The Role of Endogenous Neurogenesis in Functional Recovery and Motor Map Reorganization Induced by Rehabilitative Therapy after Stroke in Rats

Takashi Shiromoto, MD,*† Naohiko Okabe, PhD,* Feng Lu, PhD,* Emi Maruyama-Nakamura, PhD,* Naoyuki Himi, PhD,* Kazuhiko Narita, PhD,* Yoshiki Yagita, MD,† Kazumi Kimura, MD,‡ and Osamu Miyamoto, MD*

Background and Objective: Endogenous neurogenesis is associated with functional recovery after stroke, but the roles it plays in such recovery processes are unknown. This study aims to clarify the roles of endogenous neurogenesis in functional recovery and motor map reorganization induced by rehabilitative therapy after stroke by using a rat model of cerebral ischemia (CI). *Methods:* Ischemia was induced via photothrombosis in the caudal forelimb area of the rat cortex. First, we examined the effect of rehabilitative therapy on functional recovery and motor map reorganization, using the skilled forelimb reaching test and intracortical microstimulation. Next, using the same approaches, we examined how motor map reorganization changed when endogenous neurogenesis after stroke was inhibited by cytosine-β-D-arabinofuranoside (Ara-C). *Results:* Rehabilitative therapy for 4 weeks after the induction of stroke significantly improved functional recovery and expanded the rostral forelimb area (RFA). Intraventricular Ara-C administration for 4-10 days after stroke significantly suppressed endogenous neurogenesis compared to vehicle, but did not appear to influence non-neural cells (e.g., microglia, astrocytes, and vascular endothelial cells). Suppressing endogenous neurogenesis via Ara-C administration significantly inhibited (~50% less than vehicle) functional recovery and RFA expansion (~33% of vehicle) induced by rehabilitative therapy after CI. *Conclusions:* After CI, inhibition of endogenous neurogenesis suppressed both the functional and anatomical markers of rehabilitative therapy. These results suggest that endogenous neurogenesis contributes to functional recovery after CI related to rehabilitative therapy, possibly through its promotion of motor map reorganization, although other additional roles cannot be ruled out.
Key Words: Cerebral ischemia—endogenous neurogenesis—motor map reorganization—motor recovery—rehabilitative therapy—cytosine-β-D-arabinofuranoside (Ara-C).

From the *Second Department of Physiology, Kawasaki Medical School, Kurashiki City, Okayama, Japan; †Department of Stroke Medicine, Kawasaki Medical School, Kurashiki City, Okayama, Japan; and ‡Department of Neurological Science, Graduate School of Medicine, Nippon Medical School, Tokyo, Japan.

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Address correspondence to Naohiko Okabe, PhD, Second Department of Physiology, Kawasaki Medical School, 577 Matsushima, Kurashiki City, Okayama 701-0192, Japan. E-mail: n-okabe@med.kawasaki-m.ac.jp.

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Introduction

Stroke is a common cause of severe disability for which functional recovery is frequently limited.[1,2] Currently, intravenous tissue plasminogen activator[3,4] and endovascular treatment[5,6] are the only treatments that improve the clinical outcome of patients with acute ischemic stroke, but their effectiveness is limited by their narrow therapeutic time window.[7] Over the long term, 25%-74% of patients have to rely on human assistance for basic activities of daily living such as feeding, self-care, and mobility.[8] To improve the long-term prognosis in stroke, it is important to clarify the mechanisms of functional recovery that have not yet been identified or completely understood.

For the many stroke survivors unable to benefit from drug therapy, rehabilitative therapy can still promote the recovery of motor deficits.[9] The mechanisms underlying this recovery are unclear but appear to be related to cortical motor map reorganization.[10,11] In humans, noninvasive imaging data indicate that changes in cortical motor map topography are relevant to functional recovery following rehabilitative therapy.[12] In several other reports, endogenous neurogenesis in the subventricular zone (SVZ) contributes to the functional recovery after stroke. SVZ-derived, newly born neural stem cells migrate to areas of cerebral ischemia (CI), where they may contribute to postischemic repair[13-15] or the enhancement of endogenous neurogenesis by rehabilitative therapy.[16,17] Notably, inhibiting endogenous neurogenesis was found to worsen the functional outcome in mice after CI.[18,19] Given these data, we hypothesized that the inhibition of endogenous neurogenesis would decrease cerebral plasticity, as represented by motor map reorganization, and lead to the suppression of functional recovery in cases of ischemic stroke.

The aim of the present experiment was to investigate this hypothesis by analyzing the role of endogenous neurogenesis in functional recovery and motor map reorganization induced by rehabilitative therapy after CI. We analyzed the effects of rehabilitative therapy in rats after CI localized to the caudal forelimb area (CFA) of the cortex, assessing functional recovery and motor map reorganization with and without pharmacological inhibition of endogenous neurogenesis.

Methods

Animals

A total of 121 adult, male, Fisher 344 rats (7-8 week old, 160-180 g; CLEA Japan Inc., Tokyo, Japan) were used for all experiments. The rats were housed within a temperature-controlled vivarium on a 12-hour : 12-hour light : dark cycle. Food intake was moderately restricted throughout the study to maintain body weight at 80% of the ad libitum weight, but water was freely available. All experimental procedures were in accordance with National Institutes of Health regulations and were approved by the Animal Research Committee of Kawasaki Medical School.

Induction of Photothrombotic Ischemia

CI was induced via photothrombosis.[20] The rats were anesthetized with an intraperitoneal injection of ketamine hydrochloride (48 mg/kg)/xylazine hydrochloride (4.8 mg/kg). A bone window was created above the dominant CFA (3.5 mm lateral from the bregma, 3-mm diameter). A green (532 nm) light source attached to a 10× objective, providing a 2-mm-diameter illumination spot, was stereotactically centered on the bone window. The brain was illuminated for 15 minutes. During the first minutes of illumination, rose bengal (60 mg/kg) was injected via a tail vein catheter to stimulate thrombosis. Sham-operated rats underwent the same experimental procedures as described above but without the infusion of rose bengal.

Skilled Forelimb Reaching Task

The rats received a food tray task to habituate them to using the forelimb for 1 week, and 4 weeks of pretraining in the standard skilled forelimb reaching task.[21] Rats were placed within a Plexiglas reaching box (25 cm high × 10 cm wide × 25 cm long), with a tall narrow window (25 cm high × 1 cm wide) located in the middle of the 10-cm-wide wall. A horizontal plastic shelf (10 cm wide × 3 cm long) was mounted 3 cm from the floor on the front of the box. The rats reached through the window for a distance of 1.5 cm to retrieve a single food pellet held in shallow indentations on the shelf. A "trial" was defined when the pellet was placed on the shelf, after which the rat either consumed or missed reaching it. If a rat consumed a pellet without missing, knocking, or dropping it on the initial limb advance, the movement was scored as a "hit." A successful reaching score was calculated as follows: motor performance = (number of hits/25 trials) × 100. One session consisted of a maximum of 25 trials, or 5 min/day. Pretraining consisted of 2 sessions per day, 5 days/week. Preinfarct motor performance and the successful reaching score were recorded on the fourth day before CI. Rats were excluded if the number of trials was fewer than 4 trials at the third week after the start of pretraining, or if preinfarct motor performance was below 20%. The preinfarct motor performance score of most rats improved approximately 40%-60% with training.

Rehabilitative therapy consisted of 2 sessions, 5 days/week, for 4 weeks. Postinfarct motor performance was tested on the fourth day after CI, and weekly thereafter, to monitor functional deficit and recovery. Rats were excluded if the motor performance rate, as a percentage of preinfarct motor performance, was above 25% by the fourth day after CI. Motor performance rate was calculated as follows: {(Preinfarct motor performance – Postinfarct motor performance) ÷ (Postinfarct motor performance)} × 100. To ensure all reaching movements were