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SPINAL CORD MATURATION AND LOCOMOTION IN MICE WITH AN ISOLATED CORTEX

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Abstract—The spinal cord plays a key role in motor behavior. It relays major sensory information, receives afferents from supraspinal centers and integrates movement in the central pattern generators. Spinal motor output is controlled via corticofugal pathways including corticospinal and cortico-subcortical projections. Spinal cord injury damages descending supraspinal as well as ascending sensory pathways. In adult rodent models, plasticity of the spinal cord is thought to contribute to functional recovery. How much spinal cord function depends on cortical input is not well known. Here, we address this question using *Celsr3/Foxg1* mice, in which cortico-subcortical connections (including corticospinal tract (CST) and the terminal sensory pathway, the thalamocortical tract) are genetically ablated during early development. Although *Celsr3/Foxg1* mice are able to eat, walk, climb on grids and swim, open-field tests showed them to be hyperactive. When compared with normal littermates, mutant animals had reduced number of spinal motor neurons, with atrophic dendritic trees. Furthermore, motor axon terminals were decreased in number, and this was confirmed by electromyography. The number of cholinergic, calbindin, and calretinin-positive interneurons was moderately increased in the mutant spinal cord, whereas that of reelin and parvalbumin-positive interneurons was unchanged. As far as we know, our study provides the first genetic evidence that the spinal motor network does not mature fully in the absence of corticofugal connections, and that some motor function is preserved despite congenital absence of the CST.

Key words: neural plasticity, corticospinal tract, rubrospinal tract, spinal cord injury, genetic animal model, Celsr3.

INTRODUCTION

Motor control and sensory processing are regulated by cerebral cortical activity via descending and ascending pathways. Corticospinal (CST) and corticobulbospinal tracts play a key role in relaying information from the cerebral cortex to the spinal cord (Lemon, 2008). Skilled movement develops following refinement of corticospinal connections, such as pruning of transient ipsilateral branches that project to inappropriate regions, a process which is complete about ten days after birth in rat (Joosten et al., 1992; Oudega et al., 1994; Eyre, 2007). Besides innervating different segments of the spinal cord, corticospinal neurons also send collateral branches to the neostriatum, red nucleus, pontine nuclei, inferior olivary nuclei and pontomedullary reticular formation (Terashima, 1995; ten Donkelaar et al., 2004). In rodents, corticospinal axons synapse on spinal interneurons, with few, if any, direct connections with motor neurons (Schieber, 2007). It has been proposed that the CST exerts an activity-dependent influence over spinal circuits (Chakrabarty et al., 2009).

CST impairment is a leading cause of motor dysfunction in spinal cord injuries (SCI), amyotrophic lateral sclerosis (ALS) and cerebral palsy. In rodents, damage to the CST can induce restructuring of spinal networks, and this is thought to contribute to the recovery of motor function (Tillakaratne et al., 2010; Ueno et al., 2012). The rubrospinal tract (RST) can partially palliate CST dysfunction (Whishaw et al., 1998; Belhaj-Saif and Cheney, 2000; Kanagal and Muir, 2009; Yeo and Jang, 2010), and the reticulospinal tract (ReST) can compensate the transmission of motor commands after the incomplete CST injury (Umeda et al., 2010; Zaaimi et al., 2012).

Although the cerebral cortex is considered a key center to process motor control and sensation, neonatal or adult rodents with a complete removal of the neocortex maintain some motor function (Bjursten et al., 1976; Kolb and Whishaw, 1981a,b). How much the absence of cortical control affects the maturation of the motor-related network is therefore not fully understood.

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Abbreviations: ALS, amyotrophic lateral sclerosis; CB, calbindin; ChAT, choline acetyl transferase; CR, calretinin; CST, corticospinal tract; DH, dorsal horn; EMG, electromyogram; GFAP, glial fibrillary acidic protein; 5-HT, serotonin; NF200, neurofilament 200; NMJ, neuromuscular junction; PKCγ, Protein Kinase C gamma; PV, parvalbumin; Reln, reelin; ReST, reticulospinal tract; RST, rubrospinal tract; SCI, spinal cord injuries; TH, tyrosine hydroxylase.

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We previously showed that the conditional inactivation of the atypical cadherin *Celsr3* in the forebrain results in early genetic ablation of all cortico-subcortical connections (corticospinal, corticobulbar, corticopontine, corticostriate), as well as thalamocortical, corticothalamic tracts and several hippocampal projections (Zhou et al., 2008, 2009, 2010; Feng et al., 2012). Here, we used the *Celsr3/Foxg1* mouse model to study spinal motor networks in the absence of cortical control.

EXPERIMENTAL PROCEDURES

Mutant mice

Animal procedures were carried out according to guidelines from, and approved by competent ethics committees at Jinan University. The production of mice with regional inactivation of *Celsr3* and control animals was described before (Zhou et al., 2008). Briefly, we crossed [Celsr3 + /−; Foxg1-Cre] males with [Celsr3f/f] females, in which exons 19 to 27 of *Celsr3* were flanked with *loxP* sites, to generate [Celsr3f/−; Foxg1-Cre] mice (*Celsr3/Foxg1* for short). *Celsr3* is inactivated in Foxg1-positive cells in those mutants. The Thy1 transgenic line (Feng et al., 2000) was used to follow some tracts such as the CST, RST and ReST. Male or female animals were used indiscriminately.

Behavioral study (Open-field test)

Spontaneous motor activity was measured in an open field made of transparent Plexiglas panels (50 × 50 × 35 cm) with an open top, under bright illumination (500 lux). Each mouse was placed in the corner of a compartment and ambulation was recorded with a video camera for 15 min. In each group, six to nine mice (aged between postnatal days 17 to 21, P17–21) were studied for three continuous days. During the 15-min test, the total moving distance and the frequency of crossing the center of the compartment were analyzed using the EnthoVision XT 7.0 software (Noldus, Netherlands).

Histology and immunohistochemistry

Five-μm-thick paraffin sections were stained with Cresyl Violet (0.1% in H_2O) to assess neuronal density and architectonics, and with Luxol Fast Blue (0.1% in H_2O) to visualize myelin sheaths. To visualize the CST, RST and ReST, we prepared 50-μm sagittal (including the midbrain, pons and medulla) and transverse sections (C5–C8) from control and mutant mice that expressed the Thy1-YFP protein; pictures were taken with a fluorescence microscope (Leica, DM6000B, Germany). For immunohistochemistry, paraffin or frozen sections were incubated with the following primary antibodies: rabbit anti-Protein Kinase C gamma (PKCγ, 1:200, Abcam), rabbit anti-serotonin (5-HT, 1:2000, Sigma), rabbit anti-tyrosine hydroxylase (TH, 1:500, Millipore), mouse anti-neurofilament (2H3, 1:1000, Developmental Studies Hybridoma Bank), rabbit anti-neurofilament 200 (NF200, 1:1000, Millipore), goat anti-choline acetyl transferase (ChAT, 1:500, Millipore), rabbit anti-glial fibrillary acidic protein (GFAP, 1:3000, Abcam), mouse anti-parvalbumin (PV, 1:1000, Millipore), mouse anti-calbindin (CB, 1:3000, Sigma), rabbit anti-calretinin (CR, 1:400, Invitrogen), mouse anti-reelin G10 (Reln, 1:2000, provided by Andre Goffinet). 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). Alpha-bungarotoxin conjugated to Alexa Fluor 546 (α-BT, 1:200, Invitrogen) was used to label acetylcholine receptors of neuromuscular junctions (NMJs).

Cell and fiber density

Serial transverse sections from C5–8 were divided into six series of alternating adjacent sections from all segments. For example, one C5–C8 sample was sectioned into 84 adjacent sections. The first series contained sections 1, 7, 13, . . . , 79. The second contained sections 2, 8, 14, . . . , 80, with a total of six series. In each animal, one series of sections were immunostained with one antibody and the mean cell counts from all sections was taken as one sample. Six animals were used in each group. Motor neurons were identified in transverse sections of C5–8 segments, based on their morphology and location, as reported (Barber et al., 1984). ChAT-positive cells were classified into two types: motor neurons located in the ventral horn, with large cell bodies, and interneurons located around the central canal. In each section, we counted all ChAT-positive motor neurons in both ventral horns, and used the mean to estimate motor neuron density in the ventral horn. ChAT-positive interneurons located around the central canal, were counted in each section. As CB immunoreactivity was highly concentrated in lamina II of the dorsal horn (DH) (Ren and Ruda, 1994), we measured the CB-immunoreactive area and calculated the cell density in the region. The width of the CB-positive area was assessed along the dorsal-to-ventral axis. CR-positive interneurons were classified into two groups: one in the dorsal horn with small cell bodies (CR-DH), and another one in the ventral horn, with large cell bodies (CR-VH), as reported (Ren and Ruda, 1994). We measured the CR distribution area in the DH and calculated the cell density. For the CR-VH, we captured areas in the gray matter with a 20× objective and calculated the cell density. PV and Reln-positive cells were distributed in the gray matter and their cell density was calculated using the same method. For pixel-based gray analysis of GFAP-positive cell density, pictures of the ventral horn region were captured with a 40× objective from one series of sections stained with GFAP immunohistochemistry, and staining density were estimated with Image J. TH and 5-HT immunoreactive fibers were mainly distributed in the three areas of the gray matter, the ventral horn, the intermediolateral column and the DH (Gimenez y Ribotta et al., 1998). However, 5-HT-positive fibers were particularly concentrated in the ventral horn, and TH-positive fibers in the intermediolateral column. In each section, we selected one field (40× objective) in the ventral horn for 5-HT fibers, and in the intermediolateral