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Neurological Soft Signs in Adolescents Are Associated with Brain Structure

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ABSTRACT

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, 14-16 years) underwent a standardized neurological examination including 28 tests grouped into six functional clusters. Non-optimal performance in at least one 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 to 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 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). While 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 to 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 two 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 two 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 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. Diffusion MRI 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 to typically developing children and adolescents (Langevin et al. 2014; Brown-Lum et al. 2020).

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 radial diffusivity in the corpus callosum (CC) (Hirjak et al. 2017). While this 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 to adolescents without NSS. The results of our study contribute to an improved understanding of NSS-related brain structure alterations.

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 14-16 years between July 2017 and April 2020 from three 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 with at least three training sessions per week and were proficient in the language of the respective country (i.e., German, Dutch, and Norwegian). 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 1) history of serious medical condition (history of encephalitis: n = 3), 2) incidental finding on MRI (periventricular gliosis: n = 1, subependymal heterotopia: n = 1), 3) premature birth (i.e., < 37 weeks of gestation) (n = 3), 4) attention deficit disorder (n = 1), 5) neurological hard signs as evident by neurological examination (n = 0), 6) MRI not performed (n = 5), or 7) 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, ***p* = .034 ***
Handedness (R/L)	(96%/ 4%)	(95%/ 5%)	X^2 = 0.083, df = 1, *p* = .774
Age *(Mean/SD*)	14.67/ 0.68	15.12/ 0.75	*t* (139) = -2.789, ***p* = .006 ***
Height (*Mean/SD*)	170.25/ 10.22	173.59/ 7.56	*t* (135) = -1.830, *p* = .070
Weight (*Mean/SD*)	57.72/ 10.27	60.44/ 8.60	*t* (134) = -1.372, *p* = .173

Note. * Indicates statistically significant difference between groups at *p* = .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* (Gregory Gruener 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 to other assessments such as the NES, CNI, HS, the MND concept considers the developmental status of a child and assess 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 six 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 one of the six 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, EK). Examiners in Norway (SBS) and Belgium (JG, SD'H, 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 three 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 one of the three study sites. See Table 2 for a detailed overview of MRI data acquisition.

Table 2. Overview of MRI Data Acquisition

	Norway	Belgium	Germany
General			
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	1×1×1 mm^3	1×1×1 mm^3	1×1×1 mm^3
Diffusion-weighted			
Sequence	2D spin EPI	2D spin EPI	2D spin EPI
Voxel size	2×2×2 mm^3	2×2×2 mm^3	2×2×2 mm^3
Gradients	20 × b = 1000 s/mm^2; 30 × b = 2500 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 less than 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.

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, USA) by trained personnel (EMB, TLTW, MG, 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 two cases were excluded due to segmentation failures resulting in 122 cases (n = 20 with NSS; n = 102 without NSS).

T1weighted (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 Figure 1 for an illustration of investigated gray and white matter measures.

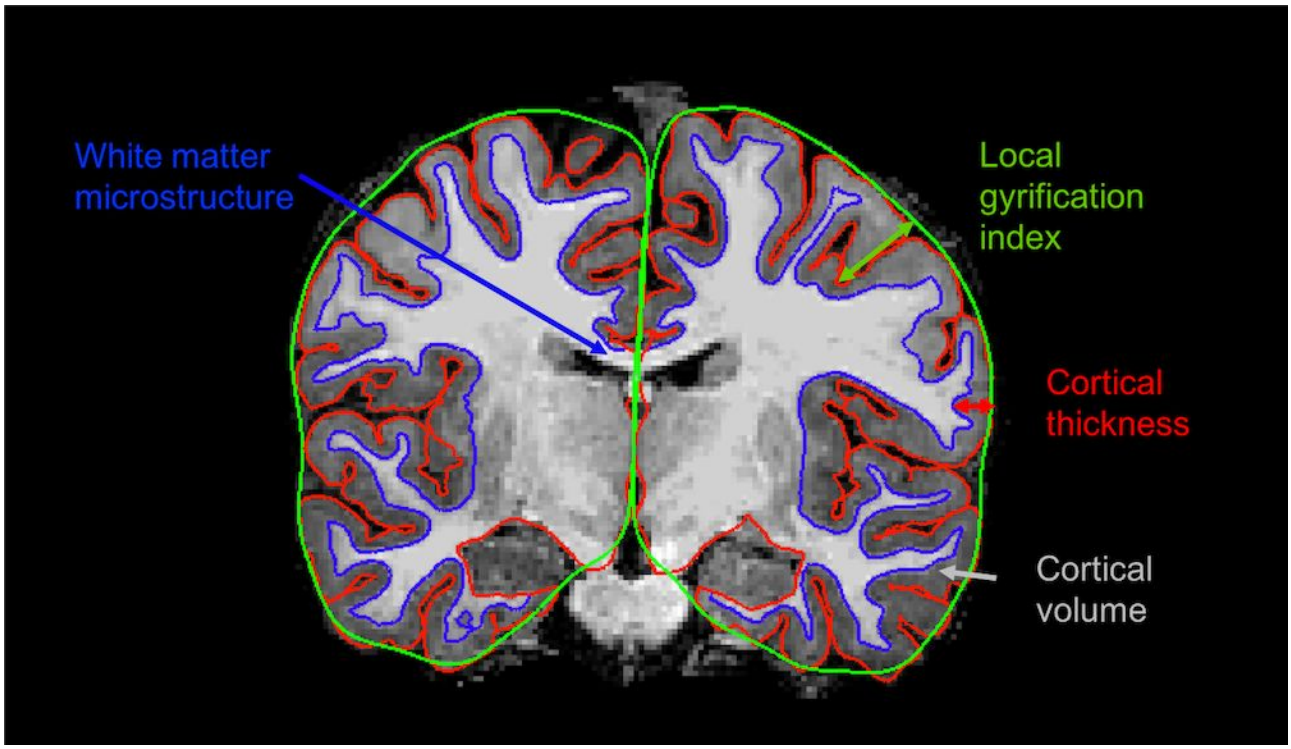

Figure 1. Overview of investigated cortical gray and white matter measures.

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 two remaining eigenvalues) which are parameters representing the magnitude (diffusivity) and direction (anisotropy) of water molecule diffusion.

Diffusion-Weighted Imaging

Preprocessing

Raw data were inspected for artifacts such as ghosting, severe 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, USA) by trained personnel (EB, TLTW, MG, AdL). Data were excluded in case of insufficient quality (n = 39). 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, eddy currents, and EPI distortions in a single step (Andersson and Sotiropoulos 2016).

Harmonization

The dMRI data were harmonized across the three 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, while 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 (FW) 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 two remaining eigenvalues). In addition, non-corrected fractional anisotropy (FA) maps were calculated by fitting a single diffusion tensor, for the purpose of the tract-based spatial statistics (TBSS) analysis (see below).

Processing and Analysis

Voxel-wise statistical analysis was carried out using the TBSS pipeline (Smith et al. 2006; Billah 2020). 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 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. 2014). 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 ($p < .05$). See **Error! Reference source not found.** 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 $p < .05$. Correction for multiple comparisons was performed using Monte Carlo cluster-wise simulation repeated 10,000 times set at $p < .05$. 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, FW), general linear models using age and handedness as additional covariates were used and corrected for family-wise error at a significance level of $\alpha < .05$. The anatomical location of resulting significant white matter clusters was identified and labeled by mapping the corrected statistical map on the JHU-ICBM-DTI-81 WM labels atlas and the JHU-WM tractography atlas in MNI space (Mori et al. 2008) 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 six clusters, 23 (16.91%) performed non-optimal in one cluster, 1 (0.74%) performed non-optimal in two clusters, and 1 (0.74%) in four 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 to 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 = .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 to 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 = .002; Figure 2).

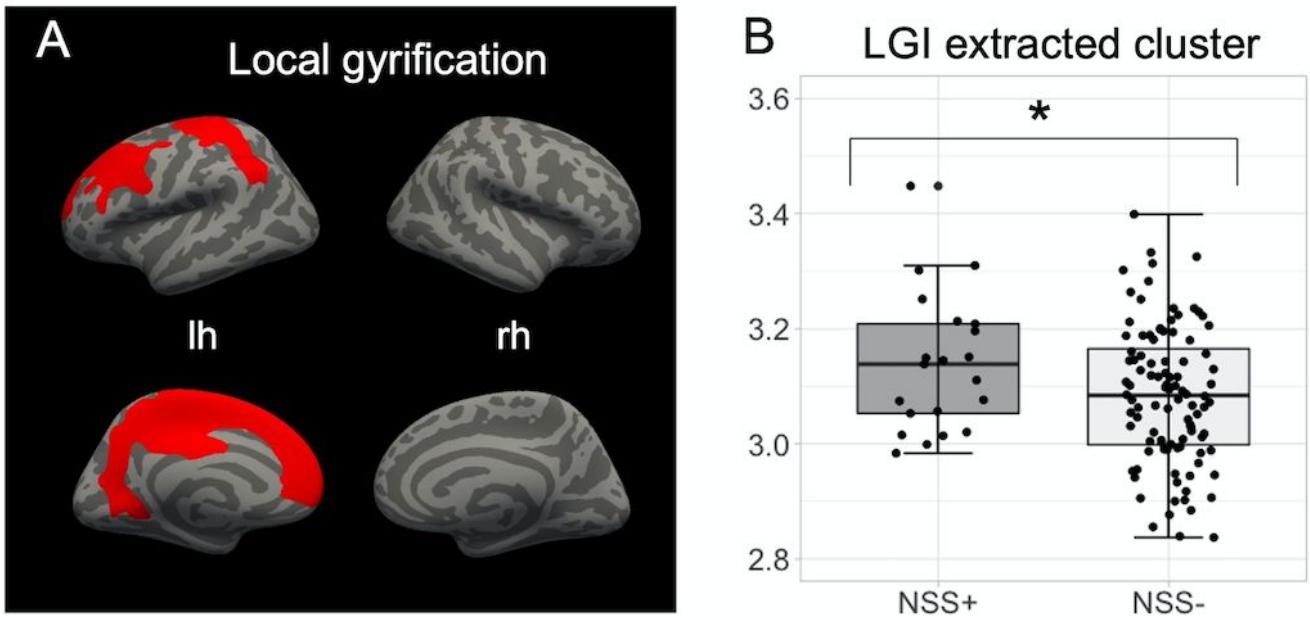

Figure 2. (A) Higher local gyrification in the left hemisphere spanning superior frontal and superior parietal lobes in the NSS+ group (red cluster) using general linear models corrected for age and handedness after cluster-wise correction for multiple comparisons. (B) Boxplots showing higher gyrification in the extracted significant cluster in the NSS+ group. Note. * Indicates statistical significance after cluster-wise correction for multiple comparisons at $\alpha < .05$.

Abbreviations. lgi = local gyrification index; lh = left hemisphere; NSS = neurological soft signs; rh = right hemisphere.

White Matter Microstructure

Participants in the NSS+ group had significantly lower FAt compared to those in the NSS- group in wide-spread white matter clusters (all $p < .05$; 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 posterior thalamic radiation (PTR), the superior longitudinal fasciculus (SLF), the corona radiata (CR), and the internal capsule (IC) (Figure 3). Moreover, participants in the NSS+ group had significantly higher RDt compared to those in the NSS- group in wide-spread white matter clusters (all $p < .05$; 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, PTR, SLF, CR, and IC. ADt and FW did not differ significantly between groups (Figure 3).

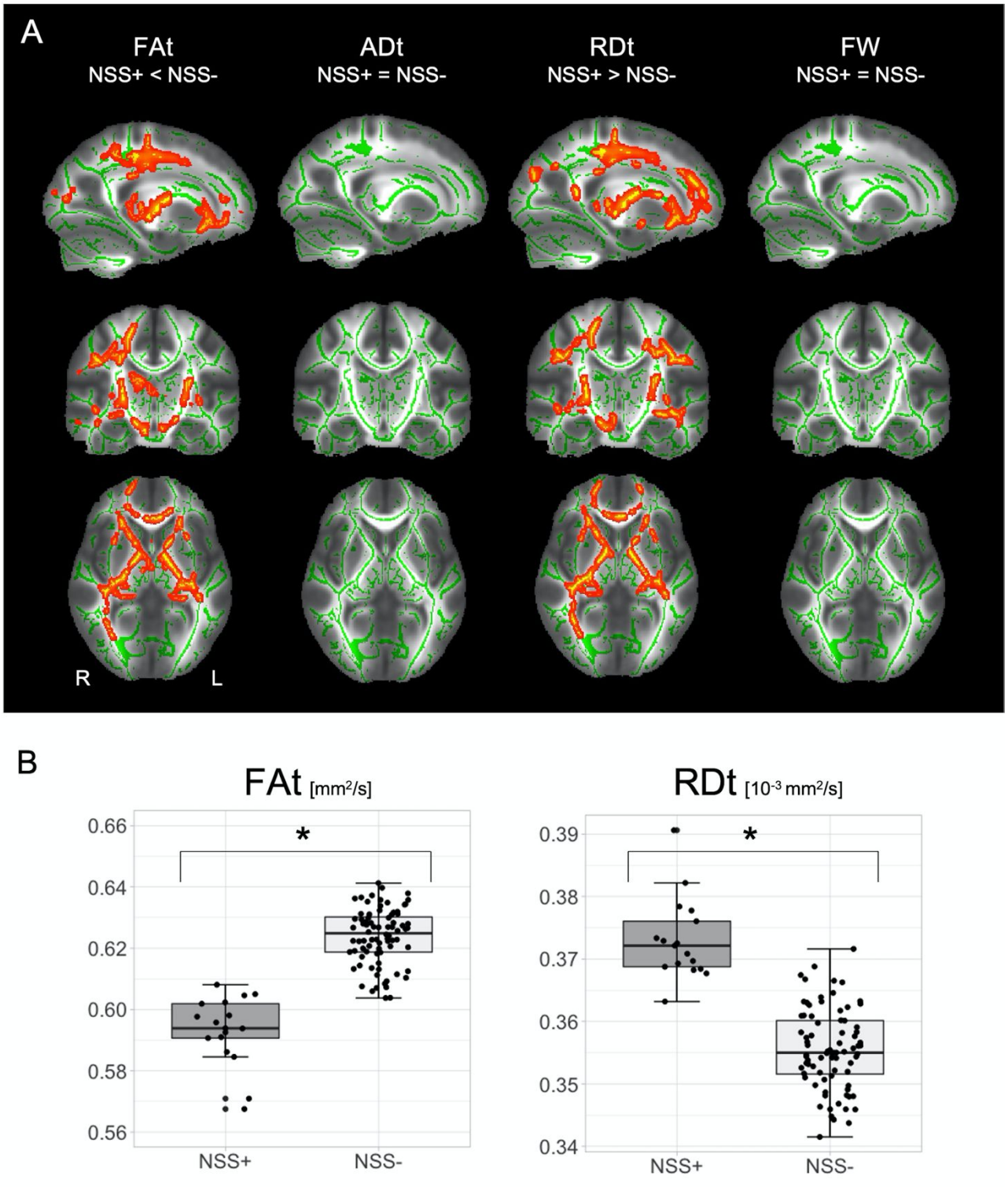

Figure 3. (A) Lower FAt and higher RDt in the NSS+ group (red-yellow clusters). No statistically significant differences in ADt, and FW between groups using general linear models corrected for age and handedness after family-wise error correction. (B) Boxplots showing individual FAt and RDt averaged across significant voxels.

Note. * Indicates statistical significance after family-wise error correction at $\alpha < .05$.

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.

DISCUSSION

This study revealed alterations in gray and white matter in physically trained adolescents with the clinical phenotype “with persistent NSS” compared to adolescents with the phenotype “without persistent 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, 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 persistent 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 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. To control for this age difference between the NSS+ and NSS- group, we included age as a covariate in all our analyses.

Moreover, we found a significant between-group difference in study site with a higher percentage of NSS+ participants from Norway compared to 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 persistent NSS compared to 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.

While 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, while 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 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, PTR, SLF, CR, and IC in adolescents in the NSS+ group compared to adolescents in the NSS- group. ADt and FW did not differ between groups.

This is the first study to use FW-corrected diffusion imaging 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 two (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 persistent NSS. Of note, the tracts covered by the identified white matter clusters 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 ADHD (Langevin et al. 2014; Brown-Lum et al. 2020).

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 two 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 to 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, 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, and the results of this study are not representative of female

adolescents and non-athlete 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 to 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 re-wiring 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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AUTHOR CONTRIBUTIONS

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.

CONFLICTS OF INTEREST

Nothing to report.

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