Investigating individual differences in brain abnormalities in autism

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Autism is a psychiatric syndrome characterized by impairments in three domains: social interaction, communication, and restricted and repetitive behaviours and interests. Recent findings implicate the amygdala in the neurobiology of autism. In this paper, we report the results of a series of novel experimental investigations focusing on the structure and function of the amygdala in a group of children with autism. The first section attempts to determine if abnormality of the amygdala can be identified in an individual using magnetic resonance imaging *in vivo*. Using single-case voxel-based morphometric analyses, abnormality in the amygdala was detected in half the children with autism. Abnormalities in other regions were also found. In the second section, emotional modulation of the startle response was investigated in the group of autistic children. Surprisingly, there were no significant differences between the patterns of emotional modulation of the startle response in the autistic group compared with the controls.

Keywords: autism; neurobiological basis; magnetic resonance imaging; voxel-based morphometry; startle response; amygdala

1. INTRODUCTION

Current understanding of the neurobiological basis of autism is limited. The diagnosis of autism is commonly made based on the child's historical and current behavioural symptomatology. The clinician is often required to make qualitative judgements about the significance of the child's difficulties. Diagnosis can therefore be highly subjective and may vary among clinicians (Howlin & Asgharian 1999).

Recently, there has been increasing evidence for the role of the amygdala in the neurobiology of autism (Baron-Cohen *et al.* 2000; Howard *et al.* 2000). For example, post-mortem studies have revealed increased cell density and abnormally small cells in the amygdala (Bauman & Kemper 1985, 1994; Raymond *et al.* 1989; Bauman 1991; Bailey *et al.* 1998). Structural imaging studies have identified abnormalities in amygdala volume (although some studies have found increased volume and others decreased volume; Howard *et al.* 2000; Pierce *et al.* 2001; Aylward *et al.* 1999). Magnetic resonance spectroscopy studies have suggested neuronal loss or damage in the amygdala-hippocampal region (Otsuka *et al.* 1999). In a recent imaging study, Baron-Cohen *et al.* (1999) reported that a group of individuals with autism did not activate the amygdala when it was activated in the controls.

We report the results of a study investigating the integrity of the amygdala in a group of children with autism. The first section addresses the issue of individual neuro-pathological profiles, and attempts to determine if it is possible to detect a structural abnormality in the amygdala in an autistic individual, as opposed to averaged data from a control group. Detection of such an abnormality might be a first step towards establishing a quantitative and objective measure for diagnostic purposes.

The second section reports on the use of a psychophysiological measure (i.e. emotional modulation of the startle response) to assess amygdala function in the group of children with autism. This independent measure of amygdala function may be useful in assessing the impact of abnormality in this brain region on emotional modulation.

2. PARTICIPANTS

Fourteen children with autism (aged between 8 and 18 years) were recruited through parental support groups (including the National Autistic Society) and from schools specializing in the education of children with autism. Each child had been diagnosed with HFA or AS by independent clinicians (including paediatricians, clinical psychologists and psychiatrists). Children were excluded from the study if they had additional neurological or psychiatric diagnoses (e.g. fragile X, epilepsy and attention deficit hyperactivity disorder), if they were taking medication or had a history consistent with a diagnosis of secondary autism (such as rubella).

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One contribution of 14 to a Theme Issue 'Autism: mind and brain'.

Table 1. Characteristics of the HFA and AS groups.

group	size	mean age (years)	diagnosis	sex
control	18	12.6	N/A	12 female; 6 male
autistic	14	12.9	3 HFA; 11 AS	1 female; 13 male

Normally developing control children (aged between 8 and 18 years) were recruited from local London schools. These children were required to meet the same inclusionary and exclusionary criteria as the children with autism, with the additional requirement that there was no family history of autism. Further details of the groups are provided in table 1. Although the control and autistic groups were not matched on sex, the results remained unchanged when analyses were restricted to males only.

(a) *Intelligence*

The age-appropriate Wechsler Intelligence Test (Wechsler 1991, 1997) was administered to provide verbal, performance and full-scale IQs. All autistic children and controls investigated in this study had verbal IQs within a standard deviation of the normal mean (85–115).

3. INDIVIDUAL NEUROPATHOLOGICAL MEASURES

This section attempts to detect a pattern of neural abnormality that is characteristic of autism, utilizing the prior hypothesis of bilateral neural abnormality as being causal. The underlying rationale is that autism is a neuro-developmental disorder with selective and chronic cognitive deficits. It is well established that in adult humans, unilateral brain damage is sufficient to produce selective cognitive impairments that are severe and chronic. By contrast, in children, bilateral lesions of a brain system appear to be necessary to produce chronic syndromes (e.g. amnesia; Gadian *et al.* 2000). The absence of such selective impairments in the face of early unilateral damage is presumed to reflect the plasticity of the immature brain, and its capacity for reorganization of a developing function to a homologous region in the undamaged hemisphere.

VBM is a technique that has been developed to characterize cerebral grey and white matter differences with uniform sensitivity throughout the entire brain. Although typically used to assess group differences, VBM has recently been validated for use in individual subjects (see Salmond *et al.* 2002). Using this technique, the presence or absence of abnormalities in the amygdala was determined for each individual. In addition, other neural areas (including the hippocampal formation, the cerebellum, the STG and the OFC) found to be abnormal in a group analysis of individuals with autism (see Salmond 2001) were investigated.

A second MRI technique, T_2 relaxometry, was used to provide a quantitative method of detecting abnormalities that are more conventionally evaluated by visual inspection of T_2 -weighted images. T_2 relaxation times have been shown to detect lesions or abnormalities that may not be identified in standard clinical imaging (Van Paesschen *et*

al. 1996). T_2 relaxation times were therefore determined for each of the participants in this study.

4. METHODS: INDIVIDUAL NEUROPATHOLOGICAL MEASURES

(a) *MRI acquisition*

All the children underwent non-sedated MRI scans in a 1.5 T Siemens Vision System. A 3D FLASH sequence was collected (TR: 16.8 ms; TE: 5.7 ms; flip angle: 21°; voxel size 0.8 mm × 0.8 mm × 1 mm) for use in the individual VBM analyses. HCT2 and AT2 maps were obtained using a 16-echo sequence as previously described (Van Paesschen *et al.* 1996, 1997; TR: 2400 ms, TE: 22–262 ms; one slice, 5 mm thick). The HCT2 map was oriented in a tilted coronal plane along the anterior border of the brainstem, perpendicular to, and at the level of, the body of the hippocampal formation. The AT2 was oriented in a tilted axial plane parallel to and above the long axis of the hippocampal formation.

(b) *Individual VBM*

The scans were analysed using VBM, according to the bilateral method described by Salmond *et al.* (2000). Briefly, the data were normalized and segmented into grey and white matter images. The grey matter images were then smoothed with 4 and 12 mm isotropic Gaussian kernels. This smoothing renders the voxel values into an index of the amount of grey matter per unit volume under the smoothing kernel. The term 'grey matter density' is generally used to refer to this probabilistic measure. Smoothing parameters of 4 and 12 mm were chosen as these correspond roughly to the cross-sectional dimensions of the hippocampal formation and amygdala, respectively, and, by the matched filter theorem, sensitized the analysis to differences at these spatial scales. Age and sex were included as covariates.

Each child in the autistic group was compared with the entire control group, searching for bilateral deficits in grey matter density using a conjunction analysis as described previously. Salmond *et al.* (2002) have reported that single-subject versus group comparisons can be subject to violations of normality assumptions at very low degrees of smoothing. This violation can render the Gaussian field correction for multiple comparisons inexact. We therefore eschewed the multiple comparisons correction by restricting our inferences to prespecified anatomical regions. These regions, which included the hippocampal formation, amygdala, OFC, STG and cerebellum, were identified on the basis of a review of the literature (Bachevalier 1994; Carper & Courchesne 2000). The exact locations of the regions were determined by a group analysis of pilot data (Salmond 2001). As a control, we also investigated an area of the visual cortex thought not to be involved in the neuropathogenesis of autism. We used an uncorrected threshold of $p = 0.001$, which corresponds roughly to a corrected p value of 0.05 having accounted for the small volume and the number of structures involved.

(c) *T_2 maps*

HCT2 and AT2 were measured by placing the largest possible circle as a region of interest within the hippocampal formation and amygdala (respectively) while avoiding

Table 2. Results from individual VBM analyses.

(Y indicates presence of abnormality at threshold uncorrected $p < 0.001$, N indicates no significant abnormality at threshold.)

autistic subject no.	hippocampal formation	amygdala	OFC	STG	cerebellum	visual cortex (V1)
1	Y	N	Y	N	Y	N
2	Y	Y	Y	Y	Y	N
3	N	N	N	Y	Y	N
4	Y	Y	Y	Y	Y	N
5	Y	Y	Y	Y	Y	N
6	N	N	Y	N	Y	N
7	Y	Y	Y	Y	Y	N
8	N	Y	Y	Y	Y	N
9	N	N	Y	N	N	N
10	N	N	Y	Y	N	N
11	N	N	Y	Y	Y	N
12	N	N	Y	Y	N	N
13	Y	Y	Y	Y	Y	N
14	Y	Y	Y	N	Y	N

Table 3. Summary of group variation according to VBM analyses.

area	number of individuals showing abnormality
hippocampal formation	7
amygdala	7
OFC	13
STG	10
cerebellum	11

boundaries where partial volume effects within cerebrospinal fluid might occur. HCT2 and AT2 values are expressed in milliseconds.

5. RESULTS: INDIVIDUAL NEUROPATHOLOGICAL MEASURES

(a) VBM

Results from the individual VBM analyses are shown in tables 2 and 3. The individual VBM analyses revealed that most children in the autistic group showed evidence of abnormality in the OFC, STG and the cerebellum. Fewer individuals showed evidence of medial temporal lobe abnormality. Representative results are shown in figure 1. Similar analyses comparing each child in the control group with the remaining controls revealed no evidence of neural abnormality.

The significance of these differing patterns of neural abnormality in relation to cognitive and behavioural function in the children with autism was explored using tests commonly thought to be associated with the functions of these areas (Salmond 2001). These included measures of episodic memory (Rivermead Behavioural Memory Test; Wilson *et al.* 1991), orbitofrontal function (number of correct responses; i.e. failure to maintain set; Stuss *et al.* 1983, 2000; Nagahama *et al.* 1996) on the Wisconsin Card Sorting Test (Heaton 1981), and motor coordination (Movement Assessment Battery for Children Checklist; Henderson & Sugden 1992) as well as ratings on the Autistic Behaviour Checklist (Krug *et al.* 1993).

Figure 1. Representative results. (a) Child 9, abnormality in OFC; child 8, abnormality in (i) amygdala, (ii) STG, (iii) OFC and (iv) cerebellum. All figures shown at uncorrected $p < 0.01$ for display purposes. Displayed in neurological convention (left is left).

Table 4. Performance scores according to presence of detected abnormality on individual VBM.

area of abnormality (performance measure)	range of raw scores and no. of children in the autistic group showing significant abnormality	range of scores and no. of children in the autistic group showing no significant abnormality
hippocampal formation (Rivermead)	13–19 (<i>n</i> = 7)	13–22 (<i>n</i> = 7)
OFC (number of correct responses)	6–50 (<i>n</i> = 13)	44 (<i>n</i> = 1)
cerebellum (Movement Assessment Battery for Children)	3–84 (<i>n</i> = 11)	0–53 (<i>n</i> = 13)

Table 5. Symptom severity according to presence of detected abnormality on individual VBM.

area of abnormality	range of scores on autistic behaviour checklist of children in autism group with significant detected abnormality in this region	range of scores on autistic behaviour checklist of children in autism group with no significant detected abnormality in this region
hippocampal formation	10–76	35–94
OFC	10–94	35 ^a
cerebellum	10–94	46–74

^a *n* = 1.

The behavioural and cognitive profiles did not distinguish between autistic children with significant abnormality in individual VBM analyses and those without significant abnormality (see tables 4 and 5).

(b) *T*₂ maps

There were no significant differences between the AT2 and HCT2 values of the two groups (AT2: *F*_{1,21} = 0.7, *p* = 0.4; HCT2: *F*_{1,23} = 1.6, *p* = 0.2). There were no effects of side, or significant interactions between side and group (*p* > 0.2). Figure 2 shows the range of *T*₂ values obtained for (a) the amygdala and (b) the hippocampal formation.

6. DISCUSSION: INDIVIDUAL NEUROPATHOLOGICAL MEASURES

The individual VBM analyses revealed abnormality in the amygdala in only half the autistic children. This is consistent with reports of heterogeneity in individuals with autism (Aylward *et al.* 1999; Howard *et al.* 2000). By contrast, the OFC abnormality was found in all but one of the autistic children, highlighting the possibility that this area is important in the pathogenesis of autism.

However, the neuropsychological tests purporting to pinpoint orbitofrontal function that we used in this study did not reflect this abnormality. Further research is required to reveal the significance of abnormality in this region in relation to other brain areas implicated in autism. Importantly, no evidence of abnormality was found in the visual cortex, suggesting that neural abnormalities are not present in every region of the autistic brain.

No neural area was found to be significantly abnormal in all of the autistic children. Additionally, there was no association between a specific area of abnormality and a selective deficit in a particular domain of cognitive or behavioural function. This suggests that autism is unlikely to be associated with abnormality in one particular location alone. Instead, the autistic phenotype may reflect

Figure 2. Scattergraph of *T*₂ values of (a) the amygdala (AT2); (b) the hippocampal formation (HCT2). Squares represent control group and triangles represent autistic group.

abnormalities within a particular neural system or, indeed, multiple systems. Five highly interconnected regions have been implicated in the neural pattern characteristic of

autism: the OFC, the cerebellum, the hippocampal formation, the amygdala and the STG (Heath & Harper 1974; Heath *et al.* 1978; Sasaki *et al.* 1979; Barbas & De Olmos 1990; Middleton & Strick 1994; Barbas & Blatt 1995; Schmahmann & Pandya 1997). However, the combination of areas detected as abnormal have shown wide individual variation.

The results from the T_2 maps revealed no group differences in either the amygdala or the hippocampal formation. Furthermore, there was no relation between an individual's T_2 values and VBM results. However, this is compatible with the VBM findings, as it is quite possible to detect a change in the volume of a structure with no change in its T_2 relaxation time. For example, cases of abnormal hippocampal volume and normal HCT2 have been reported in the literature (Van Paesschen *et al.* 1997; Gadian *et al.* 2000). The null result of the T_2 data emphasizes the difficulties inherent in detecting abnormalities in an individual using the methods currently available in clinical practice.

Our results suggest that VBM can reveal abnormalities in the amygdala, as well as in other brain regions, in particular OFC, in at least some of the autistic individuals. This raises the possibility that different etiologies may be identifiable in different individuals with autism. In the future, it may be appropriate to use cluster analyses in a larger sample of children with autism to investigate factors contributing to the homogeneity and heterogeneity of the disorder.

It is clearly premature to suggest that the results of this study have definitively determined the neural pattern characteristic of autism. First, this study investigated only five sites of anatomical abnormality. Whilst these areas are the most frequently reported sites associated with abnormality in autism, other areas may also be affected. Second, this study investigated only autistic children with verbal IQs within the normal range. It is possible that lower functioning children with autism may show more extensive neural abnormalities or may show a different pattern. Third, this study has not demonstrated specificity of the pattern of neural abnormality: children with other developmental disorders may show a similar pattern (e.g. amygdala abnormalities have also been reported in anxious and depressed children; De-Bellis *et al.* 2000; Thomas *et al.* 2001).

7. EMOTIONAL MODULATION OF THE STARTLE RESPONSE

An independent behavioural measure of the amygdala is the emotional modulation of the startle response. The startle response is a brainstem-mediated motor response that occurs following the presentation of a sudden and intense stimulus. The vigour of the startle response varies systematically with the emotional state of the individual (Lang *et al.* 1990). This emotional modulation of the startle response has been shown to be dependent on the amygdala (e.g. Rosen *et al.* 1996; Davis *et al.* 1999).

In humans, the fastest and most stable element of the startle response is the sudden closure of the eyelids (Anthony 1985). This is the traditional experimental measure of the startle response in humans. The vigour of the startle response varies systematically with the affective

status of the individual (Lang *et al.* 1990). In adult humans, the startle response is facilitated by unpleasant arousal and inhibited by pleasant arousal (e.g. Davis 1989; Lang *et al.* 1990, 1992). Studies of the normative development of emotional modulation of the startle response have produced mixed results. Two studies with school-aged children have reported non-significant trends *opposite* to those reported in adults: smaller responses to fearful than pleasant stimuli (Cook *et al.* (1995) for boys and girls; McManis *et al.* (1995) for boys). These results cannot be due to delayed maturation of the emotional modulation of the startle response, since infants in the first year of life show enhanced startle to angry, compared with happy, faces (Balaban 1995) and to a stranger approaching compared with baseline (Schmidt & Fox 1998).

One possible explanation for the negative findings in school-aged children is that these studies used fearful and pleasant stimuli that were categorized as such according to adult normative ratings. Children may not perceive the valence of the stimuli in the same way as adults, and individual differences in perception of fear may be particularly evident during childhood. One way to address this problem, adopted in the present study, is to have children themselves rate pictures as fearful or pleasant, and tailor the analysis of emotional modulation of response to their individual responses. This individual tailoring maximizes the possibility of detecting emotional modulation, which might otherwise be masked by inclusion of pleasant stimuli in the fearful category, and vice versa. This approach also prevents 'false' findings of abnormal response patterns merely due to any idiosyncratic fears or preferences of children with autism.

8. METHODS: EMOTIONAL MODULATION OF THE STARTLE RESPONSE

(a) Stimuli

More than 50 pictures were chosen from a variety of websites and rated as pleasant or unpleasant by 10 adults. From these, the 15 pictures rated as most pleasant and the 15 pictures rated as most unpleasant were selected. An additional nine pictures were selected with varying ratings for use as filler stimuli in non-probed trials.

A pilot study with adults using these stimuli demonstrated the expected pattern of augmented startle to the unpleasant compared with the pleasant pictures.

The pictures were deliberately selected to exclude facial stimuli. Children with autism have been shown to have impaired recognition of facial expression (Hobson *et al.* 1988; Teunisse & Gelder 2001). This impairment is therefore likely to confound any intended affective modulation in the paradigm (i.e. if the child does not recognize a fearful expression, such a face cannot be assumed to engender a fearful emotional response).

An acoustic startle probe (consisting of a 50 ms burst of white noise with instantaneous rise time) was presented binaurally over headphones. The intensity of the probe was chosen to be within a comfortable range for the children (at *ca.* 70 dB, sound pressure level).

(b) Paradigm

Each picture was presented for 7 s and startle probes were presented at 1300 ms after slide picture onset. The

pictures were presented on a Dell 1500 FP computer screen. Picture offset was followed by a blank screen.

The child was instructed that a series of slides would be presented and that each slide should be viewed for the whole time it was on the screen. Each child was encouraged to pay attention by being told that questions would be asked about the pictures at the end of the assessment. The child was told that occasional noises would be heard over the headphones that could be ignored.

After the startle probe series had been completed, the child was shown each picture again and asked to rate it as 'nice' or 'scary'. In order to check comprehension of these concepts, the child was asked to give an example of something 'nice' and 'scary' on a previous assessment day. These ratings were used to produce individual categorizations of the stimuli (pleasant and unpleasant) for each child.

(c) Data recording

Unilateral right blink magnitude was measured by vEOG measurements using a pair of bipolar AgCl electrodes placed just above and below the orbit in a vertical line through the pupil. Sampling rate was 500 Hz and the data were recorded with a 50 Hz notch filter. Impedances were kept below 10 k Ω . Although it is more traditional to measure blink magnitude from EMG, when both EMG and vEOG were recorded they yielded highly similar results (Sugawara *et al.* 1994). The raw vEOG signal was epoched and baseline corrected (interval: 200 to 0 ms).

(d) Data analysis

The peak of the blink was defined as the point of maximum deflection before a return towards baseline that continued for 5 ms. Latency of response was defined as the latency of peak amplitude. When multiple blinks occurred, the response whose latency was closest to the mean latency for that condition was scored. Trials were deemed non-scorable (and therefore rejected) if a blink was in progress at reflex stimulus onset or if the blink did not recover within the sampling period of 250 ms (return to at least 25% of peak amplitude).

9. RESULTS: EMOTIONAL MODULATION OF THE STARTLE RESPONSE

All the children were able to give appropriate examples of items or events that were scary or nice. Responses given included going on holiday, pets and chocolate (nice), and spiders, the dark and horror films (scary). There was no qualitative difference between the responses of the two groups.

The children's individual ratings of the pictures were used to determine the categorization of visual stimuli. There was no group difference in the number of stimuli labelled as scary ($F_{1,29} = 0.1$, $p = 0.7$). As table 6 shows, there was a large number of trials that did not elicit a blink response, but there was no significant difference in the number of no-response trials between the groups ($F_{1,29} = 0.4$, $p = 0.5$). Trials with no blink response were also evenly distributed across the 'nice' and 'scary' categories. There was no significant difference between the groups on the number of trials rejected ($F_{1,29} = 2$, $p = 0.2$).

Table 6. Numbers of trials with no blink response and numbers of rejected trials (means $\pm$ s.e.m.; total number of trials: 30).

group	no. of trials with no response	no. of trials rejected
control	14.2 $\pm$ 2.1	1.7 $\pm$ 0.5
autistic	12.1 $\pm$ 2.7	2.9 $\pm$ 0.6

Figure 3. Blink response according to individual child's picture categorization (mean $\pm$ s.e.m.). Squares represent control group and triangles represent autistic group.

Figure 4. Mean latency of blink response by picture category (mean $\pm$ s.e.m.). Squares represent control group and triangles represent autistic group.

(a) Control group

In order to characterize the pattern of responses elicited by this paradigm in control children, prior to the group analysis the results from the control group were explored. Statistical analysis was carried out with a paired *t*-test.

Analysis of the results from the control group showed that there was a significant difference between the blink amplitudes to the two picture categories ($t = -3$, d.f. = 15, $p = 0.006$). This was due to increased amplitude responses to pictures categorized as 'nice' (see figure 3). There was no significant difference between blink latencies to the two picture categories (nice–scary: paired *t*-test: $t = 1$, d.f. = 15, $p = 0.3$) (see figure 4).

(b) Group analysis

To compare responses of the control group with those of the autistic group, a mixed ANOVA was computed with

Group (autism, control) as the between-subjects factor and Picture Type (scary, nice) as the within-subjects factor. The amplitude analysis revealed a main effect of Picture Type, ($F_{1,26} = 9.79$, $p < 0.01$), which occurred because the response to 'nice' pictures was larger than for 'scary' pictures in both groups (autism: scary = 65.7 μ V, nice = 108.5 μ V; control scary = 56.8 μ V, nice = 102.06 μ V). There was no main effect of Group or Group by Picture Type interaction (see figure 3). There were no group differences in latency responses (see figure 4).

There was no relationship between the amplitude of the startle response and the detection of amygdala structural abnormality in the autistic group.

10. DISCUSSION: EMOTIONAL MODULATION OF THE STARTLE RESPONSE

In this study, both the autistic and the control groups showed a greater startle response to pleasant compared with unpleasant stimuli. To the extent that modulation of the startle response is regulated by the amygdala, the present results provide no evidence to suggest functional abnormality of this structure in the autistic group.

The results are consistent with those of two prior studies reporting trends towards larger startle responses to pleasant compared with unpleasant stimuli in school-aged children (Cook *et al.* 1995; McManis *et al.* 1995). Neither of the two previous studies used the children's individual ratings of the stimuli, which might explain why the trends were not statistically significant in those studies. This pattern of emotional modulation of the startle response is opposite to the one reported in adults and adolescents, who show fear potentiation of the response. It is not well understood why children show a reverse pattern of modulation of the startle response when visual stimuli are used to alter emotional state (Cook *et al.* 1995; McManis *et al.* 1995). In our study, this cannot be a peculiarity of the stimuli used, because adults tested with the same procedure showed the typical pattern of larger startle response to scary stimuli. Further research on the normative development of emotional modulation of the startle response will help to address this question and to understand abnormalities of the startle response in clinical populations.

One possible explanation for the reverse pattern of modulation seen in the children compared with the adult data involves attentional resource allocation. It has been previously reported that the modulation of the startle response in humans can reflect differing attentional processes (Bradley *et al.* 1993). When more attention is paid to arousing stimuli, less attention is paid to the startle probe and the response to the latter is therefore reduced. With respect to the current study, it is possible that the 'scary' stimuli were more arousing than 'nice' stimuli. This theory could be tested by having children rate stimuli on arousal, as has been done with adults (e.g. the International Affective Picture System; Lang *et al.* 1995). Measurement of galvanic skin responses may also help address this question.

One consideration in interpreting the results of our study is that only about half of the children with autism showed evidence of amygdala abnormalities in the MRI analysis. It is thus possible that modulation of the startle

response would differ for children with and without autism if we restricted our analysis to include only the subgroup children with autism who had evidence of amygdala abnormalities. However, this explanation can be ruled out because the results of the analysis with only this subgroup were the same as those for the whole group. Another consideration when interpreting the results of our study is the low response rate in both groups. Although it is reported that up to 10% of subjects fail to show the startle response to even very intense stimuli (Ornitz 1999), the response rate in this study fell below this level. This may, at least in part, be due to the low decibel level of the startle probe (see Berg & Balaban 1999). This was chosen to minimize subjects failing to complete the paradigm due to discomfort or dislike of the probe.

In conclusion, affective modulation of the startle response was not found to be different in controls and in the autistic group. We cannot rule out that emotional modulation of the startle response differs for autistic children compared with controls either early or later in the development of this response. This possibility is worth pursuing, because the non-verbal nature of the paradigm potentially lends itself to the study of all individuals with autism, regardless of their intellectual abilities.

11. SUMMARY

This paper has explored both structural and functional approaches in an attempt to uncover the role of the amygdala in autism. Individual VBM analyses revealed abnormality in a number of different regions of the brain and substantial heterogeneity in the pattern of abnormality. Only half the group showed structural abnormalities in the amygdala. The startle response paradigm thought to reflect aspects of amygdala function was used for the first time with autistic children. Unexpectedly, the emotional modulation of the startle response was not found to differ significantly between the two groups. The results suggest that abnormality in the amygdala may not be a core feature of autism. These results need to be confirmed in a larger sample of autistic children and extended to investigate the precise combinations and extents of abnormalities associated with the disorder.

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GLOSSARY

- AT2: amygdala T_2
AS: Asperger syndrome
HCT2: hippocampal T_2
HFA: high-functioning autism
IQ: intelligence quotient
MRI: magnetic resonance imaging
OFC: orbitofrontal cortex
STG: superior temporal gyrus
TE: echo time
TR: repetition time
VBM: voxel-based morphometry
vEOG: vertical electro-oculogram