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Delayed Anti-Nogo-A Therapy Improves Function After Chronic Stroke in Adult Rats

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Background and Purpose—We have shown that anti-Nogo-A immunotherapy to neutralize the neurite growth inhibitory protein Nogo-A results in functional improvement and enhanced plasticity after ischemic stroke in the adult rat. The present study investigated whether functional improvement and neuronal plasticity can be induced by this immunotherapy when administered to the chronic stroke-impaired rat.

Methods—Adult rats were trained to perform the skilled forelimb reaching test, followed by permanent middle cerebral artery occlusion to impair the preferred forelimb. Nine weeks after stroke, animals showing a profound deficit were randomly distributed to 3 groups: no treatment, control antibody, or anti-Nogo-A antibody (11C7). Animals were tested weekly after stroke surgery and daily after antibody treatment until the end of the study. Biotin dextran amine tracing was injected into the nonlesioned forelimb motor cortex at the end of behavioral testing to determine axonal plasticity.

Results—All rats showed similar forelimb impairment before treatment. Animals treated with anti-Nogo-A immunotherapy started to show improvement 3 weeks after treatment. Such improvement became significantly better than stroke-only control and control Ab-treated animals, and persisted to the end of the study. Biotin dextran amine-labeled axonal fiber analysis also showed significant enhanced corticorubral axonal sprouting from the contralesional forelimb motor cortex to the deafferented red nucleus in the anti-Nogo-A immunotherapy rats.

Conclusions—These results indicate that improvement of chronic neurological deficits and enhancement of neuronal plasticity can be induced in the adult rat with anti-Nogo-A immunotherapy, and that this therapy may be used to restore function even when administered long after ischemic brain damage has occurred. (*Stroke*. 2011;42:186-190.)

Key Words: neuroplasticity ■ Nogo-A ■ stroke recovery

Stroke is the nation's third leading killer and the most common cause of adult neurological disability. Each year, nearly 800 000 Americans are stricken by ischemic stroke, during which blood flow to the brain is interrupted and neurons are permanently damaged. More than 232 000 Americans die from stroke-related causes each year, and there are close to 4 million survivors living with a permanent neurological disability.¹ There is currently no specific treatment for improving functional recovery after stroke except for rehabilitative strategies, which have real but limited success. The only approved treatment for acute ischemic stroke is tissue plasminogen activator, which must be administered within the first hours after stroke, thus limiting its usefulness. Neurite outgrowth inhibitory proteins in the central nervous system, such as Nogo-A, play important roles to block fiber regeneration, sprouting, and new network formation, and hence prevent the regaining of lost function after central nervous system damage.^{2,3} We have shown that treatment with anti-Nogo-A immunotherapy improved functional out-

come and was associated with neuroanatomical plasticity when administered immediately,⁴ 24 hours after,⁵ or 1 week after ischemic stroke in adult^{6,7} or aged rats.⁸ Because many stroke victims survive the acute stroke period and suffer with their disability for many subsequent years,⁹ treatments to improve functional outcome beyond the acute and into the chronic stroke stage would be of great benefit.

The purpose of the present study was to examine the effectiveness of anti-Nogo-A immunotherapy as a treatment to restore lost functions in the chronic stage after ischemic stroke. Our results indicate that anti-Nogo-A immunotherapy is effective as a treatment, even when administered up to 9 weeks after stroke in adult rats.

Materials and Methods

Adult male, Long Evans black-hooded rats (mean weight of 300 grams) were first trained and tested on the skilled forelimb reaching task (Figure 1A) as previously described.⁷ This task requires fine digit movement and intact motor and sensory neural pathways.¹⁰ Animals that successfully retrieved 15 out of 20 pellets during 3

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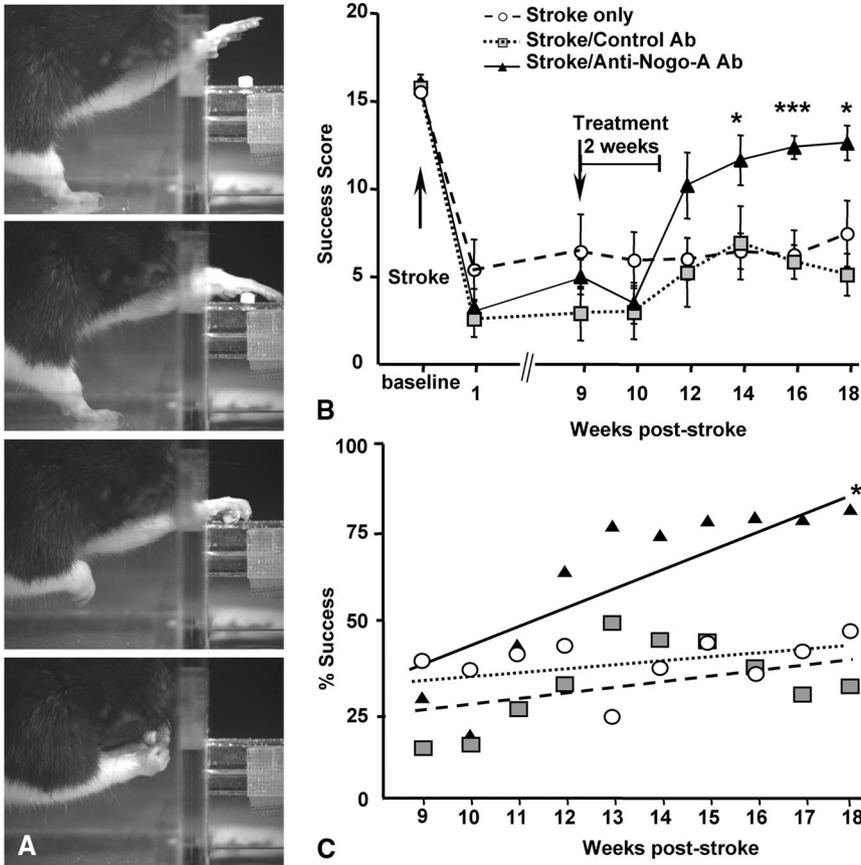


Figure 1. Behavioral assessment of animals. A, Sequential snap frames of the skilled forelimb reaching task performed by an adult rat grasping a pellet. B, All groups showed marked deficits in successfully obtaining pellets with the stroke-impaired limb before treatment, with no significant difference between groups. Animals treated with anti-Nogo-A Ab exhibited significant improvement greater than the stroke/control Ab group and the stroke-only group at 5 weeks after treatment that persisted until the end of the study. There was no difference between 2 control groups at any time point (1-way ANOVA with Tukey post hoc analysis, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; error bars indicate \pm SEM). C, Animals receiving anti-Nogo-A Ab treatment demonstrated a significant rate of improvement in comparison with stroke only (* $P < 0.05$) and stroke/control Ab treatment (* $P < 0.05$) animals. There was no difference between the 2 control groups (repeated-measures 1-way ANOVA).

consecutive training sessions received permanent middle cerebral artery occlusion to impair the preferred forelimb as described.⁷ After middle cerebral artery occlusion, animals continued to use their impaired forelimb to reach, despite showing deficits in performing the task. To reduce possible effects of rehabilitation through daily testing, animals were tested Monday through Friday for the first week after stroke and once per week until time of treatment, ie, 9 weeks after stroke. Any animal that improved spontaneously to retrieve >10 pellets in any testing session before the 9-week treatment point was removed from the study.

Nine weeks after stroke, animals received the purified monoclonal function blocking mouse anti-Nogo-A antibody 11C7¹¹ (n=6), an inactive control Ab (IgG1 against wheat auxin; n=4), or no treatment (n=5). Antibody was delivered for 2 weeks using the intracerebroventricular route through an osmotic minipump (2ML2; Alzet) as described.⁸

After a 9-week period of behavioral testing after treatment, the neuroanatomical tracer, biotin dextran amine (BDA), was injected into the forelimb motor cortex contralateral to the stroke lesion as described.⁴ Two weeks after BDA injection, animals were perfused, brains were removed, cryosectioned, and processed for BDA-positive crossing corticorubral fibers as described,⁴ and stroke lesion analysis was performed using the method of Kawamata et al.¹² Because behavioral testing showed no difference between stroke only and stroke/control Ab animals (see Results), data from these 2 groups of animals were pooled for BDA-positive fiber analysis.

Personnel performing behavioral testing or histological analyses were blinded to the treatments. For behavioral analysis, a repeated-measures ANOVA was used to compare the rate of improvement on the skilled forelimb reaching task and a 1-way ANOVA with Tukey post hoc analysis was used to compare the mean success scores. Midline crossing corticorubral fibers were analyzed using a Student *t* test. Stroke lesion size was analyzed using a 1-way ANOVA.

Results

Skilled forelimb reaching, which is a complex motor cortex-dependent behavior, was analyzed in the single pellet retrieval task. Before stroke surgery, animals in all groups showed excellent skilled reaching and no difference in performance. One week after stroke, all animals had significant deficits in obtaining pellets with the stroke-impaired limb, and there was no spontaneous improvement over the subsequent 8 weeks (before treatment; Figure 1B). However, animals that received anti-Nogo-A Ab treatment began to exhibit improvements in the pellet reaching success rate at 3 weeks after treatment (ie, 12 weeks after stroke) and showed a significant difference starting 5 weeks after treatment when compared to stroke-only animals ($P < 0.01$) or stroke/control Ab-treated animals ($P < 0.05$; Figure 1B). At the end of the study, animals treated with the anti-Nogo-A Ab demonstrated an improvement up to 78% of their prestroke performance, which was significantly different from the stroke only animals (47%; $P < 0.05$) or stroke/control Ab animals (33%; $P < 0.01$). There was no significant difference in success scores between the 2 control groups at any time point. Furthermore, animals receiving anti-Nogo-A Ab treatment demonstrated a significant increased rate of improvement when compared to the stroke-only group ($P < 0.05$) and stroke/control Ab group ($P < 0.05$; Figure 1C), with no difference between the 2 control groups. These results demonstrate that neutralization of Nogo-A with a specific function blocking antibody started at 9 weeks after ischemic

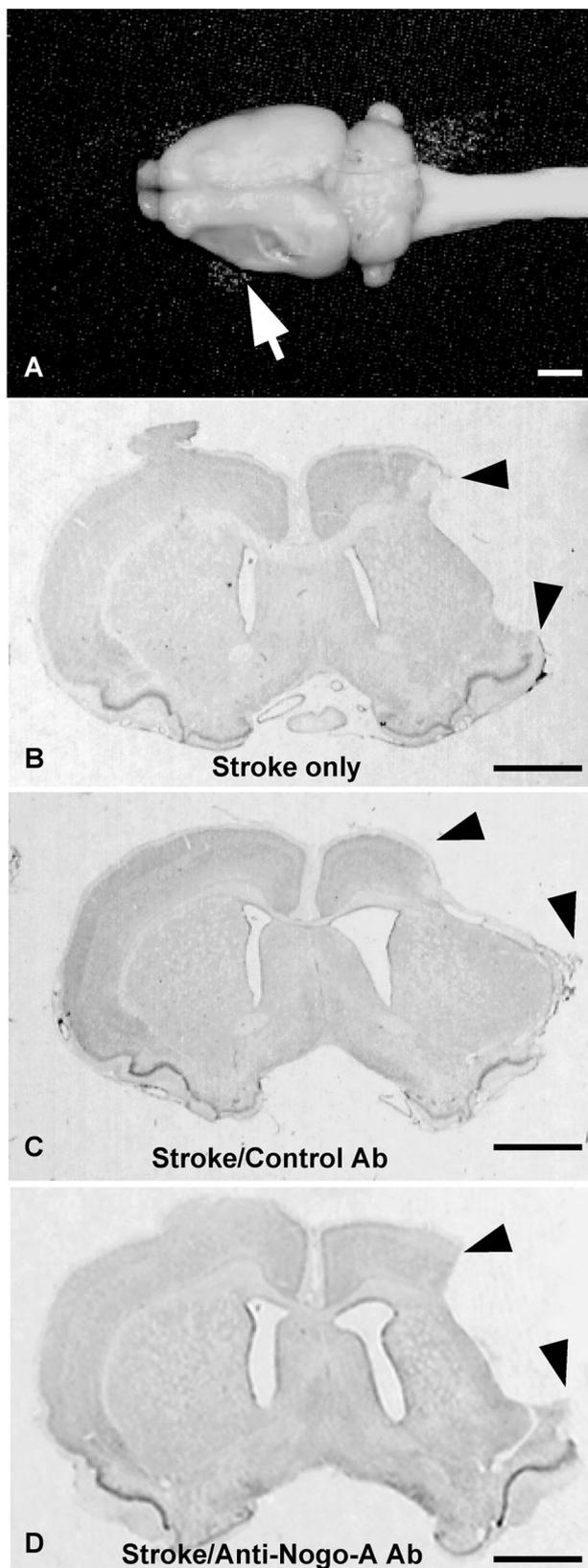


Figure 2. Representative stroke lesions. A, Gross brain of an animal in the stroke/anti-Nogo-A Ab-treated group 20 weeks after stroke. This animal showed marked improvement in skilled forelimb reaching despite the large cortical lesion, as shown here. Low-power Nissl stain photomicrographs of stroke only (B), stroke/control Ab (C), and stroke/anti-Nogo-A Ab (D). Arrow (A) and arrow heads (B, C, D) indicate stroke lesion. Bars indicate 500 μm .

infarction can result in remarkable improvement on the skilled forelimb reaching task.

Lesion analysis showed that all stroke lesions included the ipsilateral sensorimotor cortex without involving subcortical structures, such as the striatum or the hippocampus (Figure 2 A–D). There was no statistical difference in stroke size between groups (stroke only, $10.2\% \pm 3.2$ SEM; stroke/control Ab, $12.63\% \pm 4.32$ SEM; stroke/anti-Nogo-A Ab, $16.49\% \pm 3.83$ SEM; 1-way ANOVA), indicating no apparent effect of anti-Nogo-A immunotherapy on lesion size.

Compensatory sprouting and neuroanatomical plasticity were analyzed for corticorubral midline crossing fibers from the contralesional forelimb cortex to the deafferented red nucleus using the anterograde neuroanatomical tracer BDA. Control stroke animals consistently showed few BDA-labeled midline-crossing fibers (253 ± 60 SEM; Figure 3A). More than twice the number of midline crossing projections into the deafferented red nucleus were observed in the animals receiving anti-Nogo-A Ab therapy (527 ± 74 ; Figure 3B–D; $P < 0.05$; Student *t* test). This result suggests that anti-Nogo-A immunotherapy administered at 9 weeks after ischemic infarction can induce remarkable compensatory sprouting and fiber growth, indicating the responsiveness of the chronically injured brain to form new neural networks under the proper growth conditions.

Discussion

The present study shows that treatment with anti-Nogo-A immunotherapy started at 9 weeks after ischemic stroke in the adult rat results in significant improvement in a chronic lesion-induced deficit of skilled forelimb reaching. Furthermore, this therapy also enhanced sprouting and midline crossing of corticorubral axons originating from the contralesional sensorimotor cortex to innervate the deafferented red nucleus, which is an important neural structure for motor control.

Studies have shown that anti-Nogo-A immunotherapy improves functional recovery, neuroregeneration, and compensatory fiber growth after central nervous system lesions in adult rats^{11,13} and primates.¹⁴ Our laboratory was the first to show that anti-Nogo-A immunotherapy administered immediately after ischemic stroke in adult rats resulted in improvement in skilled forelimb reaching.⁴ Further studies using different function blocking anti-Nogo-A antibodies confirmed this result and showed that when anti-Nogo-A immunotherapy was delayed for either 24 hours⁵ or 1 week⁶ after stroke, significant improvement of sensorimotor function was still observed. This treatment was also effective in improving functional outcome when applied in a lesion-induced neglect model in the rat.¹⁵ Therapy targeting a Nogo-A-related receptor, NgR, also resulted in beneficial effects in rats when administered at 1 week after stroke.¹⁶ A recent report further showed that motor rehabilitation facilitated the effect of NEP1-40, which is a NgR competitive antagonist, in functional improvement after ischemic stroke in rats.¹⁷ All these findings suggested that blocking Nogo-A activities is an important intervention to restore lost function after central nervous system lesions.

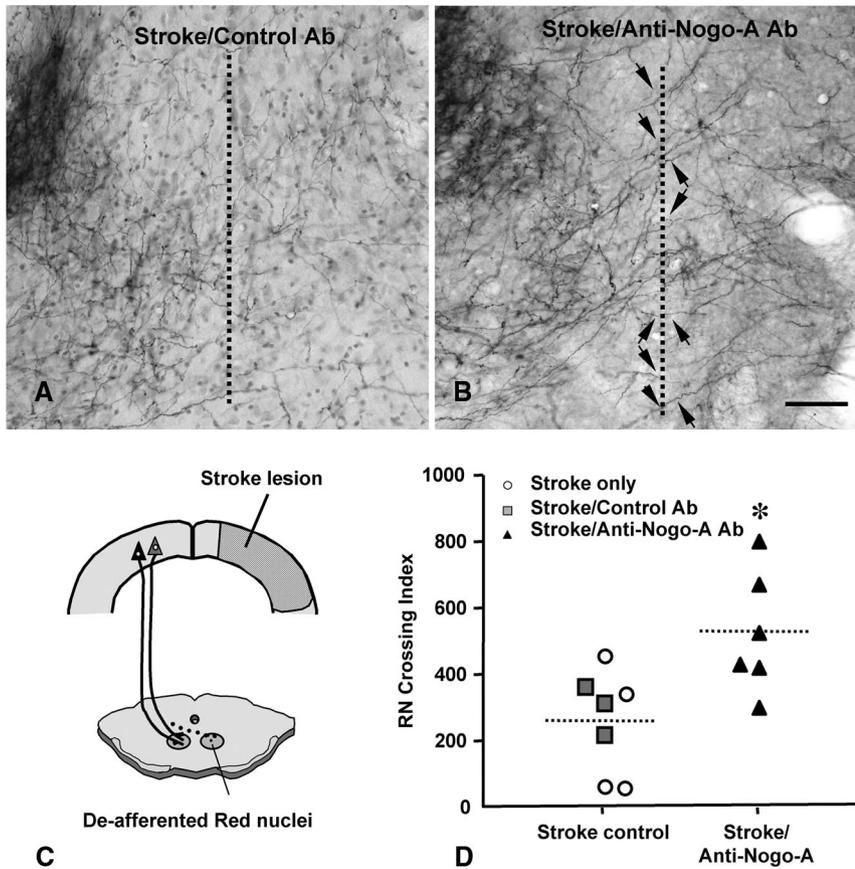


Figure 3. Corticorubral plasticity. Representative photomicrographs show obvious differences of corticorubral midline crossing fibers (arrows) between a stroke/control Ab animal (A) and a stroke/anti-Nogo-A Ab animal (B). Dotted lines indicate the midline. C, Schematic diagram showing corticorubral plasticity after stroke and anti-Nogo-A Ab treatment (adapted from Seymour et al⁶). D, Stroke/anti-Nogo-A Ab-treated animals exhibit significant increase in the midline crossing corticorubral fibers in comparison with control animals (**P*<0.05; Student *t* test). Bars indicate 50 μ m.

In the present study, although treatment was delayed for 9 weeks after stroke, animals improved significantly in a skilled forelimb reaching task by 5 weeks after the start of Ab treatment and reached a mean of 78% of their baseline performance at the end of the study. This result closely paralleled our earlier reports with acute or 1-week delayed antibody infusions. In our earlier studies, animals receiving immediate treatment showed significant improvement at 6 weeks after the start of anti-Nogo-A Ab infusion and improved to 77% of baseline performance at the end of the study.⁴ In our other study, animals received treatment 1 week after stroke and showed significant improvement at 5 weeks after the start of anti-Nogo-A Ab treatment, and these animals reached 75% of the baseline level at the end of the study.⁶ Therefore, even when starting treatment at a much longer time point after stroke, such as in the present study, similar mechanisms may be underlying the improved functional outcome.

We investigated the possibility of neuroanatomical plasticity in the improved animals and found enhanced corticorubral axonal fiber sprouting originating from the contralesional cortex and targeting the deafferented red nucleus. This result is consistent with our previous reports that anti-Nogo A immunotherapy enhanced corticorubral plasticity when administered immediately,^{4,6} 24 hours after,⁵ or 1 week after stroke.⁶ The similarity in the corticorubral crossing index between the present study (527 ± 74) and that of the immediate treatment study (469 ± 81)⁴ and the similarity of control groups at 263 ± 60 and 232 ± 37 , respectively, suggest that

anti-Nogo-A immunotherapy administered at 9 weeks after stroke is as effective as immediate therapy in inducing corticorubral axonal plasticity.

The critical window for inducing plasticity and improving functional outcome after central nervous system lesions, particularly ischemic stroke, has not been established. Enhancing neuronal plasticity by modulation of the inhibitory environment has become an important mechanism to improve lost function in the adult brain after injury. Interestingly, blocking Nogo-A function alone (no lesion) induces transient expression of various trophic factors, growth-associated factors, and neuronal cytoskeletal components, and results in transient axonal^{18–22} and dendritic²³ arborization. Also, chondroitinase ABC treatment degraded chondroitin sulfate proteoglycans, reactivated visual cortical plasticity, and, when paired with reverse environmental manipulation, completely restored ocular dominance, visual acuity, and dendritic spine density in adult rats with shifted ocular dominance attributable to early monocular deprivation.²⁴ These studies suggest that modulation of neuronal plasticity can be achieved even in the unlesioned mature brain that has sustained an earlier insult. This further supports our findings that appropriate interventions, even when administered at a chronic stage after stroke, may be sufficient to induce neuronal plasticity in a stable, chronically lesioned brain with a “fixed” neurological deficit and may ultimately improve functional outcome.

Conclusion

In conclusion, this study demonstrates that the adult brain is capable of improved functional outcome and enhanced com-

pensatory fiber growth and circuit plasticity induced by anti-Nogo-A immunotherapy, even long after ischemic stroke. Our findings are of great clinical importance because anti-Nogo-A immunotherapy may benefit not only patients with spinal cord injury or patients in the early stage of stroke recovery but also patients in later stages who have neurological disability attributable to brain damage from stroke or other causes.

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Disclosure

None.

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