The Structural Brain Correlates of Neurological Soft Signs in Healthy Individuals

It has yet to be established whether neurological soft signs (NSS), which include poor motor coordination, sensory perceptual difficulties and difficulties in sequencing of complex motor tasks, result from specific or diffuse brain structural abnormalities. Studying the neuroanatomical basis of NSS in healthy individuals may help to identify which brain areas are specifically associated with these signs, while excluding the potential confounding effects of psychiatric and neurological disorders. We investigated the relationship between brain structure and NSS in 43 healthy individuals, using the Neurological Evaluation Scale for neurological assessment, and high resolution MRI and voxel-based methods of image analysis to investigate brain structure. Higher rates of NSS were associated with a reduction of inferior frontal gyrus, middle and superior temporal gyrus, and anterior cingulate gyrus. It is of note that in a previous study of patients with psychosis we found that an excess of NSS was associated with a reduction of similar cortical areas. Therefore, we suggest that these cortical brain structural changes represent a common neuroanatomical substrate of NSS, across healthy individuals and patients with psychosis.

Keywords: grey matter, MRI, neurological soft signs, psychosis, voxel-based morphometry

Introduction

The neuroanatomical basis of neurological soft signs (NSS) remains poorly understood, and it has yet to be established whether they result from specific or diffuse brain abnormalities (Dazzan and Murray, 2002). NSS are minor abnormalities like poor motor coordination, sensory perceptual difficulties, and difficulties in sequencing of complex motor tasks. Although NSS have been described in excess in psychosis, they are also present in healthy individuals, with prevalence rates reported as varying between 0 and 50% (Kennard, 1960; Hertzig and Birch, 1968; Rochford et al., 1970; Cox and Ludwig, 1979). There are very few data on the neuroanatomical correlates of NSS in healthy individuals, and even in psychiatric populations surprisingly few studies have investigated the anatomical substrate(s) of NSS (Dazzan and Murray, 2002). Data from our and other research groups suggest that NSS in subjects with psychotic disorders are associated with reduced basal ganglia volume and reduced volume of areas that are part of the heteromodal cortex (which comprises primarily the prefrontal, superior temporal and inferior parietal cortices) (Schroeder et al., 1991; Keshavan et al., 2003; Dazzan et al., 2004). In particular, while motor coordination signs seem to be associated with reduced basal ganglia volume, sensory integration signs seem to be associated with reductions of both basal ganglia and cortical areas involved in the integration of information from different sensory modalities (Keshavan et al., 2003; Dazzan et al., 2004). However, studying the neuroanatomical basis of NSS in these disorders may be confounded by the underlying pathogenic process, and by the use of psychotropic medications (Dazzan et al., 2005). Instead, studying NSS in healthy individuals may help to identify which brain areas are specifically associated with these signs, independently of these confounders. In the present study, we have used the same methodological approach that we used in patients with psychosis to investigate brain structure and NSS in healthy individuals.

Our sample was drawn from an epidemiological study of general population, thus limiting the potential bias of selecting subjects with atypically low rates of NSS, as some recruiting strategies can lead to samples with relatively high IQ and low prevalence of NSS (Kennard, 1960; Mosher et al., 1971; Manschreck and Ames, 1984; Sanders et al., 2000). Furthermore, as in our previous study, we have used a validated method to evaluate NSS, and we have investigated brain structure using high-resolution magnetic resonance imaging (MRI) and voxel-based methods of image analysis. Voxel-based analysis of magnetic resonance images allows the evaluation of the entire brain, rather than a few pre-selected regions (Dazzan et al., 2004, 2005). On the basis of our previous findings in patients with psychosis, we hypothesized that in healthy individuals, the presence of NSS would be accompanied by reductions of frontal and temporal association brain areas, and of subcortical brain structures such as the basal ganglia, which are involved in the integration of sensory perceptions and in motor coordination.

Materials and Methods

Study Population

This sample of healthy individuals was recruited as part of an epidemiological study (ESOP: Aetiology and Ethnicity in Schizophrenia and Other Psychoses) carried out in London, UK. As part of this study, a sample of healthy subjects aged 17–65 years was recruited from the resident population of South London, using household visits, local press advertisements, and advertisement for hospital staff for a minority of cases. Exclusion criteria were: (i) evidence of any psychotic disorder (past or present); (ii) a history of head trauma resulting in loss of consciousness for >1 h; (iii) the presence of a disease of the central nervous system; and (iv) poor fluency in English language. In the MRI arm of the study, a total of 46 healthy subjects (aged 17–53 years) underwent both a NSS evaluation and an MRI scan. We excluded three MRI scans (one due to subject motion, one because the full image acquisition had not been achieved and one because it failed to segment). Ethical approval for the study was granted by the local Ethical Committee, and the participants gave written informed consent.

Clinical and Neurological Assessments

We used the Psychosis Screening Questionnaire (Bebbington and Nayani, 1995) to screen for the presence of psychosis, including...
information on any contact or treatment for any mental or psychological disorder. Current IQ was estimated by the WAIS-R (Wechsler, 1981) (shortened version consisting of: Vocabulary, Comprehension, Block Design and Digit Symbol tests).

We assessed neurological function with an expanded, previously validated, version of the Neurological Examination Scale (NES) (Buchanan and Heinrichs, 1989; Griffiths et al., 1998), as we have described before (Dazzan et al., 2004). This expanded version is composed of 4 subscales, reflecting different functional areas and showing good construct validity (Buchanan and Heinrichs, 1989; Sander et al., 2000). The first subscale represents 'Primary neurological dysfunction' and reflects a dysfunction that can be identified by a standard neurological examination. It includes abnormal function of cranial nerve, abnormal eye movement, lateralisering limb pyramidal signs and frontal release signs. The remaining three subscales can be considered together to represent 'Integrative neurological dysfunction'. They represent dysfunctions which are likely to depend on integration within or between the motor and sensory systems, and thus on anatomically distributed processing. Three subscales constitute Integrative dysfunction: 'Sensory integration dysfunction' reflects a dysfunction in the integration of sensory information, and includes signs such as right/left confusion, astereognosis, aphagruhestasia and audio-visual integration; 'Motor coordination dysfunction' reflects signs of motor incoordination, and includes tests such as tandem walk, dysdiadochokinase and finger-to-nose test; and 'Motor sequencing dysfunction' reflects the ability to perform complex motor sequences, and includes signs such as the fast-ring and the fast-edge-palm tests.

We administered the schedule in a standardised manner, as specified for each item, and according to a fixed order. The scores for the items present in the original NES (those included in the three subscales: sensory integration, motor coordination, motor sequencing) (Buchanan and Heinrichs, 1989) were left unchanged (items scored on a three-point scale, from 0 = no abnormality to 2 = marked impairment, except for the snout and suck reflexes which are scored either as a 0 or a 2). For the remaining items (included in the primary signs subscale), we used the scores as in Griffiths et al. (1998) with a three-point scale: 0 = no abnormality; 1 = intermediate criterion; 2 = a score at or above a reference criterion regarded as clearly abnormal/marked impairment. Assessment of NSS was always performed by a physician. The inter-rater reliability was evaluated with examiners rating the same subjects on a videotape (agreement rates for the four subscales were: primary, r = 0.94; motor coordination, r = 0.96; sensory integrative, r = 0.87; motor sequencing, r = 0.92). We analysed the 'Integrative score' separately from the 'Primary score', to better distinguish between those signs that have been previously described as more soft (Integrative) and those that broadly correspond to hard neurological signs (Primary) (Griffiths et al., 1998).

We wanted to examine the relationship between brain structure and NSS scores. NSS scores were not normally distributed but strongly negatively skewed, with a high number of subjects scoring 0; the data remained skewed even after logarithmic transformation. We therefore decided to use the value of the median for the Primary and Integrative subscales at the NES to divide subjects in 'High' NSS and 'Low' NSS, a method successfully used previously (Dazzan et al., 2004; Ismail et al., 1998). In the MRI analysis, this division would permit optimal identification of the brain structural changes associated with the presence of a neurological dysfunction. Hand preference was assessed according to the Annett Hand Preference Questionnaire (Annett, 1970).

### Structural MRI Acquisition
Scans were acquired with a General Electric Signa 1.5 T system (GE Medical Systems, Milwaukee), at the Maudsley Hospital, London. Contiguous, interleaved proton-density- and T2-weighted images, with a 3 mm slice thickness, were acquired in the coronal plane, to provide whole brain coverage. A repetition time (TR) of 4000 ms and effective echo times (TE) of 20 and 85 ms were used with a eight-echo train length. The matrix size was 256 × 192, collected from a rectangular field-of-view of 22 cm × 16.5 cm, giving an in-plane resolution of 0.859 mm. The total acquisition time was 10 min 12 s.

### Structural MRI Processing
The methods used for segmentation and registration of each fast spin echo dataset have been extensively validated and described in detail elsewhere (Bullmore et al., 1999; Suckling et al., 1999a,b; Shapleske et al., 2002). Briefly, extracerebral tissues were initially removed, using an automated algorithm. Manual editing of the skull-stripped images was necessary only to remove brainstem and cerebellum from the cerebral hemispheres and diencephalon. The probability of each intracerebral voxel belonging to each of four possible tissue classes [grey matter, white matter, cerebrospinal fluid (CSF) or dura/vascular] was then estimated with a modified fuzzy clustering algorithm (Suckling et al., 1999b). This type of segmentation assigns to each voxel a value in the range 0-1 assumed to indicate the fraction of the voxel comprised by each tissue type (for example, a grey matter value of 0.7 means that 70% of the tissue represented by that voxel is grey matter).

Probabilistic tissue class maps were then mapped into the standard space of Talairach and Tournoux (1988) using a nine-parameter affine registration, scaling them to the same gross dimensions. The optimum mapping was found for subjects by registering the corresponding proton density image to a template image, minimising the grey-level difference between them. The template image was constructed using the AFNI program from six proton-density images acquired from six healthy subjects and then averaging these images (Dazzan et al., 2004). The derived mapping was then applied to the corresponding probabilistic tissue class maps.

Between-group differences in grey and white matter volume were estimated by fitting an analysis of covariance model at each intracerebral voxel in standard space covarying for age at scan, and total grey or white matter volume. Permutation testing was used to assess statistical significance, and regional relationships were tested at the level of voxel clusters (Bullmore et al., 1999; Sigmundsson et al., 2001). Given that structural brain changes are likely to extend over a number of contiguous voxels, test statistics which incorporate spatial information, such as 3D cluster mass (the sum of voxel statistics above an initial probabilistic threshold), are generally more powerful than other possible test statistics, which are informed only by data at a single voxel. We set the statistical threshold for cluster significance in all analyses so that the expected number of false positive clusters (P-value times number of tests) was <1. We conducted additional analyses, correlating the size of the resulting grey matter or white matter clusters with the total NSS score using Pearson correlation coefficient. This clarifies the relationship between cluster mass and increased severity of neurological signs.

### Results

#### Group Scores for NSS
A total of 19 subjects scored above the median value of 2 on the Primary subscale (range of scores, 0–9; quartiles 1, 2 and 4) (Table 1). Nineteen subjects scored above the median value of 1 on the Integrative subscale (range of scores, 0–8; quartiles 0, 1 and 3) (Table 2). However, the score for the integrative signs almost entirely reflected deficits in sensory integration tests, as the majority of subjects did not show any abnormality in tests of motor coordination (n = 34; 79%) or motor sequencing (n = 34; 79%) or in both (n = 27; 63%). The NSS scores and the socio-demographic characteristics (gender, age, years of education, IQ) of subjects with 'High' and 'Low' NSS at the two subscales are shown in Table 1 and 2.

There were no differences in gender, age, and years of education between subjects 'High' and 'Low' at either the Primary and the Integrative subscale. However, subjects scoring 'High' on the Integrative subscale had a significantly lower Full Scale IQ and Performance IQ than subjects scoring 'Low'. Finally, subjects of the African/African-Caribbean ethnic group were more frequently represented in the group 'High' for Integrative signs.
Relationship between NSS and Brain Structure

Primary Subscale
There were no significant differences in total or regional grey or white matter volumes between subjects ‘High’ and ‘Low’ on the Primary scale.

Integrative Signs
Subjects ‘High’ on the Integrative scale had a significantly smaller total grey matter volume than subjects ‘Low’ on this subscale (566.1 versus 600.5; \( t = 2.2, df = 41, P = 0.03 \)) (Table 2), while there were no significant differences in total white matter volume.

At a regional level, and after adjusting for the difference in total grey matter volume, the ‘High’ score group showed a reduction of grey matter volume at three clusters (\( P < 0.002 \)) (Table 3; Fig. 1, upper): a bilateral cluster centred on the anterior cingulate; a cluster centred on the right middle temporal gyrus and extending into the inferior frontal gyrus; and finally, a cluster centred on the right superior temporal gyrus. In the white matter, and after adjusting for total white matter volume, subjects with ‘High’ scores showed a reduction of volume of a region involving the superior longitudinal fasciculus, which also extended to involve the internal and external capsules (\( P < 0.002 \)) (Table 3; Fig. 1, lower). Moreover, there was a negative correlation between score on integrative signs and volume of all the grey and white matter deficit clusters (Pearson \( r \) range = −0.69 and −0.46; \( P \leq 0.002 \)), indicating that the higher the integrative score (that is, the more severe the NSS), the smaller the volume of these regions.

We then compared subjects High and Low at each of the three subscales that compose the Integrative scale: Sensory Integration, Motor Coordination and Motor Sequencing. When the analysis was conducted for the Sensory Integrative subscale alone, the results remained similar to those identified in the main analysis using the Integrative scale. The analyses for the Motor Coordination and the Motor Sequencing scales could not be carried out as the vast majority of subjects (34 (79%) in each scale) was negative for these signs.

Potential Confounders
As there were significant differences in NSS scores among ethnic groups, we ran these analyses again, including only subjects of White British ethnic origin (\( n = 20 \)). The clusters previously identified in association with High NSS remained significant even when only White British subjects were included (data not shown).

Furthermore, since IQ has previously been reported as associated with NSS, we also run a third set of confirmatory analyses additionally covarying for IQ. However, the addition of IQ as a covariate did not affect the results (data not shown).

Discussion
To our knowledge, this is the first study that has investigated the brain-wide grey and white matter anatomical correlates of NSS in healthy subjects. We have shown that higher rates of integrative NSS are associated with a reduction of grey matter volume of cortical areas (anterior cingulate, inferior frontal cortex, superior temporal cortex, middle temporal cortex).
gyrus, middle and superior temporal gyri). Our original hypothesis was therefore only partially confirmed. In fact, we did not find any association between higher rates of NSS and reductions of subcortical areas. This is in contrast with our previous study in patients with first episode psychosis, where higher rate of NSS were associated with a reduction of similar cortical areas, but also with a reduction of subcortical areas (Dazzan et al., 2004).

In the present study, an excess of integrative signs was associated with cortical grey matter reductions in frontal and temporal areas. Theoretically, integrative signs include sensory integrative, motor coordination and motor sequencing signs. However, in this group of healthy individuals, the score for the integrative signs almost entirely reflected deficits in sensory integration tests, as the majority of subjects did not show any abnormality in tests of motor coordination or motor sequencing. This was confirmed by our additional analysis using only the score on sensory integrative tests, which identified the same clusters as the main analysis of integrative signs. Therefore, these findings are comparable to those that we have reported in first episode psychosis subjects, where an excess of sensory integration signs is also associated with a volume reduction of these frontal and temporal areas (inferior frontal, middle temporal and superior temporal gyri) (Dazzan et al., 2004). It is possible that frontal and temporal abnormalities are the anatomical substrate for sensory integrative deficits in both psychotic and non-psychotic individuals. These areas are normally involved in attention, in auditory, tactile and language processes or in audio-visual integration (Calvert, 2001). They are part of a multimodal network involved in the integration of stimuli from different sensory modalities (Banati et al., 2000; Calvert et al., 2000; Downar et al., 2000), which may be impaired in subjects displaying more sensory integrative signs.

We found no association between an excess of either primary or integrative signs and any reductions in basal ganglia or other subcortical regions. This is in contrast with our previous findings in first episode psychosis patients, where an excess of all types of NSS was associated with basal ganglia volume reductions (Dazzan et al., 2004). This difference further suggests the involvement of the basal ganglia in psychosis, as indicated by neuropathological, neuropsychological and neuroimaging studies (Bilder et al., 1993; Hecker, 1997; Keshavan et al., 1998; Lawrie et al., 2001). Consistent with this, the patients with psychosis in our previous study also presented a much higher rate of motor dysfunction compared to the present healthy individuals (mean score: 2.0 in patients versus 0.3 in healthy subjects), and this was unrelated to antipsychotic use (Dazzan et al., 2004). Furthermore, in the healthy individuals we found that NSS were associated with white matter reductions (in a region involving the superior longitudinal fasciculus, extending to the internal and external capsules), which may reflect a disorganisation of the fibres connecting

**Figure 1.** Brain changes in subjects with 'High' soft signs in comparison to subjects with 'Low' soft signs for the 'Integrative' subscale. Regions of tissue deficit in patients with 'High' soft signs are shown in blue for grey matter (upper) and white matter (lower). Results are displayed on an averaged grey and white matter map. The left side of the image corresponds to the right side of the brain. Numbers refer to the approximate coordinate in the standard space of Talairach and Tournoux.
perisylvian frontal, parietal and temporal cortices, areas that work together to integrate sensory information (Catani et al., 2002). In contrast, in patients with psychosis we found that NSS were associated with an increase of the white matter of the internal capsule, which could represent a compensatory response to the reduction in basal ganglia volume (Lawrie and Abukmeil, 1998; Gaser et al., 1999; Dazzan et al., 2004).

Only one previous study has looked at brain correlates of NSS in healthy individuals and in patients with psychosis (Keshavan et al., 2003), using a region of interest approach. Consistent with our previous study (Dazzan et al., 2004), these investigators showed an association between sensory integrative signs and frontal and temporal volume reductions in patients. However, the study by Keshavan et al. (2003) did not find any association between NSS and brain changes in healthy individuals. This inconsistency may be related to methodological differences. First, we used automated voxel-based analysis rather than manual region of interest measurements. Inter-individual variations in anatomical landmarks may have made the partition of the heteromodal cortex difficult in the study of Keshavan et al. (2003), as they noted themselves. Second, sensory integration scores were higher in our study. This discrepancy may be related to differences between the two samples, in ethnic composition or IQ. Indeed, in the study by Keshavan et al. (2003) there were more Caucasian subjects than in our group. Caucasian healthy individuals have been reported previously as showing lower NSS rates than other ethnic groups (Buchanan and Heinrichs, 1989), and this was also observed in our healthy individuals, with White British subjects presenting lower rates of Integrative signs. However, in our study, even when only White British subjects were included in the analyses, an excess of integrative signs was associated with reductions of cortical grey matter in the same frontal and temporal areas. In both our and Keshavan and colleagues’ studies, some controls were recruited through advertisement, a method that may have selected a particularly motivated group with good levels of functioning. However, as we additionally used household visits, we may have recruited subjects more representative of the general population and with a more average functioning and IQ.

A major strength of our study is the use of high resolution MR images analysed with a voxel-based method that evaluates the entire brain as opposed to a few preselected regions. Voxel-based morphometry (VBM) offers several advantages over the region of interest approach. Although there has been some debate on the methodology (Ashburner and Friston, 2000, 2001; Bookstein, 2001), VBM methods have been extensively validated (Suckling et al., 1999a,b; Wright et al., 1999). Moreover, studies using our and other VBM approaches have produced relatively consistent results in studies of patients with psychosis and neurological disorders (Sigmundsson et al., 2001; Good et al., 2002; Kubicki et al., 2002; Shapleske et al., 2002), and are becoming more extensively used in the study of healthy individuals (Good et al., 2001; Tisserand et al., 2004; Kaasinen et al., 2005; Suzuki et al., 2005). However, some caution must be applied in the interpretation of our findings. First, using VBM one can localise a white matter area in standard anatomical space, expressing differences as number of voxels included in that region. Therefore, although the differences are expressed as number of voxels, they can be considered to indirectly provide a measure of volume. Indeed, this has been confirmed by studies that have used both VBM methods and more traditional region of interest approaches to measure volumes, the results of which have shown good correlation (Wright et al., 1999). Finally, the analysis cannot establish what the pathophysiological mechanisms underlying these volume changes are. In fact, what is detected as a change in volume could be, for example, the consequence of changes in tissue perfusion, fat or water content (Weinberger and McClure, 2002). Future studies should use also other methods of brain analysis, such as regions of interest (Keshavan et al., 2003) and evaluation of cortical surface area (Barta and Dazzan, 2003). In particular, since motor regions are thicker than association regions, and association regions are thicker than sensory regions, the latter method would clarify whether variations in surface area in these regions are associated with an excess of specific types of NSS.

A possible limitation of this study is its relatively small sample size. Reassuringly, our rates of NSS are similar to those reported in the literature on this topic in healthy subjects (Sanders et al., 1994; Ismail et al., 1998; Ross et al., 1998; Arango et al., 1999; Sevincok et al., 2004), and therefore our sample, even in its limited size, is representative of the general population.

In conclusion, this is the first study evaluating the brain-wide anatomical correlates of NSS in healthy individuals. We suggest that the previously described association between the excess of NSS and the smaller basal ganglia volume in psychosis is particularly informative of the brain abnormalities underlying this disorder, in which NSS are represented in excess; in contrast, the cortical brain structural changes associated with NSS may represent a common neuroanatomical substrate across healthy individuals and patients with psychosis.

Notes
The £ESOP study was funded by the Medical Research Council (UK). P.D. holds a NARSAD Young Investigator Award. We thank the Stanley Medical Research Institute for their support. We wish to thank the £ESOP researchers who helped with the data collection and the patients who took part to the study.

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References
Brain Correlates of NSS


