Recovery and Brain Reorganization after Stroke in Adult and Aged Rats

Tiffanie M. Markus, PhD,1,2 Shih-Yen Tsai, MD, PhD,2 Melanie R. Bollnaw, BS,2 Robert G. Farrer, PhD,2 Timothy E. O’Brien, PhD,3 Diana R. Kindler-Baumann, PhD,4 Martin Rausch, PhD,4 Markus Rudin, PhD,5 Christoph Wiessner, PhD,4 Anis K. Mir, PhD,4 Martin E. Schwab, PhD,5,6 and Gwendolyn L. Kartje, MD, PhD1,2,7,8

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Cerebrovascular disease, or stroke, is one of the leading causes of death and is the most common cause of adult disability with increasing prevalence in the aged population.1 Neuronal loss due to stroke may result in permanent deficits in sensory, language, and motor capabilities, leading to profound economic and emotional costs. Despite tremendous research efforts and success with experimental animal models for stroke recovery, no specific treatment has emerged for improving functional recovery in patients beyond the acute stage of stroke. In many clinical settings, it often is not possible to treat stroke patients soon after the event due to delays in seeking medical care, delays in diagnosis, and relevant serious comorbidities that preclude treatment. Another problem that may affect the outcome of animal studies and their application to human outcomes after stroke is the common use of young adult animals in experimental studies, rather than the more appropriate use of aged animals.

Studies have shown that strategies to improve neuronal plasticity, that is, the reorganization of neuronal circuits from undamaged central nervous system (CNS) areas, could lead to enhanced rehabilitative potential.2,3 We have shown that a unique way to improve functional recovery and neuronal plasticity after focal ischemic stroke in the young adult rat is through neutralization of the neurite inhibitor protein Nogo-A. Here, we show, in a clinically relevant model, improved functional recovery and brain reorganization in the aged and adult rat when delayed anti–Nogo-A therapy is given after ischemic injury. These results support the efficacy of Nogo-A neutralization as treatment for ischemic stroke, even in the aged animal and after a 1-week delay, and implicate neuronal plasticity from unlesioned areas of the central nervous system as a mechanism for recovery.

Materials and Methods

This study has been approved by the Joint Institutional Animal Care and Use Committee of Loyola University and Hines Veterans Affairs Hospital and the veterinary authorities of Kanton Basel-Stadt, Switzerland.

Aged rats (25 months of age at the time of stroke) were first tested on the skilled forelimb reaching test, a sensorimotor task requiring fine digital manipulation and the integrity of sensory and motor neuronal pathways8 (Fig 1A). Animals then underwent permanent middle cerebral artery occlusion resulting in a focal ischemic stroke that impaired the trained limb (see Fig 1B). One week after stroke, animals received intracerebroventricular administration of purified anti–Nogo-A Ab 7B12 (monoclonal mouse IgG), a purified control mouse IgG Ab, or no treatment. Using Alzet miniosmotic pumps, (Durect Corporation, Cupertino, CA) as described previously9 (model 2ML2; 5μl/hour), we infused 5mg of the Ab (2.5mg/ml) at a rate of 15μg/hour for 2 weeks, and then removed the pumps. Reaching performance was analyzed using a repeated-measures analysis of variance.

To examine whether reorganization of neural circuits occurred as a result of anti–Nogo-A therapy, we evaluated adult rats (age, 3–4 months) using functional magnetic resonance imaging (fMRI) 8 weeks after stroke and delayed anti–Nogo-A therapy. Experimental groups included stroke/anti–Nogo-A Ab (7B12), stroke/control Ab, and normal rats. Each animal was anesthetized and artificially ventilated while fMRI experiments were performed using a PharmaScan 70/16 system (PharmaScan, Bruker, Germany) operating at 7 Tesla. For functional imaging, a two-dimensional rapid acquisition with relaxation (RARE) sequence with the following parameters was used: matrix (MTX) = 128 × 128; 3 slices; field of view = 40 × 40mm; slice thickness = 1mm; slice spacing = 1mm; repetition time (TR) = 2,350 milliseconds; Echo time (TE) = 76.8 milliseconds; RARE fac-

Stroke is a prevalent and devastating disorder, and no treatment is currently available to restore lost neuronal function after stroke. One unique therapy that improves recovery after stroke is neutralization of the neurite inhibitory protein Nogo-A. Here, we show, in a clinically relevant model, improved functional recovery and brain reorganization in the aged and adult rat when delayed anti–Nogo-A therapy is given after ischemic injury. These results support the efficacy of Nogo-A neutralization as treatment for ischemic stroke, even in the aged animal and after a 1-week delay, and implicate neuronal plasticity from unlesioned areas of the central nervous system as a mechanism for recovery.

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tor = 32; sweep width (SW) = 50kHz; number of excitation (NEX) = 4. Acquisition time was 20 seconds per multislice volume, and a horizontal image orientation was used. Before statistical analysis, data from all animals were coregistered, and volumes were resliced using three-dimensional linear interpolation. Sensory activation, during fMRI acquisition, was induced by electrical stimulation of both forepaws independently (constant current pulse-train: I = 10mA, 2Hz; pulse duration = 1 millisecond). Statistical analysis parametric maps were calculated using the general linear model for the two conditions (stimulation of the right/left forepaw) and for the two groups (stroke/control Ab and stroke/anti–Nogo-A Ab).

Results
Reaching performance demonstrated marked deficits in successfully obtaining pellets with the stroke-impaired limb up to 1 week after stroke, with no significant difference between groups, indicating that before treatment all animals showed similar functional impairments in skilled reaching. Importantly, aged animals with stroke and treatment with anti–Nogo-A Ab showed dramatic improvement so that by 9 weeks after stroke, they were significantly different from the stroke/control Ab group (p < 0.05) and the stroke only group (p < 0.001, respectively). Animals treated with the anti–Nogo-A Ab (n = 5) continued to improve from 2 weeks after stroke, so that by 9 weeks they were significantly different from the stroke/control Ab group (n = 6) and the stroke only group (n = 6) (p < 0.05 and p < 0.001, respectively). Animals treated with the anti–Nogo-A Ab had a significantly increased rate of recovery (p < 0.001) compared with the control Ab-treated group and animals with no treatment, with no significant difference between control groups. Error bars denote ± standard error of the mean. *p < 0.05; **p < 0.01; ***p < 0.001.
cantly increased rate of recovery compared with the stroke/control Ab-treated group and stroke only animals, again with no significant difference between the two control groups (see Fig 1D).

fMRI indicated that in normal animals, stimulation of either forepaw resulted in activation of the contralateral somatosensory cortex only, as reported previously\(^\text{11}\) (Fig 2A). After stroke and control Ab, stimulation of the nonimpaired forepaw resulted in primarily contralateral activation of the somatosensory cortex as expected (see Fig 2B). Furthermore, stimulation of the nonimpaired paw led to bilateral thalamic activation (see Fig 2C). In this same group of animals, stimulation of the impaired forepaw resulted in activation seen in the ipsilateral somatosensory cortex (see Fig 2D). This shift in activation has been reported in other fMRI studies on rats with unilateral ischemic infarction.\(^\text{12,13}\) In addition, stimulation of the impaired forepaw resulted in bilateral thalamic activation (see Fig 2E).

In animals receiving stroke and anti–Nogo-A Ab treatment, stimulation of the nonimpaired forepaw led to activation in the cortical areas contralateral to the stimulation, as expected (see Fig 2F). Bilateral thalamic activation was present (see Fig 2G) in the same pattern as seen in the stroke/control Ab animals. After stimulation of the impaired forepaw, activation was again seen in the ipsilateral somatosensory cortex (see Fig 2H). This activation pattern was similar to that seen in the stroke/control Ab group. However, a statistically significant increase (\(p < 0.05\)) in activation was found in the thalamus during stimulation of the impaired forepaw in animals that had recovered with anti–Nogo-A therapy compared with stroke/control Ab animals (see Fig 2I).

**Discussion**

Our behavioral data demonstrate that neutralization of Nogo-A 1 week after ischemic infarction in the aged animal results in excellent recovery on a skilled sensorimotor task. Although the overall time course for recovery was more prolonged than seen in our previous studies with young adult rats, our results indicated the continued responsiveness of the aged CNS to cellular mechanisms of neural repair. Our fMRI results implicate subcortical regions, that is, the thalamus, as important areas for neuronal reorganization and functional recovery after anti–Nogo-A treatment. Basal ganglia and thalamic relays contribute significantly to motor planning, sensory perception, and sensorimotor integration. The loss of thalamocortical connections in the affected hemisphere after cortical injury combined with limited plasticity to reshape interhemispheric input–output somatotopy are therefore likely to restrict recovery. The observed alterations in fMRI activation patterns after stroke have been hypothesized to be due to two possible mechanisms of bihemispheric reorganization: (1) disinhibition of existing pathways, and (2) plasticity-induced changes in neuronal circuitry.\(^\text{12}\) Because our previous work has shown increased axonal plasticity to subcortical regions with anti–Nogo-A therapy after stroke, the changes in activation patterns seen

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**Fig 2.** Functional magnetic resonance imaging (fMRI) data acquired 8 weeks after stroke in adult rats. (A) Stimulation of either forepaw in normal animals led only to activation of the primary somatosensory cortex contralateral to the stimulated paw. (B) In the stroke/control antibody (Ab) group, stimulation of the nonimpaired paw led to contralateral cortical activation. (C) Bilateral thalamic activation was present after stimulation of the nonimpaired paw. (D) When the impaired paw was stimulated, major activation was seen in the thalamus during stimulation of the impaired forepaw in animals that had recovered with anti–Nogo-A Ab treatment compared with stroke/control Ab animals. (E) Again, bilateral thalamic activation was seen. (F) In the stroke/anti–Nogo-A Ab group, stimulation of the nonimpaired paw led to contralateral cortical activation. (G) Bilateral thalamic activation was present after stimulation of the nonimpaired paw. (H) When the impaired paw was stimulated, major activation was seen in the ipsilateral somatosensory cortex with minor activation around the stroke lesion. (E) Again, bilateral thalamic activation was seen. (I) In the stroke/anti–Nogo-A Ab group, stimulation of the nonimpaired paw led to contralateral cortical activation. (G) Bilateral thalamic activation was present after stimulation of the nonimpaired paw. (H) When the impaired paw was stimulated, activation was seen in the ipsilateral somatosensory cortex. (I) Importantly, in the stroke/anti–Nogo-A Ab group, a statistically significant increase in activation was found in the thalamus during stimulation of the impaired forepaw compared with the stroke/control Ab group.
on fMRI in this study are most likely due to new neuronal connections after anti–Nogo-A Ab treatment.

There is now a growing body of literature documenting the effects of Nogo-A neutralization on neuronal regeneration and plasticity after CNS lesions in adult rats and primates leading to recovery of function after cortical lesions or corticospinal tract injury. The Nogo-A protein is expressed in oligodendrocytes and myelin and is also found in certain neuronal populations and in regenerating neurons. Our work here demonstrates that the aged CNS retains a similar growth potential as in the young adult animal and can respond to specific therapeutic interventions even if given 1 week after injury. These results support the use of therapies to neutralize Nogo-A as adjuncts to rehabilitation in elderly stroke patients and suggest that the window of opportunity for treatment is much longer compared with neuroprotective therapeutic approaches.

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References