NEUROLOGICAL SOFT SIGNS IN ADOLESCENTS ARE ASSOCIATED WITH BRAIN STRUCTURE AND POSTURAL CONTROL

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LIST OF ABBREVIATIONS

ADHD	Attention-deficit-hyperactivity disorder
AD(t)	(Tissue) Axial diffusivity
AP	Anterior-posterior
BTrackS	Balance Tracking System
CC	Corpus callosum
CR	Corona radiata
CST	Cortico-spinal tract
DTI	Diffusion tensor imaging
FA(t)	(Tissue) Fractional anisotropy
FW	Free-water
IC	Internal capsule
ML	Medial-lateral
MND	Minor neurological dysfunction
MRI	Magnetic resonance imaging
NSS	Neurological soft signs
RD(t)	(Tissue) Radial diffusivity
REPIMPACT	Repetitive Subconcussive Impacts – Brain Alterations and Clinical Consequences
SLF	Superior longitudinal fasciculus
TBSS	Tract-based spatial statistics
TR	Thalamic radiation

SUMMARY

Neurological soft signs (NSS) are minor deviations from the norm in sensory and motor performance. NSS exist in the general population but are more frequently found in cohorts with neurodevelopmental disorders. NSS are considered a diffuse and unspecific marker of altered neurodevelopment but receive increasing attention since the presence of NSS in children has been found to be predictive of psychiatric disorders in late adolescence. To date, only little is known about potential neurodevelopmental alterations that may underlay the presence of NSS. The prevalence of NSS has been shown to decrease during adolescence as part of continued neural development and brain re-wiring processes. Therefore, adolescence has been proposed as an important phase for the manifestation or outgrowing of NSS. The underlying mechanisms that may underly this process, however, are largely unknown. As NSS are subtle signs and commonly identified by subjective observer-based neurological examinations, quantitative tools may help to objectively investigate functional and structural correlates associated with NSS.

For the work included in this dissertation, healthy adolescent athletes from three European countries were investigated. All participants underwent a neurological examination, resulting in a categorization of participants into groups with and without NSS (NSS+/NSS-). A total NSS score was calculated to provide a continuous measure spanning the whole spectrum of NSS. Two quantitative tools were used to investigate functional and structural correlates of NSS in healthy adolescents: Study I) Instrumented force plate measures to investigate postural control (Bonke et al., 2023), and Study II) Structural magnetic resonance imaging to investigate brain morphology and white matter microstructure (Bonke et al., 2022).

Study I aimed to investigate the incremental value of instrumented force plate measures in addition to observer-based neurological examinations. Such associations have not been assessed before but are important for capturing subtle alterations in postural control. This will help to acquire a more comprehensive assessment of motor development. We found no statistically significant differences in postural control between NSS+ and NSS- group. However, participants performing non-optimal in the diadochokinesis sub-test measuring pronation/supination of forearms showed significantly reduced postural control in the medial-lateral (ML) direction. Moreover, the total NSS score correlated significantly with postural control performance in the ML direction. Findings from this study reveal that adolescents with NSS, and in particular adolescents that perform non-optimal in pronation/supination movements of the forearms also perform worse in ML postural control assessed by force plate

assessments. As pronation/supination movements of forearms and ML postural control continue to mature until adolescence, it can be assumed that these functions are related and may indicate altered motor development.

Study II aimed to identify and characterize NSS-related brain structure alterations using structural magnetic resonance imaging. NSS-related brain structure alterations have not yet been investigated in healthy adolescents. However, this investigation is of high relevance to better understand potential alterations in adolescent brain-rewiring processes related to NSS. Using T1-weighted imaging, we found significantly higher gyrification in the left superior frontal and parietal lobe in the group of adolescents with NSS, likely reflecting alterations in synaptic pruning. We did not find differences in cortical volume or thickness. Using diffusion tensor imaging, we found lower tissue fractional anisotropy (FAt) and higher tissue radial diffusivity (RDt) in widespread white matter clusters in the group of adolescents with NSS, likely indicating alterations in myelination. Findings from this study reveal that NSS in healthy adolescents are associated with brain structure alterations that can be objectively quantified using magnetic resonance imaging. As of now, the relevance of NSS-related brain structure alterations in otherwise healthy adolescents is not fully understood. Future studies should assess whether these alterations may explain the described association between NSS and psychiatric disorders.

In summary, the work presented in this doctoral dissertation uses two different quantitative measures to objectively investigate functional and structural differences between adolescents with and without NSS. Insights derived from this work show the beneficial use of instrumented tools to complement neurological examinations for a better understanding of functional and structural correlates of NSS. This work will help to generate a more complete picture of NSS-related developmental alterations and potentially related psychiatric vulnerabilities. Future research should make use of larger and more representative datasets to replicate, as well as extend our findings. Specific attention should be drawn on the investigation of factors that contribute to the development of NSS, longitudinal studies that allow to capture NSS-related alterations in developmental trajectories, as well as on investigating the underlying neural mechanisms of NSS.

1 GENERAL INTRODUCTION

"Neurological Soft Signs: From Soft Signs to Hard Science" (Sanders & Keshavan, 1998)

Neurological soft signs (NSS) are minor, or also called *soft* deviations from the norm in sensory and motor performance that are commonly investigated by using neurological examinations (Dazzan & Murray, 2002). NSS typically present as a combination of signs such as slowed motor sequencing and associated movements, also referred to as functional domains (Dazzan & Murray, 2002). Compared to hard neurological signs such as spasticity or hyperreflexia, which are considered pathological, NSS are regarded as subtle cerebral dysfunction without known focal morphological correlates (Neeper & Greenwood, 1987). The term *soft signs* was chosen based on the initial assumption that NSS are subclinical, non-localized signs of neurological deviations from the norm. Thus, the presence of NSS was considered a diffuse and unspecific marker (Bombin et al., 2005).

While NSS are frequently found in individuals with neurodevelopmental or psychiatric disorders, to a smaller extent, NSS also exist in the general population (Heinrichs & Buchanan, 1988). Since the presence of NSS in healthy children was found to be predictive of psychiatric disorders in late adolescence (Shaffer et al., 1985), the investigation and detection of NSS became more and more relevant. Of note, the prevalence of NSS was shown to decrease during adolescence as part of continued brain-rewiring processes (Martins et al., 2008). However, to date, still little is known about neurodevelopmental alterations that may underlay the presence of NSS. Based on the methodologies available at that time, it was assumed that NSS would not coincide with measurable brain structure alterations (Sanders & Keshavan, 1998). However, over the past years, more sensitive tools, such as advanced magnetic resonance imaging (MRI), have been developed. Such tools of *hard science* allow to objectively quantify alterations in brain structure (Grover et al., 2015). Similarly, while the examination of NSS is subjective and requires the presence of a clinician, the use of instrumented postural control tools allows to objectively quantify subtle functional alterations that may be related to NSS (Paillard & Noé, 2015). The availability of such tools enables a detailed and objective analysis of NSS-related alterations which in the past might not have been captured due to methodological constraints.

In the work included in this dissertation, I provide two examples of quantitative tools to investigate functional and structural alterations associated with NSS: Study I) Instrumented force plate measures to investigate postural control, and Study II) Structural MRI to investigate brain structure. These tools provide the opportunity to evaluate NSS objectively and quantitatively which is of importance to understand their clinical relevance.

1.1 Neurological Soft Signs

In the following, I describe the prevalence and etiology as well as structural and functional correlates of NSS. Moreover, I provide an overview of neurological examinations to assess NSS.

1.1.1 Prevalence and Etiology of Neurological Soft Signs

The prevalence of NSS in the general adult population is estimated to be approximately 5% (Heinrichs & Buchanan, 1988). This prevalence varies throughout the lifespan and follows an U-shaped trajectory (Chan et al., 2016). For a rough outline of this lifespan variation, see **Figure 1.** This U-shaped trajectory comprises higher rates of NSS in childhood, lower rates in early and middle adulthood, and another increase in late adulthood. Investigations regarding potential underlying mechanisms for this lifespan variation are ongoing. It is hypothesized that during puberty brain rewiring processes and hormonal changes are responsible for the declining prevalence of NSS (Hadders-Algra, 2002; Martins et al., 2008). At a later age, the prevalence is rising again, matching with the general aging-related decline of human physical and cognitive development (Chan et al., 2016).

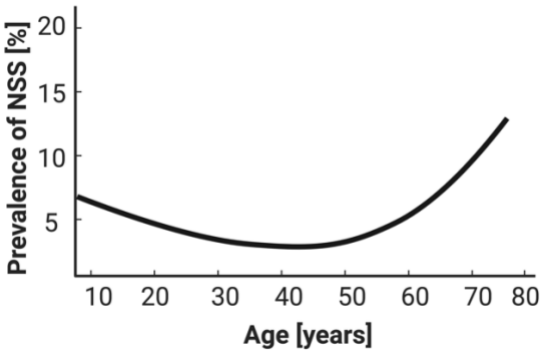

Figure 1. Outline of the development of NSS prevalence over the lifespan with higher rates in childhood, lower rates in early and middle adulthood and another increase in late adulthood adapted from Chan et al. (2016) (created with BioRender.com).

Interestingly, NSS are more frequently observed in adults with psychiatric disorders, as well as children and adolescents with neurodevelopmental disorders compared to the general population (D'Agati et al., 2018; De Jong et al., 2011; Malviya et al., 2022; Patankar et al., 2012). See **Figure 2** for examples of commonly investigated cohorts with NSS. In individuals with schizophrenia, for example, reported rates range from 50 to 65% (Heinrichs & Buchanan, 1988). For other psychiatric and neurodevelopmental disorders, rates were reported to be in

between those of healthy individuals and those with schizophrenia (Heinrichs & Buchanan, 1988).

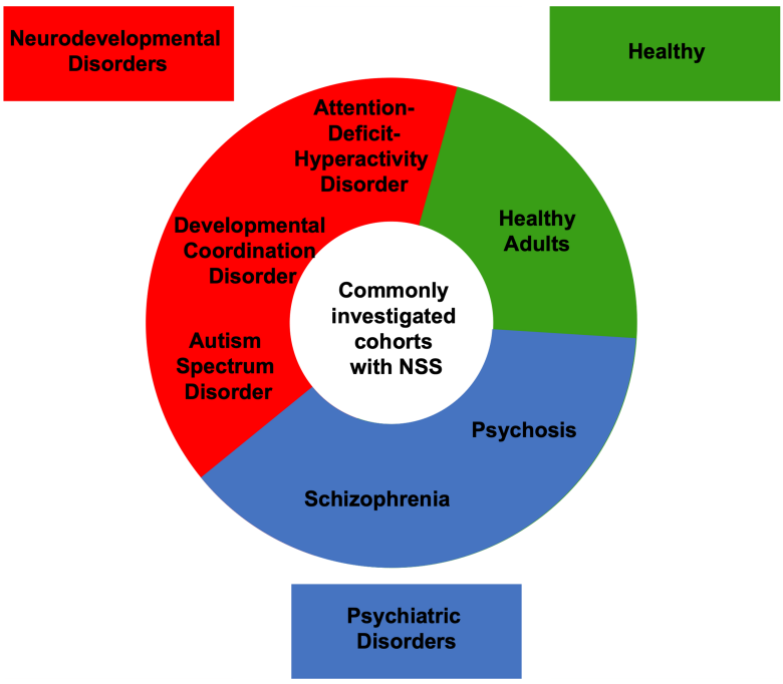

Figure 2. Overview of commonly investigated cohorts with neurological soft signs (created with BioRender.com).

Furthermore, in contrast to the typical U-shaped trajectory in the general population, the developmental trajectory of NSS in individuals with schizophrenia was shown to be flat, but with a consistently higher prevalence (Chan et al., 2016).

The etiology of NSS is not yet fully understood. A neurodevelopmental component behind the pathophysiology of NSS is assumed (Bombin et al., 2005). Multiple factors such as genetic predispositions, neonatal and perinatal events, as well as environmental causes are assumed to play a role (Hadders-Algra et al., 1988; Iannetti et al., 2005; Touwen & Huisjes, 1984). The hypothesis regarding a genetic predisposition is based on work showing that NSS are significantly more common in individuals with schizophrenia than in their first-degree relatives and more common in first-degree relatives than in healthy individuals (Neelam et al., 2011). Regarding neonatal and perinatal events, low birth weight, preterm birth, intrauterine growth retardation, and perinatal ischemia and/or asphyxia have been identified as most unfavorable adversities for the development of NSS (Hadders-Algra et al., 1988; Iannetti et al., 2005; Touwen & Huisjes, 1984). Examples of environmental causes that favor the development of NSS are low social and economic conditions (Mechri et al., 2009).

NSS have been shown to frequently co-occur with other quantifiable functional correlates such as cognitive functioning (Alamiri et al., 2018; Kikkert et al., 2013) and problem

behavior (Shaffer et al., 1985). Evidence from a study assessing 341 children at the age of nine showed that particularly coordination problems and fine manipulative disability were associated with lower scores on memory, attention, learning and language (Kikkert et al., 2013). These results were replicated by a larger study investigating over 35,000 children at the age of seven (Alamiri et al., 2018). Another study compared 90 children with NSS at the age of seven with 90 children without NSS and followed them up longitudinally until the age of 17 years (Shaffer et al., 1985). The authors discovered that the presence of NSS at age seven was a strong predictor of persistent psychiatric disorders such as anxiety and withdrawal at age 17. Of note, all girls and 80% of the boys with an anxiety-withdrawal diagnosis at the age of 17 already showed NSS at the age of seven (Shaffer et al., 1985). The results of these studies suggest that NSS are associated with lower cognitive performance and may predict the development of psychiatric disorders, which is of high clinical relevance. It is hypothesized that the presence of NSS is linked to other quantifiable structural and functional correlates, which may be helpful to better understand the relevance and clinical picture of NSS. Examples include the quantification of postural control performance, as well as brain structure alterations, which are the focus of Study I and II included in this dissertation.

1.1.2 Assessment of Neurological Soft Signs

The assessment of NSS originates in the context of investigating psychiatric disorders (Bender, 1947). However, over the past decades, it has also found increasing use in the field of pediatric neurology with a specific neurodevelopmental focus (Alamiri et al., 2018). The most commonly used test batteries to assess NSS are described in two review articles (Bombin et al., 2003; Chrobak et al., 2021) and are summarized in **Table 1.** Even though fine motor skills, coordination and postural control are included in most test batteries, there are variations between testing batteries regarding how NSS are defined and classified. Thereby, categorization approaches (e.g., Prechtl, 1980) and spectrum approaches (e.g., Schröder et al., 1991) exist. Whereas categorization approaches separate groups in optimal and non-optimal performing groups, spectrum approaches classify the presence of NSS along a spectrum. While some categorization approaches refer to the presence of NSS already when only one neurological soft sign is present (Ismail et al., 1998), other more restrictive systems require the presence of multiple neurological signs (King et al., 1991). Therefore, a comprehensive understanding of the different test batteries and classification systems is essential to interpret NSS findings properly.

Table 1. Overview of neurological examination test batteries based on Chrobak et al. (2021) and Bombin et al. (2003).

Test battery	Origin	Reference
PANESS = Physical and neurological examination for soft signs	Psychiatry	(Guy, 1976)
NES = The neurological evaluation scale	Psychiatry	(Buchanan & Heinrichs, 1989)
Rossi Scale	Psychiatry	(Rossi et al., 1990)
Hollander Scale	Psychiatry	(Hollander et al., 1990)
Heidelberg Scale	Psychiatry	(Schröder et al., 1991)
CNI = The Cambridge Neurological Inventory	Psychiatry	(E. Y. H. Chen et al., 1995)
KS = Krebs Scale	Psychiatry	(Krebs et al., 2000)
TINE = Touwen Infant Neurological Examination	Pediatrics	(Touwen & Sporrel, 1979)
HINE = Hammersmith Infant Neurological	Pediatrics	(Haataja et al., 1999)
Zürich Neuromotor Assessment	Pediatrics	(Largo et al., 2001)
Amiel-Tison neurological examination	Pediatrics	(Gosselin et al., 2005)
BOT-2 = Bruininks-Oseretsky test of motor proficiency	Pediatrics	(Bruininks & Bruininks, 2005)
Neurofunctional assessment of Picciolini	Pediatrics	(Picciolini et al., 2006)
M-ABC = Movement assessment battery for children	Pediatrics	(Henderson et al., 2007)
MND = Minor neurological dysfunction	Pediatrics	(Hadders-Algra, 2010)

Minor neurological dysfunction as example of a test battery used in the pediatric context

A widely known neurological examination test battery to assess NSS in the pediatric context is the minor neurological dysfunction (MND) concept (Hadders-Algra et al., 2010; Touwen & Sporrel, 1979). The wording MND was used to make the investigation of NSS more appropriate to its pediatric application. The concept was first invented by Touwen and Sporrel (1979) and later modified by Hadders-Algra et al. (2010). It is an age-dependent assessment performed by a pediatric neurologist and includes 54 tests classified into 8 functional domains: (1) Fine Motor Skills, (2) Associated Movements, (3) Coordination & Balance, (4) Posture & Tone, (5) Involuntary Movements, (6) Sensory Function, (7) Reflexes, and (8) Cranial Nerves. The MND concepts differentiates between simple and complex MND forms. Simple MND reflects a normal, but non-optimal functioning of the nervous system. Complex MND reflects a more severe form of MND which is assumed to be related to pre- and perinatal events and behavioral disorders. The classification scheme is based on the pubertal status of the investigated child. Before puberty onset, the classification is based on the number of dysfunctions. After puberty onset, it is based on the type of dysfunction (Hadders-Algra, 2002). Since its invention, the MND assessment has been used in several studies assessing children and adolescents (De Jong et al., 2011; Galić et al., 2018; Kikkert et al., 2013; Tripi et al., 2018).

In the work published in this dissertation, we used a modified form of the MND concept (see **Figure 3**). We included 6 of the 8 suggested functional domains consisting of 28 tests. We did not differentiate between simple and complex MND as the pubertal status of the participants was unknown. Following a dichotomous approach, in alignment with the classification system used for after puberty onset, we considered a participant to have NSS (NSS+) when at least one of the functional domains was rated as non-optimal and as NSS- if this was not the case, respectively. Moreover, following a spectrum approach, we calculated a metrical total NSS score where each optimal-performed test resulted in one point, so that a total NSS score of 28 reflects the best possible neurological performance for the respective age of the participant. For more details on the methodological set-up, see Bonke et al. (2022, 2023).

Taken together, the MND assessment is a standardized, age-dependent concept that has high clinical potential for the investigation of NSS in developing individuals, such as children and adolescents.

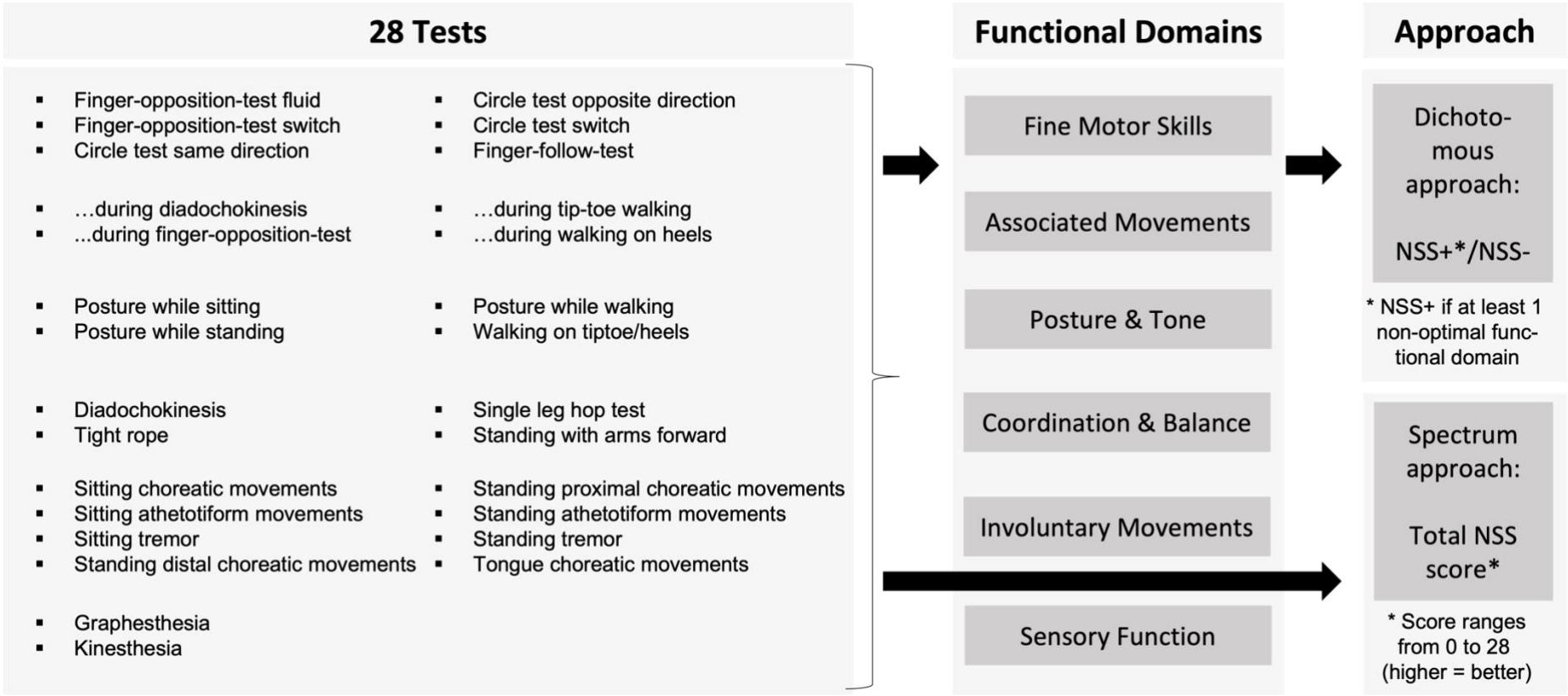

Figure 3. Overview of neurological examination used in Study I and II included in this dissertation.

Note. The examination included 28 tests grouped into 6 functional domains for the dichotomous approach categorizing the group into an NSS+/NSS- group and for the spectrum approach, adding up one point for every optimal performed test.

1.2 Postural Control as Functional Marker of Neurological Soft Signs

Postural control is defined as the ability to maintain internal stability (e.g., to stand upright) and to foresee and prevent instability following external perturbations (Bartlett & Birmingham, 2003). Postural control is an important part of (motor) development and overall brain health and therefore plays a significant role when investigating NSS. Observer-based assessments of postural control as it is the case in neurological examinations may be subjective and not sensitive enough to pick up on very subtle alterations (Mancini & Horak, 2010). It is still unknown if and to what extent instrumented assessments can complement observer-based neurological examinations. It is assumed that instrumented assessments may help to capture a more complete picture of NSS-related developmental alterations. This knowledge gap motivated Study I (Bonke et al., 2023).

1.2.1 Assessment of Postural Control

Alterations in postural control can be assessed with instrumented as well as non-instrumented tools, each of which possesses strengths and weaknesses (Paillard & Noé, 2015). Examples of instrumented tools are force plates, whereas non-instrumented tools typically include tests such as the Balance Error Scoring System (Guskiewicz et al., 2001). Instrumented tools are known to measure kinetic and/or kinematic variables (e.g., postural sway) precisely and objectively. Even though instrumented tools are more expensive and difficult to be implemented in clinical settings, they possess a higher level of sensitivity, are objective and less dependent on observation compared to non-instrumented tools (Riemann et al., 1999).

In the work included in this dissertation, the Balance Tracking System (BTrackS) was used as example of an instrumented tool to investigate postural control performance (Goble et al., 2018; O'Connor et al., 2016; Richmond et al., 2018). Postural control was assessed on a rigid and a compliant (foam) surface. For more details on the methodological set-up, see Bonke et al. (2023). Commonly used variables are listed and explained in **Table 2** (Palmieri et al., 2002).

Table 2. Overview of postural control variables based on Palmieri et al. (2002).

Postural control variables	Explanation
Path length (Mean, AP, ML)	Magnitude of two-dimensional displacement based on total distance travelled
Root mean square (Mean, AP, ML)	Amplitude deviations representing standard deviation of center of pressure displacement
Sway area	Center of pressure displacement between beginning and end of trials across axes

Abbreviations. AP = anterior-posterior, ML = medial-lateral

1.2.2 Postural Control Development during Childhood and Adolescence

A general understanding of postural control development is important to interpret NSS-related functional alterations. Evidence shows that behavioral postural control strategies develop in developmental periods (Kirshenbaum et al., 2001). Thus, the type of quantified functional alterations may provide insight into vulnerable developmental periods for the manifestation and/or outgrow of NSS.

The development of postural control takes place in two stages (Blanchet et al., 2019; Kirshenbaum et al., 2001). Young children, approximately until the age of five, use an open-loop strategy, also known as ballistic strategy, in which they explore all possible motor actions. The behavior in this strategy is characterized by a larger center of pressure displacement and, hence, more instability. Approximately at the age of eight, children begin to use a closed-loop strategy, also known as sensory strategy (Blanchet et al., 2019; Kirshenbaum et al., 2001). The behavior in this strategy is characterized by using several movement possibilities based on the situation and environment, resulting in better postural control. As children get older, their nervous system and related functionality adjust further, leading to better multisensory integration (Kirshenbaum et al., 2001; Peterka, 2002). More specifically, older children can manage postural control by combining information from several modalities (e.g., the visual and somatosensory system). This combination of information allows to operate in a dynamic environment (Kirshenbaum et al., 2001; Peterka, 2002). Of note, postural control was demonstrated to mature later along the medial-lateral (ML) than the anterior-posterior (AP) axis, meaning that children learn earlier how to control their posture in the AP than the ML direction. These different maturation processes likely are due to (1) daily life experience

expected to be greater in the AP direction, (2) antigravity muscles responsible for AP control developing earlier, and (3) the ankle strategy involved in AP postural control developing earlier than the hip strategy involved in ML postural control (Blanchet et al., 2019; Kirshenbaum et al., 2001). Disruptions of maturation process in these different developmental phases likely result in non-optimal postural control performance. Accordingly, alterations in ML and AP postural control may reflect alterations in different stages of motor development, which is of relevance when investigating NSS.

1.2.3 Postural Control Alterations Associated with Neurological Soft Signs

To date, the incremental value of using instrumented motor assessments in addition to observer-based examinations to investigate NSS is largely unknown. Particularly, postural control performance, e.g., assessed by using force plates, has rarely been examined in this context. It is assumed, however, that such tools may help to quantitatively and objectively assess subtle motor alterations that may not be visible to the observer.

To my knowledge, only two studies to date used instrumented tools to quantitatively and objectively assess motor function in children (with NSS) (Kaneko et al., 2015, 2016). In their studies, Kaneko et al. (2015, 2016) used a mechanical set-up including four wearable sensors measuring the pronation and supination of the forearms. The assessment of pronation/supination movements in the context of neurological examinations is known as diadochokinesis test. In their first study, they investigated 223 typically developing children aged 4-12 years (Kaneko et al., 2015). The authors revealed age-related developmental changes in pronation-supination movements which may be used as quantitative marker for the evaluation of neurological functioning. In their second study investigating 33 children with attention-deficit-hyperactivity-disorder (ADHD), the authors found that the pronation supination function in children with ADHD lagged behind the one from typically developing children by several years (Kaneko et al., 2016).

Taken together, these studies demonstrate that instrumented motor assessments can measure the development of neurological function and suggest that such assessments have the potential to detect atypical or non-optimal neurological performance related to NSS.

1.3 Magnetic Resonance Imaging as Structural Marker of Neurological Soft Signs

MRI is a technique which allows a non-invasive visualization of soft tissues in the brain (McRobbie et al., 2017). The underlying physical mechanism of MRI is called nuclear magnetic resonance. Thereby, a strong magnetic field temporarily realigns water molecules inside the body. Radio waves lead to a relaxation of these molecules, which are picked up by receivers and can be measured. Compared to conventional neuroimaging techniques such as computed tomography, advanced MRI has been proven useful especially for detecting subtle changes of brain (micro)structure. Moreover, MRI does not require ionizing radiation and thus, can therefore be used safely without sedation even in children and adolescents (McRobbie et al., 2017). Thus, MRI offers immense potential for increasing our understanding of NSS-related alterations in the anatomy and physical properties of brain structures which motivated Study II (Bonke et al., 2022).

1.3.1 Assessment of Magnetic Resonance Imaging

T1-weighted imaging

T1-weighted MRI is an MRI pulse sequence that is widely used to investigate brain morphology because it generates a good contrast between different tissues such as cerebrospinal fluid, gray and white matter (Symms et al., 2004). More specifically, gray matter with high water content produces a lower signal and appears as darker areas, whereas white matter with high fat content produces higher signals and appears as lighter areas (Symms et al., 2004).

While in the initial stages of MRI use, radiologists measured parameters of interest on MRI scans manually, advanced MRI as it is known today allows to extract quantitative markers of brain structure automatically (Fischl et al., 2002; Whitwell, 2009). Examples of such quantitative markers are global and regional brain volumes, and cortical thickness (Backhausen et al., 2021) (for explanation and visualization see **Figure 4**). In the last few years, also other more complex parameters such as the local gyrification index measuring the folding of the cortex (for explanation and visualization see **Figure 4**) have been developed (Schaer et al., 2008, 2012) and used in the context of investigating alterations in brain development (Bos et al., 2015; Kohli et al., 2019; Shaw et al., 2012). Such quantitative markers are calculated by using (automated) image processing pipelines and thus, are independent of the clinician rating

the scan. Thereby, FreeSurfer is a commonly used software which parcellates the brain into regions of interest based on an atlas (Fischl et al., 2002, 2004).

Taken together, T1-weighted imaging allows to objectively quantify morphological brain structure alterations in vivo and thus, gives insight into the underlying pathophysiology of clinical conditions such as NSS.

Diffusion tensor imaging

Diffusion-weighted MRI is an MRI sequence based on which mathematical models such as diffusion tensor imaging (DTI) can be applied (Soares et al., 2013). DTI indirectly examines the microstructure of the white matter by quantifying the movement of water molecules based on Brownian motion. This movement of water molecules is expressed as magnitude and directionality of diffusion (Mori & Zhang, 2006; Pierpaoli et al., 1996). Thereby, a diffusion tensor consisting of three eigenvalues ($\lambda1$, $\lambda2$, $\lambda3$) along three axes is estimated for each voxel of the brain (Basser & Pierpaoli, 1996) (see **Figure 4**). The diffusion is called *isotropic* or *spherical* if the diffusivity is similar along all axes ($\lambda1 = \lambda2 = \lambda3$) as for cerebrospinal fluid. Likewise, it is called *anisotropic* or *ellipsoidal* when the diffusivity is larger along one axis (e.g., $\lambda1 > \lambda2 > \lambda3$) as for restricted diffusion along white matter fiber tracts.

Based on this physical principle, diffusion markers can be estimated and clinically interpreted. Commonly investigated markers are fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) (Basser & Jones, 2002) (see **Figure 4**). FA measures the directionality of water molecule diffusion. FA is often considered as overall marker of white matter microstructure with higher FA reflecting better integrity (Soares et al., 2013). MD is a marker of the average magnitude of diffusion along all axes in a voxel (($\lambda1 + \lambda2 + \lambda3$)/3). AD describes the magnitude of diffusion parallel to the main axis ($\lambda1$) suggested to indicate axonal integrity and RD perpendicular to the main axis (($\lambda2 + \lambda3$)/2) suggested to indicate myelin integrity. An enlarged extracellular space for example, would lead to lower FA and higher MD. Given that changes in FA and MD are considered unspecific, additional markers like AD and RD help for a more specific interpretation of underlying alterations (Winklewski et al., 2018).

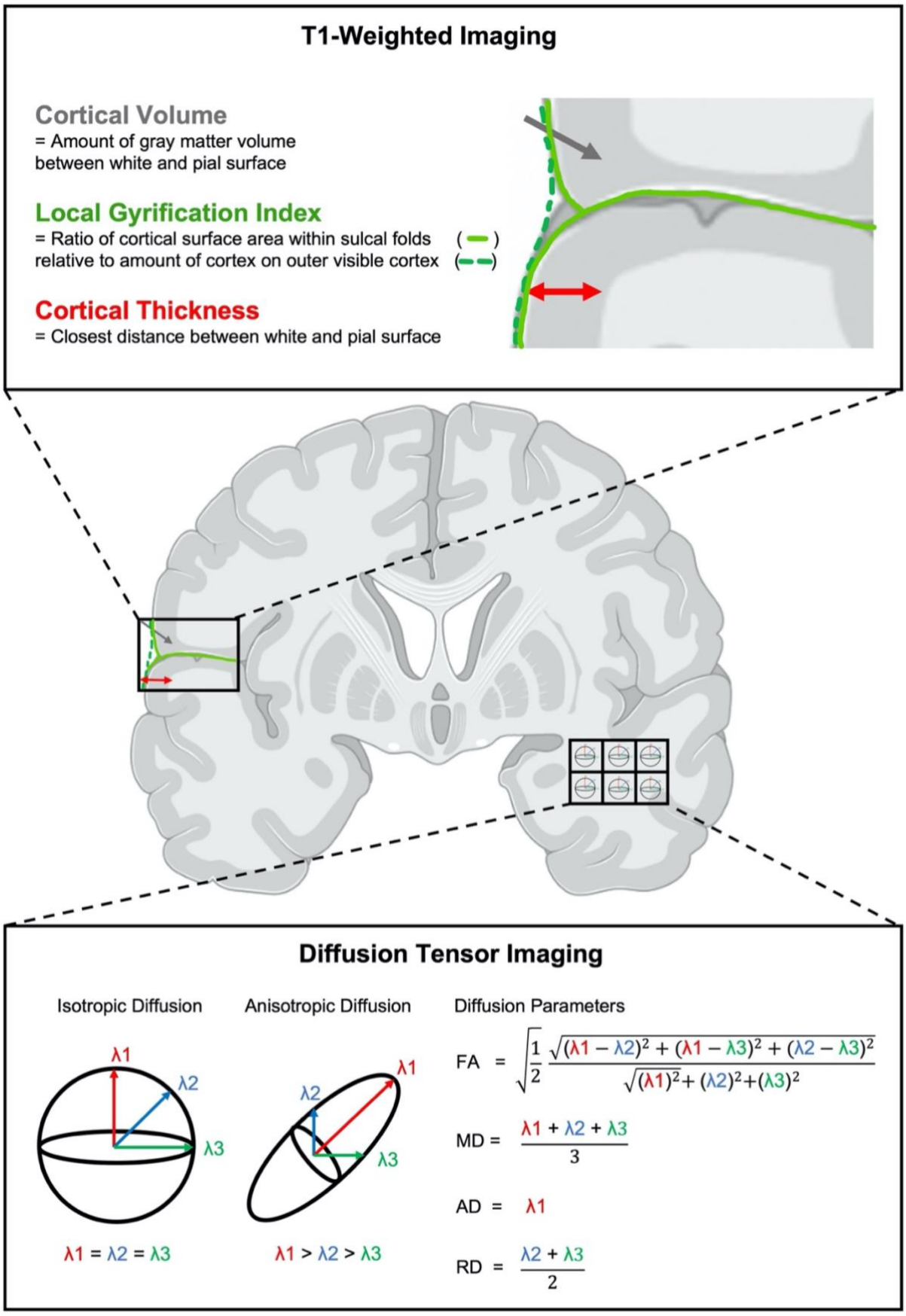

Figure 4. Visualization of T1-weighted and diffusion tensor imaging markers (created with BioRender.com).

After the computation of parametric maps (e.g., FA, MD, AD, RD), the next step is to calculate individual or group statistical analyses. To do so, summary measures either for the whole brain or for specific anatomical regions are extracted. Commonly applied approaches are region of interest quantifications, voxel-based analysis, or tract-based spatial statistics (TBSS) (Hess et al., 2013). Regions of interest-based analysis requires a manual delineation of a priori defined brain regions or can be based on automated parcellations. Voxel-based analysis includes the registration of diffusion maps into a standard space to achieve correspondence between subjects across voxels, allowing the comparison of diffusion measures between groups (Soares et al., 2013). TBSS is an automated method for detecting group voxel-wise differences along a white matter skeleton in the whole brain (Smith et al., 2006). TBSS is widely used and powerful as it captures the whole brain, no spatial smoothing is required, and the statistical power is increased due to the reduced number of voxels tested (Soares et al., 2013).

While the use of DTI is very promising, it also goes along with limitations. Typical limitations include the use of different MRI scanners leading to non-accurate diffusion outcomes (Mirzaalian et al., 2016), and the missing specificity in interpreting diffusion imaging findings (Basser & Jones, 2002). Particularly, in DTI, scanner differences should not be neglected because inter-site variability has been shown to be high and non-uniform across the white matter of the brain (Grech-Sollars et al., 2015; Vollmar et al., 2010). Variability of up to 5% of diffusion measures has been reported which is similar to what would be expected for changes related to certain pathology (Pinto et al., 2020). One approach to overcome scanner-related differences in the data is called data *harmonization*. Harmonization approaches retain the intra-subject variability at each study site and each scanner while accounting for scanner-specific variations such as spatial variability of the diffusion signal (Cetin Karayumak et al., 2019; De Luca et al., 2022; Mirzaalian et al., 2016). One approach to interpret diffusion alterations more specifically is called *free-water (FW) imaging* (Pasternak et al., 2009). FW imaging allows to differentiate between diffusion alterations in the extracellular space and tissue microstructure alterations by decomposing the diffusion signal into a FW and a FW-corrected tissue compartment (Pasternak et al., 2009). This differentiation allows for the correction of partial volumes, which is a well-known problem in DTI (Alexander et al., 2001). Commonly derived markers are FW, as well as tissue FA, MD, AD, and RD (FAt, MDt, ADt, RDt). FW imaging is particularly useful when diffusion alterations in the extracellular space are expected as it is the case for atrophy in neurodegenerative disorders (Bergamino et al., 2021; Duering et al., 2018; Dumont et al., 2019) and for acute potentially neuroinflammatory brain responses in reaction to psychiatric disorders (Carreira Figueiredo et al., 2022; Guo et al., 2021).

Its use to assess potential neurodevelopmental alterations in the context of NSS so far is largely unknown.

Taken together, while T1-weighted imaging allows to investigate brain morphology, DTI allows to investigate the white matter microstructure of the brain. DTI analyses, including harmonized FW imaging, allow a more accurate and specific interpretation of white matter microstructure alterations associated with NSS compared to DTI analyses without harmonization and FW imaging.

1.3.2 Brain Structure Development during Childhood and Adolescence

The presence/persistence of NSS is hypothesized to be linked to brain developmental processes during childhood and adolescence (Hadders-Algra et al., 2010; Soorani-Lunsing et al., 1993). Longitudinal MRI studies conducted over the past two decades have shown that the human brain, particularly during childhood and adolescence, undergoes a prolonged course of brain developmental changes (Blakemore, 2012; Mills et al., 2021). Special attention is drawn towards the phase of adolescence, which is considered to being a period of continued neural development and brain re-wiring processes including synaptic pruning and myelination (Blakemore, 2012; White et al., 2010). Thereby, different brain regions, tissue types, and circuits are considered to having distinct developmental trajectories (Giedd & Rapoport, 2010). A general understanding of typical brain developmental processes is needed to interpret NSS-related structural brain alterations. Thus, in this paragraph, I summarize important literature on typical brain structure development, including knowledge from T1-weighted MRI and DTI.

Development of brain morphology using T1-weighted magnetic resonance imaging

The development of brain morphology is complex and influenced by evolutionary (Geschwind & Rakic, 2013), genetic (C. H. Chen et al., 2013), cellular, and many other processes (Chenn & Walsh, 2002). Studies have mapped normalized trajectories of brain structure development (Giedd & Rapoport, 2010; Klein et al., 2014; Lebel & Beaulieu, 2011; Lebel & Deoni, 2018; Lenroot et al., 2007; Li et al., 2014; Tamnes et al., 2017). They consistently report a non-linear, inverted U-shaped trajectory in gray matter volume, cortical thickness and gyrification (Giedd & Rapoport, 2010; Li et al., 2014) The increase in gray matter volume and thickness in early childhood is assumed to reflect processes like neurogenesis and synaptogenesis (Gilmore et al., 2018; Stiles & Jernigan, 2010). The increase in gyrification already peaks earlier in childhood and allows a higher volume of brain mass to fit inside the skull (Rakic, 2009). In turn, the decrease in gray matter volume, thickness and gyrification in

late childhood and adolescence reflects the elimination of unnecessary connections in the brain, also known as synaptic pruning (White et al., 2010). In contrast, the white matter volume is consistently shown to increase until early adulthood (Grydeland et al., 2013; Lebel & Deoni, 2018).

Development of white matter microstructure using diffusion tensor imaging

Similarly, as for brain morphology markers derived from T1-weighted imaging, DTI studies have also mapped normalized trajectories of white matter microstructure (Barnea-Goraly et al., 2005; Blakemore, 2012; Lebel & Deoni, 2018; Tamnes et al., 2018). Findings show a rapid increase in FA during infancy and a continuous increase into early adulthood. MD often is reversely associated with FA and decreases during childhood and adolescence. Most DTI studies find this age-related increase in FA and decrease in MD to be associated with decreases in RD, indicating ongoing myelination, axon coherence, and increasing fiber density (Bava et al., 2010; Krogsrud et al., 2016; Lebel & Beaulieu, 2011). Only very few studies report an age-related increase in AD potentially related to reduced straightening of axons (Takahashi et al., 2012). FW imaging as of now has not yet been used in the context of investigating NSS-related brain structure alterations. However, as it allows to interpret pathologies such as inflammation that may also exist in association with neurodevelopmental alterations, it is of relevance when investigating children and adolescents with NSS.

Taken together, T1- and diffusion-weighted MRI are valuable to capture typical brain developmental trajectories. While in the previous paragraphs I described typical brain development, in the following paragraph I capture literature on NSS-related brain structure alterations.

1.3.3 Brain Structure Alterations Associated with Neurological Soft Signs

To date, research applying MRI to investigate NSS in healthy adolescents is sparse. Initial evidence is based on studies that investigated either adults with psychiatric disorders or children with neurodevelopmental disorders (D'Agati et al., 2018). Only a handful of studies investigated healthy adults (Dazzan et al., 2006; Hirjak et al., 2017; Hirjak, Wolf, Kubera, et al., 2016). Therefore, little is known about potentially underlying neural mechanisms of NSS and its clinical relevance in healthy adolescents.

Brain structure alterations related to neurological soft signs in psychiatric disorders

NSS are highly prevalent in populations with psychiatric disorders such as schizophrenia and psychosis (Herold et al., 2020; Thomann et al., 2009). Evidence from a meta-analysis summarizing six MRI studies that evaluated individuals with schizophrenia and other psychotic disorders shows that the presence of NSS is associated with lower volumes of the precentral gyrus, the inferior frontal gyrus, the cerebellum, and the thalamus (Zhao et al., 2014). Other smaller found similar results and additionally reported smaller volumes in the superior and middle temporal and lingual gyri, as well as the putamen, globus pallidus (Dazzan et al., 2004), and the caudate (Janssen et al., 2009). Moreover, lower cortical thickness and local gyrification in association with NSS in individuals with schizophrenia were reported (Hirjak, Wolf, Paternoga, et al., 2016). Regarding alterations in white matter microstructure, one study found a higher neurological examination scale in individuals with schizophrenia to be associated with higher RD, MD, and AD, but not with FA in major white matter tracts such as the corpus callosum (CC), the internal capsule (IC), thalamic radiation (TR), the corticospinal tract (CST) and the superior longitudinal fasciculus (SLF) (Viher et al., 2022). In summary, studies investigating individuals with psychiatric disorders such as schizophrenia found alterations in the morphology and microstructure of brain regions that play an important role in higher-order sensorimotor control.

Brain structure alterations related to neurological soft signs in neurodevelopmental disorders

Interestingly, while in adults with psychiatric disorders, NSS have been associated with decreased cortical volumes, thickness and gyrification, in children with neurodevelopmental disorders studies have reported reverse effects with higher gyrification in children with autism spectrum disorder compared to typically developing children (Wallace et al., 2013). Similarly, while in individuals with schizophrenia, the presence of NSS was associated with higher RD, MD and AD (Viher et al., 2022), Brown-Lum et al. (2020) found children with developmental coordination disorder to have lower FA and AD in similar white matter tracts such as the CC, CST, TR and the IC compared to typically developing children. It remains open whether these differences between studies are related to the investigated participant age (adults versus children/adolescents) or whether they are based on distinct clinical presentations and underlying mechanisms (psychiatric versus neurodevelopmental disorders).

Brain structure alterations related to neurological soft signs in healthy populations

To my knowledge, only three studies to date investigated NSS-related brain structure alterations using MRI in healthy cohorts (Dazzan et al., 2006; Hirjak et al., 2017; Hirjak, Wolf, Kubera, et al., 2016). However, such investigations are important to better understand potentially underlying altered neurodevelopmental or brain-rewiring processes in the context of NSS.

The first study investigated 43 healthy adults with a mean age of 30 years (Dazzan et al., 2006). NSS were assessed using the Neurological Evaluation Scale. They found a higher NSS score to be associated with lower volume in the inferior frontal, middle and superior temporal, and anterior cingulate gyrus. The second study investigated 68 healthy adults with a mean age of 24 years (Hirjak, Wolf, Kubera, et al., 2016). NSS were assessed using the Heidelberg Scale. This study reported an association between higher NSS scores and lower cortical thickness and lower gyrification in superior frontal, middle temporal, and postcentral regions. The third study investigated the same sample using DTI and found higher NSS scores to be associated with altered RD in the CC (Hirjak et al., 2017).

Taken together, NSS-related brain structure alterations are present in individuals with neurodevelopmental, and with psychiatric disorders. Preliminary evidence also shows the presence of NSS-related brain structure alterations in otherwise healthy cohorts. However, what remains unclear from existing work is the role of potential brain-rewiring processes in adolescence that are hypothesized to lead to the manifestation or outgrowing of neurodevelopmental alterations.

1.4 Thesis Aims

With the work included in my dissertation I aim to assess functional and structural alterations associated with NSS. The data included in my dissertation was derived from the prospective study REPIMPACT (Repetitive Subconcussive Impacts – Brain Alterations and Clinical Consequences). In the REPIMPACT study, we pre-selected and acquired data from a cohort of healthy male adolescent athletes aged 13-16 consisting of primarily soccer players born on term and without history of neurodevelopmental or psychiatric disorders (Koerte et al., 2022). By investigating healthy adolescents, we extend previous work assessing adults with psychiatric disorders, children with neurodevelopmental disorders or healthy adults with NSS. By using objective and quantitative measures, we show the benefit of using instrumented tools beyond subjective neurological examinations.

1.4.1 Study I

To date, the incremental value of instrumented postural control measures, in addition to neurological examinations to investigate NSS is largely unknown. It is assumed that instrumented tools may help to quantitatively and objectively assess subtle motor changes that may not be visible to the observer but may point towards subtle developmental alterations.

To address these questions, Study I aims to identify and characterize alterations in postural control performance associated with NSS in healthy adolescent athletes. Moreover, Study I intends to investigate the additional use of force plate measures in a clinical setting. We hypothesized that the neurological performance derived from an observer-based neurological examination would also be reflected in reduced postural control performance. We further hypothesized that the use of force plates would allow for a more detailed and objective quantification of motor functioning in addition to clinical observer-based assessments.

1.4.2 Study II

Another quantitative tool to investigate potentially underlying neural mechanisms of NSS is MRI. Initial evidence is based on studies that investigated either adults with psychiatric disorders, children with neurodevelopmental disorders, or healthy adults. Little is known, however, about potentially underlying neural mechanisms of NSS and its clinical relevance in healthy adolescents.

To address this, Study II aims to identify and characterize brain structure alterations in the gray and white matter of the brain associated with NSS in healthy adolescent athletes. Thereby, it investigates potentially underlying neural mechanisms of NSS that may be of developmental relevance. We hypothesized that adolescents with NSS would show alterations in cortical volume, thickness, gyrification, and white matter microstructure compared to adolescents without NSS.

2 CUMULATIVE THESIS

2.1 Study I: Neurological Soft Signs Are Associated With Reduced Medial-Lateral Postural Control in Adolescent Athletes

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Neurological soft signs are associated with reduced medial-lateral postural control in adolescent athletes

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ABSTRACT

Introduction: Neurological soft signs (NSS) are minor deviations from the norm in motor performance that are commonly assessed using neurological examinations. NSS may be of clinical relevance for evaluating the developmental status of adolescents. Here we investigate whether quantitative force plate measures may add relevant information to observer-based neurological examinations.

Methods: Male adolescent athletes (n = 141) aged 13–16 years from three European sites underwent a neurological examination including 28 tests grouped into six functional clusters. The performance of tests and functional clusters was rated as optimal/non-optimal resulting in NSS+/NSS- groups and a continuous total NSS

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score. Participants performed a postural control task on a Balance Tracking System measured as path length, root mean square and sway area. ANCOVAs were applied to test for group differences in postural control between the NSS+ and NSS- group, and between optimal/non-optimal performance on a cluster- and test-level. Moreover, we tested for correlations between the total NSS score and postural control variables.

Results: There was no significant overall difference between the NSS+ and NSS- group in postural control. However, non-optimal performing participants in the diadochokinesis test swayed significantly more in the medial-lateral direction than optimal performing participants. Moreover, a lower total NSS score was associated with reduced postural control in the medial-lateral direction.

Conclusion: Our findings demonstrate that NSS are related to postural control in adolescent athletes. Thus, force plate measures may add a quantitative, objective measurement of postural control to observer-based qualitative assessments, and thus, may complement clinical testing.

1. Introduction

Neurological soft signs (NSS) are minor deviations from the norm in motor performance and sensory-motor integration [1]. In contrast, major (also called *hard*) signs are rated as pathological and include findings such as hyperreflexia and spasticity [2,3]. Even though not considered pathological, and also present in healthy adults [4], investigating NSS in adolescents is clinically relevant because it was found to be associated with an increased vulnerability for mental disorders [5,6]. More specifically, there is initial evidence for NSS being a non-specific marker for developing developmental deficiencies such as attention-deficit hyperactivity disorder and schizophrenia spectrum disorder [7–9].

NSS are typically assessed using a clinical neurological examination [10]. Commonly used rating systems are qualitative and include the Neurological Examination Scale (NES) [11], the Cambridge Neurological Inventory (CNI) [12], the Heidelberg NSS Scale (HS) [13], and the age-dependent assessment of Minor Neurological Dysfunction (MND) [14,15] (for review of NSS rating systems see [16]). With some minor differences, most rating systems comprise tests of coordination, fine motor skills, and postural control [17]. Importantly, these assessments are based on the examiners' observation and therefore are qualitative rather than quantitative by nature. Particularly when it comes to subtle alterations as it may be the case for physically trained adolescent athletes, the addition of instrumented investigations such as force plate measures assessing postural control, which are considered to be the "gold standard" for assessing postural stability [18], may provide valuable insights by quantitatively detecting variability in performance that might not be detected visually.

Postural control is considered a milestone of physiological development and allows individuals to engage in various static and dynamic activities [19]. It can be described as the maintenance of the center of pressure inside the base of support and is defined by Bartlett and Birmingham as the ability to maintain internal stability (e.g., to stand upright) and to anticipate and avoid instability in response to an external perturbation [20]. Generally, motor performance and postural control development take place according to two stages. First, young children, approximately until the age of five years, follow an *open-loop strategy*, characterized by the explorative activity of the nervous system exploring all motor possibilities. This strategy is also referred to as the ballistic strategy and is characterized by a greater displacement of the center of pressure (COP) and thus greater instability [21]. Around the age of eight years, children transfer to using a *closed-loop strategy* (or sensory strategy) where they use various movement possibilities in each specific situation, resulting in better postural control [21,22]. As children age further, their nervous system adapts, and multisensory integration is improved. More specifically, older children integrate information from different modalities (e.g., visual and somatosensory system) more successfully to control posture, making them better equipped to function in a changing environment [22,23]. However, any disturbances of this maturation process will likely result in less optimal or non-optimal postural control. Of note, postural control has been shown to mature later along the medial-lateral (ML) compared to the anterior-posterior (AP) axis [21–23]. Currently, there is no clear consensus in the literature regarding at what age adolescents reach their peak performance in postural control. Some studies suggest that adolescents have not yet reached an adult-like performance in postural control at the age of 14 years [24] while others found that processes underlying the maintenance of an optimal postural stability are mature much earlier [25]. In adolescent athletes, this developmental process was reported to be ongoing until the age of 19 years as shown by normative Balance Tracking System (BTrackS) data of more than 10,000 adolescent athletes between 8 and 21 years [26].

To the best of our knowledge, no study has yet used a combined approach of observer-based neurological examination and quantitative assessment of postural control to comprehensively assess motor maturation between the ages of 13 and 16 years. Thus, to date it remains unknown whether the findings of both assessments are interrelated. Therefore, this study aims to provide information on the additional use of force plate measures in a clinical setting. It is expected that a lower qualitative NSS score derived from a neurological examination is reflected in reduced postural control performance. The use of force plates as an objective measure will allow for a detailed quantification of higher-order motor functioning in addition to clinical observer-based assessments. This will add relevant information when investigating motor functioning in a clinical setting.

2. Materials and methods

2.1. Study design and participants

Participant data were extracted from the multi-site study REPIMPACT (Repetitive Subconcussive Head Impacts – Brain Alterations and Clinical Consequences; 2017–2020). The study enrolled male adolescent athletes aged 13–16 years between July 2017 and April 2020 from three study sites (Oslo, Norway; Leuven, Belgium; Munich, Germany). Included athletes participated in various sports that were primarily soccer, but also swimming, track and field, cross-country skiing, cycling, tennis, biathlon, and rowing. Participants and their legal guardians at all three data acquisition sites provided written informed consent in accordance with the local ethics board approvals and the Declaration of Helsinki.

In short, inclusion criteria for participating in the study were: (1) 13–16 years of age, (2) male, (3) participation in sports at a competitive level with training at least three times per week in the respective sport, and (4) fluent language skills of the respective country of citizenship (i.e., Norwegian, Dutch, or German). For more details on the REPIMPACT study design, see [27].

Participants were excluded from the analysis in case of (1) history of serious medical condition (history of encephalitis: $n = 3$), (2) incidental finding on a magnetic resonance imaging scan (periventricular gliosis: $n = 1$, subependymal heterotopia: $n = 1$), (3) premature birth (i.e., < 37 weeks of gestation) ($n = 3$), (4) diagnosed attention deficit disorder ($n = 1$), (5) neurological hard signs as evident by neurological examination ($n = 0$), or (6) no performed neurological examination as part of the REPIMPACT project ($n = 17$). This resulted in an included sample of 141

participants (Table 1). The neurological examination and the postural control assessment were performed at the same visit.

2.2. Neurological examination assessing NSS

A standardized pediatric neurological examination based on the *William DeMyer's Neurological Examination* was performed [28]. This examination included the evaluation of NSS in line with the distinct age-related rating framework published within the MND concept [14]. We decided to follow the MND concept because it has proven useful when investigating developmental cohorts [29–31]. Of note, compared to other assessments such as the NES, CNI, HS, the MND concept considers the developmental status of a child and assesses performance with respect to age [14,32]. Using an age-dependent concept allows to detect even subtle changes which is particularly helpful in a cohort of physically trained adolescent athletes that are expected to perform well in tasks assessing motor performance.

The MND examination consists of 64 tests grouped as eight functional clusters. More details are published elsewhere [15]. In our examination, 28 out of these 64 tests were performed, assessing six functional clusters: (1) *Fine motor skills* (e.g., *finger-opposition test*: tapping the tip of the thumb with each finger of the same hand in a specific sequence), (2) *Coordination & balance* (e.g., *diadochokinesis test while standing*: quick antagonistic pro- and supination movements of the forearm), (3) Posture & tone (e.g., posture while sitting, standing, or walking), (4) Involuntary movements (e.g., sitting/standing choreatic movements or tremor) (5) Associated movements (e.g., in didochokinesis or finger-opposition test), and (6) Sensory function (e.g., graphesthesia, kinesthesia).

The performance of every test was rated as either optimal or non-optimal based on the neurological optimality score (NOS), indicating how many items are performed in an optimal way [15,33]. Optimal performance is based on predefined criteria, meaning that failing a test indicates not meeting the specific predefined criteria of the respective test.

A non-optimal performed cluster is defined as a cluster that includes a number of non-optimal performed tests that exceeds a specific threshold. If one or more clusters were rated as non-optimal, a participant was assigned to the group with NSS (NSS+ group), and otherwise in the group without NSS (NSS- group). In addition to the overall group categorization, participants were categorized as optimal/non-optimal performing on the cluster-level and on the test-level, respectively.

In a second step, an additional continuous NSS score was calculated because this qualitative clinical categorization approach does not allow to differentiate between participants with higher and lower NSS scores within a specific group. This score is in alignment with the NOS and is referred to as the *total NSS score*. For instance, a score of 26 indicates that the participant performed optimal in 26/28 tests. Consequently, a higher NSS score indicates better neurological functioning.

In Germany, the assessment was performed by experienced pediatric neurologists. Examiners from Norway and Belgium were trained by experienced pediatric neurologists from Germany before performing the examination independently. In addition, examinations from Norway and Belgium were videotaped and recorded and were then re-assessed independently by three pediatric neurologists from Germany.

2.3. Assessment of postural control using BTrackS

Postural control was assessed at all study sites using a validated portable force platform, BTrackS (Balance Tracking Systems Inc., San Diego, CA, USA) [18,34,35], consisting of a 0.4 m × 0.6 m force platform. A 25 Hz sampling rate was used to capture the ground reaction forces of the postural control assessments, which was registered as resulting COP and was filtered with a second-order, low-pass Butterworth filter (cut-off frequency of 4 Hz) prior to export. Postural control was tested both on a rigid and a compliant (foam) surface. The compliant surface consisted of a 6 cm thick Airex Balance Pad (Alusuisse Airex AG, Switzerland) placed on the force plate.

Participants performed all postural assessments without shoes and with socks. Following plate calibration, participants were instructed to stand on the force plate with their head in an upright position and as still as possible with eyes closed, hands on the hips, and feet apart at shoulder width. The start and end of each trial were indicated with an auditory tone. Each trial lasted 20 s and was repeated to obtain a total of three trials per condition (i.e., rigid and foam) per participant. All participants performed the rigid trials first, followed by the foam trials. A trial was repeated if a participant did not perform the task correctly (e.g., when a participant kept his eyes open).

The mean/total displacement of the COP, in addition to AP and ML directional components, were used to quantify postural stability among participants. Utilizing COP-based equations developed by Thomas Prieto and colleagues in a custom MATLAB [36] script [37], three traditional postural metrics were derived for each axis (Mean/AP/ML): (1) path length (PL), which informs about the COP trajectory magnitude, (2) root mean square (RMS) amplitude deviations which represents the standard deviation of the displacement of the COP, and (3) sway area, or the COP displacement between the beginning and end of trial across axes [38]. The three dependent variables were chosen to describe the spatial-temporal and variability aspects of postural performance, respectively. For all measurements, higher values are indicative of poorer static postural control.

2.4. Statistical analyses

Statistical analyses were performed using the software R (version 4.0.1) [39]. In the first analysis, a categorical approach using a cut-off criterion based on clinical diagnostic practices was used. Participants were categorized into two groups: a group with and without NSS (NSS+

Table 1
Cohort characteristics.

	NSS+ ($n = 26$)	NSS- ($n = 115$)	Statistical test
Study site (n)	B (2), G (5), N (19)	B (32), G (38), N (45)	$X^2 = 8.540$, $p = .014$ *
Age [years] *Mean (SD)*	14.70 (0.67)	15.14 (0.74)	$t(139) = -2.798$, $p = .006$ *
Height [cm] *Mean (SD)*	170.32 (10.01)	173.62 (7.50)	$t(135) = -1.861$, $p = .065$
Weight [kg] *Mean (SD)*	58.19 (10.35)	60.39 (8.55)	$t(134) = -1.130$, $p = .260$
Total NSS score *Median (SE)*	21.50 (0.55)	25.30 (0.19)	$t(139) = -7.912$, $p < .001$ *

Note. * Indicates significant difference between groups at $p < .05$.
Abbreviations. B = Belgium, G = Germany, N = Norway, NSS = neurological soft signs, SD = standard deviation, SE = standard error, X^2 = Chi Square.

and NSS-) and groups with optimal/non-optimal performance in the *fine motor skills* and *coordination & balance* cluster, as well as in the *finger-opposition test* and the *diadochokinesis test*. We selected these two clusters and two tests (1) because based on clinical experience, the performance of fine motor skills and coordination is highly relevant and commonly assessed, and (2) they were the most often non-optimal performed clusters/tests in our cohort. The sample size of participants that performed non-optimal in the other clusters/tests was too small ($n < 20$) to categorize reasonable groups. It was evaluated whether these groups differ in performance on the postural control tasks. However, while clinically relevant, this approach does not allow for a differentiation between participants with higher and lower NSS scores within a specific group. Therefore, for the second analysis, the continuous total NSS score was correlated with the postural control performance.

2.4.1. Differences in demographical variables between the NSS+ and NSS- group

Chi-Square tests were used to evaluate whether the distribution of participants from the three study sites was similar in the NSS+ and NSS- group. Moreover, to test whether the NSS+ and NSS- group include similar populations, demographical variables such as age, height, and weight and the total NSS score were assessed using independent *t*-tests.

2.4.2. Differences in postural control between groups

Data from the three acquired trials for each condition (rigid and foam) and each postural control variable (PL, RMS, sway area) were averaged. Outliers were visually inspected and removed if they were most likely caused by a technical error, resulting in an exclusion of one trial of $n = 1$ participant in the rigid condition. In this case, the remaining two trials were averaged.

Between-group differences (NSS+ and NSS-) in postural control performance in the rigid and foam condition for the variables of interest path length, root mean square, and sway area were calculated using ANCOVAs with age at the time of the neurological examination as a covariate. Additional analysis including study site as a covariate (not shown here), did not change the results. Multiple comparisons were corrected for 14 variables (PL (Mean/AP/ML), RMS (Mean/AP/ML) and sway area in both rigid and foam condition) using the Bonferroni method. The level of significance was set to $\alpha = 0.05$.

2.4.3. Correlation between postural control and total NSS score

Spearman correlation coefficients were used to test whether the total NSS score was associated with postural control performance in the rigid and the foam condition. Spearman correlation was used because the total NSS score was not normally distributed, with more participants scoring on the higher end, meaning better overall performance.

3. Results

3.1. Demographic differences between the NSS+ and NSS- group

Table 1 summarizes the demographic information for both groups (NSS+ and NSS-).

Based on the neurological examination, 26 (18.4%) participants were categorized as NSS+ and 115 (81.6%) participants as NSS-. Of the 141 participants, 115 (81.6%) participants performed optimal in all six clusters (NSS- group), 24 (17.0%) performed non-optimal in one cluster, 1 (0.7%) performed non-optimal in two clusters, and 1 (0.7%) in four clusters. The cluster that most often was performed non-optimal was *fine motor skills,* performed non-optimal by 24 participants (17.0%), followed by a non-optimal performance of 3 participants in *associated movements* (2.1%), of 2 participants in *coordination & balance* (1.4%) and of 1 participant (0.7%) in *posture & tone*. Of note, none of the participants performed non-optimal in the *sensory function* cluster.

There was a significant between-group difference for study site ($p = .014$) with a greater proportion of participants in the NSS+ group coming from Norway compared to Belgium and Germany. Furthermore, there was a significant between-group difference for age ($p = .006$), with the NSS+ group being significantly younger than the NSS- group. In addition, there was a significant between-group difference for the total NSS score ($p < .001$). Height and weight did not differ significantly between the NSS+ and NSS- group (all $p > .05$). Moreover, a-priori analyses revealed no statistically significant between-group differences regarding the type of sports (soccer versus non-contact) and concussion history (no previous concussion, probable concussion, physician-diagnosed concussion) in the prevalence of NSS (all $p > .05$) and in the postural control performance (for each dependent variable and condition; all $p > .05$).

3.2. No difference in postural control between the NSS+ and NSS- group

There were no significant differences in postural control for any of the variables of interest, neither in the rigid nor the foam condition between NSS+ ($n = 26$) and NSS- group ($n = 115$) (all $p > .05$; see Table A.1).

3.3. No difference in postural control between groups performing optimal/non-optimal on the cluster-level

There were no significant differences in postural control for any of the variables of interest, neither in the rigid nor the foam condition between the groups of participants with optimal and non-optimal performance in the *fine motor skills* cluster or the *coordination & balance* cluster (all $p > .05$).

3.4. Significant difference in postural control between groups performing optimal/non-optimal on the test-level

There were no significant differences in postural control for any of the variables of interest, neither in the rigid nor the foam condition, between the groups of participants with optimal ($n = 105$) and non-optimal ($n = 36$) performance in the *finger-opposition test* (all $p > .05$). The group that performed non-optimal in the *diadochokinesis test* ($n = 33$), however, had significantly higher sway area ($p = .003$), mean path length ($p = .022$), path length in the ML direction ($p = .002$), mean root mean square ($p = .044$), and root mean square in the ML direction ($p = .002$) in the rigid condition compared to the group that performed optimal in this test ($n = 108$). Path length and root mean square postural performance in the AP direction, as well as all variables in the foam condition, were not significantly different between groups after correcting for multiple comparisons ($p > .05$). Differences in postural control between the groups performing optimal/non-optimal in the *diadochokinesis test* are illustrated in Fig. 1 and listed in Table 2.

3.5. Significant correlation between postural control and total NSS score

Fig. 2 and Table 3 show the correlations between postural control and the total NSS score. The correlations were statistically significant for path length ($p = .016$) and root mean square in the ML direction in the rigid condition ($p = .011$), and for path length in the ML direction in the foam condition ($p = .025$). No significant correlations between postural control in the other variables were detected (all $p > .05$).

4. Discussion

This study aimed to investigate the association between an objective quantitative assessment of postural control using force plates and an observer-based neurological examination in a cohort of adolescent athletes. Such associations have not been assessed before, but are of critical importance for clinicians as it provides a more comprehensive assessment of motor maturation between the ages of 13 and 16 years. It was assumed that lower NSS scores would be reflected in reduced

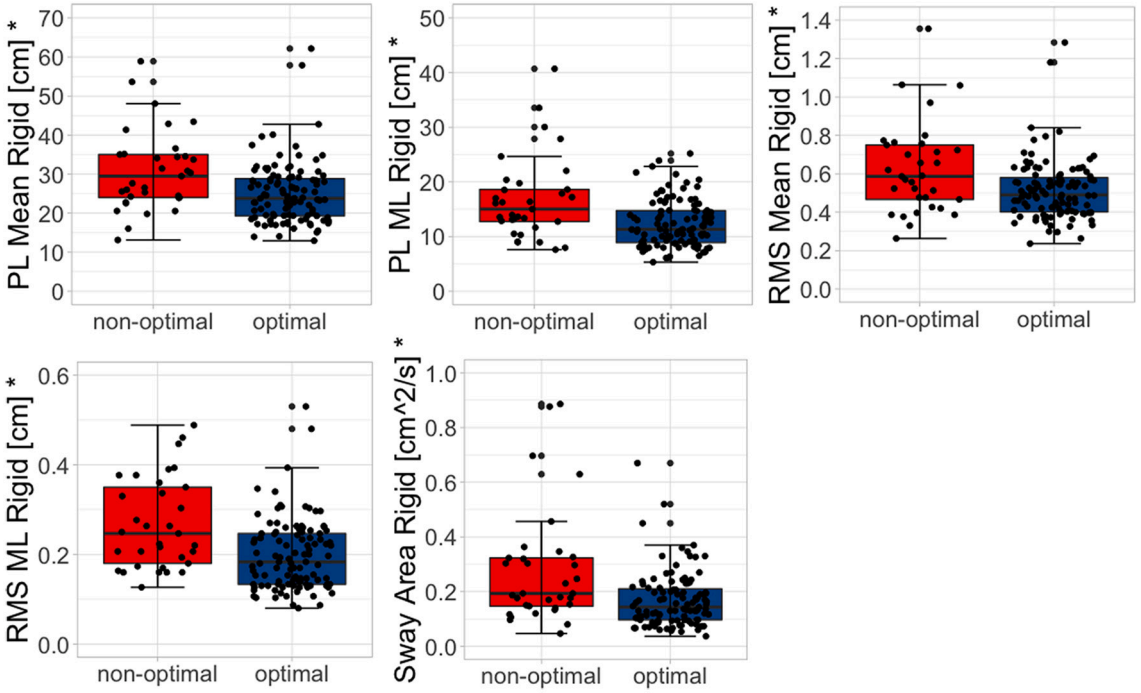

Fig. 1. Significant difference between groups performing optimal/non-optimal in the *diadochokinesis test* in the rigid condition. Higher values indicate worse performance in postural control. Variable y-axis scaling was used for better visualization.
Note. * All *p*-values were corrected for 14 variables at $p < .05$.
Abbreviations. ML = medial-lateral, PL = path length, RMS = root mean square.

Table 2
Difference in postural control between groups performing optimal/non-optimal in the diadochokinesis test.

Postural control	Non-optimal diadochokinesis	Optimal diadochokinesis		
	Mean (*SD*)	*Mean* (*SD*)	*F*	*p*
Rigid				
PL	30.82 (10.34)	25.11 (7.93)	$F(1, 136) = 10.429$	$p = .022$ *
PL ML	16.77 (7.55)	12.34 (4.28)	$F(1, 136) = 15.651$	$p = .002$ *
PL AP	21.79 (6.97)	18.99 (6.67)	$F(1, 136) = 4.410$	$p = .526$
RMS	0.63 (0.24)	0.51 (0.16)	$F(1, 136) = 9.060$	$p = .044$ *
RMS ML	0.27 (0.10)	0.20 (0.08)	$F(1, 136) = 15.185$	$p = .002$ *
RMS AP	0.56 (0.23)	0.47 (0.15)	$F(1, 136) = 6.279$	$p = .187$
Sway area	0.28 (0.21)	0.17 (0.10)	$F(1, 136) = 14.756$	$p = .003$ *
Foam				
PL	66.41 (20.72)	60.96 (13.95)	$F(1, 136) = 2.093$	$p > .999$
PL ML	30.73 (8.90)	27.71 (7.89)	$F(1, 136) = 2.515$	$p > .999$
PL AP	52.46 (17.34)	48.38 (11.17)	$F(1, 136) = 1.754$	$p > .999$
RMS	1.31 (0.36)	1.25 (0.26)	$F(1, 136) = 0.652$	$p > .999$
RMS ML	0.63 (0.17)	0.61 (0.18)	$F(1, 136) = 0.170$	$p > .999$
RMS AP	1.14 (0.34)	1.07 (0.24)	$F(1, 136) = 0.781$	$p > .999$
Sway area	1.17 (0.62)	1.00 (0.43)	$F(1, 136) = 2.313$	$p > .999$

Note. * All p-values were corrected for 14 variables at $p < .05$.
Abbreviations. AP = anterior-posterior, ML = medial-lateral, PL = path length, RMS = root mean square, SD = standard deviation.

postural control performance.

Our results revealed that participants performing non-optimal in the *diadochokinesis test* showed significantly reduced postural control. More specifically, this effect was only observed in the rigid condition of the postural control measurement, and primarily in the ML direction. No significant between-group differences in postural control were found in the foam condition.

Moreover, we found that the continuous total NSS score was, indeed, correlated with postural control performance. The results revealed a significant correlation between total NSS score and postural control in the ML direction, suggesting that those with lower performance in the neurological examination also tended to produce more ML COP movement in the force plate assessment.

4.1. A multi-method approach to NSS evaluation

In the clinical context, postural control is commonly assessed using observer-based tools such as classical neurological tests (i.e., Romberg stance, one-legged stance, tight rope walk). Particularly when it comes to subtle alterations, instrumented tools such as force plates may add additional and objective information to what even an experienced clinician can detect by eye. Using force plates in addition to a neurological examination may, thus, support clinicians by providing a more comprehensive and personalized assessment of motor maturation.

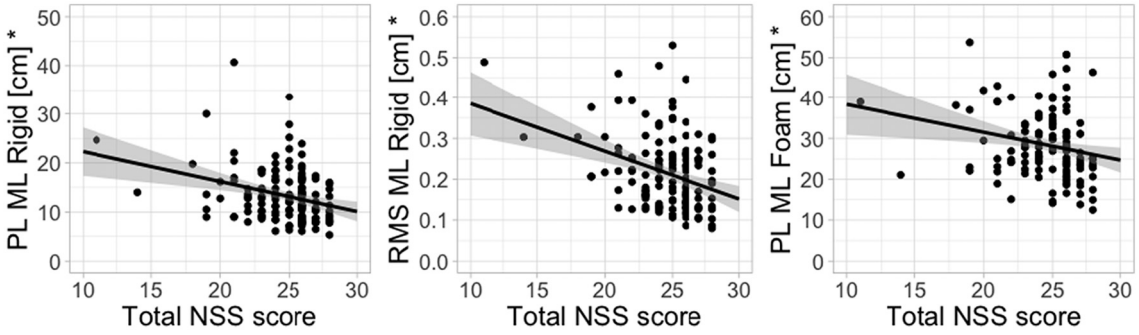

Fig. 2. Scatterplots showing negative correlations between postural control and the total NSS score. Lower NSS scores and higher postural control variables indicate lower performance.
Note. * All *p*-values were corrected for 14 variables at $p < .05$. Variable y-axis scaling was used for better visualization.
Abbreviations. AP = anterior-posterior, ML = medial-lateral, NSS = neurological soft signs, PL = path length, RMS = root mean square.

Table 3
Correlation between postural control and total NSS score.

NSS	Postural control	*Rho*	*p*
Rigid			
PL	Total NSS score	r_s (139) = −.195	p = .290
PL ML	Total NSS score	r_s (139) = −.271	p = .016 *
PL AP	Total NSS score	r_s (139) = −.132	p > .999
RMS	Total NSS score	r_s (139) = −.156	p = .906
RMS ML	Total NSS score	r_s (139) = −.279	p = .011 *
RMS AP	Total NSS score	r_s (139) = −.097	p > .999
Sway area	Total NSS score	r_s (139) = −.222	p = .114
Foam			
PL	Total NSS score	r_s (139) = −.141	p > .999
PL ML	Total NSS score	r_s (139) = −.260	p = .025 *
PL AP	Total NSS score	r_s (139) = −.189	p = .357
RMS Foam	Total NSS score	r_s (139) = −.064	p > .999
RMS ML	Total NSS score	r_s (139) = −.093	p > .999
RMS AP	Total NSS score	r_s (139) = −.055	p > .999
Sway area	Total NSS score	r_s (139) = −.182	p = .424

Note. * All p-values were corrected for 14 variables at $p < .05$.
Abbreviations. AP = anterior-posterior, ML = medial-lateral, NSS = neurological soft signs, PL = path length, RMS = root mean square.

Indeed, in the current study, the instrumented assessment of postural control revealed that reduced performance in postural control along the ML axis was associated with a lower total NSS score. Nonetheless, more research is needed to determine the common underlying neurodevelopmental substrates of the quantitative and qualitative findings and whether other instruments or test conditions can quantitatively reflect other tasks derived from the NSS assessment.

4.2. Association of NSS with postural control in medial-lateral direction

Our findings of lower postural control in the ML direction being significantly correlated with a lower total NSS score, and being significantly reduced in the group of participants performing non-optimal in the *diadochokinesis test* is in alignment with previous literature on motor development [21,22,40]. It has been shown that AP and ML postural adjustments follow different maturation processes. More specifically, there is evidence that postural control along the ML axis matures later than along the AP axis, possibly being caused by (1) earlier maturation of antigravity muscles responsible for AP control, (2) daily life experience that is expected to be greater in the AP direction, and (3) earlier development of the ankle strategy involved in AP postural control than the hip strategy involved in ML postural control [21,22,40]. We did not observe an association between postural control in the AP axis and NSS scores. While the existence of such an association cannot be ruled out with certainty based on these data, the pattern that we observed is compatible with the possibility that the maturation along the AP axis is already fully developed in our sample.

Moreover, among the neurological tests, the performance of antagonistic forearm movements in quick succession, as examined in the *diadochokinesis test*, has been shown to mature later than other tests assessing upper-limb motor proficiency [41]. Of note, the two tasks are based on similar underlying functional mechanisms. Both tasks involve higher-order motor networks that control the alternation and timing of muscle activity in agonist and antagonist muscles as a result of cortical excitatory and inhibitory neuronal activity [41]. Thus, our unique finding of reduced postural control along the ML axis in a group of participants that performed non-optimal in the *diadochokinesis test* aligns with already existing knowledge. The performance in postural control and diadochokinesis movements may thus serve as indicators of motor maturation.

Adolescent competitive athletes receive extensive physical training during a crucial period of physiological motor development. Accordingly, one may expect that adolescent athletes will be more skilled in motor tasks, including postural control, than the general adolescent population. Indeed, it has been demonstrated that increased sensorimotor experience in sensitive developmental periods can improve motor proficiency [42,43]. Interestingly, Bieć & Kuczyński have shown that in sports disciplines such as soccer which requires bodyweight transfers along the ML axis, 13-year old soccer players had better postural control in the ML direction in comparison with a control group [43]. Thus, differences in postural control performance depending on the participants' athletic background may be assumed. Unfortunately, we were unable to compare sport disciplines (e.g., soccer, swimming, etc.) in our sample due to insufficient sizes of subsamples. It may be interesting for future studies to evaluate the association between postural control along the ML axis and NSS in athletes from different disciplines and compare with a non-athlete population.

4.3. Group differences in the rigid but not foam condition

We detected group differences only in the rigid, but not the foam condition. Moreover, fewer variables in the foam condition compared to the rigid condition were significantly correlated with the total NSS score. While the rigid condition is a well-validated method that is often used in research [35], the foam condition may have resulted in a greater variability, or in other words random noise, of postural control performance e.g., due to differences in the ability to deal with less stability. Indeed, our data showed a greater variance of postural sway between participants and within the trials of individual participants in the foam condition compared to the rigid condition. Thus, the effects detected in the standardized, rigid condition may have been absent in the foam condition due to individual differences in response to the alteration of tactile inputs (decrease plantar pressure sensations) and stimulation of proprioceptive inputs (instability). Moreover, the foam condition was

always performed after the rigid condition and thus, may have been affected more by potential training effects. However, we do not anticipate strong learning effects as only three trials of the rigid condition were performed.

4.4. Strengths and limitations

It is important to note that in our sample, the NSS+ group was significantly younger than the NSS- group. Preliminary evidence shows that by reaching puberty, the prevalence of NSS decreases due to several factors, including hormonal changes and brain maturation processes [32,44]. Therefore, we explicitly controlled our group analyses for a potential age effect. Correcting or not correcting for age resulted in similar findings. This additional analysis confirmed that in our sample, the age difference between groups did not explain the differences in postural control. Moreover, we replicated our analysis in a sub-sample only including participants from Germany and Norway and excluding participants from Belgium that turned out to be older (~5 months) than the rest of the cohort. Again, our results did not change compared to our main analyses, further confirming that our findings were not merely the result of an age difference between groups.

The fact that we performed an adapted version of the neurological examination to maximize the cost-benefit ratio, leaves the possibility that we may have missed potentially relevant tests in one of the clusters, in particular, the *coordination & balance* cluster. Such tests, in addition to the performance in the *diadochokinesis test,* may have correlated with the objective postural control assessment. Moreover, given that the sample size of some non-optimal performed clusters and tests was too small for further analyses, we cannot interpret the association of these motor functions with postural control performance.

In addition to investigating an understudied population, another strength of the current study is that we were able to recruit athletes from various disciplines and three different study sites, resulting in a comparably large sample of this specific population. While this multi-site approach required differences in the NSS assessment setup (i.e., video vs. in-person evaluation), this decision allowed for relying on a comprehensive multi-rater approach to score the video examinations.

5. Conclusion

The current study demonstrated that the presence of NSS, and in particular non-optimal performance in the *diadochokinesis test*, is related to objective postural control performance in male adolescent athletes. Our findings reveal new knowledge by showing that a quantitative measurement of postural control may complement observer-based qualitative neurological examinations of NSS by capturing more detailed quantitative information. Future research should include larger samples of the general (healthy) population, with the inclusion of female participants, and participants of additional age ranges. This will ensure to capture a more complete picture of motor development, its variance, and its value to identify at-risk individuals with altered developmental trajectories.

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Author's contribution

CS, YT, SBS, DK, AL, SPS, RB, OP, FH, IKK and JG were involved in the study planning. EMB, AC, SMH, UT, CS, SBS, TLTW, DK, EK, FH, IKK, MVB and JG were involved in the data acquisition. EMB, AC, SMH, UT, EY, YT, SBR, FH, MVB, and JG were involved in data analysis and/or statistical analysis. EMB, AC, MVB, and JG drafted the manuscript and created figures and tables. EMB, AC, SMH, UT, CS, EY, YT, SBS, TLTW, DK, EK, SBR, MG, JSH, AL, SPS, RB, OP, FH, IKK, MVB, and JG critically edited the manuscript, and approved the final version of the manuscript.

Declaration of Competing Interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jns.2022.120516.

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Study I: Supplementary Material

Table A 1. Differences in Postural Control between the NSS+ and NSS- Group

Postural control	NSS+ *Mean* (*SD*)	NSS- *Mean* (*SD*)	*F*	*p*
Rigid				
PL	27.27 (10.95)	26.32 (8.34)	F (1, 138) = 0.078	p > .999
PL ML	15.20 (7.86)	12.98 (4.79)	F (1, 138) = 1.347	p > .999
PL AP	19.02 (6.69)	19.85 (6.87)	F (1, 138) = 0.299	p > .999
RMS	0.56 (0.23)	0.54 (0.18)	F (1, 138) = 0.052	p > .999
RMS ML	0.25 (0.12)	0.21 (0.08)	F (1, 138) = 3.074	p > .999
RMS AP	0.49 (0.21)	0.49 (0.17)	F (1, 138) = 0.034	p > .999
Sway area	0.23 (0.21)	0.19 (0.12)	F (1, 138) = 1.098	p > .999
Foam				
PL	65.05 (20.83)	61.54 (14.53)	F (1, 138) = 0.247	p > .999
PL ML	29.92 (8.71)	28.02 (8.08)	F (1, 138) = 0.317	p > .999
PL AP	51.37 (17.97)	48.85 (11.51)	F (1, 138) = 0.178	p > .999
RMS	1.30 (0.34)	1.26 (0.27)	F (1, 138) = 0.033	p > .999
RMS ML	0.62 (0.16)	0.62 (0.18)	F (1, 138) < 0.001	p > .999
RMS AP	1.13 (0.33)	1.08 (0.25)	F (1, 138) = 0.073	p > .999
Sway area	1.14 (0.61)	1.01 (0.45)	F (1, 138) = 0.489	p > .999

Note. All p-values were corrected for 14 variables and conditions at $p < .05$.

Abbreviations. AP = anterior-posterior, ML = medial-lateral, PL = path length, RMS = root mean square, SD = standard deviation

2.2 Study II: Neurological Soft Signs in Adolescents Are Associated With Brain Structure

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Original Article

Neurological soft signs in adolescents are associated with brain structure

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Neurological soft signs (NSS) are minor deviations in motor performance. During childhood and adolescence, NSS are examined for functional motor phenotyping to describe development, to screen for comorbidities, and to identify developmental vulnerabilities. Here, we investigate underlying brain structure alterations in association with NSS in physically trained adolescents. Male adolescent athletes ($n = 136$, 13–16 years) underwent a standardized neurological examination including 28 tests grouped into 6 functional clusters. Non-optimal performance in at least 1 cluster was rated as NSS (NSS+ group). Participants underwent T1- and diffusion-weighted magnetic resonance imaging. Cortical volume, thickness, and local gyrification were calculated using Freesurfer. Measures of white matter microstructure (Free-water (FW), FW-corrected fractional anisotropy (FAt), axial and radial diffusivity (ADt, RDt)) were calculated using tract-based spatial statistics. General linear models with age and handedness as covariates were applied to assess differences between NSS+ and NSS− group. We found higher gyrification in a large cluster spanning the left superior frontal and parietal areas, and widespread lower FAt and higher RDt compared with the NSS− group. This study shows that NSS in adolescents are associated with brain structure alterations. Underlying mechanisms may include alterations in synaptic pruning and axon myelination, which are hallmark processes of brain maturation.

Key words: brain development; gyrification; minor neurological dysfunction; motor development; neuroimaging.

Introduction

Neurological soft signs (NSS) are minor deviations from the norm in motor performance and sensory-motor integration (Dazzan and Murray 2002). NSS can be determined using developmental assessments via clinical neurological examination. Commonly used rating systems include the Neurological Examination Scale (NES; Buchanan and Heinrichs 1989), the Cambridge Neurological Inventory (CNI; Chen et al. 1995), the Heidelberg NSS Scale (HS; Schröder et al. 1991), and the age-dependent assessment of Minor Neurological Dysfunction (MND; Hadders-Algra 2010; Hadders-Algra et al. 2010; for review of NSS rating systems; see Chrobak et al. 2021). With minor differences, most rating systems

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comprise tests of coordination, fine motor skills, and postural control (Bombin et al. 2003). Typically, NSS present as a combination of signs such as associated movements and slowed motor sequencing (Dazzan and Murray 2002; Alamiri et al. 2018). Although major or also called hard neurological signs such as hyperreflexia and spasticity are rated as pathological, NSS are considered as subtle cerebral dysfunction without known focal morphological correlates. The clinical relevance of the presence of NSS is dependent on the child's age (Hadders-Algra 2002). With increasing age from childhood to adolescence, NSS have been shown to "outgrow" (Soorani-Lunsing et al. 1993; Hadders-Algra 2002; Martins et al. 2008). The presence of NSS in adolescents has been assumed as unspecific but sensitive marker of atypical neurodevelopment (D'Agati et al. 2018). Indeed, NSS are more commonly found in children and adolescents with history of premature birth (Breslau et al. 2000; Allin et al. 2006) and children and adolescents with neurodevelopmental and psychiatric disorders such as developmental coordination disorder (Sueda et al. 2022), autism spectrum disorder (Malviya et al. 2022), attention deficit hyperactivity disorder (Patankar et al. 2012), and psychosis (Mayoral et al. 2012) compared with normally developing children and adolescents (for review see D'Agati et al. 2018).

As of now, the underlying structure-function relationship of NSS, especially in adolescence, remains, largely unknown. Whereas most brain maturation processes such as proliferation, neurogenesis, and synaptogenesis peak between prenatal phases and 2 years of age (Stiles and Jernigan 2010; Gilmore et al. 2018), few processes are known to continue during adolescence and early adulthood and thus, play a central role when investigating adolescents with NSS. The 2 most common hallmark processes of adolescent brain maturation are synaptic pruning, a process in which unnecessary connections in the brain are eliminated, as well as the development of white matter myelination, which ensures a fast processing of information flow (White et al. 2010). Investigating brain structural characteristics associated with NSS in physically trained adolescents is a way to improve our understanding of sensorimotor maturation in the steps from adolescence to adulthood.

Magnetic resonance imaging (MRI) non-invasively provides information about brain structure such as global and regional volume, cortical thickness and cortical gyrification. In particular the quantification of a local gyrification index (LGI) has been developed for measuring brain developmental processes (Schaer et al. 2008). Local gyrification was shown to decrease during adolescence, which is commonly interpreted as a typical brain developmental process related to synaptic pruning (White et al. 2010). Importantly, local gyrification was shown to be increased in children and adolescents with neurodevelopmental disorders as consequence of atypical brain development (Wallace et al. 2013; Libero et al. 2019). Moreover, advanced neuroimaging techniques such as diffusion MRI (dMRI) allow for the characterization of brain microstructure. DMRI allows the estimation of the direction and magnitude of water molecule diffusion along white matter tracts (Alexander et al. 2007). Commonly derived measures are fractional anisotropy (FA), as well as axial and radial diffusivity (AD and RD), purported to reflect axonal integrity and myelination. Previous studies have reported altered white matter microstructure in major white matter tracts that play a crucial role in motor functioning in children with neurodevelopmental disorders compared with typically developing children and adolescents (Langevin et al. 2014; Brown-Lum et al. 2020) and in adults with schizophrenia and other psychotic disorders (Zhao et al. 2014; Viher et al. 2021).

To date, research applying neuroimaging to study NSS in children and adolescents is, sparse. Initial evidence is based on a cohort of 68 healthy adults (mean age ~24 years) that showed higher NSS scores to be associated with lower cortical thickness and lower gyrification in superior frontal, middle temporal, and postcentral regions (Hirjak et al. 2016). In the same sample, higher NSS scores were shown to be associated with altered RD in the corpus callosum (CC; Hirjak et al. 2017). Although previous research constitutes preliminary evidence of brain structure alterations in adults with NSS and in children with neurodevelopmental disorders, to date, there are currently no imaging studies in typically developing children and adolescents. Thus, in this study, we investigate a cohort of physically trained adolescents without history of neurodevelopmental disorders and without known risk for atypical neurodevelopment such as prematurity.

The aim of this study is to identify and characterize potential alterations in brain structure (gray and white matter) associated with NSS. We hypothesize that NSS can be identified in physically trained adolescents. We further hypothesize that adolescents with NSS show alterations in cortical thickness, cortical gyrification, and white matter microstructure compared with adolescents without NSS. The results of our study contribute to an improved understanding of NSS-related brain structure alterations.

Materials and methods

Participants

Data were drawn from the longitudinal multi-site study REPIMPACT (Repetitive Subconcussive Head Impacts—Brain Alterations and Clinical Consequences; 2017–2020). REPIMPACT recruited male youth athletes aged 13–16 years between July 2017 and April 2020 from 3 study sites (Oslo, Norway; Leuven, Belgium; Munich, Germany).

Details on the REPIMPACT study design have previously been published (Koerte et al. 2022). Study participants were participating in competitive sports ($n = 88$ soccer, $n = 15$ swimming, $n = 5$ cycling, $n = 5$ tennis, $n = 4$ biathlon, $n = 4$ track and fields, $n = 2$ cross-country skiing, $n = 2$ kayak, $n = 2$ orienteering, $n = 2$ rowing, $n = 2$ table tennis, $n = 1$ badminton, $n = 1$ gymnast, $n = 1$ judo, $n = 1$ roller-skating, and $n = 1$ triathlon) with at least 3 training sessions per week. For being included in the study, the participants had to be proficient in the language of the respective country (i.e. Norwegian, Dutch, and German). Participants and their legal guardians provided informed written consent in accordance with the local ethics boards and the Declaration of Helsinki.

Participants were excluded from the analysis in case of (i) history of serious medical condition (history of encephalitis: $n = 3$), (ii) incidental finding on MRI (periventricular gliosis: $n = 1$, subependymal heterotopia: $n = 1$), (iii) premature birth (i.e. < 37 weeks of gestation; $n = 3$), (iv) attention deficit disorder ($n = 1$), (v) neurological hard signs as evident by neurological examination ($n = 0$), (vi) MRI not performed ($n = 5$), or (vii) neurological examination not performed ($n = 17$). The total sample included 136 adolescents (Table 1). Every included participant underwent a neurological examination on one of the study time points ($n = 62$ from Norway at time point 1, $n = 30$ from Belgium at time point 3, $n = 40$ from Germany at time point 1, $n = 1$ at time point 2 and $n = 3$ at time point 3). For cross-sectional analyses, neuroimaging data acquired at the time point of the neurological examination were used.

Table 1. Cohort characteristics.

	NSS+ ($n = 25$)	NSS− ($n = 111$)	Statistical test
Study site (n)	N (17), B (2), G (6)	N (45), B (28), G (38)	$X^2 = 6.780$, $df = 2$, $\mathbf{P = 0.034}$ *
Handedness (R/L)	(96%/ 4%)	(95%/ 5%)	$X^2 = 0.083$, $df = 1$, $P = 0.774$
Age (Mean/SD)	14.67/ 0.68	15.12/ 0.75	t (139) = −2.789, $\mathbf{P = 0.006}$ *
Height (Mean/SD)	170.25/ 10.22	173.59/ 7.56	t (135) = −1.830, $P = 0.070$
Weight (Mean/SD)	57.72/ 10.27	60.44/ 8.60	t (134) = −1.372, $P = 0.173$

Note. * Indicates statistically significant difference between groups at $P = 0.05$. Abbreviations. B = Belgium; G = Germany; L = left; N = Norway; NSS = neurological soft signs; R = right; SD = standard deviation; X^2 = chi-square.

Neurological examination

A standardized pediatric neurological examination was performed based on "William DeMyer's Neurological Examination" (Biller et al. 2016) and the framework of the concept of "Minor Neurological Dysfunction" (MND; Hadders-Algra et al. 2010). We decided to follow the MND concept because it has proven useful when investigating developmental cohorts (De Jong et al. 2011; Kikkert et al. 2013; Galić et al. 2018). Of note, compared with other assessments such as the NES, CNI, HS, the MND concept considers the developmental status of a child and assesses performance with respect to age (Hadders-Algra 2002; Hadders-Algra et al. 2010).

Here, 28 tests of the MND framework were performed and grouped into 6 clusters: "Fine Motor Skills" (e.g. finger-opposition test), "Coordination & Balance" (e.g. diadochokinesis), "Posture & Tone" (e.g. posture while standing), "Involuntary Movements" (e.g. spontaneous motor activity during other tests), "Associated Movements" (e.g. associated movements during diadochokinesis), and "Sensory Function" (e.g. kinesthesia). Detailed information on the performed tests has been published elsewhere (Hadders-Algra 2010).

Each test performance was rated as "optimal" or "non-optimal" based on criteria defined in the neurological optimality score (De Jong et al. 2010; Hadders-Algra 2010). Each cluster was then rated as "optimal" or "non-optimal" based on predefined thresholds (De Jong et al. 2010; Hadders-Algra 2010). Study participants were categorized into a group with NSS (NSS+ group) if at least 1 of the 6 clusters was rated as non-optimal. Otherwise, participants were categorized into the group without NSS (NSS− group).

In Germany, the assessment was performed by experienced (pediatric) neurologists (FH, MVB, and EK). Examiners in Norway (SBS) and Belgium (JG, SD'H, and CS) were trained by the most experienced pediatric neurologist from Germany (FH) before performing the examinations independently. Examinations from Norway and Belgium were audio- and video-recorded and assessed by 3 independent raters from Germany with 0.5 (SMH), 15 (MVB), and 24 (UT) years of experience.

Neuroimaging

MRI data acquisition

Study participants underwent MR imaging at 1 of the 3 study sites. See Table 2 for a detailed overview of MRI data acquisition.

T1-weighted imaging

Preprocessing

Raw data were visually inspected for artifacts such as ghosting, motion artifacts, or signal drops using 3D Slicer (http://www.slicer.org; version 4.5, Surgical Planning Laboratory, Brigham and Women's Hospital, Boston, MA, United States) by trained personnel (EMB, TLTW, MG, and ADL), and excluded in case of insufficient quality (i.e. susceptibility artifacts caused by braces, severe motion, or cut-off images). This visual inspection for quality resulted in the exclusion of $n = 12$ cases. A total of 124 cases were included in the analysis of cortical thickness and volume ($n = 20$ with NSS; $n = 104$ without NSS). For the LGI analysis, an additional 2 cases were excluded due to segmentation failures resulting in 122 cases ($n = 20$ with NSS; $n = 102$ without NSS).

T1-weighted (T1w) images were automatically processed using the recon-all processing stream (https://surfer.nmr.mgh.harvard.edu/fswiki/recon-all) of Freesurfer version 7.1.0. For calculating the LGI, the "recon-all -localGI" processing stream, described in a step-by-step tutorial by Schaer and colleagues (Schaer et al. 2012), was performed. Subsequent steps included surface inflation, registration to a common spherical atlas, and cortical parcellation of the cortex with regard to sulcal and gyral patterns according to the Desikan–Killiany atlas (Dale et al. 1999). The parcellations were again visually inspected for quality and if necessary, edits to the white and pial surface were made by setting control points and by manually removing dura inclusions.

Processing and analysis

Cortical volume maps were obtained by determining the amount of gray matter volume between the white and the pial surface (Dale et al. 1999). Cortical thickness maps were obtained by calculating the closest distance between the white and the pial surface at each vertex of the cortical mantle (Fischl and Dale 2000). Gyrification maps were determined as the ratio of cortical surface area within the sulcal folds relative to the amount of cortex on the outer visible cortex for each point of the cortical surface (Schaer et al. 2012). Thereby, a higher gyrification index indicates a highly folded cortex and a lower gyrification index indicates a smoother cortex with less folding.

Surface-based smoothing with a full-width half-maximum default Gaussian kernel approximation of 10 mm, as recommended by Schaer and colleagues (Schaer et al. 2012), was applied to the cortical volume, thickness, and gyrification maps to improve the signal-to-noise ratio. See Fig. 1 for an illustration of investigated gray and white matter measures.

Diffusion-weighted imaging

Preprocessing

Quality control and exclusion of data with insufficient quality was performed as described above for the T1-weighted imaging data. This resulted in inclusion of data from 97 participants ($n = 17$ with NSS; $n = 80$ without NSS).

A brain mask was derived for each subject using FMRIB Software Library brain extraction tool (FSL BET; Smith 2002). Signal drift correction was performed using ExploreDTI v4.8.6 (Leemans et al. 2009). To mitigate echo-planar imaging (EPI) distortions, FSL TOPUP was applied on the mask, together with the non-weighted images of both phase encodings (Andersson et al. 2003). Subsequently, FSL EDDY 5.11 was used to correct for subject motion,

Table 2. Overview of MRI data acquisition.

	Norway	Belgium	Germany
MRI machine	3T Philips Ingenia	3T Philips Achieva dStream	3T Philips Ingenia
Head coil	32 Channels	32 Channels	32 Channels
T1-weighted			
Sequence	3D GE	3D GE	3D GE
Voxel size	$1 \times 1 \times 1$ mm^3	$1 \times 1 \times 1$ mm^3	$1 \times 1 \times 1$ mm^3
Diffusion-weighted			
Sequence	2D spin EPI	2D spin EPI	2D spin EPI
Voxel size	$2 \times 2 \times 2$ mm^3	$2 \times 2 \times 2$ mm^3	$2 \times 2 \times 2$ mm^3
Gradients	$20 \times b = 1{,}000$ s/mm^2; $30 \times b = 2{,}500$ s/mm^2 in addition to 7 non-weighted images; 4 non-weighted images with identical parameters but reversed phase encoding to correct for EPI-related geometrical distortions; Additional shells including <15 gradient directions required for data harmonization were omitted		
Multi-band	No TE = 113 ms, TR = 12 s, SENSE = 2	Yes multi-band factor 2, parallel acceleration SENSE 1.5, TE = 113 ms, TR = 7.2 s	Yes ($n = 15$) multi-band factor 2, parallel acceleration SENSE 1.5, TE = 113 ms, TR = 7.2 s No ($n = 29$) SENSE = 2 TE = 113 ms, TR = 12 s

Abbreviations. EPI = echo-planar imaging; GE = gradient echo; MRI = magnetic resonance imaging; SENSE = SENSitivity Encoding; TE = echo time; TR = repetition time.

eddy currents, and EPI distortions in a single step (Andersson and Sotiropoulos 2016).

Harmonization

The dMRI data were harmonized across the 3 data acquisition sites using a validated harmonization algorithm with rotational invariant spherical harmonics (RISH; De Luca et al. 2022). Harmonization approaches account for scanner-specific differences such as spatial variability of the diffusion signal in different brain areas, whereas at the same time maintaining the inter-subject variability at each study site and scanner. The used harmonization algorithm has recently been validated using this dataset (De Luca et al. 2022). In short, 20 scans were selected per study site for training harmonization and the study site Norway was selected as a reference. Individual sites were harmonized by computing RISH features. The RISH features were then applied to each individual dataset (De Luca et al. 2022).

Free-water imaging

Harmonized dMRI data were fitted to the free-water (FW) imaging diffusion model (Pasternak et al. 2009), which attempts to separate diffusion into tissue-specific and FW diffusion components. The volume fraction of the FW compartment provides a FW map. The tissue-specific compartment was modeled with a diffusion tensor, and diffusivity maps that are corrected for FW partial volume were derived from its eigenvalues, including tissue-specific fractional anisotropy (FAt), axial diffusivity (ADt; the principal eigenvalue) and radial diffusivity (RDt; the average of the 2 remaining eigenvalues). In addition, non-corrected FA maps were calculated by fitting a single diffusion tensor, for the purpose of the tract-based spatial statistics (TBSS) analysis.

Processing and analysis

Voxel-wise statistical analysis was carried out using the TBSS pipeline (Smith et al. 2006; Billah 2019). In this process, we generated a study-specific template to account for the young age of the subjects, which may not match the neuroanatomy of adult samples included in the Montreal Neurological Institute (MNI) standard template (Yoon et al. 2009). The study-specific template was created using an iterative procedure using advanced normalization tools (ANTS; Avants et al. 2022). Then, all individual FA maps were registered to this template and a FA skeleton map was created using the *tbss_skeleton* function, from FSL (Smith et al. 2006), with a threshold of 0.2. Each participant's aligned FA image was then projected onto the skeleton. FAt, ADt, RDt, and FW were projected onto the FA skeleton using the same projection as FA. The resulting maps were used for calculating voxel-wise statistics on the skeleton ($P < 0.05$). See Fig. 1 for an illustration of investigated gray and white matter measures.

Statistical analysis

Descriptive statistics of MRI and demographical data, as well as demographical differences between the NSS+ and NSS− group, were calculated using the software R (R version 4.0.1; R Core Team 2021). Chi-square tests were applied to assess between-group differences in study site and handedness. Independent *t*-tests were used to assess between-group differences in age, height, and weight.

For all gray matter morphology analyses, whole-brain voxel-wise analyses were performed using Freesurfer's general linear modeling tool *mri_glmfit*. Statistical surface maps were created using a vertex-wise statistical threshold of $P < 0.05$. Correction for multiple comparisons was performed using Monte Carlo cluster-wise simulation repeated 10,000 times set at $P < 0.05$. To test for differences between the NSS+ and NSS− group in cortical volume, cortical thickness, and gyrification, general linear models using age and handedness as additional covariates were used.

For the TBSS analysis, voxel-wise permutation tests for each voxel on the white matter skeleton were performed using *Randomise* in FSL with 10,000 permutations and a Threshold-Free-Cluster Enhancement with 2D optimization (Winkler et al. 2014). To assess voxel-wise differences between the NSS+ and NSS− group in white matter microstructure (FAt, ADt, RDt, and FW), general linear models using age and handedness as additional covariates were used and corrected for family-wise error at a significance level of $\alpha < 0.05$. The anatomical location of resulting significant white matter clusters was identified and labeled by mapping the corrected statistical map on the Johns Hopkins University white matter (JHU-WM) tractography atlas and the JHU-ICBM-DTI 81 WM labels atlas in the MNI space (Mori et al. 2008)

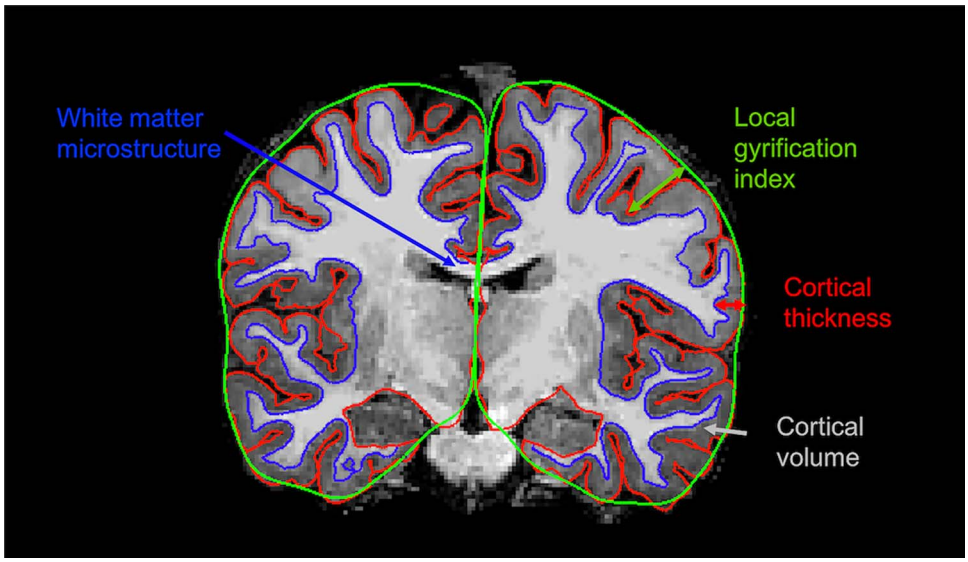

Fig. 1. Overview of investigated cortical gray and white matter measures. Cortical volume, derived by using T1-weighted imaging, is determined as the amount of gray matter volume between the white and the pial surface. Cortical thickness, derived by using T1-weighted imaging, is determined as the closest distance between the white and pial surface at each vertex of the cortical mantle. The local gyrification index, derived by using T1-weighted imaging, is determined as the ratio of cortical surface area within the sulcal folds relative to the amount of cortex on the outer visible cortex for each point of the cortical surface. White matter microstructure, derived by diffusion-weighted imaging, is assessed by calculating free-water (FW)-corrected fractional anisotropy (FAt), axial diffusivity (ADt; the principal eigenvalue), and radial diffusivity (RDt; the average of the 2 remaining eigenvalues), which are parameters representing the magnitude (diffusivity) and direction (anisotropy) of water molecule diffusion.

as previously described by Brown-Lum and colleagues (Brown-Lum et al. 2020).

Results

Cohort characteristics

Based on the neurological examination, 25 (18.38%) participants were categorized as NSS+ and 111 (81.62%) participants as NSS−. Of the 136 participants, 111 (81.62%) participants performed optimal in all 6 clusters, 23 (16.91%) performed non-optimal in 1 cluster, 1 (0.74%) performed non-optimal in 2 clusters, and 1 (0.74%) in 4 clusters. The cluster that most often was performed non-optimal was "fine motor skills," performed non-optimal by 23 participants (16.91%).

There was a statistically significant difference between the NSS+ and NSS− group regarding study site. More specifically, there was a significantly greater proportion of participants in the NSS+ group with 17/62 (27.42%) in Norway compared with 2/30 (6.67%) in Belgium and 6/44 (13.64%) in Germany ($P = .034$). Across study sites, participants in the NSS+ group were on average 6 months younger (NSS+: Mean = 14.67; SD = 0.68) than those in the NSS− group (NSS−: Mean = 15.12; SD = 0.75) ($P = 0.006$). The NSS+ and NSS− group did not differ regarding handedness, height, or weight (Table 1).

Cortical volume and cortical thickness

Neither cortical thickness nor cortical volume differed significantly between the NSS+ and NSS− group.

Local gyrification

Participants in the NSS+ group had significantly higher local gyrification compared with those in the NSS− group in the left hemisphere spanning the superior frontal lobe including the supplementary motor area, and the superior parietal lobe (NSS+: Mean = 3.17, SD = 0.24; NSS−: Mean = 3.12, SD = 0.10; $P = 0.002$; Fig. 2).

White matter microstructure

Participants in the NSS+ group had significantly lower FAt compared with those in the NSS− group in widespread white matter clusters (all $P < 0.05$; FAt values averaged across all significant voxels: NSS+: Mean = 0.59, SD = 0.01; NSS−: Mean: = 0.62, SD = 0.01), particularly spanning the CC, the CST (corticospinal tract), the posterior thalamic radiation (PTR), the superior longitudinal fasciculus (SLF), the corona radiata (CR), and the internal capsule (IC; Fig. 3). Moreover, participants in the NSS+ group had significantly higher RDt compared with those in the NSS− group in widespread white matter clusters (all $P < 0.05$; RDt values averaged across all significant voxels: NSS+: Mean = 0.37, SD = 0.01; NSS−: Mean: = 0.36, SD = 0.01), particularly spanning the CC, CST, PTR, SLF, CR, and IC. ADt and FW did not differ significantly between groups (Fig. 3).

Discussion

This study revealed alterations in gray and white matter in physically trained adolescents with the clinical phenotype "with NSS" compared with adolescents with the phenotype "without NSS". More specifically, we found significantly higher gyrification in the left superior frontal and superior parietal lobe as well as lower FAt and higher RDt in widespread clusters spanning the CC, CST, PTR, SLF, CR, and IC associated with NSS. The groups did not differ in either cortical volume or cortical thickness. Findings from this study suggest that NSS, in typically development adolescents, are associated with distinct alterations in brain structure that can be objectively quantified using neuroimaging.

Cohort characteristics

When comparing between-group differences in demographical variables, the NSS+ group turned out to be slightly younger (6 months) than the NSS− group. Previous studies report a decreasing prevalence of NSS during adolescence between the age of 12

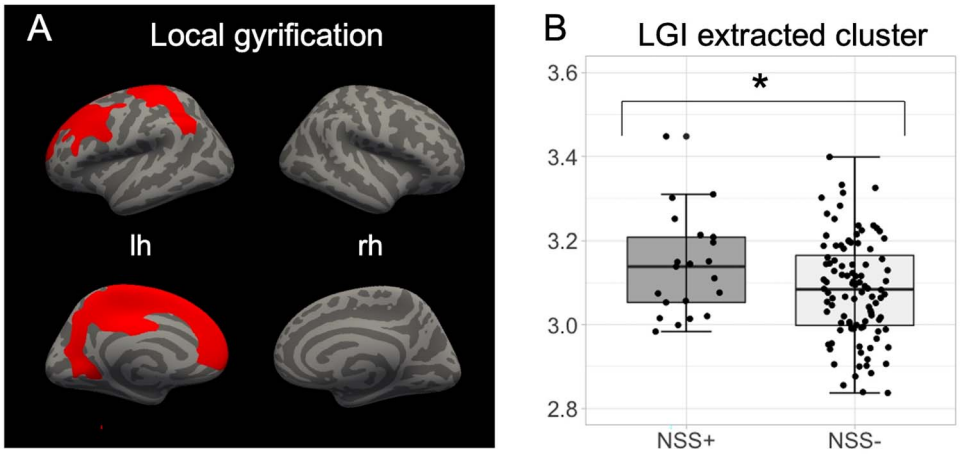

Fig. 2. A) Higher local gyrification in the left hemisphere spanning superior frontal and superior parietal lobes in the NSS+ group (red cluster) using general linear models corrected for age and handedness after cluster-wise correction for multiple comparisons. B) Boxplots showing higher gyrification in the extracted significant cluster in the NSS+ group. Note. * Indicates statistical significance after cluster-wise correction for multiple comparisons at $\alpha < 0.05$. Abbreviations. lgi = local gyrification index; lh = left hemisphere; NSS = neurological soft signs; rh = right hemisphere.

and 14 years (Soorani-Lunsing et al. 1993; Hadders-Algra 2002). The maturation of motor function has been shown to occur predominantly between childhood and adolescence with only smaller changes beyond the age of 14 years (Fietzek et al. 2000; Koerte et al. 2010). However, one study investigating longitudinal changes of NSS beyond the age of 14, demonstrated that the prevalence of NSS further decreases between the age of 13 and 17 (Martins et al. 2008). Thus, we cannot conclude with certainty to what extent our cohort was still undergoing neurodevelopmental alterations that may be related to the presence of NSS.

Evidence from previous studies unrelated to NSS, demonstrates an effect of age on dMRI measures (Nagy et al. 2004; Tamnes et al. 2010). To estimate the effect of age in this sample and to inform the interpretation of the difference in diffusion measures between the NSS+ and NSS− group, we performed an additional analysis (Supplementary Material; Supplementary Fig. 1). Results of this analysis revealed that the mean difference between the NSS+ and NSS− group in FAt and RDt values was approximately 10 times greater than the change in FAt and RDt estimated for adolescents between 14.67 (NSS+ Mean age) and 15.12 years (NSS− Mean age). Thus, although age has an effect on diffusion measures, this effect does not fully explain the difference between the study groups.

Moreover, we found a significant between-group difference in study site with a higher percentage of NSS+ participants from Norway compared with Belgium or Germany. This is surprising given previous reports that found NSS prevalence to be remarkably similar across countries and ethnicities (Bachmann and Schröder 2018). Of note, it is unlikely that the difference is due to an effect of the assessment of NSS since neurological assessments from Belgium and Norway were video-taped and later independently rated by raters from Germany. Future studies investigating large cohorts across countries are needed to better understand differences in the prevalence of NSS between regions and ethnicities.

Local gyrification

We found higher gyrification in adolescents with NSS compared with those without NSS in a large cluster spanning the left superior frontal lobe and the left superior parietal lobe. This finding substantially improves our existing knowledge on NSS by providing evidence of structural alterations in cortical folding potentially underlying NSS.

Although the quantification of local gyrification has been increasingly applied to the investigation of neurodevelopmental disorders (Schaer et al. 2012), to date, only one study has investigated local gyrification in association with NSS in young adults (Hirjak et al. 2016). This study reported an association between higher NSS scores (worse) with lower cortical gyrification (Hirjak et al. 2016). Of note, our finding of higher gyrification associated with NSS in adolescents, contrasts with this previous report in adults. Interestingly, however, higher gyrification has previously been found in adolescents with developmental disorders such as autism spectrum disorder, or schizophrenia (for review see Sasabayashi et al. 2021). Moreover, although studies assessing healthy adults found higher gyrification to be associated with better cognitive functioning (Gautam et al. 2015), this association has not been found in adolescents with developmental disorders, potentially suggesting altered brain maturation processes (Wallace et al. 2013). Similar to our finding, studies on adolescents with developmental disorders report higher gyrification located particularly in the frontal and parietal lobes (Sasabayashi et al. 2021). Of further note, those areas play a central role in higher-order sensorimotor control (Luppino and Rizzolatti 2000). Thus, our finding of higher gyrification in these cortical areas may be functionally linked to the subtle alterations in fine motor skills detected in our cohort.

Gyrification takes place when a continuously increasing cortical surface meets restricted space, as is the case for the developing brain inside the skull (Rakic 2009). However, the neural mechanisms underlying the increase in gyrification during early childhood followed by a decrease in gyrification during adolescence, are not fully understood. A widely accepted theory suggests a link between gyrification and brain connectivity (Van Essen 1997). This theory postulates that regions with greater neural connectivity are tied together with axonal tension allowing them to remain in proximity during brain growth. This early maturation process allows a faster information transfer between more densely connected brain regions and results in the formation of gyri (White et al. 2010). During adolescence, the developing brain undergoes targeted elimination processes of these neural connections, also referred to as synaptic pruning, which in turn change the morphology of gyri and sulci (White et al. 2010). Thus, measuring cortical gyrification

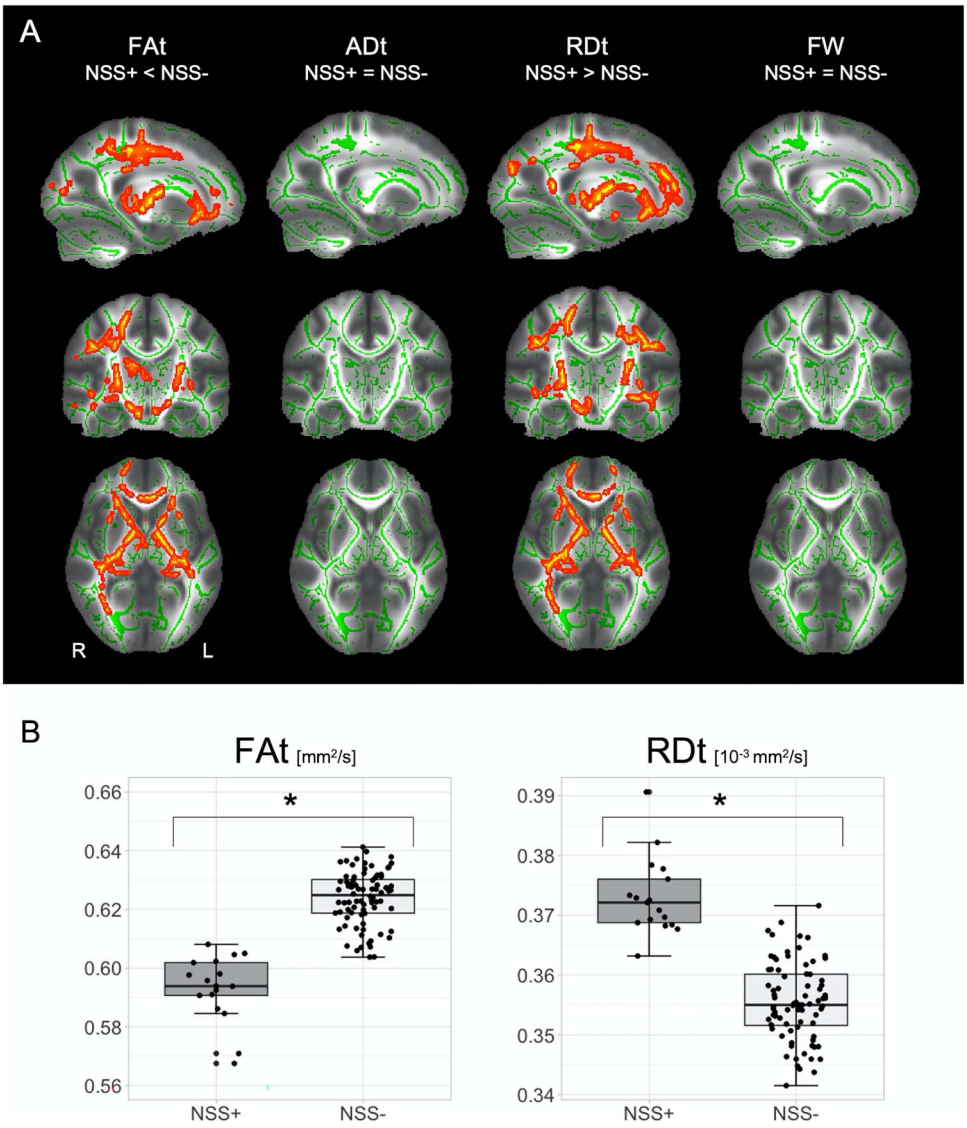

Fig. 3. A) Lower FAt and higher RDt in the NSS+ group (red–yellow clusters). No statistically significant differences in ADt, and FW between groups using general linear models corrected for age and handedness after family-wise error correction. B) Boxplots showing individual FAt and RDt averaged across significant voxels. Note. * Indicates statistical significance after family-wise error correction at $\alpha < 0.05$. Abbreviations. ADt = free-water-corrected axial diffusivity; FAt = free-water-corrected fractional anisotropy; FW = free-water; L = left; NSS = neurological soft signs; RDt = free-water-corrected radial diffusivity; R = right.

during adolescence may provide insight into the process of elimination of axonal connections taking place during synaptic pruning.

Taken together, higher gyrification in adolescents associated with the presence of NSS suggests potential alterations in synaptic pruning processes. Whether higher gyrification in adolescents with NSS is linked to alterations in synaptic pruning processes occurring during adolescence, or whether alterations in the trajectory of brain maturation may have their origin in early brain developmental phases (i.e. prenatal or perinatal), remains to be elucidated.

White matter microstructure

We found significantly lower FAt and higher RDt in widespread clusters comprising the CC, CST, PTR, SLF, CR, and IC in adolescents in the NSS+ group compared with adolescents in the NSS− group. ADt and FW did not differ between groups.

This is the first study to use FW-corrected dMRI to investigate NSS-related brain alterations. The estimation of FAt is considered more specific than FA because it separates diffusion in each voxel into a tissue compartment (FAt) and an extracellular compartment (FW). This is of importance to disentangle extracellular processes from tissue-related processes when investigating the underlying neural mechanisms of brain disorders (Pasternak et al. 2012). Our finding of group differences in FAt in the absence of differences in FW suggests that diffusion alterations reflect differences in the tissue, but not in the extracellular space which would suggest e.g. neuroinflammation (Pasternak et al. 2009).

In addition to lower FAt, we also detected higher RDt in largely overlapping clusters in the brain. This result of higher RDt is in line with a previous study that demonstrated voxel-wise correlations between NSS scores and radial diffusivity (RD) in the CC in adults with NSS (Hirjak et al. 2017). Although FAt is highly sensitive for detecting microstructural alterations in the tissue, it is not specific to the type of changes. For instance, FAt can be

reduced because of reduced ADt reflecting changes to parallel diffusivity, such as alterations in axonal shapes, or because of higher RDt reflecting changes to perpendicular diffusivity, such as myelination alterations, or a combination of the 2 (Winklewski et al. 2018). Thus, the higher RDt is more aligned with alterations in myelination, which may occur as part of adolescent brain development. In addition to regressive processes like synaptic pruning, the adolescent brain also undergoes growth processes such as myelination which ensures high speed and efficiency of information flow between brain regions. Alterations or delays in myelination during white matter maturation may lead to impaired sensory-motor function. Consequently, alterations in myelination processes during adolescence may play an important role in the context of NSS. Of note, the tracts covered by the identified white matter clusters, in particular CST and CC, play a central role in motor functioning and alterations in these tracts have previously been reported in developmental disorders such as developmental coordination disorder and attention deficit hyperactivity disorder (Langevin et al. 2014; Brown-Lum et al. 2020) and in adults with schizophrenia (Viher et al. 2021).

Taken together, we report white matter microstructure alterations in a group of adolescents with NSS, including lower FAt and higher RDt in major white matter tracts that play an important role in motor functioning. Lower FAt and higher RDt may potentially reflect alterations in axonal myelination which is a key process of brain maturation.

Cortical volume and cortical thickness

Neither cortical volume nor cortical thickness differed between the NSS+ and NSS− group. This result is partly in alignment with the results of 2 previous studies in adults with NSS. More specifically, Hirjak and colleagues (Hirjak et al. 2016) reported no alterations in cortical volume in association with NSS, whereas an earlier study (Dazzan et al. 2006) reported reduced volume in several clusters of the brain. Of note, the latter study is based on data acquired at 1.5T instead of 3T MRI and used a threefold larger voxel size which limits comparability to our study.

Compared with the study by Hirjak and colleagues that reported lower cortical thickness in the superior temporal, middle frontal, and superior frontal regions in association with NSS (Hirjak et al. 2016), we did not detect significant alterations in cortical thickness. Given that until the early twenties, cortical thickness decreases with increasing age (Tamnes et al. 2017), it may be the case that cortical thickness in our cohort still decreases, whereas the cohort by Hirjak and colleagues was already fully matured. Future longitudinal studies investigating NSS-related developmental trajectories are needed to confirm this hypothesis.

Limitations and future directions

There are limitations to this study that need to be considered. First, the results are based on cross-sectional data. Longitudinal analyses across larger age ranges are needed to elucidate the origins and trajectory of NSS-related structural brain alterations. Second, the investigated sample included male adolescent athletes only, which limits generalizability of findings from this study to the general population. More specifically, the investigated individuals participated in competitive sports which may constitute a selection bias with regard to motor coordination, meaning that individuals who choose to participate in competitive sports may be more likely to demonstrate above average motor coordination. Moreover, previous studies have reported training-related effects on white matter microstructure in children and adolescents (Chaddock-Heyman et al. 2018; Ruotsalainen et al. 2020). Given that we only investigated male adolescents, no conclusion can be drawn regarding female adolescents. The homogenous sample composition, however, allowed us to identify and characterize NSS in healthy and physically active adolescents without neurological, psychiatric, or developmental disorders. Our findings demonstrate that NSS may be of relevance beyond the commonly investigated at-risk populations. Third, 39 dMRI scans had to be excluded due to insufficient data quality which leads to lower statistical power. Of note, MRI motion artifacts are common when investigating pediatric cohorts. Thus, to ensure high data quality, rigorous quality assessment is essential.

Conclusion

This study revealed higher gyrification in left superior frontal and parietal areas and widespread alterations in white matter microstructure in adolescents with NSS compared with those without NSS. This finding suggests a structure-function relationship between NSS phenotype and brain microstructure. Potential underlying mechanisms include alterations in synaptic pruning and axon myelination, which are known as hallmark rewiring processes of brain maturation. Longitudinal neuroimaging studies investigating NSS across childhood, adolescence, and young adults are needed to elucidate brain maturation trajectories related to NSS phenotyping.

Results from this study contribute to an improved understanding of NSS-related brain alterations. This insight may pave the way for an objective and quantitative life-span assessment of NSS, its related brain structure and its association with comorbidities that are of developmental and functional relevance.

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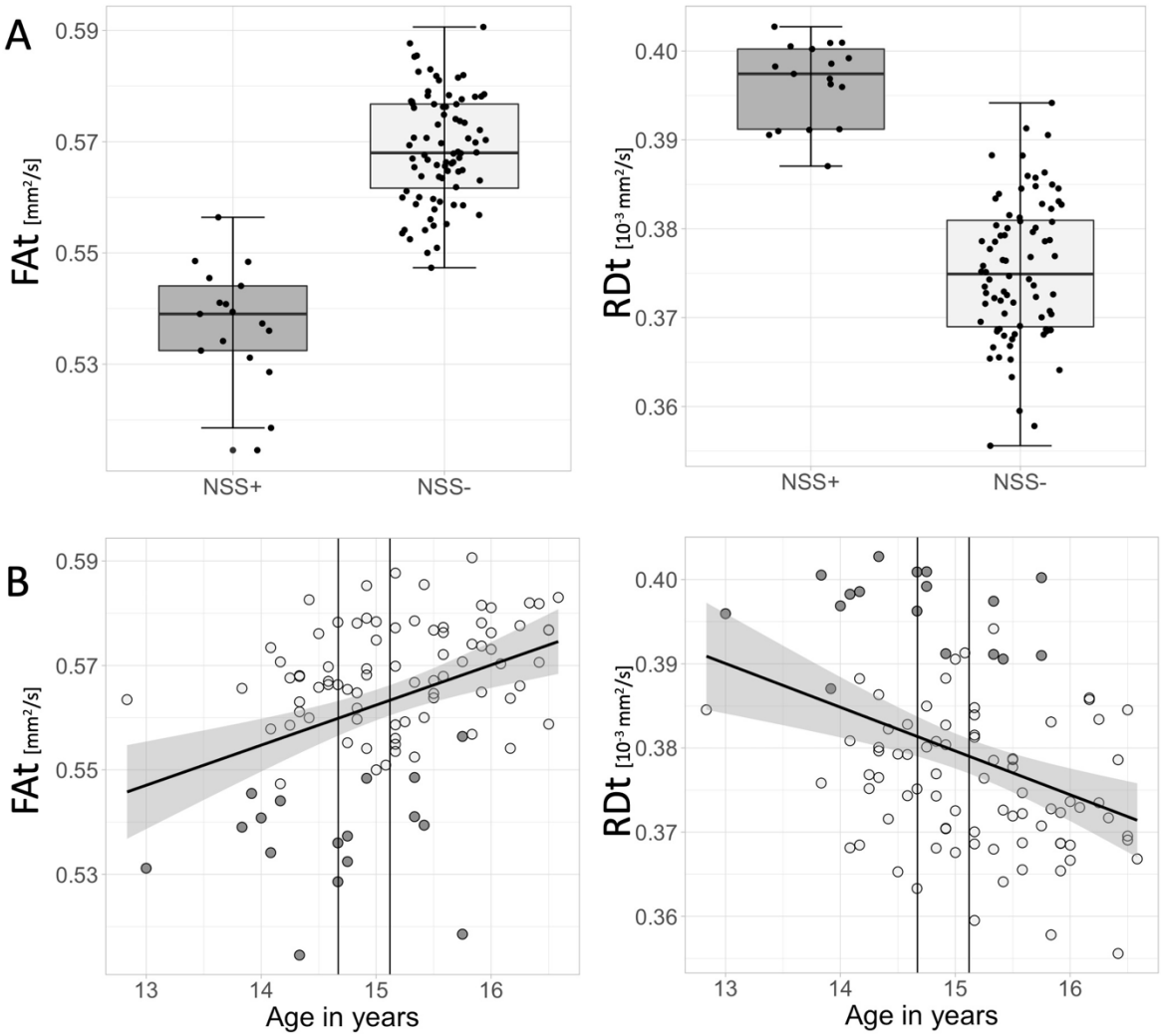
Study II: Supplementary Material: Effect of age on diffusion measures

Evidence demonstrates an effect of age on diffusion measures (Nagy et al. 2004; Tamnes et al. 2010). There is a difference in mean age between the NSS+ and NSS- group (NSS+: *Mean* = 14.67 years; NSS-: *Mean* = 15.12 years). To assess the effect of age on the difference in diffusion measures between the groups, we performed an additional analysis.

First, we extracted the individual FAt and RDt values averaged across significant voxels (without covariate correction) and calculated mean values for the NSS+ and NSS- group, respectively (NSS+: FAt: 0.537 [mm^2/s], RDt: 0.396 [10^{-3} mm^2/s] and NSS-: FAt: 0.569 [mm^2/s], RDt: 0.375 [10^{-3} mm^2/s]; **Supplementary Figure A**). This resulted in a mean difference between the NSS+ and NSS- group in FAt of |0.537-0.569| = 0.032 [mm^2/s] and in RDt of |0.396-0.375| = 0.021 [10^{-3} mm^2/s]. Second, we plotted individual diffusion measures against age (**Supplementary Figure B**). In line with the literature, FAt is positively correlated with age while RDt is negatively correlated with age. More specifically, results from the additional analysis demonstrate that between 14.67 and 15.12 years of age, the average change in FAt is +0.003 [mm^2/s] and the average change in RDt is -0.002 [10^{-3} mm^2/s]. Importantly, these results show that the group difference between the NSS+ and NSS- group in FAt and RDt is about 10 times greater than the average change in FAt/RDt over 6 months.

Taken together, results from this additional analysis further support the conclusion that while age has an effect on diffusion measures, it only explains a small fraction of the difference between the NSS+ and NSS- group.



Supplementary Figure 1. (A) Boxplots illustrating the mean group difference in individual FAt and RDt values averaged across significant voxels without covariate correction for the NSS+ and NSS- group. (B) Scatterplots illustrating the association between individual FAt and RDt values and age in years. Vertical black lines indicate the age range of interest that separates the two study groups (14.67 – 15.12 years).

Note. Individuals of NSS+ group are depicted in dark gray while individuals in the NSS- group are depicted in light gray.

Abbreviations. FAt = free-water-corrected fractional anisotropy; NSS = neurological soft signs; RDt = free-water-corrected radial diffusivity.

3 GENERAL DISCUSSION

Below, I discuss the findings of Study I and II (see **Figure 5**), their key implications, limitations, strengths, and future directions.

3.1 Main Findings

3.1.1 Study I

Study I revealed no difference in postural control performance between NSS+ and NSS- group. However, participants performing non-optimal in the diadochokinesis test showed significantly reduced postural control. This effect was observed only in the rigid condition and the ML direction. Moreover, the total NSS score correlated significantly with postural control performance, only in the rigid condition and the ML direction. Findings from this study reveal that adolescents with NSS perform worse in ML postural control assessed by force plate measures. As postural control along the ML direction continues to mature up until adolescence, lower performance in ML postural control may be indicative of delayed postural control development. Moreover, the results reveal new knowledge by demonstrating that instrumented postural control measures may complement observer-based neurological examinations.

3.1.2 Study II

Study II revealed higher gyrification in the left superior frontal and superior parietal areas in the group of adolescents with NSS, likely reflecting alterations in synaptic pruning. Cortical volumes and thickness did not differ between groups. We found lower FAt and higher RDt in widespread white matter clusters spanning the CC, corona radiata (CR), CST, IC, TR, SLF in the NSS+ group, likely reflecting alterations in myelination. Findings from this study suggest that NSS in otherwise healthy adolescents are associated with underlying brain structure alterations that can be objectively quantified using MRI. Insights derived from this work show the benefit of using MRI for objective and quantitative assessments of NSS-related brain structure alterations that may be of clinical and developmental relevance. Future studies should assess whether NSS-related brain structure alterations may be a structural correlate of the described association between NSS and neuropsychiatric disorders.

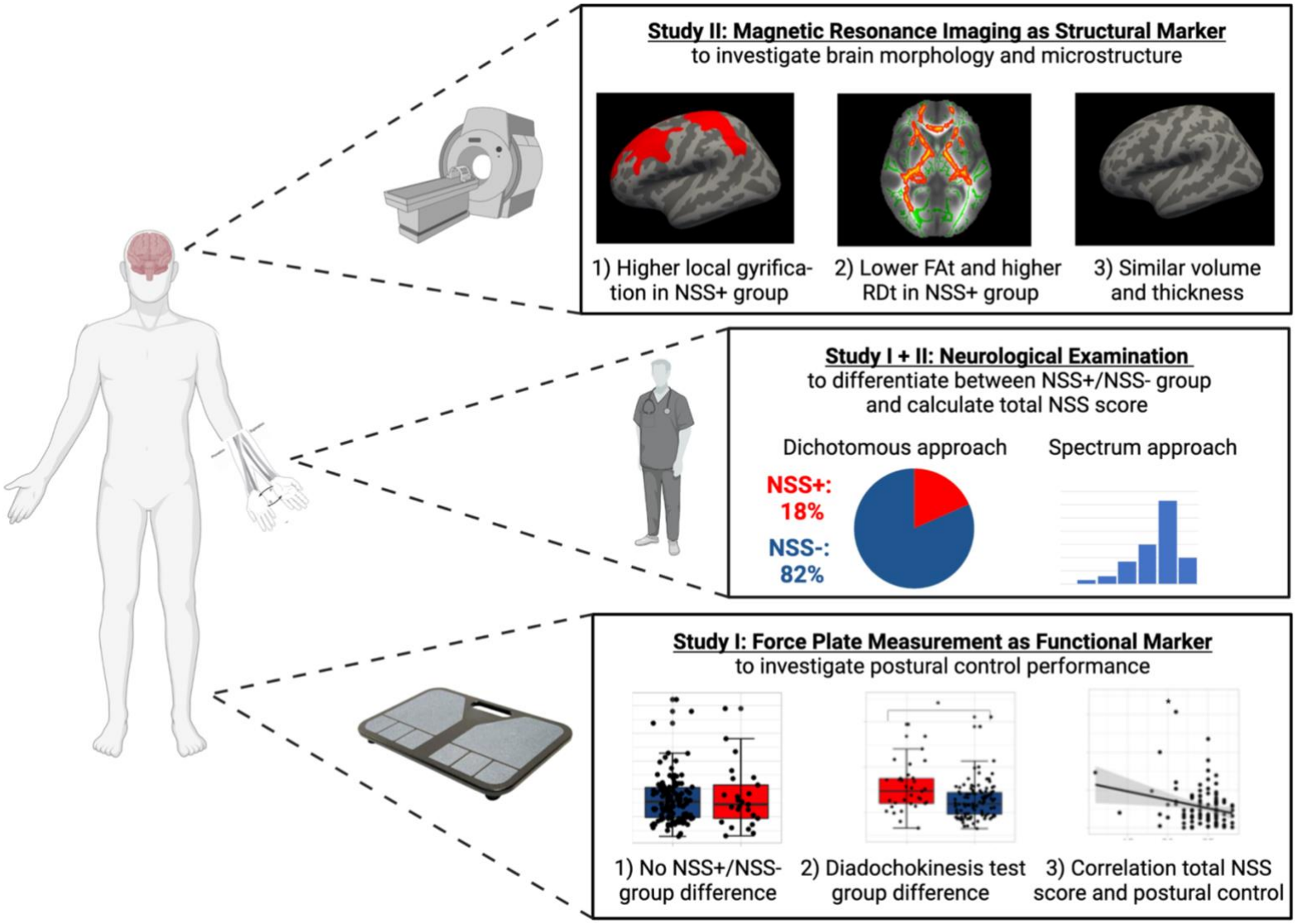


Figure 5. Graphical abstract of work included in Study I and II (created with Biorender.com).

3.2 Key Implications across Studies

The work included in this dissertation aims to identify and characterize alterations in postural control performance as well as alterations in brain structure associated with NSS in healthy adolescent athletes. The results derived from the two studies show that adolescents with NSS tend to perform worse in ML postural control (Study I) and have higher regional gyrification and altered white matter microstructure (Study II) which may point towards alterations in hallmark processes of adolescent brain maturation. Insights derived from this work help to better understand the prevalence and relevance, as well as related functional and structural correlates of NSS in healthy adolescents. Moreover, it helps to generate a more complete picture of NSS-related developmental alterations.

3.2.1 Presence of Neurological Soft Signs in Healthy Adolescent Athletes

Until now, the prevalence of NSS in healthy adolescent athletes was largely unknown. We found approximately 18% of our investigated cohort to have NSS. With this finding, we were the first to show that NSS are also present in healthy, physically fit adolescents that are expected to have good neurological functioning. The detected prevalence is higher as assumed, based on what is known from existing literature reporting a prevalence of approximately 5% in adults (Heinrichs & Buchanan, 1988). However, of note, most participants with NSS in our cohort had non-optimal performance only in one functional domain. Whereas the sole presence of non-optimal fine motor skills using our classification system was already considered as having NSS, other classification systems may have not yet considered the presence of non-optimal performance in one functional domain as NSS. This finding shows that the investigation of NSS may be important not only within commonly investigated populations at risk for psychiatric disorders, but also among healthy physically fit adolescents.

3.2.2 Relevance of Neurological Soft Signs as Sensitive but Unspecific Marker

Even though the percentage of 18% with NSS in our cohort seems relatively high, it is important to note that the presence of NSS is considered as an unspecific condition. Thus, it does not necessarily mean that all adolescent athletes with NSS in our cohort will e.g., develop a psychiatric disorder. Instead, NSS should be considered as screening marker that helps to

detect individuals with potentially altered neurodevelopmental trajectories that may eventually put them at an increased risk to develop psychiatric disorders later in life. Beyond the limited specificity, yet another open question is how relevant and how interfering with daily life the presence of NSS for affected individuals may be. Interestingly, in alignment with previous studies (Alamiri et al., 2018; Kikkert et al., 2013), in unpublished work investigating the same cohort, we also found adolescents with NSS to perform significantly worse in working memory performance. However, whether only the presence of NSS significantly affects their daily life is unclear, but unlikely. In our cohort, we predominantly found non-optimal performance in fine motor skills, which may not be crucial for athletes like soccer players that perform well in gross motor skills but are not interested in optimizing their fine motor skills. However, from our work it remains unknown whether the results would have been different for other athletes like racket sport athletes or biathletes that need good fine motor skills. Thus, based on our work, it remains unclear what relevance the detected subtle alterations in fine motor skills in our investigated adolescent athletes may have for the individual. Future work should investigate NSS in combination with multimodal outcome measures.

3.2.3 Dichotomization Versus Spectrum Approach to Investigate Neurological Soft Signs

A crucial aspect when investigating NSS is the used test battery and classification scheme (Bombin et al., 2003; Chrobak et al., 2021). Dichotomous and spectrum approaches each go along with advantages and disadvantages and require different interpretation. While the categorization approach allows for a diagnosis-like interpretation, it neglects the fact that NSS may not be black and white. Instead, the spectrum approach allows to investigate gradings of NSS but does not provide a cut-off value that helps to interpret the degree of severity.

Findings of Study I revealed no between-group differences (NSS+/NSS-) in postural control performance, but a significant correlation with the total NSS score. In contrast, Study II did reveal between-group differences (NSS+/NSS-) in brain structure. Thus, we speculate that the group differences in postural control between NSS+ and NSS- groups may have been so subtle that they were not statistically significant when using a dichotomization approach but only when using a spectrum approach. Furthermore, we hypothesize that the brain structure alterations that we detected as part of Study II were more pronounced than the results of Study I and thus, were also significant when using a dichotomous approach. Future studies should

elaborate on the differences between dichotomous and spectrum approaches and consider this in the interpretation of their results.

3.2.4 Postural Control as Functional Marker of Neurological Soft Signs

In Study I of this dissertation, we used a Balance Tracking System (BTrackS) with which we were able to objectively quantify alterations in postural control performance. The use of the device is clinically validated and normative data for healthy athletes between 8 and 21 years is available (Goble et al., 2019). Our detected performance of athletes with an average path length on a rigid surface of 26 is in alignment with normative values for 14–15-year-old male athletes (Goble et al., 2019).

Our first finding revealed no between-group differences (NSS+/NSS-) in path length or any other variable measuring postural control performance. Of note, most of the included participants performed non-optimal in the fine motor skills domain, but not the posture & tone domain. It might be assumed that non-optimal performance in the posture & tone domain as part of the neurological examination may be more closely related to instrumented postural control measures than the performance in fine motor skills. Thus, the complementation of instrumented force plate measures may be mostly helpful when expecting non-optimal performance in gross motor skills such as postural control.

Our second finding revealed that participants with non-optimal performance in the diadochokinesis test performed significantly worse in, i.e., path length compared to participants with optimal performance in this test. Based on this, we can conclude that also the performance of other tests, such as the diadochokinesis test, is functionally linked with postural control performance. Interestingly, the postural control performance of the group with non-optimal performance in the diadochokinesis test was way below average (Mean path length = 30 cm). Based on published normative data on male adolescent athletes, a path length of 30 would be comparable to the average postural control performance of an 11–12-year-old and not of a 14–15-year-old male athlete (Goble et al., 2019). The difference between groups was stronger in the ML direction of postural control. Previous studies have found postural control along the ML axis maturing later than postural control along the AP axis (Blanchet et al., 2019; Kirshenbaum et al., 2001). Thus, our detected group differences along the ML axis likely reflect a delay in later occurring developmental processes. Of note, participants with non-optimal performance in all other tests, including e.g., the finger-opposition test did not perform worse in the instrumented force plate assessments compared to participants performing optimal in these tests. Thus, a functional relation between pronation/supination movements of forearms as

needed for the diadochokinesis test and postural control may be assumed. One potential explanation for this functional relation may be the involvement of cerebellar structures for both maintaining postural control and for pronation/supination movements (Bodranghien et al., 2016).

Beyond investigating between-group differences, our third finding revealed significant correlations between the total NSS score and variables indicating ML sway. Interestingly, we detected significant correlations, but did not find significant between-group differences between NSS+ and NSS- groups. Again, a potential reason for this may have been the different approach (dichotomous versus spectrum) to assess NSS as well as the less pronounced significance of the results. Future studies should take the necessity to differentiate between NSS investigation approaches into consideration.

The results of Study I emphasize the complementary benefit of using instrumented tools such as force plates in addition to observer-based tools. Moreover, it demonstrates the importance of different classification schemes such as dichotomous versus spectrum approaches.

3.2.5 Magnetic Resonance Imaging as Structural Marker of Neurological Soft Signs

In study II, we used MRI to objectively quantify alterations in gray matter and white matter microstructure in adolescents with NSS. MRI has found increasing use over the past several years (Smith-Bindman et al., 2008). In the context of investigating brain structure alterations related to NSS, however, especially in healthy cohorts, studies including the use of MRI are sparse.

Our first finding revealed increased local gyrification in a cluster spanning the superior frontal and superior parietal areas in the group with NSS. Our finding contrasts with the findings of the only existing study that investigated cortical gyrification in association with NSS in healthy young adults (Hirjak, Wolf, Kubera, et al., 2016). While we found higher gyrification in the group with NSS, Hirjak and colleagues (2016) found a higher NSS score (worse) in healthy adults to be associated with lower cortical gyrification. This discrepancy of findings may be explained by the investigated age of the participants. Based on the literature, the cortical gyrification of our cohort (mean age of ~15 years) was still decreasing potentially reflecting a delay in cortical gyrification, while the gyrification of the young adults (mean age of ~25 years) may have already leveled off (Cao et al., 2017). Interestingly, other studies investigating

adolescents with neurodevelopmental and psychiatric disorders where NSS are common also reported increased cortical gyrification (for review see Sasabayashi et al., 2021). These studies, similar to our results, have predominantly reported the superior frontal and parietal areas to be affected (Sasabayashi et al., 2021). As these areas play a major role in higher-order sensorimotor control (Luppino & Rizzolatti, 2000), we conclude that the higher gyrification in superior frontal and parietal areas detected in our sample may reflect alterations in fine motor skills. As of now, the exact interpretation of increased gyrification in adolescents with non-optimal neurological functioning is not fully understood. Gyrification is known to increase in the first months of life (Li et al., 2014) and decrease again during childhood and adolescence (Hogstrom et al., 2013). The decrease in gyrification is assumed to reflect synapse elimination processes, also known as synaptic pruning. Synaptic pruning is known to cause alterations in the morphology of sulci and gyri and a maintenance of only necessary connections in the brain (White et al., 2010). Thus, increased gyrification during adolescence, as indicated here for adolescents with NSS, may be interpreted as delayed maturation in synaptic pruning, which is a hallmark process of adolescent brain development.

Our second finding revealed no difference in cortical volumes or thickness between NSS+ and NSS- group. This result partly aligns with the results of previous work (Dazzan et al., 2006; Hirjak, Wolf, Kubera, et al., 2016). While Hirjak and colleagues (2016) also did not detect alterations in cortical volume, Dazzan et al. (2006) found lower volumes in several clusters of the brain in the group with NSS. One important methodological difference to consider here is the use of MRI field strength and related sequence parameters. While Dazzan et al. (2006) used a 1.5T MRI and a voxel size of 3×3×3mm, Hirjak and colleagues (2016) and our study used 3T machines and a voxel size of 1×1×1mm. This finding is in alignment with previous studies reporting differences in measured brain volumes between 1.5 and 3T machines likely due to improved-tissue-CSF contrast at 3T (Chu et al., 2016). This methodological difference limits the comparability of the studies. Furthermore, while Hirjak and colleagues (2016) reported an association between NSS and lower cortical thickness in the superior temporal, superior frontal, and middle frontal regions, we did not find significant between-group differences in cortical thickness. As reported above for cortical gyrification, one reason for this discrepancy in findings may be the different brain maturation patterns of cortical thickness between 15 and 25 years (Tamnes et al., 2017). Another reason may, again, be the difference in NSS investigation approach (dichotomous versus spectrum approach) and statistical models (ANOVA versus correlation) used. Thus, as mentioned above, the approach

used here may not cover the middle spectrum of NSS. Future studies should additionally calculate correlation coefficients between the total NSS score and brain structure alterations.

Our third finding revealed lower FAt and higher RDt in widespread white matter clusters spanning the CC, CST, PTR, SLF, CR, and IC in the NSS+ group. The finding that RD is related to the presence of NSS is not new. Hirjak and colleagues (2017) also reported higher RD in the CC to be associated with higher NSS scores. We additionally found lower FAt in the NSS+ group in clusters that almost perfectly overlapped with the detected higher RDt clusters. The finding of higher RDt and an absence of ADt alterations in the NSS+ group allows to speculate about the underlying neural mechanisms of NSS. While ADt is assumed to reflect changes to parallel diffusivity such as alterations in axonal shapes (λ1), RDt is assumed to reflect changes to perpendicular diffusivity (λ2 and λ3) potentially indicating alterations in fiber tract myelination (Song et al., 2002, 2005). In both groups, the diffusivity parallel to the axon is normal (λ1), while the diffusivity perpendicular to the axon (λ2 and λ3) is increased in the NSS+ group potentially indicating alterations in fiber tract myelination (Song et al., 2002, 2005). A schematic illustration of the described underlying mechanisms is shown in **Figure 6.**

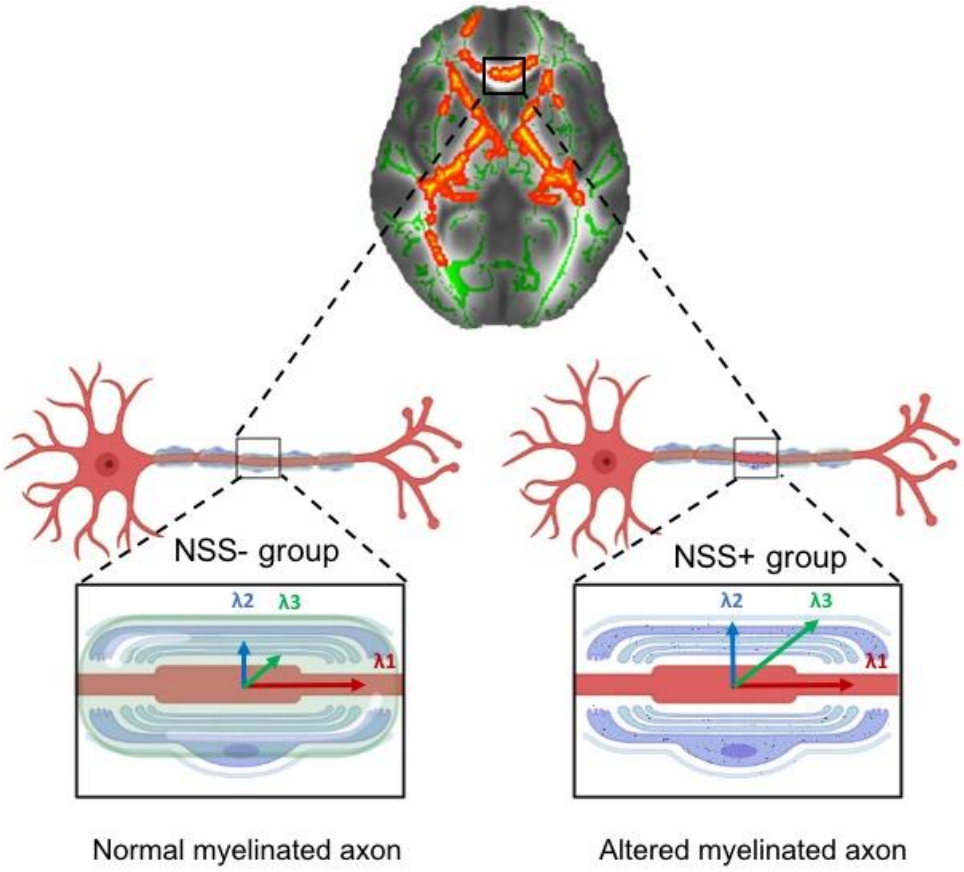


Figure 6. Schematic illustration of white matter microstructure alterations in the NSS+ group potentially indicating myelination alterations (created with BioRender.com).

Of note, we are the first to use FW-corrected diffusion parameters to investigate NSS-related brain structure alterations. Our finding of lower FAt and higher RDt in the absence of alterations in FW suggests that NSS-related diffusion alterations are present in the tissue, but not in the extracellular space, which would imply e.g., neuroinflammation (Pasternak et al., 2009). Given that FW imaging has not yet been used for investigating NSS, the exclusion of neuroinflammatory processes as underlying mechanisms for NSS in otherwise healthy adolescents reveals new knowledge. It is important to emphasize that most MRI sequences, including DTI, are not specific enough to interpret MRI-based findings to reflect a single neurobiological process. The interpretation of increased RD as altered myelination is a probable interpretation given that white matter myelination is a major hallmark process of adolescent brain maturation (White et al., 2010). However, a change in the axonal caliber may also depict a possible underlying mechanism that cannot be examined explicitly (Perrin et al., 2008).

Taken together, findings from Study II suggest that NSS in healthy adolescent athletes are associated with brain structure alterations that can be objectively quantified using neuroimaging. These brain structure alterations likely reflect NSS-related alterations in hallmark processes of adolescent brain maturation including synaptic pruning and white matter myelination.

3.3 Limitations and Strengths across Studies

As Study and Study II were conducted in the same project, I summarize the strengths and limitations regarding cohort, study design, and methods in one paragraph.

Study cohort not generalizable to general population

The project REPIMPACT includes a pre-selected cohort of healthy male adolescent athletes (Koerte et al., 2022). As described above, all participants were born on term and without history of neurodevelopmental or psychiatric disorders. Based on these strict inclusion criteria, the results of this work are not generalizable to females, non-athletes, other age groups, and the general population. Particularly, the fact that we investigated athletes may constitute a selection bias in terms of proficiency in overall motor coordination.

The homogenous group of participants, however, also comes with advantages. It allowed us to identify and characterize NSS in healthy, psychically fit adolescents. Prior to our work, it was not known whether athletes would also have NSS. We demonstrate that NSS are

present even in athletes and thus should be investigated within and beyond commonly investigated populations at risk for psychiatric disorders.

Study design not capturing longitudinal developmental trajectories

The work included in this dissertation is based on cross-sectional data. Thus, the study design does not allow to investigate the origin and developmental trajectories of NSS. To date, the interaction between age, the progression of NSS and related functional and structural correlates is largely unknown. Future longitudinal studies following children over several years until adulthood should address these open questions.

However, our study design also has benefits. For the first time, NSS were investigated in a multi-site study including adolescent athletes from three different European countries. This makes our findings more generalizable to other European countries.

Multi-site approach requiring differences in neurological examination set-up

Data in the REPIMPACT project was acquired in three European sites. To ensure consistency between study sites regarding the conductance of the neurological examination, the most experienced pediatric neurologist from Germany trained the personnel from the other two study sites. To ensure consistency in rating, the conductance of the neurological examinations was video-taped and later evaluated by three independent raters from Germany with different levels of experience. Our video-taping approach does not allow e.g., for instructing the participant to repeat a certain task when in doubt about the performance.

However, using this approach, we were able to ensure a consistent rating of neurological performance in a multi-site study. Moreover, our results, which are currently in preparation for a publication, showed a high inter- and intra-rater reliability between the three raters. Moreover, our results demonstrate that less experienced raters like medicine students may already perform similarly well as experienced pediatric neurologists. This finding indicates that the conduction of neurological examinations can be learned by less experienced staff.

3.4 Future Directions

In the following, I present future directions of this work, including a systematic investigation of factors that contribute to the development of NSS and longitudinal studies that allow to capture potential NSS-related alterations in developmental trajectories. Further, I suggest characterizing the underlying neural mechanisms, specificity, and relevance of NSS more extensively by using multimodal imaging and by investigating diverse cohorts (see **Figure 7**).

Variety of measures to investigate etiology and risk factors for neurological soft signs

Complications during pregnancy and birth are assumed to constitute risk factors for the development of NSS (Hadders-Algra, 2002). However, studies systematically assessing the etiology and risk factors of NSS are still missing. Therefore, future research should include a variety of different measures to elucidate the complex factors that may contribute to the development of NSS. Possible measures could include genetic information, pre- and perinatal adverse events, as well as demographic and environment factors such as sex, socioeconomic status, and pubertal development.

Longitudinal data to measure developmental trajectories of neurological soft signs

As of now, the exact course of NSS progression over childhood and adolescence is unknown. Ideally, future studies should follow-up with children for several times until they reach adulthood. This will contribute to a more comprehensive understanding of the interaction between age, the progression of NSS, and related functional and structural correlates. Further, longitudinal studies will enable researchers to determine which underlying developmental changes in the brain (e.g., synaptic pruning, myelination) and which external factors (e.g., demographic, environmental, and risk factors) influence the manifestation or outgrowing of NSS.

Multimodal neuroimaging for more specific interpretation of brain alterations

The findings presented in this dissertation provide a starting point for investigating the underlying neural mechanisms of NSS. For a more comprehensive understanding of NSS-specific alterations, structural neuroimaging may not be sufficient. Thus, future studies should use multimodal neuroimaging, meaning the combination of multiple neuroimaging sequences. Additional sequences could include magnetic resonance spectroscopy to quantify potential

metabolic brain alterations and functional magnetic resonance imaging to investigate potential alterations in brain connectivity. Determining what brain alterations or what combination of brain alterations underlay the presence of NSS is crucial to differentiate NSS from other neurodevelopmental conditions that may require a different clinical follow-up.

Comparison of different cohorts to better understand underlying pathology and relevance

The work included in this dissertation demonstrates that NSS are also present in healthy, physically fit adolescents. However, it remains unknown whether NSS in healthy and diseased cohorts have the same underlying neural mechanisms and the same clinical relevance. Thus, future studies should investigate and compare healthy cohorts with at-risk cohorts (e.g., prematurely born children) and diseased cohorts (e.g., children with neurodevelopmental disorders). This knowledge will further help to understand to what extent NSS-related brain structure alterations may be generalizable and of clinical and developmental relevance.

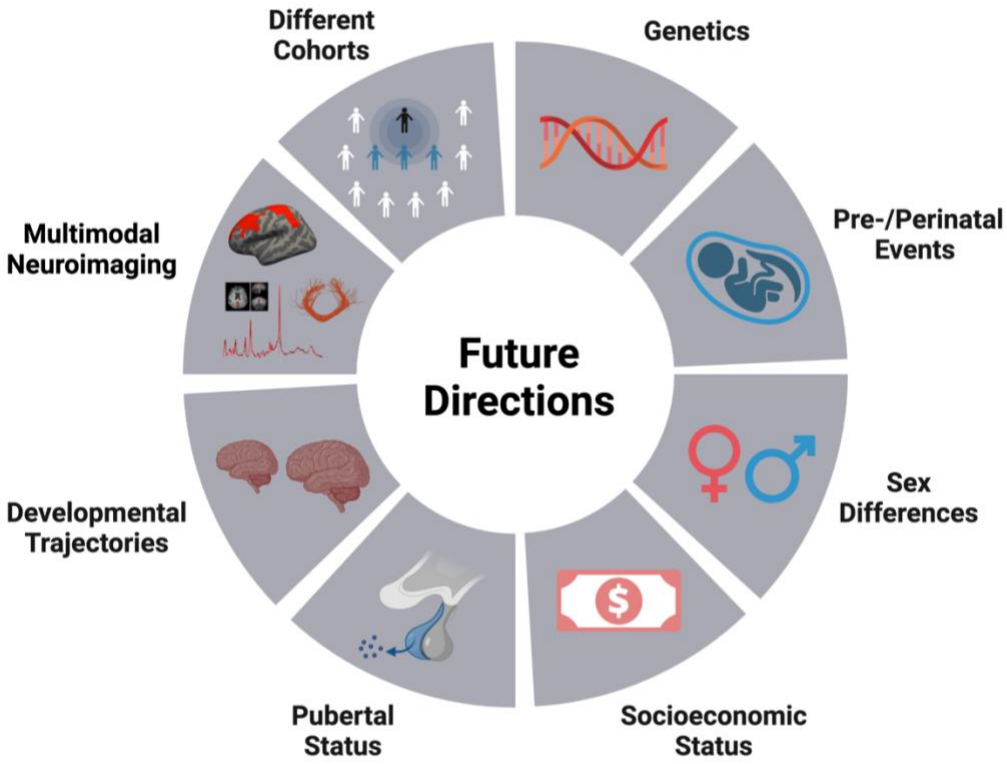


Figure 7. Overview of possible future directions (created with BioRender.com).

4 CONCLUSION

Findings from the work included in this dissertation show the beneficial use of quantitative functional and structural tools to investigate NSS in otherwise healthy adolescent athletes. Instrumented tools like force plates and MRI are particularly useful when neurological alterations are expected to be subtle as it is the case for the cohort investigated in this dissertation.

Study I revealed that participants performing non-optimal in a neurological examination also show significantly reduced postural control performance in the rigid condition, and the ML direction. This was particularly the case for adolescents performing non-optimal in pronation/supination movements of the forearms. As pronation/supination movements of the forearms and ML postural control are known to continue maturing until adolescence, it can be speculated that these functions are related and may indicate delayed motor development. Future studies should explore the use of other instrumented tools beyond force plates to capture a more complete clinical picture of NSS.

The results of Study II revealed higher gyrification and altered white matter microstructure in otherwise healthy adolescents with NSS. This finding may point towards alterations in hallmark processes of adolescent brain maturation such as synaptic pruning and white matter myelination. Our findings support the use of MRI to objectively and quantitatively assess NSS-related brain structure alterations that are of clinical and developmental relevance. Future studies should further investigate NSS-related brain structure alterations with more advanced methods and investigate its association with other clinical measures such as cognitive functioning and problem behavior.

Taken together, the work included in this dissertation contributes to generate a more complete picture of quantitative, objective functional and structural correlates of NSS in healthy adolescents. Future research should make use of larger and more representative datasets to replicate, as well as extend our findings. Specific attention should be put on the investigation of factors that contribute to the development of NSS, longitudinal studies that allow to capture NSS-related alterations in developmental trajectories, as well as on investigating the underlying neural mechanisms of NSS.

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7 AUTHOR CONTRIBUTION

Study I:

Neurological soft signs are associated with reduced medial-lateral postural control in adolescent athletes

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Contribution of co-authors:

CS, YT, SBS, DK, AL, SPS, RB, OP, FH, IKK and JG were involved in the study planning. EMB, AC, SMH, UT, CS, SBS, TLTW, DK, EK, FH, IKK, MVB and JG were involved in the data acquisition. EMB, AC, SMH, UT, EY, YT, SBR, FH, MVB, and JG were involved in data analysis and/or statistical analysis. EMB, AC, MVB, and JG drafted the manuscript and created figures and tables. EMB, AC, SMH, UT, CS, EY, YT, SBS, TLTW, DK, EK, SBR, MG, JSH, AL, SPS, RB, OP, FH, IKK, MVB, and JG critically edited the manuscript, and approved the final version of the manuscript.

My contribution to this publication in detail:

Together with other co-authors from the three data acquisition sites, I acquired the data. I performed all statistical analyses, interpreted the results, drafted, and revised the manuscript.

Munich, March 27, 2023

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Study II:

Neurological Soft Signs in Adolescents Are Associated with Brain Structure

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AdL, SBS, YT, DK, JG, APL, AL, SPS, RB, MES, OP, FH, and IKK were involved in the study planning. EMB, MVB, SMH, TLTW, SBS, CS, DK, EK, MM, JG, UT, FH, and IKK were involved in the data acquisition. EMB, MVB, JS-H, MG, TLTW, AdL, KIKC, EY, YT, UT, FH, and IKK were involved in data analysis and/or statistical analysis. EMB, MVB, FH, and IKK drafted the manuscript and created figures and tables. EMB, MVB, SMH, JS-H, MG, TLTW, ADL, KIKC, SBS, EY, YT, CS, DK, EK, MM, JG, APL, AL, SPS, RB, MES, OP, UT, FH, AND IKK critically edited the manuscript, and approved the final version of the manuscript.

My contribution to this publication in detail:
Together with other co-authors from the three data acquisition sites, I acquired the data. I performed the quality check of the raw T1-weighted and diffusion-weighted neuroimaging data and processed the data. I performed all statistical analyses, interpreted the results, drafted, and revised the manuscript.

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8 AFFIDAVIT

I hereby confirm that the dissertation “Neurological Soft Signs Are Associated with Brain Structure and Postural Control” is the result of my own work and that I have only used sources or materials listed and specified in the dissertation.

Hiermit versichere ich an Eides statt, dass ich die vorliegende Dissertation “Neurological Soft Signs Are Associated with Brain Structure and Postural Control” selbstständig angefertigt habe, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

Munich, March 27, 2023

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