Amygdala dysfunction in men with the fragile X premutation

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Premutation alleles (55–200 CGG repeats) of the fragile X mental retardation 1 (*FMR1*) gene are associated with autism spectrum disorder in childhood, premature ovarian failure, and the neurodegenerative disorder, fragile X-associated tremor/ataxia syndrome (FXTAS). FXTAS, and perhaps the other clinical presentations among carriers, are thought to be due to toxic gain-of-function of elevated levels of the expanded-repeat *FMR1* mRNA. Previous structural MRI studies have implicated the amygdala as a potential site of dysfunction underlying social deficits and/or risk for FXTAS. As a preliminary investigation of this possible association, adult males with the premutation, and male controls matched for IQ, age and education, completed three protocols that probe amygdala and sympathetic function: (i) a functional MRI paradigm that measures brain response to fearful faces; (ii) a fear-potentiated startle paradigm that differentiates responses to fearful faces and fearful non-social images and (iii) measurement of skin conductance level during a brief social encounter. Compared with controls, men with the *FMR1* premutation demonstrated diminished brain activation in the amygdala and several brain areas that mediate social cognition while viewing fearful faces. The reduced amygdala activation in the premutation group was significantly associated with self-report of psychological symptoms on the Symptom Checklist-90—Revised. These men also displayed a lack of startle potentiation while viewing fearful faces and showed reduced skin conductance response when greeting an unfamiliar experimenter in comparison with the control group. The current findings may be related to social cognition deficits reported previously in children and adults with the premutation. The aetiology for this dysfunction may be elevated *FMR1* mRNA or reduced *FMR1* protein that occurs in carriers with higher premutation CGG repeat alleles.

Keywords: *FMR1* gene; FXTAS; fragile X; face perception; social cognition

Abbreviations: ASD = autism spectrum disorder; fMRI = functional MRI; FMR1 = fragile X mental retardation 1; FMRP = fragile X mental retardation protein; FXTAS = fragile X-associated tremor/ataxia syndrome; FXS = fragile X syndrome; GSI = global severity index; ROI = region of interest; SCL-90-R = Symptom Checklist-90—Revised; STS = superior temporal sulcus

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Introduction

Until recently, the importance of premutation alleles (55–200 CGG repeats) of the fragile X mental retardation 1 (*FMR1*) gene was thought to be limited to their propensity for expansion to the full mutation range (>200 CGG repeats) during transmission, with the consequent development of fragile X syndrome (FXS). However, it is now clear that a portion of carriers of premutation alleles have significant social, emotional and cognitive problems along the FXS spectrum, including autism spectrum disorder (ASD) (Dorn *et al.*, 1994; Franke *et al.*, 1998; Tassone *et al.*, 2000*b*; Johnston *et al.*, 2001; Hagerman and Hagerman, 2002; Borghgraef *et al.*, 2004; Moore *et al.*, 2004*a, b*; Cornish *et al.*, 2005; Farzin *et al.*, 2006). In contrast to the prevalence of FXS (1 in 3000–5000 live births), the

prevalence of the *FMR1* premutation in the general population is ~1 per 813 males and 1 per 259 females (Rousseau *et al.*, 1995; Dombrowski *et al.*, 2002).

In addition to the developmental problems reported in children, male and (occasional) female premutation carriers are at significant risk for a late-onset neurodegenerative disorder, fragile X-associated tremor/ataxia syndrome (FXTAS), with principal features of intention tremor and gait ataxia (Hagerman *et al.*, 2001; Jacquemont *et al.*, 2003; Hagerman and Hagerman, 2004*a*). Additional clinical features include brain atrophy with white matter disease and characteristic hyperintensity in the middle cerebellar peduncles, autonomic dysfunction including high blood pressure and impotence, sensory neuropathy in a stocking distribution in the lower extremities and cognitive decline beginning with memory deficits (Brunberg *et al.*, 2002; Jacquemont *et al.*, 2004; Bacalman *et al.*, 2006). The late-onset neurological phenotype has never been observed or reported in FXS and has a different molecular mechanism, now believed to be a toxic gain-of-function effect resulting from elevated *FMR1* mRNA (Hagerman and Hagerman, 2004*b*). Post-mortem studies of brain tissue from premutation males with FXTAS show the presence of distinct intranuclear, eosinophilic inclusions in neurons and astrocytes (Greco *et al.*, 2002, 2006).

Recently, we documented that abnormal elevation of *FMR1* mRNA is associated with psychiatric problems, predominantly schizoid and obsessive-compulsive symptoms, in males with the premutation who have developed FXTAS as well as in younger adult males with no evidence of this neurodegenerative disease (Hessl *et al.*, 2005). *FMR1* expression was not associated with IQ, suggesting that the RNA gain-of-function has a greater impact on neuropsychiatric than on cognitive status.

Limbic brain regions, including the amygdala and hippocampus, may be especially impacted by increased CGG repeat size and the consequent abnormal elevation of *FMR1* mRNA (Abitbol *et al.*, 1993; Jäkälä *et al.*, 1997; Greco *et al.*, 2002; Moore *et al.*, 2004*b*). In a structural brain MRI study, Jäkälä *et al.* (1997) showed that, compared with controls, males and females with the premutation had significantly reduced hippocampal volumes and associated memory deficits. In a recent study of 20 male premutation carriers and 20 age and IQ matched controls, Moore *et al.* (2004*b*) demonstrated significantly reduced grey matter density in several brain regions in the premutation group, including the cerebellum, caudate, insula, amygdalo–hippocampal complex, brainstem and thalamus. Within this group, increased age, increased CGG repeat size and decreases in the percentage of blood lymphocytes expressing fragile X mental retardation protein (FMRP) were associated with decreased grey matter density in the amygdalo–hippocampal complex.

Dysfunction of the amygdala is implicated in psychological problems experienced by individuals with the premutation because of its role in emotion (see Phelps and LeDoux, 2005), social cognition (see Adolphs, 2003)

and ASD (Baron-Cohen *et al.*, 2000; Howard *et al.*, 2000; Sweeten *et al.*, 2002; Amaral and Corbett, 2003; Schultz, 2005; Bachevalier and Loveland, 2006; Dziobek *et al.*, 2006). Interestingly, Cornish and colleagues recently reported that men with the premutation, compared with matched family and non-family controls, displayed largely normal basic facial recognition ability (including recognition of fearful expressions), but significant impairment in recognizing more complex mental and emotional states (Reading the Mind in the Eyes Test; Baron-Cohen *et al.*, 2001), obsessive-compulsive traits, and executive function problems including inhibitory control (Cornish *et al.*, 2005). Based on clinical reports of ASD associated with the premutation (Tassone *et al.*, 2000*b*; Aziz *et al.*, 2003; Borghgraef *et al.*, 2004; Goodlin-Jones *et al.*, 2004; Farzin *et al.*, 2006) and the CGG- and FMRP-dependent changes reported in the amygdalo–hippocampal complex (Jäkälä *et al.*, 1997; Moore *et al.*, 2004*b*), we hypothesized that dysfunction of the limbic system contributes to the neuropsychiatric phenotype of the fragile X premutation.

To begin to address this hypothesis, we conducted a series of three experiments designed to probe amygdala and autonomic nervous system responses to social and emotional stimuli in a group of men with the premutation (without FXTAS) and a comparison group of men without the premutation matched on age, IQ and level of education. The experiments were (i) measurement of amygdala activation during exposure to fearful facial expressions by functional MRI (fMRI); (ii) potentiation of the eye blink startle reflex to fearful faces and non-social fearful stimuli, a biobehavioural response mediated by the amygdala (Hitchcock and Davis, 1986; Hitchcock and Davis, 1987; Vrana *et al.*, 1988; Bradley *et al.*, 1996; Cuthbert *et al.*, 1996); and (iii) measurement of skin conductance activity during a brief social stressor (a greeting and semi-structured interview with an unfamiliar experimenter). Given the reduction of grey matter density in the amygdalo-hippocampal region in premutation males reported previously, we also completed detailed measurement of the amygdala to examine potential volumetric differences between groups that might help inform our interpretation of the experimental data.

Material and methods
Participants

Participants included 12 men with the *FMR1* premutation (mean age = 42.9 years) and a comparison group of 13 men without the premutation (mean age = 39.8 years) (Table 1); allele status was confirmed for all participants by *FMR1* DNA testing. None of the participants with the premutation was mosaic for either repeat size or methylation. Participant descriptive statistics and *FMR1* data are shown in Table 1. The two groups were matched for age (t = 1.02, P = 0.32), IQ (premutation, 116.3; control, 113.3; t = 0.42, P = 0.68) and level of education (premutation, 15.4 years; control, 14.8 years; t = 0.55, P = 0.59). All participants except one control were right

Table 1 Participant descriptive statistics and *FMR1* measures

	Control (*n* = 13)			Premutation (*n* = 12)			
	Mean	SD	Range	Mean	SD	Range	*P*-value
Age	39.8	7.7	26–55	42.9	7.8	28–56	0.32
WAIS-III full scale IQ	113.3	16.7	84–148	116.3	19.6	83–152	0.68
Education level	14.8	2.6	11–18	15.4	3.3	10–20	0.59
SCL-90-R GSI	59.4	11.5	41–81	54.8	10.4	42–81	0.31
Handedness	12 right, 1 left			12 right, 0 left			0.33
Psychoactive medication	1 On venlafaxine and buproprion			2 On amitriptyline			0.49
FMR1 CGG repeat size	27.0	5.3	17–32	94.4	37.6	57–166	<0.0001
FMR1 mRNA	1.2	0.3	0.9–1.6	3.1	1.1	1.0–5.1	<0.0001

handed. Two participants with the premutation and one control were using psychoactive medication at the time of participation ($\chi^2 = 0.48$, $P = 0.49$). Four individuals were Hispanic, one East Indian and the remaining participants were Caucasian (self-reporting). Males with the premutation were recruited through screening of fragile X pedigrees of probands with FXS. Controls were recruited from within the medical centre community or were normal males in families affected by fragile X. No participants were referred to clinic or ascertained due to clinical symptoms. Neurological examinations on all participants were normal, including absence of tremor and ataxia. One participant with the premutation and one control had missing startle data due to equipment failure and EMG noise artefact, respectively. One participant with the premutation and three control participants had missing fMRI data due to equipment failure.

Psychological assessment
Intelligence
Cognitive ability was based on full scale IQ using the Wechsler Adult Intelligence Scale, Third Edition (WAIS-III; Wechsler, 1997).

Psychological symptoms
The Symptom Checklist-90—Revised (SCL-90-R; Derogatis, 1994) was used as a standardized self-report inventory of current psychological symptoms. Ninety items, each rated on a 5-point scale of distress, are clustered into the following symptom dimensions: somatization, obsessive–compulsive, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, and psychoticism. The global severity index (GSI) is an indicator of overall level of psychological disturbance.

Molecular genetic measures
CGG repeat size
Genomic DNA was isolated from peripheral blood lymphocytes (5 ml of whole blood using standard methods; Puregene Kit; Gentra Inc.). For Southern blot analysis, 5–10 μg of isolated DNA was digested with *Eco*RI and *Nru*I. Hybridization was performed using the *FMR1* genomic digoxigenin-labelled StB12.3 probe. Genomic DNA was also amplified by PCR using primers c and f (Fu *et al.*, 1991). Hybridization was performed with a digoxigenin-labelled oligonucleotide probe (CGG)$_{10}$. Analysis and calculation of the repeat size for both Southern blot and PCR analysis were carried out using an Alpha Innotech FluorChem 8800 Image Detection System.

FMR1 mRNA
All quantitative *FMR1* mRNA measurements involved real-time fluorescence RT–PCR performed using a 7700 Sequence Detector (PE Biosystems) as described previously (Tassone *et al.*, 2000*a*).

Brain volume and function
Brain image acquisition
Images were acquired on a 1.5T GE Signa scanner with Echospeed gradients and a standard GE whole head coil. A custom-built head holder was used to prevent head movement. fMRI was performed using a single-shot gradient recalled echo-echo planar imaging sequence with TR 2000 ms, TE 32 ms, Flip angle 90°, FOV 22 cm, 4 mm slice thickness, 1 mm slice gap, 64×64 matrix, 27 slices, 194 NEX and 62.5 kHz bandwidth and coronal orientation. To aid in localization of functional data, we also acquired a high-resolution T$_1$-weighted spoiled grass gradient recalled (SPGR) 3D MRI sequence. Structural and functional images were acquired in the same scan session. The functional tasks were programmed using Presentation™ software on an IBM compatible computer. Initiation of scan and task were synchronized using a TTL pulse delivered to the scanner timing microprocessor board from a microprocessor connected to the computer. Stimuli were presented visually using a head-coil mounted mirror and projection to a screen at the participant's feet.

Image preprocessing
Images were reconstructed, by inverse Fourier transform, for each of the time points into $64 \times 64 \times 18$ image matrices (voxel size: $3.75 \times 3.75 \times 7$ mm). fMRI data were pre-processed using SPM99. Images were corrected for movement using least squares minimization without higher-order corrections for spin history, and normalized to stereotaxic Talairach coordinates. Images were then resampled every 2 mm using sinc interpolation and smoothed with a 4 mm Gaussian kernel to decrease spatial noise.

Amygdala volume
Amygdala volumes were quantified by operator-guided tracing using Analyze 6.1. These guidelines, used at the UC Davis MIND Institute Computational Neuroimaging Laboratory, were developed from the anatomical analysis of post-mortem human brains using histological sections of tissue cut perpendicular to the hippocampus axis. For a detailed description of this protocol, see Schumann *et al.* (2004).
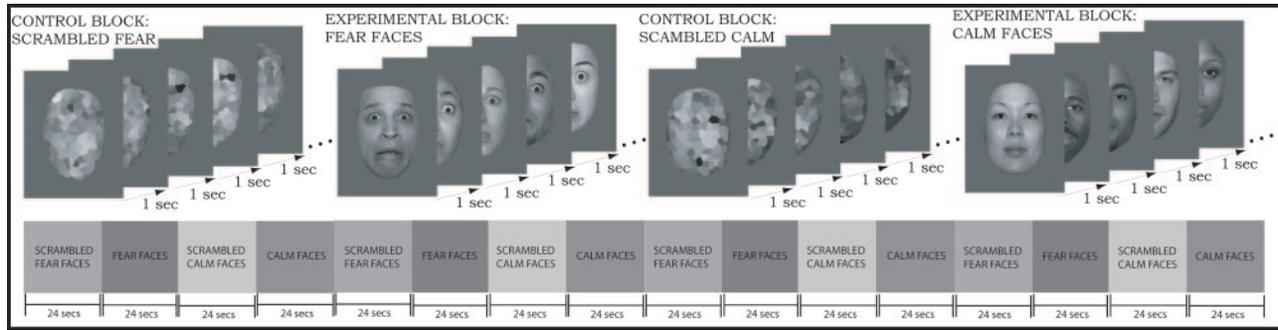
Fig. 1 Images and presentation timing used in the fMRI protocol.

Total cranial volume

To obtain a measure of total cranial volume (TCV), non-brain elements were manually removed from the image by operator-guided tracing of the dura mater within the cranial vault using a custom-written computer program operating on a UNIX, Solaris platform (Quanta 6.1). The middle cranial fossa, the posterior fossa and the cerebellum were included.

Total brain volume

The TCV was automatically segmented into CSF and brain matter components according to previously published methods in order to obtain a measure of total brain volume (DeCarli et al., 1992, 1995, 1996).

Interrater reliability for these methods is good, with intraclass correlation coefficients of 0.99 for TCV, 0.92 for left amygdala and 0.93 for right amygdala. A single rater performed all of the analyses and was blind to the participant's experimental condition and demographic information.

fMRI face processing task

We followed a design similar to that used by Thomas et al. (2001) to evaluate the activity of the amygdala in response to emotional faces. In alternating 24 s blocks, we presented greyscale fearful and calm faces (both from the NimStim Face Stimulus Set, MacArthur Foundation Research Network; Tottenham et al., 2002) as well as scrambled versions of each type (see Fig. 1). Hair and ears were stripped from each image to remove any non-facial features. Each picture was presented for 200 ms followed by an 800 ms interstimulus interval containing a central fixation point. The four block types were presented four times for a total of 16 blocks, or 6.4 min of scan time. Each face and its scrambled counterpart were presented twice during the functional run. No overt response was required. Participants were instructed to keep their eyes open and to look carefully at each picture.

fMRI analysis

Statistical analysis was performed on both individual and group data using the modified General Linear Model and the theory of Gaussian random fields as implemented in SPM99 (Friston et al., 1995). For both within-group and between-group comparisons, significant voxels were defined as those that exceeded a threshold value x equivalent to a one-tailed $P < 0.05$ (corrected for multiple comparisons). Once thresholded, the activation was superimposed on the normalized high-resolution SPGR and localized using atlases of the human brain and cerebellum (Duvernoy and Bourgouin, 1999; Talairach and Tournoux, 1998). Group analyses were overlaid on images created by averaging all individuals' normalized SPGR images.

A standard within-subjects procedure was used to model all effects of interest for each participant by contrasting experimental and control blocks (e.g. blocks of fear faces–blocks of control faces). Models for individuals were identical across participants. Group analyses were performed using a random-effects model incorporating a two-stage hierarchical procedure, which estimates the error variance for each condition of interest across participants rather than across scans (Holmes and Friston, 1998). In the first step, the contrast images for each participant for each effect of interest were generated (described above). In the second step, these contrast images were analysed using a general linear model to determine voxel-wise t-statistics. One contrast image per participant, per effect of interest was generated.

Within-group analyses of each contrast were performed to identify voxels/brain regions showing similar response modulation across participants in each group for a given contrast (e.g. fear–control). In addition, between-group analyses were performed to determine how the two groups differed in their average activation in response to each contrast of interest (i.e. to examine which regions were more active in fragile X premutation participants than in controls, and vice versa).

Region of interest (ROI) analyses were carried out using Marsbar (Brett et al., 2002), a MATLAB toolbox written to be implemented within SPM. Contrasts were first defined as described above. Each contrast of interest was then analysed only in voxels that fell within the MNI template of the amygdala provided within Marsbar. A t-statistic termed 'contrast value' is then calculated as the average of the contrast values of the voxels falling within the defined ROI. The contrast value in these analyses is comparable to the Z-score reported in the whole-brain analyses tables shown below.

Fear-potentiated startle

Stimuli and experimental paradigm

Eighteen colour pictures of human faces (6 fearful, 6 neutral and 6 happy; Tottenham et al., 2002) and 18 colour pictures of non-social scenes (6 unpleasant/fearful, 6 neutral, 6 pleasant; Center for the Study of Emotion and Attention-NIMH, 1998) were presented to the participants seated in a comfortable chair ∼30–36″ from a 19″ computer screen. IAPS images were slide numbers: 1321, 1930, 1300, 1050, 1200 and 7380 (unpleasant/fearful); 7090, 7130, 7030, 7170, 7010 and 7040 (neutral); and 7330, 1710, 7410, 5030, 1920 and 1750 (pleasant). The emotional pictures were chosen to be high on negative/positive valence and arousal
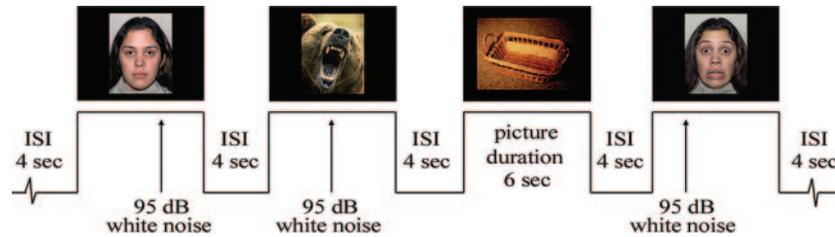
Fig. 2 Examples of images and presentation timing used in the potentiated startle experiment.

according to the normative ratings. Each picture was presented for 6 s and separated by a 4 s inter-picture interval (black screen; Fig. 2). Pictures were presented in three different random orders, with random assignment of order across participants. To elicit the startle reflex, an acoustic startle probe (95 db, 50 ms white noise burst with instantaneous rise and fall) was delivered through headphones during presentation of two-thirds of the picture presentations evenly distributed across picture category. Startle probes were presented randomly between 2.5 and 5.0 s after picture onset. Four baseline startle probes (also at 95 db set 15 s apart) were also administered, two prior to and two following the series of images. Startle probe volume was checked prior to each participant's assessment using a sound level meter. This procedure has been used in multiple studies examining potentiated startle and amygdala function in humans (see Buchanan *et al.*, 2004).

Eye blink response measurement and data analysis

The eye blink response was measured by EMG activity of obicularis oculi and stored offline for later analysis (Biopac Systems, Inc., Santa Barbara, CA). Biopac EL254 electrodes (Ag/AgCl) were placed (i) below the lower eyelid in line with the pupil, and (ii) ~2 cm lateral to the first electrode, centre-to-centre, following the curvature of the muscle. Participants were grounded via the electrodermal transducer used for the skin conductance (described below). Prior to analysis, the raw EMG signal was digitally filtered (90–250 Hz bandpass) and then fully rectified and integrated. The startle eye blink response was defined as the difference between the preblink baseline, taken as the mean EMG activity in the 50 ms prior to the startle probe, and the peak amplitude occurring in the 120 ms following the startle probe. Startle responses occurring during the course of natural eye blinks, muscle or other artefacts, or when participants did not attend to stimuli, were removed from the analysis. Visual attention to the stimuli was recorded live on the physiological record using a toggle switch by a researcher with direct view of the participant's face, positioned behind a one-way mirror and the computer screen. EMG startle responses were first averaged across image category. Startle potentiated by emotional faces was calculated for each participant as the difference between EMG startle response to fearful faces from the mean response to (i) happy faces and (ii) neutral faces. Potentiation of non-social emotional stimuli were calculated as the difference between mean EMG startle response to fearful/unpleasant images from the mean response to (i) unpleasant images and (ii) neutral images. Baseline EMG startle was taken as the mean response to the four baseline startle probes. At least three of four valid, artefact-free responses per slide category were required for a summary value. Non-social startle values for one participant with the premutation were missing due to movement artefact.

Table 2 Total brain and amygdala volumes for males with the *FMR1* premutation and matched controls

	Control (*n* = 13)		Premutation (*n* = 12)		P-value
	Mean	SD	Mean	SD	
Total brain (cc^3)	1260.58	109.66	1214.42	157.70	0.40
Amygdala (cc^3)					
Right	1.77	0.26	1.72	0.16	0.61
Left	1.76	0.29	1.81	0.26	0.66
Total	3.53	0.51	3.54	0.35	0.98

Skin conductance

Biopac Ag–AgCl electrodermal electrodes (Model TSD203) filled with Biopac GEL101 electrode paste were placed on the palmar surface of the second and third fingers of the right hand. The electrodes were connected to a Biopac GSR100C skin conductance amplifier. Data were sampled at a rate of 1000 Hz, with a gain set at 2 $\mu\Omega$/V, with a low pass filter at 1 Hz. Skin conductance was collected for an initial resting baseline period of 30 s prior to the potentiated startle protocol (above). A second resting baseline of 30 s was collected after the startle protocol and prior to the social challenge. Following the second baseline, an unfamiliar adult experimenter knocked, entered the room, greeted the participant and engaged him in a brief, semistructured 2 min interview. In order to compare the social challenge of the interview with the 30 s baseline periods, the analysis focused on the first 30 s of the interview for consistency, and in order to examine the initial social–emotional and physiological response to meeting an unfamiliar person. The experimenter was kept blind with respect to participant group. The data were scored using Acknowledge software (Biopac Systems, Inc., Santa Barbara, CA). During data collection, a second experimenter noted any unusual events in the session or movement-associated artefact. Any events resulting in artefactual responses were removed from the file prior to analysis.

Results

Total brain and amygdala volume

Total brain and amygdala volume descriptive statistics are shown in Table 2. Independent samples *t*-tests revealed no differences in total brain volume [$t(23) = 0.86$, $P = 0.40$], or in right [$t(23) = 0.51$, $P = 0.61$], left [$t(23) = -0.45$, $P = 0.66$] or total amygdala [$t(23) = -0.03$, $P = 0.98$] volumes (Fig. 3). Neither CGG repeat size nor *FMR1* mRNA was significantly correlated with total brain volume in

either group. In the premutation group, *FMR1* mRNA was significantly correlated with left amygdala volume ($r = -0.59$, $P < 0.05$), however this effect disappeared when using amygdala volumes normalized to total brain volume. Also, in the premutation group, psychological symptoms on the SCL-90-R GSI were significantly associated with reduced right amygdala volume (adjusted for total brain volume; Spearman's rank $r_s = -0.72$, $P < 0.01$), and the analogous correlation for right amygdala volume approached significance, $r_s = -0.50$, $P < 0.10$. Psychological symptoms were not significantly associated with amygdala volumes in the control group. Finally, higher IQ was significantly associated with larger total brain volume in the

premutation group, $r_s = 0.68$, $P < 0.05$, but not in the control group, $r_s = 0.28$, $P = 0.38$.

Amygdala and associated brain activation
Within-group analyses
When viewing fearful facial expressions compared with viewing scrambled faces (fear–control contrast), the premutation group showed both less overall activation as well as markedly different patterns of activation compared with controls (Table 3). Of particular interest, premutation carriers failed to activate the amygdala while the control group showed robust bilateral amygdala activation (see Fig. 4A). Even in a targeted analysis where images for the premutation group were thresholded at $P < 0.05$ (uncorrected), there was no evidence of significant amygdala activation (see Fig. 4A). This difference in amygdala activation was evident in the whole-brain analysis and confirmed in an ROI analysis focused on the amygdala (see Fig. 4C). In addition, the control group showed strong activation in bilateral superior temporal sulcus (STS), bilateral orbital gyrus, and bilateral insula. These areas, usually associated with social cognition or emotional processing, were not activated in the premutation group. Most activation within the premutation group was confined to parietal and occipital areas.

In direct contrast to the activation results from the fear-control contrast, the premutation group showed greater overall activation than controls in response to calm facial expressions when compared with viewing scrambled faces
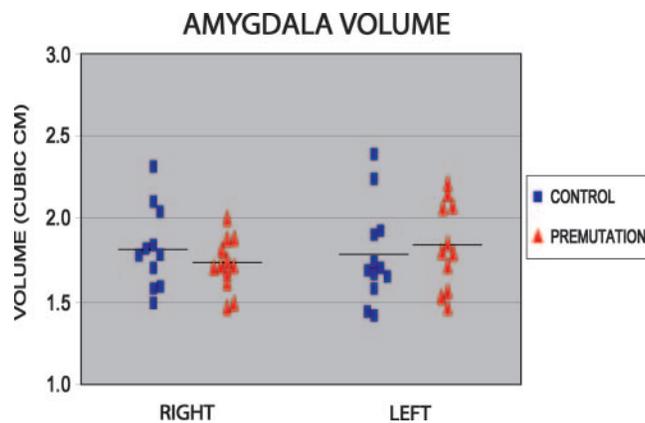

Fig. 3 Absolute left and right amygdala volume in men with the fragile X premutation and matched controls.

Table 3 Stereotaxic locations and Z-scores of activation peaks in the within-group maps

Group	Area	No. of voxels in cluster	Z max	Peak coordinates		
Fear–control contrast						
Control	L orbital gyrus; thalamus; caudate	6230	4.54	−38	32	8
	R middle temporal gyrus; STS; amygdala	2039	4.49	60	−34	−10
	L middle temporal gyrus; STS; amygdala	498	4.29	−42	−56	0
	L occipital sulcus; cerebellum VI	1105	4.05	−36	−86	−16
	L middle and inf. frontal gyrui; insula; orbital gyrus	645	3.74	−48	−8	56
	R middle and inf. frontal gyri; insula; orbital gyrus	437	3.73	50	−8	56
Premutation	R angular gyrus; temporal–parietal junction	1455	4.00	54	−58	22
	R&L cuneus; occipital gyrus	869	3.83	0	−56	20
	L middle occipital gyrus; fusiform gyrus	704	3.61	−52	−74	2
Calm–control contrast						
Control	L occipital gyrus; occipital–polar sulcus; cerebellum (VI)	977	3.94	−20	−102	−6
	R fusiform gyrus; cerebellum (VI)	581	3.71	44	−48	−22
	R inf. frontal sulcus; sup. precentral sulcus	424	3.20	42	20	30
	R amygdala**	119	3.08	14	6	−18
Premutation	R inf occipital sulcus; sup. temporal sulcus	1889	5.27	46	−78	−10
	L inf. occipital sulcus and gyrus; cerebellum (VI)	538	4.48	−42	−86	−12
	L precentral and central gyri; intraparietal sulcus (IPS); inf. parietal lobule	792	4.19	48	−18	50
	R inf. frontal gyrus; orbital gyrus	527	3.72	52	38	16
	R amygdala; hippocampus	748	3.66	14	−4	−14
	L precentral gyrus	433	3.65	−48	−14	46
	L sup. frontal gyrus	787	3.35	−28	−4	52

All clusters significant at $P < 0.05$ corrected except **significant at $P < 0.05$, uncorrected.

(calm–control contrast). When viewing calm faces, premutation carriers showed bilateral amygdala activation (Fig. 4B). Controls showed no amygdala activation to calm faces at the $P < 0.05$, corrected level (see Fig. 4B). Much weaker right amygdala response was seen in the control group when examined at the $P < 0.05$ uncorrected level. These differences in amygdala activation were confirmed in the ROI analysis (Fig. 4C). Premutation carriers also showed activation in the right STS and right orbital gyrus, similar to the control group in the fear–control contrast. Additional activation was seen in the premutation group in parietal and occipital areas as well as left prefrontal areas (Table 3). Activation in the control group was strongest in inferior temporal and occipital regions, including the fusiform gyrus.

Between-groups analysis

As predicted by the within-groups analyses, the control group showed greater activation than the premutation group in several brain areas (Table 4) for the fear–control contrast. These areas included bilateral amygdala, bilateral insula and left STS. Other areas showing greater activation in the control group were bilateral intraparietal sulcus and regions in the left basal ganglia. The calm–control contrast, however, garnered very different results, with the premutation group showing greater activation than controls in left STS and left insula, as well as bilateral cingulate gyrus and bilateral precuneus (Table 4). However, for calm faces, neither the between-group whole-brain analysis nor the ROI analysis yielded significant group differences in amygdala activation (Fig. 4C).

Correlation between psychological symptoms and amygdala activation

We previously reported a significant association between *FMR1* mRNA and psychological symptom severity in males with the premutation ($n = 54$), with Pearson correlations ranging from 0.24 for phobic anxiety to 0.47 for obsessive–compulsive symptoms and 0.50 for psychoticism (Hessl *et al.*, 2005). For the current, much smaller sample ($n = 12$), the correlations were similar in magnitude, ranging from 0.28 for hostility to 0.55 ($P = 0.06$) for obsessive–compulsive symptoms. Since we expected that abnormal amygdala activation might be associated with *FMR1* measures and/ or reflected in psychological symptoms, we investigated amygdala activation as a function of CGG repeat size, mRNA expression and the SCL-90-R GSI score. To conduct this analysis, we entered these variables as covariates of interest in analysing activation during both the fear–control and the calm–control contrasts in all study participants. In this whole-brain covariate of interest analysis (threshold $P < 0.05$, corrected), a negative correlation between SCL-90-R GSI score and amygdala activation to the fear–control contrast was evident in the premutation group but not in the control group. In order to more directly quantify this, we then conducted a targeted analysis of the amygdala through ROI analysis. In the premutation group but not in the control group, severity of psychological symptoms as measured by the SCL-90-R GSI were negatively correlated with both left ($t = 4.45$, $P < 0.001$) and right ($t = 3.84$, $P < 0.01$) amygdala activation. *FMR1* mRNA expression was negatively correlated with right amygdala activation in the premutation group ($P = 0.05$). These effects, however, were primarily driven by the most psychologically affected premutation carrier with highest mRNA expression (CGG repeat size = 103). When this participant was removed from the data set and the ROI analyses were re-run, a weaker but still significant negative correlation remained between SCL-90-R GSI score and left amygdala activation only ($t = 1.95$, $P = 0.04$).

Fear-potentiated startle

Due to violations of normality in several of the startle measures and the small sample sizes, groups were compared using non-parametric Wilcoxon's rank tests. In comparison with controls, participants with the premutation did not demonstrate the expected potentiation of the startle response to fearful faces (contrasted with happy faces, $t = 2.57$, $P = 0.009$; contrasted with neutral faces, $t = 1.78$, $P = 0.08$; see Fig. 5). The groups did not differ significantly in their baseline startle responses, $t = 1.12$, $P = 0.28$, nor in their potentiation to unpleasant non-social stimuli (contrasted with pleasant images, $t = 0.50$, $P = 0.65$; contrasted with neutral images, $t = 1.28$, $P = 0.22$). CGG repeat size and *FMR1* mRNA were not significantly associated with potentiated startle measures in either group (Ps > 0.20). IQ was not significantly correlated with startle measures in either group; however, SCL-90-R GSI was correlated with potentiation of startle to fearful faces in the premutation group only, $r_s = 0.64$, $P < 0.05$.

Skin conductance

A repeated measures analysis of variances, utilizing the Greenhouse–Geisser correction for variance non-homogeneity, with condition (baseline 1, baseline 2 and social challenge) as the repeating dependent variable and group (premutation versus control) as the independent variable, yielded significant effects of condition, $F(1.52) = 29.15$, $P < 0.001$, and group, $F(1) = 8.68$, $P < 0.01$, and a group by condition interaction approaching significance, $F(1.52) = 3.18$, $P = 0.07$. Skin conductance data presented in Fig. 6 show that both groups demonstrated a significant increase from baseline in skin conductance during the social greeting; however, in comparison with controls, participants with the premutation had a diminished skin conductance response. CGG repeat size and *FMR1* mRNA were not significantly associated with skin conductance in any condition or with skin conductance change from baseline to social challenge (Ps > 0.20). There was a strong association between skin conductance change and age in the premutation group only ($r_s = 0.85$, $P < 0.001$) such that
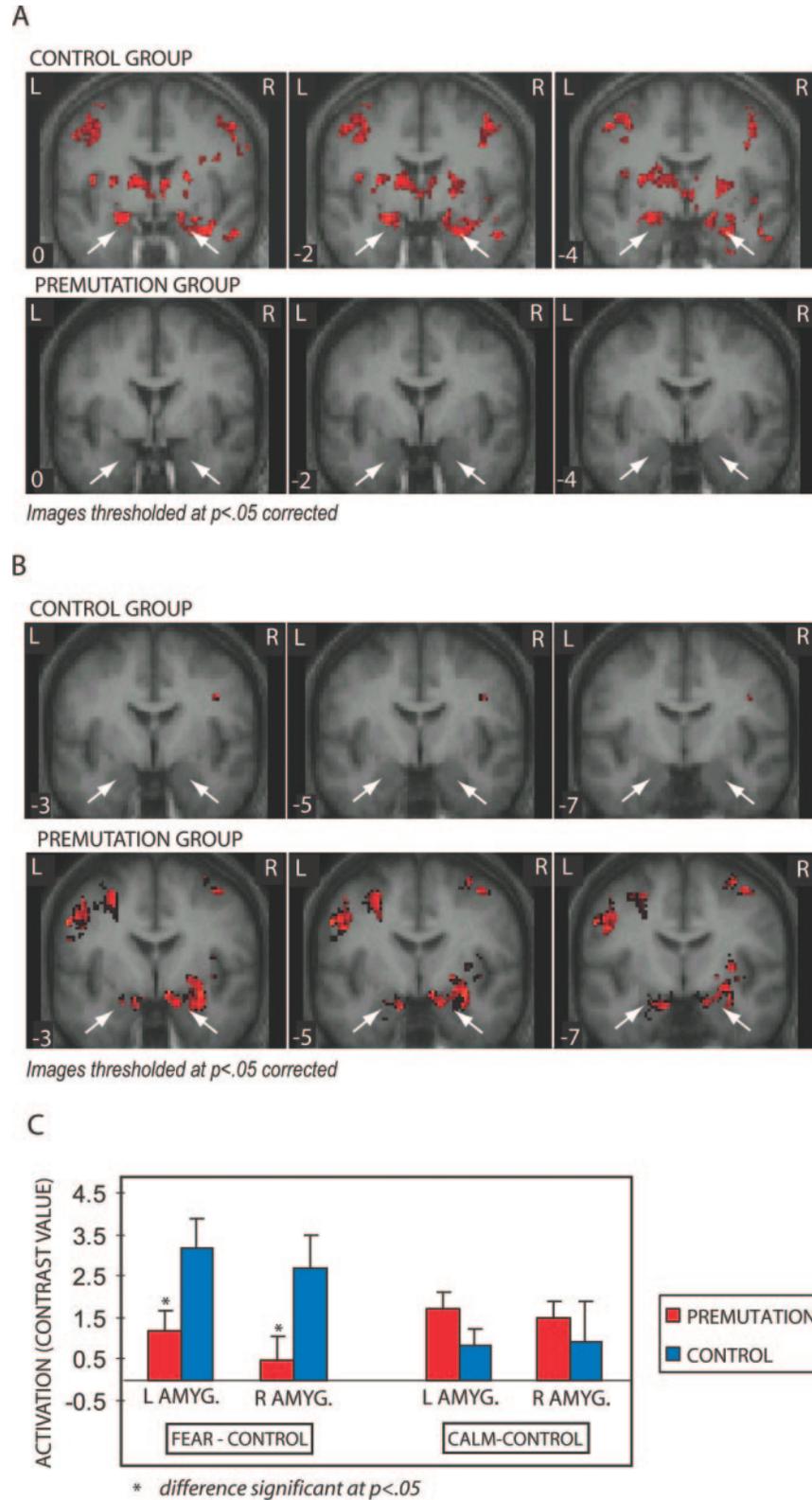
Fig. 4 Regions of activation overlaid on the average normalized T1 structural images. (A) Bilateral amygdala regions were more active in response to fearful faces when compared with scrambled faces in the control group but not in the premutation group. (B) Bilateral amygdala regions were more active in response to calm faces when compared with scrambled faces in the premutation group, only. (C) Results of the ROI analysis focused on the amygdala for both the fear–control and the calm–control contrasts. The control group showed significantly more bilateral amygdala activation than the premutation group in response to fearful faces. There were no significant differences in amygdala activation between groups in response to calm faces. (Error bars show 1 SEM.)

older men demonstrated a stronger skin conductance response to the challenge. Neither IQ nor SCL-90-R GSI was associated with skin conductance in the premutation or control groups.

Discussion

This study utilized different physiological methods to provide convergent evidence that adult males with the fragile X premutation have diminished amygdala and autonomic responsivity to social–emotional stimuli. Relative to IQ-, age- and education level-matched controls, males with the premutation showed reduced amygdala responses to fearful faces as measured directly by fMRI, and indirectly as shown by a lack of eye blink potentiation to fearful faces in the psychophysiology laboratory. In the fMRI experiment, reduced amygdala activity in response to fearful faces was also accompanied by a lack of activation in 'social cognition' areas (orbital-frontal cortex and STS), which was robust in the control group. Furthermore, during a less structured, naturalistic social greeting and interaction with an unfamiliar experimenter, men with the premutation demonstrated reduced sympathetic activation as measured by skin conductance level. The reduced skin conductance may in fact be due to reduced sympathetic outflow that is normally mediated by the amygdala. Interestingly, in the fMRI experiment probing responses to fearful faces, this group also had reduced responses in the insula, a region known to regulate the autonomic nervous system and to be involved in conveying a cortical representation of fear to the amygdala (Phelps *et al.*, 2001; Wright *et al.*, 2003).

Males with the premutation did not differ from controls in detailed measurements of left or right amygdala volume. The lack of significant group differences shows that the diminished BOLD activation found in this region during the face processing task in participants with the premutation was not simply due to differences in volume. In addition, these results are in contrast to the study by Moore *et al.* (2004*b*) demonstrating reduced grey matter density in the combined amygdalo–hippocampus complex, and negative correlations between grey matter density in this region and both CGG repeat size and FMRP in males with the premutation. We did find a marginally significant effect of elevated *FMR1* mRNA on left amygdala volume; however, this did not survive adjustment for total brain volume. Thus, the diminished amygdala and autonomic responses to social–emotional stimuli in individuals with the premutation may be due to morphological differences within the amygdala rather than due to gross volume reductions. Also, the findings by Moore and colleagues in the amygdalo–hippocampal region also may have been driven prominently by structural changes in the hippocampus, a morphological abnormality that has been reported previously in premutation carriers (Jäkälä *et al.*, 1997).

However, despite a lack of structural group differences in the amygdala, psychological symptom severity was associated with decreased adjusted amygdala volume and reduced amygdala activation to fearful faces in men with the premutation. These patterns were not observed in the control group despite similar variation in symptom severity and brain measures. We reported previously that abnormal elevation of *FMR1* mRNA is associated with psychological symptom severity on the same measure (SCL-90-R) in a similar group of men with the premutation who do not have FXTAS (Hessl *et al.*, 2005). We have hypothesized that chronic elevation of *FMR1* mRNA leads to functional and perhaps structural brain changes in limbic regions that contribute to psychological difficulties in males with the premutation. In this preliminary study, we found partial support of this hypothesis; larger and more detailed neurogenetic studies are needed to test this model, to examine the potential impact of reduced FMRP, and to identify the specific phenotype of the premutation.

The decreased reactivity of the amygdala observed in premutation carriers cannot simply be reflective of amygdala damage that prevents any recruitment of this brain region. Indeed, when viewing calm faces, premutation carriers were shown to activate the amygdala as much, if not more, than the control group. In addition, the activation of social cognition areas (STS, orbito-frontal areas) to the calm but not the fearful faces in the premutation group suggests that the dysfunction is more complex in nature, perhaps reflective of mild deficits in social cognition, or a more specific deficit in the processing of emotional or fearful stimuli. Perhaps our participants with the premutation perceived the neutral faces as more ambiguous or threatening than controls, as has been suggested by previous studies of the amygdala response to viewing novel versus familiar faces in normal adults (Schwartz *et al.*, 2003). Interestingly, males with the premutation do have difficulty recognizing neutral facial expressions (Cornish *et al.*, 2005).

Although more definitive studies are needed, there is an indication from several reports that boys with the premutation are at increased risk for developing ASD (Aziz *et al.*, 2003; Borghgraef *et al.*, 2004; Goodlin-Jones *et al.*, 2004; Farzin *et al.*, 2006). These studies suggest that the abnormalities reported here could be developmental in origin and represent the broader phenotype of ASD. ASD in those with the premutation may be due to the abnormal elevation of *FMR1* mRNA, or it may be due to reduction of FMRP, which is the cause of FXS and is known to occur in some male premutation carriers, especially those with high CGG repeat alleles (Tassone *et al.*, 2000*b*). In these experiments, we may have detected subclinical, or endophenotypic traits that may not be evident by routine exam.

Especially relevant to our line of reasoning regarding amygdala dysfunction is research conducted on amygdala function in individuals with ASD. Baron-Cohen *et al.* (2000) have shown that patients with ASD do not activate the amygdala while judging from the expressions of another person's eyes what that other person might be thinking or

Table 4 Stereotaxic locations and Z-scores of activation peaks in the between-group maps

Comparison	Area	No. of voxels in cluster	Z max	Peak coordinates		
Fear-control contrast						
Control > premutation	L IPS; supramarginal gyrus	299	4.35	−30	−48	40
	R cingulate sulcus; insula; amygdala	2441	4.20	16	30	30
	L insula; putamen; caudate	1996	3.98	−46	4	−2
	R orbital gyrus	262	3.78	26	38	−8
	R IPS	357	3.53	38	−56	42
	L sup. temporal gyrus and sulcus; amygdala	259	3.36	−48	−24	4
Premutation > control	R & L transverse parietal sulci	820	4.18	6	−54	30
	R angular gyrus	226	3.81	54	−58	24
Calm-control contrast						
Control > premutation	L cerebellum VI; occipital gyrus descendens; lingual gyrus	587	3.62	−36	−60	−28
Premutation > control	L insula; sup. frontal sulcus and gyrus	996	4.69	−38	12	8
	L sup. temporal sulcus; precuneus; cingulate gyrus	1296	4.39	−44	−52	4
	L thalamus; central sulcus; postcentral gyrus	8319	4.28	−32	−26	0
	R cingulate gyrus; precuneus	472	3.47	10	−48	16
	R occipital sulcus; calcarine sulcus; medial occipital gyrus	498	3.38	50	−74	−12

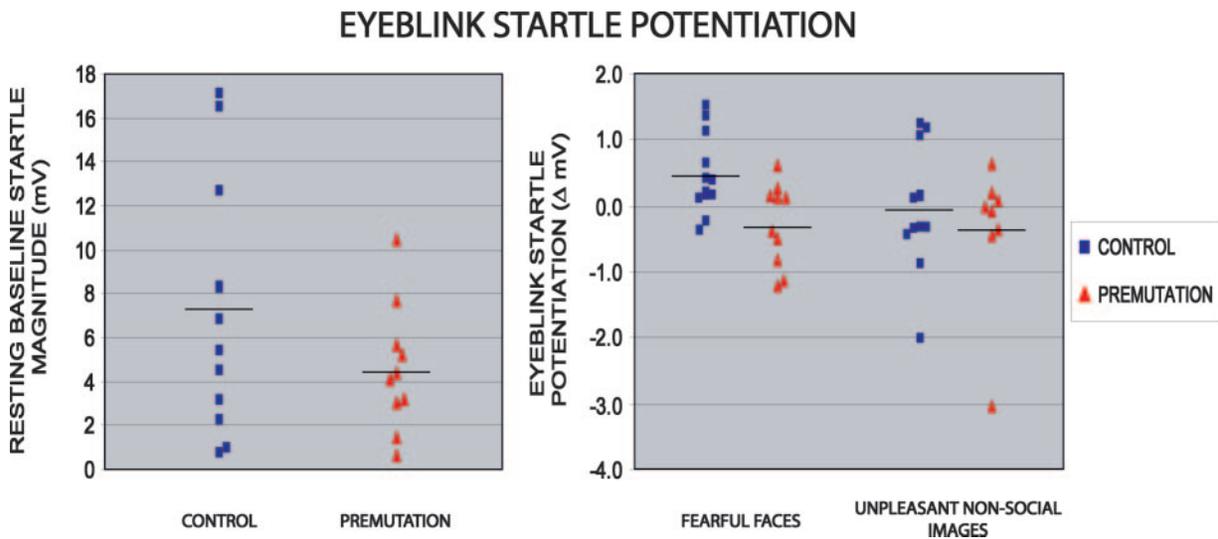All clusters significant at $P < 0.05$ corrected.

Fig. 5 Eye blink startle potentiation to fearful social and non-social images in men with the fragile X premutation and matched controls. (*Left panel*) The premutation and control groups did not differ significantly in resting baseline startle responses (P = 0.28). (*Right panel*) In comparison with controls, males with the premutation did not demonstrate potentiation of the startle reflex to fearful faces (P = 0.009), whereas they showed no significant differences from controls in their startle modulation to unpleasant/fearful non-social images (P = 0.65).

feeling. Consistent with this finding is a study by Howard *et al.* (2000), which found that people with high-functioning autism show neuropsychological profiles characteristic of the effects of amygdala damage, in particular selective impairment in the recognition of facial expressions of fear, perception of eye-gaze direction and recognition memory for faces. Interestingly, Adolphs *et al.* (2002) demonstrated that patients with amygdala damage are not impaired in recognizing basic emotions such as happiness and anger but perform more poorly than brain-damaged controls and normal controls in recognizing complex mental states,

especially social emotions (e.g. arrogant, guilty, admiring and flirting states). The authors concluded that the amygdala is necessary for processing recognition of complex mental states, and argue that deficits in complex social behaviour exhibited by people with ASD may be attributable, in part, to dysfunction in circuits involving the amygdala.

In this study, none of the 12 men with the premutation demonstrated social deficits consistent with ASD. However, several of the participants reported a preference for social isolation, and the individual with the highest *FMR1* mRNA (five times above normal) and most abnormal reduction
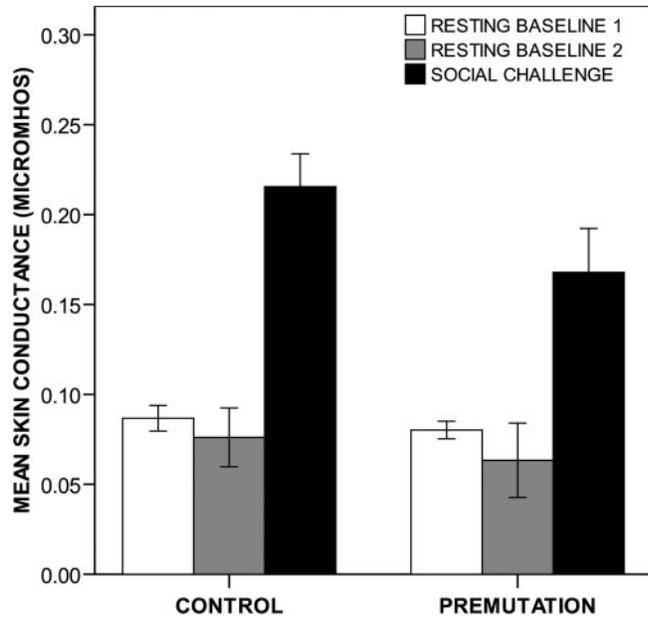
Fig. 6 Mean skin conductance level (+/- 1 SEM) during two resting baseline conditions and a brief social challenge with an unfamiliar experimenter in men with the fragile X premutation and matched controls. Men with the premutation demonstrated a diminished skin conductance response to the challenge as compared with controls. The groups did not differ in their baseline skin conductance levels.

in amygdala response to fearful faces reported chronic social isolation and the highest self-report of psychological problems. This is consistent with our previous study demonstrating that abnormal elevation of *FMR1* mRNA is associated with psychological symptoms including schizoid/avoidant behaviour in adult males with the premutation (Hessl *et al.*, 2005). It is reasonable to hypothesize that more substantial dysfunction of the amygdala and other social cognition brain areas would be present in males with the premutation who meet full diagnostic criteria for ASD. More detailed clinical psychiatric investigations, in conjunction with the brain and physiological measures, are needed to fully address this hypothesis. In addition, studies of children with the premutation and longitudinal studies of adults with the premutation in transition from late adulthood to old age are clearly needed.

Alternatively, or in addition to a developmental effect, our findings could represent early presymptomatic brain changes associated with FXTAS. In post-mortem brain studies of men with FXTAS, Greco *et al.* (2006) reported the presence of intranuclear neuronal and astrocytic inclusions, with the highest rate of inclusions found in the hippocampus. However, more recent examinations of these brains demonstrate that inclusions also are present in the amygdala (C. M. Greco, personal communication). The post-mortem studies involved much older individuals than those reported here; it is not clear whether RNA toxicity impacts the limbic system before onset of FXTAS.

The present set of experiments had several important limitations. In the fMRI and startle experiments, the tasks involved passively viewing images and no behavioural data were obtained. It is possible that the two groups of men differed in their visual attention to fearful social stimuli, leading to differential brain activation and startle responses. Indeed, Dalton *et al.* (2005) demonstrated that activation in the fusiform gyrus and amygdala was positively correlated with the time spent fixating the eyes, in a group of individuals with autism. The lack of amygdala and social cognition area activation in response to fearful faces could be reflective of eye/gaze avoidance, a prominent behavioural abnormality in individuals with FXS. In addition, it is possible that deficits in social cognition in the premutation group could affect their ability to understand the more ambiguous calm faces, potentially causing them to misinterpret them as threatening. Future studies utilizing eye tracking technology would help to confirm that decreased activation of the amygdala in premutation carriers reflects a true diminished response rather than a reduced visual attention to social–emotional stimuli in general or the eye region of faces specifically. It is important to emphasize that the measures of CGG repeat size and *FMR1* mRNA were ascertained from blood samples and may not necessarily reflect what would be found in brain tissue, a possible explanation for the lack of significant correlation between genetic and amygdala function measures. Post-mortem studies performed on male and female carriers, reported so far, have demonstrated inter-tissue somatic stability with regard to CGG repeat length (Tassone *et al.*, 2004); however, it is known that *FMR1* mRNA expression varies across tissue type and between different brain regions (Tassone *et al.*, 2004). Finally, the small sample sizes and missing data in this study limit the conclusions that can be drawn and generalized to the larger population of males with the premutation, and as such the results should be considered preliminary and await replication.

This study demonstrates amygdala dysfunction associated with psychological problems that have been reported previously in fragile X premutation carriers, particularly males (Dorn *et al.*, 1994; Moore *et al.*, 2004a; Cornish *et al.*, 2005; Hessl *et al.*, 2005; Farzin *et al.*, 2006). The psychological difficulties, particularly obsessive–compulsive traits, autism spectrum symptoms and executive function deficits may represent a mild form of RNA toxicity that in some may progress to a more severe form of neurological disease, FXTAS, in later life.

Acknowledgements

References

Abitbol M, Menini C, Delezoide A, Rhyner T, Vekemans M, Mallet J. Nucleus basalis magnocellularis and hippocampus are the major sites of FMR-1 expression in the human fetal brain. Nat Genet 1993; 4: 147–53.

Adolphs R. Cognitive neuroscience of human social behaviour. Nat Rev Neurosci 2003; 4: 165–78.

Adolphs R, Baron-Cohen S, Tranel D. Impaired recognition of social emotions following amygdala damage. J Cogn Neurosci 2002; 14: 1264–74.

Amaral DG, Corbett BA. The amygdala, autism and anxiety. Novartis Found Symp 2003; 251: 177–87, discussion 187–97; 281–97.

Aziz M, Stathopulu E, Callias M, Taylor C, Turk J, Oostra B, et al. Clinical features of boys with fragile X premutations and intermediate alleles. Am J Med Genet 2003; 121: 119–27.

Bacalman S, Farzin F, Bourgeois JA, Cogswell J, Goodlin-Jones BL, Gane LW, et al. Psychiatric phenotype of the fragile X-associated tremor/ataxia syndrome (FXTAS) in males: newly described fronto-subcortical dementia. J Clin Psychiatry 2006; 67: 87–94.

Bachevalier J, Loveland KA. The orbitofrontal-amygdala circuit and self-regulation of social-emotional behavior in autism. Neurosci Biobehav Rev 2006; 30: 97–117.

Baron-Cohen S, Ring HA, Bullmore ET, Wheelwright S, Ashwin C, Williams SC. The amygdala theory of autism. Neurosci Biobehav Rev 2000; 24: 355–64.

Baron-Cohen S, Wheelwright S, Hill J, Raste Y, Plumb I. The 'Reading the Mind in the Eyes' Test revised version: a study with normal adults, adults with Asperger syndrome or high-functioning autism. J Child Psychol Psychiatry 2001; 42: 241–51.

Borghgraef M, Steyaert J, Deroo S, Maes B, Fryns JP. Preliminary findings in boys with fragile X premutation: is there a distinct behavioral phenotype? In: International Fragile X Conference, Washington, D.C. 2004.

Bradley MM, Cuthbert BN, Lang PJ. Picture media, emotion: effects of a sustained affective context. Psychophysiology 1996; 33: 662–70.

Brett M, Anton JL, Valabregue R, Pauline JB. Region of interest analysis using an spm toolbox. Neuroimage 2002; 16: S497.

Brunberg JA, Jacquemont S, Hagerman RJ, Berry-Kravis EM, Grigsby J, Leehey MA, et al. Fragile X premutation carriers: characteristic MR imaging findings of adult male patients with progressive cerebellar and cognitive dysfunction. AJNR Am J Neuroradiol 2002; 23: 1757–66.

Buchanan TW, Tranel D, Adolphs R. Anteromedial temporal lobe damage blocks startle modulation by fear and disgust. Behav Neurosci 2004; 118: 429–37.

Cornish K, Kogan C, Turk J, Manly T, James N, Mills A, et al. The emerging fragile X premutation phenotype: evidence from the domain of social cognition. Brain Cogn 2005; 57: 53–60.

Center for the study of Emotion and Attention (1998). The International Affective Picture System. University of Florida: Gainsville, FL.

Cuthbert BN, Bradley MM, Lang PJ. Probing picture perception: activation and emotion. Psychophysiology 1996; 33: 103–11.

Dalton KM, Nacewicz BM, Johnstone T, Schaefer HS, Gernsbacher MA, Goldsmith HH, et al. Gaze fixation and the neural circuitry of face processing in autism. Nat Neurosci 2005; 8: 519–26.

DeCarli C, Maisog J, Murphy DG, Teichberg D, Rapoport SI, Horwitz B. Method for quantification of brain, ventricular and subarachnoid CSF volumes from MR images. J Comput Assist Tomogr 1992; 16: 274–84.

DeCarli C, Murphy DG, Tranh M, Grady CL, Haxby JV, Gillette JA, et al. The effect of white matter hyperintensity volume on brain structure, cognitive performance, and cerebral metabolism of glucose in 51 healthy adults. Neurology 1995; 45: 2077–84.

DeCarli C, Murphy DG, Teichberg D, Campbell G, Sobering GS. Local histogram correction of MRI spatially dependent image pixel intensity nonuniformity. J Magn Reson Imaging 1996; 6: 519–28.

Derogatis LR. Symptom Checklist-90-R: administration, scoring and procedures manual, 3rd edn. Minneapolis: National Computer Systems, Inc.; 1994.

Dombrowski C, Levesque S, Morel ML, Rouillard P, Morgan K, Rousseau F. Premutation and intermediate-size FMR1 alleles in 10572 males from the general population: loss of an AGG interruption is a late event in the generation of fragile X syndrome alleles. Hum Mol Genet 2002; 11: 371–8.

Dorn MB, Mazzocco MM, Hagerman RJ. Behavioral and psychiatric disorders in adult male carriers of fragile X. J Am Acad Child Adolesc Psychiatry 1994; 33: 256–64.

Duvernoy HM, Bourgouin P. The human brain: surface, three-dimensional sectional anatomy with MRI, blood supply. New York: Springer; 1999.

Dziobek I, Fleck S, Rogers K, Wolf OT, Convit A. The 'amygdala theory of autism' revisited: linking structure to behavior. Neuropsychologia 2006; 44: 1891–9.

Farzin F, Perry H, Hessl D, Loesch D, Cohen J, Bacalman S, et al. Autism spectrum disorders and attention-deficit/hyperactivity disorder in boys with the fragile X premutation. J Dev Behav Pediatr 2006; 27: S137–44.

Franke P, Leboyer M, Gansicke M, Weiffenbach O, Biancalana V, Cornillet-Lefebre P, et al. Genotype-phenotype relationship in female carriers of the premutation and full mutation of FMR-1. Psychiatry Res 1998; 80: 113–27.

Friston KJ, Holmes AP, Worsley J-P, Poline CD, Frith CD, Frackowiak RSJ. Statistical parametric maps in functional imaging: a general linear approach. Hum Brain Mapp 1995; 2: 189–210.

Fu YH, Kuhl DP, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, et al. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. Cell 1991; 67: 1047–58.

Goodlin-Jones BL, Tassone F, Gane LW, Hagerman RJ. Autistic spectrum disorder and the fragile X premutation. J Dev Behav Pediatr 2004; 25: 392–8.

Greco CM, Hagerman RJ, Tassone F, Chudley AE, Del Bigio MR, Jacquemont S, et al. Neuronal intranuclear inclusions in a new cerebellar tremor/ataxia syndrome among fragile X carriers. Brain 2002; 125: 1760–71.

Greco CM, Berman RF, Martin RM, Tassone F, Schwartz PH, Chang A, et al. Neuropathology of fragile X-associated tremor/ataxia syndrome (FXTAS). Brain 2006; 129: 243–55.

Hagerman RJ, Hagerman PJ. The fragile X premutation: into the phenotypic fold. Curr Opin Genet Dev 2002; 12: 278–83.

Hagerman PJ, Hagerman RJ. Fragile X-associated tremor/ataxia syndrome (FXTAS). Ment Retard Dev Disabil Res Rev 2004a; 10: 25–30.

Hagerman PJ, Hagerman RJ. The fragile-X premutation: a maturing perspective. Am J Hum Genet 2004b; 74: 805–16.

Hagerman RJ, Leehey M, Heinrichs W, Tassone F, Wilson R, Hills J, et al. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. Neurology 2001; 57: 127–30.

Hessl D, Tassone F, Loesch DZ, Berry-Kravis E, Leehey MA, Gane LW, et al. Abnormal elevation of FMR1 mRNA is associated with psychological symptoms in individuals with the fragile X premutation. Am J Med Genet B Neuropsychiatr Genet 2005; 139: 115–21.

Hitchcock J, Davis M. Lesions of the amygdala, but not of the cerebellum or red nucleus, block conditioned fear as measured with the potentiated startle paradigm. Behav Neurosci 1986; 100: 11–22.

Hitchcock JM, Davis M. Fear-potentiated startle using an auditory conditioned stimulus: effect of lesions of the amygdala. Physiol Behav 1987; 39: 403–8.

Holmes AP, Friston KJ. Generalisability, random effects and population inference. Hum Brain Mapp 1998; 7: S754.

Howard MA, Cowell PE, Boucher J, Broks P, Mayes A, Farrant A, et al. Convergent neuroanatomical and behavioural evidence of an amygdala hypothesis of autism. Neuroreport 2000; 11: 2931–5.

Jacquemont S, Hagerman RJ, Leehey M, Grigsby J, Zhang L, Brunberg JA, et al. Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. Am J Hum Genet 2003; 72: 869–78.

Jacquemont S, Hagerman RJ, Leehey MA, Hall DA, Levine RA, Brunberg JA, et al. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. JAMA 2004; 291: 460–9.

Jäkälä P, Hanninen T, Ryynanen M, Laakso M, Partanen K, Mannermaa A, et al. Fragile-X: neuropsychological test performance, CGG triplet repeat lengths, and hippocampal volumes. J Clin Invest 1997; 100: 331–8.

Johnston C, Eliez S, Dyer-Friedman J, Hessl D, Glaser B, Blasey C, et al. Neurobehavioral phenotype in carriers of the fragile X premutation. Am J Med Genet 2001; 103: 314–9.

Moore CJ, Daly EM, Schmitz N, Tassone F, Tysoe C, Hagerman RJ, et al. A neuropsychological investigation of male premutation carriers of fragile X syndrome. Neuropsychologia 2004a; 42: 1934–47.

Moore CJ, Daly EM, Tassone F, Tysoe C, Schmitz N, Ng V, et al. The effect of pre-mutation of X chromosome CGG trinucleotide repeats on brain anatomy. Brain 2004b; 127: 2672–81.

Phelps EA, LeDoux JE. Contributions of the amygdala to emotion processing: from animal models to human behavior. Neuron 2005; 48: 175–87.

Phelps EA, O'Connor KJ, Gatenby JC, Gore JC, Grillon C, Davis M. Activation of the left amygdala to a cognitive representation of fear. Nat Neurosci 2001; 4: 437–41.

Rousseau F, Rouillard P, Morel ML, Khandjian EW, Morgan K. Prevalence of carriers of premutation-size alleles of the FMRI gene—and implications for the population genetics of the fragile X syndrome. Am J Hum Genet 1995; 57: 1006–18.

Schultz RT. Developmental deficits in social perception in autism: the role of the amygdala and fusiform face area. Int J Dev Neurosci 2005; 23: 125–41.

Schwartz CE, Wright CI, Shin LM, Kagan J, Whalen PJ, McMullin KG, et al. Differential amygdalar response to novel versus newly familiar neutral faces: a functional MRI probe developed for studying inhibited temperament. Biol Psychiatry 2003; 53: 854–62.

Schumann CM, Hamstra J, Goodlin-Jones BL, Lotspeich LJ, Kwon H, Buonocore MH, et al. The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. J Neurosci 2004; 24: 6392–401.

Sweeten TL, Posey DJ, Shekhar A, McDougle CJ. The amygdala and related structures in the pathophysiology of autism. Pharmacol Biochem Behav 2002; 71: 449–55.

Talairach J, Tournoux P. Co-planar stereotaxic atlas of the human brain: a 3-dimensional proportional system, an approach to cerebral imaing. New York: Thieme Medical Publishers; 1998.

Tassone F, Hagerman RJ, Taylor AK, Gane LW, Godfrey TE, Hagerman PJ. Elevated levels of FMR1 mRNA in carrier males: a new mechanism of involvement in the fragile-X syndrome. Am J Hum Genet 2000a; 66: 6–15.

Tassone F, Hagerman RJ, Taylor AK, Mills JB, Harris SW, Gane LW, et al. Clinical involvement and protein expression in individuals with the FMR1 premutation. Am J Med Genet 2000b; 91: 144–52.

Tassone F, Iwahashi C, Hagerman PJ. FMR1 RNA within the intranuclear inclusions of fragile X-associated tremor/ataxia syndrome (FXTAS). RNA Biol 2004; 1: 103–5.

Thomas KM, Drevets WC, Dahl RE, Ryan ND, Birmaher B, Eccard CH, et al. Amygdala response to fearful faces in anxious and depressed children. Arch Gen Psychiatry 2001; 58: 1057–63.

Tottenham N, Borscheid A, Ellersten K, Marcus DJ, Nelson CA. Categorization of facial expressions in children and adults: establishing a larger stimulus set. Cogn Neurosci Soc, 2002.

Vrana S, Spence E, Lang PJ. The startle response: a new measure of emotion? J Abnorm Psychol 1988; 97: 487–91.

Wechsler D. Wechsler Adult Intelligence Scale, 3rd edn. Manual. San Antonio: Psychological Corporation; 1997.

Wright CI, Martis B, McMullin K, Shin LM, Rauch SL. Amygdala and insular responses to emotionally valenced human faces in small animal specific phobia. Biol Psychiatry 2003; 54: 1067–76.