Quantitative comparison of corticospinal tracts arising from different cortical areas in humans

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ABSTRACT

The corticospinal tract (CST), which plays a major role in the control of voluntary limb movements, arises from multiple motor- and somatosensory-related areas in monkeys. Although the cortical origin and quantitative differences in CSTs among the cortical areas are well-documented in monkeys, they are unclear in humans. We quantitatively investigated the CSTs from the cerebral cortex to the cervical cord in healthy volunteers using fiber tractography of diffusion-weighted magnetic resonance imaging. The corticospinal (CS) streamlines arose from nine cortical areas: primary motor area (mean ± SD = 49.71 ± 1.61%), dorsal (11.02 ± 0.90%) premotor cortex, supplementary motor area (5.14 ± 0.36%), pre-supplementary motor area (2.46 ± 0.26%), primary somatosensory cortex (11.06 ± 0.91%), Brodmann area 5 (0.88 ± 0.09%), caudal cingulate zone (1.70 ± 0.30%), and posterior part of the rostral cingulate zone (1.70 ± 0.34%). In all cortical areas, the number of CS streamlines gradually decreased from the rostral to caudal spinal segments, but the proportion was maintained throughout the cervical cord. Over 75% of CS streamlines arose from the lateral surface of the frontal lobe, which may explain the voluntary control of dexterous and flexible limb movements in humans. (197/200 words)

1. Introduction

The corticospinal (CS) tract (CST) has evolved in mammals and reached a climax in humans. CST is associated with manual dexterity and voluntary movements (Heffner and Masterton, 1975, 1983). Using retrograde transport of tracers injected into the spinal cord, several studies investigated the origin of CS neurons from the cerebral cortex in neurologically intact non-human primates (Toyoshima and Sakai, 1982; Dum and Strick, 1991, 1996; He et al., 1993, 1995; Galea and Darian-Smith, 1994), and found that CSTs arise widely from multiple motor-related areas of the frontal lobe and somatosensory-related areas of the parietal lobe. In these studies, the retrogradely-labeled neurons of the cerebral cortex were quantified to determine the proportion of CS neurons in the cerebral cortex. Although the cortical origin and quantitative differences in CSTs among the cortical regions are well-documented in non-human primates, they remain obscure in healthy humans.

In humans, quantitative assessment of the cortical origin of CST has only been investigated in pathological conditions, such as stroke (Weil and Lassek, 1929), and patients who underwent cortical ablation (Jane et al., 1967). Because these studies were conducted many years after the damage, extensive anatomical reorganization could have occurred in the cortical and subcortical structures, including the CST. Furthermore, an accurate assessment of the proportion of CSTs among the cortical regions was not possible due to the damaged brain regions are different in each patient. Intraoperative cortical electrical stimulation is the gold standard investigation for functional mapping to identify motor-related cortical areas, including the CST origins (Penfield and Boldrey, 1937). However, quantitative assessment of the cortical origin of CST cannot be performed using this method because of the possibility that descending pathways other than CSTs or indirect activation of the CSTs are involved in the evoked movements or motor arrest (Foerster, 1931). Thus, there is no consensus on the origin of the CSTs other than the primary motor cortex (M1) in humans, either by anatomical or electrophysiological methods. Therefore, an alternative method, using a non-invasive measurement, is required to perform a quantitative analysis of the CST.
structures in healthy individuals. Fiber tractography is a three-dimensional (3D) reconstruction technique used to evaluate the course and location of white matter pathways based on the data collected by diffusion-weighted imaging (DWI). This allows a non-invasive assessment of the anatomical connectivity in humans (Conturo et al., 1999; Catani et al., 2002; Mori and Zhang, 2006; Assaf et al., 2013). The quantification of tractography-derived strength of connectivity between distant brain regions has been increasingly used in the last decade (Hagmann et al., 2007, 2008, 2010; Gallien et al., 2013; Jeong et al., 2014). Using diffusion tensor tractography, some studies (Seo and Jang, 2013; Dalamagkas et al., 2020) performed a quantitative assessment of CST volume among frontal motor-related areas in healthy humans. However, an assessment of the tract volume is biased due to the size of the volume of interest, especially in the areas from which fibers pass.

The aim of the current study was to evaluate the origin of CS fibers and estimate the proportion of CS fibers arising from each cortical area. We hypothesized that the cortical origins of CSTs based on diffusion tractography would be consistent with the combination of previous anatomical and electro-physiological evidences obtained from humans. To test this hypothesis, we reviewed previous studies that examined the degenerated cells in cortical areas following spinal cord injury and investigated the functional mapping using intraoperative cortical electrical stimulation. Then, we investigated the CSTs from each cortical area to C1-Th1 in healthy volunteers and quantified the detected CS streamlines from the candidates of CST origins using fiber tractography based on DWI measurement using a 3 T magnetic resonance imaging (MRI) scan.

2. Materials and methods

2.1. Participants

Thirty-one healthy volunteers (10 females and 21 males) aged 18–35 years (mean ± standard error: 24.61 ± 0.79 years) participated in this study. None of the volunteers reported a history of major neurological or musculoskeletal disease. Thirty volunteers were right-handed and one was left-handed, as verified by the Edinburgh Handedness Inventory (Oldfield, 1971). This study was conducted in accordance with the Declaration of Helsinki. All volunteers provided written informed consent prior to participation. The study protocol was approved by the Ethics Committees at Tokyo Metropolitan Matsuzawa Hospital, and the study procedures were performed in accordance with the existing guidelines.

2.2. Data acquisition

MRI data were acquired using a 3 T MAGNETOM Skyra (Siemens, Erlangen, Germany) with a 20-channel head/neck coil. To reduce the geometric distortion in DWI images of the cervical spinal cord, pads containing rice were placed around the neck (Moriya et al., 2011). These pads stabilized the head and neck of participants during DWI scanning. In addition, to reduce head motion by providing tactile feedback, a medical tape was attached to the head/neck coil and the participant’s forehead (Krause et al., 2019). Therefore, these procedures maintained the participants’ head and neck in a stable position during DWI scanning.

T1-weighted images were obtained in the sagittal plane using a magnetization-prepared rapid gradient-echo (MPRAGE) sequence (Mugler and Brookesman, 1990; repetition time [TR] = 1710 ms, echo time [TE] = 3 ms, field of view [FoV] = 352 × 352 mm², matrix size = 352 × 352, slice thickness = 1 mm, in-plane resolution = 1 × 1 mm², flip angle = 9°, GRAPPA = 2). The T1-weighted image covered the areas from the brain to at least the lower level of the Th2 vertebrae. The images were analyzed to obtain detailed anatomical information and estimate the transforming parameters from the native space to the template space.

T2-weighted images were obtained in the coronal plane using sampling perfection with application optimized contrast using different flip angle evolution (SPACE) sequence (Mugler et al., 2000; TR = 1490 ms, TE = 233 ms, FoV = 200 × 200 mm², matrix size = 256 × 238, slice thickness = 0.4 mm, in-plane resolution = 0.39 × 0.39 mm², interpolation = on, flip angle = 100°, GRAPPA = 2). This image covered the areas from the pontomedullary junction (PMJ) to the lower level of the Th2 vertebrae. T2-weighted images were used to identify the segmental level of the spinal cord based on the position of the ventral and dorsal spinal roots.

DWI images were obtained in the transaxial plane using a multiband echo planar imaging (EPI) sequence (Moeller et al., 2010; multiband factor = 3; TR = 3510 ms, TE = 89.80 ms, FoV = 208 × 208 mm², matrix size = 104 × 104, slice thickness = 4 mm, in-plane resolution = 2.0 × 2.0 mm, flip angle = 90/180°, GRAPPA = 2, phase partial Fourier = 6/8). The diffusion scheme contained two shells (b = 700 and 2000 s/mm²), and 120 directions were uniformly distributed over the sphere. An additional 15 scans with b = 0 s/mm² (8 scans in AP) were distributed across the diffusion scheme. To correct for the geometric distortions, the diffusion scheme was obtained using two runs with reversed phase encoding directions (AP and PA) and different diffusion directions (60 scans in AP; Andersson and Sotiropoulos, 2016). The images spanned from the brain to at least the bottom of the C7 vertebrae. In addition, to apply eddy-topup tools in FSL (Andersson et al., 2003; Smith et al., 2004; Andersson and Sotiropoulos, 2016), two spin echo (SE) EPI images with reversed phase encoding directions were acquired (TR = 12,000 ms, TE = 72.80 ms, FoV = 208 × 208 mm², matrix size = 104 × 104, slice thickness = 4 mm, in-plane resolution = 2.0 × 2.0 mm, flip angle = 78/160°, phase partial Fourier = 6/8). DWI images were measured as anisotropic voxels to preserve the signal intensity in the spinal cord by increasing the slice thickness (Cohen-Adad et al., 2021).

2.3. Defining the volumes of interest (VOIs)

2.3.1. VOIs for cortical origin of the CSTs

There is no consensus on the cortical origins of the CSTs in humans. Therefore, we determined the candidate areas of CST origin in humans on the basis of anatomical and electro-physiological evidence from previous studies.

To identify the anatomical evidence, we searched studies that examined the degenerated cells in the cortical areas following spinal cord injury. We identified four studies that reported 30 cases (Table 1; Marinescu, 1900; Holmes and May, 1909; Marinesco, 1910; Wohlfarth, 1932). These anatomical studies showed degeneration in the cortical areas, absence of the giant cells that characterize BA4, and presence of degenerated cells in the precentral gyrus, paracentral lobule, and post-central gyrus. These areas roughly correspond to Brodmann areas (BAs) 1–5 (Table 1) and were selected as candidates for CST origin based on the anatomical evidence (Fig. 1 A).

The anatomical evidence from neurologically intact macaque monkeys documented that CSTs arise widely from not only the precentral and post-central gyr as well as the paracentral lobule, which correspond to BAs 1–5 in humans, but also higher order motor-related areas located in the rostral portion of the precentral gyrus, medial wall of frontal lobe, and parietal lobe (Toyoshima and Sakai, 1982; Dum and Strick, 1991, 1996; He et al., 1993, 1995; Galea and Darian-Smith, 1994). Thus, anatomical evidence alone is insufficient to determine the origin of CSTs. Therefore, we used the results of functional mapping obtained from intraoperative cortical electrical stimulation in humans as electrophysiological evidence to determine the candidate CST origins. For electrophysiological evidence, we searched for studies that investigated the cortical areas where electrical stimulation elicited motor responses or inhibited movements of body other than the face, without damage to the cerebral cortex and/or CSTs. We identified ten studies that reported...
anatomically distant to the spinal cord. These cortical areas were functionally and occupiess the most anterior part of the prefrontal cortex and lies atop a control. To the best of our knowledge, no studies have reported an for CST origin (Fig. 1C). In addition, BA10 was selected as the negative described above, BAs 1–509 cases (Table 2; Barthlow, 1874; Horsley, 1909; Foerster, 1931; Penfield and Boldrey, 1937; Scarff, 1940; Penfield and Welch, 1951; Talairach et al., 1973; Caruana et al., 2018; Rech et al., 2019; Fornia et al., 2020). The electro-physiological studies showed that the cortical areas that could elicit motor responses in the body (except face) by electrical stimulation were the precentral gyrus, posterior parts of su–3, 1, and 2 (Zilles et al., 1995; Sanchez-Panchuelo et al., 2012; Roux et al., 2018). BA10 is also known as the frontal pole (FP) (Boschin et al., 2015).

Table 1

| Reference          | Subject number | Sex | Age (years) | Duration after CST damage | Lesion site        | Cause of SCI damage | Anatomical degeneration | Brodmann area |
|--------------------|----------------|-----|-------------|---------------------------|--------------------|----------------------|-------------------------|---------------|
| Marinescu, 1901    | 1              | N/A | N/A         | 5 months–2 years          | Spinal cord        | Sarcoma              | Precentral gyrus        | 4             |
|                    | 1              |     |             |                           | Spinal cord        | Multiple echnococcal blisters | Precentral gyrus        | 4             |
|                    | 1              |     |             |                           | Lumbar             | Pachymeningitis      | Precentral gyrus        | 4             |
|                    | 1              |     |             |                           | Lower thoracic     | Myelitis             | Precentral gyrus        | 4             |
|                    | 1              |     |             |                           | C7                 | Lift accident        | Paracentral lobule and precentral gyrus | 4 |
|                    | 1              |     |             |                           |                    |                      | Paracentral lobule and precentral gyrus | 4 |
| Holmes and May     | 1              | Male | 17         | 229 days                  |                    |                      | Paracentral lobule and precentral gyrus | 4 |
| (1909)             | 1              | Female | 68        | 108 days                  | C6–7               | N/A (completely destroyed) | Paracentral lobule and precentral gyrus | 4 |
| Marinesco (1910)   | 1              | Female | 40        | 30 days                   | Th10–11            | Fracture of spine    | Precentral lobule and precentral gyrus | 4 |
|                    | 1              | Male | 28         | 1 month                   | L2                 | Fracture of spine    | Precentral lobule and precentral gyrus | 4 |
|                    | 1              | Female | 28        | 68 days                   | C6                 | N/A                  | Precentral lobule and precentral gyrus | 4 |
|                    | 1              | Male | 36         | 3 months                  | Lower lumbar       | Fracture of spine    | Precentral lobule and precentral gyrus | 4 |
|                    | 1              | Male | 20         | 4 months                  | Lower thoracic     | Multiple scars       | Precentral lobule and precentral gyrus | 4 |
|                    | 1              | Male | 35         | 6 months                  | Th9                | Pott disease         | Precentral lobule and precentral gyrus | 4 |
|                    | 1              | Male | 17         | 6 months                  | L3-Cauda equina    | Pott disease         | Paracentral lobule and precentral gyrus | 4 |
|                    | 1              | Male | 19         | 8 months                  | Th10               | Hydatid cyst         | Paracentral lobule and precentral gyrus | 4 |
|                    | 1              | Female | 56        | 1 year                    | Below L1           | Tumor                | Precentral lobule and precentral gyrus | 4 |
|                    | 1              | Male | 26         | 19 months                 | Th12               | Compression of the marrow | Precentral lobule and precentral gyrus | 4, 5 |
|                    | 1              | Male | 45         | 2 years                   | L3-Filum terminale | Pott disease         | Precentral lobule and precentral gyrus | 4 |
|                    | 1              | Male | 62         | 2.5 years                 | Lumbar             | Myelitis             | Precentral lobule and precentral gyrus | 4 |
|                    | 1              | Male | 41         | 4 years                   | N/A                | Sclerosis            | Precentral lobule and precentral gyrus | 4 |
|                    | 1              | Male | 45         | 3 years                   | N/A                | Amyotrophic lateral sclerosis | Precentral lobule and precentral gyrus | 4 |
|                    | 1              | Male | 48         | 3 years                   | N/A                | Amyotrophic lateral sclerosis | Precentral lobule and precentral gyrus | 4 |
| Wohlfahrt, 1932    | 1              | Female | 41        | 10 days                   | Cervical           | Malacia              | Pre and post central gyrus | 1–5 |
|                    | 1              | Male | 36         | 7 months                  | C5-Th1             | Malacia              | Precentral gyrus        | 4 |
|                    | 1              | Male | 62         | 13 months                 | Lower cervical-    | Pachymeningitis      | Post central gyrus       | 1–5 |
|                    | 1              | Female | 52        | More than 1 year          | C4–C6, upper thoracic | Pachymeningitis      | Post and precentral gyrus | 1–5 |
|                    | 1              | Male | 44         | 6 days                    | Th7–8              | Myelitis             | Precentral gyrus        | 4 |
|                    | 1              | Female | 21        | 11–12 days                | Th11               | Epidural abscess     | Precentral gyrus (central regions) | 4 |
|                    | 1              | Male | 19         | 15 months                 | Th4                | Tumor                | Pre and post central gyrus | 1–5 |
| Total              | 30             |     |             |                           |                    |                      |                         |               |

C: Cervical, CST: corticospinal tract, L: Lumbar, N/A: not available, SCI: spinal cord injury, Th: Thoracic.

509 cases (Table 2; Barthlow, 1874; Horsley, 1909; Foerster, 1931; Penfield and Boldrey, 1937; Scarff, 1940; Penfield and Welch, 1951; Talairach et al., 1973; Caruana et al., 2018; Rech et al., 2019; Fornia et al., 2020). The electro-physiological studies showed that the cortical areas that could elicit motor responses in the body (except face) by electrical stimulation were the precentral gyrus, posterior parts of superior, middle, and inferior frontal gyri, post-central gyrus, superior parietal lobule, paracentral lobule, and the cingulate gyrus. These areas roughly correspond to BAs 1–7, 24, 31, 32, and 44 (Table 2), and were selected as candidates for CST origin based on the electro-physiological evidence (Fig. 1B).

Based on the anatomical and electro-physiological evidences described above, BAs 1–7, 24, 31, 32, and 44 were selected as candidates for CST origin (Fig. 1C). In addition, BA10 was selected as the negative control. To the best of our knowledge, no studies have reported an anatomical connection of BA10 to the spinal cord in any species. BA10 occupies the most anterior part of the prefrontal cortex and lies atop a prefrontal hierarchy (Boschin et al., 2015). BA10 is functionally and anatomically distant to the spinal cord. These cortical areas were
defined using the “Atlas of the human brain” (Mai et al., 2015).

2.3.2. VOIs for cortical parcellation (parcels)
The cortical areas were divided into sub-regions on the basis of their functional roles. The primary motor area (M1) corresponds to BA4 (Zilles et al., 1995). The premotor (PM) area corresponds to BAs 6 and 44 (Picard and Strick, 1996, 2001; Grézes and Decety, 2001; Tomassini et al., 2007). BA44 is a part of Broca’s area and lies anterior to BA6, the premotor area (Dronkers et al., 2007). Neuroimaging studies showed that human BA44 is activated during imitation, observation of finger movements (Iacoboni et al., 1999), and manipulation of complex objects with hands (Binkofski et al., 1999). These activation profiles are consistent with the observations in macaque F5, which is a part of the PMv (Picard and Strick, 2001). Therefore, we
defined BA44 as part of PM, which is involved in voluntary movements of the upper limb. The primary somatosensory cortex (S1) corresponds to BAs 3, 1, and 2 (Zilles et al., 1995; Sanchez-Panchuelo et al., 2012; Roux et al., 2018). BA10 is also known as the frontal pole (FP) (Boschin et al., 2015).
Fig. 1. Candidate areas for the cortical origin of the corticospinal tract (CST). (A) The cortical areas showing degeneration in SCI patients. The cortical areas have been reported to show absence of the giant cells, which characterize BA4, and presence of the degenerated cells in the precentral gyrus, paracentral lobule, and post-central gyrus after spinal cord injury (see also Table 1). (B) The cortical areas evoking or inhibiting body movements by electrical stimulation (see also Table 2). (C) The candidate areas of CST origin with control area in Brodmann’s classification. Summation of the cortical areas selected based on anatomical (A) and electrophysiological (B) evidences were defined as the candidates for cortical origin of CSTs. Volumes of interest (VOIs) corresponding to these cortical areas were defined in terms of Brodmann areas (BA) based on the “Atlas of the human brain” (Mai et al., 2015). In addition, BA10 was included as the negative control area to identify the false positive results. Each row shows the lateral (left), medial (middle), and superior (right) views of the left hemisphere. The numbers of areas represent the BA number.
Table 2
Cortical areas evoking body movements by electrical stimulation.

| Reference                  | Subject number | Sex | Age (years) | Stimulation sites | Motor response                                      |
|----------------------------|----------------|-----|-------------|-------------------|-----------------------------------------------------|
|                            |                |     |             | Anatomical labels | Brodmann area                                      |
|                            |                |     |             |                   |                                                     |
| Barthlow (1874)            | 1              | Female | 30         | Posterior lobe    | 7                                                  |
|                           |                |       |             |                   | Movements of neck, upper limbs, fingers, and lower limbs. |
| Horsley (1909)            | 1              | Male | 14          | Precentral lobe   | 4, 6                                                |
|                           |                |       |             |                   | Finger, wrist, elbow, and shoulder movements.       |
| Foerster (1931)          | N/A            | N/A | N/A         | Area 4 in Vogt’s myeloarchitectonic map             | 4                                                   |
|                           |                |       |             |                   | Isolated movement of a single part of the limbs or trunk. |
|                           |                |       |             | Area 6as in Vogt’s myeloarchitectonic map           | 6                                                   |
|                           |                |       |             |                   | Isolated movement of a single part of the limbs or trunk. |
|                           |                |       |             | Areas 1–3 in Vogt’s myeloarchitectonic map          | 1, 2, 3                                             |
|                           |                |       |             |                   | Isolated movement of a single part of the limbs or trunk. |
|                           |                |       |             | Area 5a in Vogt’s myeloarchitectonic map            | 5                                                   |
|                           |                |       |             |                   | Complex movements of arm and leg.                    |
| Penfield and Boldrey (1937) | 163           | N/A | N/A         | Area 6 in Vogt’s myeloarchitectonic map             | 4                                                   |
|                           |                |       |             |                   | Movements of fingers, hand, arm, shoulder, ankle, thigh, knee, leg, neck, and toe. |
|                            |                |       |             | Area 6as in Vogt’s myeloarchitectonic map           | 6                                                   |
|                            |                |       |             |                   | Movements of fingers, hand, arm, shoulder, ankle, thigh, knee, leg, neck, and toe. |
|                            |                |       |             | Area 6d in Vogt’s myeloarchitectonic map            | 6                                                   |
|                            |                |       |             |                   | Movements of hand, shoulder, and leg.                |
|                            |                |       |             | Area 8y in Vogt’s myeloarchitectonic map            | 44                                                  |
|                            |                |       |             |                   | Movements of ankle, thigh, and knee.                |
| Scarff (1940)            | 1              | Male | 21          | Area 5 in Vogt’s myeloarchitectonic map             | 1, 2, 3                                             |
|                           |                |       |             |                   | Movements of fingers, hand, arm, shoulder, ankle, thigh, knee, leg, and neck. |
| Penfield and Welch (1951) | 24             | N/A | N/A         | Superior and mesial intermediate precentral cortex | 6                                                   |
|                           |                |       |             |                   | Complex movements of fingers, hand, and upper limb. |
| Talairach et al. (1973)   | 52             | Both | 8–42        | Anterior cingulate gyrus                            | 24, 32                                              |
|                           |                |       |             |                   | Twitches and tremors of the upper or lower body parts. Atonia of the upper and lower limbs. Goal-oriented behavior using hand and arm. |
| Caruana et al. (2018)     | 114            | N/A | N/A         | pACC            | 24                                                  |
|                           |                |       |             |                   | Twitches and tremors of the upper or lower body parts. Atonia of the upper and lower limbs. Goal-oriented behavior using hand and arm. |
|                           |                |       |             | aMCCv            | 24                                                  |
|                           |                |       |             |                   | Twitches and tremors of the upper or lower body parts. Atonia of the upper and lower limbs. Goal-oriented behavior using hand and arm. |
|                           |                |       |             | pMCCv            | 31                                                  |
|                           |                |       |             |                   | Twitches and tremors of the upper or lower body parts. Atonia of the upper and lower limbs. Goal-oriented behavior using hand and arm. |
| Rech et al. (2019)        | 117            | N/A | 39 ± 10     | Precentral gyrus                                      | 4, 6                                                |
|                           |                |       |             |                   | Goal-oriented behaviors using hand and arm.         |
|                           |                |       |             | Postcentral gyrus                                    | 1–3                                                |
|                           |                |       |             |                   | Inhibition of upper limb movements.                 |
|                           |                |       |             | Posterior part of inferior frontal gyrus            | 44                                                 |
|                           |                |       |             |                   | Contraction of upper limb or hand muscles. Inhibition of upper limb movements. |
|                           |                |       |             | Dorsal premotor                                       | 6                                                  |
|                           |                |       |             |                   | Inhibition of the hand motor task.                  |
| Fornia et al. (2020)      | 36             | N/A | 25–75       | Ventral premotor                                      | 6, 44                                               |
|                           |                |       |             |                   | Inhibition of the hand motor task.                  |

N/A: not available.

et al., 2015). Therefore, in the present study, we defined BA4 as M1, BAs 6 and 44 as PM, BAs 3, 1, and 2 as S1, and BA10 as FP. We defined BAs 24, 31, and 32 as the cingulate motor area (CMA). Parietal lobe was defined as BAs 5 and 7 (Fig. 2 A).

A meta-analysis of the functional neuroimaging studies in humans reported seven subdivisions in PM and CMA (Picard and Strick, 1996, 2001). In the present study, as proposed elsewhere, PM was divided into four sub-regions: dorsal premotor cortex (PMd), ventral premotor cortex (PMv), supplementary motor area (SMA), and presupplementary motor area (preSMA). To define the four VOIs in PM, we referred to the “Human Motor Area Template” (Mayka et al., 2006). The overlapping regions between PM (BAs 6 and 44) and four subdivisions were defined as subdivisions of PM (Fig. 2B). CMA was divided into three sub-regions: caudal cingulate zone (CCZ) and anterior and posterior parts of the rostral cingulate zone (RCZa and RCZp). To define the three VOIs in CMA, we referred to the “Cingulate and orbitofrontal parcellation atlas” (Beckmann et al., 2009; Neubert et al., 2015). The overlapping regions between CMA (BAs 24, 31, and 32) and three subdivisions were defined as the three subdivisions of CMA (Fig. 2C).

Based on the aforementioned definitions, we classified the candidate cortical areas for CST origin into twelve cortical areas in the MNI space (Fig. 2D).

2.3.3. VOIs for waypoints of CSTs

Anatomical studies have reported that CST axons pass through PLIC, MCP, pons, and MP in humans (Hoche, 1990; Pfiefer, 1934) and non-human primates (Morecraft et al., 2002). To identify the CS streamlines delineated by fiber tractography, four waypoint VOIs were used: posterior limb of internal capsule (PLIC), cerebral peduncle in midbrain (MCP), pons, and medullary pyramid (MP) on the left side (brown; Fig. 3A). The VOIs of PLIC, MCP, pons, and MP were defined on the basis of the “JHU-white matter tractography atlas” (ICBM-DTI-81 white-matter labels atlas) (Mori et al., 2008; Oishi et al., 2008). The VOI of PLIC was defined as the part of the “posterior limb of internal capsule” located from \( z = 9 \) to \( z = -7 \) in the MNI space. The VOI of MCP was defined as the part of “cerebral peduncle” located from \( z = -12 \) to \( z = -14 \) in the MNI space. The VOIs of the pons and MP were defined as the part of CSTs located from \( z = -34 \) to \( z = -36 \) and from \( z = -50 \) to
A. Parcellation of the cortical areas

B. Subregions of premotor area

C. Subregions of cingulate motor area

D. The candidate areas of CST origin with control area

(caption on next page)
was segmented into white matter, grey matter, and cerebrospinal fluid (CS). The histological examination of CSTs in humans (Weil and Lassek, 1929; Nathan and Smith, 1955; Nathan et al., 1990) showed that the majority of CS fibers pass through the posterior part of the lateral funiculus in the spinal cord. Thus, to further identify the CS streamlines at the spinal cord level, the VOI of the posterior part of the lateral funiculus in the spinal cord was defined on the basis of the MNI Poly AMU spinal cord template (Fonov et al., 2014). CS fibers are uniformly distributed on the posterior part of the lateral funiculus area in humans (Nathan et al., 1990) and are mixed in the lateral reticulospinal tract (Nathan et al., 1996), rubrospinal tract (Nathan and Smith, 1982), spinthalamic tract passing through the spinal lemniscus (Nathan et al., 2001), and spinocerebellar tract (Nathan et al., 1990). Hence, in the current study, we defined the VOI of the contralateral posterior part of the lateral funiculus as the sum of the lateral CST, lateral reticulospinal tract, rubrospinal tract, spinal lemniscus, and spinocerebellar tract in the right-sided half of the MNI-Poly-AMU spinal cord atlas (Fonov et al., 2014).

2.3.4. Defining the spinal cord segmental level

We used the T2-weighted image to identify the border of each spinal segment. Fig. 3D shows the definitions of spinal segments. In the standard space, ventral and dorsal rootlets were identified by visual inspection and manually traced from the intervertebral foramen to the spinal cord. The lowest points of the ventral and dorsal rootlets at each spinal segment were defined as the lowest z-coordinate of the spinal cord segment. The volume from the lowest z-coordinate of a segment (e.g., C6) to the lowest z-coordinate of the segment above (e.g., C5) was defined as the VOI of the segment (e.g., C6). Unlike the other segments, the VOI of C1 was defined as the region from the lowest z-coordinate of C1 to the upper end of the segmented spinal cord region. These VOIs of spinal segments from C1 to Th1 were merged into one spinal cord mask in the standard space.

2.3.5. Defining the inclusion mask

To restrict the region of interest (ROI) for fiber tractography, the inclusion mask was defined by combining the segmented white and gray matter regions of the whole brain and the posterior part of the lateral funiculus in the right-sided area of the spinal cord.

2.3.6. Preprocessing

To preprocess the brain structures, the brain region (A-2 in Supplementary Fig. 1; Sfig. 1) was cut out from the raw T1-weighted image (A-1 in Sfig. 1) for each participant at the obel level, which is the junction of the brainstem and spinal cord in the superoinferior axis. The brain image was segmented into white matter, grey matter, and cerebrospinal fluid (A-2 in Sfig. 1) using Mrtrix3 Sttgen function (https://www.mrtrix.org/ ) (Tournier et al., 2004, 2008). Non-brain tissues in the image were removed using the Mrtrix3 Stt2vis function (A-2 in Sfig. 1). The brain was registered to the Montreal Neurological Institute standard space with the FMRIB Software Library (FSL, http://www.fmrib.ox.ac.uk/fsl) (A-3 in Sfig. 1) to inversely transform the template VOIs into the native image (A-4 in Sfig. 1). The brain image (A-4 in Sfig. 1) was registered to transform the brain mask in the cropped brain space (A-5 in Sfig. 1) into raw data image space (A-1 in Sfig. 1).

For the analysis of spinal cord imaging, spinal cord segmentation was performed using raw T1-weighted images (A-6 in Sfig. 1). Then, the vertebral levels were manually labelled. The deformation field from the native space to the template space was calculated based on the segmented spinal cord and the labels of vertebral levels (A-7 in Sfig. 1). Using the deformation field, the VOIs defined in template space were inversely transformed into the native space (A-8 in Sfig. 1).

The segmentation masks of the brain (A-2 in Sfig. 1) and spinal cord (A-6 in Sfig. 1) were combined to create a merged mask of the brain and spinal cord (A-9 in Sfig. 1). If the parts of the mask above and below the obex were not reasonably continuous, the obex level was modified manually by painting using fsleyes (FSL, http://www.fmrib.ox.ac.uk/fsl) to connect the brain and spinal cord. Using the integrated brain and spinal mask, the brain and spinal cord data were extracted from the raw T1-weighted images (A-10 in Sfig. 1).

In addition, to define the VOIs in the cervical and thoracic spinal cord segments, T2-weighted images (B-1 in Sfig. 1) were segmented and spatially normalized from the native space to the standard space (B-2 in Sfig. 1) using the Spinal Cord Toolbox (De Leener et al., 2017). It is easier to identify spinal nerve roots on template space than native space due to spinal cord straightening. The VOIs of spinal segments in the template space (B-3 in Sfig. 1) were converted to the T1-weighted native space (B-5 in Sfig. 1) using a deformation field (B-4 in Sfig. 1), which was estimated during the spatial normalization process of the spinal cord on T1-weighted images (A-7 in Sfig. 1).

2.4. Fiber tractography

The preprocessing of DWI images involved the following steps (Sfig. 1 C): (1) removal of non-Gaussian noise based on the random matrix theory (Verava et al., 2016a, 2016b) (C-2 in Sfig. 1), (2) removal of Gibb’s ring artifacts by optimal shifting of the pixels to minimize the oscillation in the surroundings of sharp edges and interpolating the pixel values (Kellner et al., 2016) (C-3 in Sfig. 1), (3) correction of the eddy-current, motion-related artifacts, and susceptibility distortions based on the eddy-topup tools using SE-EPIs (Anderson et al., 2003; Smith et al., 2004; Andersson and Sotiropoulos, 2016) (C-4 in Sfig. 1 and 2); the distortion in DWI images was corrected (Sfig. 2) (4) bias field correction using the N4ITK algorithm (Tustison et al., 2010) (C-5 in Sfig. 1), (5) up-sampling DWI images with 1-mm isotropic voxel-size (C-6 in Sfig. 1) to enhance the reliability and robustness of fiber tractography based on the interpolation methods (Dyrby et al., 2014), and (6) registering DWI images into native T1-weighted images using rigid-body transformation matrix calculated by registering the mean b0 image into a native T1-weighted image (C-7 in Sfig. 1 and 2). To confirm the registration quality, the consistency of tissue edges between DWI and T1 weighted images was evaluated by visual inspection (Sfig. 2). One volunteer was excluded from analysis because of failure of registration from the mean b0 image to the T1-weighted image. The signal intensity around the upper cervical cord was low in the DWI image obtained from this volunteer, which may explain the registration failure. Therefore, further analyses were performed for the data from 30 volunteers (29 right-handed and 1 left-handed volunteers).

In the native space, the orientation distribution of the streamlines in each voxel under the inclusion mask was estimated using the multi-shell...
and multi-tissue version of the constrained spherical deconvolution (CSD). (Tournier et al., 2004, 2008) (D-1, 2, and 3 in Sfig. 1). We delineated 50,000 streamlines from the VOIs of candidates for the CST origin to the VOI of the posterior part of the lateral funiculus located from the C1 to Th1 levels on the right side, based on the fiber orientation distribution (FOD) using MRtrix3 (https://www.mrtrix.org/) (Tournier et al., 2004, 2008) (step size $= 0.25$ mm, max length $= 350$ mm, cutoff value $= 0.1$, maximum angle $= 15^\circ$) (D-4 in Sfig. 1 and 3 A). To exclude the fibers passing through the corpus callosum, the exclusion mask was set on the vertical plane to separate both hemispheres along the
longitudinal fissure of the cerebrum. Then, the streamlines passing through the four waypoint VOIs (i.e., PLIC, MCP, pons, and MP) were preserved, while the others were removed (D-5 in Sfig. 1 and 3A-C). The resulting streamlines were classified by their terminal spinal segments (C1-Th1) (D-6 in Sfig. 1). To remove the streamlines with unreasonable results, the terminal spinal segments through the four waypoint VOIs (i.e., PLIC, MCP, pons, and MP) were classified by their terminal spinal segments. Streamlines that were longer than 3 SD of the mean were deleted as outliers. Finally, the resulting streamlines were classified into the candidate cortical areas of CST origin and area of negative control (D-7 in Sfig. 1). To focus on the resulting streamlines, we eliminated the streamlines passing through more than one cortical area. Thus, when the streamlines arising from one cortical area were illustrated, the other cortical areas were set as the exclusion mask.

2.5. Quantitative analysis of CST origin from cortical areas

To quantitate the streamlines, we counted the number of streamlines arising from the candidate cortical areas for CST origin and an area of negative control (FP). To normalize for the individual differences, the proportion of streamlines was calculated as the number of streamlines from a candidate CST origin divided by the total number of streamlines. To normalize for the variation in the volumes of the cortical VOIs, the density of streamlines (lines/cm³) in each cortical area was calculated as the number of streamlines arising from each cortical area divided by the volume of each cortical VOI.

Cortical areas where the density of streamlines was not significantly different from that of streamlines from FP were defined as false positives for CST origin and excluded from analysis. Using the data that excluded the FP and false positives for CST origin, we calculated the proportion of streamlines in the remaining cortical areas.

2.6. CST passing areas in PLIC

To characterize the trajectory of CSTs, we investigated the passing area of CSTs from each cortical origin at the PLIC and MCP levels. We defined the voxels that included more than one streamline from each cortical area as the passing area for each volunteer. Then, we assessed the number of volunteers in whom the streamlines from each cortical origin passed through the voxel. We summed the binary individual images of passing area for each cortical origin, which was divided by the total number of volunteers (i.e., 30). Based on this passing ratio, the mean passing area for each cortical origin was defined as the number of voxels through which the streamlines passed in more than half of the volunteers (i.e., ratio of 0.5 or higher).

2.7. Proportion of CS streamlines passing through each spinal segment

We quantified the distribution of CS streamlines in the spinal cord. The number of CS streamlines arising from each cortical area was counted for spinal segments from C1 to Th1.

The proportion of streamlines for each cortical origin was calculated for each spinal segment. The proportion of streamlines at the C1 segment was the sum of the mean proportion of streamlines from the cortical origin of CSTs for each volunteer. The proportion of streamlines from each cortical origin at each spinal segment was calculated by dividing the number of streamlines from each cortical origin at each spinal segment by the number of streamlines from each cortical origin at the C1 segment for each volunteer.

In addition, the proportion of the number of CS streamlines at each spinal segment arising from each cortical origin was calculated. The number of streamlines arising from each cortical area was divided by the total number of streamlines at each spinal segment for individual volunteers, and then averaged for all volunteers.

2.8. Statistical analyses

One-way repeated measures analysis of variance (rmANOVAs) was performed to test the difference in the number of streamlines arising from different cortical areas. If there was a significant main effect, post hoc paired t-tests were conducted to explore the differences between the cortical areas. Bonferroni’s method was applied to correct for multiple comparisons. Furthermore, rmANOVA was performed to assess the difference in the number of streamlines among the spinal cord segments. If there was a significant main effect, post hoc paired t-tests with Bonferroni’s method were performed. Statistical significance was set at p < 0.05.

3. Results

We investigated the CSTs arising from the cerebral cortex in 29 right-handed and 1 left-handed volunteer by fiber tractography.

3.1. Trajectory of the detected streamlines arising from the cortical areas

Fig. 4 illustrates a typical example of CS streamlines arising from the candidate cortical areas with FP as the negative control in 3D native space. M1 (number: 1125, proportion: 43.02%) gave rise to an abundant number of streamlines. The streamlines originated from the lateral convexity to the medial wall, thereby covering the entire M1, including the whole-body representation. Although PMd (432, 16.52%), PMv (302, 11.55%), SMA (154, 5.89%), and S1 (328, 12.54%) gave rise to an abundant number of streamlines, their proportions were lower than those for M1. A few streamlines originated from preSMA (85, 3.25%), BA5 (23, 0.88%), BA7 (62, 2.37%), CCZ (27, 1.03%), RCZp (44, 1.66%), and RCZa (2, 0.08%), as well as FP (31, 1.19%), which was considered the negative control region.

3.2. Quantitative comparison of the detected streamlines among the candidate cortical areas and negative control area

To quantify the detected streamlines, we counted the number of streamlines originating from each candidate cortical area of CST origin and the negative control area in each volunteer (Fig. 5A). Despite considerable inter-individual variation in the number of streamlines originating from the cortical VOIs (max-min: 5401–50), M1 (1854–32) gave rise to the highest number of streamlines, followed by PMd (858–5), S1 (603–5), PMv (419–2), SMA (231–2), preSMA (104–1), BA5 (45–0), BA7 (247–0), CCZ (97–0), RCZp (73–0), and RCZa (25–0). Streamlines were also detected to arise from FP (53–0), the negative control area. There was a significant difference between FP and candidate cortical areas, including M1, PMd, PMv, SMA, and S1, in terms of the number of streamlines. However, the number of streamlines originating from preSMA, BA5, BA7, CCZ, RCZp, and RCZa was not different from that from FP [rmANOVA: F(11,319) = 41.68, p < 2.0 × 10⁻¹⁶, Fig. 5A].

To normalize the number of streamlines arising from each cortical area, the proportion of streamlines from each cortical area was calculated (Fig. 5B). 47.69 ± 1.54% of the streamlines arose from M1 (max = 64.00, min = 23.80). Furthermore, 15.71 ± 1.35%, 10.59 ± 0.87%, 10.58 ± 0.87%, and 4.94 ± 0.35% were observed to arise from PMd (max = 37.30, min = 4.58), S1 (max = 24.45, min = 2.11), PMv (max = 22.69, min = 3.23), and SMA (max = 8.02, min = 0.50), respectively. The remaining streamlines were seen to arise from preSMA (max = 5.83, min = 0.40), BA5 (max = 2.16, min = 0.00), BA7 (max = 10.26, min = 0.00), CCZ (max = 7.26, min = 0.00), RCZp (max = 7.57, min = 0.00), and RCZa (max = 3.24, min = 0.00). Importantly, 1.24 ± 0.22% of the streamlines were observed to arise from FP (max = 4.44, min = 0.00). There was no significant difference between FP and BA5, BA7, CCZ, RCZp, and RCZa in terms of the proportion of streamlines arising from the cortical areas [rmANOVA: F(11,319) = 311.9, p < 2.0 × 10⁻¹⁶, Fig. 5B].
There was a difference in the volume of the VOIs between the cortical areas (mean ± SE; M1: 15.14 ± 0.28 cm³, PMd: 9.38 ± 0.17 cm³, PMv: 7.32 ± 0.14 cm³, SMA: 2.79 ± 0.05 cm³, preSMA: 2.69 ± 0.05 cm³, S1: 13.33 ± 0.25 cm³, BA5: 2.25 ± 0.04 cm³, BA7: 25.10 ± 0.46 cm³, CCZ: 3.91 ± 0.07 cm³, RCZp: 2.31 ± 0.04 cm³; RCZa: 2.33 ± 0.05 cm³, FP: 9.84 ± 0.18 cm³). To normalize for this difference, the density of streamlines was calculated for each cortical area. M1 (mean ± SE: 41.99 ± 5.96 lines/cm³) showed the highest density among the cortical areas. The density of PMd (23.21 ± 4.16 lines/cm³), PMv (20.29 ± 3.17 lines/cm³), and SMA (24.11 ± 4.03 lines/cm³) were similar [rmANOVA: F(11,319) = 32.28, p < 2.0 × 10⁻¹⁶, Fig. 5C]. The density of streamlines originating from BA7 and RCZa were not significantly different from that from the negative control area FP (Fig. 5C); therefore, the streamlines detected from BA7 and RCZa were considered false positives and excluded from further analysis. Based on these results, we determined that CS fibers arise from nine cortical areas: M1, PMd, PMv, SMA, preSMA, S1, BA5, CCZ, and RCZp (Fig. 6A).
3.3. Quantitative comparison of streamlines among the cortical origins of CST

We calculated the proportion of CS streamlines originating from the nine cortical areas that were determined to be the origin of CSTs (Fig. 6). The proportion of CS streamlines differed significantly between the cortical areas [rmANOVA: \(F(8,232) = 293, p < 2.0 \times 10^{-16}\), Fig. 6 A and B]. The proportion of CS streamlines arising from M1 was 49.72 ± 1.61% (mean ± SE) (max-min: 65.31–24.21). Furthermore, 16.33 ± 1.37%, 11.06 ± 0.91%, and 11.01 ± 0.90% of the CS streamlines were distributed in PMd (37.94–4.84), S1 (25.85–2.14), and PMv (23.09–3.44), respectively, with no significant differences between them. The proportion of CS streamlines from SMA (5.14 ± 0.36%, 8.22–0.51) was significantly greater than those from preSMA (2.46 ± 0.26%, 5.98–0.42), RCZp (1.70 ± 0.34%, 7.91–0.42), CCZ (1.70 ± 0.30%, 7.72–0.5), and BA5 (0.88 ± 0.09%, 2.26–0.26) (Fig. 6B). The proportion of streamlines arising from preSMA was significantly higher than those from BA5, CCZ, and RCZp. However, there were no significant difference among BA5, CCZ, and RCZp.

These results demonstrated that, in humans, almost 50% of CS fibers arise from M1 and most of the remaining arise from PMd, PMv, SMA, and S1. A few CS fibers arise from preSMA, BA5, CCZ, and RCZp. Thus, over 75% of CS fibers arise from the lateral surface of the frontal lobes, including M1, PMd, and PMv.

3.4. Passing areas of CSTs in the subcortical waypoints

Fig. 7 shows the spatial distribution of CS streamlines in the subcortical waypoints. Fig. 7A-D shows an example of CS streamlines in a voxel (1.0 × 1.0 × 1.0 mm\(^3\)) at the PLIC level. This example voxel contained 48 streamlines from multiple cortical origins, indicating that the descending streamlines from each cortical area were substantially overlapped at the voxel level.

Figure E–G shows the spatial distribution of CS streamlines at the PLIC level. CS streamlines from the cortical areas were spread widely and partially overlapped. CS streamlines from PMd, PMv, and M1 on the

Fig. 6. Cortical origins of corticospinal tract (CST) and the proportion of corticospinal (CS) streamlines. (A) Cortical areas of CST origin in the lateral, medial, and superior views of left hemisphere. These nine areas were determined as CST origin based on statistical comparison of streamline density with FP as a negative control (see results in Fig. 5). Bars indicate mean proportion of streamlines from cortical areas of CST origin (n = 30). Dot represent data obtained from each participant. (B) The proportion of CS streamlines arising from these areas. Dots and bars represent the individual and mean proportion of streamlines from each area, respectively. (C) Matrix shows the results of post hoc pair-wise comparisons among areas. Black square indicates significant difference in proportion between the two cortical areas (p < 0.05, Bonferroni’s correction).
Fig. 7. Spatial distribution of corticospinal (CS) streamlines. (A) Location of an example single voxel (brown voxel, very small) in the posterior limb of the internal capsule (white-framed area) (subject: K.H.) and (B) magnified view of the same image. (C) CS streamlines passing through this example voxel and (D) magnified view of the same image. The numbers of streamlines are noted below the name of each cortical CST origin. (E) The spatial distributions of CS streamlines from the cortical areas were evaluated in the posterior limb of the internal capsule (white boxed region) on the template space (MNI coordinate $z = 8$) and in the cerebral peduncle of the midbrain (MNI coordinate $z = -13$) (H). Passing regions of CS streamlines from (F and I) lateral and (G and J) medial cortical areas. The colored frame represents the passing regions where CS streamlines from each cortical area passed through in more than half of volunteers. The lower and right line plots show the ratio of subjects whose CS streamlines from each cortical area passed through that MNI coordinate.
lateral cortical surface were ordered from anterior to posterior in the PLIC. The passing areas of CSTs from BA5 were completely overlapped by those from S1 (Fig. 7 F). CSTs from preSMA, RCZp, SMA, and CCZ in the medial wall occupied anterior to posterior positions in the PLIC. Additionally, CS streamlines from preSMA occupied the most anterior portion, and those from CCZ passed through the most posterior area on PLIC. RCZp occupied the front of the middle region and SMA occupied the back of the middle region (Fig. 7G).

Figure H–J shows the spatial distribution of CS streamlines at the MCP level. CS streamlines from the cortical areas were spread from

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**Fig. 8.** Corticospinal (CS) streamlines passing through the cervical spinal cord. (A) Typical example of 3D view of CS streamlines from the cortical areas to the spinal cord (subject: A.Y.). Brown volumes of interest (VOIs) represent C1, C4, and C7 segments. The passing streamlines from the nine CST origins in the C1, C4, and C7 segments are shown to the left of the 3D view. (B–D) The passing streamlines from each cortical area are illustrated at the C1, C4, and C7 segments. In these panels, gray and white areas indicate white and grey matter of the spinal cord on the trans-axial view, respectively.
lateral to the middle part of the cerebral peduncle and mostly overlapped at the MCP level (Fig. 7H–J). CS streamlines from PMd, PMv, and M1 on the lateral cortical surface were ordered from middle to lateral in the MCP. The passing areas of CSTs from S1 were in the most lateral part of MCP and completely overlapped those from BA5 (Fig. 7I). CSTs from preSMA, RCZp, SMA, and CCZ in the medial wall occupied middle to lateral positions in the MCP (Fig. 7J).

3.5. Trajectory of CS streamlines in the spinal cord

Fig. 8 shows the CS streamlines passing through the cervical cord in the native space (Fig. 8A). CS streamlines from each cortical area were intermingled in the posterior part of the lateral funiculus of the spinal cord (Fig. 8A). As seen in the transaxial spinal cord view in Fig. 8B–D, M1, PMd, PMv, and S1 gave rise to an abundant number of CS streamlines that passed through the C1 segment. CS streamlines from SMA and pre-SMA tended to be less than those arising from the areas mentioned previously. A few streamlines were found to arise from BA5, CCZ, and RCZp. In all cortical areas, the number of CS streamlines gradually decreased from C1 to C7 segments.

Throughout the cervical cord, CS streamlines were distributed uniformly in the posterior part of the lateral funiculus, regardless of the CST origins (Fig. 8), which was observed in all volunteers.

3.6. Quantification of CSTs in the spinal cord

To quantify the CS streamlines from each cortical area that descend within the spinal cord, we counted the number of CS streamlines from each cortical area at each spinal segment. The proportion of CS streamlines passing through each spinal segment was calculated (Fig. 9). In general, the number of CS streamlines gradually decreased in the lateral CST bundles from C1 to Th1 segments. The numbers of CS streamlines from M1 \(F(8232) = 38.46, p < 2.00 \times 10^{-16}\), PMd \(F(8232) = 26.29, p < 2.00 \times 10^{-16}\), and PMv \(F(8232) = 33.99, p < 2.00 \times 10^{-16}\) were significantly decreased in the lateral CST bundles from C1 to Th1 segments. The CS streamlines from SMA \(F(8232) = 27.61, p < 2.00 \times 10^{-16}\), S1 \(F(8232) = 25.73, p < 2.00 \times 10^{-16}\), and BA5 \(F(8232) = 21.97, p < 2.00 \times 10^{-16}\) were significantly decreased from the rostral to the caudal segments until C5. CS streamlines from preSMA \(F(8232) = 27.95, p < 2.00 \times 10^{-16}\), CCZ \(F(8232) = 20.46, p < 2.00 \times 10^{-16}\), and RCZp \(F(8232) = 21.37, p < 2.00 \times 10^{-16}\) were significantly decreased from the rostral to the caudal segments until C6 (Fig. 9B).

To understand the inter-segmental differences in CS streamlines, we investigated the proportion of CS streamlines from each cortical area at each spinal segment (Fig. 10). The proportion of streamlines at the C1 segment was the sum of the mean proportion of streamlines from each CST origin, which was the same as the proportion of CSTs among all CST origins (Fig. 6B). The number of CS streamlines at each spinal segment was normalized to that at the C1 segment. The proportion of CS streamlines gradually decreased from the rostral to the caudal segment (Fig. 10B), indicating that the CS fibers gradually terminated into the gray matter from the rostral to the caudal segment, regardless of the cortical origin.

To understand the differences in CST origins between the spinal segments, we investigated the intra-segmental proportion of CS streamlines within each spinal segment. The intra-segmental proportion of CS streamlines within each spinal segment was calculated by...
normalizing the number of CS streamlines arising from each cortical origin in each spinal segment to the total number of CS streamlines in each segment. This proportion was similar for all spinal segments (Fig. 10 C).

4. Discussion

In present study, we quantified the CSTs arising from multiple cortical areas in humans using fiber tractography with 3 T MRI. Since the densities of CS streamlines from M1, PMd, PMv, SMA, preSMA, CCZ, RCZp, S1, and BA5 were higher than that of the negative control site, FP, we concluded that these nine cortical areas are the CST origins in humans. The results showed that 49.71% of CS streamlines arise from M1, while the remaining 16.33%, 11.06%, 11.02%, and 5.14% of the CS streamlines arise from PMd, S1, PMv, and SMA, respectively. The CS streamlines from preSMA, BA5, and cingulate motor areas, such as the CCZ and RCZp, were sparse. The features of CS streamlines identified by our method are consistent with those of previous evidence obtained from necropsy and non-human primates in the following respects. 1) CS streamlines arise widely from multiple motor-related areas of the frontal lobe and somatosensory-related areas of the parietal lobe (Fig. 6). 2) At the subcortical level, the location of CS streamlines arising from the CST origins overlapped substantially (Fig. 7). 3) At the subcortical level, the antero-posterior spatial locations of CS streamlines bundles of each cortical origin were identical to those of the CS origins of cortex (compare Fig. 6 A and 7). 4) At the spinal cord level, the CS streamlines from each cortical area were uniformly distributed in the posterior part of the lateral funiculus (Fig. 8). 5) The CS streamlines reached the cervical cord, and their number were gradually decreased from the rostral to the caudal end (Fig. 9). Based on these results, we conclude that our method could reliably depict the CS streamlines from multiple cortical CS origins to the cervical cord in humans. Herein, we discuss the reliability of the quantitative assessment of the delineated CS streamlines using fiber tractography as well as the cortical origin of CSTs and proportions thereof.

4.1. Applicability of quantitative assessment of the delineated CS streamlines in fiber tractography

To non-invasively quantify the distribution of CS fibers from each CST origin, we counted the number of CS streamlines detected by fiber tractography. We assumed that the number of CS streamlines detected by fiber tractography reflected the relative number of CS axons. This concept is supported by previous studies, which reported that the number of CS streamlines detected by fiber tractography correlated with the level of motor dysfunction. Patients with postoperative motor deficits due to the resection of epileptic cortical tissue had significantly decreased number of streamlines from M1 (Jeong et al., 2014). Patients showing mirror movements showed an abnormally increased number of CS streamlines on the ipsilateral side of the spinal cord (Gallea et al., 2013; Weiniarz et al., 2017). The cortical origins of human CSTs have been investigated using necropsy. Jane et al. (1967) counted the number of residual pyramidal axons at the MP level after cortical ablation of the precentral gyrus and paracentral lobule, which includes M1, S1 and BA5, and concluded that 60% of CS fibers arise from these areas. This proportion is consistent with the total proportion of the sum of CS streamlines from M1, S1, and BA5 obtained in the present study (Fig. 6). The majority of CS axons in humans are 1–3 µm in diameter (Lassek, 1942), which means that each voxel in present study contained a very large number of axons. Thus, these results indicate that the number of CS streamlines reflects the “relative” number of CS axons.

Using diffusion tensor tractography, some previous studies (Seo and
study, none of participants showed significantly low FA (Sfig. 3D). However, in the present research, we investigated the relationship between the reduced number of streamlines and topographic distribution (compare Fig. 6 A and 7). On the other hand, at the MCP level, the CS streamlines arising from each cortical origin mostly overlapped, but were clustered (Fig. 7H–J). These results are consistent with the direct observation of corticofugal axons in macaque monkeys (Bucy et al., 1966; Morecraft et al., 2002) and humans (Pfeifer, 1934). Meanwhile, at the spinal cord level, the CS streamlines from each cortical area were uniformly distributed in the contralateral posterior part of the lateral funiculus (Fig. 8), which is consistent with the anatomical evidence from previous studies. At the spinal cord level, anterograde degenerated axons resulting from partial lesions of the motor cortex were uniformly distributed in the posterior part of the lateral funiculus in monkeys (Sherrington, 1889) and humans (Hoche, 1900; von Szinyethy, 1925). Labeled CS axons from M1 (Rosezweig et al., 2009; Yoshino-Saito et al., 2010), PMv (Borra et al., 2010), SMA (Maier et al., 2002), S1, and BA5 (Ralston and Ralston, 1985) are scattered in the lateral funiculus of the cervical cord of monkeys. These suggests that the CSTs are topographically distributed at the subcortical levels, but were intermingled in the spinal cord.

We found considerable inter-individual variations in the number, proportion, and density of streamlines originating from cortical VOIs (Figs. 5, 6, and 9). This inter-participant variability occurred due to the segregation process at each waypoint (i.e., PLIC, MCP, pons, and MP) (Sfig. 3A–C). A substantial number of streamlines were excluded at the levels of PLIC, MCP, and pons in most cases (Sfig. 3B and 3 C). The significant variations among participants can occur due to the poor image quality at each waypoint; therefore, the image quality at each waypoint was assessed. To assess the image quality, the fractional anisotropy (FA) values were calculated at each waypoint (Sfig. 3D). The FA value indicates fiber integration and density in the brain (Assaf and Pasternak, 2008). Participants with significantly poor image quality should have low FA values for the waypoints. However, in the present study, none of participants showed significantly low FA (Sfig. 3D). Moreover, we investigated the relationship between the reduced number of streamlines and FA values of the waypoints, and did not find any significant correlation (Sfig. 3E) at the four waypoints, indicating that the image quality was not the primary factor for the inter-participant variability in the number of the streamlines.

Inter-individual variation may contribute to the ability of dexterous body control. Further investigations should clarify the relationships between the parameters of CS streamlines and motor functions in healthy individuals. However, cortical geometry varies between individuals (Dale et al., 1999), which complicates the detection of connections by tractography (Abdali and Johansen-Berg, 2011). The geometric differences in the cortices of individuals may also affect the number of streamlines.

Based on our results, we concluded that counting the number of streamlines detected by fiber tractography is a reasonable method to estimate the relative number of CS axons.

### 4.2. Validation of the cortical origins of the CSTs

Although it is well-known that CSTs also originate from cortical areas other than the M1 in non-human primates, the cortical origins of CSTs in healthy humans are unclear. Therefore, we identified the candidates of CST origins. We selected the candidate CST origins (Fig. 2D) on the basis of anatomical (Table 1 and Fig. 1 A) and electro-physiological (Table 2 and Fig. 1B) evidence provided by previous studies. We selected FP as the negative control because there are no reports of anatomical connection between FP and spinal cord in any species. Cortical areas with significantly high density of streamlines compared to that of the negative control area were defined as the CST origins. As a result, we identified M1, PMd, PMv, SMA, preSMA, S1, BA5, CCZ, and RCZp as the CST origins. Because the densities of streamlines from BA7 and RCZa were identical to that of the negative control area (Fig. 5 C), indicating a false negative result, we excluded them from further analysis (Fig. 6). Because the results obtained using this procedure were consistent with the previous anatomical and electro-physiological evidences, which were used for selecting the CST origin candidates, we concluded that the procedure used in the present study was appropriate to determine the CST origins.

Anatomical evidence after spinal cord injury has shown that M1, S1, and BA5 are the CST origins (Fig. 1 and Table 1; Marinescu, 1901; Holmes and May, 1909; Marinesco, 1910; Wohlfarth, 1932), which is in line with our study results. The remaining cortical areas (i.e., PMd, PMv, SMA, preSMA, CCZ, and RCZp) were not identified as CST origins by previous anatomical evidence, but rather selected on the basis of electro-physiological evidence (Fig. 1 and Table 2; Horsey, 1909; Foerster, 1931; Penfield and Boldrey, 1937; Penfield and Welch, 1951; Talairach et al., 1973; Caruana et al., 2018; Rech et al., 2019; Fornia et al., 2020). Ablation of PM, including PMd, PMv, SMA, and preSMA, causes degeneration of the CSTs (Minckler et al., 1944), thereby suggesting that these areas are the CST origins. Additionally, electrical stimulation of these areas induces isolated, simple limb movements or motor task arrest (Table 2), suggesting that PM has direct access to the motor-related circuits in the spinal cord. In contrast, we did not find any evidence of retrograde or anterograde CST degeneration after spinal cord or brain injury to CCZ and RCZp. However, these areas contain the gigantopyramidal cells (Paus, 2001), which are known to be the origin of CSTs (Holmes and May, 1909; Lasek, 1940). Considering the results of this study and previous studies, the number of CS axons from these areas is considered to be quite small.

Although BA7 and RCZa were suggested to be CST origin candidates based on the electro-physiological evidence (Fig. 1B), they were excluded as CST origins (Fig. 6). Bartholomew (1874) reported that electrical stimulation of BA7 (Harris and Almerigt, 2009) requires a strong current to induce hand movements, but was accompanied by generalized convulsions. This description indicates that the current spread beyond the stimulation target. Electrical stimulation of the RCZa, located in the anterior part of the cingulate gyrus, elicits complex hand movements, such as opening or closing, rubbing, and/or scratching (Talairach et al., 1973), but mainly produced emotional responses, such as mirthful and mirthless laughter (Caruana et al., 2018). RCZa is activated during the response inhibition task, which requires the subject to stop an on-going response (Rubia et al., 2001). RCZa may project to the prefrontal region and M1, which are involved in cognitive and motor functions (Picard and Strick, 2001). Taken together, the aforementioned evidence suggests that BA7 and RCZa are either not the origins of CST or contribute a small part to CST origin.

### 4.3. Comparison of the proportion of CSTs between non-human primates and humans

The highly developed CS projections of primates play an important role in the relay of motor commands from the sensorimotor cortex to the spinal cord (Heffner and Masterton, 1975, 1983; Lemon and Griffiths,
gradually decreased from the cervical to sacral segments, indicating that of patients with pontine lesions. They found that degenerated CS fibers the number of degenerated CS fibers in the spinal cord during necropsy the number of CSTs from PMv leads to movement suppression is also reported in humans (Rech et al., 2019; Fornia et al., 2020). The ability to inhibit movements is essential for flexible and strategic movements (Filevich et al., 2012). These evidences indicate that the increment in the number of CSTs from PMv leads to enhanced inhibitory function, which may explain the dramatic evolution of behavioral imitation and motor learning in humans. Conversely, our results showed that few streamlines arise from CMA (Fig. 6), which has dense projections to the spinal cord in macaque monkeys. In macaque monkeys, almost 20% of the CSTs arise from CMAs, especially CMAd (He et al., 1995), which is thought to be equivalent to CCZ in humans (Picard and Strick, 2001). However, degenerated cells in CCZ and RCZp were not found following spinal cord injury in humans (see Table 1; Marinescu, 1906; Holmes and May, 1909; Marinesco, 1910; Wohlfarth, 1932). These anatomical findings in humans support our present results, which showed few streamlines arising from these areas (Fig. 6). The cingulate sulcus in humans often has anatomical variations (Paus et al., 1996). Indeed, 25 participants in the present study had a paracingulate sulcus (red line in Sfig. 4) in addition to the cingulate sulcus (white line in Sfig. 4). In 12 out of 25 participants, the paracingulate sulcus overlapped with RCZp VOI, and did not overlap with CCZ VOI (green colored VOIs in Sfig. 4) in any of the participants. However, there was no relationship between the overlapping VOIs and the number of streamlines from RCZp (Figs. 6 and Sfig. 4). These evidences may reflect the interspecies differences, and imply that the cingulate cortex is well-developed in humans, while the corticospinal pathway from the cingulate cortex has de-evolved. Although no direct evidence takes into account this interspecies difference, it is possible that in humans these cortical areas develop the cortico-cortical connections observed in macaque monkeys (Wang et al., 2001, 2004; Luppino et al., 2003; Takada et al., 2004).

4.4. CS projection patterns to the spinal segments

Yoshino-Saito et al. (2010) quantitatively assessed the CS projections from the digit region of M1 to C2-Th2 spinal segments using anterograde tracers in macaque monkeys. Labeled CS axons arising from M1 gradually decreased from the rostral to the caudal segment of the cervical cord, indicating that the majority of CS axons from the digit region of M1 terminate in the cervical enlargement. Well and Lassek (1929) counted the number of degenerated CS fibers in the spinal cord during necropsy of patients with pontine lesions. They found that degenerated CS fibers gradually decreased from the cervical to sacral segments, indicating that CS axons gradually terminate from the rostral to the caudal cord. These direct observations from previous studies in macaque monkeys and humans are consistent with our results obtained from tractography, which showed a decrement in the number of CS streamlines from the rostral to the caudal end (Fig. 9). Therefore, the mechanism underlying the decrement in the CS streamlines at the spinal level may be related to the direct observation in previous studies that suggested that CS axons gradually terminate in the gray matter in the spinal cord from the rostral to the caudal segments.

Although the VOIs included a whole-body representation of M1, including the trunk and lower limb territories (Fig. 2D), less than 10% of streamlines reached Th1 (Fig. 10B). Based on the aforementioned evidence, we expected a greater number of CS streamlines in the thoracic and lumbar cords, even in the caudal segment. Therefore we cannot rule out the possibility that the small number of streamlines toward the caudal cord reflect the reduced signal-to-noise ratio at the lower level of the cervical cord (Karbassirouzhan et al., 2019) and difficulty in reconstructing the longer streamlines due to uncertain tangent estimation (Jones, 2010).

Our results showed that the intra-segmental proportion of CS streamlines from different cortical areas was maintained across the spinal segments (Fig. 10). This novel finding indicates that the pattern of CS projections from different cortical origins is similar throughout the cervical cord. In macaque monkeys, PMv, especially the F5 hand motor area, has a weaker direct access and a stronger indirect access to the cervical enlargement, where hand muscle motoneurons are located (He et al., 1995; Borra et al., 2010), which is consistent with the present results in humans. This discrepancy may be due to phylogenetic interspecies differences. It is suggested that the CS neurons from PMv in macaque monkeys innervate the propriospinal neurons in the upper cervical cord (He et al., 1995; Borra et al., 2010). The propriospinal system is a phylogenetically older system (Alstermark et al., 2007, 2011). In humans, the growing need to control the skilled movements has led to the evolution of direct CS connections and weakened the indirect propriospinal connections (Nakajima et al., 2000).

4.5. Clinical perspective

Fiber tractography is a valuable tool for preoperative planning and postoperative follow-up of surgically treated tumors, vascular malformations, and epilepsy (Jeong et al., 2014; Auria et al., 2015; Mormina et al., 2015). The goal of surgical treatment is to maximize the extent of focused resection while minimizing the postoperative neurological impairments resulting from damage to the intact, functioning brain. The surgical outcomes can be improved by preoperative mapping of the tumor and its relationships to functional structures, including cerebral cortex and CSTs. While the presence of CSTs from other cortical areas besides the M1 is well-known in non-human primates, they have received less attention in humans. Clinical studies (Kennard et al., 1934; Freund and Hummelshelm, 1985; Fries et al., 1991; Binkofski et al., 1996) and animal studies (Passingham, 1988; Murata et al., 2015) of focal brain lesions and pharmacological inactivation (Hikosaka et al., 1985; Matsumura et al., 1991; Kermadi et al., 1997; Fogassi et al., 2001; Nishimura et al., 2007) have reported that the motor-related areas have separate functional roles in the control of voluntary movements. Although the functional roles of CSTs from different cortical areas are still unknown, it is likely that CSTs from each cortical area play a different role in voluntary movements. Our results showed that the CS streamlines from different CST origins are separated at the rostral aspect of the cortex (Fig. 4). Focal brain damage in this region can selectively damage CSTs from specific cortical origins and result in the loss of specific functions of those CSTs. The methods used in the present study may detect the damaged CSTs from a specific cortical area as well as the residual CSTs. Residual CSTs may compensate for the function of damaged CSTs. The methods reported in this study may provide an opportunity to evaluate whether residual CSTs can compensate for the
function of damaged CSTs after cortical or subcortical damage, thereby contributing to the recovery of motor functions. Such an assessment would enhance our understanding of the relationship between brain injury and functional impairment observed in clinical diseases and predict the prognosis.

In summary, we quantified the human CSTs arising from the cerebral cortex using fiber tractography based on DWI images. By comparing the density of streamlines between candidate CST origins and a negative control area, we identified nine cortical areas as CST origins: M1, PMd, PMv, SMA, preSMA, S1, BAS, CCZ, and RCZp. The densities of CS streamlines were higher for those arising from the frontal lobe compared to those arising from the parietal lobe or medial wall. These results have enhanced our understanding of the mechanisms underlying the cortical control of movements and can serve as the basis for future studies of the reorganization of CSTs after neural damage.

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Author contributions

NU, SS, HF, KA, and YN conceived and designed the experiments. NU, SS and HF performed the experiments. NU and SS analyzed the data. NU, SS, HF, KN, KA, and YN interpreted the results. NU, SS, and YN wrote the manuscript. All authors contributed to the article and approved the submitted version.

Declaration of interests

The authors declare no conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.neures.2022.06.008.

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