Motor recovery after grafting a peripheral nerve to bridge denervated skeletal muscle with health spinal cord

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Spinal cord injury is the leading cause of permanent paraplegia due to the fact that injured axons cannot regenerate into the CNS (1). However, a number of evidence indicated that PNs directly grafted into the CNS could constitute a suitable environment for regeneration of severed central axons (2). In order to bypass a spinal cord lesion, muscular nerve branches were inserted into the severed lateral bundle of spinal cord in primates which recovered motor function (3). We repeated experiments in rats. An autologous sural nerve graft was implanted into the acutely severed lateral white matter of the rat spinal cord and connected to the transected motor nerve of the internal obliquus abdominis muscle. We found that the regenerated axons derived from glutamatergic supraspinal neurons located in red nucleus and brainstem nuclei (4). The glutamatergic reinnervation specified the type of postsynaptic receptor at the muscular level.
Reinnervated muscle fibers expressed increased amounts of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptor subunits that cluster at the junctional postsynaptic site. At 2 months after nerve grafting, nerve-evoked contraction of the skeletal muscles became insensitive to curare but was efficiently blocked by the AMPA receptor antagonist GYKI 52466. It was well-known that when cholinergic motor axons regenerate after peripheral nerve injury, the cholinergic terminals of regrowing motoneurons reoccupy precisely the original endplate sites. However, it was unknown whether the cholinergic apparatus was still preserved when skeletal muscle was reinnervated by supraspinal glutamatergic neurons, and whether regenerating glutamatergic axons reached the cholinergic endplates rather than new areas to form a postsynaptic glutamatergic apparatus. The possibility that functional AMPA receptors assemble at the cholinergic postsynaptic membrane recently became more plausible as glutamate receptors were immunolocalized at the neuromuscular junction (NMJ) of mouse quadriceps.

To investigate the presence of cholinergic endplates and the localization of newly formed glutamatergic synapses in rat abdominal muscles, we first analyzed the structure of NMJs in the internus obliquus and transversus abdominis muscles. Both denervated muscle and muscle reinnervated by supraspinal neurons displayed stable clusters of acetylcholine receptors (AChRs) at all times examined. We studied the ultrastructure of the newly formed neuromuscular synapses. Immunoelectron microscopy analysis showed that the NMJs in muscles surgically reconnected to the spinal cord by the nerve graft express markers of glutamatergic synapses. The AMPA receptors clustered at the postsynaptic membrane partially colocalized with AChRs by interacting with anchoring proteins of the cholinergic postsynaptic apparatus (5).

Overall, these data suggest that under appropriate surgical manipulation supraspinal neurons can target skeletal muscle, while the latter retains the plasticity to generate a functional glutamatergic NMJ located at the preexisting cholinergic endplates.

1. Silver J, Miller JH: **Regeneration beyond the glial scar**. *Nat Rev Neurosci* 2004, **5:**146-156.
2. Richardson PM, McGuinness UM, Aguayo AJ: **Axons from CNS neurons regenerate into PNS grafts.** *Nature* 1980, **284:**264-265.
3. Brunelli GA, Brunelli GR: **Experimental surgery in spinal cord lesions by connecting upper motoneurons directly to peripheral targets**. *J Peripher Nerv Syst* 1996, **1:**111-118.
4. Brunelli G, Spano PF, Barlati S, Guarneri B, Barbon A, Bresciani R, Pizzi M: **Glutamatergic reinnervation through peripheral nerve graft dictates assembly of glutamatergic synapses at rat skeletal muscle.** *Proc. Natl. Acad. Sci. USA* 2006, **102**: 8752-8757
5. Francolini M., Brunelli G., Cambianica I., Barlati S., Barbon A., Luca L., Guarneri B., Boroni F., Lanzillotta A., Baiguera C., Ettorre M., Buffelli M., Spano P., Clementi F., Pizzi M. Glutamatergic reinnervation and assembly of glutamatergic synapses in adult rat skeletal muscle occurs at cholinergic endplates. *J Neuropathol Exp Neurol*. 2009, 68 (10) 1103-1115