Investigating the white matter structure of the sensorimotor system in children with cerebral palsy

Julia Jaatela
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A doctoral thesis completed for the degree of Doctor of Science (Technology) to be defended, with the permission of the Aalto University School of Science, at a public examination held at the lecture hall F239a (Otakaari 3) of the school on 9 February 2024 at 13.00.

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Aalto University publication series
DOCTORAL THESES 23/2024

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ISBN 978-952-64-1653-3 (printed)
ISBN 978-952-64-1654-0 (pdf)
ISSN 1799-4934 (printed)
ISSN 1799-4942 (pdf)

Images: Cover image: Paula Ikonen

Unigrafia Oy
Helsinki 2024

Finland
Abstract

Cerebral palsy (CP) is the leading motor disorder in childhood, primarily caused by a brain insult occurring before birth. CP is typically characterized by spasticity, affecting one side of the body (hemiplegia) or both sides, predominantly the lower limbs (diplegia). The advancements in diffusion-weighted magnetic resonance imaging (dMRI) and tractography, have allowed the identification of altered white matter structure of the brain in CP. However, our understanding of how these alterations differ between hemiplegic and diplegic subtypes, and their relationship with sensorimotor deficits, such as balance, gait and manual dexterity, remains limited.

In this thesis, we investigated a cohort of children and adolescents aged 10–18 years diagnosed with hemiplegic (N = 16) or diplegic CP (N = 11) alongside their typically developed peers (N = 31). Using tractography, we investigated the interhemispheric commissural pathways, i.e. corpus callosum, and thalamocortical pathways connecting to the representational areas of the upper and lower limbs. To address the cortical abnormalities seen in CP and enhance the functional relevance of the studied tracts, we introduced a novel seeding approach using proprioceptive simulation (passive movement) of the limbs together with functional neuroimaging. The derived dMRI metrics were compared between the three groups, and their association with behavioral measures was investigated.

Our results showed significant alterations in the diffusion properties of the investigated pathways between children with and without CP, indicating changes in the axonal organization. Specifically, participants with hemiplegic CP seemed to have more severe structural changes that were relatively localized when compared to those with diplegic CP. The white matter involvement reflected, to some extent, the topographic presentation of the functional deficit. While we observed some associations between the dMRI-metrics and sensorimotor function, they were weak, and the directionality was non-conclusive, underscoring the complexity of the structure-function relationships.

The dMRI holds promising potential as an objective tool for guiding the diagnosis and treatment of CP in the future. By highlighting the differences in location and severity of white matter alterations between hemiplegic and diplegic CP, our findings contribute significantly to the existing literature. Further, our research emphasizes the importance of along-tract analysis and specific outlining of investigated tracts in future studies on both CP and typical development. Research on CP not only enhances our understanding of the disorder itself but also sheds light on the development and plasticity of the human sensorimotor system.

Keywords tractography, cerebral palsy, diffusion-weighted MRI, MEG, fMRI
Tiivistelmä
CP-vamma on motorinen oireyhtymä, joka on yleisimmin seurauksena ennen syntyä tapahtuneesta aivoavamasta. CP-vamman tavalisiin oireyyppeihin on poikkeava lihasjärjestys eli spastisuus, joka voi ilmetä pääasiassa toisella puolella kehoa (hemiplegia) tai vaikuttaa molemminpuolisesti, usein painottuen alaraajoihin (diplegia). CP-vamman vaikutuksia aivojen valkean aineen rakenteeseen voidaan nykyään tutkia hyödyntäen diffuusiopainotettua magneettikuvantamista (dMRI) ja traktografiaa. Kuitenkin ymmärrysemme siitä, miten nämä valkean aineen muutokset eroavat hemiplegisessä ja diplegisessä CP-vammassa, ja miten ne liittyvät sensorimotorisiin häiriöihin, kuten tasapainon, kävelyn ja käsin käytön haasteisiin, on edelleen rajallinen.


Tulevaisuudessa dMRI voi mahdollisesti toimia objektiivisena työkaluna CP-vamman diagnoosinnossa sekä kuntoutuksen ohjaamisessa. Tutkimuksen löydökset osoittivat selkeitä eroja valkean aineen muutosten sijainnin ja vakavuuden suhteen hemiplegisen ja diplegisen CP-vamman välillä, mikä tuo merkittävän lisän aiempaan tietoon. Lisäksi väittöskirja osoittaa, että tulevissa tutkimuksissa on tärkeää kiinnittää huomiota hermoratamallin tarkkaan rajaamiseen sekä sen pitkittäisanalyysiin. CP-tutkimus lisää paitsi ymmärrystämme itse oireyhtymästä, se antaa lisäksi tärkeää tietoa ihmisen sensorimotorisen järjestelmän kehittymisestä ja muovautumiskyvystä.

Avainsanat traktografia, CP oireyhtymä, diffuusiopainotettu MRI, MEG, fMRI
ISSN (painettu) 1799-4934 ISSN (pdf) 1799-4942
Julkaisupaikka Helsinki Painopaikka Helsinki Vuosi 2024
Preface

This research project would not have been possible without the support of several institutions. First, I would like to thank the Department of Neuroscience and Biomedical Engineering, Aalto University School of Science, for providing the facilities and scientific support to conduct the research. I thank the Academy of Finland, Jane and Aatos Erkko Foundation, Emil Aaltonen Foundation and the Doctoral Programme Brain & Mind for the financial support. Moreover, I thank the Department of Neurology and Motion Analysis Laboratory of the Helsinki University Central Hospital for their valuable co-operation.

The Thesis has not been a solo endeavor. My greatest gratitude goes to my supervisor, Prof. Harri Piitulainen. Thank you for trusting me with this topic and for all the guidance throughout the years. I could not have hoped for a better mentor for this path. Your enthusiasm towards the world of research is something to look up to. I also want to thank Prof. Lauri Parkkonen for the valuable input to the Thesis and all the practical support especially during the final stages of finalizing this work. Thank you for always finding the time for me in your fully booked calendar.

I am deeply grateful for the co-authors who have offered their expertise to this project. Helena Mäenpää, this project would not have happened without your passion and knowledge. Thank you for all the insightful discussions that will undoubtedly continue in the future. Thank you Viljami Sairanen for introducing me to the fascinating world of diffusion imaging and medical physics. Moreover, I want to thank Dogu Baran Aydogan for his irreplaceable support in this project.

I would like to extend my gratitude to all the people I have had the privilege working with throughout these years. First and foremost, Timo Nurmi and Jaakko Vallinoja, thank you for all the conversations and memorable moments in the past years. I feel fortunate to have shared this path with both of you. Thank you, Mia Illman, for the friendship. I was lucky to be pointed to a workstation next to you. All the great people from Aalto NeuroImaging, thank you for the support. This project would not have been possible without you. Thank you to all those mentioned and the countless others who may not be explicitly named but have played a part in this endeavor. Importantly, I also want to extend my deepest appreciation to the children and families who participated in this research project. Your contributions are invaluable.

Furthermore, I want to thank the pre-examiners Dr. Lisa Mailleux and Prof. Miika Nieminen for taking the time to review my Thesis. I am grateful for the
encouraging comments and valuable feedback that undoubtedly enhanced the quality of the work. I also want to express my gratitude to Prof. Christos Papadelis for accepting the request to act as the opponent in the public defence of my Thesis.

Finally, I wish to extend my appreciation to all those outside academia who have stood by me during this process. Your unwavering support and encouragement have been my constant motivators. Whether it was lending an empathetic ear during moments of frustration or celebrating small victories, your presence made this academic endeavor all the more meaningful. Through your encouragement, I have grown to trust myself and believe in my ability to achieve what I set out to accomplish. To my friends, thank you for being there to get my head out of the research world and remind me of all the great things I have in my life. To my family, thank you for the never-ending support. You are the pillars I can always lean on. Last but not least, I thank my loving husband for always having my back and being my biggest cheerleader. Tatu, I am infinitely grateful for our journey so far, and I look forward to the continued adventures we will face together.

Espoo, December 16, 2023

Julia Jaatela
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADF</td>
<td>Apparent diffusion coefficient</td>
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<tr>
<td>AFD</td>
<td>Apparent fiber density</td>
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<tr>
<td>AI</td>
<td>Asymmetry index</td>
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<tr>
<td>BOLD</td>
<td>Blood-oxygen-level-dependent response</td>
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<tr>
<td>CC</td>
<td>Corpus callosum</td>
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<tr>
<td>CP</td>
<td>Cerebral palsy</td>
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<tr>
<td>CSD</td>
<td>Constrained spherical deconvolution</td>
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<tr>
<td>dMRI</td>
<td>Diffusion-weighted magnetic resonance imaging</td>
</tr>
<tr>
<td>DP</td>
<td>Diplegic cerebral palsy</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion tensor imaging</td>
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<tr>
<td>EPI</td>
<td>Echo planar imaging</td>
</tr>
<tr>
<td>FA</td>
<td>Fractional anisotropy</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
</tr>
<tr>
<td>FOD</td>
<td>Fiber orientation distribution</td>
</tr>
<tr>
<td>GMFCS</td>
<td>Gross motor functioning classification scale</td>
</tr>
<tr>
<td>HP</td>
<td>Hemiplegic cerebral palsy</td>
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<tr>
<td>IMU</td>
<td>Inertial measurement unit</td>
</tr>
<tr>
<td>MACS</td>
<td>Manual ability classification scheme</td>
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<tr>
<td>MD</td>
<td>Mean diffusivity</td>
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<tr>
<td>MEG</td>
<td>Magnetoencephalography</td>
</tr>
<tr>
<td>MI</td>
<td>Primary motor cortex</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>PTT</td>
<td>Parallel transport tractography</td>
</tr>
<tr>
<td>PVL</td>
<td>Periventricular leukomalacia</td>
</tr>
<tr>
<td>PWM</td>
<td>Periventricular white matter</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>ROI</td>
<td>Region-of-interest</td>
</tr>
<tr>
<td>SI</td>
<td>Primary somatosensory cortex</td>
</tr>
<tr>
<td>SMI</td>
<td>Primary sensorimotor cortex</td>
</tr>
<tr>
<td>sMRI</td>
<td>Structural magnetic resonance imaging</td>
</tr>
<tr>
<td>TD</td>
<td>Typically developed</td>
</tr>
<tr>
<td>TE</td>
<td>Echo time</td>
</tr>
<tr>
<td>TMS</td>
<td>Transcranial magnetic stimulation</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition time</td>
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List of Publications

This doctoral dissertation is based on the following original publications:


Author’s Contribution

Publication 1 “Identification of proprioceptive thalamocortical tracts in children: comparison of fMRI, MEG, and manual seeding of probabilistic tractography”

HP, TN, JV and JJ conceptualized the work, designed the methodology and performed the measurements. TN was responsible for the fMRI analysis. JV was responsible for the MEG analysis. JJ did the preprocessing of structural and diffusion-weighted MRI data. JJ planned and carried out the tractography analysis together with DBA. JJ interpreted the analysis results with input from other authors. JJ prepared the illustrations and wrote the initial version of the manuscript. All authors reviewed and edited the manuscript.

Publication 2 “Altered corpus callosum structure in adolescents with cerebral palsy: connection to gait and balance”

HM, HP, TN, JV and JJ conceptualized the work. HP, TN, JV and JJ designed the methodology and performed the measurements. JJ did the preprocessing of the experimental data and planned the analysis. JJ carried out the data analyses with software input from VS. JJ interpreted the analysis results with input from other authors. JJ prepared the illustrations and wrote the initial version of the manuscript. All authors reviewed and edited the manuscript.

Publication 3 “Limb-specific thalamocortical tracts are impaired differently in hemiplegic and diplegic subtypes of cerebral palsy”

HM, HP, TN, JV and JJ conceptualized the work. HP, TN, JV and JJ designed the methodology and performed the measurements. TN was responsible for fMRI analysis. JJ did the preprocessing of structural and diffusion-weighted MRI data. JJ planned and carried out the tractography analysis together with DBA. JJ interpreted the analysis results with input from other authors. JJ prepared the illustrations and wrote the initial version of the manuscript. All authors reviewed and edited the manuscript.
1. Introduction

The sensorimotor system enables us to move and interact with the environment in everyday life. The brain structures responsible for sensorimotor processing are among the first to develop during the fetal period, undergo profound changes after birth and continue to mature through childhood and adolescence (Bear et al. 2007). The unceasing sensorimotor experiences drive this maturation that coincides with the emergence of new motor skills and motor control.

The developing brain possesses remarkable abilities to reorganize and adapt itself after an insult. Cerebral palsy (CP), a motor disorder resulting from a lesion or abnormality in the fetal or neonatal brain, offers thus a unique view on the brain’s compensatory abilities. By gaining a deeper understanding of the pathophysiological mechanisms underlying CP, we can enhance our knowledge of the sensorimotor system organization not only in CP but also in typical development. Moreover, advances in this field can lead to the development of objective tools that can guide both diagnosis and therapeutic interventions in individuals affected by this lifelong disability.

Half a century has passed since the emergence of magnetic resonance imaging (MRI), a technique that allows safe, non-invasive imaging of the brain structure. In the last few decades, modelling of the white matter pathways based on MRI data, i.e., tractography, has allowed researchers to visualize and study the underlying structure of specific connections between brain areas. So far, tractography has demonstrated an altered structure of multiple white matter pathways in children with CP (for a review, see Scheck et al. 2012; Franki et al. 2020). However, much uncertainty still exists about the relationship between the altered white matter structure and the range of sensorimotor difficulties in CP. Moreover, specifying functionally relevant white matter pathways reliably in typical as well as abnormal brain remains a challenge.

The primary objective of this thesis was to provide new insights into the structural organization of the sensorimotor system in children and adolescents, both with and without CP. Specifically, we aimed to investigate if the sensorimotor white matter pathways are affected differently in hemiplegic (affecting one side of the body) and diplegic (affecting predominantly lower limbs) subtypes of CP. In addition, we aimed to examine how these structures are linked to the key sensorimotor functions such as gait, balance and manual dexterity. Furthermore, we aimed to improve the specificity of tractography by targeting the analysis on the white matter pathways connecting to functionally relevant cortical locations.
2. Aims of the Thesis

The overall aim of this Thesis was to enhance understanding of the structural deficits of the sensorimotor brain network in CP and their association with motor performance. The specific objectives were:

I. To compare different seeding methods in constructing thalamocortical white matter tracts carrying proprioceptive information. (Publication 1)

II. To evaluate if the commissural pathways are differently affected in hemiplegic and diplegic CP and assess the role of the corpus callosum structure in standing and gait performance. (Publication 2)

III. To study the structural deficits of limb-specific thalamocortical pathways in hemiplegic and diplegic CP and evaluate their functional relevance. (Publication 3)
3. Background

3.1 Sensorimotor system

3.1.1 Sensorimotor integration

Precise motor control requires not only focused activation of the muscles but also efficient processing and integration of multimodal sensory information. Information from the surrounding world (touch and pain) and from the body (proprioception) travel to the central nervous system and is used to adjust bodily movements and posture. This processing of both motor and sensory information, i.e., sensorimotor processing, requires the interplay of a large network of cortical areas, but most importantly the primary sensorimotor cortex (SMI) area. The loop between peripheral receptors, cortical neurons and activation of the muscle cells forms the basis of our sensorimotor function.

Peripheral receptors are unique for each sensory modality (Bear et al. 2016a). Tactile sensations are conveyed via several specialized mechanoreceptors located in the skin, whereas thermoreceptors and nociceptors detect temperature and pain. The location and movement of the limbs are sensed with proprioceptors, such as muscle spindles and Golgi organs. Proprioceptors provide information on muscle force, heaviness and movement limits, enabling the adaptation of the motor plan and informing the internal model of movement. It has become evident that especially the proprioceptive feedback is essential for smooth motor actions, although the exact mechanisms of proprioception are still inadequately understood (Riemann and Lephart 2002).

Figure 1 shows a schematic illustration of the main pathways responsible for the loop between sensory experiences and motor execution. The afferent (i.e., carried from the periphery to the central nervous system) tactile and proprioceptive information is conveyed to the SMI cortex mainly through the afferent dorsal column-medial lemniscus pathway (Bear et al. 2016a). From the dorsal root of the spinal cord, the pathway crosses the body’s midline and travels up to the brainstem along the medial lemniscus, finally reaching the ventro-postero-lateral nucleus of the thalamus and, from there, the cortex. The efferent (i.e., carried from the central nervous system to the periphery) motor commands travel from the SMI cortex along the lateral column of the spinal cord, most importantly along the corticospinal pathway (Bear et al. 2016b). The corticospinal pathway bypasses the thalamic nuclei through the posterior limb of the internal capsule, continues towards the spinal cord through the cerebral peduncle,
travels along the ventral surface of the medulla (medullary pyramid) and decussates to the contralateral side of the body. Finally, the motor command reaches the motoneuron and interneuron pools through the ventral horn of the spinal cord. Other important pathways include, for example, the trigeminal pathways that are responsible for somatosensory information of the face, the spinothalamic pathway carrying temperature and pain signals and the ventromedial pathways mediating posture and balance through the brainstem (Bear et al. 2016a).

3.1.2 Primary sensorimotor cortices

The SMI cortices, located on both sides of the central sulcus, can be divided into distinct cytoarchitectural areas, i.e., Broadmann areas (Brodmann 1909), as illustrated in Figure 2. The postcentral gyrus, also known as the primary somatosensory (SI) cortex, is mainly responsible for the processing of sensory information. Area 3a is the most relevant for proprioceptive and 3b for cutaneous input, both receiving dense input from the ventro-postero-lateral nucleus of the thalamus (Bear et al. 2016a). Areas 1 and 2 are responsible for higher-order processing and information integration (Hsiao 2008). The precentral gyrus, i.e., the primary motor (MI) cortex or Area 4, is mainly responsible for motor processing. The corticospinal pathway originates mostly from Area 4 and Area 6 on its rostral side.
As illustrated in Figure 2, the SMI cortices have a well-established somatotopic organization, meaning that the limbs, trunk and face have representative areas along both the precentral and postcentral gyri. On the cranial end of the gyri are the areas for the lower limb and on the other end, more caudally and laterally, are the representative areas for the hand, fingers and face (Penfield and Jasper 1954). The size of the somatotopic area corresponds to the density of the received sensory input, resulting in larger areas for the face and fingers.

In addition to the SMI cortex, several other cortical and subcortical structures contribute to the sensorimotor system functioning. The secondary sensorimotor cortex, on the upper bank of the Sylvian fissure, is important for the integration of sensorimotor information of the whole body (Simões and Hari 1999), while the supplementary motor area and premotor cortex participate in motor planning and coordination. Furthermore, the posterior parietal cortex, including Areas 5 and 7, is responsible for the perception of body image and spatial relationships (Hyvärinen 1982) and the prefrontal cortex contributes to higher-order motor planning. Subcortical basal ganglia, such as the thalamus, putamen and globus pallidus, form several cortico-subcortical essential for motor control. Outside the two cerebral hemispheres, the cerebellum has a crucial role in motor learning, balance and unconscious movements.

Figure 2. The primary sensorimotor (SMI) cortex organization. (Based on Penfield and Jasper 1954; Bear et al. 2016a)

3.1.3 Neural structure of the brain

Neurons, the basic functional units of the brain, are formed by the main body (soma), multiple dendrites and typically a single axon as illustrated in Figure 3. The dendrites act as antennae receiving electrical impulses from other neurons via specialized chemical junctions, i.e., synapses. These tree-like structures extend from the soma by a maximum of one or two millimetres whereas the axon can stretch vast distances, even over a meter in the lower extremities (Bear et al. 2016c). The axon is responsible for the output of the neuron, carrying the signal, i.e., axon potential, from the soma to other neurons via synapses.
The white matter of the brain is almost entirely comprised of axons, whereas the cerebral cortex is formed by somas and layers of differentiated neurons, such as stellate cells or spiny pyramidal cells (Catani and de Schotten 2012). Therefore, it is thought that the cortex is responsible for higher-order processes, and the white matter accounts for conveying and modulating information to, from and between cortical areas. Efficient information transport is essential for all brain functions, and thus the calibration of signal propagation along the white matter axons of varying lengths, diameters and branching structures has a highly important role.

The conduction velocity of the axon can be greatly increased by myelinization. The sheaths wrap around the axon and are arranged in a periodical manner separated by approximately 1-µm long gaps called nodes of Ranvier (Fields 2015; Bear et al. 2016c) as illustrated in Figure 3. This discontinuous structure enables saltatory conduction, where the action potential “leaps” from node to node instead of sequential depolarization and repolarization of the axon membrane. Moreover, myelin insulates the axon from external interference (Bear et al. 2016c). Besides the thickness and coverage of the myelin sheaths, the propagation speed is influenced also by the diameter and ion-channel density of the axon.

The multilayered myelin sheaths are formed by specialized cells called oligodendrocytes that send protrusions that spiral around the axon. It has been suggested that oligodendrocytes also interact with neurons in other ways, influencing neuronal growth and survival (Jakovcevski et al. 2009). Oligodendrocytes belong to glial cells, which are as numerous as neurons in the brain (Bear et al. 2016c). Other glial cells include astrocytes, which fill most of the extraneuronal space in the brain and are thought to regulate the chemical environment and modulate neuronal growth and interactions. Small microglial cells act as phagocytes, removing damaged neurons and infectious material.

![Figure 3. Schematic presentation of the neuronal structure. (Based on Rowe et al. 2016)](image)
3.1.4 White matter pathways

In the brain, the myelinated axons travel in bundles from one location to another, forming a network of connections. The white matter bundles can connect neighbouring gyri (e.g., between the SI and MI) or reach long distances across the brain (e.g., between the occipital and frontal lobe). As proposed by Meynert (1885), these pathways can be classified into three categories: projection, commissural and association pathways.

Projection pathways carry neuronal signals from the cerebral cortex to subcortical structures, spinal cord and cerebellum, and back. They can be divided into ascending (afferent) or descending (efferent) pathways, meaning that the information travels to or from the cortex, respectively. The most important descending pathway from the SMI cortex is the corticospinal tract, as discussed in Section 3.1.1 and illustrated in Figure 1 (green). The primary ascending pathway, the dorsal column-medial lemniscus pathway, synapses in the thalamus, and thus the corresponding white matter bundles are referred to as thalamocortical tract or thalamic projections (Figure 1, blue). Although this section describes the structure of the sensorimotor system, it must be noted that the thalamus is widely connected to other cortical areas as well.

Commissural pathways connect the two cerebral hemispheres with each other (Figure 1, red). The most important commissure is the corpus callosum, the biggest white matter structure of the brain. The corpus callosum can be divided into different subparts using anatomical, histological, geometrical, or connectional approaches (Friedrich et al. 2020). The sensorimotor areas connect mainly through the body (the mid-section) or isthmus (posterior to body) of the corpus callosum, whereas more anterior brain areas connect through the genu and rostrum, and more posterior areas through the splenium.

Associative pathways connect different cortical areas of the same hemisphere. In the sensorimotor cortex, there are many short reciprocal connections between S1 and M1 cortices. These short U-fibers typically connect neighbouring gyri. Longer association pathways that connect to the sensorimotor areas include the arcuate fasciculus involved in visuospatial processing and the cingulum that connects the four lobes.

3.1.5 Neurotypical development

The developmental process of the sensorimotor cortices begins in the early embryonic period with the formation of the neural tube. Neurons start to form shortly after, approximately at gestational week 6 (Bear et al. 2016d). The walls of the rostral neural tube continue to develop to the forebrain, midbrain and hindbrain, and later to neural structures such as the cortex and thalamus. At the same time, neurons of the forebrain reach their axons to communicate with each other, forming the essential white matter networks. The arrangement of bundles, i.e., fasciculation, is guided by genes and intrinsic activity of the neurons and is thought to be also influenced by glial cell structures (Bear et al. 2016d). The major commissural and descending white matter bundles develop between gestational weeks 13–18, followed by maturation of the thalamocortical
pathways, approaching their cortical destination during the third trimester, and even later emergence of the long association pathways (Takahashi et al. 2012; Vasung et al. 2017). By the end of the second trimester, the central sulcus is formed.

This intrinsic formation of synapses is followed by a critical period of activity-dependent refinement, growth and differentiation of the connections. The synapse density peaks at the age of two or three and then ceases, presumably due to the diminished neuronal growth or maturation of synaptic communication and cortical processing (Bear et al. 2016c). For example, the corticospinal tract shows both ipsilateral and contralateral projection postnatally, and by two years of age, most ipsilateral corticospinal pathways are gradually withdrawn, and the contralateral tracts are reinforced.

In addition to synaptogenesis, the fetal period is a starting point for myelination and is also characterized by the proliferation and maturation of glial cells, accompanied by high expression of microglia (Billiards et al. 2006). The myelination follows caudal–cranial and posterior–anterior directions: first to mature are the sensorimotor areas, then the temporal and parietal areas, and last, the prefrontal areas responsible for higher-order processing (for a review, see Lebel & Deoni, 2018).

Although the most impressive neuronal plasticity ends after infancy, the refinement of brain connections continues through childhood and adolescence. For example, the size of the corpus callosum, which is thought to relate to myelination, seems to peak on average during the third decade of life (Danielsen et al. 2020). Despite the fact that sensorimotor areas are the first to develop, maturation has been seen to continue into adulthood in both SI and MI areas, with distinct maturational patterns (Tamnes et al. 2010).

3.2 Diffusion-weighted MRI

3.2.1 Basic principles

The development of magnetic resonance imaging (MRI) has significantly contributed to our understanding of human brain structure and function. In short, MRI relies on the interaction between the magnetic field and atomic nuclei, typically protons of hydrogen (H¹) atoms in water molecules. Atomic particles have an intrinsic quantum property called spin. The spin interacts with the electromagnetic field, giving rise to the phenomenon of nuclear magnetic resonance, where the precession of the nuclei in an applied magnetic field enables them to absorb and emit electromagnetic energy at a field-dependent frequency called Larmor frequency.

In MRI, first, the strong static magnetic field causes the angular momenta of the nuclear spins within the sample to align mostly with the field. Then, the net magnetization, the sum of multiple spins, is tipped from this equilibrium using a radiofrequency pulse perpendicular to the main magnetic field. After this tipping, the relaxation process gradually restores the net magnetization to its initial alignment both in longitudinal and transverse directions. During relaxation,
the nuclei in the sample emit radiofrequency signals, which can be picked up outside the sample. Finally, the spatially-encoded signals from multiple voxels in the sample are collected into an array of spatial frequencies (k-space) and the final image is reconstructed by applying an inverse Fourier transform.

The hydrogen of water molecules acts as the primary source of the nuclear magnetic resonance signal in biological tissue. Due to their inherent thermal energy, water molecules constantly move and randomly collide with each other (Einstein 1905). This movement, diffusion, gives rise to an imaging technique called diffusion-weighted MRI (dMRI). With a special line-up of pulsed magnetic field gradients (Stejskal and Tanner 1965), it is possible to detect the diffusion occurring within the sample. In brief, two strong diffusion-sensitizing gradients are introduced, which causes signal loss from the diffusing spins while the stationary spins remain unaffected. The strength and duration of these paired pulsed gradients, i.e., the degree of diffusion weighting, is quantified with a b-value (Rowe et al. 2016).

The movement of water molecules is affected by the surrounding structures. In an obstruction-free environment, such as the fluid-filled ventricles, the movement is isotropic, whereas in tissue, the diffusion is restricted or hindered as the molecules move within and between cellular structures. In the white matter, where myelinated axons are parallelly organized into bundles, the water molecules move more easily along the axonal direction than perpendicular to it, causing the diffusion to be anisotropic. If the diffusion-weighting is introduced in at least six spatial directions, it is possible to construct a mathematical model that describes the diffusion taking place within a voxel. The simplest three-dimensional model is called a tensor, which has been the most frequently used diffusion model in past research.

The ellipsoidal tensor can be presented mathematically by its three eigenvalues $\lambda_1$, $\lambda_2$, and $\lambda_3$, which describe the shape and size of the tensor (Dhollander 2016). Typical tensor metrics, such as fractional anisotropy (FA) and mean diffusivity (MD), are still commonly reported even if more sophisticated models might be used for tractography purposes or fiber-based metrics (Dhollander et al. 2021). MD describes the average water molecular displacement within a voxel and represents the size of the tensor, invariant of its orientation or shape. Both cortical and white matter voxels have a high number of cellular structures restricting free diffusion, therefore showing similar (low) MD values. Mathematically, MD is defined as the average of the eigenvalues:

$$MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$$

FA is used to describe the directionality of the diffusion, i.e., the shape of the tensor. FA is defined as:

$$FA = \sqrt{\frac{3((\lambda_1 - MD)^2 + (\lambda_2 - MD)^2 + (\lambda_3 - MD)^2)}{2(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)}}$$
In other words, FA is a normalized measure of how much the eigenvalues differ. FA values range between 0 (indicating perfect isotropy) and 1 (indicating perfect anisotropy). FA maps of the brain show regions with uniformly oriented axons, such as the corpus callosum, bright, whereas regions with complex neuronal structure, such as the cortex, appear dark.

Besides FA and MD, other metrics, such as the radial and axial diffusivity, have been introduced to describe the voxel-specific diffusion properties (Curran et al. 2016). Recently, there have been advances in creating more sophisticated measures that are not restricted to individual voxels but also describe the underlying neuronal pathways. For example, the apparent fiber density (AFD) describes the diffusion along a certain fiber direction (Raffelt et al. 2012). AFD, often referred also as FD, is therefore considered to be more applicable than voxel-based metrics in regions with crossing fibers (Raffelt et al. 2012, 2017).

Interaction between water molecules and cell membranes forms the foundation of dMRI imaging. Thus, the derived metrics can reveal interesting aspects of the underlying microstructure, including axon count and density, water content within a tissue, membrane permeability, viscosity of subcellular structures and the degree of myelination. Although the microstructural configuration is reflected by changes in dMRI metrics, there are numerous other biological and methodological factors influencing the measures, making the interpretation challenging (Curran et al. 2016). Moreover, a dMRI voxel is always an approximation over many heterogeneous microstructural environments that are summarized in the obtained signal. Diffusion imaging has, nevertheless, proven a useful tool in the attempt to understand the role of white matter pathways introduced for example by maturation or different brain disorders.

### 3.2.2 Tractography

Modelling of the white matter pathways, i.e., tractography, allows us to visualize and quantify the average trajectories of certain axonal bundles. It is important to remember, however, that the resolution of dMRI (millimeters) is far from the axonal diameter ranging from less than 1 µm to about 25 µm in the brain (Bear et al. 2016c). Single dMRI voxel can thus include not only countless axons but also several smaller bundles that cross, kiss, merge or diverge each other, which has been an issue especially in DTI-based tractography (Farquharson and Tournier 2016). Newer, more sophisticated mathematical approaches address these issues better but are still limited by the voxel resolution and data quality, including noise, intrinsic MRI artefacts, movement artifacts and imaging sequence design (such as b-weighting and the number of spatial directions).

In its simplest form, tractography can be implemented by selecting a starting voxel (seed), continuing to the next voxel pointed by the orientation of the longest axis of a tensor, and repeating this again in the following voxels until specific stopping criteria are filled (Caan 2016). The resulting paths are referred to as streamlines or fibers. Nowadays, the tensor model has been widely replaced by models that can better account for the crossing fibers. To model the fiber orientation distributions (FODs), a popular approach has been to use constrained spherical deconvolution (CSD, Tournier et al. 2004; Dell’Acqua et al. 2013),
which uses spherical harmonic functions to represent the diffusion data. The compartment model approach (Tran and Shi 2015) also enables approximations of intra- and extra-axonal signal components. Some approaches incorporate biophysical models, such as neurite orientation dispersion and density imaging, i.e., NODDI (Zhang et al. 2012).

In addition to different diffusion models, also the tractography algorithms are numerous. More traditional, deterministic tractography approaches model the fibers step by step following the voxel-wise diffusion orientation estimate, as described earlier. In contrast, probabilistic methods choose the fiber direction randomly from a distribution of possible directions and construct probability estimates of each tract (Caan 2016). It is generally acknowledged that all tractography algorithms can introduce both false positive (Schilling et al. 2019) and false negative (Aydogan et al. 2018) streamlines although the ratio might differ between approaches and depends highly on the data acquisition approach used.

All tractography approaches require several preset parameters and constraints, such as thresholds for fiber length and curvature, FA or FOD. Most importantly, the definition of tract endpoints is required, which can be solved in several ways. The tract can be aligned manually by drawing specific regions-of-interest (ROIs) in the individual anatomy. The ROIs can also be derived using automated segmentation and parcellation protocols, which are thought to decrease the intra-rater and inter-rater variability (Caan 2016). The outlining of ROIs can be, however, challenging especially in clinical populations (Rheault et al. 2020). An emerging approach to overcome this issue is to define the cortical endpoints for tractography using functional brain imaging, i.e., functional seeding, which has the possibility to improve the functional relevance and validity of the studied white matter tracts. Different functional seeding pipelines have been recently introduced by using transcranial magnetic stimulation (TMS; Frey et al. 2012; Weiss et al. 2015; Sollmann et al. 2020), magnetoencephalography (MEG; Gaetz et al. 2010; Meng et al. 2010; Papadelis et al. 2018) and functional magnetic resonance imaging (fMRI; Guye et al. 2003; Gschwind et al. 2012; Bernier et al. 2014; Reid et al. 2016; Oechslin et al. 2018).

3.2.3 dMRI and sensorimotor system

The development of the sensorimotor system is reflected in diffusion metrics. In short, the MD tends to decline and FA to increase through childhood and adolescence, which is considered to reflect the maturation and myelination of the axons (Tamnes et al. 2010). Moreover, recent studies measuring fiber density (Raffelt et al. 2012, 2017) have indicated that axonal density increases in childhood (Dimond et al. 2020; Gene et al. 2020). Distinct brain areas seem to reach the peak or plateau at different time points (Lebel et al. 2012). For example, FA in the body of corpus callosum seems to peak in the mid-thirties, whereas the genu matures already in the early twenties (Lebel et al. 2012). Tractography studies indicate that measures of connectivity also undergo age-related changes. In a study by Alkonyi and colleagues (2011), adolescents showed an increase in overall thalamocortical connectivity with age, although specific connections, such as the thalamic tracts to the right SMI cortex, seemed to
decrease with age. The maturation of sensorimotor white matter tracts has also been linked to manual dexterity (Fuelscher et al. 2021).

Besides age, also sex and handedness seem to be reflected in the white matter structure. The effect of gender on white matter maturational trajectories appears small but significant, with earlier development in females (for a review, see Lebel & Deoni, 2018). Handedness has been seen to relate with the FA values of the association and commissural pathways, but not with the corticospinal pathway (reviewed by Budisavljevic et al. 2021). Interestingly, the projection pathways seem to show left-ward lateralization in both left- and right-handed individuals (Wilde et al. 2009; Alkonyi et al. 2011; Budisavljevic et al. 2021).

Moreover, physical training can result in measurable changes in the white matter structure. For example, physical activity in children has been related to higher FA in the genu of the corpus callosum (Chaddock-Heyman et al. 2018) and decreased MD of several pathways, including the corticospinal and thalamocortical tracts (Rodriguez-Ayllon et al. 2020). In adults, training of the non-dominant hand has been shown to increase the FA values of the corresponding corticospinal tract (Reid et al. 2017). Furthermore, adults with a long background of specialized training, such as professional golfers, musicians, gymnasts and ballet dancers, seem to show structural alteration of the sensorimotor pathways compared to controls (Imfeld et al. 2009; Jäncke et al. 2009; Hänggi et al. 2010; Wang et al. 2016).

More support for the functional relevance of dMRI metrics is provided by their correlation to functional brain imaging. The fMRI blood-oxygen-level-dependent (BOLD) response following somatosensory stimulation or motor task seems to be positively correlated with FA (for a review, see Warbrick et al. 2017). Furthermore, the strength of resting-state functional connectivity seems to correlate positively with structural connectivity strength (for a review, see Damoiseaux and Greicius 2009; Garcés et al. 2016). A recent TMS study also demonstrated a direct relationship between the corticospinal tract structure and its excitability and conductivity (Betti et al. 2022).

To summarize, dMRI offers many possibilities to gain an understanding of the sensorimotor system organization and functional state. The measures of brain structure and function often go hand-in-hand and reflect physical sensorimotor abilities. Although the interpretation of dMRI can be difficult due to the complex microstructural and methodological aspects influencing the results, it offers a unique view into the arrangement of white matter pathways.

### 3.3 Cerebral palsy

#### 3.3.1 Clinical picture

The human brain can display staggering plasticity and adaptation after an injury, especially in the critical period of prenatal and postnatal brain development (Ismail et al. 2017). Hence, early brain lesions offer a unique opportunity to gain an understanding of the brain’s compensatory capabilities and the interplay between brain structure and function. As follows, neuroimaging studies in
children with cerebral palsy (CP) have shed light not only on the pathophysiology of the disorder but on the organization of the sensorimotor system in the developing brain. By definition, “CP describes a group of permanent disorders of the development and posture, causing activity limitations that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, perception, cognition, communication, and behaviour, by epilepsy, and by secondary musculoskeletal problems” (Rosenbaum et al. 2007). CP is therefore an umbrella term for a heterogenous clinical population that differs in terms of aetiology as well as in quality and severity of sensorimotor impairments.

CP has a prevalence of approximately 2–3 per 1000 live births in Western countries (Hagberg et al. 2001; Yeargin-Allsopp et al. 2008), ranking as the most common motor disorder in children. CP is typically diagnosed in early childhood, usually between 12–18 months of age, after the observation of abnormal movement and posture, delayed gross motor development or the persistence of primitive reflexes (Panteliadis et al. 2015). The clinical finding is confirmed with brain imaging, albeit the gross brain structure can appear normal in about 10% of children with CP (Krägeloh-Mann and Horber 2007; Horber et al. 2020). The motor symptoms can be accompanied by hearing and vision impairments, epileptic seizures, cognitive and attentional deficits, and problems with perception, such as touch, pain, and proprioception (Rosenbaum et al. 2007).

The CP diagnosis is usually based on the predominant neuromotor abnormality, which can be of spastic, dyskinetic or ataxic type (Krigger 2006). Over 70% of individuals with CP show spasticity, i.e., increased muscle tone or stiffness, which is accompanied by increased deep tendon reflexes and tremors. Dyskinetic CP accounts for 10–20% of the diagnoses and is characterized by slow, twisting movements. The remaining 5–10% of CP diagnoses are of ataxic type, displaying unsteadiness of movements and impairments in balance and coordination. Typically, the clinical diagnoses also incorporate topographical classification: the neuromotor abnormalities can be lateralized (hemiplegia), locate predominantly in the lower limbs (diplegia) or affect all four limbs (tetraplegia) (Upadhyay et al. 2020). As an example, spastic hemiplegic CP can be characterized by a bent, spastic arm and abnormal lower-limb use on the affected side, whereas spastic diplegic CP is commonly displayed by toe walking and flexed, inward-oriented knees due to the excessive muscle contraction of the lower limbs.

The most prominent activity limitations in CP are related to postural control and movement of the limbs. The severity of mobility restrictions is commonly evaluated with the gross motor functioning classification scale (GMFCS; Palsano et al. 1997), ranging from minor challenges in running and jumping (level I, mild) to inability to move independently (level V, severe). Upper-limb performance is typically classified using a manual ability classification scheme (MACS; Eliasson et al. 2006) where level I accounts for mild impairments in speed and accuracy of hand use and level V for total inability to handle objects.

Although CP is a permanent and incurable disorder, the clinical manifestations may change. Therapeutic interventions aim to improve the mobility, functionality and independence of individuals with CP. Rehabilitation approaches include physiotherapy, the use of orthosis, electrical stimulation, medical interventions such as botulinum injections, and surgeries (Krigger 2006; Upadhyay et al. 2020). As reviewed recently by Santana and colleagues (2022), the somatosensory and motor impairments in CP are often linked together, suggesting the need for incorporating both in rehabilitation approaches. Moreover, the increasing knowledge of the relationship between neuroimaging and motor outcome in CP (for a review, see Franki et al. 2020; Mailleux, Franki, et al. 2020) suggests the possible benefit of including structural brain imaging more closely in the process of making precise prognoses, and in evaluating the usefulness of different rehabilitation approaches.

3.3.2 MRI and aetiology

CP is caused by damage in the developing brain, which varies by cause, location, extent and timing (Rosenbaum et al. 2007). The majority of CP cases arise from a prenatal cause, and the rest during gestation or in the postnatal period (Krigger 2006). The risk for CP is increased in preterm-born babies (< 32 gestational weeks). Although the exact timing of the injury is often unknown, some assumptions can be derived from the pathological patterns seen in cranial MRI (Krägeloh-Mann and Horber 2007).

Structural MRI (sMRI) classification introduced by Krägeloh-Mann and Horber (2007) divides the brain findings in CP into five groups: normal, maldevelopment, periventricular white matter (PWM) lesions, cortical or deep grey matter lesions and miscellaneous, i.e., not fitting any other description (see Figure 4). In their review, predominant PWM lesions accounted for over half (56%) of the abnormal findings, followed by predominant grey matter lesions (18%) and maldevelopments (6%) (Krägeloh-Mann and Horber 2007). In a more recent European population study, the distributions were comparable: 49% PWM lesions, 21% grey matter injury and 11% maldevelopments (Horber et al. 2020). Maldevelopments of the brain are considered to originate from the embryonic or early fetal periods when the development of brain gross architecture takes place. The causes of malformations range from genetic factors to specific in-utero infections (Krägeloh-Mann and Horber 2007; Upadhyay et al. 2020). After the development of the basic neural architecture, brain damage is usually manifested as local lesions or defects. Typically, PWM lesions are associated more frequently with the early third trimester, whereas grey matter lesions are more typical in the late third trimester of pregnancy (Krägeloh-Mann...
Furthermore, PWM lesions are strongly related to prema-
turity and the most common lesion pattern is white matter injury around the
ventricles, periventricular leukomalacia (PVL) (Krägeloh-Mann and Horber
2007; Franki et al. 2020). Grey matter lesions, in turn, typically originate from
local unilateral infarction or haemorrhage (Krägeloh-Mann and Horber 2007;
Upadhyay et al. 2020).

Review papers by Anfield and colleagues (2013) and Franki and colleagues
(2020) have demonstrated relationships between sMRI abnormalities, CP type
and GMFCS levels. The most prominent association appears to be between
PWM lesions and mild (GMFCS I-II) spastic bilateral CP, whereas grey matter
involvement is typically related to spastic hemiplegic CP, spastic quadriplegic
CP, or dyskinetic CP (Arnfield et al. 2013; Franki et al. 2020). Moreover, the
lesion location and extent seem to correlate with upper-limb dysfunction in chil-
dren with hemiplegic CP (Fiori et al. 2015; Gupta et al. 2017; Mailleux et al.
2017) and with GMFCS level in children with symmetric white matter injury
(Reid et al. 2015). Overall, it seems that the severity of the motor impairment is
more dependent on the extent and topography of the brain injury and less on
the timing or type of the injury (Horber et al. 2020).

However, similar sMRI classifications can still lead to a wide range of different
clinical outcomes and pathophysiological models are far from absolute (Upadh-
yay et al. 2020). The classification of the predominant lesion does not take into
account detailed topography or secondary degenerative changes that might fol-
low the primary injury. For example, irrespective of the original cause, the cell
loss is followed by similar cellular responses, such as excess production of pro-
flammatory signalling molecules, oxidative stress and decrease of growth fac-
tors, that have a broad impact on the myelination process (Marret et al. 2013).
Thus, more sophisticated imaging of the brain structure might better explain
the severity of the impairments in CP.
3.3.3 Sensorimotor pathways

A growing body of literature suggests that the white matter pathways play an important role in CP (reviewed by Scheck et al. 2012) and impact the sensorimotor outcome (reviewed by Mailleux, Franki, et al. 2020). The most often reported pathway is the corticospinal tract, which may preserve its ipsilateral organization after unilateral brain insult (Kuo et al. 2017; Weinstein et al. 2018; Bleyenheuft et al. 2020). Moreover, the corticospinal tract appears to show decreased FA and/or increased MD bilaterally in diplegic and quadriplegic CP (Trivedi et al. 2010; Koerte et al. 2011; Lee et al. 2011; Chang et al. 2012; Wang et al. 2014; Arrigoni et al. 2016; Jiang et al. 2019) and particularly on the more affected side in hemiplegic CP (Thomas et al. 2005; Pannek et al. 2014; Kim and Son 2015; Lennartsson et al. 2015; Scheck et al. 2016; Kuczynski et al. 2018; Papadelis, Ahtam, et al. 2019; Papadelis, Kaye, et al. 2019; Azizi et al. 2021). Higher FA values of the corticospinal tract appear to correlate with better mobility (GMFCS) (Trivedi et al. 2010; Yoshida et al. 2010; Lee et al. 2011; Wang et al. 2014; Arrigoni et al. 2016; Jiang et al. 2019), and the corticospinal tract injury has been linked with impaired gait (Azizi et al. 2021) and upper-limb function (Pannek et al. 2014; Reid et al. 2016; Scheck et al. 2016; Gupta et al. 2017; Kuczynski et al. 2018; Mailleux, Simon-Martinez, et al. 2020).

Compared to corticospinal tracts, thalamocortical tracts seem more capable of bypassing severe lesions and retaining the contralateral organization (Guzzetta et al. 2007; Wilke et al. 2009; Nevalainen et al. 2012). Several studies have, however, highlighted the importance of thalamic projections in the

Furthermore, the involvement of corpus callosum in CP has been demonstrated with both conventional sMRI (i.e., Hayakawa et al. 1996; Kulak et al. 2007; Hawe et al. 2013; Reid et al. 2015) and dMRI (Thomas et al. 2005; Koerte et al. 2011; Lee et al. 2011; Weinstein et al. 2014; Arrigoni et al. 2016; Scheck et al. 2016; Jiang et al. 2019; Papadelis, Ahtam, et al. 2019). The structural changes seem to be more prominent in the body and splenium of the corpus callosum, overlapping with the location of the sensorimotor commissural pathways (Lee et al. 2011; Arrigoni et al. 2016). The integrity of callosal pathways seems especially important for bimanual upper-limb performance (Weinstein et al. 2014; Hung et al. 2019; Robert et al. 2021) and mirror movements (Hawe et al. 2013; Weinstein et al. 2014, 2018) and possibly for gait (Meyns et al. 2016; Papageorgiou et al. 2020).

Several pathways seem to mediate the motor outcome of children with CP, but their exact contributions are not fully understood. The corticospinal tract has drawn the most attention in past studies, thus leaving much uncertainty regarding the role of thalamocortical and transcallosal connections in CP. The importance of proprioception in the clinical picture of CP has been recently apprehended, highlighting the necessity for more research on the neural pathways mediating the afferent input. Due to the heterogeneity of the CP population and the variability in methodological approaches, there is a need for objective and detailed tractography studies. Although structural connectivity studies of the white matter indicate that the changes exceed the abovementioned pathways in diplegic (Lee et al. 2017), hemiplegic (Craig et al. 2020, 2022) and dyskinetic CP groups (Ballester-Plañé et al. 2017), the nature of these changes might be unravelled by studying specific neural tracts and their relationship with functional outcomes.
4. Materials and methods

4.1 Participants

Altogether, 88 children and adolescents aged between 10 and 18 years were recruited for the study. Due to schedule challenges, the burden of the extensive measurements or MRI contraindications (i.e., metal in the body, claustrophobia, anxiety or inability to stay still), 58 of these volunteers finally participated in the MRI session. Of the 58 volunteers, 27 were diagnosed with either hemiplegic (HP: \( N = 16, 11 \) females, age mean ± sd = 13.4 ± 2.3 years) or diplegic (DP: \( N = 11, 5 \) females, age = 13.2 ± 2.1 years) spastic CP and the rest were typically developed controls (TD: \( N = 31, 18 \) females, age = 14.0 ± 2.4 years). One hemiplegic and one diplegic participant interrupted the measurement before the dMRI scan, and one control participant was excluded from the analysis due to excessive movement during the sMRI scan. Furthermore, Publications 2 and 3 included only right-handed controls with successful functional imaging. Detailed demographics are presented in Table 1.

In Publication 1, the participants were all typically developed adolescents whereas Publications 2 and 3 included individuals both with and without CP. The inclusion criteria for participants with CP were the following: GMFCS level I or II (corresponding to limited or minimal ability to perform gross motor skills such as running and jumping), no diagnosed cognitive or co-operative deficiencies, no hearing deficit, no visual deficit other than refractive error, and no condition (other than CP) or medication known to affect gait and balance. The control participants had no known history of neurological deficits, diagnosis or medication.

For participants with hemiplegic CP, the dominant hand was specified as the non-paretic hand. For controls and participants with diplegic CP, hand dominance was assessed with the Edinburgh Handedness Inventory (Oldfield 1971). The questionnaire was filled out together with the researcher, and the participants were either asked to specify which side they preferred to perform the given activity or if unsure, to perform the activity. The test scale ranges from –100 (purely left-handed) to 100 (purely right-handed).

The research was conducted according to the recommendations of the Declaration of Helsinki and was approved by the Helsinki University Hospital ethics committee (HUS/2318/2016). Before the experiments, an informed written consent was obtained from all participants and their guardians. During the brain imaging, the participants were repeatedly asked if they wished to continue.
the experiment or take a break, and they had the possibility to stop the recording at any point. Participants were encouraged to ask questions and voice out any discomfort.

Table 1. Participant demographics.

<table>
<thead>
<tr>
<th>Publication 1</th>
<th>Hemiplegic cerebral palsy</th>
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<tr>
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<td>-</td>
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<td>-</td>
<td>14.2 ± 2.5</td>
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<td>30</td>
</tr>
<tr>
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</table>

* maldevelopment, miscellaneous or normal sMRI finding; ? unknown
GMFCS = gross motor function classification system; MACS = manual ability classification system

4.2 Measurements

4.2.1 Structural and diffusion-weighted MRI

All MRI measurements were performed at the Advanced Magnetic Imaging Centre of Aalto NeuroImaging (Aalto University, Espoo, Finland) using a 3 Tesla MRI scanner (Magnetom Skyra™, Siemens Healthcare, Erlangen, Germany) with a 32-channel head coil. All MRI measurements were performed by an experienced radiographer or laboratory technician together with one or two researchers. The whole MRI session, including sMRI, dMRI and fMRI, lasted for
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~75 minutes with a short break in the middle. All participants were familiarized with the measurements beforehand by watching an introductory video (https://www.aalto.fi/fi/neurotieteen-ja-laaketieteellisen-teknikan-laitos/proprioseptiikka-terveilla-ja-cp-vammaisilla). During the structural scans, they were instructed to lay as still and relaxed as possible and fixate their eyes on a cartoon show of their choosing, which was presented using a projector outside the MRI room, back-projection screen and mirrors. Earplugs and additional foam pillows were used for hearing protection.

The sMRI protocol included a T1-weighted magnetization-prepared rapid gradient-echo pulse sequence with 1-mm³ voxel resolution [field of view (FOV) = 256 x 256 mm²; reconstructed matrix = 256 x 256; slices = 176; repetition time (TR)/echo time (TE) = 2.53 s/3.3 ms; flip angle = 7°]. The dMRI sequence was a spin-echo-based single-shot echo-planar imaging (EPI) sequence with 2.5-mm³ resolution [FOV = 240 x 240 mm²; reconstructed matrix = 96 x 96; slices = 70; TR/TE = 8.3 s/81 ms; flip angle = 90°]. The dMRI protocol consisted of 64 gradient directions with diffusion weighting $b = 1000$ s/mm² and 8 acquisitions with $b = 0$ s/mm². Half of the images with $b = 0$ were gathered in the posterior–anterior phase-encoding direction and half in the anterior–posterior direction.

4.2.2 Functional brain imaging using MEG and fMRI

Functional brain imaging was utilized in Publication 1 and Publication 3 to specify cortical endpoints for tractography. The experimental setup is presented in Figure 5. Both MEG and fMRI measurements included proprioceptive stimulation of the index fingers and ankle joints performed with custom-made non-magnetic pneumatic movement actuators (Aalto NeuroImaging, Aalto University, Espoo, Finland) described previously by Nurmi and colleagues (2018) and Piitulainen and colleagues (2015, 2018). The feet were secured to the actuators with elastic straps and the index fingers with surgical tape. Tactile stimulation of the fingers was minimized by adding a layer of surgical tape around the fingertips. The seated (MEG) and supine (fMRI) positions were both supported by pillows for comfortability. In both measurements, the participants were instructed to keep their head as still as possible and fixate their eyes on a screen presenting slowly moving landscape video (MEG) or space images (fMRI).

MEG was recorded with a whole-scalp 306-channel neuromagnetometer (Elekta Neuromag™, MEGIN Oy, Helsinki, Finland) at MEG Core of Aalto NeuroImaging (sampling rate 1 kHz, passband 0.1–330 Hz). MEG was recorded by two or three researchers together with an experienced laboratory technician. During the recording, participants were either alone or together with the researcher inside a magnetically shielded room (Imedco AG, Hägendorf, Switzerland). The head position was tracked with five active head position indicator coils located on fixed anatomical positions on the scalp (Fastrak, Polhemus, Colchester, VT, USA). The electro-oculogram signal was recorded with a pair of electrodes placed vertically below and above the left eye. In total, 60 stimuli were delivered for each limb (index finger extensions of ~5 mm, ankle joint
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dorsiflexions of ~12°) in a random order, with an inter-stimulus-interval of 4000 ms and jitter of 250 ms.

The fMRI measurement was done with a standard spin-echo-based EPI sequence with a voxel size of 3 mm³ and a duration of ~30 minutes. Respiration and pulse were simultaneously recorded with a BIOPAC MP150 system (BIOPAC Systems Inc., Goleta, USA). Block-designed stimulation (20-s stimulation block followed by a 20-s rest block) included continuous passive movement of index fingers (3 Hz) and ankle joints (1 Hz) and was repeated 12 times.

Figure 5. Experimental setups in MEG and fMRI. Proprioceptive activation was induced by using custom-made pneumatic-movement actuators to evoke index finger and ankle joint movements. In MEG, visual stimulation was blocked by a cardboard screen. Fig. 1 in Publication 1.

4.2.3 Sensorimotor performance

The sensorimotor performance was assessed in terms of static standing stability and dynamic gait stability in Publications 2 and 3 and in terms of manual gross and fine motor skill in Publication 3. These measurements were conducted on a separate day either before or after the neuroimaging studies (with a maximum of 2.5 months apart) at the Motion Analysis Laboratory of the New Children’s Hospital (Helsinki University Hospital, Helsinki, Finland). The testing was performed by an experienced occupational therapist.

The static stability was quantified by measuring the average center-of-force velocity (mm/s) during four separate tasks. The center-of-force was recorded with a plantar-pressure plate (0.5 m Hi-End Foot-scan® system, RSscan international, Brussels, Belgium) with a rate of 33 samples/second. The participants were instructed to stand still to the best of their ability for 30 seconds at a time. The task was either to (1) stand with eyes open and feet pelvis-width apart, (2) stand eyes closed and feet pelvis-width apart, (3) stand with eyes open and feet together or (4) stand with eyes closed and feet together. The pelvis width was
measured as the distance between the anterior superior iliac spines. To ensure the absence of visual stimuli in the eyes-closed tasks, the participants wore goggles with opaque black lenses.

The dynamic gait stability was measured with an inertial measurement unit, i.e., IMU, (NGIMU, x-io Technologies Limited, Bristol, UK) placed on the mid-back with an elastic strap (Piitulainen et al. 2021). The refined-compound-multiscale entropy indices (https://github.com/tjrantal/javaMSE; Ihlen et al. 2016) were calculated separately for horizontal and vertical acceleration recorded with the IMU. The participants walked at a preferred speed on a 10-m walkway either 1) without any specific task (normal condition), (2) carrying an empty mug with a tray (motor dual task), or (3) listing aloud semantic or phoneme class words as fast as possible (cognitive dual task, in accordance with the NEPSY-II assessment by Korkman et al. 2007). Initial and final portions of the 10-m walkway cycle were removed from the analysis.

The gross-manual dexterity was measured with the Box and Block test (developed by Hyres and Buhler in 1957; Mathiowetz, Federman, et al. 1985) and fine-manual dexterity with the Nine-Hole Peg test (Mathiowetz, Weber, et al. 1985). In the Box and block test, the participant moves as many small wooden blocks from one compartment to another within 60 seconds using only one hand. In the Nine-Hole Peg test, the task is to place nine small pegs into nine small holes on the board and then move them back to the container as fast as possible using only one hand. Both tasks were first performed with the dominant hand and then with the non-dominant hand without any training. Two hemiplegic and one diplegic CP participants were unable to perform the task with the more affected hand.

Figure 6. Sensorimotor tests used in the studies. The tests included static standing stability, dynamic gait stability (arrow shows the IMU device position on the lower back), Box and Block and Nine-Hole Peg tests. Modified from Fig. 1 in Publication 2.

4.3 Data processing

4.3.1 Sum variables of sensorimotor performance

To achieve robust performance estimates and reduce the number of comparisons in the correlation analysis, the sensorimotor test results were presented as
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Sum variables. In Publication 2, the sum variables were constructed separately for the static and dynamic stability. In Publication 3, the sum variables were defined separately for the upper (including unimanual gross and fine dexterity, pooled over both hands) and lower limbs (including static and dynamic stability).

Sum variables were constructed as follows. First, each measure was normalized across the whole studied population to a scale from 0 to 1 (min-max normalization). Then, the normalized values of individual tests were averaged for each participant to obtain the sum variable. In Publication 3, we used imputation (replacing the missing value with the average value of the correct participant group) if less than half of the test scores were missing.

4.3.2 Parcellation and segmentation of the T1-weighted images

The structural T1-weighted images were used for volumetric analysis in Publication 2, for MEG analysis in Publication 1 and for dMRI analysis in all publications. The automatic parcellation and segmentation of the T1 images were performed with the Freesurfer image analysis program (http://surfer.nmr.mgh.harvard.edu/; Dale et al. 1999; Fischl et al. 2002; Segonne et al. 2007; Fischl 2012). For cortical parcellation, the Desikan-Killiany Atlas was used (Desikan et al. 2006). The cortical and white matter surface reconstructions, SMI cortex parcellation and thalamic segmentations were all visually checked and corrected if necessary.

In Publication 2, the corpus callosum was outlined manually due to the imprecise performance of the automatic pipeline in the patient group. Outlining was done at one sagittal slice at the midline of the brain using the MRIcron software (version 2018; http://www.mricron.com by Chris Rorden). This cross-sectional corpus callosum area was then geometrically parcelled into seven subparts (Witelson 1989) using a custom script on Matlab (R2021b, Mathworks, Natick, Massachusetts, United States).

4.3.3 Limb-specific ROIs for thalamocortical tracts

In Publication 1, the thalamocortical tracts were constructed using the functionally (fMRI and MEG) and manually selected cortical ROIs specific for the index finger and ankle proprioceptive cortical areas. The manual-ROIs (based purely on the anatomical landmarks) and fMRI-ROIs (based on the proprioceptive BOLD signals) were also used in Publication 3. To study the lateralization of the thalamocortical tracts, only right-handed controls were included in the analysis.

The individual T1 images and surface reconstructions were utilized to specify the finger and ankle specific manual-ROIs. The ROI for the index finger was placed in the proximity of the ‘hand-knob’ landmark (Yousry et al. 1997) on the postcentral sulcus of the contralateral hemisphere. The ROI for the ankle was set on the medial wall of the paracentral lobule of the contralateral hemisphere. Locations were consistent with the SI cortex homunculus (Penfield and Jasper 1954) and earlier fMRI studies using proprioceptive stimulation (for example, Francis et al. 2009; Piitulainen et al. 2015; Lolli et al. 2019; Hakonen et al. 2019).
The placement of the left and right finger and ankle ROIs was done manually with 3D Slicer software (version 4.8.0; https://www.slicer.org/; Fedorov et al. 2012) by carefully selecting a single voxel on the white matter surface.

Preprocessing of the fMRI data was performed using the SPM12 software (Wellcome Department of Imaging Neuroscience, University College London, UK) with a custom script on Matlab (R2016b, Mathworks, Natick, Massachusetts, United States). The preprocessing included slice-, time- and motion-corrections, co-registration to the T1 image, correction for physiological signals (Drifter tool, Särkkä et al. 2012), smoothing with a 6-mm kernel and temporal high-pass filtering between 334 and 658 seconds. The general linear model was used to estimate the relevance of voxel-specific signals in response to the stimuli. To find relevant activity locations for each limb, the contrast image threshold was adjusted to show activation in the contralateral SMI cortex (Marsbar toolbox: MARSeille Boîte À Région d’Intérêt; Marseille, France; version 0.44). The primary activation location was specified as the center-of-mass (including only the activation inside an 8-mm sphere fixed around the local maximum). These center-of-mass locations for all stimulated limbs were finally manually projected on the individual white matter surface in order to obtain fMRI-ROIs for tractography.

To obtain MEG-ROIs, the raw MEG signal was denoised using an oversampled temporal projection method (Larson and Taulu 2017) and corrected for artefacts with temporal-signal-space separation (MaxFilter™, MEGIN Oy, Helsinki, Finland; Taulu & Simola, 2006) and independent component analysis methods (fastICA; Hyvärinen & Oja, 1997). Then the data was further band-pass filtered (1–40 Hz) and then averaged over all baseline corrected epochs (−0.5–0.5 seconds with respect to stimulus onset) for each stimulus type. Using MNE-Python software (Gramfort et al. 2014), the MNE forward solution (Gramfort et al. 2013) was created for the surface-based source space obtained from Freesurfer. Finally, source location estimation was done using a normalized minimum norm estimate sLORETA algorithm (Pascual-Marquina 2002). For each limb, the single voxels at the peak response locations were selected as MEG-ROIs for tractography analysis.

**4.3.4 Diffusion-weighted MRI preprocessing**

First, the dMRI data was converted to 4D NIFTI format using dcm2nii software (version 2016, https://people.cas.sc.edu/rorden/mricron/dcm2nii.html) and checked visually for any pronounced artefacts. Next, the FMRIB’s Software Library tools topup and eddy (FSL version 6.0; Andersson et al. 2003; Smith et al. 2004; Jenkinson et al. 2012; Andersson and Sotiropoulos 2016) were used for motion and eddy current corrections. The estimate of susceptibility induced off-resonance field was created based on the \( b=0 \) images obtained in both anterior–posterior and posterior–anterior directions. The \( b \)-vector directions were updated at each step.

Voxel-wise diffusion tensors were then estimated with robust extraction of kurtosis indices with a linear estimation algorithm (REKINDLE; Tax et al. 2015) in ExploreDTI software (version 4.8.6; Leemans et al. 2009). The left-right
orientation of the images and the color-coded tensor directions were ensured visually for correct gradient component order and directionality. Any remaining geometric deformations were corrected using the T1-weighted image as an undistorted reference modality, and the dMRI data was interpolated to 1-mm³ voxel resolution. Finally, the outlier profiles, data quality and the alignment of the color-coded dMRI data with the T1 image were verified visually.

4.4 Tractography

In Publication 2, the tractography analysis of the commissural sensorimotor tract was done with the ExploreDTI software. A CSD model with 8th-order spherical harmonics was used for the FOD estimation with a recursive calibration of the response function (Tax et al. 2014). Compared to DTI, the CSD model can more accurately represent complex white matter structures, such as crossing fibers. Then, using a deterministic algorithm implemented in the software (Jeurissen et al. 2011), streamlines were created for the whole brain. The seed point resolution and step size were both set to 1 mm, the maximum angle threshold was selected to be 30°, the fiber length range was restricted to 20–250 mm, and the FOD threshold (roughly corresponding to FA threshold) was set to 0.1. Streamlines were then filtered to include only those travelling between the corpus callosum cross-sectional area and the dominant SM1 cortical area, i.e., FreeSurfer parcellations of the precentral gyrus, postcentral gyrus and paracentral lobule. The cortical reconstruction was used to exclude fibers jumping across any sulcus. Finally, the bundles were trimmed manually to include only streamlines reaching the lower-limb cortical repetitional areas (Penfield and Jasper 1954), i.e., the most superior parts of the SMI gyri or the medial wall of the paracentral lobule. The analysis of these transcallosal sensorimotor fibers was restricted to one hemisphere in order to achieve high robustness and consistency also in the presence of large unilateral lesions.

In Publication 1 and Publication 3, tractography was used to study the thalamocortical tracts specific for the left and right finger and ankle. In these analyses, a compartment model approach (Tran and Shi 2015) was used for the FOD estimation, allowing the separation of intra- and extra-axonal signal components. Streamlines were generated in Trekker software (https://dmritrekker.github.io/) with a probabilistic parallel transport tractography algorithm (PTT; Aydogan & Shi, 2020), which propagates by predicting a future streamline segment using a geometrically smooth fiber model, i.e., the probe. The following tracking parameters were used: probeLength = 0.2 mm, minRadiusOfCurvature = 0.4 mm, minFODamp = 0.01 and dataSupportExponent = 0.25, other parameters were set to default. First, one million streamlines were initiated from random starting points within the thalamus. Then, streamlines were filtered to reach either (1) a manually specified anatomy-based cortical ROI or (2) a functionally obtained ROI representing the cortical activation location following proprioceptive stimulation (both MEG and fMRI in Publication 1, fMRI in Publication 3). To address possible registration errors, all ROIs (originally sized 1 mm³) were dilated using a spherical kernel of 3 mm. Dipy
(Garyfallidis et al. 2014) was used to remove any spurious streamlines by thresholding the fiber to bundle coherence measure (Meesters et al. 2016), and cortical reconstruction was used to exclude fibers jumping across any sulcus.

Figure 7. Examples of the studied tracts. The left panel shows the ROIs and the resulting lower-limb sensorimotor commissural tract in a hemiplegic participant. The right panel presents the limb-specific thalamocortical tracts obtained with manual (green), fMRI (red) or MEG (blue) based ROIs in a control participant. Modified from Fig. 4 in Publication 1 and Fig. 4 in Publication 2.

4.4.1 Reported metrics

All tractography analyses were done in the individual anatomy of the participants. In all publications, the tracts were evaluated in terms of FA and MD, which are the most often reported dMRI metrics in literature. In Publication 1 and Publication 3, the AFD value (Raffelt et al. 2012) was also reported. These variables were reported both as the average tract values and as individual data points along the tract bundle trajectories (Publications Publication 2 and Publication 3). Furthermore, in Publication 2, the cross-sectional areas (mm²) were reported alongside the average FA and MD values for each corpus callosum subpart.

Publication 1 also evaluated the Euclidean distances between the cortical ROIs obtained either using manual, fMRI or MEG seeding approach and their depth (ROI distance from the brain envelope, i.e., a smoothed outer surface of the cortex). Moreover, the publication reported the overlap percentages of the thalamocortical tracts obtained using manual, fMRI or MEG approaches as follows:

\[
\text{Overlap} \, (\%) = 100 \times \frac{A_{\text{overlap}}}{A_1 + A_2 - A_{\text{overlap}}},
\]

where \(A_1\) and \(A_2\) represent either fMRI-, MEG- or manual-ROI based tract volumes and \(A_{\text{overlap}}\) their overlap, i.e., the number of voxels shared by both tracts. In addition, Publication 3 included an evaluation of the hemispheric symmetry of the thalamocortical tracts, calculated as:
Asymmetry index (\%) = 100 × \frac{D_{\text{non-dominant}} - D_{\text{dominant}}}{(D_{\text{non-dominant}} + D_{\text{dominant}})/2},

where \( D \) describes the average dMRI metric (FA, MD or AFD) of the thalamocortical tract located either in the dominant or non-dominant hemisphere.

### 4.5 Statistical analysis

All statistical testing was performed with R statistical software (version 4.0.4; R Development Core Team, 2019). To compare the hemiplegic CP, diplegic CP and control groups, the Kruskal-Wallis H-test (Kruskal and Wallis 1952) was applied. It is a rank-based nonparametric test that can determine if there are statistically significant differences between the groups in the given parameter. If a statistically significant difference (\( p < 0.05 \)) was present, the Conover post-hoc test was used to study pair-wise differences (Conover 1999). Post-hoc tests were FDR-corrected for multiple comparisons (Benjamini and Hochberg 1995). Publication 1 further included the F-test (variance of ROI MNI coordinates) and the Mann-Whitney U-test (comparison of left and right hemispheres; Mann & Whitney, 1947).

To study the associations between the white matter metrics and sensorimotor performance, a multiple linear regression model (\texttt{lm[-]} function, R statistical software; Wilkinson and Rogers 1973; Chambers 1992) was used in Publication 2. It is a generalization of simple linear regression with the possibility to evaluate the existence of linear relationships between several explanatory variables and the response variable. In addition, associations were explored using two-tailed Spearman (Publication 2) or Pearson (Publication 3) correlation, which were corrected for age (Kim 2015). Pearson correlation assumes a Gaussian-like distribution for the data, whereas Spearman is a non-parametric version of the test. Multiple comparisons in correlation analysis were addressed with Bonferroni correction.
5. Summary of Results

5.1 Publication 1: Comparison of seeding approaches

The aim of Publication 1 was to compare different seeding approaches for constructing proprioceptive thalamocortical tracts specific for fingers or ankles. The tracts were created using either manual cortical ROIs, based purely on anatomical landmarks of the SMI cortex, or functional cortical ROIs, based on the cortical activation following the passive movement of the limbs in MEG or fMRI. The participants included in the study were all right-handed typically developed children with no history of neurological disorders or brain injuries (N = 19).

All approaches succeeded in extracting anatomically plausible thalamocortical tracts. As presented in Figure 8, the MEG-based ROIs were located deeper in the brain, whereas the fMRI-based ROIs were located closer to the brain surface. The volume, anisotropy (FA) and diffusivity (ADC/MD) values of the resulting tracts differed between the three seeding approaches, as seen in Figure 9. For example, the finger-specific thalamocortical tracts had lower ADC values when defined with MEG-based ROIs ($p < 0.05$ for MEG-based vs. manual-based left finger tracts; $p < 0.05$ for MEG-based vs. fMRI-based right finger tracts). The MEG-based thalamocortical tracts for ankles had, in turn, higher FA values ($p < 0.01$ for MEG-based vs. manual-based left ankle tracts; $p < 0.05$ for MEG-based vs. manual-based right ankle tracts). Thus, the results indicate that the selected seeding approach might influence the studied thalamocortical tract properties.

![Figure 8](image.png)

**Figure 8.** Locations of fMRI, MEG and manual ROIs for fingers and ankles. The left and middle panels show the individual locations, and the right panel shows the average locations on an MNI template brain. Modified from Fig. 2 in Publication 1.
5.2 Publication 2: Corpus callosum in CP

The second publication aimed to investigate if the corpus callosum structure differs between hemiplegic \((N = 16)\) and diplegic \((N = 11)\) CP subtypes when compared to controls \((N = 30)\) and whether the callosal structure is associated with static and dynamic stability. The study included ROI-based analysis of the corpus callosum cross-section and tractography analysis of the sensorimotor tract travelling from the corpus callosum to the lower-limb SMI cortical area of the dominant hemisphere.

As can be seen in Figure 10, the corpus callosum cross-section was smallest for participants with hemiplegic CP \((p < 0.001 \text{ for HP vs. TD}; p < 0.05 \text{ for HP vs. DP})\). Furthermore, the FA values were decreased and MD values increased in both CP groups compared to controls. These structural alterations were observed across the body and splenium of the corpus callosum. Similarly, tractography analysis revealed increased MD and decreased FA in both CP groups, but in diplegics these differences seemed to continue further along the tract when moving towards the cortex. Tractography results are presented in Figure 11. Significant correlations were found between the white matter structure and the motor stability performance both in participants with CP and in typically
developed controls, although the associations found failed to show any conclusive directionality.

Figure 10. Cross-sectional areas of total corpus callosum and its geometrically divided subparts. Areas were the smallest for participants with hemiplegic CP (HP) when compared to diplegic CP (DP) or typically developed participants (TD). Modified from Fig. 3 in Publication 2.

Figure 11. Diffusion properties of the commissural tract connecting the corpus callosum cross-section to the dominant SMI cortex. The diplegic CP group (DP) also appeared to show impairments in the middle part of the tract whereas the hemiplegic group (HP) differed more from controls (TD) closer to the brain's midline. Modified from Fig. 4 in Publication 2.
5.3 Publication 3: Thalamocortical tracts in CP

Publication 3 aimed to clarify how the thalamocortical tracts are affected in CP. Using the manual and fMRI-based approaches introduced in Publication 1, the thalamocortical tracts were created separately for the four limbs and compared between hemiplegic ($N = 15$), diplegic ($N = 10$) and control ($N = 19$) groups. The fMRI was selected over MEG because of the data amount and quality in our sample, the results of Publication 1 and the easy accessibility to fMRI in future tractography studies. We also studied the correlations between sensorimotor performance of the upper and lower extremities and the corresponding tract properties.

Figure 12 presents the tractography results obtained with manual, i.e., anatomy-based approach. The results from fMRI-based tractography with a lower number of CP participants appeared comparable at the group level. The hemiplegic participants showed increased MD values especially in the non-dominant (lesioned) hemisphere, whereas diplegic participants had more symmetrically affected thalamocortical tracts. In both CP groups, the tracts for upper limbs appeared to be similarly affected as the tracts for lower limbs when measured with MD. The typically developed participants showed higher AFD asymmetry than participants with CP (opposite direction for the upper and lower limb tracts). Correlations between tract properties and sensorimotor performance did not show statistically significant results, but more affected thalamocortical tracts appeared to be associated with worse sensorimotor performance.

![Figure 12](image-url.png)

**Figure 12.** Diffusion properties of the limb-specific thalamocortical tracts obtained using manually selected anatomy-informed cortical ROIs in hemiplegic (HP), diplegic (DP) and control (TD) participants. Fig. 2 in Publication 3.
6. Discussion

The objective of this dissertation was to contribute new knowledge of the sensorimotor system structure both in typical development and in CP, as well as to enhance the methodology of tractography. Specifically, we aimed to explore the disparities in the white matter changes between hemiplegic and diplegic subtypes of CP, focusing the investigation on limb-specific tracts and their association with behaviour. In Publication 1, we compared the feasibility of different seeding approaches to construct thalamocortical tracts carrying proprioceptive information in typically developed children. This new seeding approach was further utilized in participants with CP in Publication 3. Publication 2 focused on the structural properties of the corpus callosum and the sensorimotor tract connecting to the dominant lower-limb cortical representational area.

Overall, children with CP showed altered structure of the studied white matter pathways, which is likely to contribute to the deficits in their sensorimotor performance. We observed no strong correlations with performance measures, presumably due to the limited number of participants and the high complexity of the sensorimotor system. The hemiplegic and diplegic CP subtypes were observed to have distinct patterns of white matter involvement, reflecting the topographical manifestation of their sensorimotor impairment. Noteworthy, previous literature was extended by introducing a novel approach of establishing tractography endpoints based on cortical activation following proprioceptive stimuli.

6.1 Altered diffusion properties in cerebral palsy

6.1.1 Interpretation of the diffusion-weighted MRI values

Publication 2 demonstrated altered diffusion properties in CP participants both at the corpus callosum cross-section and along the transcallosal tract connecting to the unaffected, dominant SMI cortex. Publication 3 showed the involvement of thalamocortical tracts travelling from the thalamus to the representational areas of the upper and lower limbs. The results were in accordance with previous studies reporting lower anisotropy (FA) and/or higher diffusion magnitude (MD) in the white matter of children with cerebral palsy (for a review, see Scheck et al. 2012; Maileux, Franki, et al. 2020). For commissural tracts, we observed changes in both parameters, and for the thalamocortical tracts only in MD when comparing participants with and without CP.
FA and MD metrics have been proposed to reflect the integrity of the axonal bundles, but it is evident that disentangling the exact contributions of different microstructural features within a voxel is not plausible (van Hecke et al. 2015). Changes in dMRI measures can, nevertheless, give indications of altered white matter organization, which can be due to changes in axonal number, diameter, density and coherence, degree of myelination, water content and membrane permeability (Curran et al. 2016). Reduced FA and increased MD observed in CP suggest that the structural barriers restricting the movement of water molecules are diminished or disorganized: FA is considered to reflect changes in axonal organization or myelination and MD changes in extra-cellular water content (Curran et al. 2016).

In both Publication 2 and Publication 3, we showed that the dMRI changes in CP were not uniform along the studied pathways. Although few studies have previously reported along-tract results in CP, it has been proposed that tracts might be differently affected at different levels of their trajectories (Lennartsson et al. 2015; Jiang et al. 2019). This region-specific behaviour of the dMRI metrics might be due to pathophysiological mechanisms but also due to methodological reasons. For example, in areas with multiple crossing fibers, damage to one sub-population of fibers can make another bundle more dominant, thus paradoxically increasing the FA (Curran et al. 2016). The MD values are, in turn, lower in the crossing fiber areas and higher within a more organized axonal structure. Furthermore, the partial volume effect can be more dominant in narrower tract sections (Curran et al. 2016). For example, in some individuals with CP, the thalamocortical tracts seem to travel on a thin bridge between the ventricle and primary lesion site, which may influence especially the MD estimate. Because the long white matter pathways encounter a mixture of different brain regions, it is thus advisable to study the dMRI metrics along their course and not only as average values over the whole tract length.

Some of the abovementioned limitations of the voxel-wise FA and MD metrics can be overcome with new fiber-specific metrics, such as the AFD. AFD can provide information about the location and orientation of fiber bundles and is considered to be approximately proportional to total intra-axonal volume (Raffelt et al. 2012). As this study was restricted by a low b-value, it may increase the possibility that the AFD measure includes also extra-axonal signal (Dhollander et al. 2021). We observed lower AFD values in dominant lower-limb specific thalamocortical tracts in participants with CP, indicating reduced axonal density or packing. Although no previous study has focused on thalamocortical pathways, the lower fiber density in other white matter areas has been shown to be associated with hemiplegic cerebral palsy (Pretzel et al. 2022), prematurity and brain abnormalities (Pannek et al. 2020), and worse motor outcome in infants (Pannek et al. 2020; Jeong et al. 2022). However, lateralization of the thalamocortical projections might influence these comparisons between participants with and without CP as discussed later in Section 6.3.
6.1.2 Aetiological considerations

As discussed in Section 3.3.2, CP results from a brain injury during a highly sensitive developmental period. Therefore, the changes observed in dMRI metrics are highly plausible to reflect changes in the brain microstructure. In addition to the cell death caused by the primary insult, disrupted myelination has been suggested to play an important role in CP. Changes in diffusion values might thus be attributable to injury of oligodendrocytes or oligodendrocyte precursor cells (Koerte et al. 2011; Lee et al. 2011; Marret et al. 2013) that can also modulate axonal growth and diameter (Jakovcevska et al. 2009). It has been further hypothesized that increased MD distant from the lesion site might indicate gliosis and cystic changes, possibly due to Wallerian degeneration. (Trivedi et al. 2010; Lennartsson et al. 2015; Papadelis et al. 2018). Hence, changes in the axonal organization could result from the primary prenatal or perinatal insult and/or from the following secondary neurodegeneration.

Alternatively, changes can also reflect the compensatory mechanisms and plasticity of the developing brain. For example, changes observed in the corpus callosum might be driven by possible ipsilateral wiring pattern of projection pathways, decreasing the need for interhemispheric communication (Hawe et al. 2013). Alternatively, it is possible that the mismatch between ipsilateral corticospinal and contralateral somatosensory wiring patterns increases the reliance on interhemispheric communication as the motor and somatosensory information of the affected limb localize in the opposite hemispheres. The distinction between maladaptive and compensatory changes might also have individual variation within the heterogeneous CP population.

Moreover, atypical motor activity and sensorimotor feedback can possibly promote atypical development of the white matter structure (Ferre et al. 2020). It has been hypothesized that CP disorder can lead to diminished spontaneous use of the affected extremities and, as a result, decrease the activity within the peripheral — central nervous system loop for the affected limbs during early development (Thomas et al. 2005). For example, decreased corpus callosum cross-sectional area in hemiplegic participants might result from the diminished communication between the hemispheres caused by the lack of bimanual activity or spontaneous use on the affected side (Ferre et al. 2020).

Maturation level is also an important factor to consider when studying the developing brain. Although we did not observe significant differences in age between CP and control groups within our studies, a possible interaction effect between age and group cannot be completely ruled out. The observed differences in dMRI values between participants with CP and their typically developed peers could, moreover, indicate alterations in the developmental trajectories of the groups. The maturation of white matter pathways might be prolonged or halted in CP compared to typical development, thus causing disparities when comparing similar-aged participants. Indeed, Papadelis and colleagues (2019) showed maturation arrests in the bilateral corticospinal tracts of children with hemiplegic CP compared to controls, indicating halted development or early closure of the developmental period in children with CP. Although we are unaware of similar results for thalamic projections or commissural tracts, it is...
possible that the observed variations are, in part, result from trajectorial differences in development. To gain an understanding of the possible differences in the maturational trajectories, longitudinal studies in CP are needed in future.

6.1.3 Hemiplegic and diplegic CP subtypes

Both Publications 2 and 3 revealed dissimilarities between hemiplegic and diplegic CP subtypes. To our knowledge, this is the first time dMRI metrics have been compared between the two CP subtypes in the same study. Statistically significant differences between the subtypes were observed in the corpus callosum cross-sectional area, transcallosal tract FA values and thalamocortical tract MD values. In general, children with diplegia appeared to show structural changes of lower magnitude when compared to those with hemiplegia that showed more prominent (although more local) changes.

The observed differences can be expected to reflect the aetiological background of the subgroups. Diplegic CP is typically characterized by bilateral brain injury, such as PVL, whereas hemiplegic CP is more often due to local unilateral infarction or haemorrhage (Upadhyay et al. 2020). A localized, abrupt injury might cause a more prominent primary injury, whereas a slower hypoxic-ischemic injury could result in more widespread primary and secondary pathological changes. The injury type and timing might also alter the developmental trajectory of the central nervous system and mediate possible reorganization (Staudt 2010). Moreover, diplegia is strongly associated with prematurity, which may, as such, affect the development of brain microstructure (Jiang et al. 2019; Pannek et al. 2020).

Although the type of injury (i.e., PWL vs. grey matter) may be an important factor modulating the white matter development, the clinical manifestation (i.e., HP vs. DP) is also highly relevant. Similar motor impairments indicate a shared brain basis and, in addition, can be thought to result in similar spontaneous motor behaviour that modulates the sensorimotor system development. In addition, as symptom-based classifications are frequently used in clinics, brain imaging studies should also comply with the same classifications in order to benefit clinical work more directly. Reflecting the topographical presentation of the symptoms, we indeed saw that participants with hemiplegic CP presented more lateralized thalamocortical injury than diplegics. In diplegics, the commissural tract connecting to dominant foot areas showed alteration over a longer distance than in hemiplegies, which could reflect the more prominent lower-limb dysfunction in diplegia. However, the thalamocortical tracts for both upper and lower limbs seemed similarly affected in diplegic CP. As diplegic participants also showed impaired hand function, changes in the upper-limb thalamocortical tracts are not, however, unexpected. Supporting the need for hemiplegic–diplegic division, the two subtypes were recently demonstrated to express different sensory and motor profiles (Guedin et al. 2018). Guedin and colleagues (2018) showed that children with hemiplegic CP presented diminished dexterity of both hands and sensory impairments only for the more affected hand, whereas diplegics presented sensory impairments for both hands, and diminished dexterity only for the dominant hand. Recent evidence has moreover shown that
hemiplegic CP is not confined to the more affected side, with somatosensory deficits observed also on the dominant side (Jovellar-Isiegas et al. 2023).

The observed results between hemiplegic and diplegic participants could be influenced by the different (and limited) sample sizes of the hemiplegic and diplegic groups. As the heterogeneity of brain findings and deviation in the dMRI metrics was notable within both groups, even a few participants can have a notable impact on the group-wise comparisons. Moreover, the commissural tract travelling to SMI cortices was studied only in the dominant hemisphere, due to methodological reasons, which does not give a full picture of how this tract is affected especially in hemiplegic CP. Given the unilateral nature of the brain injury in children with hemiplegic CP, the transcallosal tract in the unaffected hemisphere may show less impaired white matter structure than its counterpart in the affected hemisphere. Notably, Pagnozzi and colleagues (2020) also demonstrated variations in the structure of corpus callosum within the hemiplegic CP population, with differences noted between those with a bilateral brain injury and those with a unilateral injury.

Lastly, despite the apparent importance of topographical categorization (hemiplegia vs. diplegia), even more insight could be obtained by including the aetiological background in the studies. For example, Kyczynski and colleagues (2017) compared the thalamocortical tract injury in hemiplegic children with either arterial ischemic stroke or periventricular venous infarction and observed that the thalamocortical tract of the lesioned hemisphere was more affected in the arterial ischemic group. Similarly, Mailleux and colleagues (2020) showed that MD of the medial lemniscus of the more affected hemisphere was significantly higher in hemiplegic children with grey matter lesion compared to those with PWM lesion. Although our sample size was insufficient for this type of analysis, such approaches can be recommended for future studies.

6.1.4 Relevance to brain function

Structural alterations of the white matter pathways have been suggested to modulate the cortical brain function in several multimodal CP studies. Altered dMRI metrics in CP have been reported together with, for example, abnormal laterality of fMRI BOLD-response (Weinstein et al. 2014), diminished functional connectivity in fMRI (Lee et al. 2011) and increased TMS-measured resting motor threshold (Koerte et al. 2011; Azizi et al. 2021). Recently, Papadelis and colleagues (2018) studied the association between MEG response following tactile stimulus and the thalamocortical tract connecting to the same functional area in participants with hemiplegic CP. Correlations were observed between the diffusion properties of the MEG-defined thalamocortical tract and MEG response latency, amplitude and gamma power change (Papadelis et al. 2018).

Although our studies did not include direct comparisons between white matter structure and functional imaging, some support for the functional importance of the altered white matter structure can be drawn from functional studies including partly the same participants as presented here. Nurmi and colleagues (2021) demonstrated that children with CP had increased BOLD responses following proprioceptive stimulation (passive movement) of the non-
dominant finger compared to controls. In a MEG study by Illman and colleagues (2023), the diplegic participants showed increased beta suppression (i.e., increased cortical activation) in the contralateral hemisphere following dominant hand proprioceptive stimulus, and decreased beta rebound (i.e., reduced cortical inhibition) in the ipsilateral hemisphere, following both dominant and non-dominant finger proprioceptive stimulation. Diplegic children also showed increased resting state functional connectivity of the SMI cortices (Vallinoja et al. 2023).

As the CP individuals showed an altered structure of the projection and commissural pathways, it can be assumed that these alterations might give rise to the abnormal cortical processing observed in other studies (Nurmi et al. 2021; Illman et al. 2023; Vallinoja et al. 2023). On the one hand, these functional changes could reflect compensatory mechanisms, where the increased activity is a result of ineffective information processing, possibly due to injury of the thalamocortical pathways (observed as increased MD). On the other hand, the functional imaging findings could reflect disrupted excitation–inhibition balance in participants with CP, possibly modulated by the affected interhemispheric connections (observed as increased MD and decreased FA in the corpus callosum). Overall, the link between structural alterations and functional imaging can be assumed to be highly complex and include multiple overlapping mechanisms.

6.1.5 Association with sensorimotor performance

An extensive body of literature has associated dMRI changes with behavioural manifestations in CP, but often neglected the performance of lower limbs (Mailleux, Franki, et al. 2020). Furthermore, to our knowledge, only one CP study has previously delineated projection pathways that are specific for the lower limbs (Chang et al. 2012). Here, we explored the association between lower-limb performance, i.e., static standing and dynamic gait stability, and thalamocortical (Publication 3) and transcallosal tracts (Publication 2) connecting to representational areas of the lower limbs. Although we saw some significant correlations, the directionality of the results was nonconclusive. Some earlier studies have successfully demonstrated associations between gait/balance performance and white matter structure (Sullivan et al. 2010; Brodoefel et al. 2013; Meyns et al. 2016; Papageorgiou et al. 2020), whereas some have failed to do so (Groeschel et al. 2019; Bleyenheuft et al. 2020; Azizi et al. 2021). A possible explanation for the inconsistent findings is that balance and gait are highly complex processes that require simultaneous functioning of a wide brain network (for a review, see Surgent et al. 2019). Thus, a single white matter pathway might be relevant for the process but insufficient to explain the variability of stability performance. For example, the cerebellum is known to play a major role in stability. This can be seen in ataxic CP, where the individuals show problems in coordinated movement and balance due to a cerebellar injury. In addition, more specific measurements of either motor, sensory or proprioceptive function of the lower extremities might provide stronger associations with the selected white matter pathways.
In general, however, higher FA and lower MD values have been associated with better sensorimotor performance in CP (for a review, see Mailleux, Franki, et al. 2020). This directionality is supported by intervention studies showing training-induced changes in the white matter pathways. For example, increased FA of the corticospinal tract following therapeutic intervention has been observed in several studies (Trivedi et al. 2008; Weinstein et al. 2015; Bleyenheuft et al. 2020). When correlating thalamocortical dMRI measures with the manual dexterity measure, we did observe some similar trends but failed to show any significance. As the structural changes appeared to vary along the tracts, stronger associations might have been obtained at a certain level of the fiber bundles. However, such an investigation was not possible with the limited sample size. Moreover, we did not explore the association between upper extremity performance and the callosal structure, even though they seem to be linked especially with bimanual coordination (for a review, see Gooijers & Swinnen, 2014).

In addition to the limited sample size, one explanation for the weak correlations in our study can be due to the decision to use sum variables. While sum variables can provide a more comprehensive and general view of the studied performance, they lack the specificity to distinct components, such as gross and fine manual dexterity or bimanual coordination. In the future, more research on the reliability and validity of the used sensorimotor assessments is therefore required. Moreover, pooling both dominant and non-dominant hand performance together might not be optimal, especially in hemiplegic CP. Although we did control for age in the analyses, the different pubertal stages in our participants might also have affected the lack of significant correlations (Genc et al. 2020).

For a more detailed view of the structure–function relationship in future, it is advisable to extend studies to limb-specific performance and a greater number of white matter pathways both in different CP subtypes and in typical development. Moreover, the role of hemispheric symmetry in modulating performance remains to be clarified. In Publication 3, we showed that increased asymmetry of the thalamocortical tracts might be associated with worse sensorimotor performance, which follows the findings by Mailleux and colleagues (2020) in children with hemiplegic CP.

6.2 Functional seeding

Publication 3 showed that functional seeding of proprioceptive thalamocortical tracts is plausible in children with CP. Functional seeding of thalamocortical tracts has been previously demonstrated in hemiplegic CP by Papadelis and colleagues (2018), utilizing MEG with tactile stimulus to fingertips, and by Reid and colleagues (2016), using block-designed motor task (hand tap, by full extension at the wrist) in fMRI. The evoked-passive movement paradigm used in our work thus extends this area of research to the proprioceptive domain for the first time. Furthermore, to our knowledge, no previous work has used functional seed points for the lower-limb-specific tractography. Moreover, we
demonstrated a MEG-based seeding approach in typically developed children, which could be replicated in the CP population in the future.

Integration of functional imaging into the tractography pipeline can substantially aid the analysis in this population, as the definition of anatomical endpoints can be challenging in a CP brain (Pagnozzi et al. 2015). Purely anatomical tractography approaches have previously led to the exclusion of some participants with large lesions or abnormal cortical folding (Pannek et al. 2014; Scheck et al. 2016; Maileux, Simon-Martinez, et al. 2020), which can distort the results and prevent translational advances. Large unilateral lesions can result in ipsilateral wiring of the corticospinal tract, but thalamocortical pathways are typically still preserved on the lesioned side (Guzzetta et al. 2007; Wilke et al. 2009; Staudt 2010; Nevalainen et al. 2012) although they may be displaced slightly (Kuczynski et al. 2017). Another reason for utilizing functional seeding is that the functional organization can be altered in CP despite the gross wiring pattern being preserved. Thus, the functional locations in typically developed brains might not correspond to the locations in the CP brain, making detailed anatomical templates or landmarks possibly misleading. For example, the tactile representational areas of fingers seem to show altered Euclidean distances (Nevalainen et al. 2012; Papadelis et al. 2014, 2018), shift to the direction of the precentral gyrus (Papadelis et al. 2018) and even have abnormal somatotopic order (Papadelis et al. 2014) in CP population.

Our proprioceptive seeding approach using fMRI resulted in plausible cortical ROIs in the sensorimotor SMI cortex for most control and CP participants. However, the locations were not consistent on the 3a area of the postcentral gyrus, which is considered responsible for proprioceptive processing. Some locations were observed in the anterior Area 4 (MI) or more posteriorly in Areas 3b, 2 and 1. As Area 3a is located in the fundus of the central sulcus, it is plausible that small anterior or posterior shifts of proprioceptive areas to the neighbouring gyri might describe true reorganization, especially in CP. Still, methodological factors may also influence the result. For fMRI-ROIs, we used the center-of-mass of the activation cluster, which ignores the wider spatial distribution of the BOLD response. Moreover, the cluster was manually selected to approximately overlay the contralateral SMI cortex, which can affect the objectivity of the pipeline. Finally, the resolution of fMRI (3 mm³) can be accountable for imprecise locations, which is why we used a spherical kernel of 3 mm for the tractography.

Although functional seeding can potentially increase the relevance and specificity of the studied tracts, it can also introduce interpretive challenges. In Publication 1, we showed that different imaging modalities might result in disparities in tractography results, as can different methodological choices within a modality (Reid et al. 2016). Furthermore, functional imaging might not be equivalent in the typically developed and in the CP brain, as, for example, different cortical folding might introduce biases in MEG. More research is therefore needed in both functional and structural domains in order to acquire the best practices for functional seeding in CP.
6.3 Hemispheric asymmetry

In Publications 1 and 3, we observed hemispheric asymmetry of the thalamocortical tracts in typically developed children. The average AFD value was left-lateralized in tracts connecting to representational areas of the fingers, but right-lateralized in tracts connecting to ankle areas, whereas no lateralization was observed in FA or MD metrics. To the best of our knowledge, no earlier research has investigated fiber density asymmetries specific to thalamocortical tracts. However, recent fixel (fiber populations within a voxel, Raffelt et al. 2017) based analyses have shown rightward lateralization of the anterior limb of internal capsule, and, in contrast, leftward lateralization of the posterior limb in both right- and left-handed healthy adults (Honnevedasthana Arun et al. 2021; Verhelst et al. 2021). Thalamic projections enter the internal capsule orderly so that most anterior parts connect to the frontal lobe, whereas most posterior parts connect to visual areas (Catani and de Schotten 2012). Thus, it seems plausible that sensorimotor thalamocortical fibers, travelling through the middle parts of the internal capsule, might show reversed lateralization, although more research is needed to specify the results in typical development.

Participants with CP did not show similar thalamocortical lateralization as controls. One explanation can be that the patient groups included both left- and right-hand-dominant children, whereas controls were all right-handed. The small number (N = 2) of left-handed controls was not sufficient for separate analysis, and the decision to include only right-handed controls enabled a more meaningful interpretation of the lateralization results. Although there is some evidence that the effect of handedness in density measures of projection fibers could be negligible in typical development (Dimond et al. 2020), such an effect cannot be ruled out. Furthermore, the effect of lesion lateralization on subsequent brain development might be important especially in hemiplegic CP. It seems that children with left hemiplegic CP (more affected right hemisphere) might show more widespread white matter alterations than right hemiplegic CP (Scheck et al. 2016; Pretzel et al. 2022). Additionally, neonatal stroke has been linked to motor system asymmetry even without CP (Dinomais et al. 2017). Future studies are therefore recommended to analyze differently lateralized patient groups separately (Dhollander et al. 2021). However, it might not be meaningful to directly compare left-handed controls with left-dominant patients due to the potentially different lateralization mechanisms in typical and atypical brain development.

Diplegic CP is typically characterized by symmetrical PWI and motor deficits and is therefore not often considered as a lateralized disorder. Thus, many neuroimaging studies have not separated dominant and non-dominant hemispheres but settled for left–right division. However, children with diplegic CP show left-handedness significantly more often than in typically developed children as shown in our sample and others (Lin et al. 2012). Despite the primary diagnosis being diplegic CP, also hemiplegic characteristics might be present, and it has been suggested that there is altogether a need for a more specific characterization of the CP disorder topography (Rosenbaum et al. 2007).
To conclude, future studies are encouraged to address the development of motor and sensory function asymmetries and hemispheric lateralization, not only in the presence of a unilateral brain lesion but also in diplegic CP and in typical development. Moreover, our observation of conflicting lateralization of upper and lower limb thalamocortical tracts points out the demand for more detailed tractography that addresses the intragyral organization, such as somatotopy.

6.4 Future perspectives and limitations

In general, the heterogeneity of CP is an abiding challenge for all research addressing the CP population. In order to study the sensorimotor domain in a more controlled manner, we excluded participants with common CP-related comorbidities such as vision, hearing or learning deficits. Still, individuals diagnosed with CP have distinct aetiological backgrounds, sensorimotor symptoms and therapeutic histories, which should be taken into account when interpreting neuroimaging results. As CP studies often have limited sample sizes and heterogeneous participant groups (as here), there might be a need to shift from group studies to single-case approaches (Dhollander et al. 2021), which may eventually provide guidance on individual prognosis or optimal therapy. The diverse brain abnormalities further introduce methodological challenges in terms of brain parcellation and segmentation, outlining and comparison of specific tracts, and interpretation of imaging findings. Some of these challenges might be addressed by multimodal approaches, such as functional seeding.

A limitation of our study was the decision to concentrate on specific tracts, i.e., thalamocortical pathways and corpus callosum. A more comprehensive view of the extent of white matter injury could have been achieved by using also global connectivity measures. However, some of the hemiplegic participants would have been excluded from this type of analysis due to the abnormal cortical pattern. Moreover, connectivity metrics are often associated with a large number of false positives, and the interpretation of the results is challenging (Maier-Hein et al. 2017). By concentrating on single white matter bundles, we were able to concentrate on the specific characteristics of the studied tracts, i.e., asymmetry, along-tract evaluations, and correlations with behaviour. Thalamocortical tracts and corpus callosum are both significant in the pathophysiology of CP and, thus, worth focusing on. However, these specific tracts represent a limited element within the extensive framework of the pathophysiology of CP. In future studies, uncovering the association between the structural alterations in various pathways and incorporating functional imaging – such as functional connectivity – along with motor performance assessments will significantly enhance our comprehension of the intricate operation of the sensorimotor system and its development. Additionally, prospective studies may elucidate the role of other comorbidities linked to CP on the modified brain structure and function.

Tractography and dMRI are powerful tools in the attempt to disentangle the structure–function relationship in CP. Although several advances have been recently introduced to address the well-known limitations of dMRI, such as crossing fibers, some fundamental restrictions remain. Most importantly, all dMRI
measures are restricted by the voxel size, which restrains the studies to large axon bundles. Even with larger bundles, tractography cannot differentiate individual fibers that converge together within a voxel (so-called bottleneck effect; for detailed description, see Rheault et al. 2020). The resolution could be improved in future with novel imaging techniques, such as combining higher field strength data (Sotiropoulos et al. 2016). Moreover, the appearance of false positives is typical in tractography (Maier-Hein et al. 2017). We aimed to minimize the effect with visual inspection and manual pruning of all the studied tracts and by using a deterministic approach when possible (Publication 2). Even with optimized, modern methodology, the interpretation of dMRI metrics is, however, still not straightforward as several biophysical and biological mechanisms can exist especially in young patient populations.

Despite the challenges, studying the complex interplay between altered brain structure and functional outcome in CP can offer substantial advances in our understanding of the sensorimotor system and its development. Multimodal neuroimaging approaches and objective measures of sensorimotor performance might be the key elements in future research. Continued efforts are needed for a more detailed view on the structure of specific white matter pathways. It is encouraged to include along-tract analysis and careful selection of cortical target locations also on future studies. In future, tractography could be used to guide diagnosis and therapeutic interventions in children with CP, even at the individual level.
7. Conclusion

Our findings confirmed significant alterations in the diffusion properties of the commissural and ascending pathways in children and adolescents with CP, indicating pathophysiological or compensatory changes in the axonal organization. Notably, we showed distinct alterations of these white matter structures in hemiplegic and diplegic subtypes of CP. Additionally, we introduced a novel approach to constructing thalamocortical tracts carrying proprioceptive information from the extremities. Extending previous research, we examined the associations between the white matter structure and lower-limb performance (balance and gait) as well as manual dexterity.

Following the topography of the sensorimotor dysfunction, hemiplegic CP was associated with more unilateral white matter alterations compared to more bilateral involvement in diplegic CP. In the non-dominant (lesioned) hemisphere, the thalamocortical tracts appeared more severely affected in participants with hemiplegic CP. In the dominant hemisphere, in turn, the thalamocortical and callosal tracts showed more alterations in diplegic CP. However, we did not observe differences between the thalamocortical tracts specific for the upper and lower limbs in diplegic CP. Interestingly, in typically developed children, the upper and lower limb specific thalamocortical tracts showed opposite laterализation, which was not evident in CP. While strong associations between white matter structure and motor performance were not observed, there were indications that the structure of corpus callosum structure could partially modulate stability performance in children with and without CP. In addition, our results suggested that the hemispheric symmetry of thalamocortical pathways might play a role in motor impairments.

These findings also highlight the need for detailed, function-specific tractography in future studies. By improving our understanding of the pathophysiological mechanisms of CP, we will be able to develop more accurate diagnostic and therapeutic approaches for this lifelong impairment. Overall, a better understanding of the role of white matter pathways in CP contributes to our knowledge regarding the compensatory and reorganizational capacities of the developing brain.
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