Experimental Neurology 267 (2015) 194–208

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Regular Article

Plasticity of motor network and function in the absence of corticospinal projection

Qi Han [a,1], Changshu Cao [a,b,1], Yuetong Ding [a,1], Kwok-Fai So [a,c,e], Wutian Wu [a,c,e], Yibo Qu [a,*], Libing Zhou [a,b,d,*]

[a] Guangdong–Hongkong–Macau Institute of CNS Regeneration, Jinan University, Guangzhou 510632, PR China
[b] Department of Human Anatomy, Medical School of Jinan University, Guangzhou 510632, PR China
[c] Department of Anatomy, The University of Hong Kong, Pokfulam, Hong Kong SAR, PR China
[d] Co-innovation Center of Neuroregeneration, Nantong University, Jiangsu, PR China
[e] State Key Laboratory of Brain and Cognitive Sciences, The University of Hong Kong, Pokfulam, Hong Kong SAR, PR China

ARTICLE INFO

Article history:
Received 14 December 2014
Revised 9 March 2015
Accepted 10 March 2015
Available online 17 March 2015

Keywords:
Corticospinal tract
Rubrospinal tract
Celsr3
Propriospinal neurons
Motor system

ABSTRACT

Despite the obvious clinical interest, our understanding of how developmental mechanisms are redeployed during degeneration and regeneration after brain and spinal cord injuries remains quite rudimentary. In animal models of spinal cord injury, although spontaneous regeneration of descending axons is limited, compensation by intact corticospinal axons, descending tracts from the brainstem, and local intrinsic spinal networks all contribute to the recovery of motor function. Here, we investigated spontaneous motor compensation and plasticity that occur in the absence of corticospinal tract, using *Celsr3|Emx1* mice in which the corticospinal tract is completely and specifically absent as a consequence of *Celsr3* inactivation in the cortex. Mutant mice had no paresis, but displayed hyperactivity in open-field, and a reduction in skilled movements in food pellet manipulation tests. The number of spinal motoneurons was reduced and their terminal arbors at neuromuscular junctions were atrophic, which was reflected in electromyography deficits. Rubrospinal projections, calretinin-positive propriospinal projections, afferent innervation of motoneurons by calretinin-positive segmental interneurons, and terminal ramifications of monoaminergic projections were significantly increased. Contrary to control animals, mutants also developed a severe and persistent disability of forelimb use following the section of the rubrospinal tract at the C4 spinal level. These observations demonstrate for the first time that the congenital absence of the corticospinal tract induces spontaneous plasticity, both at the level of the motor spinal cord and in descending monoaminergic and rubrospinal projections. Such compensatory mechanisms could be recruited in case of brain or spinal cord lesion or degeneration.

Introduction

Limb movement is driven by spinal motoneurons which receive multiple inputs, including propriospinal, corticospinal, rubrospinal, vestibulospinal, recticulospinal and monoaminergic. The corticospinal tract (CST), in particular, controls fine voluntary movement in rodents through indirect connections with spinal motoneurons, via segmental interneurons (Schieber, 2007). Damage of the CST at the level of the motor cortex or descending tracts occurs in neurological conditions such as spinal cord injury (SCI), amyotrophic lateral sclerosis or cerebral palsy, and is a leading cause of motor disability.

Both in animal models and human disorders, damage of the spinal cord and CST leads to spontaneous reorganization of the motor network and some functional recovery, usually associated with sprouting of spared axons to denervated targets (Nishimura and Isa, 2012; Raineteau and Schwab, 2001; Thuret et al., 2006). For example, following neonatal hemi-decortication or unilateral adult traumatic brain injury, CST fibers originating from the intact hemisphere are able to reach the denervated spinal cord (Ueno et al., 2012; Umeda et al., 2010). In monkeys with C7 spinal cord hemisection, corticospinal descending axons from the intact side cross the midline to innervate the gray matter below the level of the lesion (Rosenzweig et al., 2010). Motor control is also partly restored by a reorganization of spinal intrinsic networks. After the spinal cord section, spontaneous walking recovers, mainly thanks to changes of intrinsic circuitry (Anderson et al., 2007; Bareyre et al., 2004; Courtine et al., 2009; Stelzner et al., 1975; Tillakaratne et al., 2010). Propriospinal neurons, which send axons to different spinal segments (Flynn et al., 2011) and are important for the modulation of

* Corresponding authors at: Guangdong–Hongkong–Macau Institute of CNS Regeneration, Jinan University, Huangpu Avenue West 601, Guangzhou 510632, PR China. Fax: +86 20 85223563.
E-mail addresses: tquyibo@jnu.edu.cn (Y. Qu), tlibingzh@jnu.edu.cn (L. Zhou).
[1] Those authors contributed equally to this work.

http://dx.doi.org/10.1016/j.expneurol.2015.03.008

skilled movements (Azim et al., 2014), can create new intraspinal circuits that bypass injury sites and relay cortical inputs to their original targets (Deng et al., 2013).

The rubrospinal tract (RST) shares many anatomical and functional properties with the CST, such as common segmental interneuron targets in the spinal cord. Both systems may substitute for each other during the execution of skilled movements (Cheney et al., 1991; Kennedy, 1990), and collateral sprouting of one tract could contribute to functional recovery when the other one is injured. Following unilateral lesion of the CST, activity changes in the red nucleus were recorded in the monkey (Belhaj-Saif and Cheney, 2000) and human (Yeo and Jang, 2010). In rats with the section of both CSTs, spontaneous sprouting from the RST was limited, but increased by the administration of neutralizing antibodies against the neurite growth inhibitory protein Nogo-A (Raineteau et al., 2001). Reciprocally, sprouting of the CST did not occur spontaneously following the section of the RST, but could be induced by exogenous neurotrophin-3 (Jeffery and Fitzgerald, 2001). In addition to the CST and RST, other descending pathways such as the reticulospinal, vestibulospinal or tectospinal tracts synapse on spinal segmental interneurons and regulate movement and locomotion (Riddle and Baker, 2010; Soteropoulos et al., 2012), and are also thought to contribute to functional recovery following CST injury (Umeda et al., 2010; Zaaimi et al., 2012).

In lesion models of descending pathways, interpretation of experimental results is difficult because it is nearly impossible to accurately damage a single tract. Here we assessed spontaneous compensation resulting from the specific and genetic deletion of the CST, using *Celsr3|Emx1* mice (Zhou et al., 2008). In these mutant mice, we analyzed motor behavior, anatomical modifications in spinal motoneurons, neuromuscular junctions and spinal segmental interneurons and plasticity of the propriospinal neurons and RST, and tested compensatory roles of the RST in motor control.

Materials and methods

Animals

Animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Laboratory Animal Ethics Committee at Jinan University (Permit Number: 20111008001). Male mice of *Emx1–Cre;Celsr3*$^{+/-}$ genotype were crossed with *Celsr3*$^{f/f}$ females to obtain *Emx1–Cre;Celsr3*$^{f/-}$ mutant mice, named *Celsr3|Emx1* for short, in which *Celsr3* is conditionally inactivated in Emx1 positive cells (Zhou et al., 2008); *Emx1–Cre;Celsr3*$^{f/+}$ or *Celsr3*$^{f/-}$ mice were used as controls. The *Thy1-YFP* transgenic line (Feng et al., 2000) was used to visualize corticospinal axonal projections. Animals of both sexes were used indiscriminately. The numbers of animals used in each experimental set are summarized in Supplementary Table 1.

Behavioral studies

Behavioral tests were carried out by experimenters blind to genotypes. Data were analyzed using EnthoVision XT 7.0 software (Noldus, Netherlands). Open-field tests were carried out as described (Feng et al., 2012). Skilled motor function was assessed by testing food pellet handling. Following deprivation of food and water for 24 h, animals (2–3 months, 25–30 g) were videotaped for food handling. IBB scores ranging from 0 to 9 were used to estimate forelimb usage, based on joint position, object support, digit movement and grasping technique (Irvine et al., 2010).

Walking was assessed using Catwalk™ (Noldus, The Netherlands). Mice walked in an enclosed walkway and images of footprints were recorded. The criteria for data collection were: completing one walk in between 0.5 s and 10 s and walking speed variation less than 60%.

Forelimb recovery after the section of the RST was assessed using rearing and grid tests (Starkey et al., 2005). To measure rearing, mice were placed in a perspex cylinder and videotaped for 5 min. For the grid test, mice explored freely a grid with 2 cm × 2 cm squares for 3 min. Foot slips during the first 50 steps were scored when the paw missed a rung and the animal lost balance, or when the paw slipped off during weight bearing. Mice were tested one day prior to surgery and on the 2nd, 7th, 14th, 28th and 56th day post-surgery.

Surgical procedure for the RST section

Adult mice (2–3 months, 25 − 30 g) were anesthetized with avertin (20 μl/g in distilled water). Under an operating microscope, a dorsal midline incision exposed C4–T2 spines and the longer spine of T2 was used for segment identification. The C4 vertebral lamina on the right side was removed, the dura matter was opened and the lateral one third of the right C4 spinal cord segment was transected with a fine scalpel. After the procedure, animals resumed drinking and eating within 24 h and recovered uneventfully. Mice were allowed to survive for 10 weeks post-surgery.

Histology and immunohistochemistry

For histology, 5 μm thick paraffin sections were stained with 0.1% cresyl violet (Nissl staining) to assess gross morphology. Immunohistochemistry was carried out on 40-μm frozen sections. Rabbit anti-protein kinase Cγ antibody (1:200, ab109539, Abcam) was used to detect the CST in the dorsal funiculus. Goat anti-choline acetyltransferase (1:500, AB144p, Millipore) immunofluorescence was performed to characterize spinal motoneurons. For neuromuscular junction studies, we used rabbit anti-neurofilament 200 (1:1000, N4142, Sigma) to label axon terminal and α-bungarotoxin conjugated to Alexa Fluor 546 (1:1000, T1175, Molecular Probes) to label acetylcholine receptors. Axonal arbors were studied within 50 μm of their distal terminus. Monoclonal antibody to nonphosphorylated neurofilament H (SMI-32, 1:1000, NE1023, Calbiochem) was used to label the red nucleus following FluoroGold tracing. Monoclonal anti-glial fibrillary acidic protein antibody (1:1000, G3893, Sigma) was used to visualize the cut of the RST section. To classify segmental interneurons and propriospinal neurons in the spinal cord, we used the following primary antibodies: rabbit anti-calretinin (1:400, AB5054, Invitrogen), mouse anti-parvalbumin (1:1000, MAB1572, Millipore), rat anti-glycine (1:2000, IG1002, ImmunoSolution), rabbit anti-GABA (1:1000, A2052, sigma), mouse anti-calbindin (1:1000, C9848, Sigma), goat anti-choline acetyltransferase (1:500, AB144P, Millipore). To study monoaminergic neurons and fibers, anti-serotonin (1:1000, S5545, sigma) and tyrosine hydroxylase (1:500, AB152, Millipore) antibodies were used. Signal was detected with a mouse-rabbit ABC kit (PK-6200, Universal, Vector) or with Alexa Fluor 546 or 488 fluorescent secondary antibodies (1:1000, Invitrogen).

Anatomical tracing

Adult mice (2–3 months, 25–30 g) were used for tracing using FluoroGold, wheat germ agglutinin lectin (WGA) and biotinylated dextran amine (BDA). Animals were anesthetized and placed in a head holder (68004, RWD Life Science Co. Ltd, China). For retrograde tracing to the brainstem, 0.6 μl FluoroGold (6% in water; 52-9400, Fluorochrome) was injected in the right side of the C6 segment, and the brains were processed after 3 days. To study propriospinal projections from C3–C4 to the cervical enlargement, we injected 0.5 μl FluoroGold in the right side of the C8–T1 segments, 700 μm lateral to the midline, at a depth of 500 μm, with a glass capillary. After 6–7 days, the brains were fixed and sections at C3–C4 were prepared for immunofluorescence using different markers (indicated above). To study monoaminergic projections, we injected FluoroGold in the C5–C6 segments and prepared sagittal