Deactivation in Anterior Cingulate Cortex during facial processing in young individuals with high familial risk and early development of depression: fMRI findings from the Scottish Bipolar Family Study

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Abstract

Background: Studies have identified perturbations in facial processing in Bipolar Disorder and Major Depressive Disorder (MDD), but their relationship to genetic risk and early development of illness is unclear.

Methods: The Scottish Bipolar Family Study is a prospective longitudinal investigation examining young individuals (age 16-25) at familial risk of mood disorder. Participants underwent functional MRI using an implicit facial processing task employing angry and neutral faces. An explicit facial expression recognition task was completed outside the scanner. Clinical outcomes obtained two years after the scan were used to categorise participants into controls (n = 54), high-risk individuals who had developed MDD (HR MDD; n = 30), and high-risk individuals who remained well (HR Well, n = 43).

Results. All groups demonstrated activation patterns typically observed during facial processing including activation of the amygdala, hippocampus, fusiform gyrus and middle frontal regions. Notably, the HR MDD group showed reduced activation of the anterior cingulate gyrus versus both the control and HR Well group for angry faces, and versus the HR Well group for neutral faces. Outside the scanner, the HR MDD group was less accurate in recognising fearful expressions than the HR Well group.

Conclusions. Here we demonstrate functional abnormalities of the anterior cingulate cortex alongside facial emotional recognition deficits in high-risk individuals in the early stages of depression compared to both controls and at risk individuals who remained well. These neural changes were associated with a current or future diagnosis of MDD, and were not simply associated with increased familial risk.

Keywords. *mood disorder, major depressive disorder, fMRI, anterior cingulate, facial recognition, familial risk*

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Introduction

Major depressive disorder (MDD) and Bipolar disorder (BD) are among the ten most disabling medical conditions worldwide. They account for approximately 12% of all days lived with disability (Ustun, Ayuso-Mateos, Chatterji, Mathers, & Murray, 2004). These disorders are highly heritable and have overlapping genetic architectures (Liu et al, 2011; Schulze et al., 2014). Offspring of BD probands have almost a ten-fold risk of developing BD and a three-fold risk of developing MDD compared with the general population (Barnett & Smoller, 2009; Hillegers et al., 2005). Familial MDD is associated with earlier onset and manifests as a more severe form with higher recurrence and complex comorbidity (Lieb, Isensee, Hofler, Pfister, & Wittchen, 2002). However, little is known about the mechanisms underlying the aetiology of psychopathology in this vulnerable population of youth. Targeting the younger at-risk group will reveal the temporal course of abnormalities, and ultimately aid the development of more effective early detection and intervention strategies, before the illness develops a recurrent or chronic pattern.

The neural correlates of genetic risk and the changes in brain function that accompany the early development of mood disorder are of particular importance. Facial expressions are salient social cues underpinning our everyday emotional experience. Facial processing has been studied extensively in healthy individuals and is mediated by a network of regions encompassing prefrontal, temporo-occipital regions, and limbic areas, including the amygdala (Atkinson & Adolphs, 2011; Fusar-Poli et al., 2009; Sabatinelli et al., 2011). Processing of facial stimuli is impaired in both BD and MDD and in vulnerable individuals defined through a positive family history or by high neuroticism (Bourke, Douglas, & Porter, 2010; Bozikas, Tonia, Fokas, Karavatos, & Kosmidis, 2006; Brotman et al., 2008; Chan, Goodwin, & Harmer, 2007; Chan, Norbury, Goodwin, & Harmer, 2009; Phillips, Drevets,

Rauch, & Lane, 2003; Getz, Shear, & Strakowski, 2003; Vederman et al., 2012). Both positive and negative facial emotions have been shown to elicit aberrant neural activity in patients with mood disorder (Almeida et al., 2009; Brotman et al., 2014; Garrett et al., 2012; Pavuluri, Passarotti, Harral, & Sweeney, 2007; Surguladze et al., 2010, Thomas et al., 2012). Previous studies have also indicated emotion-specific deficits or biases for anger recognition in BD and MDD (Guyer et al., 2007; Leyman, De Raedt, Schacht, & Koster, 2007) and abnormal neural response to angry stimuli (Pavuluri et al., 2007; Perlman et al., 2013; Rich et al., 2006) possibly mediated by irritability (Shankman, Katz, Passarotti, & Pavuluri, 2013). Notably, anger is also an emotion that is often present in the clinical symptoms of young individuals with BD (Pavuluri, Birmaher, & Naylor, 2005).

The majority of the above studies have however been conducted in patients with longstanding illness. It is therefore unclear whether these abnormalities are related to the effects of illness or treatment, or whether they are vulnerability mechanisms conferring the effects of genetic risk.

In order to study the neural basis of mood disorder, we conducted a prospective longitudinal investigation of young, initially unaffected individuals with familial risk for mood disorder due the presence of a close relative with BD (Whalley et al., 2011, 2015); the Scottish Bipolar Family Study (BFS). So far the BFS has conducted assessment at three time points. The current study reports functional neuroimaging and behavioural findings during a facial processing task collected at Time 2. We compared high-risk individuals who had a MDD diagnosis either at the time of the imaging experiment or within 2 years after the scan versus high-risk individuals who had remained well and a healthy control group. This enabled us to

investigate neural abnormalities underpinning facial processing relating to the presence of familial vulnerability and / or early development of illness.

We hypothesised that abnormal activation patterns previously implicated in facial processing and in mood disorders would be seen in those with familial liability, and that these would be seen to a greater extent in those with familial liability *and* a current or imminent mood disorder diagnosis.

Methods

Participants

Individuals at high familial risk of bipolar disorder type I (BD) and control subjects were recruited as part of the BFS, a prospective cohort study described in detail elsewhere (Whalley et al., 2011, 2015). Individuals diagnosed with BD were identified through psychiatrists' caseloads across Scotland. Diagnoses were confirmed with the OPCRIT symptom checklist using data from clinical notes and the structured clinical interview for DSM IV (SCID; First et al., 2002). These individuals were asked to identify a first or second degree relative aged 16-25 years who have no personal history of mental illness. These unaffected individuals with at least one first degree or two second degree relatives suffering from BD were recruited as 'high-risk group'. Specifically, the majority (~90%) of the high-risk individuals were first degree relatives of the ill family member, the remainder had two or more second degree relatives. Control participants with no family history of BD or other mood disorders, matched for age, gender, and IQ, were identified from the social networks of the high-risk participants to minimise environmental differences between the two groups. All participants were interviewed by one of the two experienced psychiatrists (AMM, JES) using the SCID. Exclusion criteria for both groups were: lifetime history of major depression, mania or hypomania, psychosis, or any major neurological or psychiatric disorder, substance dependence, learning disability, or any history of head injury that included loss of consciousness and any contraindications to MRI. Written informed consent was obtained after a full explanation of the study protocol. The study was approved by the Multi-Centre Ethics Committee for Scotland.

The BFS is an ongoing longitudinal study; so far data have been available at recruitment (T1) and two follow-up assessments (T2, T3) approximately two years apart. At T2, face-to-face

SCID interviews were repeated (by the same psychiatrists AMM and JES) to determine if any mood disorders have emerged since recruitment. At T3, any presence of mood disorders since T2 were determined by face-to-face assessment (by psychiatrists AMM, JES and research assistants TS and AM) or access to clinical records at the National Health Service (NHS). All interviewers received appropriate training prior to data collection. To achieve reliability standard, all SCID assessments were reviewed by at least two interviewers; all uncertainties and disagreements were discussed and resolved at each stage. However, it was not feasible for the interviewers to be blind to the proband status as families were initially seen together at recruitment.

The final sample here included 127 participants categorised into three groups: The Control group consisted of 54 participants with no familial risk and have remained unaffected throughout the three time points; the 'HR Well' group consisted of 43 high-risk participants who have remained well throughout; and the 'HR MDD' group consisted of 30 high-risk participants who have developed clinically diagnosed MDD during the study period (22 developed MDD between T1 and T2; 8 between T2 and T3).

The primary hypothesis concerned the functional brain differences assessed by fMRI scanning at T2. However, the most up-to-date clinical data collected at T3 were used in group categorisation. Individuals who developed clinically diagnosed MDD *after* T2 were included in the HR MDD group as they were highly likely to be at an early stage of psychopathology at the time of the scanning (two of them were on antidepressants at T2), and in order to keep the HR Well group as 'pure' as possible. Of note two HR individuals developed BD over the course of the study, and seven control individuals developed MDD; due to the small group sizes, these individuals were excluded from the current sample.

Clinical Assessments

In addition to the SCID interview, current manic and depressive symptoms were indexed by self-reported questionnaires including Young Mania Rating Scale (YMRS; Young, Biggs, Ziegler, & Meyer, 1978) and Hamilton Rating Scale for Depression (HRSD; Hamilton, 1960). Variation in affective temperaments (cyclothymic, dysthymic, irritable, hyperthymic, and anxious) was measured by the Temperament Evaluation of Memphis, Pisa, Paris and San Diego - autoquestionnaire (TEMPS-A; Akiskal et al., 2005). Personality trait liability to mood disorders (neuroticism, extraversion, openness, agreeableness, conscientiousness) was also measured by NEO –Five Factor Inventory (NEO-FFI; McCrae & John, 1992).

Facial Expression Recognition Experimental Paradigm

Inside Scanner: At T2, participants performed an *implicit* facial emotion processing task in the scanner based on that used previously but adapted for angry faces (Hall et al., 2008). An implicit task was chosen for the imaging experiment to avoid any neural differences being confounded by performance differences. An explicit task was used outside the scanner to further examine behavioural differences (see below). This implicit task consisted of two runs, each comprising three angry blocks, three neutral and seven interleaved baseline conditions. During the angry blocks, six greyscale Ekman faces (three male, three female) expressing the emotion of anger (100% intensity) were presented for 3.5 s each in random order. During neutral blocks, the same six faces were presented displaying neutral expressions. During baseline a fixation cross was displayed. This task was designed to assess implicit processing, as such participants were asked to select the gender of the face presented ('male' or 'female'). Standardised verbal and written instructions were given prior to scanning. Reaction time and

the percentage of correct responses were recorded as behavioural measures. The paradigm is illustrated in Figure 1 (Appendix). An emotional memory task was used within the same scanning session (see Whalley et al., 2015).

[Insert Figure 1 about here]

Post-scan behavioural task: After scanning, participants were asked to perform an *explicit* behavioural test of facial emotion recognition, Ekman 60. Images of 10 faces were shown on a computer screen depicting each of the six basic emotions (happiness, surprise, sadness, fear, anger and disgust) at 100% intensity. In a psuedo-random order the 60 images were individually presented for 3s and participants were requested to choose the emotion that best described the facial expression with no time limit for responding. Both accuracy and reaction time were recorded.

Scanning Procedure

Imaging was carried out at the Brain Imaging Research Centre for Scotland on a 1.5 T Signa scanner (GE Medical, Milwaukee, USA). The functional imaging protocol consisted of axial gradient echo planar images (EPI) (TR/TE = 2500/40 ms; matrix = 64 x 64; field of view (fov) = 24 cm) acquired continually during the experimental paradigm. Thirty contiguous 5 mm slices were acquired within each TR (2.5 s). There were 2 sessions and each EPI sequence was run for 96 volumes, the first 4 of which were discarded. The T1 sequence yielded 180 contiguous 1.2 mm coronal slices (matrix = 192 x 192; fov = 24 cm; flip angle = 8°. Visual stimuli were presented using a screen (OFIS, MRI Devices, Waukesha, WI, USA) in the bore of the magnet.

Image Processing

The EPI and T1 weighted images were reconstructed into nifti format (Mayo Foundation, Rochester, MN, USA) using DICOM convert functions in SPM8 (Statistical Parametric Mapping: The Wellcome Department of Cognitive Neurology and collaborators, Institute of Neurology, London) running in Matlab (The MathWorks, Natick, MA, USA). Images were pre-processed using standard protocols available in SPM8. All EPI images were realigned to the mean volume in the series. The functional images were then normalised according to the standard co-registration procedures using the individuals' structural scan. Finally, all images were smoothed with an 8 x 8 x 8 mm full width half maximum Gaussian filter.

MRI Analysis

First level statistical analysis was performed using the general linear model approach. At the individual subject level the data was modelled with two conditions, angry and neutral, each by a boxcar convolved with a synthetic haemodynamic response function. Estimates of the subjects' movement during the scan were entered as 'covariates of no interest'. Contrast images were generated for each participant representing angry faces versus baseline, neutral faces versus baseline and angry faces versus neutral faces.

Contrast images generated by the steps above were entered into a second level random-effects analysis examining angry faces vs. baseline, neutral faces vs. baseline, and angry faces vs. neutral faces using *t* tests. We initially conducted the analysis using the ANOVA approach with flexible factorial repeated measures designs (modeling Emotion versus Baseline and Neutral versus Baseline as the repeated component per subject and then a contrast of the two to determine Emotion versus Neutral). While this repeated-measures model is appropriate for analysis of interaction effects, the design is not valid for the analysis

of group effects for a single task as SPM does not apply the appropriate degrees-of-freedom adjustment. Since we did not find any significant Group x Condition interaction using this fuller model, standard *t* tests were deemed to be the most appropriate statistical approach for analysis of group effects in this dataset. Based on our prior hypothesis, small volume corrections were applied for the amygdala, hippocampus and anterior cingulate cortex all created using the WFU PickAtlas (Maldjian, Laurienti, Kraft, & Burdette, 2003; Tzourio-Mazoyer, 2002). For completeness we also report results of the whole brain analysis. Statistical maps were thresholded at a level of $p<0.001$ (uncorrected) and regions were considered significant at a cluster level, with family-wise error correction (FWE), of $p<0.05$. All coordinates are quoted in Montreal Neurological Institute (MNI) convention (http://www.mni.mcgill.ca) and images are overlaid onto standard brain in MNI space using Mango software package (http://ric.uthscsa.edu/mango).

Relationship to symptom severity and trait-liability measures

Correlation analysis was run between the extracted neural signals from significant clusters and measures of current depressive symptoms (HDRS scores) as well as selected measures of trait-liability to mood disorder (cyclothymia, neuroticism and extraversion).

Analysis of Potential Confounders

Seven individuals (all in the HR MDD group) were prescribed antidepressants at the time of scanning. These individuals were included in all initial analyses, but were subsequently excluded to determine findings without the potentially confounding medication effects.

Analysis of Demographic, Clinical, and Behavioural Measures

Normality assumption was verified using histograms and Kolmogorov-Smirnov test. One-way or two way ANOVAs (for continuous variables), chi-squared (for categorical variables) or Kruskal-Wallis tests (for non-parametric variables) were performed in SPSS. Significant interactions were followed up by post hoc *t* tests or Mann-Whitney U Tests.

Results

Demographic and Clinical Characteristics

Descriptive data and full statistics are presented in Table 1. The three groups were well matched with no differences in age, gender, handedness, or NART IQ. As expected there were group differences in depressive symptoms with HR MDD reporting higher HDRS scores than HR Well (z=3.58, p<.001) and control (z=3.60, p <.001). There was no group difference in YMRS scores.

In terms of temperamental markers of mood disorder, we found group differences for cyclothymia, depression and irritability, but not hyperthymia or anxiety. These contrasts were driven by HR MDD reporting higher scores than both HR Well (Cyclothymia: z=3.76, p< .001; Depression: z=2.82, p=.005; Irritability: z=2.15, p=.03) and control group (Cyclothymia: z=3.32, p=.001; Depression: z= 2.73, p=.006; Irritability: z =1.05, p=.024).

The three groups differed on all personality traits except openness. The HR MDD group reported higher neuroticism (t=5.60, p<.001), lower extraversion (t=4.34, p<.001), lower agreeableness (t= 2.91, p=.005), and lower conscientiousness (t=2.97, p=.004) than the control group. HR MDD also had higher neuroticism (t = 4.58, p <.001) and lower agreeableness (t=2.67, p=.01) than the HR Well group. The HR Well group did not differ from controls apart from having lower extraversion scores (t = 2.81, p=.006).

[Table 1 about here]

Behavioural Measures of Facial Expression Experimental Paradigm

Inside the scanner, the implicit facial expression paradigm yielded high accuracy level (>95%) across all participants suggesting that the task was appropriately performed. The groups did not differ in accuracy or reaction time, although all participants were faster in responding to the angry than neutral faces. Outside the scanner, Ekman 60 task yielded a significant group x emotion interaction. *Post hoc* one-way ANOVAs suggested a group difference for fearful faces (F=3.48, p =.04) driven by HR MDD group having poorer accuracy than HR Well group (t =2.20, p =.04).

Task-related Brain Activation Patterns

All participants demonstrated the expected patterns of brain activation in areas typically associated with facial processing, including prefrontal areas, temporo-occipital regions and limbic areas including the amygdala and hippocampus (see Table 2, Figure 2) for both angry faces versus baseline and neutral faces versus baseline. There was limited activation within groups for effects of the emotional component (i.e. angry versus neutral faces), however the controls did demonstrate a significant activation for this contrast in the middle temporal gyrus. Activation for all contrasts in controls is additionally depicted in Figure 2.

[Table 2 and Figure 2 about here]

Between-group Differences in Activation

We found significantly reduced activation of the sub-genual anterior cingulate cortex (sgACC) in the HR MDD group versus the HR Well and Control groups for the angry faces versus baseline, and versus the HR Well for the neutral face versus baseline (see Table 3, Figure 3). Graphical depiction of the extracted data indicated that this region demonstrated deactivation during both the angry and neutral face conditions, with greater deactivation

across all groups for the emotional condition, and the degree of deactivation for both conditions was greatest in the HR MDD group (see Figure 4).

[Table 3, Figure 3 & Figure 4 about here]

Relationship to Symptom Severity and Trait Liability Measures

Across all individuals there were negative associations between activation in the anterior cingulate in the neutral condition and measures of cyclothymia (ρ =-0.214, p=0.015) and neuroticism (ρ =-0.235, p=0.018). These associations would not however survive controlling for multiple comparison testing. There were no associations found for measures of depression severity from the HDRS, nor for extraversion. Further there were no significant relationships with anterior cingulate activation for the angry condition.

Analysis of Potential Confounders

After removing the seven individuals prescribed antidepressants at the time of the scan, the main finding remained significant suggesting that medication was not a confounding factor.

Discussion

This study involved participants from a prospective longitudinal study of young individuals at familial risk of mood disorder, some of whom had developed MDD over the course of the study. This provided a valuable opportunity to examine neurobiological changes that are associated with risk as well as those that accompany early phases of the development of psychopathology. Here we report disruptions in the predicted neural network implicated in both mood disorders and mood regulation. Specifically, we found decreased activation of sgACC in the HR MDD group during the processing of facial stimuli, regardless of emotional content. This finding was not related to the severity of current depressive symptoms, nor were they confounded by medication or within-scanner behavioural differences between groups. Outside scanner, high-risk individuals with MDD were less accurate in recognising fearful facial expression. The overall pattern of results suggests marked differences between the HR MDD and HR Well group in terms of facial emotion processing, with the latter resembling control participants across most clinical measures and neural responses. The neural changes and behavioural differences observed here are therefore related to the presence of illness itself, or imminent diagnosis, rather than the presence of familial risk.

In this study we report regions of activation typically seen in this type of facial processing task (Atkinson & Adolphs, 2011; Fusar-Poli et al., 2009; Sabatinelli et al., 2011), namely visual, prefrontal and limbic regions. Our main finding within the anterior cingulate cortex is particularly noteworthy given the well-documented role of this area in depression and emotional regulation (Drevets, Price, & Furey, 2008; Phillips et al., 2003; Pizzagalli, 2011). Here, neural abnormalities were related to task *deactivation* rather than task *activation*. Although this appears to be at odds with studies that reported increased cingulate activity during the processing of negative emotional stimuli (e.g. Elliott, Rubinsztein, Sahakian, &

Dolan, 2002; Gotlib et al., 2005), these were however typically more caudal ACC areas (pre-genual or anterior dorsal) than those reported here. Further, many other studies have observed hypoactivity or no effects using different task batteries of negative emotion processing (e.g. Davidson, Pizzagalli, Nitscke, & Putnam, 2002; George et al., 1997; Okada, Okamoto, Morinobu, Yamawaki, & Yokota, 2003; Wagner et al., 2006). The meta-analysis by Fusar-Poli *et al* (2008) reported that activation of the ACC to angry faces was located around the coordinates [4, 24, 18], whereas our deactivation was located more ventrally at [4, 38, 2]. Another study of young individuals with BD also reported enhanced deactivation of the ACC in response to angry facial stimuli (Thomas et al., 2013). This region is also considered part of the default mode network involved in the monitoring of internal states and in the shifting of attention toward or away from internal cues (Raichle & Snyder, 2007). Resting state studies have consistently reported differences in activation in such regions in patients with MDD and BD (Sundermann, Beverbotg, & Pfleiderer, 2014). The current findings therefore suggest abnormal modulation of interoceptive cues during the processing of facial stimuli regardless of emotional content (Thomas et al., 2013). It is however important to acknowledge a limitation that our initial ANOVA did not find a significant Group x Condition interaction, the group difference reported here was based on post hoc *t* tests and should therefore be interpreted with caution.

It should also be considered that the differences in ACC activation were found in both angry and neutral face stimuli. Group effects in the absence of group by condition interactions could indicate dysfunction in more basic facial processing pathways than those involved in the more subtle emotional modulation component. An alternative explanation could be that neutral faces have been perceived by some participants to have an emotional content (Bourke et al., 2010; Hall et al., 2008) or attributable to sample characteristics or other methodological

details. For example, although other studies have reported deficits in anger versus neutral face processing, these have tended to involve paediatric bipolar samples (Pavuluri et al., 2007; Perlman et al., 2013), which is typically considered a more severe form of the disorder.

We only found significant group differences in ACC but not in the other two *a priori* regions, amygdala and hippocampus. We propose there are several possible reasons for the lack of medial temporal lobe differences which relate to specifics of the task. Based on our previous functional imaging findings in the cohort where we reported significant over-activation of the amygdala in an executive function task (Whalley et al., 2011) but not in an emotional processing task (Whalley et al., 2015), we propose that over-activation of such limbic regions in high-risk individuals may be *more* evident in tasks where there is little emotional content.

In this study, only angry faces with 100% intensity were used because of its association with BD and MDD in the literature, constraints of scanner time, and also because our initial analysis of pilot behavioural data indicated a deficit in this emotion. However, our findings generated limited activations in the emotional modulation indicated by the contrast between angry vs. neutral faces; these could be related to angry faces being less emotionally intense than, for example, fearful faces, which have been more commonly used in the literature (e.g. Chan et al., 2009; Hall et al., 2008, Phillips et al., 2003). Inclusion of fearful faces in the paradigm within scanner would also allow us to interpret the imaging results in conjunction with the behavioural findings. Future studies should use a wider range of emotional stimuli with varying intensity to decrease the tendency for habituation and increase sensitivity for detecting subtle differences in emotional processing.

In the post-scan facial expression recognition task, we found behavioural deficits in fear recognition amongst the high-risk individuals with depression compared with those without. This finding is noteworthy, given that depression was often associated with *increased* perception of negative expressions (e.g. Bouhuys, Geerts, & Gordijn, 1999; Bhagwagar, Cowen, Goodwin, & Harmer, 2004; Hayward, Goodwin, Cowen, & Harmer, 2005) rather than a *decrease*, as seen here. Reduction in fear recognition has particularly been associated with manic symptoms (Lembke, & Ketter, 2002). It is possible that this behavioural difference was driven by some individuals within this group whose depressive symptoms may later be manifested into BD.

While the differences between HR MDD and HR Well groups appear to be related to the presence of illness, it is also possible that the HR Well individuals may represent a resilient group. Previous studies have indeed proposed that individuals could be considered as 'resilient' if they have a high risk factor but have 'avoided' illness beyond the typical onset age (e.g. Chan et al., 2016, Peterson et al., 2014). However, it should be noted that our current sample consisted of individuals with mean age ~23 years, which was younger than the high risk samples in the above studies as well as the median onset age for BD and MDD previously reported (Kessler et al., 2005). As such, they were still at considerable risk of developing illness. Further follow-up studies will be able to elucidate the mechanisms underlying risk vs. resilience in high risk populations.

Finally, in some cases the development of MD may herald an ultimate onset of BD. It may also be the case that some HR Well individuals, and indeed control participants, may still develop a mood disorder. Within the timeframe of this study, only eight high risk individuals fell ill between T2 and T3; therefore we do not have enough statistical power to examine if

neural responses predict subsequent illness onset. We are currently conducting a new wave of follow-up assessment of this cohort, which will allow us to address these important issues. A repeated assessment of the functional brain scan and the behavioural measure of emotional facial processing in follow-up studies will also help investigate if these neural-behavioural functioning changes with time and if so whether these changes are associated with the development of illness.

Conclusion

Longitudinal imaging studies of young at risk populations are scarce. Here we report decreased activation of the anterior cingulate cortex in individuals at familial risk for mood disorder with a recent diagnosis of MDD based on the cohort study of the Scottish Bipolar Family Study. We demonstrate that neural abnormalities are present early on in the illness and are unrelated to medication effects. Our ongoing longitudinal follow-up assessment will clarify to what extent such neuroimaging markers together with genetic and behavioural measures of trait-liability for illness contribute to vulnerability to mood disorders. If these early neural-cognitive changes are shown to be predictive of subsequent illness onset, they could potentially be developed into screening tools to facilitate development of preventative strategies and early intervention.

Key points:

- Abnormalities in facial processing have been implicated in major depressive disorder (MDD) and other mood disorders.
- The Scottish Bipolar Family Study is a prospective longitudinal study examining young individuals at familial risk of mood disorder comparing with healthy controls.
- At-risk individuals with current or imminent MDD diagnosis showed reduced activations in anterior cingulate while processing angry and neutral faces comparing with at-risk individuals without MDD and controls. This group also showed deficit in fear recognition outside the scanner.
- These differences were related to the presence of MDD and not merely baseline familial risk alone.
- Differentiating neural mechanisms underlying risk versus current illness will help advance clinical development of prevention and early intervention strategies for the young vulnerable population.

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Table 1 Demographics, clinical, temperament, personality, and behavioural measures

	Controls (n=54)		**High Risk Well** (n=43)		**High Risk MDD** (n=30)		**Significance**
	Mean/median	St dev/ IQR	Mean/median	St dev/ IQR	Mean/median	St dev/ IQR	F/χ^2 (P value)
Demographics							
Mean age (yrs)	23.0	2.40	23.8	2.45	23.4	3.07	0.91 (0.41)
Gender (%M)	38.9	-	55.8	-	36.7	-	3.66 (0.16)
Handedness (%R)	83.3	-	88.4	-	86.7	-	1.28 (0.53)
Mean NART IQ	110.8	5.60	111.2	8.53	108.2	6.87	1.77 (0.18)
Clinical measures							
YMRS	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (1.00)
HAM-D #	0.00	2.00	0.00	1.00	2.00	6.50	16.66 (< .001) *
Temperament measures (TEMPS-A)							
Cyclothymia #	1.00	3.00	1.00	2.00	4.00	7.00	16.18 (< .001) *
Depressive #	0.00	1.75	0.00	1.00	1.00	3.25	9.95 (.007) *
Irritability #	1.00	2.00	1.00	2.00	1.00	3.00	6.18 (.045) *

Hyperthymia #	2.00	3.00	1.00	2.00	1.50	3.00	4.16 (.13)
Anxious #	0.00	1.00	0.00	1.00	1.00	1.00	3.48 (.18)
Personality Traits (NEO – Five Factor Inventory)							
Neuroticism	17.98	7.25	19.75	7.01	28.81	9.02	17.94 (< .001) *
Extraversion	31.38	5.02	28.15	5.72	25.88	5.48	9.47 (< .001) *
Openness	28.53	6.27	28.55	5.31	29.08	6.02	0.08 (0.92)
Agreeableness	33.68	4.56	33.58	4.71	30.19	5.50	5.06 (< .01) *
Conscientious	31.62	6.53	28.95	7.41	26.38	8.30	4.49 (0.01) *
Within scanner behavioural measures							
% correct responses anger #	95.83	16.67	94.44	16.67	97.22	11.27	0.85 (0.65)
%correct responses neutral#	100.00	5.56	100.00	5.56	100.00	2.78	0.01 (0.99)
Reaction time: anger	1.61	0.23	1.55	0.22	1.54	0.33	Valence: 14.27 (<.001)

Reaction time: neutral	1.56	0.21	1.51	0.21	1.49	0.33	Group: 1.10 (0.34) V x G: 0.11 (0.89)
Post-scan behavioural measures (% correct responses)							
Fear	65.8	22.61	74.1	21.36	57.0	31.30	
Happy	98.9	3.83	96.2	16.16	97.5	5.50	
Sad	76.40	18.97	75.4	19.17	70.0	22.71	Valence: 53.40 (<.001)†
Anger	80.9	15.50	74.4	19.97	74.5	17.61	Group: 2.17 (0.02) * V x G: 0.72 (0.18)
Disgust	75.3	1.97	77.7	21.21	67.5	17.73	
Surprise	91.3	9.68	85.9	18.60	87.5	12.93	

[#] Kruskal-Wallis tests, median and interquartile range presented for skewed variables. Asterisk * denotes statistical significance $p < .05$

Table 2: Within group task activation (Appendix)

P value	K_E	Z	Co-ords	Region
Controls				
Angry faces versus baseline				
<0.001	21491	inf	16 -92 2	R lingual gyrus
<0.001	8912	7.71	52 20 42	R middle frontal gyrus
<0.001	7655	6.95	-58 -20 52	L postcentral gyrus
<0.001	2389	6.45	8 24 44	R superior frontal gyrus
0.001	455	5.43	22 -4 -18	R amygdala*
0.003	251	4.59	-26 4 -10	L amygdala*
0.002	346	7.06	24 -28 -6	R hippocampus*
0.014	132	6.50	-22 -30 -6	L hippocampus*
Neutral faces versus baseline				
<0.001	17105	inf	18 -96 8	R cuneus/lingual gyrus
<0.001	6604	7.11	52 34 26	R middle frontal gyrus
<0.001	2019	6.47	-58 -20 52	L postcentral gyrus
<0.001	1626	6.25	34 -56 38	R superior parietal lobule
0.001	1100	5.79	8 26 44	R medial frontal gyrus
0.001	1033	4.90	-26 4 -4	L lentiform
0.006	726	4.82	-30 -56 42	L superior parietal lobule
0.002	314	4.73	26 -2 -10	R amygdala*
0.038	19	3.92	--24 -4 -10	L amygdala*
0.019	105	6.33	-26 -30 -4	L hippocampus*
0.006	219	6.18	-24 -30 -4	R hippocampus*

P value	K_E	Z	Co-ords	Region
Angry faces versus neutral faces				
0.041	1002	3.59	52 -60 4	R middle temporal gyrus
0.133	33	2.97	-28 0 -20	L amygdala*
High Risk Well				
Angry faces versus baseline				
<0.001	16387	inf	16 -84 2	R lingual gyrus
<0.001	5589	6.77	52 36 26	R middle frontal gyrus
<0.001	1819	6.25	40 -50 40	R Inferior Parietal Lobule
<0.001	2304	5.87	-32 -52 40	L Inferior Parietal Lobule
<0.001	4715	5.72	-34 -28 -4	L caudate
0.002	891	4.77	8 28 42	R medial frontal gyrus
0.001	332	4.70	24 -4 -16	R amygdala*
0.003	256	4.68	-22 -6 -14	L amygdala*
0.005	238	5.08	24 -28 -6	R hippocampus*
0.018	114	5.53	-24 -30 -4	L hippocampus*
Neutral faces versus baseline				
<0.001	11698	6.90	40 -78 -12	R fusiform gyrus
<0.001	2095	5.66	42 30 16	R middle frontal gyrus
0.016	564	4.51	8 32 48	R superior formal gyrus
0.048	8	3.48	26 -10 -10	R amygdala*
0.012	150	5.25	28 -28 -6	R hippocampus*
0.029	71	4.63	-26 -3 -4	L hippocampus*
Angry faces versus neutral faces				

P value	K_E	Z	Co-ords	Region
n/s				
High Risk MDD				
Angry faces versus baseline				
<0.001	16296	inf	36 -82 -12	R inferior occipital gyrus
<0.001	4048	6.09	52 18 36	R middle frontal gyrus
<0.001	1627	5.36	-54 22 34	L middle frontal gyrus
0.011	624	4.69	-34 -62 52	L superior parietal lobule
0.005	182	4.03	20 -4 -16	R amygdala*
0.003	256	4.94	-24 4 -14	L amygdala*
0.039	50	4.32	24 -60 -4	R hippocampus*
0.025	85	5.27	-24 -30 -4	L hippocampus*
Neutral faces versus baseline				
<0.001	14265	7.45	34 -84 -12	R inferior occipital gyrus
<0.001	3092	6.26	44 28 18	R middle frontal gyrus
0.004	792	4.80	-52 28 32	L middle frontal gyrus
0.047	409	3.99	4 28 44	R medial fontal gyrus
0.001	1100	5.79	8 26 44	R medial frontal gyrus
0.026	39	3.44	18 -6 -12	R amygdala*
0.039	17	3.44	--22 -2 -12	L amygdala*
0.031	68	4.68	24 -30 -4	R hippocampus*
0.036	57	4.35	-26 -30 -4	L hippocampus*
Angry faces versus neutral faces				
n/s				

Note. Asterisk * denotes statistical significance using svcs (for amygdala, hippocampus, and anterior cingulate), thresholded p<0.001 considered significant at p<0.05 FWE, except for angry versus neutral which was thresholded at <0.005 and then considered significant at p<0.05 FWE

Table 3: Between group task activation differences

p	K_E	*z*	Co-ords	Region
Angry faces versus baseline:				
Controls > HR MDD				
0.046	49	3.39	4 38 2	R Anterior cingulate*
HR Well > HR MDD				
0.016	163	4.10	-4 34 2	L Anterior cingulate*
Neutral faces versus baseline				
HR Well > HR MDD				
0.029	93	3.58	6 34 0	R Anterior cingulate*
Angry faces versus neutral faces				
Controls > HR Well				
0.006	688	3.77	36 -70 8	R middle occipital gyrus

Note. Asterisk * denotes statistical significance using svcs (for amygdala, hippocampus, and anterior cingulate), Statistical maps were thresholded at a level of $p<0.001$ (uncorrected) and regions were considered significant at a cluster level, with family-wise error correction (FWE), of $p<0.05$. Only significant contrasts are shown here, remainder n/s

Figure 1 Experimental paradigm of the implicit facial emotion processing task used inside the fMRI scanner (Appendix)

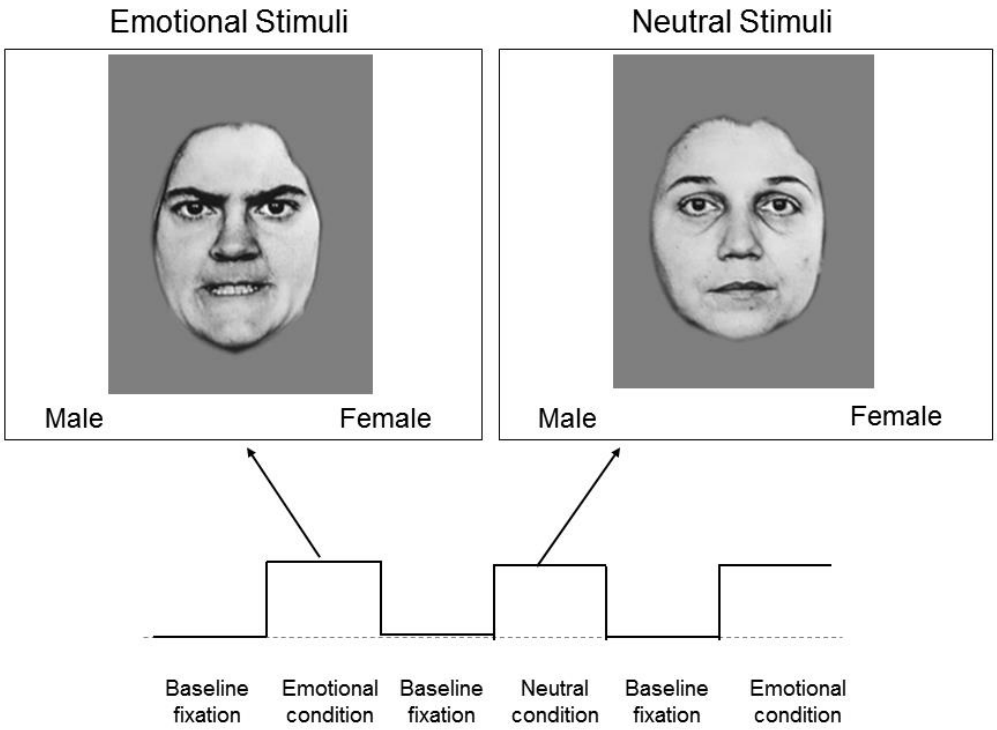

Figure 2 Within group activation map for controls (Appendix)
Figure 1 depicts angry faces > baseline shown in red, neutral faces > baseline shown in blue and angry > neutral faces shown in green, cross hairs centred on left amygdala for angry versus neutral face. Images are overlaid onto standard brain in MNI space using Mango software package (http://ric.uthscsa.edu/mango). Map represents T-statistic images threshold equivalent to *p*=0.001 uncorrected and significant clusters corrected for cluster-level significance are presented in Table 2, See methods for further details.

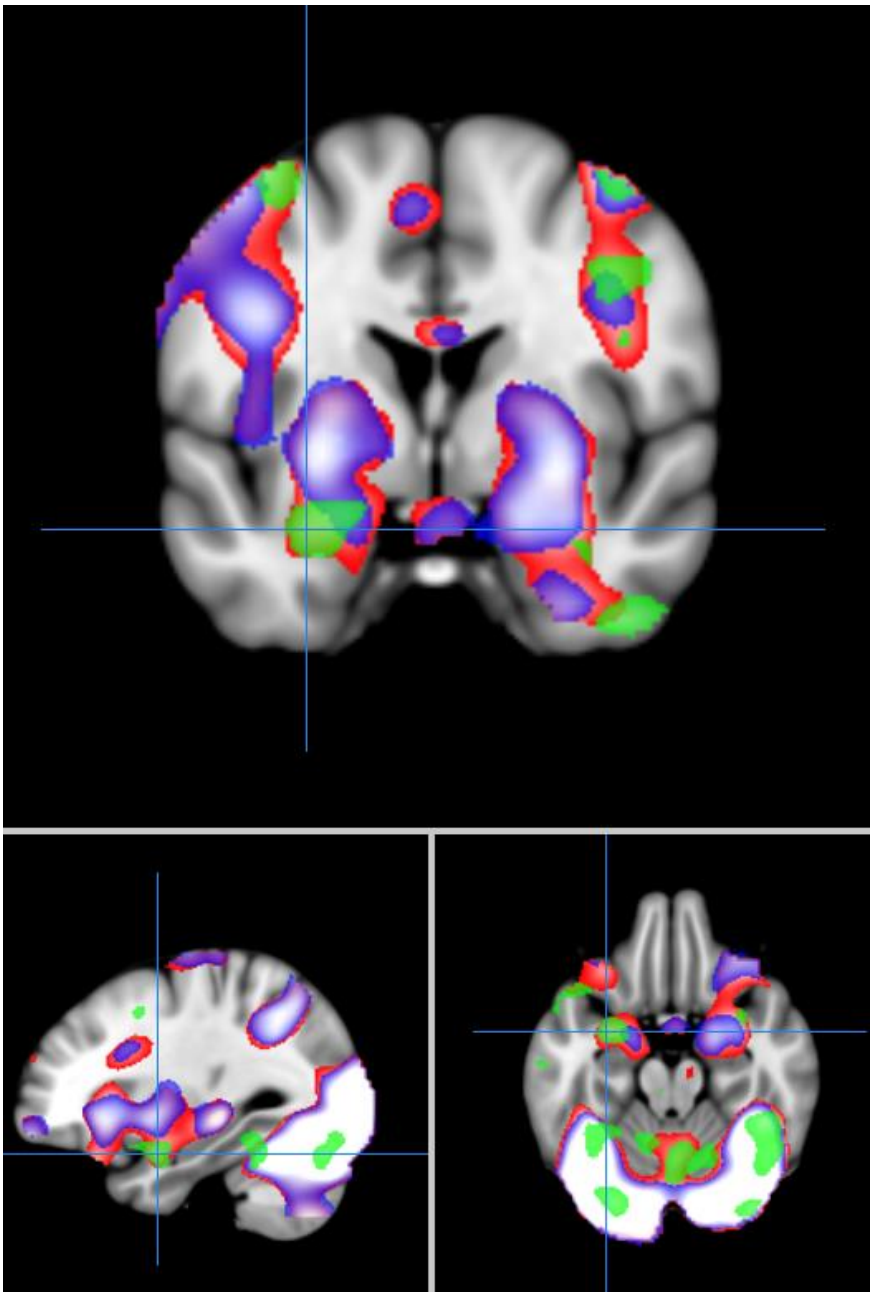

Figure 3 Between group differences in anterior cingulate cortex
Figure 2 depicts reduced activation in the anterior cingulate cortex in the HR MDD versus the HR Well group; red depicts angry faces > baseline, blue depicts neutral faces > baseline. Statistical maps were thresholded at a level of $p<0.001$ (uncorrected) and regions were considered significant at a cluster level, with family-wise error correction (FWE), of $p<0.05$

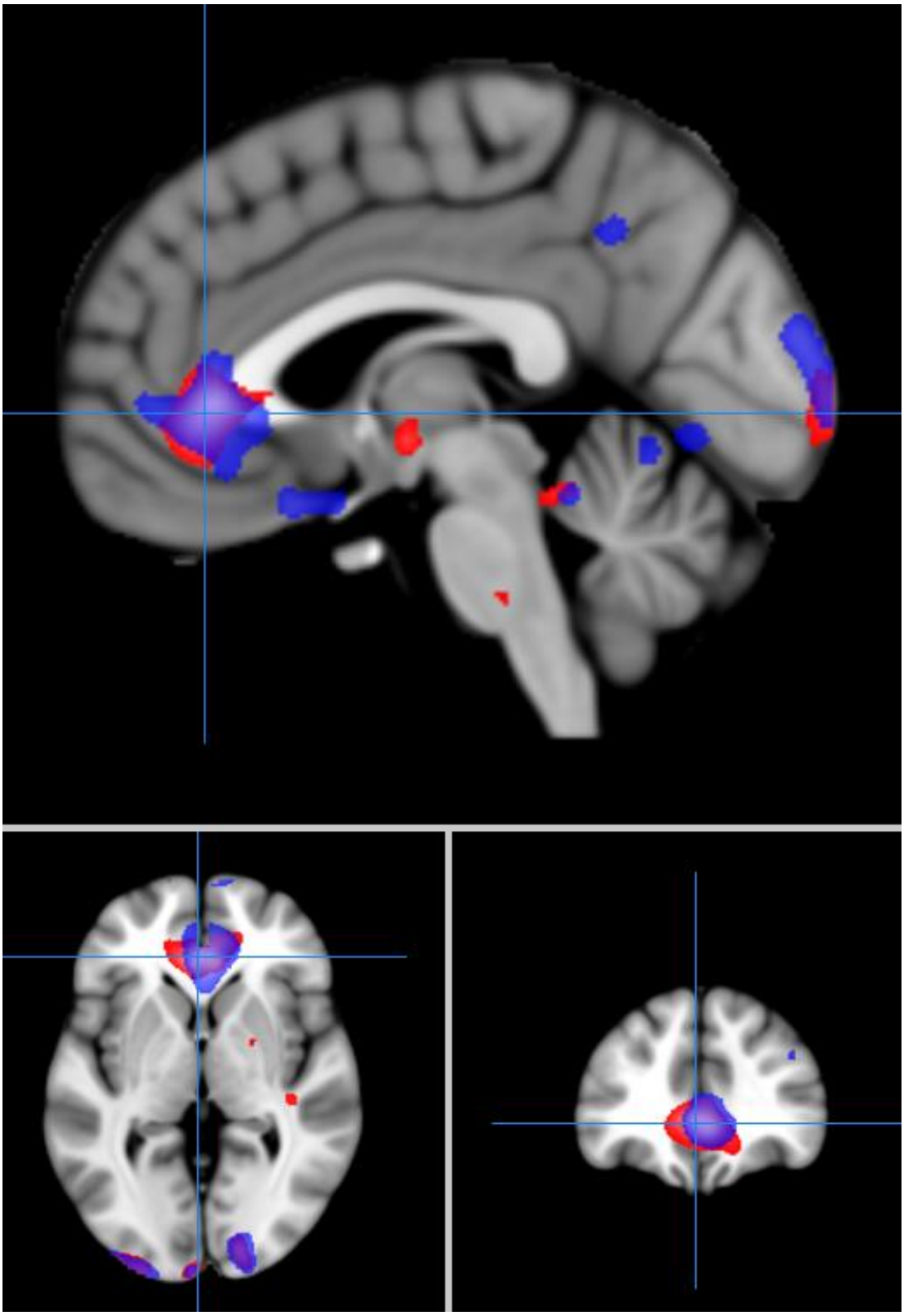

Figure 4 Extracted data for Anterior Cingulate Cortex
Red – angry faces versus baseline, Blue – neutral faces versus baseline