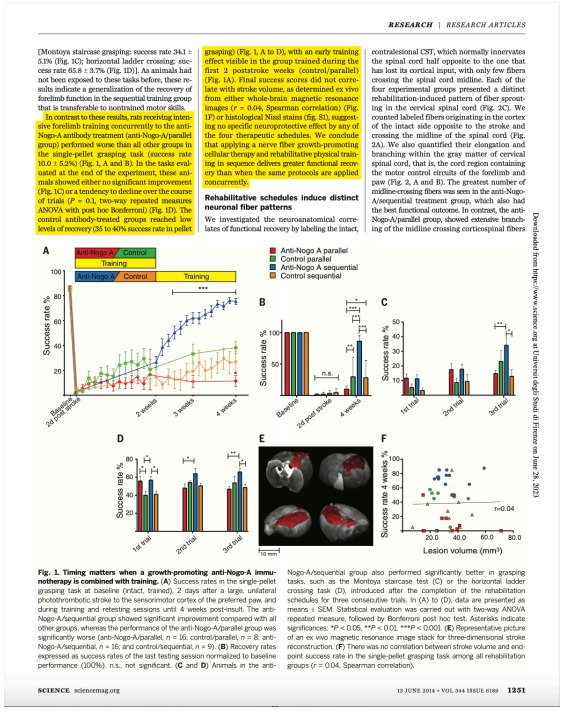
PUBBLICAZIONE, AI SENSI DELL'ART. 19 DEL D.LGS N. 33 DEL 14 MARZO 2013, MODIFICATO DALL'ART. 18 DEL D.LGS N. 97 DEL 25 MAGGIO 2016 COME INTEGRATO DALL'ART.1 C. 145 DELLA LEGGE 27 DICEMBRE 2019 N. 160, DELLE DOMANDE DELLA PROVA COLLOQUIO STABILITE DALLA COMMISSIONE ESAMINATRICE DELLA SELEZIONE DI SEGUITO INDICATA NELLA RIUNIONE IN DATA 28/06/2023

BANDO N. 400.21 PNRR CNR-INO IR0006 IPHOQS CUP B53C22001750006

Selezione per titoli e colloquio ai sensi dell'art. 8 del "Disciplinare concernente le assunzioni di personale con contratto di lavoro a tempo determinato", per l'assunzione, ai sensi dell'art. 83 del CCNL del Comparto "Istruzione e Ricerca" 2016-2018, sottoscritto in data 19 aprile 2018, di una unità di personale con profilo professionale di **Ricercatore III livello**, presso il CNR-Istituto Nazionale di Ottica, Sede di Sesto Fiorentino.

BUSTA 1

1. la candidata descriva un esperimento di imaging non lineare per lo studio della funzionalità corticale
2. la candidata faccia degli esempi, presi dalla letteratura, di utilizzo dell'imaging non lineare per studiare le lesioni del tessuto neuronale in vivo
3. la candidata legga e traduca il brano estratto dal seguente articolo: Wahl, Anna-Sophia, et al. "Asynchronous therapy restores motor control by rewiring of the rat corticospinal tract after stroke." Science 344.6189 (2014): 1250-1255.



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[Montoya staircase grasping: success rate 34.1 ± 5.1% (Fig. 1C); horizontal ladder crossing: success rate 65.8 ± 3.7% (Fig. 1D)]. As animals had not been exposed to these tasks before, these results indicate a generalization of the recovery of forelimb function in the sequential training group that is transferable to nontrained motor skills.

In contrast to these results, rats receiving intensive forelimb training concurrently to the anti-Nogo-A antibody treatment (anti-Nogo-A/parallel group) performed worse than all other groups in the single-pellet grasping task (success rate 10.0 ± 5.2%) (Fig. 1, A and B). In the tasks evaluated at the end of the experiment, these animals showed either no significant improvement (Fig. 1C) or a tendency to decline over the course of trials ($P = 0.1$, two-way repeated measures ANOVA with post hoc Bonferroni) (Fig. 1D). The control antibody-treated groups reached low levels of recovery (35 to 40% success rate in pellet grasping) (Fig. 1, A to D), with an early training effect visible in the group trained during the first 2 poststroke weeks (control/parallel) (Fig. 1A). Final success scores did not correlate with stroke volume, as determined ex vivo from either whole-brain magnetic resonance images ($r = 0.04$, Spearman correlation) (Fig. 1F) or histological Nissl stains (fig. S1), suggesting no specific neuroprotective effect by any of the four therapeutic schedules. We conclude that applying a nerve fiber growth-promoting cellular therapy and rehabilitative physical training in sequence delivers greater functional recovery than when the same protocols are applied concurrently.

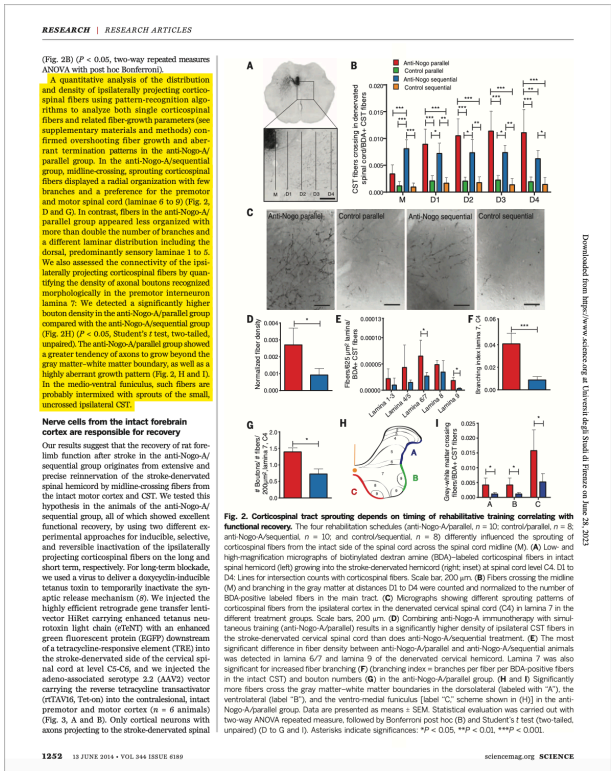
Rehabilitative schedules induce distinct neuronal fiber patterns

We investigated the neuroanatomical correlates of functional recovery by labeling the intact, contralesional CST, which normally innervates the spinal cord half opposite to the one that has lost its cortical input, with only few fibers crossing the spinal cord midline. Each of the four experimental groups presented a distinct rehabilitation-induced pattern of fiber sprouting in the cervical spinal cord (Fig. 2C). We counted labeled fibers originating in the cortex of the intact side opposite to the stroke and crossing the midline of the spinal cord (Fig. 2A). We also quantified their elongation and branching within the gray matter of cervical spinal cord, that is, the cord region containing the motor control circuits of the forelimb and paw (Fig. 2, A and B). The greatest number of midline-crossing fibers was seen in the anti-Nogo-A/sequential treatment group, which also had the best functional outcome. In contrast, the anti-Nogo-A/parallel group, showed extensive branching of the midline crossing corticospinal fibers Nogo-A/sequential group also performed significantly better in grasping tasks, such as the Montoya staircase test (C) or the horizontal ladder crossing task (D), introduced after the completion of the rehabilitation schedules for three consecutive trials. In (A) to (D), data are presented as means ± SEM. Statistical evaluation was carried out with two-way ANOVA repeated measure, followed by Bonferroni post hoc test. Asterisks indicate significances: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. (**E**) Representative picture of an ex vivo magnetic resonance image stack for three-dimensional stroke reconstruction. (**F**) There was no correlation between stroke volume and end-point success rate in the single-pellet grasping task among all rehabilitation groups ($r = 0.04$, Spearman correlation).

Fig. 1. Timing matters when a growth-promoting anti-Nogo-A immunotherapy is combined with training. (**A**) Success rates in the single-pellet grasping task at baseline (intact, trained), 2 days after a large, unilateral photothrombotic stroke to the sensorimotor cortex of the preferred paw, and during training and retesting sessions until 4 weeks post-insult. The anti-Nogo-A/sequential group showed significant improvement compared with all other groups, whereas the performance of the anti-Nogo-A/parallel group was significantly worse (anti-Nogo-A/parallel, $n = 16$; control/parallel, $n = 8$; anti-Nogo-A/sequential, $n = 16$; and control/sequential, $n = 9$). (**B**) Recovery rates expressed as success rates of the last testing session normalized to baseline performance (100%). n.s., not significant. (**C** and **D**) Animals in the anti-

BUSTA 2 (estratta)

1. La candidata descriva delle possibili applicazioni che prevedano l'utilizzo di microscopia non lineare per lo studio delle lesioni neuronali
2. La candidata descriva le componenti principali di un esperimento di microscopia non lineare in vivo.
3. La candidata legga e traduca il brano estratto dal seguente articolo: Wahl, Anna-Sophia, et al. "Asynchronous therapy restores motor control by rewiring of the rat corticospinal tract after stroke." Science 344.6189 (2014): 1250-1255.



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(Fig. 2B) ($P < 0.05$, two-way repeated measures ANOVA with post hoc Bonferroni).

A quantitative analysis of the distribution and density of ipsilaterally projecting corticospinal fibers using pattern-recognition algorithms to analyze both single corticospinal fibers and related fiber-growth parameters (see supplementary materials and methods) confirmed overshooting fiber growth and aberrant termination patterns in the anti-Nogo-A/parallel group. In the anti-Nogo-A/sequential group, midline-crossing, sprouting corticospinal fibers displayed a radial organization with few branches and a preference for the premotor and motor spinal cord (laminae 6 to 9) (Fig. 2, D and G). In contrast, fibers in the anti-Nogo-A/parallel group appeared less organized with more than double the number of branches and a different laminar distribution including the dorsal, predominantly sensory laminae 1 to 5. We also assessed the connectivity of the ipsilaterally projecting corticospinal fibers by quantifying the density of axonal boutons recognized morphologically in the premotor interneuron lamina 7. We detected a significantly higher bouton density in the anti-Nogo-A/parallel group compared with the anti-Nogo-A/sequential group (Fig. 2H) ($P < 0.05$, Student's t test, two-tailed, unpaired). The anti-Nogo-A/parallel group showed a greater tendency of axons to grow beyond the gray matter–white matter boundary, as well as a highly aberrant growth pattern (Fig. 2, H and I). In the medio-ventral funiculus, such fibers are probably intermixed with sprouts of the small, uncrossed ipsilateral CST.

Nerve cells from the intact forebrain cortex are responsible for recovery

Our results suggest that the recovery of rat forelimb function after stroke in the anti-Nogo-A/sequential group originates from extensive and precise reinnervation of the stroke-denervated spinal hemicord by midline-crossing fibers from the intact motor cortex and CST. We tested this hypothesis in the animals of the anti-Nogo-A/sequential group, all of which showed excellent functional recovery, by using two different experimental approaches for inducible, selective, and reversible inactivation of the ipsilaterally projecting corticospinal fibers on the long and short term, respectively. For long-term blockade, we used a virus to deliver a doxycyclin-inducible tetanus toxin to temporarily inactivate the synaptic release mechanism (8). We injected the highly efficient retrograde gene transfer lentivector HiRet carrying enhanced tetanus neurotoxin light chain (eTeNT) with an enhanced green fluorescent protein (EGFP) downstream of a tetracycline-responsive element (TRE) into the stroke-denervated side of the cervical spinal cord at level C5-C6, and we injected the adeno-associated serotype 2.2 (AAV2) vector carrying the reverse tetracycline transactivator (rtTAV16, Tet-on) into the contralesional, intact premotor and motor cortex ($n = 6$ animals) (Fig. 3, A and B). Only cortical neurons with axons projecting to the stroke-denervated spinal

Fig. 2. Corticospinal tract sprouting depends on timing of rehabilitative training correlating with functional recovery. The four rehabilitaton schedules (anti-Nogo-A/parallel, $n = 10$; control/parallel, $n = 8$; anti-Nogo-A/sequential, $n = 10$; and control/sequential, $n = 8$) differently influenced the sprouting of corticospinal fibers from the intact side of the spinal cord across the spinal cord midline (M). (**A**) Low- and high-magnification micrographs of biotinylated dextran amine (BDA)–labeled corticospinal fibers in intact spinal hemicord (left) growing into the stroke-denervated hemicord (right; inset) at spinal cord level C4. D1 to D4: Lines for intersection counts with corticospinal fibers. Scale bar, 200 μm. (**B**) Fibers crossing the midline (M) and branching in the gray matter at distances D1 to D4 were counted and normalized to the number of BDA-positive labeled fibers in the main tract. (**C**) Micrographs showing different sprouting patterns of corticospinal fibers from the ipsilateral cortex in the denervated cervical spinal cord (C4) in lamina 7 in the different treatment groups. Scale bars, 200 μm. (**D**) Combining anti-Nogo-A immunotherapy with simultaneous training (anti-Nogo-A/parallel) results in a significantly higher density of ipsilateral CST fibers in the stroke-denervated cervical spinal cord than does anti-Nogo-A/sequential treatment. (**E**) The most significant difference in fiber density between anti-Nogo-A/parallel and anti-Nogo-A/sequential animals was detected in lamina 6/7 and lamina 9 of the denervated cervical hemicord. Lamina 7 was also significant for increased fiber branching (**F**) (branching index = branches per fiber per BDA-positive fibers in the intact CST) and bouton numbers (**G**) in the anti-Nogo-A/parallel group. (**H** and **I**) Significantly more fibers cross the gray matter–white matter boundaries in the dorsolateral (labeled with "A"), the ventrolateral (label "B"), and the ventro-medial funiculus [label "C," scheme shown in (H)] in the anti-Nogo-A/parallel group. Data are presented as means ± SEM. Statistical evaluation was carried out with two-way ANOVA repeated measure, followed by Bonferroni post hoc (B) and Student's t test (two-tailed, unpaired) (D to G and I). Asterisks indicate significances: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

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