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Differential contributions of brainstem structures to neurological soft signs in first- and multiple-episode schizophrenia spectrum disorders

Stefan Fritze [a], Alina L. Bertolino [a], Katharina M. Kubera [b], Cristina E. Topor [a], Mike M. Schmitgen [b], Robert C. Wolf [b,1], Dusan Hirjak [a,*,1]

[a] Department of Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany
[b] Center for Psychosocial Medicine, Department of General Psychiatry, University of Heidelberg, Heidelberg, Germany

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ABSTRACT

Neurological soft signs (NSS) are frequently found in patients with schizophrenia spectrum disorders (SSD) at any stage of the disease. Brainstem structures are crucial for motor control, integration of sensory input and co-ordination of automatic motor actions. It is unclear whether disease duration has an impact on NSS/brainstem volume relationships. We tested the hypothesis that volumes of brainstem structures differ between first-episode psychosis (FEP) and multiple-episodes psychosis (MEP) patients with SSD, and that alterations of these structures are associated with NSS. T1-weighted structural MRI data at 3 T were obtained from 92 right-handed SSD patients (27 FEP and 65 MEP). FreeSurfer vers. 6.0 was used for segmentation of brainstem structures including the medulla oblongata, pons, superior cerebellar peduncle (SCP), and midbrain. Multiple regression analyses were used to describe the relationship between brainstem structures and distinct NSS subdomains. In FEP, pons volume had a significant effect on NSS total score ($p = 0.001$, Bonferroni corr.). Further, medulla oblongata ($p = 0.001$, Bonferroni corr.) and pons ($p = 0.001$, Bonferroni corr.) volumes had a significant effect on NSS motor coordination score. In MEP, significant associations between brainstem structures and NSS levels were not found. The present data support the notion that brainstem structures play an important role in the expression of NSS in SSD individuals with FEP, in contrast to individuals with MEP. Our study also emphasizes the need of better characterizing episode-specific brainstem correlates of NSS in SSD.

1. Introduction

As early as the end of the 19th century, Emil Kraepelin had observed that patients with schizophrenic psychoses show clinically subtle sensorimotor abnormalities in comparison to manifest movement disorders (Jahn, 1999; Kreapelin, 1899; Schroder et al., 1991). In addition, he also postulated a putative etiological relevance of these discrete motor signs (Jahn, 1999; Jahn et al., 2006). Today, these phenomena are referred to as neurological soft signs (NSS); notably subtle neurological abnormalities in the execution of motor or sensory tasks (Jahn, 1999; Jahn et al., 2006; Schroder et al., 1991). NSS cannot be fully observed in patient's everyday behavior but can only be detected in the context of a detailed structured clinical examination. Recent studies have shown that NSS are observed in approximately 60–70% of patients with schizophrenia spectrum disorders (SSD) and show familial clustering (Bombin et al., 2005; Chan and Gottesman, 2008). It has been robustly shown that NSS levels are higher in acutely ill in contrast to remitted patients (Bombin et al., 2005; Jahn et al., 2006). In addition, an association between NSS severity and executive deficits or negative symptoms has been demonstrated (Cuesta et al., 2014). According to recent Magnetic Resonance Imaging (MRI) studies, especially alterations of parallel organized cortico-cerebello-thalamo-cortical networks (CCTCC) are involved in their neuropathogenesis (for review see also (Hirjak et al., 2017a; Hirjak et al., 2018a; Hirjak et al., 2018b)).

However, unlike other brain regions, the role of brainstem and its structures in the pathogenesis of NSS in SSD is poorly understood. Therefore, we employed an automated segmentation method relying on a probabilistic atlas of the brainstem and its neighboring brain structures (Iglesias et al., 2015) to assess alterations of the medulla oblongata, pons, superior cerebellar peduncle (SCP), and midbrain, as well as relationships between these regions and NSS in SSD. Since patients with multiple-episodes psychosis (MEP) might show more pronounced structural abnormalities compared to SSD patients with first-episode psychosis (FEP) (Dietsche et al., 2017; Li et al., 2017), a major aim of this study was to examine putative stage-dependent brainstem pathology, and its association with NSS scores. In specific, we predicted that (1) Volumes of brainstem structures will differ between SSD

* Corresponding author at: Department of Psychiatry and Psychotherapy, Central Institute of Mental Health, D-68159 Mannheim, Germany.
E-mail address: dusan.hirjak@zi-mannheim.de (D. Hirjak).
[1] Both authors contributed equally to this work.

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patients with FEP and MEP. (2) In accordance with one of our previous studies conducted in an independent patient sample (Hirjak et al., 2013), we also expected pons and midbrain volumes to be significant predictors of NSS levels in FEP patients. (3) NSS levels will be related to abnormal volume of the brainstem structures in SSD patients with MEP as well.

2. Methods

2.1. Participants

All patients were consecutively recruited from the Department of Psychiatry and Psychotherapy at the Central Institute of Mental Health in Mannheim, Germany. Patients were diagnosed with SSD using the German version of the Structured Clinical Interview for DSM-IV-TR (SCID-I) (Wittchen et al., 1997) and examination of the case notes. The present sample included 92 right-handed patients with schizophrenia ($n = 84$), schizoaffective disorder ($n = 3$) or schizotypal personality disorder ($n = 5$). All patients diagnosed with schizotypal personality disorder according to DSM-IV-TR criteria were patients experiencing their first full-blown psychotic episode that required treatment in a psychiatric hospital setting. The first-episode psychosis was confirmed by the patient's relatives. After a remission of the full-blown psychotic symptoms, the diagnosis of a schizotypal disorder was confirmed. Clinical evaluation included ascertainment of personal and family history and detailed physical and neurological examination. Patients were excluded if: (i) they were aged<18 or >65 years, (ii) they had a history of brain trauma or neurological disease, or (iii) they fulfilled diagnostic criteria for substance use disorder (alcohol and illicit drugs) within 12 months prior to participation. At the time of inclusion, six patients were antipsychotic free and other patients were receiving treatment with antipsychotic agents according to their psychiatrists' choice. Antipsychotic dosage remained unchanged 2 weeks prior to the study inclusion. For statistical purposes, daily doses of antipsychotic medication were converted to olanzapine equivalents (OLZ) according to the classical mean dose method presented by Leucht and colleagues (Leucht et al., 2015). 'Five FEP patients and one MEP patient (overall 6.5%) were not taking any antipsychotics, 58 patients (63.0%; 18 FEP and 40 MEP) received one antipsychotic and 28 patients (30.4%; 4 FEP and 24 MEP) received two antipsychotics. Of note, the number of MEP patients treated with first-generation antipsychotics was very low ($n = 3$). Patients were treated with amisulpride ($n = 21$; 22.8%), clozapine ($n = 19$; 20.6%), aripiprazole (n = 21; 22.8%), olanzapine ($n = 17$; 18.4%), quetiapine ($n = 12$; 13.0%), risperidone ($n = 9$; 9.7%), and paliperidone palmitate ($n = 7$; 7.6%). All participants were right-handed (Oldfield, 1971). The study was approved by the ethics committee of the Medical Faculty of the University of Heidelberg, Germany, and written informed consent was obtained from all participants after the procedures of the study had been fully explained.

2.2. Clinical assessments

Patients were recruited and examined as soon as possible after partial remission within one week. We considered at least partial remission (>25% reduction of symptoms according to PANSS) as necessary for the motor assessment, since acute psychotic symptoms, agitation and severe formal thought disorder may considerably influence patients' cooperation as well as their ability to understand the instructions. NSS were examined with the Heidelberg Scale (Schroder et al., 1991), which consists of five items assessing motor coordination (MOCO) [Ozeretski's test, diadochokinesia, pronation/supination, finger-to-thumb opposition, speech articulation], three items assessing integrative functions (IF) [station and gait, tandem walking, two-point discrimination], two items assessing complex motor tasks (COMT) [finger-to-nose test, fist-edge-palm test], four items assessing right/left and spatial orientation (RLSPO) [right/left orientation, graphesthesia, face-hand test, stereognosis], and two items assessing hard signs (HS) [arm holding test, mirror movements]. Ratings are given on a 0 (no prevalence) to 3 (marked prevalence) point scale. A sufficient internal reliability and test-retest reliability have been established previously (Bachmann et al., 2005; Schroder et al., 1991).

2.3. Structural MRI data acquisition

MRI scans were acquired parallel to the anterior commissure-posterior commissure line using 3.0 Tesla Siemens Trio whole-body imaging system (Siemens Medical Systems, Erlangen, Germany), using T1-weigthed magnetization-prepared rapid gradient-echo (MP-RAGE) with following parameters: Repetition time (ms): 2530; Echo time (ms): 3.8; Inversion time (ms): 1100; Flip angle: 7°; Number of averages: 1; Slice thickness (mm): 1; Image columns: 256; Image rows: 256; Phase encoding direction: ROW; Voxel size x (mm): 1; Voxel size y (mm): 1; Number of volumes: 1; Number of slices: 176; Number of files: 176.

2.4. Image processing

The data were processed with Freesurfer vers. 6.0 (Dale et al., 1999; Fischl and Dale, 2000; Fischl et al., 1999; Khan et al., 2008). For detailed description of the method see (http://surfer.nmr.mgh.harvard.edu/) and supplementary material. Volumes of four brainstem structures (medulla oblongata, pons, SCP and midbrain) were calculated from T1 images using the automated procedure for volumetric segmentation, as implemented in Freesurfer vers. 6.0. This segmentation technique is based on a probabilistic atlas of the brainstem and neighboring structures and makes use of a robust and very accurate Bayesian algorithm (Iglesias et al., 2015). The four different brainstem structures from the input T1 scan are computed upon soft segmentations (i.e. a voxel can be assigned to multiple structures/tissues), which ameliorates the problems with partial volume effects of the surrounding cerebrospinal fluid (Iglesias et al., 2015).

2.5. Statistical analyses

SPSS for Windows version 22 was used. Initially, a descriptive analysis for demographic, clinical and volumetric data in FEP and MEP patients was performed. Then, homogeneity of variances of all brainstem structures and NSS scores in FEP and MEP patients was asserted using Levene's test. In a next step, we performed multiple regression analyses with all the predictors entered simultaneously and included the following predictors: age, gender, education, OLZ, medulla oblongata, pons, superior cerebellar pedunculus, midbrain and eTIV (estimated total intracranial volume). Previous studies on gender-specific NSS level differences in schizophrenia showed contradictory results (Chan et al., 2016): While Bjorck et al. (Bjorck et al., 2000) could not identify any gender-specific differences in their patient sample, the study by Duggal et al. (Duggal et al., 2005) showed that motor sequencing task may be modulated by gender. In addition, gender has an impact on brainstem structure as well (Xie et al., 2012). Therefore, we included gender as a covariate in all analyses. To account for false positive findings within the regression analyses, p-values were corrected after each step for the number of applied statistical tests using the Bonferroni method. To this end, p was set to $p = 0.05/N$, where $N = 4$ (4 brainstem structures) which resulted in $p = 0.0125$.

3. Results

3.1. Clinical, demographic and volumetric characteristics

Clinical, demographic and volumetric characteristics are shown in Table 1. The FEP group was operationally defined as the first contact with psychiatric services or SSD patients receiving treatment for an

Table 1
Clinical, demographic and morphological variables in first-episode and multiple-episode schizophrenia spectrum disorders patients.

	First-episode patients (n = 27)	Multiple-episodes patients (*n* = 65)	*T*[1]	*Df*[1]	*Sig. (2-tailed)*[1]
Age[1]	31.41 ± 11.15	41.48 ± 11.14	−3.946	90	**0.0001**
Gender (m/f)[2]	15/12	39/26	–	1	0.693
Education (years)[1]	12.52 ± 2.54	13.25 ± 2.92	−1.128	90	0.262
Olanzapine equivalents[1]	11.94 ± 9.94	19.7 ± 9.54	−3.507	90	**0.001**
Duration of illness[1]	0.11 ± 0.32 (0–1 year)	16.11 ± 9.89	−8.37	90	<**0.0001**
PANSS total score[1]	72.19 ± 25.8	67.54 ± 17.68	0.997	90	0.322
PANSS positive score[1]	17.41 ± 9.39	15.4 ± 6.48	1.178	90	0.242
PANSS negative score[1]	18.41 ± 8.63	16.66 ± 7.37	0.983	90	0.328
PANSS global score[1]	36.37 ± 12.41	35.48 ± 9.62	0.371	90	0.711
BPRS[1]	40.15 ± 16.02	37.78 ± 10.76	0.825	90	0.412
GAF[1]	65.19 ± 19.28	68.6 ± 16.68	−0.853	90	0.396
SAS total score[1]	1.93 ± 1.75	2.82 ± 2.46	−1.701	90	0.092
AIMS total score[1]	0.52 ± 1.08	1.75 ± 3.13	−1.991	90	**0.049**
NSS total score[1]	17.59 ± 7.62	21.51 ± 9.44	−1.907	90	0.06
NSS MOCO score[1]	6.85 ± 3.51	8.78 ± 4.07	−2.152	90	**0.034**
NSS IF score[1]	2.04 ± 1.22	3.05 ± 1.65	−2.859	90	**0.005**
NSS COMT score[1]	3.26 ± 2.26	3.55 ± 2.3	−0.561	90	0.576
NSS RLSPO score[1]	2.67 ± 2.84	2.91 ± 2.65	−0.388	90	0.699
NSS HS score[1]	2.78 ± 1.67	3.22 ± 1.79	−1.088	90	0.279
Medulla[3]	4801.57 ± 503.02	4731.44 ± 625.15	F = 0.253	92	0.617
Pons[3]	14,751.5 ± 1904.26	14,813 ± 2076.7	F = 0.005	92	0.943
SCP[3]	290.32 ± 68.02	271.83 ± 60.88	F = 4.1	92	**0.046**
Midbrain[3]	5932.29 ± 598.17	5981.36 ± 603.76	F = 0.013	92	0.910
Whole brainstem[3]	25,775.7 ± 2913.59	25,779.96 ± 3176.65	F = 0.047	92	0.829
eTIV[3]	$1.45 \times 10^6 \pm 2.55 \times 10^5$	$1.46 \times 10^6 \pm 2.02 \times 10^5$	−0.192	92	0.848

Data are mean ± standard deviation.
Abbreviations: PANSS=Positive and Negative Symptoms Scale (p = positive, n = negative, g = global), BPRS=Brief Psychiatric Rating Scale, GAF = Global Assessment of Functioning, SAS=Simpson and Angus Scale, AIMS = Abnormal involuntary movement scale, MOCO = motor coordination, IF = integrative function, COMT = complex motor tasks, RLPSO = right/left and spatial orientation, HS = hard signs, SCP=Superior Cerebellar Peduncle, eTIV = estimated total intracranial volume.
[1] Independent samples *t*-test (significant p-values are in bold).
[2] The *p*-values for distribution of gender were obtained by chi-square test.
[3] Data are mean ± standard deviation (in mm^3). The F- and p-values were obtained using analysis of covariance (ANCOVA) adjusted for age, gender, medication and eTIV as covariates.

episode of psychosis for the first time. Levene's test showed that the variances for all brainstem structures volumes and NSS scores in FEP and MEP patients were equal (*p*-values>0.05). FEP and MEP patients significantly differ in age, OLZ and duration of illness (DOI), but they did not differ in terms of gender, education level, eTIV and volumes of medulla oblongata, pons and midbrain. MEP patients showed smaller

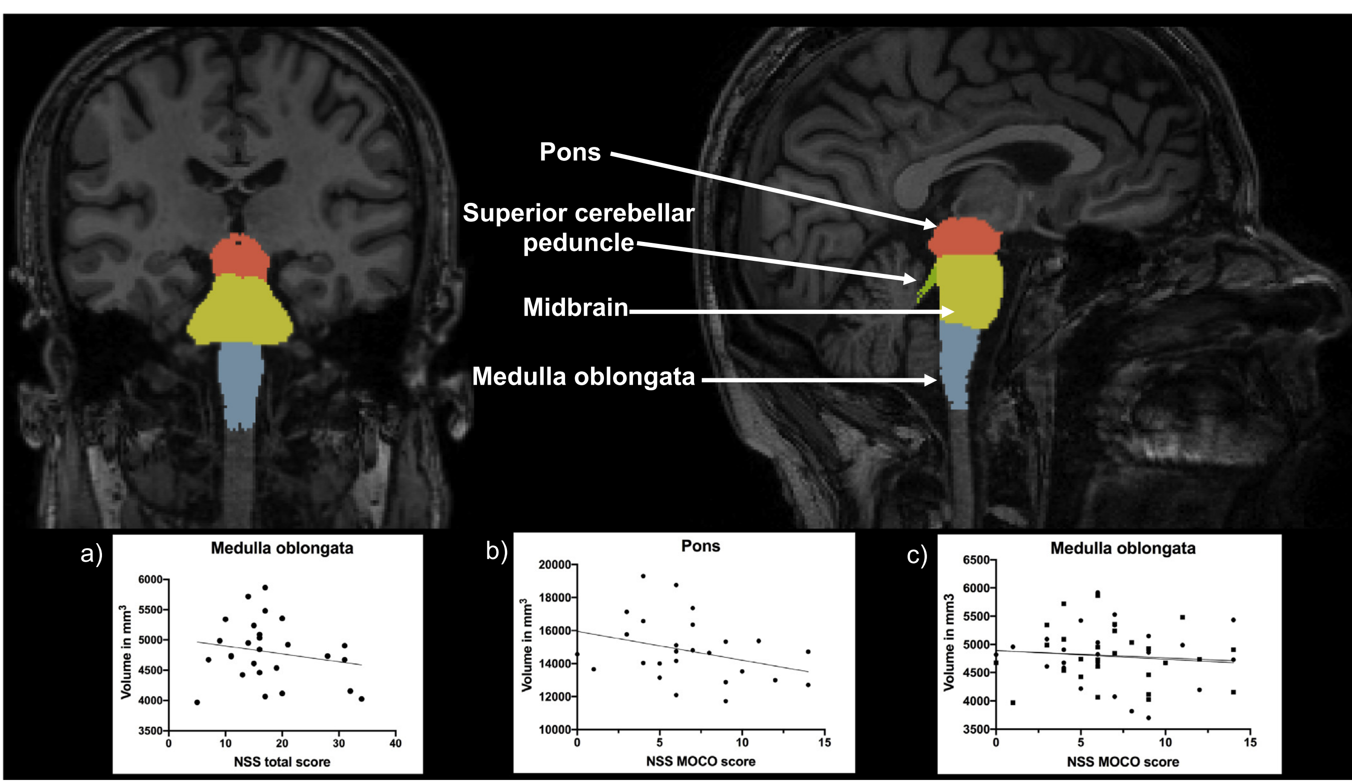

Fig. 1. Scatter plots of linear regression analyses of NSS total scores and medulla oblongata volumes (a) as well as NSS MOCO scores and pons (b) and medulla oblongata (c) volumes in FEP patients (*n* = 27).

SCP volumes when compared to FEP participants (one-way ANCOVA; $p = 0.046$). There was also a significant difference between NSS MOCO and IF scores between FEP and MEP ($p = 0.034$ and $p = 0.005$).

3.2. FEP patients ($n = 27$)

A multiple regression was run to predict NSS total, MOCO, COMT, IF, RLSPO and HS score from age, gender, education, OLZ, AIMS total score, medulla oblongata volume, pons volume, SCP volume, midbrain volume, and eTIV. First, these variables significantly predicted NSS total score [$F(10, 16) = 10.206$, $p < 0.001$, $R^2 = 0.864$, adjusted $R^2 = 0.780$], although only medulla oblongata ($p = 0.015$), pons ($p = 0.001$), gender ($p = 0.002$), education ($p < 0.001$), OLZ ($p = 0.01$) and AIMS total score ($p < 0.001$) significantly contributed to the prediction ($p < 0.05$) (Fig. 1). All predictors survived Bonferroni correction for multiple comparisons ($p < 0.0125$). Second, the above mentioned variables significantly predicted NSS MOCO score [$F(10, 16) = 5.108$, $p = 0.002$, $R^2 = 0.761$, adjusted $R^2 = 0.612$], although only medulla oblongata ($p = 0.001$), pons ($p = 0.001$), gender ($p = 0.035$), education ($p = 0.003$), OLZ ($p = 0.032$) and AIMS total score ($p = 0.006$) significantly contributed to the prediction ($p < 0.05$) (Fig. 1). Only medulla oblongata, pons, education and AIMS total score survived correction for multiple comparisons ($p < 0.0125$) (Fig. 1). Third, the above mentioned variables did not significantly predict NSS IF [$F(10, 16) = 1.397$, $p = 0.266$, $R^2 = 0.466$, adjusted $R^2 = 0.132$], COMT [$F(10, 16) = 2.157$, $p = 0.082$, $R^2 = 0.574$, adjusted $R^2 = 0.308$], and RLSPO score [$F(10, 16) = 1.341$, $p = 0.29$, $R^2 = 0.456$, adjusted $R^2 = 0.116$]. Finally, the above mentioned variables significantly predicted NSS HS score [$F(10, 16) = 3.624$, $p = 0.011$, $R^2 = 0.694$, adjusted $R^2 = 0.502$], but only eTIV ($p = 0.004$), age ($p = 0.046$) and AIMS total score ($p = 0.005$) significantly contributed to the prediction ($p < 0.05$). ETIV and AIMS total score survived Bonferroni correction for multiple testing ($p < 0.0125$).

A partial correlation (two-tailed) was run to determine the relationship between an individual's OLZ and AIMS total score, SAS total score, BARS global score and NSS scores while controlling for age, gender and education. There was no significant partial correlation between OLZ and motor symptoms in FEP patients ($p > 0.05$). Finally, we run a partial correlation (two-tailed) to determine the association between an individual's AIMS total score and NSS scores on all subscales while controlling for age, gender, education and OLZ. There were no significant associations ($0.05/6 = p > 0.008$, Bonferroni corr.).

In addition, we re-analyzed the data in a smaller group of FEP patients ($n = 22$) after the exclusion of individuals with schizotypal personality disorder ($n = 5$). For detailed information please see supplementary material.

3.3. MEP patients ($n = 65$)

A multiple regression was run to predict NSS total, MOCO, IF, COMT, RLSPO and HS scores from age, gender, education, OLZ, AIMS total score, medulla oblongata volume, pons volume, SCP volume, midbrain volume, and eTIV in MEP. These variables significantly predicted NSS total score [$F(10, 54) = 3.211$, $p = 0.003$, $R^2 = 0.373$, adjusted $R^2 = 0.257$], but only age ($p = 0.012$) and AIMS total score ($p = 0.048$) significantly contributed to the prediction ($p < 0.05$). Further, the above mentioned variables significantly predicted NSS MOCO score [$F(10, 54) = 3.662$, $p = 0.001$, $R^2 = 0.404$, adjusted $R^2 = 0.294$], but only age ($p = 0.015$) significantly contributed to the prediction ($p < 0.05$). The above mentioned variables significantly predicted NSS IF score [$F(10, 54) = 2.646$, $p = 0.011$, $R^2 = 0.329$, adjusted $R^2 = 0.205$], but only AIMS total score ($p = 0.005$) significantly contributed to the prediction ($p < 0.05$). None of the above mentioned variables did significantly predict NSS scores on the subscale COMT [$F(10, 54) = 1.252$, $p = 0.281$, $R^2 = 0.188$, adjusted $R^2 = 0.038$], RLSPO [$F(10, 54) = 1.066$, $p = 0.404$, $R^2 = 0.165$, adjusted $R^2 = 0.01$] and HS [$F(10, 54) = 1.107$, $p = 0.374$, $R^2 = 0.170$, adjusted $R^2 = 0.016$]. Finally, the individual brainstem structures did not significantly contribute to the prediction of NSS scores in MEP nor did they survive the Bonferroni correction for multiple comparisons ($p < 0.0125$). Only age (NSS total score) and AIMS total score (IF score) survived Bonferroni correction for multiple comparisons ($p < 0.0125$).

A partial correlation (two-tailed) was computed to determine the relationship between an individual's OLZ and AIMS total score, SAS total score, BARS global score and NSS scores while controlling for age, gender and education. There was no significant relationship between OLZ and motor symptoms in MEP patients ($p > 0.05$). Finally, we calculated a partial correlation (two-tailed) to determine the association between an individual's AIMS total score and NSS scores on all subscales while controlling for age, gender, education and OLZ. There were no significant associations ($0.05/6 = p > 0.008$, Bonferroni corr.).

4. Discussion

In this study, we examined volumes of specific brainstem subregions in 92 right-handed SSD patients with FEP and MEP using a brainstem-optimized segmentation technique. Three main findings emerged: (1) Patients with MEP showed lower SCP volume compared to FEP patients. (2) In FEP patients, medulla oblongata and pons volume predicted NSS total and MOCO scores. (3) In MEP patients, brainstem volumes did not significantly predict NSS.

4.1. Comparison of brainstem regions between FEP and MEP

In line with our expectations, we found lower SCP volumes in MEP patients when compared to FEP patients. This finding is in line with a previous study on brainstem correlates of NSS in FEP (Hirjak et al., 2013) and at the same time corroborates the view that patients with longer duration or chronic form of illness show more severe structural brain alterations than patients with FEP (Lawrie and Abukmeil, 1998; Pantelis et al., 2005). Although aberrant brainstem structure has been implicated in psychotic disorders (Nopoulos et al., 2001), previous MRI studies showed inconsistent results regarding the SCP structure in SSD patients. It is noteworthy that three earlier diffusion tensor imaging (DTI) studies found reduced fractional anisotropy (FA) in SCP in SSD patients compared to healthy controls (Magnotta et al., 2008; Okugawa et al., 2006; Park et al., 2004). However, two DTI studies found no significant reduction of FA in SCP of SSD patients when compared to healthy controls (Freitag et al., 2013; Wang et al., 2003). To date, MRI studies comparing SCP morphology between SSD patients with different durations of illness in a longitudinal setting have not been conducted.

Contrary to our prediction, there were no significant differences in medulla oblongata, pons and midbrain volumes between FEP and MEP SSD patients. A possible explanation for these findings might be the rate of brainstem volume loss over time, which depends on tissue compartment, brainstem region, age and disease stage. While white matter volume changes are not detectable at age of 35, the estimated physiological average global percentage grey matter (GM) volume loss per year starts from 0.32% at age of 35 and increase to 0.55% at age of 70 (Schippling et al., 2017). According to another study conducted by Opfer and colleagues the mean brain volume loss per year was 0.15%, 0.30%, 0.46%, and 0.61% at ages 45, 55, 65, and 75 years, respectively (Opfer et al. 2017; Schippling et al., 2017). In the present study, we cannot fully rule out the possibility that the lack of difference between the patient groups may reflect a low sensitivity of the imaging method. This said, only more pronounced structural changes and/or atrophy might be detected when using Freesurfer 6.0 brainstem toolbox. Further, magnets at higher field strength may have the potential to reveal subtle differences that may not be detectable at 3 T. While the above mentioned explanations appear plausible, we acknowledge that brainstem changes in manifest SSD have not been longitudinally studied so far.

The present findings build upon a previous study on white matter integrity that found no significant difference in the brainstem and cerebellum between FEP patients and chronic schizophrenia patients (White et al., 2011). Furthermore, our study supports the hypothesis of progressive developmental pathology in SSD, which does not affect the whole brain uniformly but is rather selective concerning specific cortical and subcortical areas (De Peri et al., 2012; Pantelis et al., 2005; Vita et al., 2012). With regard to brainstem, this is not true for all subregions, but only for those structures that are part of the CCTCC. While SCP is the smallest structure and susceptible to subtle structural changes, medulla oblongata, midbrain and pons are more robust structures in which morphological alterations can only be noticed when more pronounced changes may have taken place.

4.2. Association between the volumes of brainstem regions and NSS

In line with our predictions and consistent with our previous study in an independent sample (Hirjak et al., 2013), the present data suggest a significant relationship between the MOCO subdomain and medulla oblongata and pons volume in FEP. Associations between NSS scores and brainstem alterations are largely consistent within the literature on NSS in ultra-high risk (Mittal et al., 2013) and SSD individuals [for reviews see (Hirjak et al., 2017a; Hirjak et al., 2018a; Hirjak et al., 2015)]. Although other authors found significant associations between brainstem morphology and NSS levels as well, this relationship mainly affected the midbrain (Heuser et al., 2011; Venkatasubramanian et al., 2008). In addition, brainstem morphology not only plays a specific role in manual motor difficulties in other neurodevelopmental disorders such as autism (Travers et al., 2015) but is also present in healthy controls exhibiting NSS (Hirjak et al., 2017b).

Anatomically, the ventral part of the medulla oblongata contains the *nuclei olivares inferiores*, which are involved in coordination and fine-tuning of precision movements (Baizer, 2014). Further, the olive receives its afferent pathways from the spinal cord, the midbrain, and the motor cortex. The efferent pathways of the olive are directed to the cerebellum. Furthermore, the pons (i.e. *Nuclei pontis*) receives its afferent pathways from the frontal lobe and its efferent pathways end in the contralateral cerebellar hemisphere. Pons receives information about the movement from cortical motor regions and sends them for further fine-tuning to the cerebellum (Baizer, 2014). This anatomical arrangement matches the individual tests of MOCO (Ozeretski's test, diadochokinesia, pronation/supination, finger-to-thumb opposition, speech articulation) that the SSD patients have to perform. All tests require coordination, fine motor skills and motor inhibition. In case of disturbances in the above-mentioned motor loops, patients are not able to execute precise/fine movements and inhibit their bodily actions. Therefore, the results suggest that aberrant structure of medulla oblongata and pons and their associated neural circuits might contribute to the development of NSS in SSD.

Although we found increased MOCO and IF scores in MEP compared to FEP patients, NSS levels were not predicted by brainstem alterations in MEP patients. This finding extends our prior knowledge by demonstrating that NSS levels deteriorate with age and duration of illness (Herold et al., 2018), but are not modulated by brainstem abnormalities. Since this is the first study investigating the association between NSS levels and brainstem structure alterations in MEP patients with SSD, the reason for the missing relationship remains speculative. One possible explanation may be the dominance of cortical regions and basal ganglia in the modulation of motor loops in SSD patients with multiple episodes of flamboyant illness. This said that brainstem regions may have only little effect on the cortico-cortical and cortico-striatal circuits and do not contribute to the development of NSS at later stages of SSD. Another possible explanation for the missing association might be the frequently and critically discussed neuroprotective effect of long term antipsychotic use. In particular, recent study by Ebdrup and colleagues (Ebdrup et al., 2016) showed an increase of FA in corticospinal tract (which pass through the brainstem) after 6 weeks of antipsychotic monotherapy with amisulpride. A protective effect of antipsychotic medication on white matter integrity via modification of neuroplasticity has been also showed in other studies using mixed antipsychotic treatment (Reis Marques et al., 2014) or clozapine (Ozcelik-Eroglu et al., 2014). In particular, Ozcelik-Eroglu and colleagues showed FA increase in cerebellar regions after 12 weeks of clozapine treatment (Ozcelik-Eroglu et al., 2014). Still, the mechanism underlying NSS in MEP patients' needs to be examined in future longitudinal and multimodal MRI studies.

4.3. Limitations

We acknowledge potential limitations of this study. First, we focused on brainstem structures only, so that we cannot fully appreciate further contributions of other CCTCC structures to NSS, particularly the basal ganglia and thalamus (Hirjak et al., 2012; Zhao et al., 2014). Second, the brainstem segmentation tool implemented in Freesurfer ver. 6.0 can't distinguish between grey and white matter so far. In the future, the possibility of distinguishing between grey and white matter would of course be of great scientific relevance, preferably including brainstem nuclei. Third, only few results survived the Bonferroni correction for multiple comparisons, and thus, our findings need further replication. Finally, the potential side effects of antipsychotic medication have to be considered as potential confounds. Although NSS are considered to be independent of medication effects (Bersani et al., 2011; Chen et al., 1995; Hirjak et al., 2013; Kong et al., 2012; Mouchet-Mages et al., 2011; Rossi et al., 1990), daily antipsychotic dose might have an influence on the brainstem volume that may not have been fully accounted for by our OLZ-adjusted analyses.

5. Conclusion

To the best of our knowledge, the present study is the first showing stage-specific differences in SSD patients (FEP vs. MEP) with respect to SCP volume. Our study further demonstrates that medulla oblongata and pons volume alterations represent further subcortical correlates of NSS in FEP patients. This study provides evidence for potentially different pathomechanisms that might lead to NSS in SSD patients with FEP and MEP. Such mechanisms might be associated with disease duration.

Declaration of Competing Interest

The authors have declared that there are no conflicts of interest in relation to the subject of this study.

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Contributors

DH and RCW designed the study and wrote the protocol. AB performed the neuropsychological testing in all study subjects. KMK and CET managed the literature searches and analyses. SF, MMS and DH undertook the statistical analysis and interpreted the results. DH wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.schres.2019.05.041.

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