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Postnatal Development of the Corticospinal Tract in the Reeler Mouse

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ABSTRACT

Corticospinal tract (CST) neurons are dislocated in the motor cortex of Reelin-deficient mouse, *reeler*. In the present study, we examined whether postnatal axonal growth arising from these dislocated CST neurons are normal or not with use of anterograde tracer, DiI and retrograde tracer, HRP. A single injection of DiI into the motor cortex of the normal and *reeler* mice was made during postnatal period and 8-24 hours later, the animals were sacrificed to examine DiI-labeled CST axons at the lower medulla and spinal cord. Both in the normal and *reeler* mice, CST axons arrived at the pyramidal decussation and entered into the contralateral spinal cord around on postnatal day (P) 0.5, and descend in the ventral area of the contralateral dorsal funiculus at C2 level on P2, at C8 level on P3, at the mid-thoracic level on P4, and at the upper lumbar level on P8. The similar results were also demonstrated by the retrograde labeling of CST neurons with injection of HRP into the C1 level or upper lumbar enlargement. Next, we examined CaMKII α expression in the CST axons of the adult normal and *reeler* mice. CaMKII α -immunopositive fibers were recognized throughout the CST pathway from the internal capsule to the dorsal funiculus of the spinal cord both in the normal and *reeler* mice. The present study has demonstrated that ectopic location of cell bodies of *reeler* CST neurons do not affect postnatal development of CST axons in the spinal cord.

INTRODUCTION

Corticospinal tract (CST) neurons are located in layer V of the motor cortex, and send their axons to the spinal motoneurons, directly (5, 23) or indirectly (2, 35). The CST forms the longest axonal projection in the mammalian central nervous system (CNS). The development of CST axons is the latest among descending and ascending spinal tracts, and in the rodents, this outgrowth occurs during the first and second postnatal weeks (9, 12, 13, 27), which enables us to find molecules concerning to path-finding and target recognition of neural circuits in the mammalian CNS (4). For example, severe defects in the development of CST are caused by cell adhesion molecule L1 (17), transcriptional factor Fezf2 (10, 16), and neural recognition molecule NB-3 (13).

The *reeler* mouse, a spontaneously occurring mutant mouse, is caused by mutated Reelin gene (6). In this mutant mouse, cytoarchitecture of the cerebral neocortex is roughly inverted: layer VI neurons occupy the uppermost layer just beneath the pial surface, and layer II and III neurons are located in the lowermost cortical layer (15, 34). In this mutant mouse, CST neurons are radially scattered in the motor cortex instead of distributing in layer V (34). In the *reeler* mouse, the course and termination of adult CST axons and their collaterals to the subcortical nuclei are identical to their normal counterparts, suggesting that radially malpositioned CST neurons can project to the subcortical nuclei in a similar manner of the normal counterparts (30). However, in this mutant mouse, there are only limited data for development of CST axons during early postnatal weeks.

Thus, in the present study, we aimed to examine whether *reeler* CST axons normally develop or do not during the early postnatal days with use of anterograde and retrograde tracers. In addition, alpha subunit of Ca²⁺/calmodulin-dependent protein kinase II (CaMKII α) is expressed by CST axons both in the rodents and primates (7, 20, 32), and therefore, we have examined CaMKII α immunoreactivity in *reeler* CST axons to elucidate whether chemical specificity of CST axons is normally developed in this mutant or not.

MATERIALS AND METHODS

1. Mice

The classical *reeler* heterozygous (*Reln*^{rl/+}) mice were originally purchased from the Jackson laboratory (Bar Harbor, Me) and raised in our animal facility. The *reeler* mutant mice (*Reln*^{rl/rl}) and littermate wild type control (*Reln*^{+/+}) were obtained from mating with *reeler* heterozygous male and female mice. All animals were housed in temperature-controlled (22 ± 0.5 °C) colony room with a 12-hour light/dark cycle in groups in acrylic cage with free access to food and water. All experiments were carried out with the approval of the Committee on Animal Care and Welfare, Kobe University Graduate School of Medicine.

2. Tracer Injections

(1) Anterograde labeling: For anterograde labeling of CST axons, we used 1,1'-diiodo-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI; Molecular Probe, D-282). The numbers of the animals used in the present DiI study were as follows: 2 normal and 4 *reeler* on P0.5, 3 normal and 5 *reeler* on P1, 2 normal and 2 *reeler* on P2, 2 normal and 3 *reeler* on P3, 3 normal and 3 *reeler* on P4, 3 normal and 4 *reeler* on P5, 3 normal and 4 *reeler* on P7, 2 normal and 4 *reeler* on P9/ P10. The experimental animals were anesthetized with 3.5% chloral hydrate by intraperitoneal injection and clamped in a stereotactic apparatus with auxiliary devices. Following an incision of skin, a small bur hole was made in the left parietal bone using a dental drill. A single injection of 0.1 µl of 10% DiI solution dissolved in N,N-dimethyl formamide was made in the left motor cortex by pressure through a glass pipette attached to the Hamilton syringe. After 8-24 hours, the animals were anesthetized with 10% sodium pentobarbital and transcardially perfused with a small volume of 0.1M PB, and then 4% paraformaldehyde in 0.1M PB for 15 min at 4°C. The brains were removed from the skull and stocked in the same fixative overnight at 4°C (post-fixation). The brains were embedded in 2% agarose, and coronally or horizontally sectioned at 50 or 100 µm thickness on a microslicer (Dosaka-EM, Kyoto, Japan). They were mounted on MAS-coated slides (Matsunami Glass Industry, Osaka, Japan), and coverslipped with FluoroGuard (Bio-Rad). Histological sections were examined under an Olympus AX80 microscope equipped with a FITC filter. The images were captured from the microscope equipped with a DP70 Digital CCD camera connected with a Windows computer using DP70 controller software (Olympus). To create photomicrographs, captured images were transferred to Macromedia Flash MX (Adobe).

(2) Retrograde labeling: For retrograde labeling of CST neurons, we used horseradish peroxidase (HRP, type VI). (Sigma-Aldrich, # F3378). The numbers of the animals used in the present HRP study were as follows: for cervical injection, 3 normal and 5 *reeler* on P6, and 5 normal and 7 *reeler* on P21, and for the lumbar injection cases, 2 normal and 3 *reeler* on P6, 3 normal and 4 *reeler* on P9, 1 normal and 3 *reeler* on P14, and 3 normal and 5 *reeler* on P21. All experimental animals were anesthetized with an intraperitoneal injection of 3.5% chloral hydrate (1ml/100g body weight) and clamped in a stereotactic apparatus (Narishige Co., Tokyo). The dorsal aspect of the upper cervical or upper lumbar cord was exposed after removal of vertebral arches and 0.1 µl of 10% HRP dissolved in distilled water was injected into both sides of the first cervical segment (C1) or upper lumbar enlargement via 1 µl Hamilton syringe under an operating microtome, as described previously (34). After 12-24 hours survival, the animals were deeply anesthetized with 3.5% chloral hydrate (1 ml/100 g body weight) and sacrificed by transcardial perfusion of 0.1 M phosphate buffer (PB; pH7.4) containing 0.9% NaCl (phosphate-buffered saline, PBS) for 5 min at room temperature, followed by a mixed solution of 1% paraformaldehyde and 1.25% glutaraldehyde in PB for 25 min at 4 °C, and then washed by buffered 10% sucrose solution for 15 minutes at 4°C. After perfusion the brain and spinal cord were immediately removed from the skull and vertebral canal, respectively, and immersed in 30% PB sucrose (pH 7.4) overnight at 4°C. The brains and spinal cords were then sectioned coronally, sagittally or horizontally at 40 µm thickness on a freezing microtome. The sections were reacted for presence of HRP by using chromogen tetramethyl benzidine (TMB) (24), mounted on glass slides pretreated with gelatin, and counterstained with 1% neutral red. Sections were dehydrated with graded ethanol, and cleared in xylene, and coverslipped. Spinal cords were cut horizontally at 40 µm thickness and reacted in diaminobenzidine (DAB) according to the method of LaVail *et al.* (21) to demonstrate the injection site. The atlas of Zilles (37) was used for definition of cortical areas.

In addition, we have examined whether the tangential distribution pattern of *reeler* CST neurons are similar to the normal counterpart or not. For this purpose, HRP was injected into the upper lumbar enlargement of the normal and *reeler* mice at the age of P19, and sacrificed one or two day(s) later with the perfusion of the same fixatives described above. The brains were removed from the skull, and immersed in 30% PB sucrose (pH 7.4) overnight at 4°C. To examine tangential distribution pattern of CST neurons, cerebral hemispheres of normal and *reeler* mice (2 normal, 3 *reeler*) were sandwiched between two slides, kept for several days, and flattened. The flattened cerebral hemispheres were frozen on the top stage of the freezing microscope, and serial tangential frozen sections of 50 µm thickness were made parallel to the pial surface of the flattened hemispheres. The

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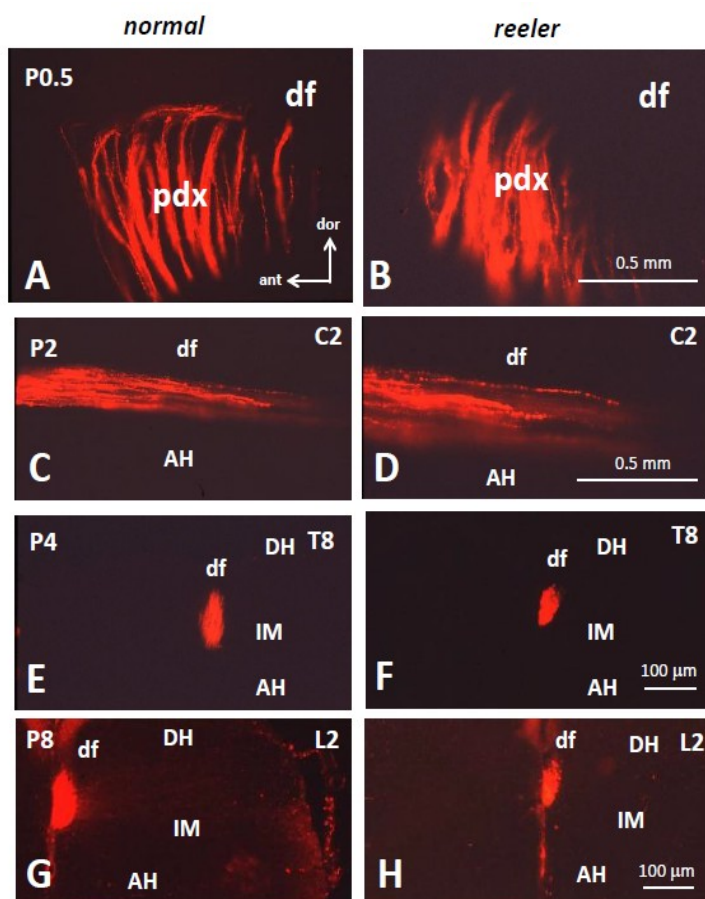


Figure 1. Anterogradely labeled corticospinal tract (CST) fibers with an injection of DiI into the motor cortex of normal (left column) and *reeler* (right column) 8 h-24 h before sacrifice. In the normal mouse, DiI-labeled CST fibers pass through the pyramidal decussation (pdx) and enter into the contralateral spinal cord on postnatal day (P) 0.5 (A), enter into the most ventral area of the dorsal funiculus (df) of the C2 level on P2 (C), and arrive at the T8 level on P4 (E) and the L2 level on P8 (G). In the *reeler*, DiI-labeled CST fibers develop in a similar manner of the normal counterparts (B, D, F, H). Sagittal (A-D) and coronal (E-H) sections. A-D: Dorsal (dor) is up, anterior (ant) to left. E-H: dorsal (dor) is up, lateral (lat) is to left. Scale bars: 0.5 mm (A-D), 100 μm (E-H).

sections were collected in 0.1M PB, and reacted by the TMB method, mounted on glass slides pretreated with gelatin, and counterstained with 1% neutral red, as described above.

3. CaMKIIα Immunohistochemistry

The adult normal and *reeler* mice at two months of age (3 normal, 5 *reeler*) were anesthetized by intraperitoneal injection of 3.5% chloral hydrate, as described above, and perfused transcardially with PBS at room temperature for 5 minutes, followed by a mixed solution of 4% paraformaldehyde and 7% saturated picric acid, buffered to pH 7.4 with PB, for 15 minutes. After perfusion, the brains and spinal cords were removed, and then cut coronally on a freezing microtome at a thickness of 50 μm. The sections were processed with

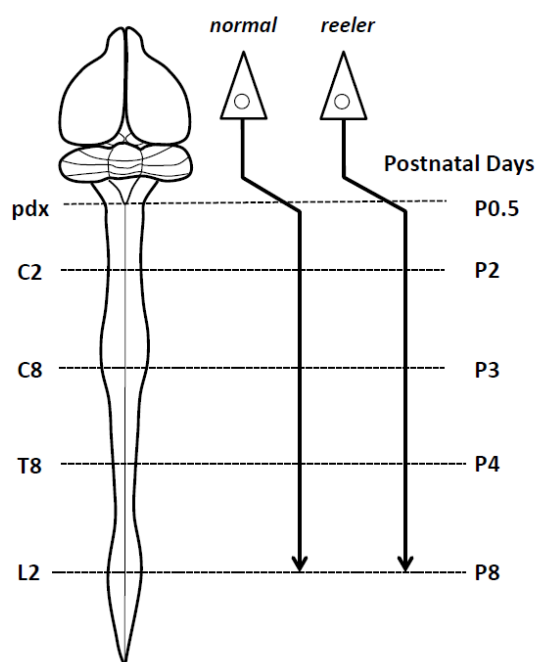


Figure 2. The schematic diagram shows the postnatal development of CST fibers both in the normal and *reeler* mice based on the anterograde DiI labeling. The time-course of development of CST axons is identical between the normal and *reeler* mice. Abbreviations are described in Figure 1.

monoclonal antibody specific for CaMKII α . The specificity of the present monoclonal antibody (6H7) was previously described in detail (32). Free-floating sections were incubated in anti-CaMKII α antibody (2 μ g/ml) for 1 hour at room temperature, followed by additional 16–40 hours at 4 °C. The sections were washed in PB (3 washes, 5 minutes each) and treated for 30 minutes with biotinylated horse anti-mouse IgG (1:200 dilution). They were then washed in PB (3 washes, 5 minutes each) and incubated for avidin-biotin peroxidase complex (ABC kit, Vector; 1:200 dilution). The reactive products were visualized by a 5–15 minute incubation with chromogen 3, 3'-diaminobenzidine (50 mg in 100 ml PB) and 0.01% hydrogen peroxide. The reaction was terminated by rinsing the sections in several changes of fresh PB. Sections were mounted on gelatin-coated slides, dehydrated with a series of graded ethanol, and cleared in xylene. Control sections were processed identically but without the addition of the primary antiserum.

RESULTS

1. Anterograde labeling of CST axons with DiI

Injection of DiI into the motor cortex resulted in anterograde labeling of CST axons and their collaterals both in the normal and *reeler* mice (Fig. 1). In the normal mouse, anterogradely labeled CST axons descended in the medullary pyramid ipsilateral to the injection site, and then entered in the pyramidal decussation on P0.5 (Fig. 1A). Two days later, *i. e.*, on P2, DiI-labeled axons entered in the ventral area of the dorsal funiculus of the C1 contralateral to the injection site and arrived at C2 level (Fig. 1C). On P4, the caudal end of DiI-labeled axons descended in the ventral area of the dorsal funiculus contralateral to the injection site. They arrived at the mid-thoracic level on P4 (Fig. 1E) and at the second lumbar segment (L2) on P8 (Fig. 1G). In the *reeler*,

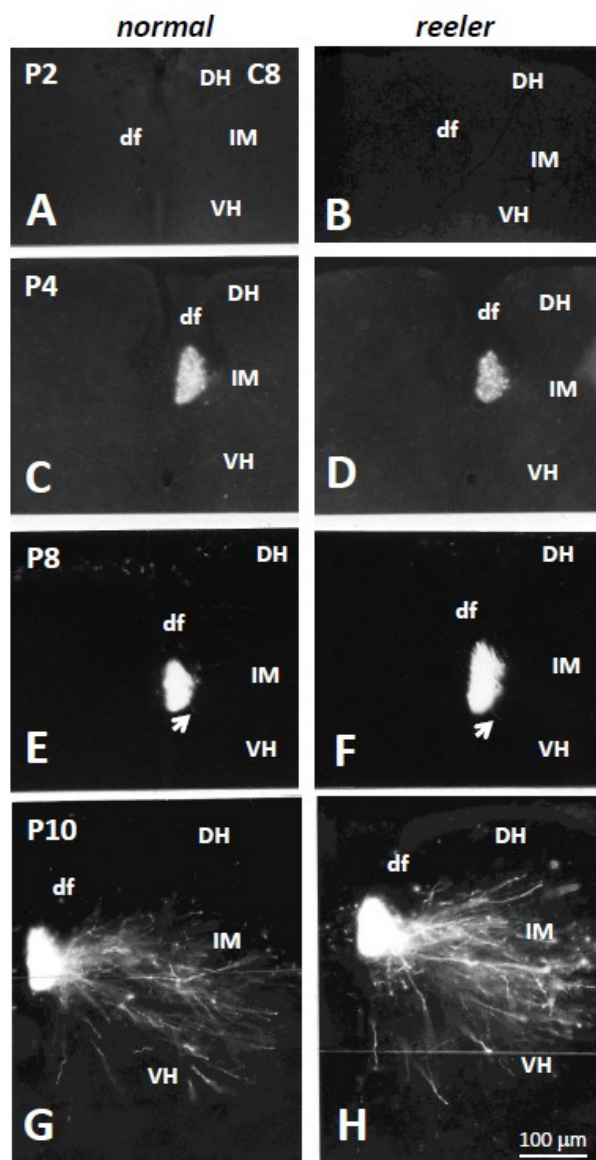


Figure 3. Formation of collateral branches from CST parent axons at the C8 level during postnatal days both in the normal (left) and *reeler* (right) mice. In the normal mouse, no labeled fibers are recognized on P2 (A). Many DiI-labeled fibers descend in the most ventral area of the dorsal funiculus on P4 (C) and then give off a short collateral branch from the side of the parent axon on P4 (arrow in E). These short collateral branches quickly elongate on P8 (E), and give off secondary and tertiary collateral branches into the intermediate substance and ventral horn on P10 (G). The similar time-course of the development of the CST collaterals is again recognized in the *reeler* (B, D, F, H). Scale bars: 100 μ m (A–H). Abbreviations: df, dorsal funiculus; DH, dorsal horn; IM, intermediate substance; VH, ventral horn.

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anterogradely labeled CST axons developed in a similar manner of the normal counterpart (Fig. 1B, D, F, H). The schematic illustration shows that the postnatal development of the *reeler* CST axons is quite similar to the normal counterpart (Fig. 2).

Next, formation of collateral branches sprouting from the parent CST axons was examined at C8 level. In the normal mouse, no labeled fibers were recognized on P2 (Fig. 3A). Many DiI-labeled fibers descended in the most ventral area of the dorsal funiculus and arrived at C8 level on P3, but no collateral sprouting was still identified at that age. DiI-labeled CST fibers at C8 level gave off a short collateral branch from the side of the parent axon on P4 (Fig. 3C). These collateral branches elongated and gave off many collateral branches into the intermediate substance and ventral horn on P8 (Fig. 3E, arrow). The collateral branches continued to elongate and formed secondary and tertiary collateral branches on P10 (Fig. 3G). The similar time course of development of CST collaterals was again confirmed in the *reeler* (Fig. 3B, D, F, H).

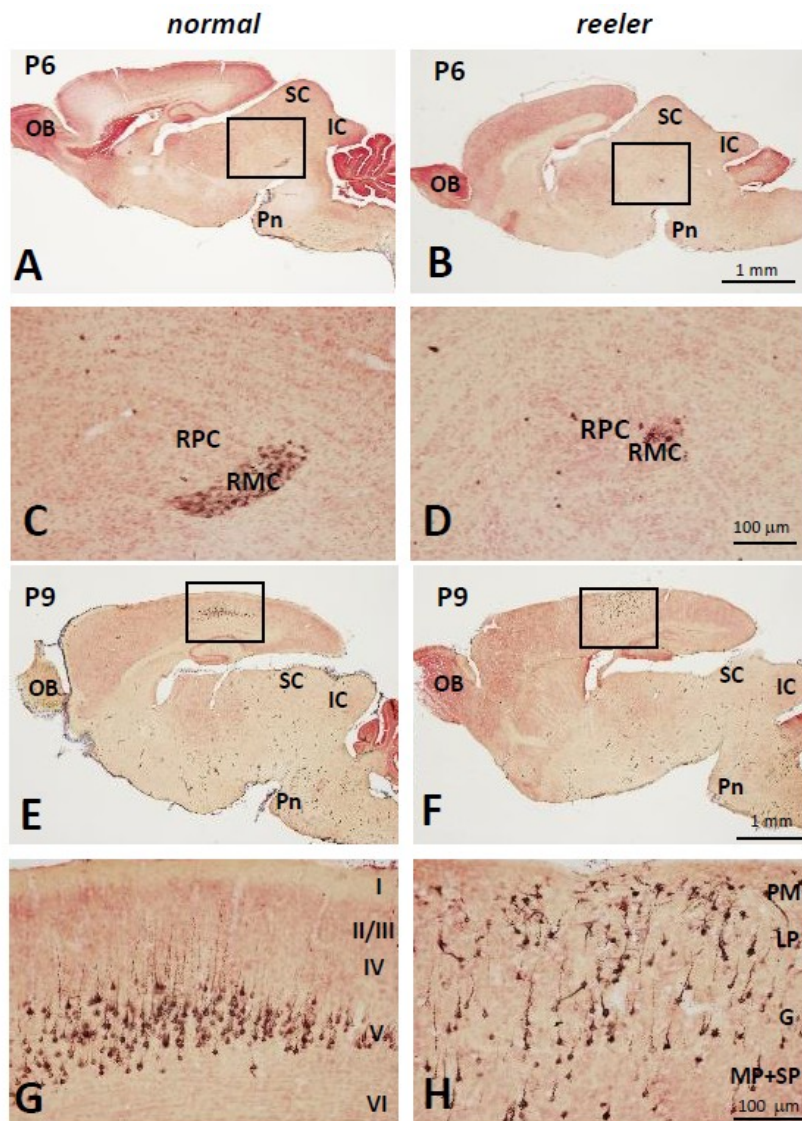


Figure 4. HRP is injected into the upper lumbar cord of the normal and *reeler* mice to label CST neurons both in the normal (left column) and *reeler* (right column) mice one day before sacrifice. **A-D:** No retrogradely labeled neurons are found in the cerebral cortex of the normal (**A**) and *reeler* (**B**) mice on P6. However, retrogradely labeled neurons are found in the ventro-caudal area of the nucleus ruber (magnocellular part; RMC) both in the normal (**C**) and *reeler* (**D**) mice. **E-F:** The similar injection of HRP results in retrogradely labeling of CST neurons in the hindlimb area of the motor cortex both in the normal (**E**) and *reeler* mice (**F**) on P9. Labeled CST neurons are exclusively located in layer V of the normal cortex (**G**), but radially scattered in the *reeler* cortex (**H**). **Other abbreviations:** I-VI, cortical layers I-VI; G, granule cell layer; IC, inferior colliculus; LP, large pyramidal cell layer; MP+SP, medium and small pyramidal cell layer; PM, polymorphic cell layer; RPC, parvocellular part of red nucleus; SC, superior colliculus. Scale bars: 1 mm (**A, B, E, F**), 100 μ m (**C, D, G, H**).

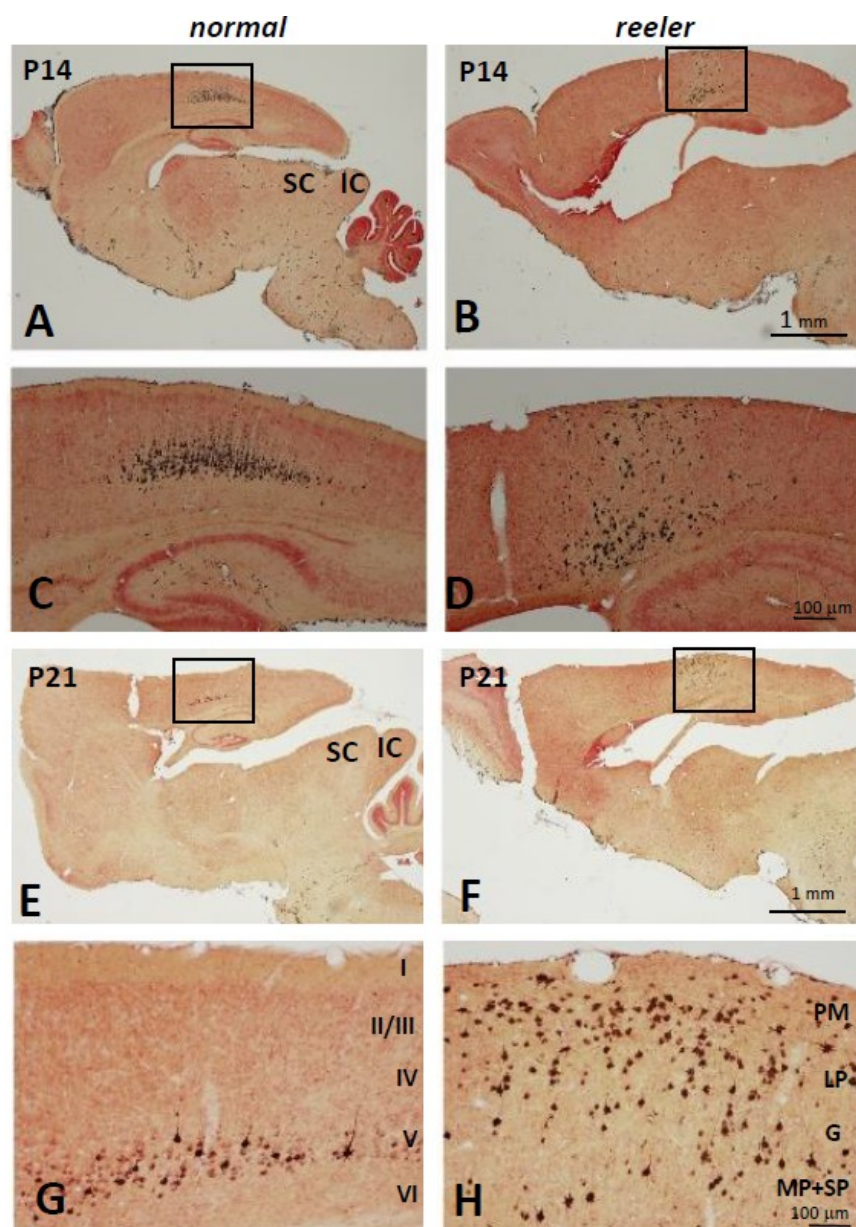


Figure 5. HRP is injected into the upper lumbar cord of the normal (left column) and *reeler* (right column) mice to label CST neurons. **A-D:** Retrogradely labeled CST neurons are found in Layer V of the normal cortex (**A, C**), but scattered radially in the *reeler* cortex (**B, D**). **E-F:** The similar injection was made, and the animal was sacrificed on P21. Retrogradely labeled CST neurons are found in Layer V of the normal cortex (**A, C**), but they are scattered radially in the *reeler* cortex (**B, D**). Scale bars: 1 mm (**A, B, E, F**), 100 μ m (**C, D, G, H**). Abbreviations are shown in **Figure 4**.

2. Retrograde labeling of CST neurons with HRP

Next, we injected HRP into the upper cervical cord (C1) or the upper lumbar enlargement (L2) to confirm the developmental profile of CST axons based on DiI anterograde labeling. The spinal cords were removed, and horizontally sectioned. The injection site was stained with DAB method. For the upper cervical cases, the injection site was mainly confined to the bilateral C1 level, but weak HRP reactive products spread into the pyramidal decussation and C2-C3 level. For the upper lumbar cases, injection sites were confined to the L2 level, but weak staining was recognized in the adjoining segments. In the case in which injection of HRP was made into the upper lumbar cord (L2) on P5 and sacrificed on P6, no retrogradely labeled CST neurons were identified in the cerebral cortex of the normal and *reeler* mice (Fig. 4A, B), whereas neurons in the ventrocaudal area of the red nucleus were retrogradely labeled both in the normal and *reeler* mice (Fig. 4C, D). These findings suggest that rubrospinal tract axons arrive at the L2 level before P6, but CST axons do not arrive at the L2 level on P6

both in the normal and *reeler* mice. These data based on the retrograde HRP method coincide with the DiI anterograde labeling of CST axons. As shown in Figure 2, the tips of CST fibers arrived at the L2 level on P8 both in the normal and *reeler* mice, and thus it is no wonder that no retrograde labeling of CST neurons occurred in the case in which injection of HRP was made into the upper lumbar cord before P8. Retrogradely labeled CST neurons were distributed in the hindlimb area of the motor cortex of the normal and *reeler* mice on P9 (Fig. 4E, F). HRP-labelled CST neurons were confined to layer V in the normal cortex (Fig. 4G), whereas they were radially scattered from the pial surface to the white matter in the *reeler* (Fig. 4H). In the normal mouse, all of HRP-labelled CST neurons were morphologically classified as pyramidal cells with an upward apical dendrite. In the *reeler* mouse, HRP-labeled CST neurons consisted of two categories: normal pyramids and abnormal pyramids. The normal pyramids were pyramidal cells with an upward apical dendrite and occupied the lower half of the motor cortex (hindlimb area) corresponding to the lower half of granule cell layer (G) and small and medium pyramidal cell layer (MP+SP) (Fig. 4H). On the contrary, abnormal pyramids were pyramidal cells with an inverted or horizontally directed apical dendrite, and occupied the upper half of the motor cortex corresponding to the polymorphic cell layer (PM) and large pyramidal layer (LP) (Fig. 4H). The similar distribution pattern of HRP-labeled CST neurons in the normal and *reeler* cortex were recognized on P14 (Fig. 5A-D) and on P21 (Fig. 5E-H).

We examined tangential distribution pattern of HRP-labeled neurons in a series of tangential sections of flattened cerebral hemispheres. HRP was injected into the upper lumbar cord on P19, and two days later the animals were sacrificed. The cerebral hemispheres were flattened, and a series of tangential frozen sections were stained according to the TMB method. Cell bodies of HRP-labeled neurons were confined to the hindlimb area

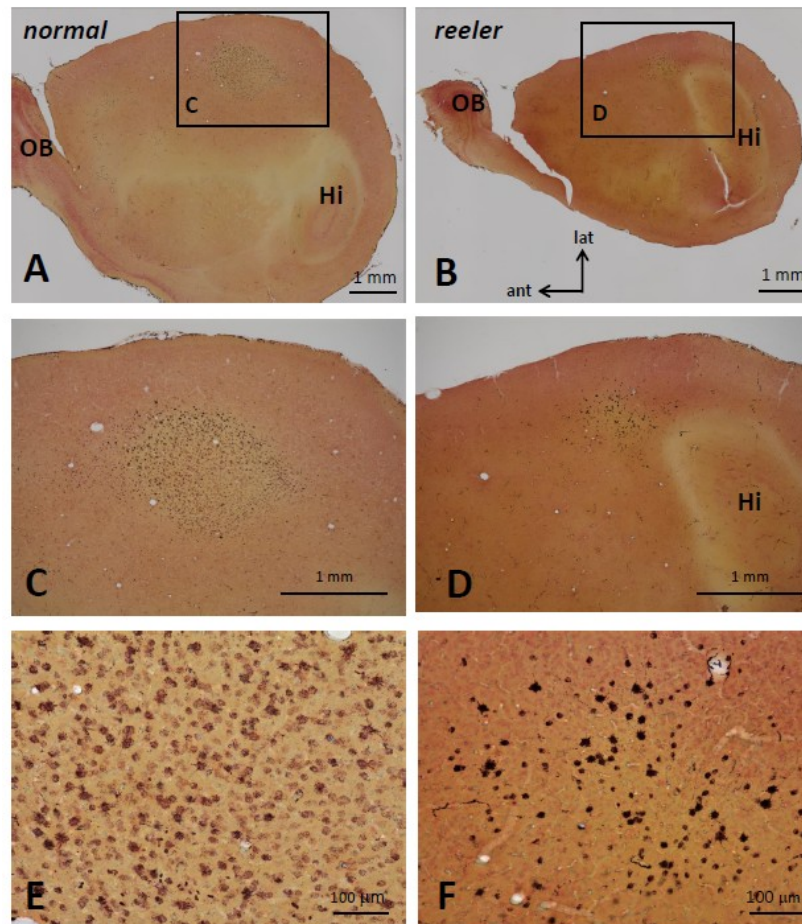


Figure 6. Distribution of retrogradely labeled CST neurons in a horizontal plane through the motor cortex of the normal (left column) and *reeler* (right column) mice of P21 after the injection of HRP into the lumbar cord. **A, B:** The cerebral hemispheres of the normal (**A**) and *reeler* (**B**) were sandwiched between two slides to be flattened before cutting. The rectangles in **A** and **B** are enlarged into **C** and **D**, respectively. Retrogradely labeled CST neurons are horizontally distributed in a similar manner between the normal (**C**) and *reeler*. (**D**) **E, F:** **E** and **F** are enlarged from **C** and **D**, respectively. In the *reeler*, labeled CST neurons are scarce (**F**) compared with the normal counterpart (**E**). **Abbreviations:** Hi, hippocampus; OB, olfactory bulb.

of the motor cortex both in the normal and *reeler* mice (Fig. 6A-D). However, the number of HRP-labeled cells in a given section was much reduced in the *reeler* compared with the normal counterpart (Fig. 6E, F). This finding is no wonder that all of labeled cells were confined to two or three sections through the layer V of the normal cortex, but in the *reeler* cortex HRP-labeled cells were contained in all of the sections from the pial surface to white matter (Fig. 7).

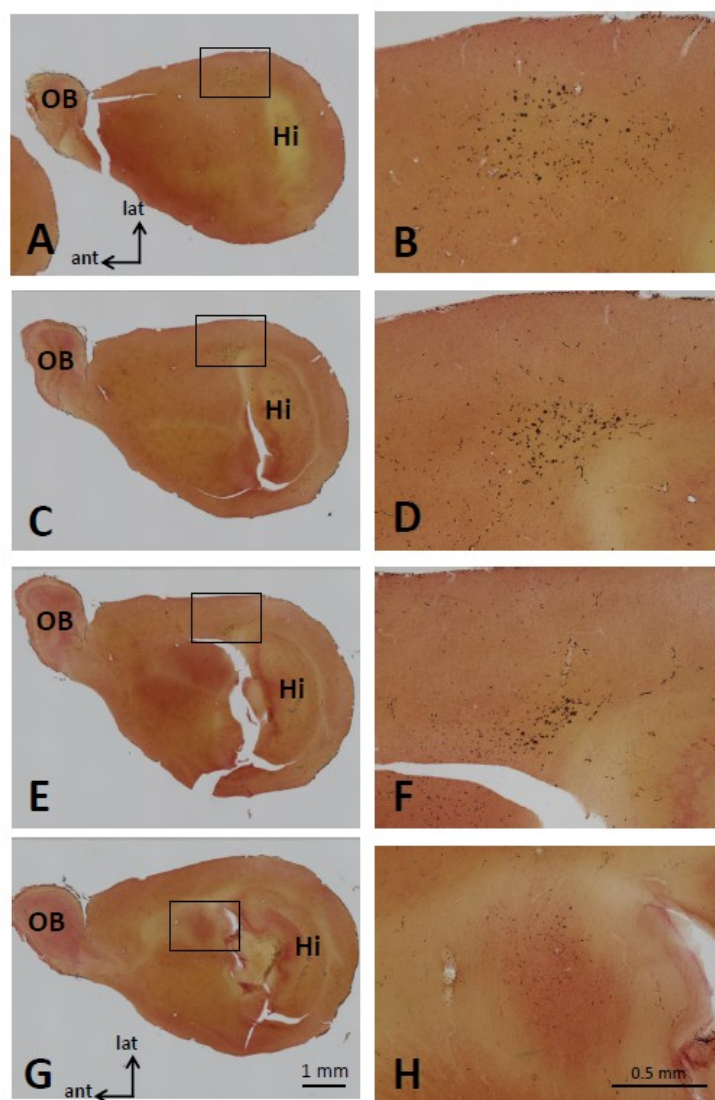


Figure 7. A series of tangential sections through the cerebral cortex of the adult *reeler* mouse to show the tangential distribution of CST neurons after the injection of HRP into the lumbar cord. Labeled CST neurons are radially scattered from the level just beneath the pial surface (A) to the level just above the white matter (G). B, D, F, and H are enlarged from the rectangle area in A, C, E and G, respectively. Scale bars: 1 mm (A, C, E, G), 0.5 mm (D, B, F, H). Abbreviations are shown in **Figure 6**.

3. CaMKII α immunohistochemistry

CST axons were immunostained with an antibody specific for CaMKII α both in the normal and *reeler* mice at 2 months of age, as shown in Figure 8. CaMKII α -immunopositive CST axons penetrated through the basal pontine gray as pontine longitudinal fibers (Fig. 8A, B), passed through the medullary pyramid, and then crossed at the pyramidal decussation (Fig. 8C, D). CaMKII α -immunopositive CST axons descended in the ventral area of the dorsal funiculus of the spinal cord (Fig. 8E, F). CaMKII α -immunoreactive products were not confined to the CST axons. Neurons in the inferior olivary nuclei and dorsal horn of the spinal cord were also immunostained with CaMKII α antibody both in the normal and *reeler* mice (Fig. 8C-F). In addition, Purkinje cells in the cerebellar cortex, pyramidal cells in the cerebral neocortex and hippocampus (CA1-CA3) were also strongly immunopositive for this antibody both in the normal and *reeler* mice (data not shown).

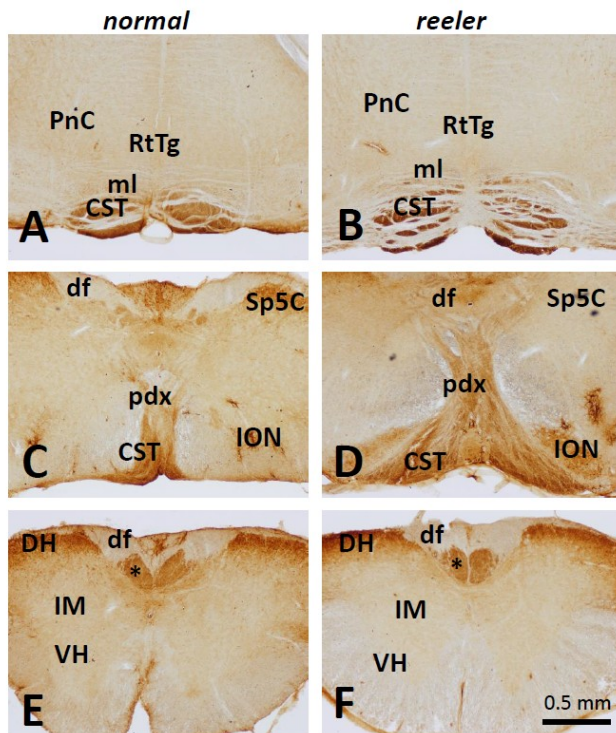


Figure 8. The CST axons express CaMKII α -immunoreactivity in pontine longitudinal fibers in the basal pons (**A**, normal; **B**, *reeler*), pyramid and pyramidal decussation (pdx) in the caudal end of the medulla oblongata (**C**, normal; **D**, *reeler*), and the ventral area (asterisk) of the dorsal funiculus (df) in the C1 level of the spinal cord (**E**, normal; **F**, *reeler*).

Other Abbreviations: ION, inferior olivary nuclei; ml, medial lemniscus; PnC, caudal pontine reticular nucleus; RtTg, reticulotegmental nucleus; Sp5C, caudal part of spinal trigeminal nucleus.

DISCUSSION

The present study has revealed that *reeler* CST axons normally develop during the early postnatal period. In the *reeler* mouse, CST neurons are radially malpositioned within the motor cortex (15, 28, 34, 36). The course of adult *reeler* CST axons and their collaterals projecting to the subcortical nuclei are similar to the normal counterpart (30), suggesting that radially dislocated CST neurons can elongate their axons along their way from the internal capsule to spinal cord, and recognize their targets, appropriately. However, it has remained obscure whether the time course of development of *reeler* CST axons is regulated in a similar manner of the normal counterpart or not. The present study based on the DiI anterograde labeling has shown that *reeler* CST axons normally develop during the postnatal period. In brief, *reeler* CST axons arrive at the pyramidal decussation and enter into the contralateral spinal cord around on P0.5, and descend in the ventral area of the contralateral dorsal funiculus at C2 level on P2. They continue to descend at C8 level on P3, at the mid-thoracic level on P4, and at the upper lumbar level on P8. Such a time course of development of *reeler* CST axons is similar to the normal counterpart (Fig. 2). This DiI anterograde labeling has been also confirmed by HRP retrograde labeling (Figs. 4, 5). Taken together with the present and previous studies (30), *reeler* CST axons normally develop in spite of dislocated CST neurons. This finding suggests that path finding and target recognition of CST axons in Reelin deficient mouse, *reeler*, are determined by birth order of cortical neurons rather than by laminar position in the cerebral cortex (28).

It is well known that CST axons project to multiple targets in different spinal segments of the rat (2) and cat (8, 29). In the rat, these spinal collaterals are formed by interstitial budding from the shaft of CST axons rather than bifurcation of growth cones of developing CST axons, as seen in formation of pontine collaterals of CST axons (3, 27). In the present study, we have examined formation of collateral branches arising from the parent CST axons at C8 level with an injection of DiI into the motor cortex of the normal and *reeler* pups. No collateral fibers arising from DiI-labeled parent CST axons were identified on P3 but one day later (P4) short collateral branches arose from the side of the parent axon both in the normal and *reeler* mice, suggesting that target recognition by the shaft of CST axons is not affected by intracortical position of CST neurons.

The present study has demonstrated that CaMKII α protein is strongly expressed by CST axons in the adult normal and *reeler* mice (Fig. 8). In the rodents and monkeys, CST axons express CaMKII α -immunoreactivity (32). Combination of anterograde DiI labeling of CST axons and CaMKII α immunohistochemistry revealed that CST axons are immunostained at all of the levels from the internal capsule to the caudal end of the spinal cord of the rat (33). In addition to CST axons, CaMKII α protein is widely distributed in pyramidal neurons in the cerebral cortex and hippocampus (25), Purkinje cells in the cerebellar cortex (14), cartwheel cells in the dorsal cochlear nucleus (26), and inferior olivary nuclei neurons (26). All of these neurons are ectopically distributed in

the *reeler*, but they are immunostained with CaMKII α antibody in a similar manner of the normal counterparts (19). These findings indicate that chemical characterization of neurons is not affected by radial displacement of cell bodies, which implies that location of neuronal cell bodies and their chemical identity are independently regulated.

As discussed above, we could not find any abnormalities in the development of CST axons during the postnatal period in spite of malpositioning of CST neurons in the motor cortex. Lee and Song (22) reported that glial limitans at the exit point of the ventral root is disrupted in the embryological day (E) 11.5 *reeler* embryos, which results in ectopic spinal motoneurons distributing outside of the neural tube. If these ectopic motoneurons outside of the neural tube persist through the postnatal period and in the adulthood, some CST axons may penetrate through the ventral horn, enter into the ventral root, and terminate with these ectopic motoneurons. We could not find any abnormal terminals of CST axons nearby the exit of the ventral root during postnatal period and in the adulthood, which suggests that such ectopic motoneurons may die before birth. Further studies must be awaited to confirm whether ectopic motoneurons outside of the neural tube persist in the *reeler* through the perinatal period or die.

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