 is developmentally regulated, concentrated in growth cones, and highly correlated with memory formation and the synaptic plasticity of LTP[9, 10].

The present study indicates that short daily sessions of skilled reaching training significantly increase GAP-43 expression in the hippocampus at 2 and 4 weeks post ICH. The present findings suggest that increases in GAP-43 expression in the hippocampus following skilled reaching training might result in enhanced cognition and neural plasticity post ICH. Although further study of therapeutic interventions is necessary to confirm their neurological mechanisms, the results of this study may nevertheless help to establish novel rehabilitative strategies.

ACKNOWLEDGEMENT

This research was supported by a Youngsan University Research Grant in 2014.

REFERENCES

1) Koo H, Kim M, Lee S, et al.: Skilled reach training induces synaptogenesis on the hippocampus after left hemorrhagic stroke in rats. J Phys Ther Sci, 2012, 24: 127–132. [CrossRef]
2) Arya KN, Pandian S, Verma R, et al.: Movement therapy induced neural reorganization and motor recovery in stroke: a review. J Bodyw Mov Ther, 2011, 15: 528–537. [Medline] [CrossRef]
3) Sacrey LA, Whishaw IQ: Development of collection precedes targeted reaching: resting shapes of the hands and digits in 1-6-month-old human infants. Behav Brain Res, 2010, 214: 125–129. [Medline] [CrossRef]
4) Klein A, Sacrey LA, Dunnett SB, et al.: Proximal movements compensate for distal forelimb movement impairments in a reach-to-eat task in Huntington's disease: new insights into motor impairments in a real-world skill. Neurobiol Dis, 2011, 41: 560–569. [Medline] [CrossRef]
5) Hiraoka K: Rehabilitation effort to improve upper extremity function in post-stroke patients: a meta-analysis. J Phys Ther Sci, 2001, 13: 5–9. [CrossRef]
6) Kempermann G, Kuhn HG, Gage FH: Experience-induced neurogenesis in the senescent dentate gyrus. J Neurosci, 1998, 18: 3206–3212. [Medline]
7) van Praag H, Schinder AF, Christie BR, et al.: Functional neurogenesis in the adult hippocampus. Nature, 2002, 415: 1030–1034. [Medline] [CrossRef]
8) Olson AK, Eadie BD, Ernst C, et al.: Environmental enrichment and voluntary exercise massively increase neurogenesis in the adult hippocampus via dissociable pathways. Hippocampus, 2006, 16: 250–260. [Medline] [CrossRef]
9) Cheon SH, Koo HM: Spontaneous and forced exercise promotes cognitive function and expression of GAP-43 and NT-3 in the hippocampus of aged rats. J Phys Ther Sci, 2013, 25: 321–324. [CrossRef]
10) Sossin WS: Isoform specificity of protein kinase Cs in synaptic plasticity. Learn Mem, 2007, 14: 236–246. [Medline] [CrossRef]
11) Cramer SC: Repairing the human brain after stroke: I. Mechanisms of spontaneous recovery. Ann Neurol, 2008, 63: 272–287. [Medline] [CrossRef]
12) Biernaskie J, Corbett D: Enriched rehabilitative training promotes improved forelimb motor function and enhanced dendritic growth after focal ischemic injury. J Neurosci, 2001, 21: 5272–5280. [Medline]
13) Paxinos G, Watson C: The rat brain in stereotaxic coordinates. Sydney: Academic Press; 1986.
14) Whishaw IQ, Pellis SM: The structure of skilled forelimb reaching in the rat: a proximally driven movement with a single distal rotatory component. Behav Brain Res, 1990, 41: 49–59. [Medline] [CrossRef]
15) Gharbawie OA, Whishaw IQ: Parallel stages of learning and recovery of skilled reaching after motor cortex stroke: "oppositions" organize normal and compensatory movements. Behav Brain Res, 2006, 175: 249–262. [Medline] [CrossRef]
16) Strbian D, Durukan A, Tatlisumak T: Rodent models of hemorrhagic stroke. Curr Pharm Des, 2008, 14: 352–358. [Medline] [CrossRef]
17) MacLellan CL, Silasi G, Poon CC, et al.: Intracerebral hemorrhage models in rat: comparing collagenase to blood infusion. J Cereb Blood Flow Metab, 2008, 28: 516–525. [Medline] [CrossRef]
18) Farmer J, Zhao X, van Praag H, et al.: Effects of voluntary exercise on synaptic plasticity and gene expression in the dentate gyrus of adult male Sprague-Dawley rats in vivo. Neuroscience, 2004, 124: 71–79. [Medline] [CrossRef]
19) Eadie BD, Redila VA, Christie BR: Voluntary exercise alters the cytoarchitecture of the adult dentate gyrus by increasing cellular proliferation, dendritic complexity, and spine density. J Comp Neurol, 2005, 486: 39–47. [Medline] [CrossRef]
20) Wurm F, Keiner S, Kunze A, et al.: Effects of skilled forelimb training on hippocampal neurogenesis and spatial learning after focal cortical infarcts in the adult rat brain. Stroke, 2007, 38: 2833–2840. [Medline] [CrossRef]
21) Mirescu C, Gould E: Stress and adult neurogenesis. Hippocampus, 2006, 16: 233–238. [Medline] [CrossRef]
22) Auriat AM, Wowk S, Colbourne F: Rehabilitation after intracerebral hemorrhage in rats improves recovery with enhanced dendritic complexity but no effect on cell proliferation. Behav Brain Res, 2010, 214: 42–47. [Medline] [CrossRef]