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BRIEF REPORT

Brief Report: Alterations in Cerebral Blood Flow as Assessed by PET/CT in Adults with Autism Spectrum Disorder with Normal IQ

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Abstract Specific biological markers for Autism Spectrum Disorder (ASD) have not yet been established. Functional studies have shown abnormalities in the anatomo-functional connectivity of the limbic-striatal "social" brain. This study aimed to investigate regional cerebral blood flow (rCBF) at rest. Thirteen patients with ASD of normal intelligence and ten IQ-, sex- and age-matched healthy controls (HC) underwent PET/CT using [1-^{11}C]butanol, a perfusion tracer. As compared to HC, ASD showed significant CBF increases in the right parahippocampal, posterior cingulate, primary visual and temporal cortex, putamen, caudatus, substantia nigra and cerebellum. No statistically significant correlation between CBF and IQ was found. The limbic, posterior associative and cerebellar cortices showed increased blood flow in ASD, confirming previous findings about the neurobiology of ASD.

Keywords High functioning autism · PET/CT · [1-^{11}C]butanol · Cerebral blood flow

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Introduction

Autism spectrum disorder (ASD) is defined by impairments in social-communicative interaction, in understanding other people's complex emotions and in automatically processing social information and responding to it (Frith and Frith 2003), in addition to restricted patterns of behaviour and interests. However, most people with ASD demonstrate abnormalities in additional areas such as perception (Andersen et al. 2011) and motor functioning, which prevail into adulthood (Sahlander et al. 2008). Altered functional connectivity within/between functional territories and pathways has been suggested as possible explanation for ASD (Gepner and Féron 2009).

Functional and anatomical studies in ASD have shown localized metabolic alterations affecting various cortical and subcortical regions. However, no common regional abnormalities have been found across cerebral blood flow (CBF) or cerebral glucose metabolism (CMR_{gl}) studies even though a consensus judgement suggests focal hypoperfused areas in the thalamus, basal ganglia, parietal, temporal lobes, and cerebellum (Rumsey and Ernst 2000). The discrepant results are possibly due to the lack of homogeneity across studies (i.e. differences in age, IQ, verbal skills, handedness, socio-economical status, working capabilities, diagnostic issues and medication) and poorly matched control groups.

The aim of this study was to investigate the functional status at rest in highly-functioning subjects with ASD as compared to an IQ-, sex- and age-matched control group. The study was performed by assessing CBF using Positron Emission Tomography/Computerised Tomography (PET/CT) with in-house produced [1-^{11}C]butanol.

Methods

Participants

Thirteen adults with ASD and normal intelligence and ten matched healthy controls were included in the study. Demographic data are shown in Table 1. The study was approved by the local Ethics and Radiation Safety Committees. Written informed consent was obtained from all participants.

ASD Group

Six females and seven males were included in the study. Ten of the thirteen subjects had a social style defined as active odd, while three were characterized as loners or aloof (Wing 1997).

At the time of the study, eight of the subjects received a disability pension or were on sick leave, whereas the others were either studying or held a job. Ten had completed upper secondary school. Mini international neuropsychiatric interview (M.I.N.I.) was used to determine psychiatric comorbidity (Sheehan and Lecrubier 2006). Eight subjects had a psychiatric co-morbidity. Six were treated with psychotropic drugs (antidepressants SSRI/SNRI, n = 5; anxiolytics/hypnotics, n = 3; stimulants, n = 2).

Control Group

Ten IQ-, sex- and age-matched individuals without physical disorders or mental disabilities according to the Structured Clinical Interview for Diagnostic Statistical Manual of Mental Disorders 4th edition (DSM-IV) Axis I Disorders (SCID-I) and SCID-II were the healthy controls (HC). The exclusion criteria for all participants were alcohol and substance-abuse, intellectual disability, epilepsy, psychosis, brain damage or neurological disorders.

Neuropsychiatric Assessment

The diagnoses of all ASD subjects were based on extensive interviews, rating scales and parental interviews at a specialized neuropsychiatric unit (Rydén and Bejerot 2008). They were further confirmed by the Autism Diagnostic Observation Schedule (ADOS-G) (Lord et al. 2000) and supported by the self-administered Ritvo Autism and Asperger Diagnostic Scale Revised (RAADS-R) (Andersen et al. 2011), and Autistic Quotient (AQ) (Baron-Cohen et al. 2001).

Functional levels were assessed with the DSM-IV Global Assessment of Functioning (GAF) (American Psychiatric Association 1994). Full scale IQ and all neuropsychiatric tests, with the exception of AQ, were administered to all participants.

Table 1 Demographics and neuropsychologic data for subjects with autism spectrum disorders (ASD) and healthy controls

Variable	ASD (n = 13)	Healthy controls (n = 10)	*p*
Sex (female:male)	6:7	5:5	0.85
Age, mean (range, SD)	31.8(20–48, 8.6)	28.5(20–42, 7.5)	0.35
Female, mean (range, SD)	27.7(20–38, 7.1)	31.8(20–42, 8.1)	0.38
Male, mean (range, SD)	35.3(25–48, 8.8)	25.2(20–34, 5.8)	0.05
WAIS-III-R, IQ			
Full scale IQ, mean (range, SD)	104.2(87–135, 17.1)	115.7(99–134, 10.8)	0.08
Verbal IQ, mean (range, SD)	105.3(83–133, 16.4)	114.6(94–135, 13.2)	0.18
Performance IQ, mean (range, SD)	101.5(82–134, 17.6)	114.2(102–130, 9.9)	0.07
Smoking (yes:no)	3:9*	2:8	0.96
Handedness (right:left)	12:1	9:1	0.85
Civil status (single:cohabit)	12:1	6:4	0.04
Have children (yes:no)	0:13	3:7	0.02
University education (yes:no)	5:8	8:2	0.02
In full time work/studies (yes:no)	3:10	10:0	<0.0001
GAF total mean (range, SD)	54(40–65, 7.5)	86(80–100, 7.4)	<0.0001
RAADS-R mean (range, SD)	110(73–163, 29)	20(1–46, 15)	<0.0001
AQ mean (range, SD)	29(18–40, 6.9)	Not assessed	

SD standard deviation, *WAIS-III-R* Wechsler Adult Intelligence Scale-III-Revised, *IQ* intelligence quotient, *GAF* General assessment of functioning, *RAADS-R* Ritvo autism and asperger diagnostic scale revised, *AQ* autistic quotient, * Missing data in one subject with ASD

Radiopharmaceutical

[1-^{11}C]Butanol was produced via the reaction of a Grignard reagent, with cyclotron-produced carbon-11 labelled carbon dioxide, followed by reduction with lithium aluminum hydride by an adaptation of the method in Brodack et al. (1988).

Scanning Protocol

The examinations were performed at rest in a lit room with eyes closed on a Siemens Biograph 64 Positron Emission Tomography/Computed Tomography (PET/CT) scanner, with a spatial resolution of about 4 mm. A bolus of [1-^{11}C]butanol (about 300 MBq) was injected manually in about 1–2 s simultaneously as the PET acquisition was started and data were acquired in the list mode for 5 min.

We identified in all patients the interval between 40 and 100 s after injection as the window with the maximal number of events from which raw data should be extracted and summed to reconstruct the images to be analysed. During this interval the [1-^{11}C]butanol uptake reached a plateau before starting to decrease with time.

The protocol implemented was chosen to keep the time in the scanner to the absolute maximum of 10 min enabling patients to comply with the demands of functional neuroimaging without any impact on the results. None of the subjects showed any anxiety spikes or panicked.

Statistical Analysis

Voxel-based analysis was performed using SPM2 (Wellcome Department of Cognitive Neurology, London, U.K.). Images of relative tracer distribution were spatially normalised into the stereotactic Montreal Neurological Institute (MNI) space to a predefined PET template. After global normalisation, images were smoothed with a Gaussian filter (8 mm FWHM) to account for individual gyral differences and brain anatomy. Correction of SPM coordinates to match the Talairach coordinates was achieved by the subroutine implemented by Matthew Brett (http://imaging.mrc-cbu.cam.ac.uk/imaging/CbuImaging). Brodmann areas were then identified, after importing the corrected coordinates, by Talairach Daemon Database (http://www.talairach.org/daemon.html) at a range of 0–2 mm from the isocenter.

Group analyses between ASD and HC and between patients with and without medication were performed by the "compare populations: one scan per subject (ANCOVA)" model. The "single subject: covariates only" model was used to correlate the CBF in all participants and their IQs adjusting for age, sex and educational level. Significant differences between the groups were thresholded at $p$corrected < 0.001 at cluster level and $p$uncorrected < 0.01 at voxel level. Only clusters containing more than 64 voxels ($8 \times 8 \times 8$ mm) were considered to be significant. Proportions of categorical variables at baseline were compared using the chi-square tests and frequency data were computed through the Fisher's exact test. Values of continuous measures were compared using the t test. The p-level was set to 0.05.

Results

As expected the scores of RAADS-R and GAF differed significantly between ASD subjects and HC (see Table 1).

Compared to HC, ASD showed CBF increases in the right limbic structures, namely the parahippocampal (Brodmann Areas, BA 28) and posterior cingulate (BA 30) cortex, as well as visual cortex (BAs 17) and parieto-temporal (BAs 37, 38, 39) cortex, putamen, caudatus, substantia nigra and cerebellum (see Table 2; Fig. 1). When ASD data were subtracted from those of controls, no statistically significant difference was found.

Analyses of the medicated versus non-medicated patients and the correlation analysis between CBF and IQ performed on all participants revealed no significant differences and did not impact on the CBF differences.

Discussion

The main finding of this preliminary study was an increased resting CBF in ASD in a relatively large portion of the right cerebral and cerebellar hemispheres including regions belonging to the so-called limbic-striatal system.

The CBF increases found in the right parieto-temporal lobes, in the parahippocampal gyrus and in the primary visual cortex may be due to a heightened emotional status, an increased processing of visual information and a more laborious engagement of cognitive functions of ASD subjects compared to HC. Though high-functioning individuals with ASD have social impairments, explicit intellectual skills processed mainly in the amygdalo-hippocampal junction are preserved (Critchley et al. 2000). Maybe our ASD subjects implemented explicit cognitive strategies to assist them during the PET examination.

These results are in agreement with previous PET studies that have shown a global increase in metabolism and abnormalities in networking across frontal cortex, parietal cortex and subcortical regions (Rumsey et al. 1985).

The increased cerebellar CBF in high-functioning ASD (Table 2) is suggestive of its role in firing neuronal activity in the brain stem and in systems involved in emotions and higher cortical functions and skills (Rumsey and Ernst

Table 2 Results for CBF increases in ASD as compared to HC

Cluster level		Voxel level		Talairach coordinates			Regions and Brodmann Areas	Range (mm)
p(cor)	K	Z-score	*p*(unc)	x	y	z		
0.001	6,408	3.12	0.001	18	−45	−12	R Cerebellum; Culmen	0
		2.84	0.002	29	−64	−16	R Cerebellum; Declive	0
		2.78	0.003	22	−14	−27	R Uncus; BA 28	1
		2.75	0.003	31	3	−4	R Putamen	1
		2.66	0.004	10	−18	−8	R Substantia Nigra	0
		2.58	0.005	46	7	−12	R Superior Temporal Gyrus; BA 38	1
		2.49	0.006	19	−76	14	R Cuneus; BA 17	0
		2.47	0.007	29	−58	−40	R Cerebellum; Cerebellar Tonsil	0
		2.46	0.007	45	−59	8	R Middle Temporal Gyrus; BA 39	2
		2.41	0.008	47	−62	−13	R Fusiform Gyrus; BA 37	1
		2.32	0.010	26	−64	9	R Posterior Cingulate; BA 30	1
		2.31	0.010	8	2	−1	R Nucleus Caudatus	1

p(cor) corrected *p*-value, *K* cluster size in voxels, *p(unc)* uncorrected *p*-value, *R* right, *BA* Brodmann area

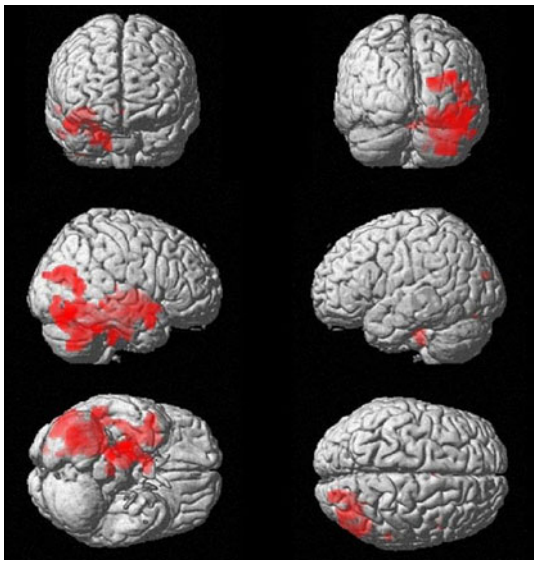

Fig. 1 3D rendering showing those regions in which CBF was significantly higher in ASD (n = 13) as compared with HC (n = 10). *Top left* frontal view, *top right* posterior view, *middle left* right-side view, *middle right* left-side view, *left down* view from below, *right down* view from above

2000). The CBF increase in the primary visual cortex is consistent with increased metabolism found in the calcarine cortex in ASD (Siegel et al. 1992).

Mean CMR_{gl} in highly functioning adult ASD was reported to be higher than in HC by Heh et al. (1989) and also in dispersed regions by Siegel et al. (1992). Other PET studies either failed to find differences between ASD and HC (Buchsbaum et al. 1992) or reported reduced metabolism in patients with intellectual disability and other comorbid conditions (DeLong and Heinz 1997).

The majority of the Single Photon Emission Computed Tomography (SPECT) studies in ASD, showing decreased or unchanged CBF distributions, have been performed on low-functioning individuals with various comorbidities and sedation (Zilbovicius et al. 1992; Chiron et al. 1995). This could explain the widely varying results of increased or normal CBF in high-functioning ASD compared to those with low IQ. This latter is suggested to play a key role in differentiating ASD subgroups (Witwer and Lecavalier 2008).

The CBF at rest reflects the baseline state of the brain, always active even during repose periods. In neuropsychiatric studies not employing specific brain activations during the scan, the subjects will process information and elaborate concepts and sensations. This resting activity might have contributed to the group differences found here.

Brain activation findings would suggest that subjects with ASD use different networks of activity and secondary strategies than HC and utilize alternative brain circuitry, possibly also at rest (Hazlett et al. 2004).

The experimental conditions during PET scanning may have caused a preoccupation and/or hypersensitivity to the external environment (Kennedy et al. 2006) in the ASD subjects, along with, consistent with the "central coherence theory" of autism (Frith and Happé 1994), a particularly high activation of visuospatial processes due to the new situation and to the amount of details perceived. This low-level enhanced perception is specifically processed in temporal-occipital regions (O'Connor and Kirk 2008) and might be absent in subjects with intellectual disability or in sedated subjects.

A CBF increase in the right hemisphere is compatible with proficiencies in visuo-spatial functions and with low scores in functions attributed to the left hemisphere. Using

quantitative ^{133}Xe-SPECT Chiron et al. (1995) found a higher CBF in the right hemisphere of ASD subjects at rest, with an inversion of hemispheric laterality compared to HC. Neuroanatomical differences between groups may also contribute to the CBF changes observed. Thicker grey matter has been reported in the temporal lobe (Hardan et al. 2006) and in the cerebellum. Just et al. (2004) hypothesized that in ASD the "under-connectivity" is due to processing centres not developing adequate connections, which causes potential association areas to develop independently and become hyper-specialized. Hence, the elevated CBF and/or CMR_{gl} found in some brain areas may derive from increased neuronal activity in redundant and poorly integrated circuits (Schwartz 1993), especially in domains most dependent on highly coordinated somato-sensory association processes (Herbert 2005).

Butanol as a blood flow tracer labelled with both ^{11}C and ^{15}O was validated two decades ago for human studies (Herscovitch et al. 1987; Quarles et al. 1993, for review see Saha et al. 1994) and was reported to more accurately measure rCBF than [^{15}O]water (Raichle et al. 1976). Additionally, the longer half-life of carbon-11 (20 min vs. 2 min for ^{15}O) facilitated transportation of multi-patient doses from the cyclotron lab to the clinical PET-CT, which was instrumental in enabling the performance of this study.

Findings in the previous ASD imaging literature have reported jeopardized cortical and subcortical abnormalities in flow and metabolism and have failed to identify any patterns specific for the disorder. This is possibly also due to inhomogeneity between patient and controls investigated across studies. Further inconsistencies might derive from the scanning and image analysis methodology used. Moreover, the high costs of functional neuroimaging often limit recruitment to an inadequate number of subjects, which increases the likelihood of Type II statistical errors.

In this investigation both the ASD and HC groups were specifically recruited for the study, and all underwent the same neuropsychological and neuropsychiatric tests. This might have reduced the contamination from confounding factors and of variables out of control. In order to investigate how our findings extend to other groups within the autistic spectrum, studies on larger and equally well-characterized cohorts of subjects will be needed.

Conclusion

In this preliminary study significant CBF differences were found between highly functioning ASD subjects and healthy controls in part of the posterior right hemisphere in limbic, posterior associative, visual and cerebellar cortices. This underscores the involvement of these regions in the phenotypic expression of the disorder and raises methodological and diagnostic issues in the evaluation of the heterogeneous findings in functional and neuroanatomical investigations on ASD.

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