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Decreased functional brain response to emotional arousal and increased psychiatric symptomology in *FMR1* premutation carriers

Stephanie S.G. Brown[a,*], Heather C. Whalley[a], Peter C. Kind[b], Andrew C. Stanfield[a]

[a] Patrick Wild Centre, Division of Psychiatry, School of Molecular and Clinical Medicine, University of Edinburgh, Royal Edinburgh Hospital, Edinburgh EH10 5HF, United Kingdom

[b] Patrick Wild Centre, Centre for Integrative Physiology, School of Biomedical Sciences, University of Edinburgh, Hugh Robson Building, 15 George Square, Edinburgh EH8 9XD, United Kingdom

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ABSTRACT

The *FMR1* premutation is an expansion of the CGG repeat island in the *FMR1* gene to between 55 and 200 repeats. Evidence suggests that as well as conferring risk for neurodegeneration, the premutation is also associated with increased risk for autistic traits and psychiatric symptoms. An emotional processing fMRI task was used to examine the response to a change in emotional arousal in 17 male carriers and 17 matched controls. A psychiatric symptom checklist (SCL-90-R), autism spectrum and empathy quotients (AQ and EQ), and the Ekman Faces Test were used to investigate clinical symptoms and emotional processing. Carriers exhibited significantly lower activation compared to controls at the bilateral superior parietal lobe, bilateral Brodmann Area (BA) 17 (V1), right intraparietal area and right BA18 (V2) when comparing high and low arousal conditions. Group by age analyses were not significant. Assessments revealed that carriers displayed significantly worse symptoms of psychiatric symptoms and higher levels of autistic traits, as well as impaired facial emotion recognition. No measurements revealed an association with age. Here, we show significantly altered emotional processing in carriers which display stability over age, suggesting that, unlike degenerative aspects, emotional symptoms may be consistent over the lifespan in carriers.

1. Introduction

The *FMR1* premutation is defined by an expansion of the CGG repeat island in the *FMR1* gene from below 55 repeats to between 55 and 200 repeats. In male carriers, this genetic change causes a 40–60% risk for the development of neurodegenerative disease known as the Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) (Dombrowski et al., 2002; Coffey et al., 2008). FXTAS is a late-onset disorder, although changes to brain function in disease relevant areas are seen in younger carriers, and we have recently identified preliminary evidence that these changes may increase over time, prior to the onset of the clinical condition (Brown et al., 2018).

In addition to FXTAS, an increased risk for psychiatric problems and higher levels of autistic traits have been identified in premutation carriers, the manifestation of which appears to be irrespective of FXTAS development. It is possible that these abnormalities are functionally separate to neurodegeneration in carriers, representing a phenotype that remains stable over time (Hagerman and Hagerman, 2013; Bourgeois et al., 2011).

Psychiatric problems identified in *FMR1* premutation carriers over their lifespan include anxiety, depression, obsessive-compulsive behaviour and irritability (Bourgeois et al., 2011; Dorn et al., 1994; Merino et al., 2016; Koldewyn et al., 2008; Lozano et al., 2014; Bacalman et al., 2006). Psychiatric checklist questionnaires have revealed that both male and female asymptomatic premutation carriers scored higher than control subjects for obsessive-compulsiveness and overall symptom severity (Hessl et al., 2005). Studies have also shown that male premutation carriers may have higher levels of autistic traits than the general population (Bailey et al., 2008; Besterman et al., 2014). Previous fMRI studies in premutation cohorts have suggested that there may be abnormalities in emotional processing which could underlie these difficulties (Brown and Stanfield, 2015). During an Ekman faces-based task, presentation of neutral faces elicited greater overall brain activation in premutation carriers compared to controls, which may be reflective of neuropsychological abnormalities (Hessl et al., 2007; Bradley et al., 2003). Additionally, an emotional processing task also utilising Ekman faces revealed that limbic activation in carriers was significantly reduced. Regression analyses indicated

* Corresponding author.
E-mail address: s0935170@sms.ed.ac.uk (S.S.G. Brown).

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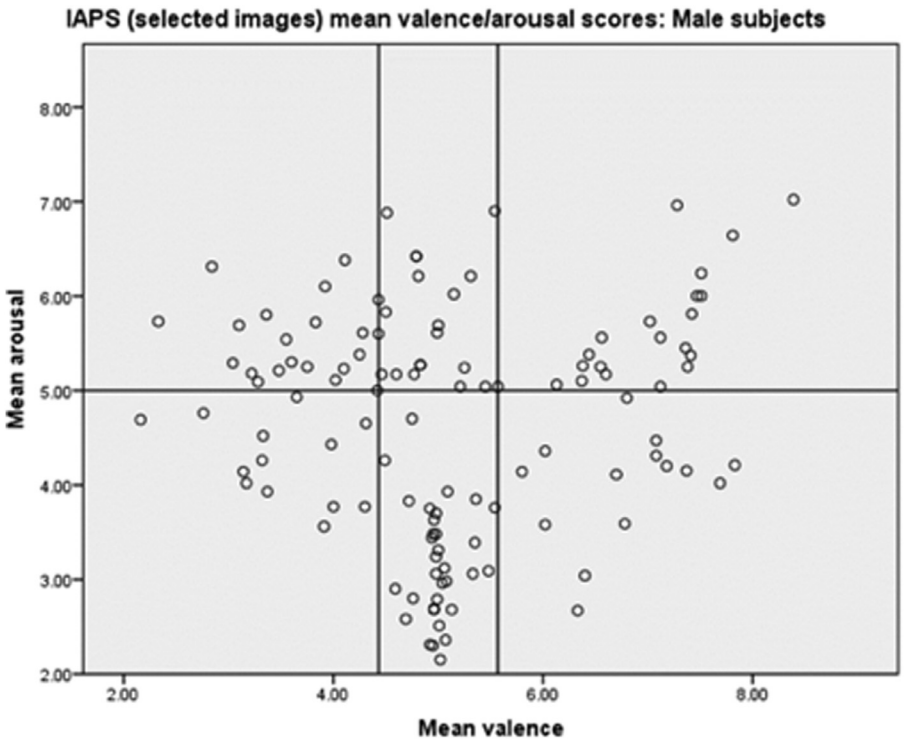

Fig. 1. Selected images from the IAPS that form the fMRI emotional processing task: Mean valence and arousal scores for male subjects from the IAPS normative data set. Reference line for arousal indicates differentiation between 'high' and 'low' arousal images and reference lines for valence indicate differentiation between 'high', 'neutral' and 'low' valence images.

that lower levels of FMRP in the premutation group were a primary factor in attenuation of amygdala BOLD response (Hessl et al., 2011).

In the present study, we aimed to determine if carriers exhibit functional brain differences in emotional processing that may be a basis for the increased rates of autistic and psychiatric symptomatology in premutation groups (Jacquemont et al., 2004b; Cornish et al., 2005; Adams et al., 2010; Seritan et al., 2016). We also aimed to determine whether these changes would be stable across a wide age range of participants. The present study utilised a cross-sectional design and an emotional processing fMRI task that used emotive photographic stimuli sourced from the International Affective Picture System (IAPS) to probe possible between group differences in emotional function. The IAPS was used as it allows for the examination of arousal in response to emotional stimuli; arousal has been found to be dysregulated in conditions which carriers are at high risk of developing, including obsessive-compulsiveness and anxiety (Hofmann et al., 2012).

We predicted that the response to emotional arousal would be altered in premutation carriers compared to controls in social and salience brain regions. We also predicted that in contrast to our findings for motor features (Brown et al., 2018), these changes will represent a stable trait across an age range as opposed to one that is involved in the neurodegenerative processes of FXTAS (Jacquemont et al., 2003; Dorn et al., 1994).

2. Materials and methods

2.1. Participants and recruitment

Seventeen male carriers of the premutation and seventeen healthy male age-matched control subjects were recruited. The age range for inclusion in the study was 20–70 years. Premutation carrier participants were required not to have received a diagnosis of FXTAS and to have been genetically confirmed as a carrier prior to taking part in the study. All participants were also screened for MRI eligibility and safety prior to taking part.

The protocol was approved by the South East Scotland Research Ethics Committee and NHS Lothian Research and Development Office.

2.2. Imaging methods

All MRI data was acquired on a 3 Tesla Siemens MRI scanner using an 8 channel head coil. For the acquisition of functional images the TR was 1560 ms, the TE (echo time) was 26 ms, the flip angle was 66°, the FOV (field of view) was 220 mm, slice thickness was 5 mm and slice number per volume was 26. Slice order was interleaved and bottom up in the axial orientation. Each participant also underwent a T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence, for the purpose of co-registration to and subsequent normalization of the functional images. For the MPRAGE acquisition the TR was 2300 ms, the TE was 2.98 ms, the flip angle was 9°, the FOV was 256 mm, slice thickness was 1 mm and slice number per slab was 160.

The emotional processing task used in the scanning protocol was designed and ran on Presentation™ software (Version 18.0, Neurobehavioral Systems, Inc., Berkeley, CA, www.neurobs.com). The task utilised 120 pictures sourced from the International Affective Picture System (IAPS), which differentiates images based on arousal and valence (pleasure) ratings. As defined by IAPS normative data, collected by Lang et al., 2005, arousal and valence are rated on a scale of 1–10, with higher scores indicating higher levels of arousal or pleasure. Normative affective ratings were obtained to form this material from approximately 50 male college students (Lang et al., 2005). In the present task, IAPS images were chosen *a priori* based on their valence and arousal normative estimation. Twenty pictures for each of the following conditions were used: high pleasure/low arousal, high pleasure/high arousal, low pleasure/low arousal, low pleasure/high arousal, neutral pleasure/high arousal and neutral pleasure/low arousal. Pictures that were judged to be too distressing, disturbing or graphic were not included in the task, meaning that arousal rating of the pictures was not above 7.02. Neutral images were chosen based on a median valence range of 4.5–5.5. Selected IAPS images for this study are plotted according to valence and arousal ratings in Fig. 1. The task utilised an event-related design, with each trial duration comprising of stimulus presentation and an inter-stimulus interval. Order of the presentation of stimulus condition was randomised, without variation between participants. Jittering of the stimuli relative to the TR was

achieved by varying inter-stimulus interval length at upper and lower truncation points of 1.5 and 2.5 s. Mean inter-stimulus interval for each condition was 2 s. All stimuli presentation durations were 1.5 s, and mean trial duration was 3.5 s for each condition. A 2 s fixation period was presented every 10 trials. Each total condition duration was 1 min 10 s. Participants were asked to indicate using left and right trigger buttons in the scanner whether or not there was a face in each of the pictures – i.e. "Face" or "No face" – as a measure of engagement with the task. Presence of a face was not considered a variable of interest for further analysis, and due to the inherent nature of visual arousal and valence, the conditions were not balanced for percentage of face-containing images. Participant button presses were monitored visually during the task to ensure engagement in the task, however task accuracy data was not directly collected, as the purpose of responsivity was primarily task participation. The task was visually presented to participants using goggles that fit onto the scanner head coil.

2.3. fMRI analysis

Statistical analysis on fMRI data was carried out using Statistical Parametric Mapping software (SPM12) (Wellcome Department of Clinical Neurology).

The functional images from all participants were preprocessed according to the following steps: 1) firstly images were realigned, estimated (for optimal transformation from individual images to the reference using SPM12 default quality, separation, smoothing, num passes, interpolation, wrapping and weighting parameters) and resliced 2) images were then slice timed, adjusting for interleaved and bottom up slice order 3) subsequent functional images were co-registered with the source structural image from the T1 MPRAGE anatomical scan 4) co-registered images were segmented into grey matter, white matter and CSF outputs 5) images were normalised to MNI space, and 6) finally the normalised images were smoothed with a 8 mm FWHM (full-width at half maximum) Gaussian smoothing kernel.

Movement was controlled for by adding realignment parameters for each participant as a multiple regressor into the first level model. For first-level analysis of the IAPS emotional processing task, activation during high arousal images were compared to those during low arousal conditions, regardless of valence. Valence conditions were not investigated as separate contrasts. For the second level analysis, explicit masking was used to exclude voxel data outside brain tissue with greater accuracy than default implicit masking. This explicit mask was comprised of an average binarised image created from the combined grey matter and white matter segmented images from all participants. Both within and between group analyses were completed. Within group analysis used a one-sample *t*-test for each task and first level contrasts to look at significant activation for the control and premutation group separately. Within group second level contrasts were defined as either [1] or [−1] to look at positive and negative activation patterns in the high/low arousal contrast. The between group analyses used a full factorial design to examine the differences between the control and premutation groups. The groups were compared using [1 −1] and the reverse [−1 1] contrasts. To examine the effects of age in the sample, age was added into the full factorial design as a regressor of interest and group x age interactions were examined for both the control and the premutation group using [1 −1] and the reverse [−1 1] contrasts. All second level contrasts were calculated using a height threshold value of $p = 0.005$ and a voxel threshold of 0 to allow for the detection of spike activations. Family-wise error correction was then carried out, with significance being indicated by cluster-wise inference ($FWE_{corr} < 0.05$). Cluster locations were anatomically defined using the SPM anatomy toolbox (Eickhoff et al., 2005; Eickhoff et al., 2006).

2.4. Clinical and neuropsychological measurements

The Symptom Checklist 90 Revised (SCL-90-R®) (Pearson) was used to evaluate participant psychiatric symptomatology. The SCL-90-R is a self-report questionnaire comprising of 90 questions. Traits measured in the SCL-90-R are somatization, obsessive-compulsiveness, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation and psychoticism. A global severity index can also be generated, which is the severity of all symptoms combined. All participants also completed the Autism Spectrum Quotient (AQ), a 50 item scale which is widely used to measure autistic traits in non-learning disabled individuals, and the Empathy Quotient (EQ), a 60 item scale designed to measure empathy. Verbal and non-verbal intelligence quotients (IQ) was assessed using the Kaufman Brief Intelligence Test (KBIT) Second Edition (Kaufman & Kaufman, 2004).

The Ekman 60 Faces Test (Version 1.0, Thames Valley Test Company) was carried out by all participants (Young et al. 2002). The Ekman 60 Faces Test asked participants to correctly identify the facial emotion in 60 pictures of males and females, which were presented for a duration of 5 s each in a random order. The facial emotions used in the test were anger, disgust, fear, happiness, sadness and surprise and 10 of each were presented during the test. Scores were reported as number of correctly identified images out of 10 per emotion, and a total of correctly identified images out of 60. Each participant was given verbal instructions and a short practice run of the test. Regression analyses were carried out between clinical and neuropsychological variables and age separately for both groups.

2.5. CGG repeat length assay

DNA was isolated from blood samples for both control and carrier participants. The assay for CGG repeat length in the *FMR1* gene was carried out using a PCR-only approach based on Triplet Repeat Primed PCR design (AmplideX® PCR/CE *FMR1* reagents).

3. Results

3.1. Participants and recruitment

The group of male premutation carriers ($n = 17$) had a mean age of 50.4 years old (SD = 15.1), age range 24–68 years and the age-matched male control group had a mean age of 47.6 years old (SD = 12.9), age range 26–68 years. Independent two-sample t-testing revealed that age did not differ significantly between the groups ($p = 0.507$). CGG repeat length testing indicated that 12 carriers were in the premutation range, 1 carrier was in the intermediate range, 3 carriers were mosaic for repeat size and one carrier was borderline for the full mutation. It was confirmed that all control subjects were within the normal CGG repeat length range. All participants were right handed, excepting one control subject and two carrier subjects, who were left handed. All participants had a composite IQ >80, as measured by the KBIT Second Edition Intelligence Test (Pearson) (Table 1). Two-sample t-testing of IQ data revealed that composite and verbal IQ as measured by the KBIT-2 were not significantly different between the groups ($p = 0.053$, $p = 0.604$), although composite IQ exhibits a trend towards being lower in carriers. Non-verbal IQ as measured by the KBIT-2 was significantly lower in carriers ($p = 0.015$). Based on visual observation at the time of scan, there were no differences across the two participant groups in terms of task engagement.

3.2. Within group imaging

One sample *t*-test within group analysis revealed that when contrasting the combined low arousal and high arousal conditions, controls exhibited four significant clusters of activation. The first and largest significant cluster ($FWE_{corr} < 0.001$, $T = 10.17$) incorporated the right intraparietal cortex and the right human intraparietal areas 1 and 3. The maximum of the cluster was located at [48 −48 34]. A second significant cluster ($FWE_{corr} < 0.001$, $T = 7.37$) was located at the left

Table 1
Participant data.

Participant ID	Age	Premutation status	Composite IQ
1	26	Normal (40 repeats)	129
2	24	Normal (20 repeats)	123
3	33	Normal (22 repeats)	110
4	30	Normal (38 repeats)	103
5	52	Normal (19 repeats)	107
6	41	Normal (20 repeats)	128
7	58	Normal (23 repeats)	112
8	53	Normal (30 repeats)	110
9	48	Normal (32 repeats)	110
10	55	Normal (32 repeats)	111
11	64	Normal (31 repeats)	108
12	52	Normal (31 repeats)	105
13	45	Normal (31 repeats)	121
14	68	Normal (23 repeats)	126
15	58	Normal (30 repeats)	127
16	55	Normal (20 +/−1 repeats)	122
17	47	Normal (30 repeats)	126
18	46	Premutation (91 +/−3 repeats)	139
19	67	Premutation (82 +/- 3 repeats)	106
20	24	Premutation (82 +/−2 repeats)	119
21	50	Premutation (88 +/- 3 repeats)	144
22	54	Premutation (85 +/- 5 repeats)	108
23	57	Premutation (185 +/- 10 repeats)	101
24	26	Premutation (mosaic 150 +/- 5; ~200 +/- 10 repeats)	86
25	33	Premutation (74 +/- 2 repeats)	111
26	68	Premutation (118 +/- 8 repeats)	104
27	68	Premutation (88 +/- 3 repeats)	97
28	43	Premutation (71 +/- 2; 119 +/- 5 repeats)	107
29	66	Premutation (mosaic 133, 156; 198 +/- 10 repeats)	109
30	52	Premutation (58 repeats)	94
31	30	Premutation (mosaic 148 +/- 5; ~200 repeats)	102
32	67	Premutation (80 +/- 2 repeats)	108
33	58	Premutation (91 +/−3 repeats)	125
34	47	Intermediate (50 repeats)	117

cerebellar lobule VIIa Crus I, left cerebellar lobule VI and the right human occipital cortex 3 dorsal (hOC3v) with the co-ordinates of the cluster maximum at [−18 −76- 26]. A third significant cluster ($FWE_{corr} < 0.001$, $T = 6.90$) was located at the bilateral superior parietal lobes, with the co-ordinates of the cluster maximum at [12 −62 40] and a final significant cluster ($FWE_{corr} = 0.001$, $T = 6.18$) was located at the right middle and superior frontal gyri, with the co-ordinates of the cluster maximum at [22 54 30] (Fig. 2a). In contrast, a one sample *t*-test within group analysis in the carrier group showed no clusters of significant activation (Fig. 2b).

3.3. Between group imaging

Full factorial between group analysis revealed that the premutation group had two clusters of significantly lower BOLD response when compared to the control group. The first, largest cluster of significantly lower activation ($FWE_{corr} = 0.001$, $T = 4.77$) was located at bilateral BA17 of the primary visual cortex, right BA18 of the visual association area and the right superior parietal lobule. The maximum of the cluster was located at [16 −88 14]. The second cluster of significantly lower activation in the carrier group ($FWE_{corr} = 0.021$, $T = 4.26$) was located at the bilateral superior parietal lobes, right BA2 of the primary somatosensory cortex and right human intraparietal area 1. The maximum of the cluster was located at [34 −40 42] (Fig. 3).

In the analysis probing presence of age related group differences, no significant group x age clusters were identified at a whole brain level. Within group regression analyses also confirmed that there was no significant association between age and activation at global maxima in either group.

3.4. Clinical and neuropsychological measurements

The psychiatric symptomatology checklist, SCL-90-R, revealed that premutation carriers had significantly worse obsessive-compulsiveness ($p = 0.006$), anxiety ($p = 0.028$), global severity index (GSI) ($p = 0.033$) and positive symptom distress index (PSDI) ($p = 0.002$) scores compared to controls. Somatisation, interpersonal sensitivity, depression, hostility, phobic anxiety, paranoid ideation, psychoticism and positive symptom total (PST) were not significantly different between the groups ($p = 0.212$, 0.389, 0.135, 0.187, 0.171, 0.161, 0.122, 0.202 respectively). Regression analyses confirmed that age was not associated with any SCL-90-R variable in either the premutation or control group. Mean carrier and control scores of the SCL-90-R are plotted in Fig. 4. The premutation group showed significantly higher levels of autistic traits as measured by the AQ than the control group ($p = 0.015$). Scores of empathy as measured by the EQ were not significantly different between groups, however, a trend towards carriers scoring lower, indicative of less empathy, was evident ($p = 0.084$). Mean AQ and EQ scores are plotted in Fig. 5.

The Ekman 60 Faces Test indicated that carriers performed significantly worse than control subjects when attempting to recognise facial emotions of anger ($p = 0.045$), disgust ($p = 0.039$) and surprise ($p = 0.027$). Controls and carriers did not perform significantly differently when asked to recognise facial emotions of fear, happiness or sadness ($p = 0.142$, 0.08, 0.161 respectively). Carriers performed worse overall on the test than the control group ($p = 0.002$). Regression analyses indicated that age was not associated with any Ekman faces variable in either the premutation or control group. Mean carrier and control scores of the Ekman 60 Faces Test are in Fig. 6. None of the clinical and neuropsychological variables correlated significantly with the BOLD activation at significant clusters.

4. Discussion

The present study identifies a robust pattern of activation for the control group during a change in arousal state, which was not replicated in the premutation carrier group. In controls, clusters of significant activation were centred in the right intraparietal cortex, left lobules VII and VI of the cerebellum, the bilateral superior parietal lobe and the right middle and superior frontal gyrus. Previous reports of investigation into emotional arousal in healthy participants utilising the IAPS images have demonstrated similar regions of interest, which map onto networks of alerting attentional control intended to peak attention in response to the presence of arousing stimuli (Prehn et al., 2015; Bradley et al., 2003; Van Overwalle et al., 2014; Lang et al., 1998).

Between group analysis of the high arousal-low arousal contrast, inclusive of various levels of valence, revealed that premutation carriers showed significantly less activation in a cluster located bilaterally at BA17 (primary visual cortex), right BA18 (visual association area) and the right superior parietal lobe, and another cluster located bilaterally at the superior parietal lobes, right BA2 (primary somatosensory cortex) and right intraparietal area. In previous studies utilising the IAPS, an increase in the extent of visual cortex activation has been found when viewing emotional images that are both pleasant and unpleasant compared to low arousal images (Bradley et al., 2003; Lang et al., 1998). The basis of such increased metabolism at the visual cortex, related to arousal, is thought to reflect increased attention towards perceived threat or positive stimuli (Bradley et al., 2003; Lang et al., 1998). Our findings of between group differences at the primary visual cortex and visual association area indicate that this response to high arousal stimuli is significantly attenuated in premutation carriers. The parietal cortex has also been implicated as an area of interest in an IAPS-based fMRI task, with images portraying sadness prompting clusters of BOLD activation at the bilateral parietal cortices including the angular and supramarginal gyri compared to neutral images (Radua et al., 2014). It is possible therefore that extensive clusters of significantly lower

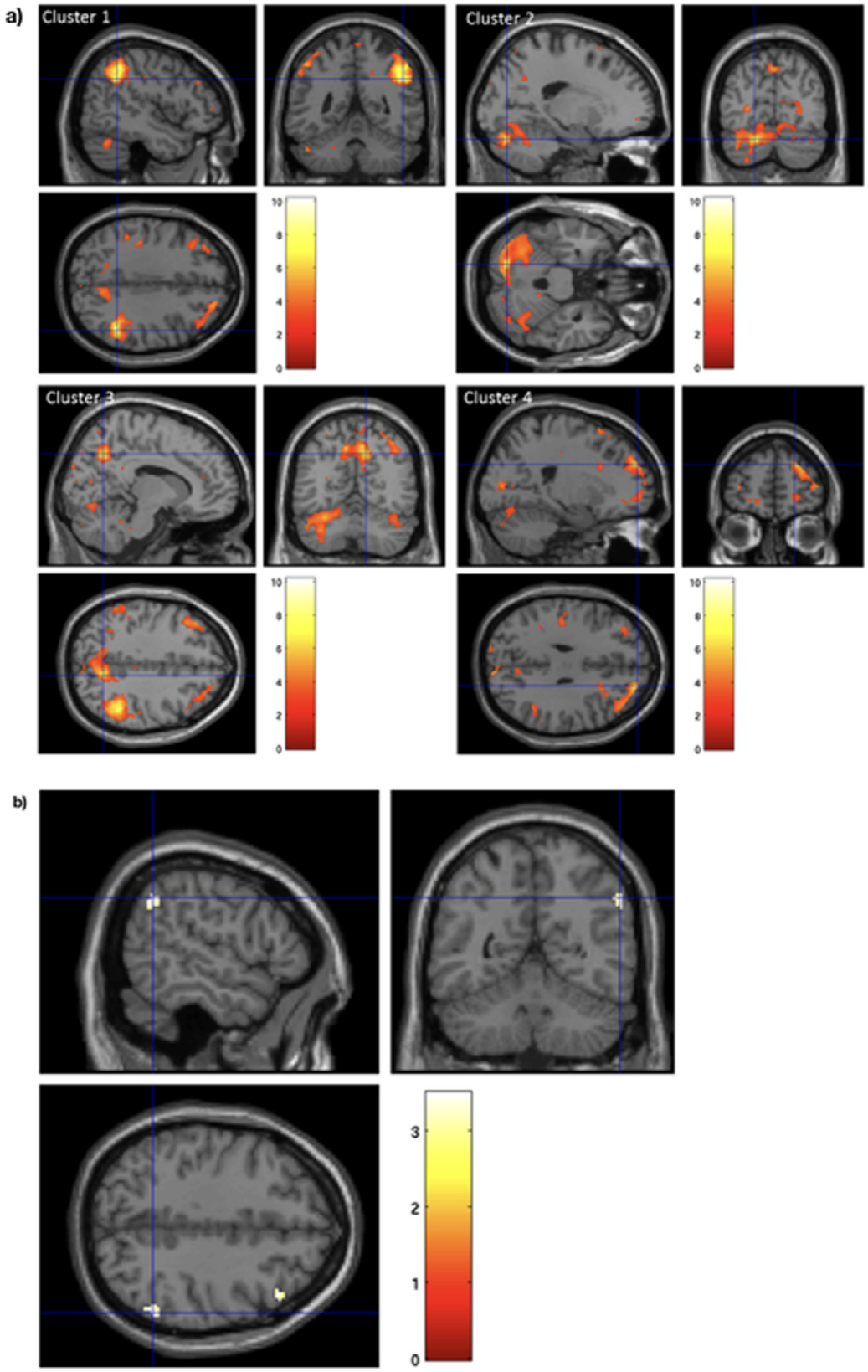

Fig. 2. a) Within group analysis of the control group for the high arousal-low arousal contrast. Significant cluster 1 ($FWE_{corr} < 0.001$, $T = 10.17$) at the right intraparietal cortex and the right intraparietal areas 1 and 3. Cluster co-ordinate of maximum voxel: 48 −48 34, $k = 1321$, normalised voxel size: 2 mm^3. Significant cluster 2 ($FWE_{corr} < 0.001$, $T = 7.37$) at the left cerebellar lobule VIIa Crus I, left cerebellar lobule VI and the right human occipital cortex 3 dorsal (hOC3v). Cluster co-ordinate of maximum voxel: −18 −76 −26, $k = 2102$, normalised voxel size: 2 mm^3. Significant cluster 3 ($FWE_{corr} < 0.001$, $T = 6.90$) at the bilateral superior parietal lobes. Cluster co-ordinate of maximum voxel: 12 −62 40, $k = 1070$, normalised voxel size: 2 mm^3. Significant cluster 4 ($FWE_{corr} = 0.001$, $T = 6.18$) at the right middle and superior frontal gyri. Cluster co-ordinate of maximum voxel: 22 54 30, $k = 754$, normalised voxel size: 2 mm^3. All significant clusters are defined by cluster-wise inference and family-wise error correction. Colour bar represents extent of BOLD activation, with white demonstrating high and red demonstrating lower relative responsivity. **b)** Within group analysis of the carrier group for the high arousal > low arousal contrast. No significant clusters of activation.

activation in carriers at the superior parietal areas is reflective of deficits in the emotional and attentional processing of arousing images.

Like the current study, the two neuroimaging studies to date utilising fMRI emotion-based tasks in premutation carriers, carried out by Hessl et al., demonstrate the existence of between group differences in BOLD response concerning emotional processing (Hessl et al., 2007; Hessl et al., 2011). In one study, when contrasting fearful and neutral face conditions, the control group showed significantly greater activation at the bilateral amygdalae, bilateral insula, left superior temporal sulcus, bilateral intraparietal sulcus and regions of the left basal ganglia

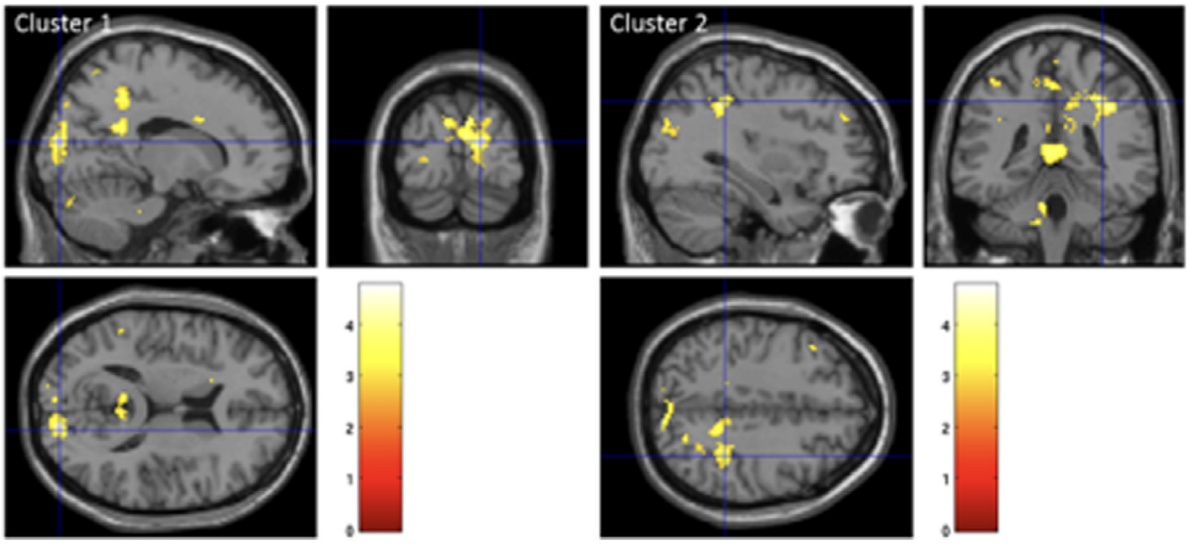

Fig. 3. Cluster 1 of significantly lower BOLD activation in carriers compared to controls ($FWE_{corr} = 0.001$, $T = 4.77$) at bilateral BA17 of the primary visual cortex, right BA18 of the visual association area and the right superior parietal lobe. Cluster co-ordinate of maximum voxel: 16 −88 14, $k = 938$, normalised voxel size 2 mm^3. Cluster 2 of significantly lower activation in the carrier group compared to controls ($FWE_{corr} = 0.021$, $T = 4.26$) at the bilateral superior parietal lobes, right BA2 of the primary somatosensory cortex and right intraparietal area. Cluster co-ordinate of maximum voxel: 34 −40 42, $k = 555$, normalised voxel size 2 mm^3. All significant clusters are defined by cluster-wise inference and family-wise error correction. Colour bar represents extent of BOLD activation, with white demonstrating high and red demonstrating lower relative responsivity.

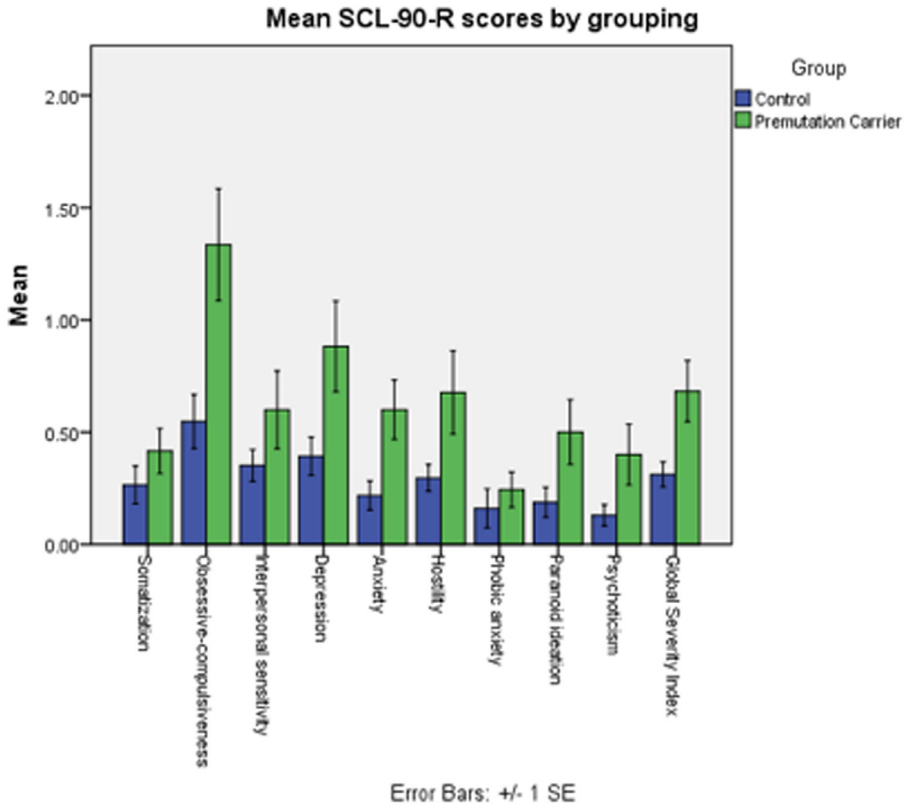

Fig. 4. Mean scores of somatization, obsessive-compulsiveness, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, psychoticism and global severity index as measured by the SCL-90-R for the control group and premutation group.

(Hessl et al., 2007). The other study demonstrated no significant differences at a whole brain level when comparing facial stimuli to inanimate stimuli, however subsequent region of interest (ROI) analysis revealed significantly lower activation in the carrier group compared to controls at the left amygdala (Hessl et al., 2011). The present study tallies with these previously identified between group differences in that activation in that carriers exhibited significantly lower activation clusters, not significantly higher. This replicated lower level of activation is indicative of significantly attenuated response to or recognition of emotional stimuli in premutation carriers. Moreover, in the present study there is concurrence with previous findings of the location of between group activation differences at the intraparietal regions during

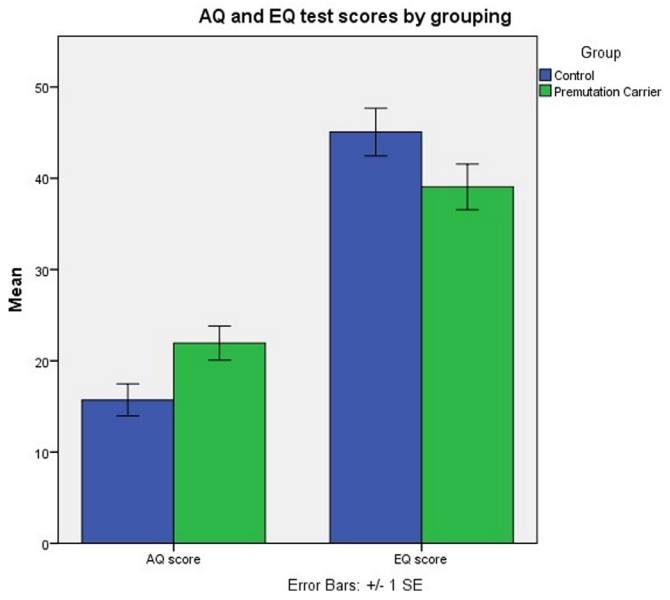

Fig. 5. Mean scores from the AQ and the EQ for the premutation carrier group and the control group.

emotional processing contrasts.

When group x age interactions were examined at a whole brain level for this emotional processing task, the analysis showed no significant clusters of BOLD activation. This indicates that no areas of the brain differ significantly between the groups in their reactivity to arousing stimuli when considering their interaction with age. This stands in contrast to our previous study in the same population where a group x age interaction was observed for brain activation using a motor task (Brown et al., 2018). The previously published evidence of a phenotypic relationship with age in premutation carriers was specific to movement-related responses, interpreted as being a potential link to FXTAS development, and cannot be directly compared to the current results due to the fundamentally different nature of the task-based analysis. However, the present study is suggestive that, in contrast to motor function, differences in emotional processing are not significantly associated with aging in premutation carriers and may represent a stable trait.

Consistent with the hypotheses of increased levels of psychiatric symptoms and autistic traits in premutation carriers compared to controls, our results show significant differences in carriers pertaining to autistic and psychiatric traits. Here, we report significantly higher neuropsychiatric symptomatology of obsessive-compulsiveness, anxiety, global psychiatric severity and positive symptom distress levels as measured by the SCL-90-R in premutation carriers compared to controls. These results strongly align with previous evidence of psychiatric symptomology in carriers, where outcomes from the SCL-90-R show that obsessive-compulsiveness and global symptom severity are significantly increased in male carriers without FXTAS (Hessl et al., 2005). Moreover, lack of an association of any variable with age is supportive of the hypothesis that these psychiatric and neuropsychological symptoms exhibit stability over age and develop irrespective to FXTAS manifestation (Wang et al., 2012; Kenneson et al., 2001; Seltzer et al., 2012; Hagerman and Hagerman, 2016). Our findings are further validated by previous research as higher rates of anxiety and obsessive-compulsive symptoms have been demonstrated in multiple studies of premutation males without FXTAS (Dorn et al., 1994; Jacquemont et al., 2004a).

Participants in the present study also carried out the Ekman 60 Faces Test as an investigation into facial emotion recognition abilities. Our results show that carriers are significantly worse at identifying emotive faces of anger, disgust and surprise, and recognising emotive facial expressions overall as reflected by the total test score. Controls

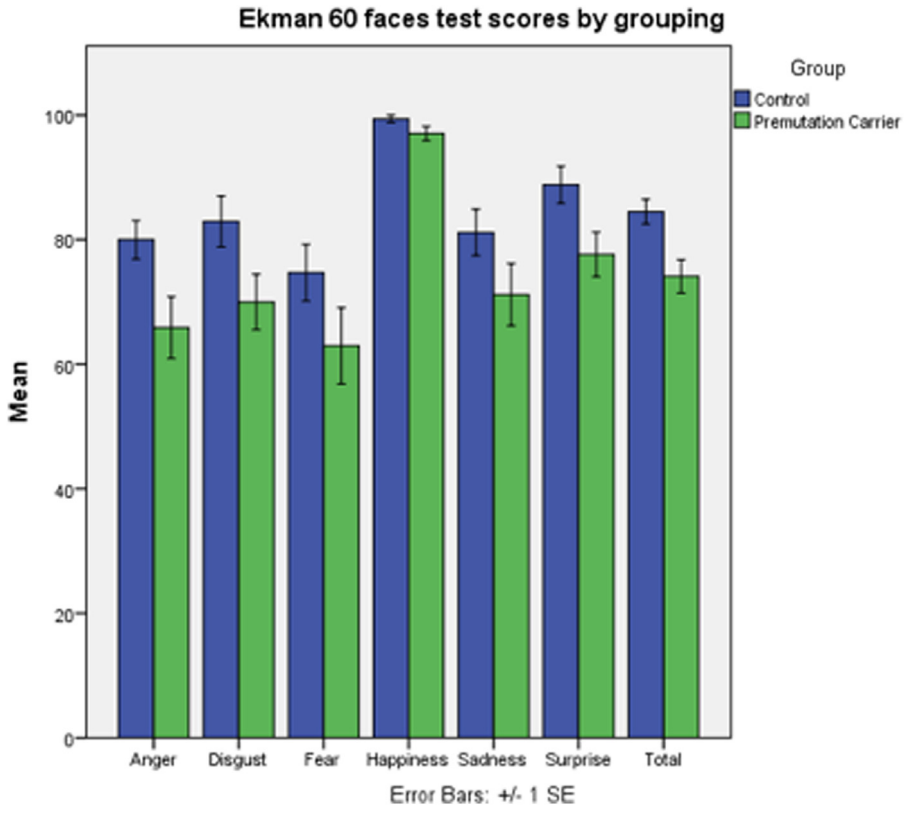

Fig. 6. Mean scores of correct recognitions of facial expressions of anger, disgust, fear, happiness, sadness, surprise and total score as measured by the Ekman 60 Faces Test for the control group and premutation group.

and carriers did not perform significantly differently when asked to recognise facial emotions of fear, happiness or sadness. Carriers have previously been shown to exhibit diminished potentiation of the startle response to fearful faces as measured by skin conductance, despite being similar to the control group in their baseline startle responses (Hessl et al., 2007). This lower level of skin conductance reaction to fearful faces in carriers may be a pertinent phenotype, and in a similar manner to the between group neuroimaging attenuated BOLD response to arousing stimuli in premutation carriers, it is possible that when observing Ekman face images, carriers possess a weakened initial alerting attentional response to facial images demonstrative of heightened arousal, which then manifests as a reduced ability to recognise such facial emotions correctly. Our results are supportive of this theory, as the variables that carriers performed significantly worse on were primarily those that can be considered as typically more threatening or arousing. Our findings of increased AQ scores in carriers are also consistent with the hypotheses presented here and the previous literature (Hagerman and Hagerman, 2013; Chonchaiya et al., 2012; Saul and Tarleton, 1993). Again, the Ekman faces variables, AQ or EQ did not demonstrate any associations with age, supporting the idea of a stable neuropsychological phenotype in premutation carriers.

Although the present results provide promising preliminary evidence of abnormalities in premutation carriers in emotional arousal and general emotionality, there are several limitations to the study which should be considered. Firstly, participant performance in detecting faces in the functional task was not directly analysed and the percentage of faces relative to total images in each arousal and valence condition were not balanced, both of which could possibly be confounding factors in the data. The premutation phenotype is comprised of multiple pathological facets, including differences in IQ and autistic traits, and these were also not modelled as confounds in the data. It is possible that significant differences in non-verbal IQ and AQ scores may be a driving factor in reduced BOLD response in carriers compared to controls, however, when considering the premutation as a composite of many phenotypes, the present results remain promising. Additionally, the samples were not matched based on handedness, which may have affected the functional analyses at a group level. Another limitation is that whilst we report a lack of a group x age interaction in the task-based analysis, it is possible that the absence of such an effect may be due to insufficient statistical power. Similarly, the absence of significant associations between neuropsychological variables and BOLD response in this study, contrary to what may be hypothesized, could also be due to constrained power.

Overall, we find in this study multiple differences in premutation carriers compared to controls in several modes of measurement. Our neuroimaging analysis revealed that carriers exhibit significantly attenuated response at bilateral BA17 (primary visual cortex), right BA18 (visual association area) bilateral superior parietal lobes, right BA2 (primary somatosensory cortex) and right intraparietal area when contrasting high arousal and low arousal images. Previous research utilising fMRI and the IAPS indicates that the visual system is an early regulator of attention and arousal response, and exhibits localised increased BOLD response during high arousal stimuli compared to low arousal (Bradley et al., 2003). Significantly lower activation in carriers at the visual cortex and visual association area therefore indicates diminution of this response and a possible reduction in the ability to heighten attentional control during arousing events. In addition, no group x age interaction was identified for this task, showing that changes to emotional processing concerning arousal in carriers are not sensitive to age in a way that deviates from the normative sample. In general, we demonstrate a significantly reduced functional emotional response in premutation carrier males which appears to be stable across a wide range of ages, supporting the hypothesis that carriers display neuropsychological and psychiatric symptoms that are separate from the neurodegenerative aspects of the *FMR1* premutation.

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Supplementary materials

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