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ORIGINAL ARTICLE

Brainstem volumetric alterations in children with autism

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Background. Although several studies have examined brainstem volume in autism, results have been mixed and no investigation has specifically measured gray- and white-matter structures. The aim of this investigation was to assess gray- and white-matter volumes in children with autism.

Method. Subjects included 22 right-handed, non-mentally retarded boys with autism and 22 gender- and age-matched controls. Magnetic resonance imaging (MRI) scans were obtained using a 1.5-T scanner and volumetric measurements were performed using the BRAINS2 software package. Gray- and white-matter volumes were measured using a semi-automated segmentation process.

Results. There were no significant differences in age and total brain volume (TBV) between the two groups but full-scale IQ was higher in controls. A decrease in brainstem gray-matter volume was observed in the autism group before and after controlling for TBV. No significant differences were observed in white-matter volume. A significant relationship was observed between brainstem gray-matter volume and oral sensory sensitivity as measured by the Sensory Profile Questionnaire (SPQ).

Conclusions. Findings from this study are suggestive of brainstem abnormalities in autism involving gray-matter structures with evidence supporting the existence of a relationship between these alterations and sensory deficits. These results are consistent with previous investigations and support the existence of disturbances in brainstem circuitry thought to be implicated in the sensory dysfunction observed in autism.

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Introduction

Autism is characterized by impairments in the development of reciprocal social interaction, verbal/non-verbal communication and the presence of stereotyped behavior (APA, 2000). In addition to these deficits, known as core symptoms, there are several associated features, including under-reactivity and/or over-reactivity to sensory stimuli and self-stimulatory behaviors (APA, 2000). These associated symptoms, together with the core disturbances, have provided impetus to the development of a hypothesis proposing that autism is a disorder of sensory modulation best explained in terms of brainstem pathology (Ornitz, 1983). However, the data from neuropathologic and neuroimaging studies do not suggest that the pathology of autism is localized to a single area of the brain. Nevertheless, the contribution of specific key regions, such as the brainstem, to the repertoire of autistic symptomatology should be investigated.

Over the past two decades, evidence for brainstem abnormalities in autism has emerged from a variety of investigations, applying widely different methodologies. With the advent of electrophysiological techniques for audiologic and neurologic assessment, several auditory response studies were reported in autism, suggesting brainstem involvement (Klin, 1993). Structural magnetic resonance imaging (MRI) studies examining the brainstem reported morphometric abnormalities. Earlier imaging studies assessed its size using area measurements calculated from midsagittal images. Using this method, reductions in brainstem size were reported in some (Gaffney *et al*. 1988; Hashimoto *et al*. 1995; Ciesielski *et al*. 1997) but not all studies (Hsu *et al*. 1991; Garber & Ritvo, 1992; Kleiman *et al*. 1992; Piven *et al*. 1992; Elia *et al*. 2000). More recently, two volumetric MRI investigations

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observed no significant differences in the brainstem volume between individuals with autism and healthy controls (Hardan *et al*. 2001; Herbert *et al*. 2003); however, neither of these studies examined gray- and white-matter differences.

In light of the mounting evidence implicating the brainstem in the pathophysiology of autism and taking into consideration the limitations of the previous morphometric studies, the primary aim of the current study was to examine gray- and white-matter volumes of the brainstem in a sample of children with autism. In line with prior studies, we hypothesized that brainstem volume would be decreased in the autism group. A secondary aim of this study was to assess the clinical relevance of imaging findings by identifying significant relationships between brainstem structures and sensory abnormalities as measured by the Sensory Profile Questionnaire (SPQ; Dunn, 1999).

Method

Participants

Quantitative volumetric analysis was performed on brain MRI scans obtained from 44 boys: 22 with autism and 22 healthy controls (age range 8–12 years). The study was confined to boys because the sample size was too small to allow for the structural variability associated with gender. Subjects with autism were referred to a research clinic from the community and met the following inclusion criteria: (1) diagnosis of autism through expert clinical evaluation and two structured research diagnostic instruments, including the Autism Diagnostic Interview – Revised (ADI-R; Lord *et al*. 1994) and the Autism Diagnostic Observation Schedule (ADOS; Lord *et al*. 1989); and (2) absence of medical/neurological disorders. Those with autistic disorder met both ADI-R and ADOS criteria for autism. Subjects with pervasive developmental disorder, not otherwise specified (PDD NOS), had ADOS scores ranging from 7 to 10 while meeting ADI-R criteria for autism.

Control subjects consisted of medically healthy individuals recruited from the community through advertisements in areas socio-economically comparable to those of the families of origin of the participants with autism. Control subjects had a full-scale IQ (FSIQ) $\geqslant$70 and were screened by face-to-face interviews, questionnaires, telephone interviews and observation during psychometric tests. Individuals with a family history of any neuropsychiatric disorder (such as autism, learning disability, affective disorders and schizophrenia) were not included. Potential subjects with a history of birth asphyxia, head injury or a seizure disorder were also excluded. All control subjects had no present or lifetime history of psychiatric disorders and no learning disability as assessed by the Schedule for Affective Disorders and Schizophrenia for School-Age Children (Kaufman *et al*. 1997) and the Wide Range Achievement Test-Revised (Jastak & Wilkinson, 1985) respectively.

Evaluation of potential subjects also included obtaining a thorough history, physical examination, and laboratory testing when indicated. The Wechsler Intelligence Scale for Children was administered to measure cognitive functioning (Wechsler, 1991). The Hollingshead method was used to assess socioeconomic status (SES) of the family of origin of all participants (Hollingshead, 1975). The SPQ (Dunn, 1999) was obtained from parents for a better characterization of sensory processing dysfunction. In brief, the SPQ is a 125-item caregiver questionnaire reporting the frequency with which the child responds to various sensory experiences. The items are written such that low scores reflect greater symptom severity. For example, the frequency of a given behavior/symptom can be rated as never (5 points), seldom (4 points), occasionally (3 points), frequently (2 points), and always (1 point). Items of the SPQ generate nine summary factors: (1) sensory seeking, (2) emotionally reactive, (3) low endurance/tone, (4) oral sensory sensitivity, (5) inattention/distractibility, (6) poor registration, (7) sensory sensitivity, (8) sedentary, and (9) fine motor/perceptual.

After the procedures were fully explained, all subjects or their legal guardians provided written informed consent. Verbal assent was obtained from all subjects. The Institutional Review Board approved the methodology of the study, including MRI scanning of minors.

Measurements

MRI scans were acquired using a 1.5-T GE Signa MR Scanner (General Electric Medical Systems, Milwaukee, WI, USA). Final images for each subject were generated by obtaining T_1-, T_2- and PD-weighted images from all participants. The T_1-weighted spoiled GRASS (SPGR) sequence was acquired using the following parameters: slice thickness = 1.5 mm, slice number = 124, echo time (TE) = 5 ms, repetition time (TR) = 24 ms, flip angle = 40°, number of excitations (NEX) = 2, field of view (FOV) = 26 cm, matrix = 256 × 192. Both PD- and T_2-weighted images were obtained with the following parameters: slice thickness = 5.0 mm, TE = 96 ms for T_2 and 36 ms for PD, TR = 3000 ms, NEX = 1, FOV = 26 cm, matrix = 256 × 192 with an echo train length = 8. All images were obtained in the coronal plane. MRI data were identified by scan number alone to retain blindness of raters.

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Table 1. *Subject characteristics*

	Autism ($n=22$)		Control ($n=22$)		t test (df = 42)	
	Mean (range)	S.D.	Mean (range)	S.D.	t	p
Age (years)	10.7 (8.1–12.9)	1.4	10.5 (7.9–13.0)	1.4	0.318	0.752
Full-scale IQ	92.3 (64–128)	17.0	116.9 (91–134)	13.1	−5.381	<0.001
SES	4.5 (3–5)	0.59	4.41 (3–5)	0.59	0.522	0.604
TBV (cc)	1347 (1132–1608)	121	1357 (1208–1549)	100	−0.309	0.759

SES, Socio-economic status; TBV, total brain volume.

Image processing was performed on an SGI workstation (Silicon Graphics Inc., Mountain View, CA, USA) using the Brain Research: Analysis of Images, Networks, and Systems 2 (BRAINS2; University of Iowa, Iowa City, IA, USA) software package (Magnotta *et al*. 2002). Six brain-limiting points (anterior, posterior, superior, inferior, left and right) were then identified to normalize the image data to the standard Talairach stereotactic three-dimensional space (Talairach & Tournoux, 1988) in which the anterior–posterior commisure line specifies the x axis, a vertical line rising from the x axis through the interhemispheric fissure specifies the y axis, and a transverse orthogonal line with respect to x and y coordinates specifies the z axis.

After fitting the image sequences to a standard three-dimensional space, the voxels representing gray matter, white matter and cerebrospinal fluid were identified using a segmentation algorithm applied to the T_1-, T_2- and PD-weighted image sequences as described elsewhere (White *et al*. 2003). Measurements were performed using the BRAINS2 masks as generated by a neural network and corrected by manual tracing [intra-class correlation (ICC) >0.9]. Total brain volume (TBV) was defined as the cerebrum, cerebellum and brainstem while excluding cerebrospinal fluid. The brainstem was defined as the infra-tentorial brain tissue volume superior to the foramen magnum and excluding the cerebellar volume (Magnotta *et al*. 2002; Pfaendner *et al*. 2005).

Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences, version 16.0 (SPSS Inc., Chicago, IL, USA). Age, FSIQ, SES and all volumetric measures were compared using Student's t test with a two-tailed statistical significance level set at $p<0.05$.

Pearson's correlations were applied to examine potential relationships between brainstem volumes (total and also gray and white matter) with TBV and FSIQ. Analysis of covariance was applied to examine volumetric differences between the two groups while controlling for any confounding factors. SPQ summary factors were compared using Student's t test with a corrected significance level of $p<0.005$ (two-tailed). Partial correlations were used to examine the relationships between brainstem volumes and sensory abnormalities while controlling for subject group and FSIQ. Spearman's rank order correlation was used to examine the existence of any relationship between clinical measures as assessed by the ADI-R and brainstem volumes. This was used because the continuous nature of the scores on the ADI-R items has not been established (e.g. ADI-R domains/items are not intended for use as scales).

Results

The autism group included 18 individuals with autistic disorder and four with PDD NOS. Group-wise comparisons between the autism and control groups revealed no significant differences in age, SES and TBV; however, FSIQ was significantly higher in controls (Table 1).

Correlations were observed between total brainstem volume and TBV in both groups (autism: $R=0.516$, $p=0.014$; controls: $R=0.613$, $p=0.002$) but not between total brainstem volume and FSIQ (autism: $R=-0.007$, $p=0.977$; controls: $R=-0.326$, $p=0.139$) (Table 2). Reductions in brainstem volumes were observed in the autism group when compared to controls (Table 3). Decreased gray-matter volume was observed before and after controlling for TBV. Differences in total brainstem volumes reached statistical significance only after controlling for TBV.

The SPQ findings are summarized in Table 4. Data from four participants were not available. All summary factors were significantly lower in subjects with autism indicating more abnormalities in this group. The relationships between brainstem volumes and sensory measures were examined. When controlling for subject groups, an association was observed

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Table 2. *Correlations between total brainstem volume and Full-scale IQ as well as total brain volume (TBV) in the autism and control groups*

		Brainstem gray matter	Brainstem white matter	Total brainstem volume
Autism group ($n=22$)				
Full-scale IQ	Pearson	0.384	−0.390	−0.007
	p (two-tailed)	0.078	0.073	0.977
TBV	Pearson	0.194	0.448*	0.516*
	p (two-tailed)	0.386	0.037	0.014
Control group ($n=22$)				
Full-scale IQ	Pearson	0.066	−0.524*	−0.326
	p (two-tailed)	0.770	0.012	0.139
TBV	Pearson	0.608**	0.353	0.613**
	p (two-tailed)	0.003	0.107	0.002

* Correlation is significant at the 0.05 level (two-tailed).
** Correlation is significant at the 0.01 level (two-tailed).

Table 3. *Gray- and white-matter brainstem volumes*

	Autism ($n=22$)		Control ($n=22$)		t test (df = 42)		TBV as covariate [df = (2, 41)]	
	Mean (range)	S.D.	Mean (range)	S.D.	t	p	F	p
Gray	19.05 (13.71–25.17)	3.39	21.44 (14.30–26.07)	2.74	−2.58	0.014	6.80	0.013
White	17.33 (11.69–27.11)	3.40	17.55 (13.35–24.96)	3.17	−0.22	0.828	0.01	0.920
Total	36.38 (28.64–41.56)	4.23	38.99 (31.43–49.33)	4.54	−1.97	0.055	4.57	0.039

TBV, Total brain volume; df, degrees of freedom; S.D., standard deviation.

Table 4. *Sensory Profile Questionnaire (SPQ) findings in children with autism and controls*

	Autism ($n=21$)		Control ($n=19$)		t test (df = 38)	
Summary factors	Mean	S.D.	Mean	S.D.	t	p
Sensory seeking	61.6	8.4	78.4	5.9	−5.711	0.000
Emotionally reactive	42.9	11.8	74.9	4.5	−8.836	0.000
Low endurance/tone	30.3	9.3	43.8	1.9	−4.930	0.000
Oral sensory sensitivity	27.1	9.1	44.1	2.5	−6.189	0.000
Inattention/distractibility	17.4	2.8	31.5	3.9	−10.406	0.000
Poor registration	28.5	2.9	38.3	1.6	−10.066	0.000
Sensory sensitivity	16.6	3.5	19.5	1.0	−2.766	0.001
Sedentary	7.6	2.84	16.3	3.8	−6.474	0.000
Fine motor/perceptual	11.3	2.4	13.1	2.1	−1.978	0.001

df, Degrees of freedom; S.D., standard deviation.
Lower scores reflect greater symptom severity.

between brainstem gray matter and oral sensory sensitivity factor ($R=0.458$, df = 37, $p=0.003$) (Fig. 1). This relationship remained unchanged when controlling for subject group and FSIQ ($R=0.443$, df = 36, $p=0.005$). No correlations were observed between brainstem white-matter volume and any sensory factors.

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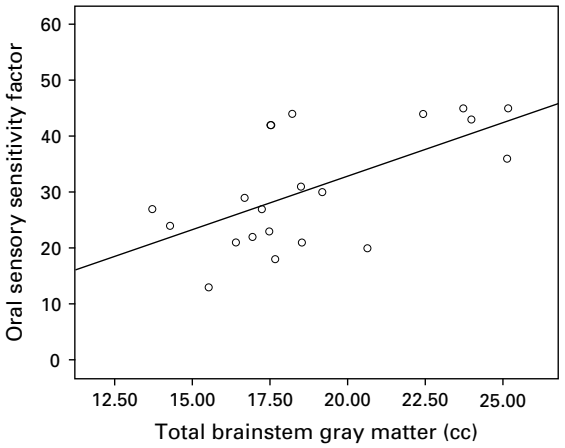

Fig. 1. Relationship between brainstem gray-matter volume and oral sensory sensitivity in the autism group as measured by the Sensory Profile Questionnaire (SPQ) ($r = 0.377$).

Finally, no associations were found between any of the three main domains of the ADI-R (qualitative abnormalities in reciprocal social interaction; qualitative abnormalities in communication; and restricted, repetitive and stereotyped patterns of behavior) and brainstem volumes.

Discussion

This study provides evidence of brainstem gray-matter volumetric reductions in a group of male children with autism and points to the existence of a relationship between this structure and sensory abnormalities. These observations are consistent with several previous neuroimaging studies that examined this structure in autism, but used only the midsagittal slice to measure the size of the brainstem and its three subdivisions: midbrain, pons and medulla. Hashimoto *et al*. (1995) reported significant hypoplasia of brainstem structures using a large sample of patients with autism (102 cases). An earlier MRI study reported that the entire brainstem and pons were significantly smaller in a group of autistic youth when compared to controls (Gaffney *et al*. 1988). A more recent investigation observed a reduction in the size of the pons but did not report on the total size of the brainstem (Ciesielski *et al*. 1997). By contrast, several studies have failed to report findings similar to that of the current investigation; however, these investigations limited their measurements to subdivisions of the brainstem (i.e. pons only or pons and midbrain), which could account for the differences in findings (Hsu *et al*. 1991; Garber & Ritvo, 1992; Kleiman *et al*. 1992; Piven *et al*. 1992; Elia *et al*. 2000).

Although many previously published studies have used the midsagittal slice to measure brainstem size, two recent reports have in fact examined the volume of this structure (Hardan *et al*. 2001; Herbert *et al*. 2003). These investigations did not detect any volumetric alterations of the brainstem in autism, but differences in sample characteristics and study methodology may explain the inconsistent findings. Hardan *et al*. (2001) reported on a sample of adolescents and adults with autism who had a mean age of 22 years, *versus* 10.5 years in the current study. In addition, volumetric measurements were performed manually and no gray-/white-matter segmentation was conducted. In the more recent study by Herbert *et al*. (2003), neuroanatomic segmentation was applied on scans obtained from a sample of 17 boys with autism (age range 7–11 years) and 15 age- and gender-matched controls. No volumetric alterations were detected between the two groups and gray/white segmentation could not be performed reliably (Herbert *et al*. 2003). In light of these inconsistencies, additional research is warranted to determine whether brainstem volumetric alterations exist in autism.

The role of the brainstem in the pathophysiology of autism has long been suspected. Specific clinical features such as under/over-reactivity to sensory stimuli, self-stimulatory behaviors (APA, 2000) and even deficits in social interactions and communication have supported a hypothesis linking autism to dysfunctional brainstem sensory modulation (Ornitz, 1983). The diversity of deficits possibly resulting from brainstem abnormalities is consistent with its complex neuroanatomy; it is the source of various neurotransmitter systems thought to be implicated in autism, including serotonergic, cholinergic and gamma-aminobutyric acid (GABA)ergic systems (Pickett & London, 2005). Moreover, the brainstem houses the nuclei of the vagus nerve, which has extensive enervation to many parts of the body such as the heart, gastrointestinal system and larynx/mouth (Nolte, 2002). This nerve controls varied tasks such as heart rate, gastrointestinal peristalsis and several muscle movements in the mouth, including speech and eating. Impairments in all these domains have been reported in autism (Volkmar *et al*. 2005). However, the most direct evidence of brainstem abnormalities comes from post-mortem investigations that have reported abnormal inferior olives (Bauman & Kemper, 1985; Bailey *et al*. 1998), reduction of neurons in the facial nucleus, shortened brainstem and absent superior olive (Rodier *et al*. 1996). Moreover, Bauman & Kemper (1985) reported that neurons appeared larger in youth with autism and smaller in adults with the disorder. Additional post-mortem investigations reported on a variety of histological abnormalities in the brainstem, including enlarged arcuate nuclei in the medulla (Bailey *et al*. 1998) and a major reduction

of neuron numbers in the facial nuclei (Rodier *et al*. 1996).

Support for brainstem abnormalities as contributors to the pathophysiology of autism also comes from the fields of teratology and embryology. Prenatal exposures to medications have also suggested the existence of a relationship between brainstem abnormalities and autism. There is an early period in embryonic development (<6 weeks) when the forebrain is absent and brainstem and cranial nerve nuclei are developing; this results in a vulnerable period when chemical contact could be neurotoxic (Rodier *et al*. 1996). For instance, exposure to thalidomide *in utero* (20–24 days of gestation) has been associated with the development of autistic features (Stromland *et al*. 1994). A report of misoprotol exposure during the sixth gestational week has also reportedly led to an increased risk of autism in children with Möbius sequence (Miller & Ventura, 2001; Bandim *et al*. 2003). This link between medication exposure early in the pregnancy and autism has led to speculation that brainstem injury sustained *in utero* plays a role in the development of autism (Rodier, 2002).

Decreased gray-matter volume in the brainstem observed in the current study provides indirect support for the potential contribution of altered cortico-cerebellar and brainstem–cerebellum networks to the pathophysiology of autism. Higher cognitive functions rely on communications between the cerebral cortex, brainstem and cerebellum; and it has been hypothesized that some core symptoms of autism are related to the disconnection between these brain regions (Skoyles, 2002). Additionally, specific behavioral abnormalities reported in autism could be related to brainstem anomalies. Studies comparing eye-blink conditioning (with individuals with autism developing the conditioned response more rapidly than controls) are suggestive for alterations of the brainstem–cerebellum loops (Sears *et al*. 1994). Although other behaviors such as visual orientation and facial expression have been linked to brainstem alterations, no associations were observed in the current study between these clinical features and structural findings. These observations could be related to the limitation of the current morphometric software, including the inability to generate midbrain, pons and medulla measurements, or to the contribution of other brain regions to gaze and facial abnormalities.

Findings reported in this study must be interpreted in the context of several methodological limitations. The two groups were not matched on FSIQ, with the autism group having lower scores than controls. The relationship of cognitive functioning and brain structures in autism is complex and it remains unclear whether matching for IQ is necessary (Jarrold & Brock, 2004). Limited evidence is available to support a relationship between cognitive functioning and brain structures in autism, as suggested by the existence of increased brain size in individuals with low IQ (Cody *et al*. 2002). Additionally, the difficulty with IQ-matching is that the cognitive profiles in individuals with autism are markedly uneven (Joseph *et al*. 2002). Therefore, matching groups on FSIQ may ensure that only the group averages are not significantly different, but matching on all subsets is clearly impractical (Hobson, 1991; Happe, 1994; Joseph *et al*. 2002; Jarrold & Brock, 2004).

In addition to the above limitations, the autism sample was relatively heterogeneous, consisting of a majority of children with autistic disorder and a minority with PDD NOS. Including these individuals in one autism group assumes that these disorders have a common neurobiological underpinning, an assertion that currently lacks strong scientific support as PDD NOS continues to be considered a separate disorder according to DSM-IV (APA, 2000). In addition, the accuracy and validity of gray- and white-matter segmentation of the brainstem have not been confirmed by post-mortem studies and the image analysis procedure used to measure these structures was semi-automated, and was not confirmed by traditional manual tracing methods. Finally, this study did not separate the brainstem into individual components (i.e. midbrain, pons and medulla), which limits more specific localization of gray-matter reductions and does not allow direct comparison with previous studies that have examined brainstem subdivisions.

Although there are several studies examining brainstem volume in autism, results have been inconsistent and no studies have measured gray- and white-matter volumes separately. The findings from this study are suggestive of brainstem abnormalities in autism involving gray-matter structures and are supported by prior neuropathologic investigations. This reduction may possibly point to disconnectivity between the brainstem and cerebrum and cerebellum. However, in light of the limitations of this investigation, additional studies are needed before any conclusions can be drawn about gray-matter volumes of the brainstem in autism. Cross-sectional and longitudinal studies are warranted in large samples of individuals with autism and a wide range of intellectual abilities to examine the three subregions of the brainstem using well-validated segmentation techniques.

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Declaration of Interest

None.

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