SPINAL CORD MATURATION AND Locomotion in Mice with an Isolated Cortex

Q. HAN,1 J. FENG,1 Y. QU,1 Y. DING,1 M. WANG,1 K.-F. SO,1,2,3 W. WU,1,2,3 AND L. ZHOU1,2,3

1Guangdong-Hongkong-Macau Institute of CNS Regeneration, Jinan University, Guangzhou 510632, PR China
2Department of Anatomy, The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region, PR China
3State Key Laboratory of Brain and Cognitive Sciences, The Guangdong University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region, PR China

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. © 2013 IBRO. Published by Elsevier Ltd. All rights reserved.

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 oliveary 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 restructing 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; Kanagai 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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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/f; 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 H2O) to assess neuronal density and architectonics, and with Luxol Fast Blue (0.1% in H2O) 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 (PKCy, 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 Reelin-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...
region for TH fibers, and their density was assessed by using a 10 × 10-mm reticle positioned in the eyepiece of the microscope and by counting intersections of fibers with two sides of the reticle (Rozsa et al., 1983).

**Electromyogram (EMG) of biceps brachii**

P20 mice were deeply anesthetized with ketamine (80 mg/kg). The musculocutaneous nerve and biceps brachii were dissected under a stereomicroscope. A stimulating bipolar electrode was placed on the musculocutaneous nerve, and a recording electrode was inserted into the middle of the muscle belly of biceps brachii at a depth of 0.2 mm. The same negative stimulating current (0.05 mA, 0.5 ms, 1 Hz frequency) was used in all animals. EMG signals were collected with a multi-channel signal acquisition and processing system (RM6240BD, Chengdu, China). Individual responses were measured three times, at 2-min intervals. Signals were amplified, bandpass filtered on-line and digitized at 2 kHz for off-line analysis, and used to measure the peak-to-peak motor amplitude of evoked potentials. Seven mice were used in each group.

**Golgi stain and morphological analysis of motor neurons**

Golgi–Cox staining was carried out on 150-μm-thick frozen sections at the level of the cervical enlargement (C5–T1), using the FD Rapid GolgiStain Kit (FD NeuroTechnologies, USA) according to the manufacturer’s protocol. For each genotype, 45 well-individualized, well-impregnated large neurons in the ventral horn were selected (Mannen, 1975). Sequential sections (1392 × 1040 pixels) were captured (20× objective) at 1-μm intervals using an upright microscope (Leica, DM6000B, Germany). Sequentially scanned sections of each selected neuron were deconvolved using AutoQuantX (Media Cybernetics, USA) to decrease noise prevalent in confocal images and improve the signal-to-noise ratio. All dendrites were traced manually from start to end, and the length and number of dendrites were calculated using the Imaris software (BitPlane AG, Switzerland).

**Electron microscopy**

Mice were deeply anesthetized with ketamine (80 mg/kg) and perfused intracardially with a solution containing 2% glutaraldehyde and 2% paraformaldehyde in 0.15 M phosphate buffer, pH 7.4. One millimeter distal segments of the musculocutaneous nerves were dissected out, kept in the same fixative overnight at 4 °C and then postfixed in 0.5% osmium tetroxide for 1 h. Following dehydration and embedding (EMbed 812, Electron Microscope Sciences), small samples (about 1 mm³) were glued on resin blocks with Crazy glue (Borden Inc., USA). Areas of interest were defined in 1-μm-thick Toluidine Blue-stained sections. Sections were collected on 200 mesh grids, and stained with 2% uranyl acetate for 30 min and 1% lead citrate for 15 min. Images were captured with a Philips 400 electron microscope. Three animals were used in each group.

**Statistics**

Data are expressed as mean ± SEM. Independent samples t-test was performed using SPSS 17.0 software. Levene’s Test was used for the equality of variances as the level of α = 0.05. When equal variances could be assumed (P > α = 0.05), the two-independent samples t-test was used for the equality of means; otherwise, the approximate t-test was used. P values < 0.05 and < 0.01 were considered significant (+) and highly significant (+/+), respectively.

**RESULTS**

**Celsr3/Foxg1 mice are able to perform some movements and are hyperactive**

Celsr3/Foxg1 mutant mice look severely hypotrophic and do not survive later than P21. They remain active until their sudden death, the cause of which is unclear, and they are able to eat food pellets, drink, swim and climb on grids, suggesting that they maintain some skilled movement. The open-field test was used to measure their spontaneous locomotion. Both control and mutant mice preferred to walk in the peripheral areas of the open-cage and they crossed the center occasionally, but Celsr3/Foxg1 mice were clearly hyperactive (Fig. 1A, B). During 15-min tests, the walking distance was 43.01 ± 2.59 m in the control versus 89.38 ± 18.11 m in the mutant (P < 0.01, n = 6), showing a significant increase in mutants (Fig. 1C). As we reported before, Celsr3/Foxg1 mice could swim (Feng et al., 2012). We counted the number of paddling mice during swimming. It was 6.49 ± 1.50 in control, versus 5.00 ± 1.06 times/s in mutant mice (n = 5), showing no significant differences. Furthermore, mutant mice could climb on grids (Movie 1). Thus, some basic

**Fig. 1.** Celsr3/Foxg1 mice are more active than control mice. In open-field tests, all mice preferred to walk in peripheral areas of the cage (A, B). Moving distance within 15 min showed a significant increase in Celsr3/Foxg1 mice compared to littermate controls (C). **P<0.01; n = 6.**
motor skills are preserved, but the locomotor rhythm is disrupted, with hyperactivity, following the absence of sensory and motor connections with the cortex.

**Abnormal network in the spinal cord of Celsr3/Foxg1 mice**

In addition to extrapyramidal tracts such as the RST, the CST projects directly to the spinal cord and is involved in fine movement control. Using PKCγ as a marker for corticospinal axons (Mori et al., 1990; Miki, 1996), the CST was visible in the posterior funiculus of the wildtype but not mutant spinal cord (Fig. 2A, B). Using the Thy1-YFP transgene, red nuclei, RST bundles, reticular nuclei and reticulospinal axons were well labeled in sagittal sections of the brain stem (Fig. 2C, D), and the RST and the ReST were visible in transverse sections of the cervical enlargements (Fig. 2E, F). In contrast, the CST was completely absent in Celsr3/Foxg1 mice (Fig. 2C–F).

As mutant mice died around P20, we could not quantify projecting fibers from red nuclei and pons by in vivo tracing. We measured the fluorescent intensity of Thy1-YFP in coronal spinal sections and found that the size of the ReST was increased in the mutant (Fig. 2O), but the size of the RST displayed a big variation from mice to mice with this method (data not shown). Thus, movement in Celsr3/Foxg1 mice is not primarily controlled by the cortex but by red nuclei, reticular nuclei and presumably other supraspinal descending tracts.

Midbrain dopaminergic and raphe serotonergic neurons send axons to all levels of the spinal cord and are known to modulate spinal locomotor rhythms (Holstege et al., 1996; Schmidt and Jordan, 2000; Qu et al., 2006). The hyperactivity of Celsr3/Foxg1 mice prompted us to study TH-positive and serotonergic fibers and terminals at spinal cervical levels C5–8, using immunohistochemistry. Dot-like TH immunoreactivity was found scattered in the white matter, presumably corresponding to axons descending to lower spinal segments. Transversal fibers were visible in the ventral gray matter, mostly concentrated in the intermediolateral column (Fig. 2G, G'). In Celsr3/Foxg1 mice, axon terminals in the intermediolateral column had a similar distribution but were more abundant than in control samples (Fig. 2H, H'). Furthermore, profuse serotonergic fibers were mainly present in the ventral horn, and their number was significantly increased in Celsr3/Foxg1 animals compared to control mice (Fig. 2I, J, P). Inasmuch as dopamine increases the excitability...
of spinal motor neurons (Han et al., 2007) whereas 5-HT exerts different actions on spinal locomotor networks via different receptors (Beato and Nistri, 1998; Schmidt and Jordan, 2000; Kao et al., 2006), changes in TH-positive and serotoninergic fiber density might contribute to the hyperexcitability of motor neurons in Celsr3/Foxg1 mice.

In addition, we observed fiber distribution using anti-neurofilament (2H3) IHC, and axon myelination with Luxol Fast Blue staining, in transverse sections of C5–C8 segments. In both genotypes, neurofilament immunoreactivity was mainly concentrated in the white matter (Fig. 2 K, L), with no differences between genotypes (data not shown, n = 3 in each group). Myelinated fibers also looked similar in both genotypes (Fig. 2 M, N).

Altered motor neuron maturation and gliosis in the cervical enlargement of Celsr3/Foxg1 mice

Forelimb movement is controlled by motor neurons of the ventral horn in cervical enlargements, which receive input from corticospinal neurons via the CST, red nuclei in midbrain, and reticular and vestibular nuclei in the medulla and pons (Hobbelen et al., 1992). In coronal Nissl-stained sections at the level of cervical enlargements (C5–8), large motor neurons were readily identified and displayed a similar distribution in a “butterfly” shape, but were less numerous in Celsr3/Foxg1 than in control mice (Fig. 3 A, B). Using anti-ChAT immunohistochemistry to label motor neurons (Fig. 3 C, D), we observed an 18% reduction in their number in Celsr3/Foxg1 compared to control samples (P < 0.01, Fig. 3 I). In the control spinal cord, GFAP immunoreactivity showed a graded expression, higher in the white matter and midline, and lower in the gray matter (Fig. 3 E). This difference was blunted in Celsr3/Foxg1 mice (Fig. 3 F), in which the density of GFAP-positive cells was significantly increased in the gray matter (Fig. 3 G, H, J), presumably indicating reactive gliosis following motor neuron degeneration. We studied dendritic arbors of motor neurons using Golgi–Cox staining at C5–8 levels (Fig. 4 A–C). Although axons are not stained by Golgi–Cox impregnation, large motor neurons in the ventral horn could be identified as reported (Mannen, 1975). Compared to control motor neurons, the total dendrite length was decreased in Celsr3/Foxg1 cells (P < 0.01, Fig. 4 D), but no difference was noted in branch number (P > 0.05, Fig. 4 E). This suggests that the survival and the dendritic maturation of spinal motor neurons are partly dependent on the direct and/or indirect input from the cortex.

Altered NMJ maturation and function in Celsr3/Foxg1 mice

Celsr3/Foxg1 mice looked smaller from birth and their mean body weight at P18 was 4.10 ± 0.23 g versus 10.48 ± 0.17 g for controls (n = 10). Mutant skeletal muscles were severely atrophic, with the weight of the biceps brachii about 60% of that in control mice (Fig. 5 A–D). This prompted us to study whether the motor innervation of muscles is affected in our mutants. To study NMJs, we examined postsynaptic AchRs clustering with α-BT staining, and motor axon terminals with anti-neurofilament staining. Although musculocutaneous nerves normally innervated biceps brachii in both genotypes, the terminal axonal arbors were diminutive in mutants, with 4.50 ± 0.48 terminals in wildtype samples, versus 2.00 ± 0.36 in mutants (Fig. 6 A, B, n = 40 NMJs in each group). With α-BT staining AchRs (Fig. 6 C, D), the total number of AchR clusters was counted in each biceps brachii, which was 592.00 ± 80.61 clusters/muscle in the mutant versus 663.50 ± 31.82 clusters/muscle in the control, showing no significant difference (P > 0.05, n = 3 mice in each group).
group). Nerve terminals contacted NMJs similarly in both genotypes (Fig. 6E, F). In mutants, using TEM analysis of musculocutaneous nerves, we found that many myelin sheaths were deformed and internalized, and that the mean perimeter of axons was increased, but the thickness of myelin sheaths was comparable to wildtype, possibly indicating incipient axon degeneration (Fig. 5E–H). To assess NMJ function, we recorded EMG from the biceps brachii upon stimulation of musculocutaneous nerves. The peak-to-peak EMG amplitude was reduced by 47% in Celsr3/Foxg1 mice compared to controls (Fig. 5I–L; $P < 0.01$, $n = 7$ in each group).

Abnormal development of spinal interneurons in Celsr3/Foxg1 mice

In rodents, corticospinal axons synapse directly with spinal interneurons (Schieber, 2007), whereas sensory information processing is dependent on different sets of spinal interneurons (Bardoni et al., 2013). Our mutant with isolated cortex provides the opportunity to test whether the absence of cortical connections has any influence on spinal interneurons. To address this, we carried out immunohistochemical studies of the established interneuron markers – CB, ChAT, CR, PV and Reln, all of which were detected in wildtype and mutant C5–8 spinal cords, with comparable distribution patterns, with the exception of CB-positive ones (Fig. 7). CB-positive interneurons were concentrated in lamina II; their density was significantly increased and they were more widely scattered along the dorsal-to-ventral axis in mutant than in control samples ($P < 0.01$, $n = 6$, Fig. 7A, B, M). Although cholinergic motor neurons were reduced in number in mutants as described above, the number of cholinergic interneurons (ChAT-INs) around the central canal was significantly higher in the mutant than the control spinal cord ($P < 0.01$, Fig. 7C, D, N). CR-positive interneurons were identified as two groups: small cells located in the DH, laminae II–III (CR-DH), and large neurons widely scattered in the ventral horn (CR-VH) (Fig. 7E–H). The number of small, but not large CR-interneurons was increased in the mutant compared to the control ($P < 0.01$, $n = 6$; Fig. 7M). No significant changes were noted in PV- and Reln-positive interneurons (Fig. 7I–L, M). These data suggest that the maturation of CB-, CR- and cholinergic interneurons is influenced by corticospinal connections.

DISCUSSION

The absence of CST and thalamocortical connections in Celsr3/Foxg1 mutant mice allowed us to study spinal cord development in the absence of cortical control. Our results show that, although the cortex is functionally less critical for movement control in rodents than in humans, it nevertheless has profound influences on the maturation of motor neurons and motor terminals, as well as on some spinal interneuronal networks.

In rodents, CST axons travel in the dorsal funiculus and connect with propriospinal and segmental interneurons, thereby regulating the activity of motor neurons indirectly (Alstermark and Ogawa, 2004; Umeda et al., 2010). Following SCI, some locomotor ability can be restored, but the supraspinal pathways are indispensable for skilled activities, such as the ability to grip or manipulate food (Anderson et al., 2005). Besides the CST, cortical motor neurons send projections to the brainstem, which can also modulate limb functions (Riddle and Baker, 2010; Fisher et al., 2012; Soteropoulos et al., 2012). For instance, the reticulospinal system is thought to mediate some functional recovery following corticospinal lesions (Riddle et al., 2009; Umeda et al., 2010; Zaaimi et al., 2012). In Celsr3/Foxg1 mutants, cortico-subcortical connections are completely absent from early developmental stages, providing a unique animal model of the isolated cortex, so that the spinal cord and brainstem never receive cortical inputs (Zhou et al., 2008, 2009, 2010), yet mutant mice retain the ability to...
move and perform some movements such as walking, swimming and gripping. This suggests that, unlike in human in whom the CST is critical for skilled movements, other supraspinal pathways or the propriospinal network itself overtakes CST function in mice. Compensatory regeneration of rubrospinal axons plays a key role in movement recovery in SCI models (Webb and Muir, 2003; Pettersson et al., 2007; Kanagal and Muir, 2009). The RST and the ReST are preserved in Celsr3/Foxg1 mice, suggesting that they may serve compensatory function.

The most evident motor trait in Celsr3/Foxg1 mutants is their hyperactivity. Rats with complete removal of the neocortex in infancy or adulthood were also reported to display increased activity, as measured in running wheels (Kolb and Whishaw, 1981a,b). Several populations of spinal ventral interneurons, including Ia inhibitory interneurons and Renshaw cells, provide major inhibitory input to motor neurons (Alvarez et al., 2005; Wilson et al., 2010). In general, corticospinal axons make direct connections with common spinal inhibitory interneurons, which are thus activated by corticospinal inputs (Alstermark et al., 1984). Lacking the corticospinal innervations in our mutants might weaken the inhibitor effect of interneurons and therefore...
generate some increase in motoneuron activity. Our genetic model provides evidence that motor rhythms are inhibited by direct or indirect cortical input. As DA midbrain afferents increase locomotor activity (Zhao et al., 2007), and DA can boost excitability by decreasing the first spike latency of spinal motor neurons (Han et al., 2007), the observed increase of TH-positive terminals may contribute to hyperactivity. Serotoninergic neurons from the raphe pallidus, raphe obscuris and raphe magnus also send long axons that terminate at all spinal levels. Depending on receptor expression, serotoninergic fibers can inhibit or stimulate the activity of locomotor networks (Schmidt and Jordan, 2000), making it difficult to assess whether the increase in 5-HT terminal density in Celsr3/Foxg1 mutants may contribute to hyperactivity. Altogether, our data suggest that the defect in corticospinal innervation upsets a delicate balance between excitatory and inhibitory stimuli, thereby probably leading to hyperactivity.

We found that Celsr3/Foxg1 mice harbor significant motor neuron anomalies. The number of motor neurons is decreased; they have atrophic dendritic trees with decreased numbers of axon terminals at NMJs, and this affects electrophysiological NMJ function. Quite similarly, upon muscimol-induced silencing of corticospinal neurons at postnatal stages, a decreased number of cholinergic neurons was found in the spinal cord (Chakrabarty et al., 2009), indicating a trophic action from corticospinal inputs on the survival and maturation of spinal motor neurons. The arrival of corticospinal axons to the spinal cord and corticospinal synaptogenesis are complete by the end of the second postnatal week, a developmental window period accompanied with changes of gene expression, such as c-Jun, and of neural activity (Clowry et al., 2004). Activity-dependent release of BDNF or NT3 may affect the survival and maturation of spinal motor neurons (Giehl, 2001). In our mutant, the absence of CST may therefore impact motor neuron maturation.

In cat, CST terminals contact various types of interneurons and exert an activity-dependent influence over the maturation of interneuronal circuits (Chakrabarty et al., 2009; Chakrabarty and Martin, 2010). ChAT-interneurons make c-bouton contacts with motor neurons where they act on muscarinic receptors (Hellstrom et al., 2003; Miles et al., 2007). Their number increases in parallel to the refinement of CST terminals (Chakrabarty et al., 2009), and the CST exerts oligosynaptic influences on motor neurons via ChAT interneurons. In the absence of CST, we found an increase of ChAT interneurons around the central canal, which might be involved in motor compensation. Perhaps, when motor neurons are deprived of CST inputs, connections between ChAT interneurons and motor neurons are strengthened. More electrophysiological studies in vivo would be required to test this hypothesis further. CB, CR and PV have differential expression patterns in the spinal cord (Ren and Ruda, 1994). CB-positive interneurons are scattered in the spinal cord gray matter before

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**Fig. 7.** CB-, ChAT- and CR- positive spinal interneurons are increased in Celsr3/Foxg1 mice. In C5-8 paraffin sections, CB immunoreactivity was distributed in the substantia gelatinosa in normal mice (A), with wider expression in the mutant (B). ChAT-positive interneurons (ChAT-INs) were distributed around the central canal (C, D). CR-positive cells were classified into two groups: small cells located in the substantia gelatinosa (CR-DH; E, F) and large cells in the ventral horn (CR-VH; G, H). PV and Reln-positive cells were present in the gray matter (I–L). CB, ChAT and CR-DH-positive interneurons were significantly increased in Celsr3/Foxg1 mice compared to littermate controls (M, N). Circled areas in A and B showed CB immunoreactive regions in the dorsal horn. Boxed areas in G', I and K' correspond to panels G and H, I and J, K and L, respectively. *P < 0.01, n = 6 mice in each group.
maturation of CST axons. During maturation, they decrease in number and are found mostly in superficial laminae, a phenomenon which is proposed to accompany the innervation shift of CST axons (Chakrabarty et al., 2009). In Celsr3/Foxg1 mice, CB-positive interneurons are more abundant and distributed more widely than in wildtype mice, possibly mimicking the immature state in the absence of CST innervation. A rather similar phenomenon was observed for CR-DH-positive interneurons. In contrast, the distribution and number of PV, CR-VH and Rein-positive interneurons were similar in both genotypes. This suggests that ChAT -, CB- and CR-DH interneurons respond to the absence of CST, perhaps due to decreased competition for postsynaptic innervations sites, which may be important for rewiring and compensation. Following a complete midthoracic spinal cord transection in neonatal rats, the reorganization of neuronal circuitry caudal to the injury plays a key role in recovering the stepping ability (Tillakaratne et al., 2010; Ichiyama et al., 2011). Whether this also happens in our mutants and how much this plasticity contributes to the locomotor phenotype needs to be studied further.

Finally, in the human disorder ALS, two hallmarks are the degeneration of lower spinal motor neurons as well as corticospinal “upper” cortical neurons, a combination which is modeled in our mutant. Our analysis of consequences of CST ablation on spinal motor neurons and aminergic innervation may prove relevant to the study of spinal cord plasticity in ALS.

CONCLUSIONS

Mice maintain basic movements in the genetic absence of corticospinal connections, but the integrity of corticospinal projections is required for locomotor rhythm. In mice with an isolated cortex, dopaminergic and serotonenergic projections to the spinal cord are affected and the locomotor networks cannot mature fully.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neuroscience.2013.08.057.