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Research report

Astrocytic changes in the hippocampus and functional recovery after cerebral ischemia are facilitated by rehabilitation training

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Abstract

In this study we examined whether astrocytic and basic fibroblast growth factor changes after cerebral ischemia can be influenced by rehabilitation training and if these changes are associated with functional improvement. After receiving either ischemia or sham surgery, male adult Wistar rats were assigned to one of two rehabilitation training group: complex environment housing (EC) or paired housing as controls (CON). Rats were tested in the water maze after 14 days of rehabilitation training. Results showed increased expression of reactive astrocytes (GFAP) in all ischemic animals and in the sham EC rats with a significant overall increased seen in the ischemia EC housed animals. The pattern of basic fibroblast growth factor (FGF-2) expression seen was somewhat similar to that of GFAP. Behavioral data showed that even though all animals learned to perform the water maze task over time, the ischemia CON rats took longer to learn the task while all the ischemia EC animals performed as well as the sham groups. Regression analysis showed that increased GFAP was able to explain some of the variances in the behavioral parameters in the water maze of the ischemia EC rats suggesting that the activation of astrocytes in this group probably mediated enhanced functional recovery. Lastly, it is possible that the favorable effect of astrocyte activation after cerebral ischemia was mediated by FGF-2.

Keywords: Enriched environment; Reactive gliosis; FGF-2; Immunohistochemistry; Fluoro–Jade; Water maze

1. Introduction

Previous studies have shown that neuronal growth and restructuring after central nervous system insult (CNS) was influenced by behavioral training post-injury (reviewed in [24]). For example, lesions in the motor cortex resulted in numerous structural changes in the contralateral and homotopic areas, including increased cortical volume, ratio of synapses per neuron, and dendritic branchings [9,20,21] (also reviewed in [26]). Behavioral training following ischemic injury also resulted in similar changes in the hippocampus; that is, increased dendritic branchings [6], synapse number per neuron, and the formation of synapses with multiple synaptic boutons were seen [5]. The neuronal growth, synaptogenesis, and changes in synaptic morphology after CNS insult reported in these studies have been found to be dependent, in part, on the rehabilitation strategies used such as motor skills training and complex environment housing. However, the neurobiologic mechanism(s) whereby this rehabilitation-induced recovery following CNS insult may be facilitated is not clear.

A possible mechanism that might contribute to post-ischemic neuronal restructuring is astrocytic activation. Reactive astrocytic changes have been studied extensively following CNS injury. Strong evidence exists that injury to the CNS resulted in an increase in the expression of glial fibrillary acidic protein (GFAP), a major constituent of astrocytic filaments and a marker for reactive astrocytes (reviewed in [40]). Others have reported that the reactive astrocytic responses seen after CNS injury formed glial scar tissue and sometimes hindered axonal growth [2,19,40]. On the other hand, reactive astrocytes can play a role in neural plasticity in intact animals evidenced by increased GFAP expression following behavioral and environmental manipulations such as complex environment housing [23,44], olfactory conditioning [30], sensory deprivation [18], and spatial learning

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and motor activity [16]. Ultrastructurally, astrocytes have also been shown to increase their contact with synaptic elements in the visual cortex of rats housed in the complex environment [22]. Furthermore, induction of long-term potentiation also resulted in increased astrocyte-synapse apposition in the dentate gyrus of intact rats [48]. These findings seen in both intact and injured animals raise the issue that although increased astrocyte activation after CNS injury may lead to glial scar formation, it is also possible that the production of neurotrophic factors by astrocytes may enhance the plasticity of surviving neurons. Thus, in this study we examined the effects of complex environment exposure on reactive astrocytic responses and basic fibroblast growth factor (FGF-2) expression after transient global cerebral ischemia. FGF-2 was chosen for this study because it is widely distributed in both neuronal and nonneuronal tissues and that its expression in models of cerebral ischemia was associated with increased GFAP immunoreactivity [36,46,49]. We also examined correlative evidence between GFAP immunoreactivity and functional recovery measured by performance in the water maze task.

2. Materials and methods

2.1. Cerebral ischemia

The four-vessel occlusion method was used to induce transient global cerebral ischemia as described previously [5]. Adult male Wistar rats 3–4 months of age (body weight of 350–375 g at the time of surgery) were used in the study. Briefly, rats were anesthetized with isofluorane/oxygen (2.5% isofluorane and 30% oxygen) mixture on the first day and an incision was made to isolate both common carotid arteries. Immediately following isolation of both carotid arteries, the vertebral arteries were electrocauterized in the alar foramina at the level of the first cervical vertebrae. Body temperature was kept at 37–37.5 °C using a heating pad during the surgical procedure and until the animals were fully recovered. The next day, both common carotid arteries were occluded for 12 min while the animals were awake. This period of carotid occlusion was used because we have previously shown that it results in damage confined to the hippocampal area [5,7]. The criteria used to determine transient global cerebral ischemia were the bilateral loss of righting reflex within 2 min of occlusion. Animals that did not loose their righting reflex within 2 min of occlusion ($n = 3$) or if the righting reflex did not return within 30 min of removal of the silastic ligatures ($n = 1$), and those that developed excessive weight loss postoperatively (>20% of preoperative body weight, $n = 3$) were excluded from the study. In addition, pain level was assessed by observing for sluggishness, extreme aversion to being touched, and weight loss. Animals were not given any post-operative analgesia but were euthanized immediately when persistent pain was observed ($n = 2$). In addition, animals that exhibited unilateral lesion ($n = 4$) were excluded from the study. A total of 44 animals were included in the study. Sham-operated animals were subjected to the same anesthesia and surgery that consisted of a neck incision without carotid manipulation and an incision behind the occipital bone without cauterization of the vertebral arteries. All efforts were made to minimize animal distress and to reduce the number of animals used. Experimental protocols in this study were approved by the Institutional Animal Care and Use Committee and in accordance with the National Institutes of Health guidelines.

2.2. Animal housing

Three days after transient global cerebral ischemia, rats were randomly assigned for 14 days to either one of two behavioral training conditions: complex environment (EC) housing or paired housing (controls). All animals were housed in the same room under a 12 h light:12 h dark cycle and had free access to food and water. Room temperature was maintained at 22 ± 2 °C and noise level was kept to a minimum. Animals in the complex environment group ($n = 11$ ischemia and $n = 11$ shams) were housed together in a sensory-rich living condition (wire cage measuring 2 m × 1 m × 1.65 m) consisting of a variety of objects such as toys, wooden blocks, running wheels, Plexiglas tunnels, ladders, plastic castles, swing, etc. In addition, these rats were placed each day in an open field (1.2 m × 1.2 m × 1.2 m) with a novel arrangement of toys and objects and allowed to explore for 30 min while the objects in their home cage were being changed. Objects in both EC housing and open field were changed daily to maintain novelty.

Animals assigned in the control group (CON) were housed in pairs in standard laboratory cages (16.5 cm × 22.5 cm × 13.5 cm). Although rats in this group ($n = 11$ ischemia and $n = 11$ shams) were able to observe ongoing activity of the room, they did not receive any stimulation and contact was limited to daily handling and routine cage changing.

2.3. Behavioral testing

Spatial learning and memory (acquisition and recall) were examined using the water maze task. This behavioral test was started after 14 days of rehabilitation training post-ischemia. The water maze apparatus consisted of a circular tub made of galvanized steel measuring 1.52 m in diameter; and the interior surface was painted white. The use of a large tub decreased the probability that the rats will find the goal/platform by chance. During testing, the tub was filled with tepid water (22 ± 2 °C) and made opaque by the addition of powdered milk. An inverted white flower pot, submerged 2 cm beneath the water's surface served as the goal/platform and the opaqueness of the water enabled the goal/platform to be concealed. Extramaze cues, such as overhead lighting, windows and room noise were held constant during testing. The pool was divided into four quadrants of equal surface area and the starting locations for testing were assigned north, south, east, and west. The goal/platform was located in the middle of the southeast quadrant approximately 22 cm from the pool rim. The day before actual testing started, rats were allowed a habituation swim for 10 s without the platform. The habituation swim and consistent water temperature throughout the test days were necessary to minimize animal stress during water maze testing. Animals received four trials a day for four consecutive days. A different starting point was used on each of the four daily trials and the order of starting points was random. If the rat failed to find the hidden platform within 3 min, they were guided to the platform and given a swim latency score of 180 s. The animals were allowed to stay on the platform for 30 s then towel-dried until the next trial. A minimum of two minutes was used between trials to provide a rest period for the animals and avoid "practice effect." During the trials, swim latency (time to reach the platform) and the path taken by the animals to reach the platform were recorded by a video camera connected to an image analyzer (Water Maze System Version 4.20, Columbus, OH) and these data were used to assess performance in the water maze task. In addition, swimming speed (path length/swim latency) was used to assess the motoric activity of the rats in performing the task. Black shoe polish was applied on top of the animals' head to facilitate video camera tracking as rats swim in the water maze. On the fifth day, a probe trial was performed wherein the animals were tested but the goal/platform was removed from the pool. All testing were done approximately 2 h prior to the onset of the dark cycle to ensure that it is close to the rats' active period.

2.4. Tissue preparation

The day after water maze testing, all rats were anesthetized with pentobarbital (100 mg/kg) and perfused according to the guidelines of the Panel on Euthanasia of the Veterinary Medicine Association. Perfusion entailed transcardial infusion with heparinized phosphate buffered saline (pH 7.3), followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3). The brains were then removed and placed in fixative for 24 h at 4 °C. Following postfixation, the brains were cryoprotected with 30% sucrose in 0.1 M phosphate buffered-saline (PBS) and stored at −20 °C until sectioned. Using a cryostat, coronal sections at 30 μm thickness were obtained throughout the entire dorsal hippocampal formation (bregma −2.30 to −4.52 mm [34]) since the ventral region is less susceptible to ischemic damage [5,7,27].