NeuroReport 10, 1647–1651 (1999)

The neuroanatomy of autism: a voxel-based whole brain analysis of structural scans

Frances Abell, Michael Krams,[1] John Ashburner,[1] Richard Passingham,[2] Karl Friston,[1] Richard Frackowiak,[1] Francesca Happé,[3] Chris Frith[1] and Uta Frith[CA]

Institute of Cognitive Neuroscience and Department of Psychology, UCL, 17 Queen Square, London WC1B 3AR; [1]Wellcome Department of Cognitive Neurology, Institute of Neurology, UCL, 12 Queen Square, London WC1N 3BG; [2]Department of Experimental Psychology, University of Oxford, South Parks Road, Oxford; [3]SGDP Institute of Psychiatry, 111 Denmark Hill, London SE5 8AF, UK

[CA]Corresponding Author

AUTISM is a biological disorder which affects social cognition, and understanding brain abnormalities of the former will elucidate the brain basis of the latter. We report structural MRI data on 15 high-functioning individuals with autistic disorder. A voxel-based whole brain analysis identified grey matter differences in an amygdala centered system relative to 15 age- and IQ-matched controls. Decreases of grey matter were found in anterior parts of this system (right paracingulate sulcus, left inferior frontal gyrus). Increases were found in posterior parts (amygdala/peri-amygdaloid cortex, middle temporal gyrus, inferior temporal gyrus), and in regions of the cerebellum. These structures are implicated in social cognition by animal, imaging and histopathological studies. This study therefore provides converging evidence of the physiological basis of social cognition. *NeuroReport* 10:1647–1651 © 1999 Lippincott Williams & Wilkins.

Key words: Amygdala; Anterior cingulate; Autism, Social cognition; Structural MRI

Introduction

Autism is a biologically based disorder with a behavioral definition, spanning a wide range of manifestations and showing characteristic impairments in social communication [1]. While the majority of patients suffer from intellectual retardation and often have little or no useful language, explorations of the cognitive impairments in autism have focused on high-functioning individuals, in whom general intellectual and linguistic impairment is not a contributory factor [2]. High-functioning individuals, often labeled as having Asperger syndrome, appear to suffer from the relatively subtle developmental consequences of a cognitive deficit in 'theory of mind', a deficit that accounts well for the more conspicuous social impairments of children with severe forms of autism.

Recently, a PET scan study contrasted tasks where reasoning about other minds and the attribution of mental states is essential with tasks where it is not [3]. A circumscribed region on the border of medial frontal cortex and anterior cingulate was found to be specifically active for theory of mind tasks in normal volunteers but was significantly less active in individuals with Asperger syndrome. However, little is known about the link between this functional deficit and the underlying neuroanatomy.

The evidence from histopathological studies suggests abnormalities in the limbic system and in the cerebellum, in both low- and high-functioning patients with autistic disorder [4,5]. Reduced numbers of Purkinje cells in the cerebellum, reduced neuronal cell size and increased cell packing density have been identified in the hippocampal complex, subiculum, entorhinal cortex, amygdala, mamillary body, medial septal nucleus and anterior cingulate gyrus. The limbic abnormalities have been related to lesion studies in primates where removal of the amygdala results in decreases in affiliative behavior, social communication and emotional response to other animals [6]. Neonatal lesions of amygdala and hippocampus in monkeys produce a pattern of social withdrawal that has led to an animal model for autism [7]. In humans, amygdala damage has been found to cause abnormal affect, impaired face recog-

nition and impaired memory for the emotional content of stories [8]. While these data do not speak directly to autism, a case study of a boy with left hemisphere temporal oligodendroglioma, reported a reduction of autistic features following tumor resection [9]. Indirect evidence for limbic system abnormalities in autism also comes from studies of tuberous sclerosis where the presence of lesions in the temporal lobe predicts autistic dysfunction [10].

Despite an impressive number of brain imaging studies attempting to isolate brain regions or pathways specifically implicated in autistic disorder, the literature remains inconclusive. A number of methodological problems limit the conclusions of previous work. First, the populations studied are heterogeneous with a wide range of severity; second, appropriate control groups matched for age, general IQ level and other medical conditions are often lacking; third, the scanning methods used have been qualitative rather than quantitative. One way forward is to collect samples of specific ages, investigated with relevant neuropsychological measures, and to use controls who are matched for age, gender, health and IQ. Furthermore it is important to use unbiased methods of quantification that are not restricted to *a priori* regions of interest. The present structural MRI study takes into account these requirements.

Materials and Methods

Participants: The participants were 15 young adults, 12 males and three females, who met the criteria of autism in childhood according to DSM-IV [1]. They also conformed in their current presentation to clinical descriptions of Asperger syndrome in showing high verbal ability and only mild signs of communication impairment. None suffered from any additional medical condition. They were matched to 15 normal volunteers on sex, handedness, age and performance on tests of verbal and non-verbal ability. The average age was 28 years and 9 months (s.d. 6.6) and 25 years and 4 months (s.d. 3.1) for autism and control groups respectively. The average raw scores on a verbal (picture vocabulary) test (maximum score = 50) [11] were 42.5 (s.d. 6.6) and 45.2 (s.d. 2.9) The average raw scores on a non-verbal (matrices) test [12] were 48.9 (s.d. 8.9) and 52.2 (s.d. 5.7). All participants gave written informed consent to participate in the project, which was approved by the local hospital ethical committee.

To check for the extent of persistent if subtle impairments in 'theory of mind' in the autism group, a battery of standard tasks was used: the Sally-Ann and Smarties test, both requiring attribution of a first-order false belief; the Ice-cream and the Birthday puppy test, both requiring attribution of a second-order false belief (e.g. Mary does not know that John knows that...). Consistent with previous findings, the high-functioning participants in the present group all passed the first-order false belief tasks, but the group's performance on second-order tasks was significantly poorer than that of the control group. Only four autism subjects passed both these tasks, compared with 14 controls ($\chi^2 = 6.21$, $p < 0.05$).

To substantiate the presence of current problems in the autism group, subjects were rated by the same observer for quality of verbal and non-verbal communication, using an experimental checklist: 11 individuals with autistic disorder showed slight or pronounced peculiarities in speech use (intonation, speed, volume); 15 in language use (social appropriateness, topic maintenance), and 12 in non-verbal communication (eye gaze, body posture, proximity, facial expression). Fourteen subjects with autism showed peculiarities in at least two of the three areas, while only one control subject was noted as showing slight peculiarities in speech and language use. Furthermore, while the social interaction and interest of these individuals had markedly improved since childhood, none had developed a close friendship, and all showed narrow and obsessively pursued interests.

Morphometric measurement: We used voxel-based morphometry, an automatic and unbiased procedure designed to identify regionally specific differences in the relative grey matter volume within a series of MRI scans. Previous morphometric studies in this area have employed highly constrained and directed regions of interest analyses. By contrast, voxel-based morphometry allows every point in the brain to be compared in an unbiased way on a spatial scale of several millimeters. It uses standard techniques (statistical parametric mapping) developed for the analysis of neuroimaging data that include tissue segmentation or partitioning, spatial normalization and the detection of regionally specific effects. This method has been developed and applied in several previous studies and validated by taking independent measurements from non-normalized MRI scans [13].

The morphometric analysis comprises four components. First, all the structural MRI scans are segmented such that the voxel values correspond to the probability of being grey matter voxels. Second, the images are spatially normalized into a common stereotactic space. Third, the images are smoothed. This local averaging procedure makes the ensuing voxel values a measure of the proportion of the local tissue volume occupied by grey matter. Finally, these regional indices of grey matter are compared

statistically at every voxel to create a statistical parametric map (SPM) of the t-statistic. Inferences about regionally specific grey matter deficits are made on the basis of these SPMs using a threshold of $p < 0.001$. The spatial scale at which grey matter differences are detected is determined by the volume of tissue considered in the local averaging or smoothing procedure. This scale is therefore defined operationally by the size of the smoothing kernel employed, which in this study was 12 mm.

T1-weighted MRI scans of the subjects were obtained with a 2T Siemens VISION System (TR 9.5 ms, TE 4 ms, flip angle 12°, field of view 25 cm, $192 \times 256 \times 256$ matrix size at a resolution of $1 \times 1 \times 1.5$ mm). The images were interpolated to yield cubic 1 mm voxels. The images were partitioned to give probabilistic grey matter segmented images [14]. They were spatially normalized [15] on the basis of a 12 parameter affine transformation into the standard space described by the Atlas of Talairach and Tournoux [16] using a template based upon the 305 brain average from the Montreal Neurological Institute [17]. The images were then smoothed with an isotropic Gaussian kernel of 12 mm. Regionally specific differences in grey matter between the groups were assessed using the general linear model. The statistical model included a measure of the total amount of grey matter in each brain as a confound. The ensuing t-statistics were transformed into a Z score and thresholded at $p = 0.01$ (uncorrected) for display. We only report maxima at $p < 0.001$ (uncorrected). The above procedures were implemented with the SPM96 software [18].

Results

A number of local differences in the relative volume of grey matter were revealed. Decreased grey matter volume was observed in the autistic group in the right paracingulate sulcus, the left occipito-temporal cortex and the left inferior frontal sulcus. Increased volume was observed in the left amygdala/peri-amygdaloid cortex, the right inferior temporal gyrus and the left middle temporal gyrus. In addition, there were bilateral increases in grey volume in the cerebellum (Table 1, Fig. 1, Fig. 2).

Discussion

The areas of abnormal grey matter volume in the autism group, with the exception of the cerebellum, form part of a circuit that is centered on the amygdala. The ventral temporal cortex (areas 20 and 21) sends connections to the amygdala, and there are projections from the amygdala to the inferior prefrontal convexity (area 12/45A) and anterior cingulate cortex (areas 24 and 32); these connections are also reciprocal [19]. The connections with the temporal lobe allow visual stimuli to be associated with

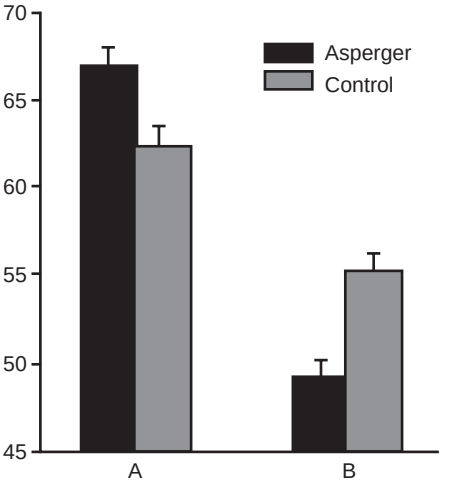

FIG. 1. Mean relative grey matter volume in the autism group (filled bar) and in the normal comparison group (hatched bar). Values are normalized to 50. Error bars indicate s.e.m. (**A**) Left amygdala/peri-amygdaloid cortex (−14, −5, −28). (**B**) Right anterior paracingulate sulcus (+14, +50, +22).

Table 1. Regions in which individuals with autistic disorder show significant decreases and increases in grey matter compared with controls ($p < 0.001$). Listed are co-ordinates in Talairach space with anatomical designations from the canonical brain interpreted using the Duvernoy atlas and *z*-scores for all peaks

Anatomical region	Co-ordinates			*z*
Decreases				
Right paracingulate sulcus	+14	+50	+22	3.85
Left inferior frontal gyrus (BA 45)	−49	+26	+05	3.95
Left occipito-temporal junction	+47	−59	−06	3.43
Increases				
Left amygdala/peri-amygdaloid cortex	−14	−05	−28	3.44
Right anterior lobe of cerebellar hemisphere	+46	−54	−33	3.82
Left anterior lobe of cerebellar hemisphere	−52	−66	−23	3.26
Pyramid of cerebellar vermis	−02	−81	−26	3.62
Left middle temporal gyrus	−66	−16	−07	4.02
Right inferior temporal gyrus	+50	−06	−47	3.74

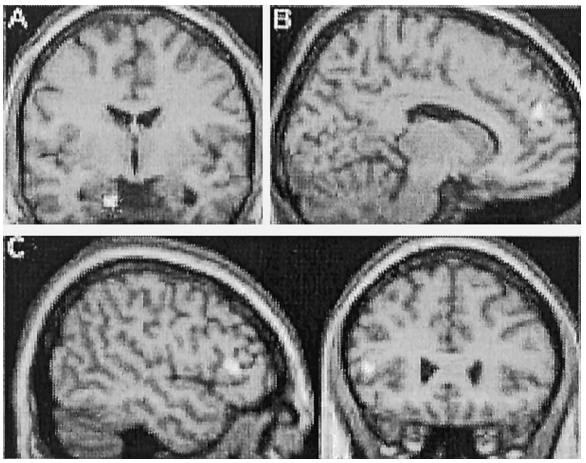

FIG. 2. Areas with significant differences of relative grey matter volume at $p < 0.001$, superimposed onto the standard template. (**A**) Left amygdala/peri-amygdaloid cortex (−14, −5, −28): increased relative grey matter volume in the autism group (coronal cut). (**B**) Right paracingulate sulcus (+14, +50, +22): decreased relative grey matter volume in the autism group (sagittal cut). (**C**) Left inferior frontal gyrus BA 45 (−49, +26, +5): decreased relative grey matter volume in the autism group (sagittal and coronal cut).

emotional significance, and the connections with the frontal lobe (ventral and orbital frontal cortex and anterior cingulate cortex) provide pathways through which mental states, such as emotions, can be monitored and modulated. The abnormalities in the cerebellum, which relate to previous anatomical studies of autism [5], may have to be accounted for separately, as they do not seem to be part of this amygdala-centered system.

The same areas that differentiated the present groups, whether in terms of increased or reduced relative volume, have shown increased cell packing density in post-mortem studies of seven autistic brains [4], including in particular, amygdala, anterior cingulate and cerebellum. The medial frontal focus in the anterior cingulate region was close to the focus found in the PET study of theory of mind by Happé *et al*. [3]. Thus the same areas have been highlighted in independent studies using entirely unrelated measurement techniques.

Since impairments of social communication, and specifically deficits in the ability to attribute mental states to self and others, can be seen as the common denominator across the spectrum of autistic disorders [2], we propose that the neural system identified in the present study is critical to self- and other-awareness. The anterior cingulate in particular is known to be implicated in the awareness of mental states and in the reportable experience of emotions [20]. The set of brain areas showing group differences in the present study fits well with the 'social brain' delineated on the basis of animal and human neuropsychological studies [6]. According to this model, the social behavior required in two-way communication is critically dependent on a specialized circuit centered on the amygdala, involving orbital frontal cortex, anterior cingulate and temporal pole cortex.

We cannot of course interpret increases and decreases of relative volumes of grey matter as mapping onto absolute increases or decreases of brain tissue, since this was not measured directly. It is not clear what relationship should be expected between cell packing density and grey matter volume. A possible explanation of greater volume of grey matter would be a failure of programmed cell death (apoptosis) in certain regions [21]. Speculatively, we suggest that the anatomical abnormalities found correspond to functional abnormalities. The anterior (executive) components of the amygdala-centered system may underlie poor monitoring and control of mental states. The posterior (sensory) components of the system may underlie the often reported overwhelming sensory overload and high anxiety levels in autism. For example, abnormality in the amygdala/peri-amygdaloid cortex might lead fear conditioning to be more swiftly established and less amenable to extinction in autism. Abnormally fast classical eye-blink conditioning in autism has indeed been shown [22]. Further, as the system pinpointed is particularly rich in opioid receptors, and since abnormalities in opioid metabolism have been found in autism [23], it is possible that some further symptoms (e.g. self-injury, high pain threshold, self-reports of sensory overload) can also be illuminated.

Conclusion

Our results and those of previous studies converge on a distributed system which shows structural abnormalities in individuals with autism. This system, which appears to be centered on the amygdala, is strongly implicated in emotional and social learning and, more speculatively, self-awareness. One theoretical formulation of brain plasticity posits a central role for the amygdala that relates directly to the current findings [24]. According to this theory, the amygdala integrates highly processed perceptual inputs that have value or salience. This synthesis is then used to modulate or consolidate adaptive changes in synaptic efficacy throughout the brain via its vicarious projections to the ascending modulatory neurotransmitter systems. A critical component of this theory is that the amygdala is responsible for consolidating its own inputs. This model suggests that a neurodevelopmental abnormality involving the amygdala, or its targets, would be sufficient to explain the impairments in emotional and social learning in autism, and can account for morphological abnormalities at the sources of amygdala inputs.

Although highly speculative, there is compelling convergence among the neuropsychological deficits of autism, the functional anatomy of an amygdala centered system, and finally, the neuroanatomical correlates of self-awareness identified by this work.

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ACKNOWLEDGEMENTS: This research was funded by the Wellcome Trust and the Medical Research Council.

Received 17 March 1999; accepted 30 March 1999