Anti-Nogo-A treatment enhances corticospinal tract sprouting and functional recovery after unilateral cervical lesion in adult primates

Eric M. Rouiller

Unit of Physiology, Dept. Medicine, Faculty of Sciences, Ch. Mus

Method Article

Keywords: monkey, primate, functional recovery, functional restitution, lesion, injury, lesion, spinal cord, paraplegia, motor control, manual dexterity, tracing, corticospinal, motor cortex, reversible inactivation, tract tracing

Posted Date: July 21st, 2006

DOI: https://doi.org/10.1038/nprot.2006.190

Abstract

Introduction

Following injury, the adult mammalian central nervous system has limited capacity to repair. Transected nerve fibers do not spontaneously re-grow, due in part to myelin-associated neurite growth inhibitors such as Nogo-A. After section of the corticospinal \(CS) tract in adult rats, neutralizing Nogo-A with monoclonal antibodies leads to enhancement of axonal re-growth and compensatory sprouting, accompanied by increased motor recovery. The neutralization of Nogo-A represents a promising approach for therapy after lesion if its enhancement of functional recovery can be transposed to primates. The aim of our study was to extend these data in rodents to macaque monkeys and assess whether neutralization of Nogo-A enhances motor recovery from spinal lesion in primates.

Reagents

Anti-Nogo-A antibodies Two monoclonal antibodies \(mAbs) against different sites of Nogo-A were used: the mouse mAb 11C7 \[1] was raised against a 18 amino acid peptide of rat Nogo-A \(aa623 – 640), close to the most inhibitory region of the Nogo-A protein2, which cross-reacts with mouse and monkey Nogo-A. The second antibody used, mAb hNogo-A is directed against the Nogo-A specific region of the human Nogo-A sequence. Both antibodies recognize primate Nogo-A monospecifically on Western blots \[2]. Both antibodies increase neurite outgrowth in vitro and penetrate deeply the CNS in vivo in monkeys \[3]. The antibodies were purified as IgGs and concentrated to 3.7-10 mg/ml in phosphate buffered saline \(PBS). **Control antibodies** Purified IgG of a mouse mAb directed against wheat auxin \(AMS Biotechnology, Oxon/UK) was used as control antibody. **Muscimol \(GABA agonist)** for reversible inactivation of motor cortex \(see Kermadi et al. 1997 for more information). **Biotinylated Dextran Amine** \(=BDA; Molecular Probes) used as anterograde anatomical tracer.

Equipment

Set-ups to perform behavioral assessment of manual dexterity in monkeys; see www.unifr.ch/neuro/rouiller/motorcontcadre.htm **Video recording system** **Osmotic pumps** to deliver the antibodies

Procedure

1) Habituate the monkey to the primate chair 2) Train the monkey to the various motor tasks to assess manual dexterity daily. Duration of a behavioral session is 30-60 minutes 3) Establish during 2 months the pre-lesion behavioral scores 4) Perform the cervical hemi-section at C7 5) Place osmotic pump to deliver the antibody during 4 weeks 6) Immediately after the lesion, test daily the motor performance to establish the post-lesion behavioral scores \(2-3 months) 7) Injection of BDA at multiple sites in the

contralesional motor cortex 8) Survival time \(60-70 days) 9) Sacrifice of the animal 10) Histology: sections \(50 microns thick) of the brain and spinal cord. Histochemistry.

Timing

6-12 months for each monkey

Critical Steps

The success of the procedure depends, among other things, on the complete transection of the dorsolateral funiculus unilaterally. Incomplete transection of the dorsolateral funiculus may dramatically affect the interpretation of the behavioral data and, even more prominently, the tracing data Make sure that the antibodies have been delivered \(check volume of antibody left at removal of the osmotic pump Although BDA is transported in intact macaque monkeys in about 20 days from motor cortex to cervical cord, after a lesion of the corticospinal tract, the speed of transport is slower. Therefore, a survival time of 60 days was considered here. A double-blind procedure is required \(the experimenter testing the monkeys behaviorally should not know which antibody was infused, e.g. anti-Nogo-A or control. Check body weight of the animals daily.

Troubleshooting

Monkeys are not food deprived but the rewards received during the behavioral tests represent the first access to food of the day. After the behavioral tests, additional food is given \(fruits, cereals).

Anticipated Results

Anti-Nogo-A treated monkeys recover manual dexterity faster and to a larger extent than control-antibody monkeys. In parallel, corticospinal sprouting is enhanced in the anti-Nogo-A treated monkeys.

References

1. Liebscher,T. _et al. Ann. Neurol._ **58**, 706-719 \(2005). 2. Oertle,T. _et al. J. Neurosci._ **23**, 5393-5406 \(2003). 3. Weinmann,O. _et al. Mol. Cell. Neurosci._ 2006 \(In press). 4. Schmidlin,E., Wannier,T., Bloch,J., & Rouiller,E.M. _Brain Research_ **1017**, 172-183 \(2004). 5. Liu,Y. & Rouiller,E.M. _Exp. Brain Res._ **128**, 149-159 \(1999). 6. Rouiller,E.M. _et al. Eur. J. Neurosci._ **10**, 729-740 \(1998). 7. Kazennikov,O. _et al. Eur. J. Neurosci._ **6**, 203-210 \(1994). 8. Kazennikov,O. _et al. Neurosci._ **85**, 703-716 \(1998). 9. Kermadi,I., Liu,Y., & Rouiller,E.M. _Somatosensory and Motor Research_ **17**, 255-271 \(2000). 10. Kermadi,I., Liu,Y., Tempini,A., & Rouiller,E.M. _Somatosens. Mot. Res._ **14**, 268-280 \(1997). 11. Kermadi,I., Liu,Y., Tempini,A., Calciati,E., & Rouiller,E.M. _Somatosens. Mot. Res._ **15**, 287-308 \(1998). 12. Wannier,T., J. Liu, A. Morel, C. Jouffrais and E.M. Rouiller \(2002) _Neuroreport_ **13**, 143-147. 13. Jenny,A.B. & Inukai,J. _J. Neurosci._ **3**, 567-575 \(1983). 14. Schmidlin,E. _et al. BMC

Neuroscience_ **6**:56, \(2005). 15. Rouiller,E.M. _et al. Eur. J. Neurosci._ **8**, 1055-1059 \(1996). 16. Rouiller,E.M. _et al. J. Comp. Neurol._ **396**, 169-185 \(1998). 17. Wannier,T., Schmidlin,E., Bloch,J., & Rouiller,E.M. _J.Neurotrauma_ **22**, 703-717 \(2005).

Acknowledgements

The authors wish to thank the technical assistance of Georgette Fischer, Véronique Moret, Christine Roulin and Françoise Tinguely \(histology and behavioral evaluations), Josef Corpataux, Bernard Bapst and Bernard Morandi \(animal house keeping), André Gaillard \(mechanics), Bernard Aebischer \(electronics), Laurent Monney \(informatics). Grant Sponsors: Swiss National Science Foundation, grants No 31-61857.00 \(EMR) and No 31-63633.00 \(MES), and 4038043918/2 \(PNR-38); Novartis Foundation; The National Centre of Competence in Research \(NCCR) on "Neural plasticity and repair", and the Christopher Reeves Foundation \(Springfield, NJ). The antibodies were provided by Novartis Pharma.