Elsevier Editorial System(tm) for Biological Psychiatry
Manuscript Draft

Manuscript Number: BPS-D-07-01136R2

Title: Overactivation of fear systems to neutral faces in schizophrenia

Article Type: Brief Report

Keywords: Fear; Amygdala; Schizophrenia; fMRI; face; emotion

Corresponding Author: Dr Jeremy Hall, MRCPsych PhD

Corresponding Author's Institution: Edinburgh University

First Author: Jeremy Hall, PhD MRCPsych

Order of Authors: Jeremy Hall, PhD MRCPsych; Heather C Whalley, PhD; James W McKirdy, PhD; Liana Romaniuk, MSc; David McGonigle, PhD; Andrew M McIntosh, MD MRCPsych; Ben J Baig, MRCPsych; Viktoria-Eleni Gountouna, MSc; Dominic E Job, PhD; David I Donaldson, PhD; Reiner Sprengelmeyer, PhD; Andrew W Young, PhD; Eve C Johnstone, MD FRCPsych; Stephen M Lawrie, MD MRCPsych

Abstract: BACKGROUND The amygdala plays a central role in detecting and responding to fear related stimuli. A number of recent studies have reported decreased amygdala activation in schizophrenia to emotional stimuli (such as fearful faces) compared to matched neutral stimuli (such as neutral faces). Here we have investigated whether the apparent decrease in amygdala activation in schizophrenia could actually derive from increased amygdala activation to the neutral comparator stimuli.

METHODS Nineteen patients with schizophrenia and 24 matched control participants viewed pictures of faces with either fearful or neutral facial expressions, and a baseline condition, during functional magnetic resonance imaging scanning.

RESULTS Patients with schizophrenia showed a relative decrease in amygdala activation to fearful faces when compared to neutral faces. However this difference resulted from an increase in amygdala activation to the neutral faces in patients with schizophrenia, not from a decreased response to the fearful faces.

CONCLUSIONS Patients with schizophrenia show an increased response of the amygdala to neutral faces. This is sufficient to explain their apparent deficit in amygdala activation to fearful faces compared to neutral faces. The inappropriate activation of neural systems involved in fear to otherwise neutral stimuli may contribute to the development of psychotic symptoms in schizophrenia.

Response to Reviewers: We thank the reviewers for their positive comments.

REVIEWER 2

1. The authors have now answered the points I raised in the previous review. There are some minor typographical errors requiring correction.

Response: We have carefully re-checked the manuscript for typographical errors.

REVIEWER 5

1. The results of a functional connectivity analysis between the amygdala and other regions (e.g., fusiform) was suggested to help explain the findings. This analysis was not performed due to space limitations. Even a 2 sentence addition of such results would be beneficial or another possibility would be to add these results in the supplemental results. Otherwise the paper is methodological in nature and does not specifically provide new information about the disorder of interest.

Response: We have now performed the suggested analysis which we have presented in Supplementary Results and Supplementary Figure 2. These results show an interesting pattern of decreased functional connectivity in schizophrenia (especially at rest), which is in line with previous reports (1). However the data do not suggest that alterations in amygdalo-fusiform connectivity account for the primary and novel finding of increased amygdala activation to neutral faces in the schizophrenia group.

2. The relevance of the findings to symptoms that characterize the disorder would strengthen the paper (i.e., relate the findings to symptoms in individuals with schizophrenia in the context of theories in the literature on amygdala function). Otherwise, the findings seem overly data driven.

Response: We have sought to relate the current data to two of the main theories of the pathogenesis of schizophrenia in the discussion (2, 3). The former of these theories (2) argues for inappropriate amygdala activation in schizophrenia, a view which is directly supported by our data. The second hypothesis (3) argues that individual with schizophrenia attribute increased affective salience to otherwise neutral events,

providing the setting for the formation of symptoms such as delusional beliefs. We believe that the present finding of increased amygdala activation to neutral faces in schizophrenia provides a potential biological basis for such a liability to psychosis. We have attempted to re-word part of the discussion to make these links more explicit, although fuller coverage is precluded by the word limit.

REFERENCES

1. Zhou Y, Liang M, Tian L, Wang K, Hao Y, Liu H, et al. (2007): Functional disintegration in paranoid schizophrenia using resting-state fMRI. Schizophrenia Research. 97:194.

2. Grace AA (2000): Gating of information flow within the limbic system and the pathophysiology of schizophrenia. Brain Research Reviews. 31:330-341.

3. Kapur S (2003): Psychosis as a State of Aberrant Salience: A Framework Linking Biology, Phenomenology, and Pharmacology in Schizophrenia. Am J Psychiatry. 160:13-23.

Fear of faces in schizophrenia

Hall et al looked at the response of the amygdala, a brain region mediating fear, to faces in control subjects and participants with schizophrenia. They found that control subjects show amygdala activation to fearful faces, but not neutral faces. However patients with schizophrenia activated the amygdala fear system to both neutral and fearful faces. These results suggest that people with schizophrenia may perceive neutral faces as fearful, potentially contributing to the development of psychotic symptoms.

Overactivation of fear systems to neutral faces in schizophrenia

Jeremy Hall[1]*, Heather C. Whalley[1], James W. McKirdy[1], Liana Romaniuk[1], David McGonigle[2], Andrew M. McIntosh[1], Ben J. Baig[1], Viktoria-Eleni Gountouna[1], Dominic E. Job[1], David I. Donaldson[3], Reiner Sprengelmeyer[4], Andrew W. Young[5], Eve C. Johnstone[1] and Stephen M. Lawrie[1]

[1]Division of Psychiatry, University of Edinburgh, UK
[2]Schools of Psychology/Biosciences, University of Cardiff, UK
[3]Psychology Department, University of Stirling, UK
[4]School of Psychology, University of St Andrews, UK
[5]Department of Psychology, University of York, UK

* Corresponding author:
Dr Jeremy Hall
Division of Psychiatry
University of Edinburgh
Kennedy Tower
Royal Edinburgh Hospital
Edinburgh
UK
EH10 5HF

T: +44 131 537 6313
F: +44 131 537 6531
E: jhall5@staffmail.ed.ac.uk

Keywords: Fear, amygdala, schizophrenia, fMRI, face, emotion

Count:

Abstract	200
Main Text	1500
Figures	3
Tables	1

Supplementary material – 2 figures, 2 text documents

Abstract

Background The amygdala plays a central role in detecting and responding to fear related stimuli. A number of recent studies have reported decreased amygdala activation in schizophrenia to emotional stimuli (such as fearful faces) compared to matched neutral stimuli (such as neutral faces). Here we have investigated whether the apparent decrease in amygdala activation in schizophrenia could actually derive from increased amygdala activation to the neutral comparator stimuli.

Methods Nineteen patients with schizophrenia and 24 matched control participants viewed pictures of faces with either fearful or neutral facial expressions, and a baseline condition, during functional magnetic resonance imaging scanning.

Results Patients with schizophrenia showed a relative decrease in amygdala activation to fearful faces when compared to neutral faces. However this difference resulted from an increase in amygdala activation to the neutral faces in patients with schizophrenia, not from a decreased response to the fearful faces.

Conclusions Patients with schizophrenia show an increased response of the amygdala to neutral faces. This is sufficient to explain their apparent deficit in amygdala activation to fearful faces compared to neutral faces. The inappropriate activation of neural systems involved in fear to otherwise neutral stimuli may contribute to the development of psychotic symptoms in schizophrenia.

Introduction

The amygdala plays a central role in detecting and responding to fear-provoking stimuli (1). Individuals with bilateral lesions of the amygdala show a marked impairment in their ability to recognise fear from faces, and neuroimaging studies have confirmed that the amygdala shows greater activation to the presentation of faces expressing fear than to matched neutral faces (1, 2).

There is evidence of structural and functional abnormalities of the amygdala in schizophrenia (3). A number of functional neuroimaging studies have reported decreased amygdala activation in patients with schizophrenia compared to control subjects (4-13). Decreased amygdala activation in schizophrenia has been particularly shown in studies in which the response of the amygdala to an emotional stimulus (such as a face expressing fear) has been compared to a matched neutral stimulus (such as a neutral face) (7-9, 12, 13). These results have been interpreted as revealing a deficit in amygdala activation in response to emotion in patients.

The finding of decreased amygdala activation in patients with schizophrenia, especially those with paranoid symptoms, poses a paradox. Why should it be that patients whose clinical presentation is characterised by increased fear and arousal have decreased activation of the key brain region involved in fear? One possibility is that the apparent decrease in amygdala activation in response to fearful stimuli in these individuals actually derives from an increase in amygdala activation to the neutral comparator stimuli (3). Amygdala activation is known to occur to the presentation of neutral stimuli (14) and there is evidence that this response may be enhanced in individuals with schizophrenia (15). In the current study we have directly addressed the hypothesis that the apparent deficit in amygdala activation in

schizophrenia to emotional stimuli actually derives from an increase in amygdala response to the neutral comparator stimuli.

Materials and Methods

Participants

Twenty-four patients meeting DSM-IV diagnostic criteria for schizophrenia and 24 matched control subjects participated in the study. One patient was excluded due to the presence of a benign cerebral cyst and four were excluded due to failure to make behavioural responses. Full details of subject recruitment and characteristics are given in the Supplementary Methods.

Experimental design

A block design was used with three conditions: fear, neutral and baseline. During fear blocks 6 faces expressing the emotion of fear were presented for 3.5s each; during neutral blocks the same faces showing a neutral emotional expression were presented, and during baseline blocks participants were instructed to look at a fixation cross. Participants were required to select the gender of the face by pressing a button. The full task consisted of two runs each comprising 3 fear blocks (25s each), 3 neutral blocks (25s) and 7 interleaved blocks of the baseline condition (12.5s). Pilot data demonstrated that maximal amygdala activation was seen in the first run, consistent with previous studies (2), so analysis was primarily focussed on this period.

Image acquisition, processing and analysis

Details are given in the Supplementary Methods. Image analysis was conducted using SPM2 (www.fil.ion.ucl.ac.uk/spm). Contrast images were generated for each participant for the contrasts of interest (fear versus neutral, fear versus baseline and neutral versus baseline). One contrast image per participant was then entered into a

second-level random effects analysis for each contrast of interest to examine regions of significant difference between groups using a *t*-test. All statistical maps were thresholded at a level of $P<0.001$ uncorrected and regions were considered significant at $P<0.05$ at the cluster level, corrected for multiple comparisons. A region of interest (ROI) analysis was conducted for the bilateral amygdala.

Behavioural tests of emotion recognition

A standardised test of recognition of the six basic facial emotions facial emotion recognition was conducted after the scanning session (see Supplementary Methods).

Results

Demographics and behavioural responses in the scanner

There were no significant differences between the groups in terms of age ($F_{1,41}$=0.9, P>0.3), NART IQ ($F_{1,41}$=1.3, P>0.2) or gender (Fisher's exact test, P=1.0). Both groups performed the incidental gender discrimination task to high degree of accuracy (patients 91% correct (SD 13%), controls 98% correct (SD 3%)), although the schizophrenia group did show a deficit in accuracy of gender judgments compared to controls ($F_{1,41}$=6.4, P<0.05).

Response to fearful faces versus neutral faces

Patients with schizophrenia showed a relative decrease in left amygdala activation compared to control participants in the contrast of fearful faces versus neutral faces (P<0.05 corrected within amygdala ROI; Table 1 and Figure 1). In addition patients showed less activation relative to controls in the right fusiform gyrus and right lingual gyrus (Table 1).

Response to neutral faces versus baseline

Patient showed greater activation of the amygdala to neutral stimuli than the control group (P<0.01 corrected within an amygdala ROI; Table 1 and Figure 1). Patients also showed relatively greater activation than controls in the right lingual gyrus, fusiform gyrus and posterior cingulate (Table 1).

Response to fearful faces versus baseline

There was no significant difference in activation of the amygdala or other brain regions between patient and control groups in the contrast of fearful faces versus baseline (Table 1).

Comparison of amygdala activation across the task conditions

We extracted data for activation in the left amygdala for both task conditions (fear and neutral) relative to baseline and performed an ANOVA with group as a between subjects factor and emotion as a within subjects factor (Figure 2). This analysis revealed a significant group by emotion interaction ($F_{1,41}$=4.9, P<0.05). Planned *post-hoc t*-tests confirmed that this derived from greater activation of the amygdala in the schizophrenia group to neutral faces (P<0.01), with no difference between groups in amygdala activation to fearful faces (P=0.4).

Correlation analyses

Correlation analyses were performed to assess whether left amygdala activation (fear versus baseline or neutral versus baseline) was related to medication dose, PANSS total score, PANSS positive or PANSS negative symptoms, PANSS anxiety and depressive symptoms or gender judgment performance. None of these correlations was significant.

Amygdala activation over time

A comparison of amygdala activation between the first and second task runs showed significant habituation of amygdala activation over time, with group differences only apparent in the first run (Supplementary Figure 1).

Functional connectivity analysis

Additional analysis of functional connectivity between the amygdala and fusiform gyrus is presented in Supplementary Results and Supplementary Figure 2.

Performance on tests of emotion recognition

Performance of the patient and control groups on the test of emotion recognition was analysed using an ANOVA with group as a between-subjects factor and emotion as a within-subjects factor and showed a significant overall effect of group ($F_{1,41}$=7.0, P=0.01) and emotion ($F_{5,205}$=32.1, P<0.001) and a trend to a group by emotion interaction ($F_{5,205}$=2.2, P=0.054). *Post-hoc t*-tests showed a significant impairment in the recognition of the emotion of fear in patients with schizophrenia relative to control participants (P<0.01), with this emotion most commonly mistaken for surprise. There was no correlation of patient IQ with fear recognition performance.

Discussion

A number of neuroimaging studies have reported decreased activation of the amygdala to emotional stimuli compared to neutral stimuli in schizophrenia (3). We have investigated this effect by examining amygdala response to fearful and neutral faces in patients with schizophrenia and control subjects. We found that whilst patients did not differ significantly from controls in their amygdala response to fearful faces, they showed increased activation of the amygdala to neutral faces.

Patients with schizophrenia experiencing psychotic symptoms, especially paranoid beliefs, characteristically have increased levels of fear and arousal. The amygdala plays a central role in mediating fear and arousal and it is therefore paradoxical that previous studies have shown a decrease in amygdala activation in schizophrenia (7-9, 12, 13). The present findings provide a resolution to this paradox by showing that the apparent deficit in amygdala activation in individuals with schizophrenia can in fact be explained by increased amygdala response to the neutral comparator stimuli. This finding is supported by other studies which have shown increased medial temporal lobe activation in response to neutral stimuli in individuals with schizophrenia (15-17). Notably enhanced amygdala activation to neutral faces is also seen in children, suggesting the current findings may have a neurodevelopmental origin (18).

The present results help explain the findings of behavioural studies, which have typically shown deficits in the recognition of facial emotions in patients with schizophrenia, especially for negative emotions such as fear. Our findings suggest that the behavioural deficits may derive from an indiscriminate activation of the amygdala

to both fearful and non-fearful facial stimuli rather than a failure to activate the amygdala to fearful faces.

Patients with psychosis ascribe affective importance to stimuli and events that would generally be considered neutral. An influential theoretical model of the origin of psychosis is that it represents a state characterized by the aberrant attribution of salience to internal and external events (19). A related hypothesis posits amygdala hyper-activation, related to increased dopamine responsiveness, as a key pathogenic process in schizophrenia causing exaggerated affective drive to govern behavioural response selection (20). The current findings support these theories by demonstrating increased activation of the amygdala to neutral stimuli in schizophrenia, which may contribute to liability for psychosis.

Acknowledgements

The work was funded by the Dr Mortimer and Theresa Sackler Foundation. JH is supported by a MRC Clinical Research Training Fellowship and AMcI is supported by The Health Foundation. HCW and SML were supported by the Sackler Foundation. Imaging was performed at the SFC Brain Imaging Research Centre in Edinburgh. We would like to express our thanks to Iona Hamilton, Elaine Sandeman and Andrew Saul for their expert assistance in this work. We would also like to thank all the participants.

Financial Disclosures

The authors have no competing financial interests to declare in relation to the current work.

Figure legends

Figure 1. (A) Statistical parametric map (SPM) showing relatively greater amygdala activation in control participants than participants with schizophrenia in the contrast of fearful faces versus neutral faces. SPM thresholded at P<0.001 uncorrected. Crosshairs show the peak voxel within the left amygdala (co-ordinates -22 -1 -12). (B) SPM showing greater amygdala activation in participants with schizophrenia than control participants in the contrast of neutral faces versus baseline. SPM thresholded at P<0.001 uncorrected, K_E=50. Crosshairs show the peak voxel within the left amygdala (co-ordinates -17 -2 -15).

Figure 2. Plot of mean within group response in the left amygdala for the main contrasts of interest. Data were extracted from a volume of interest centered on the voxel of peak difference in the contrast of neutral versus baseline (8mm sphere of centre -22 -1 -12) for each of the three contrasts: Neutral versus Baseline (N vs B); Fear versus Baseline (F vs B) and Fear versus Neutral (F vs N). CON = control group. SCH = schizophrenia group. Error bars show SEM.

Figure 3. Recognition of basic facial emotions. CON = control group. SCH = schizophrenia group. ANG = anger, DIS = disgust, FEAR = fear, HAP = happiness, SAD = sadness, SUR = surprise. ** indicates a significant difference between groups at P < 0.01. Error bars show SEM.

References

1. Phelps EA, LeDoux J (2005): Contributions of the amygdala to emotion processing: from animal models to human behaviour. *Neuron*. 48:175-187.

2. Breiter HC, Etcoff NL, Whalen PJ, Kennedy WA, Rauch SL, Buckner RL, et al. (1996): Response and Habituation of the Human Amygdala during Visual Processing of Facial Expression. *Neuron*. 17:875-887.

3. Aleman A, Kahn RS (2005): Strange feelings: Do amygdala abnormalities dysregulate the emotional brain in schizophrenia? *Progress in Neurobiology*. 77:283-298.

4. Gur RE, McGrath C, Chan RM, Schroeder L, Turner T, Turetsky BI, et al. (2002): An fMRI Study of Facial Emotion Processing in Patients With Schizophrenia. *American Journal of Psychiatry*. 159:1992-1999.

5. Schneider F, Weiss U, Kessler C, Salloum JB, Posse S, Grodd W, et al. (1998): Differential amygdala activation in schizophrenia during sadness. *Schizophrenia Research*. 34:133-142.

6. Johnston PJ, Stojanov W, Devir H, Schall U (2005): Functional MRI of facial emotion recognition deficits in schizophrenia and their electrophysiological correlates. *European Journal of Neuroscience*. 22:1221-1232.

7. Phillips ML, Williams L, Senior C, Bullmore ET, Brammer MJ, Andrew C, et al. (1999): A differential neural response to threatening and non-threatening negative facial expressions in paranoid and non-paranoid schizophrenics. *Psychiatry Research*. 92:11-31.

8. Williams LM, Das P, Harris AWF, Liddell BB, Brammer MJ, Olivieri G, et al. (2004): Dysregulation of Arousal and Amygdala-Prefrontal Systems in Paranoid Schizophrenia. *American Journal of Psychiatry*. 161:480-489.

9. Williams LM, Das P, Liddell BJ, Olivieri G, Peduto AS, David AS, et al. (2007): Fronto-limbic and autonomic disjunctions to negative emotion distinguish schizophrenia subtypes. *Psychiatry Research: Neuroimaging*. 155:29-44.

10. Taylor SF, Liberzon I, Decker LR, Koeppe RA (2002): A functional anatomic study of emotion in schizophrenia. *Schizophrenia Research*. 58:159-172.

11. Paradiso S, Andreasen NC, Crespo-Facorro B, O'Leary DS, Watkins GL, Boles Ponto LL, et al. (2003): Emotions in Unmedicated Patients With Schizophrenia During Evaluation With Positron Emission Tomography. *American Journal of Psychiatry*. 160:1775-1783.

12. Takahashi H, Koeda M, Oda K, Matsuda T, Matsushima E, Matsuura M, et al. (2004): An fMRI study of differential neural response to affective pictures in schizophrenia. *NeuroImage*. 22:1247-1254.

13. Hempel A, Hempel E, Schonknecht P, Stippich C, Schroder J (2003): Impairment in basal limbic function in schizophrenia during affect recognition. *Psychiatry Research: Neuroimaging*. 122:115-124.

14. Schwartz CE, Wright CI, Shin LM, Kagan J, Whalen PJ, McMullin KG, et al. (2003): Differential amygdalar response to novel versus newly familiar neutral faces: a functional MRI probe developed for studying inhibited temperament. *Biological Psychiatry*. 53:854-862.

15. Holt DJ, Kunkel L, Weiss AP, Goff DC, Wright CI, Shin LM, et al. (2006): Increased medial temporal lobe activation during the passive viewing of emotional and neutral facial expressions in schizophrenia. *Schizophrenia Research*. 82:153-162.

16. Taylor SF, Luan Phan K, Britton JC, Liberzon I (2005): Neural Response to Emotional Salience in Schizophrenia. *Neuropsychopharmacology*. 30:984-995.

17. Surguladze S, Russell T, Kucharska-Pietura K, Travis MJ, Giampietro V, David AS, et al. (2006): A Reversal of the Normal Pattern of Parahippocampal Response to Neutral and Fearful Faces Is Associated with Reality Distortion in Schizophrenia. *Biological Psychiatry*. 60:423-431.

18. Thomas KM, Drevets WC, Whalen PJ, Eccard CH, Dahl RE, Ryan ND, et al. (2001): Amygdala response to facial expressions in children and adults. *Biological Psychiatry*. 49:309-316.

19. Kapur S (2003): Psychosis as a State of Aberrant Salience: A Framework Linking Biology, Phenomenology, and Pharmacology in Schizophrenia. *Am J Psychiatry*. 160:13-23.

20. Grace AA (2000): Gating of information flow within the limbic system and the pathophysiology of schizophrenia. *Brain Research Reviews*. 31:330-341.

Figure1

Click here to download high resolution image

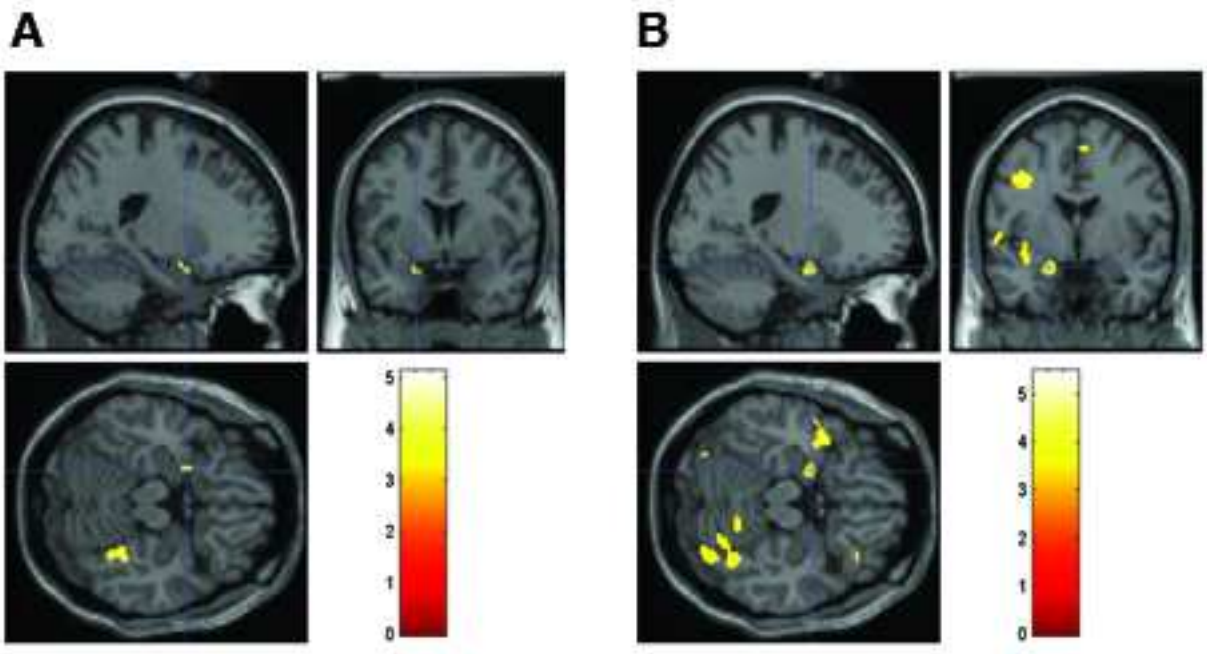


Figure 1

Figure2

Click here to download high resolution image

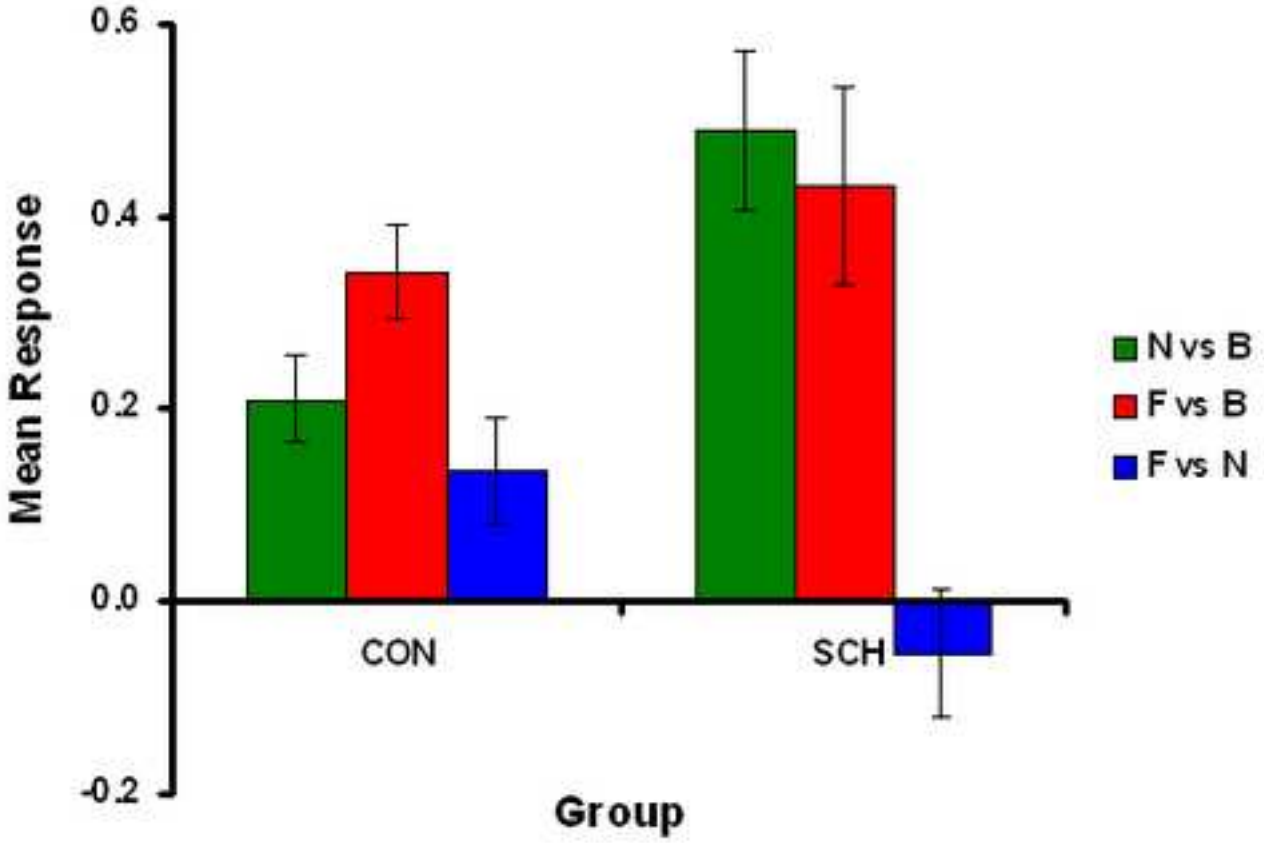


Figure3

Click here to download high resolution image

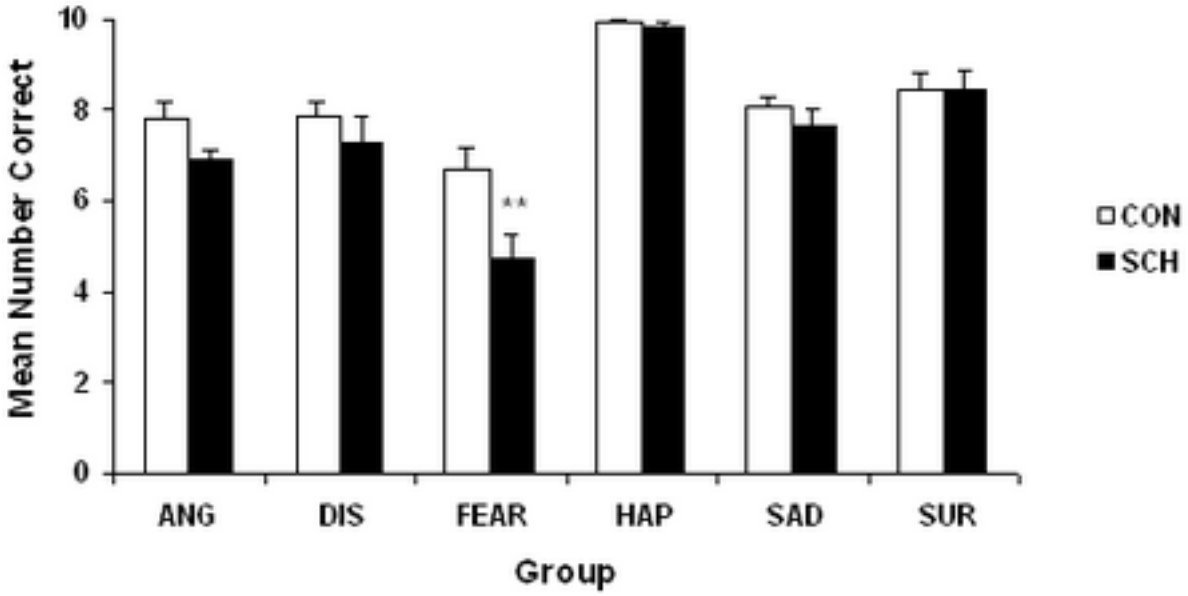


Table 1

Cluster P (Corrected)	Extent (Voxels)	Z	Peak voxel	Region
Fear versus Neutral:				
Controls > Patients				
0.004	419	4.34	14 -55 -2	R lingual gyrus/calcarine sulcus
0.022	283	4.17	33 -39-11	R fusiform gyrus/parahippocampal gyrus
0.026*	18	3.33	-22 -1 -12	L amygdala
Neutral versus Baseline:				
Controls < Patients				
0.021	312	4.03	10 -53 -10	R lingual gyrus/posterior cingulate
<0.001	766	3.92	10 -76 -7	R fusiform gyrus/lingual gyrus
0.009*	61	4.11	-17 -2 -15	L amygdala
Fear versus Baseline:				
No significant differences between groups				

*within amygdala ROI (small volume correction)

Table 1. Random effects analysis for main contrasts of interest. Corrected cluster level P values are shown.

Supplementary Methods

Participants

Twenty-four patients meeting DSM-IV diagnostic criteria for schizophrenia participated in the study, recruited from both inpatient and outpatient populations. Diagnoses were made by clinical consensus amongst clinicians highly experienced in working with patients with psychosis and was confirmed from case-notes using OPCRIT criteria (1). Exclusion criteria were age under 18 or over 65, neurological disease, dependence on alcohol or non-prescribed drugs, and other concomitant axis I or axis II disorder. Sixteen were men and eight were women. One subject was excluded due to the presence of a benign cerebral cyst and four individuals were excluded prior to analysis due to a failure to make any behavioural responses in the scanner. The remaining 19 individuals in the patient group (twelve men and seven women) had a mean age of 37.7 years (SD 8.4) and a mean pre-morbid IQ as assessed by the National Adult Reading Test (NART) (2) of 111.6 (SD 10.1). All patients were Caucasian, 17 were right handed and 2 were left handed. All were treated with antipsychotic medication (16 with atypical antipsychotics) with a mean chlorpromazine equivalent dose of 496mg (SD 377mg). Symptoms were rated on the day of the scanning session using the positive and negative syndrome scale (PANSS) (3) and the mean PANSS score was 46.8 (SD 8.5). Mean positive syndrome score on the PANSS was 12.3 (SD 4.5) with 15 out of the 19 individuals scoring 3 or greater on one or more positive syndrome items. Mean negative syndrome score on the PANSS was 11.8 (SD 3.4).

Twenty-four healthy control volunteers were recruited from the same regions and communities as the patients themselves. None of these subjects reported any

personal history of psychiatric illness or any family history of psychosis. Sixteen were men and eight were women; mean age was 35.1 years (SD 9.7), and mean IQ was 114.5 (SD 6.5). All control participants were Caucasian and right handed. Control participants were screened for any family or personal history of psychiatric illness and were additionally subject to the same exclusion criteria as the patients.

The study was approved by the local ethics committee and after complete description of the study written informed consent was obtained from all participants.

Task design

During fear and neutral blocks participants were initially presented with a 1s visual prompt ("Gender?") followed by six greyscale pictures of faces from the Ekman and Friesen series (4) each presented for 3.5s in a random order with a 0.5s inter-stimulus interval. Three of the faces were male and three were female in each block. Participants were required to select the gender of the face by pressing a button. The alternative response choices ("Male" and "Female") were shown on the screen. Pictures of the same individuals were shown in both the fear blocks and neutral blocks, differing only in the emotion shown (fearful or neutral expressions respectively). The order of presentation of fear and neutral blocks was counterbalanced across subjects. A block design was chosen because of the statistical power afforded by this approach, and the fact that it has proven effective in demonstrating amygdala activation in previous studies (5, 6). An implicit task was selected because increasing cognitive task demands has previously been shown to decrease limbic, and in particular amygdala activation (7).

Image acquisition

Imaging was performed at the SFC Brain Imaging Research Centre in Edinburgh using a GE 1.5TE Signa scanner (GE Medical, Milwaukee, WI, USA). After a localiser scan participants underwent functional scanning (99 volumes; Field of View 22cm; Time to Echo (TE) 40ms; Volume acquisition time (TR) 2.5s). Interleaved axial slices were acquired with a thickness of 5mm with no gap and matrix size of 64 x 64. The first four EPIs were discarded to avoid T1 equilibrium effects.

Image processing and analysis

The EPI images were reconstructed offline in ANALYZE format (Mayo Foundation, Rochester, MN, USA). Image analysis was conducted using SPM2 (Statistical Parametric Mapping; Wellcome Department of Cognitive Neurology and collaborators, Institute of Neurology, London, UK). Pre-processing consisted of re-orientation of the images and realignment to the mean EPI image, followed by normalisation to the standard Montreal Neurological Institute EPI template and spatial smoothing using a Gaussian kernel ($8mm^3$ full-width at half-maximum). Images from all subjects were inspected to exclude susceptibility artefacts in the medial temporal lobe. Within-scanner movement data was examined for all subjects and no subject was found to have moved more than 3.0mm in any axis across the duration of the scan. Before fitting the model, the subject's data were filtered in time using a high pass filter (150 s cut-off) and temporal autocorrelations were accounted for by imposing an AR(1) model.

Statistical analysis was performed using the general linear model approach as implemented in SPM2. At the individual participant level the data for each task were

modelled with three conditions (fear, neutral and baseline) each modelled by a boxcar convolved with a canonical haemodynamic response function. Parameters representing the participants movement during the scan were also entered into the model as covariates of no interest. Contrast images were generated for each participant for the contrasts of interest (fear versus neutral, fear versus baseline and neutral versus baseline) representing the pair-wise comparison of parameter estimates for the conditions. One contrast image per participant was then entered into a second-level random effects analysis for each contrast of interest to examine regions of significant difference between groups using a two sample *t*-test.

All statistical maps were thresholded at a level of $P<0.001$ uncorrected and regions were considered significant at $P<0.05$ at the cluster level, corrected for multiple comparisons. Co-ordinates were converted from MNI to Talairach co-ordinates using a non-linear transformation (http://www.mrc-cbu.cam.ac.uk/Imaging). A region of interest (ROI) analysis was conducted for the bilateral amygdala using a mask derived from the automated anatomical labelling atlas in WFU_PickAtlas v2.0 (8, 9).

Functional connectivity analysis

Time series were extracted from spheres (radius 5mm) centred on voxels of peak difference in the amygdala and fusiform gyrus for the fear versus neutral contrast. These data were regressed against motion parameters, and time series extracted from white matter and CSF, to reduce the effects of noise unrelated to the task. Pearson correlation coefficients were then calculated between the regions of interest and transformed to normal space using Fisher's r-to-z. The conditions of interest were considered separately by truncating the timecourses according to condition blocks.

Independent t-tests were then performed between the patient and control groups to compare connectivity across the conditions.

Behavioural tests of emotion recognition

A well charactersised behavioural test of facial emotion recognition was conducted after the scanning session (10-13). Photographs of the faces of 10 people used in this test were taken from the Ekman and Friesen series (4). For each face, there were poses corresponding to each of 6 basic emotions (happiness, surprise, fear, sadness, disgust, and anger), giving a total of 60 photographs (10 for each emotion). These were shown one at a time on a computer screen in pseudo-random order, for 3 seconds each. The task involved deciding which of the emotion names (happiness, surprise, fear, sadness, disgust, or anger) best described the facial expression shown. All participants were asked if they understood the meanings of the emotional names. If not, brief standardised descriptions were given. The names of the six emotions were displayed at the bottom of the screen and they could be consulted throughout the test. The order of the emotions on the screen was varied between tests. Responses were made by clicking the computer mouse over the name of the selected emotion. There was no time limit for responding. The next face was not shown until the subject had made a response. No feedback was given as to the appropriateness of any responses. Results were analysed using SPSS for Windows (version 14.0, SPSS Inc., US).

References

1. McGuffin P, Farmer A, Harvey I (1991): A polydiagnostic application of operational criteria in studies of psychotic illness. Development and reliability of the OPCRIT system. *Arch Gen Psychiatry*. 48:764-770.

2. Nelson HE, Willison JR (1991): *The Revised National Adult Reading Test – Test Manual*.

3. Kay SR, Fiszbein A, Opler LA (1987): The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophrenia Bulletin*. 13:261-276.

4. Ekman P, Friesen WV (1976): Pictures of facial affect. Palo Alto, CA: Consulting Psychologists Press.

5. Breiter HC, Etcoff NL, Whalen PJ, Kennedy WA, Rauch SL, Buckner RL, et al. (1996): Response and Habituation of the Human Amygdala during Visual Processing of Facial Expression. *Neuron*. 17:875-887.

6. Phillips ML, Young AW, Senior C, Brammer M, Andrew C, Calder AJ, et al. (1997): A specific neural substrate for perceiving facial expressions of disgust. *Nature*. 389:495.

7. Hariri A, Bookheimer SY, Mazziotta JC (2000): Modulating emotional responses: effects of a neocortical network on the limbic system. *Neuroreport*. 11:43-48.

8. Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH (2003): An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *NeuroImage*. 19:1233-1239.

9. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, et al. (2002): Automated Anatomical Labeling of Activations in SPM Using a Macroscopic Anatomical Parcellation of the MNI MRI Single-Subject Brain. *NeuroImage*. 15:273-289.

10. Young AW, Perrett DI, Calder AJ, Sprengelmeyer R, Ekman P (2002): Facial emotional expressions: stimuli and tests (FEEST). Bury St. Edmunds: Thames Valley Test Company.

11. Hall J, Harris JM, Sprengelmeyer R, Sprengelmeyer A, Young AW, Santos IM, et al. (2004): Social cognition and face processing in schizophrenia. *Br J Psychiatry*. 185:169-170.

12. Sprengelmeyer R, Young AW, Calder AJ, Karnat A, Lange H, Homberg V, et al. (1996): Loss of disgust: Perception of faces and emotions in Huntington's disease. *Brain*. 119:1647-1665.

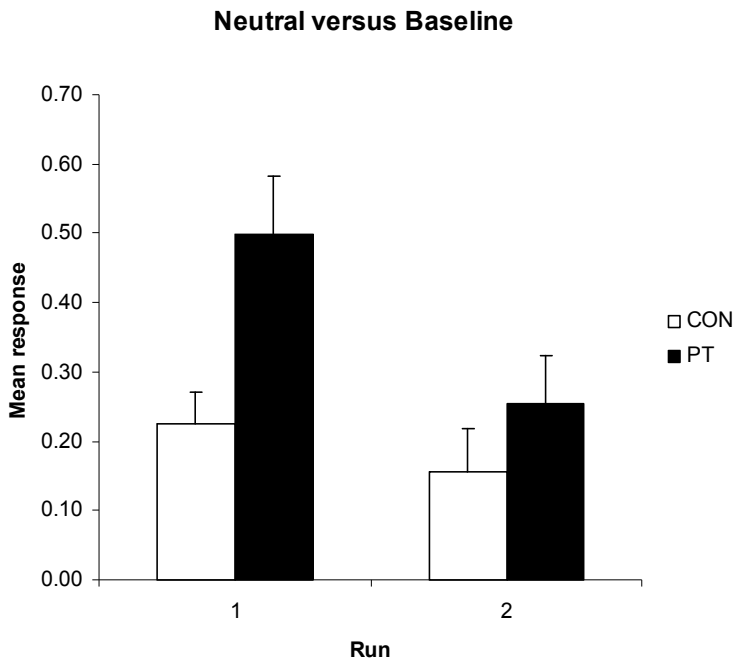
13. Sprengelmeyer R, Young AW, Schroeder U, Grossenbacher PG, Federlein J, Buttner T, et al. (1999): Knowing no fear. *Proc Biol Sci*. 266:2451-2456.

Supplementary Results

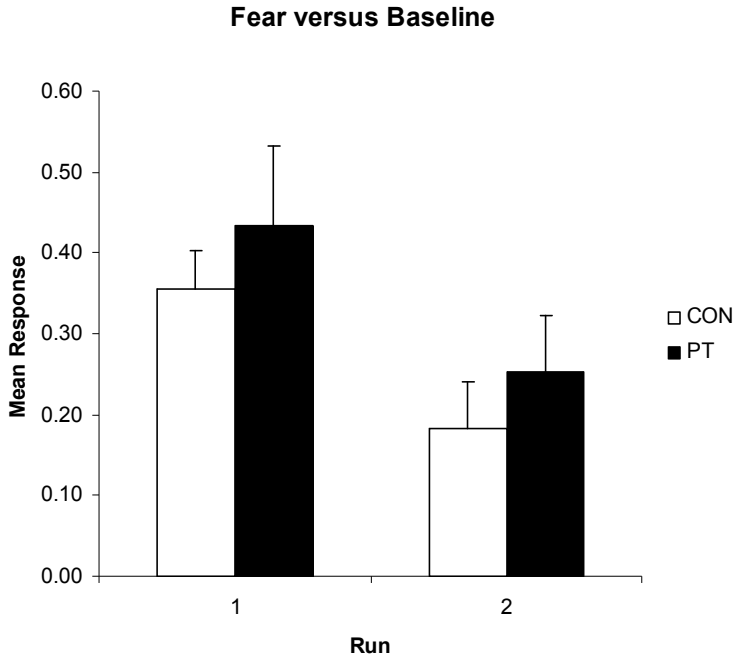
Examination of the pattern of functional connectivity between the amygdala and fusiform gyrus within the healthy control group across task conditions revealed greater connectivity in the baseline condition than in either the neutral or fear conditions (Supplementary Figure 2). The same overall pattern of connectivity was also seen within the patient group (Supplementary Figure 2). The patient group showed lower overall connectivity between the amygdala and fusiform gyrus that reached significance in the comparison of baseline connectivity ($t(41)=2.4$ $p=0.021$), although this effect would not survive correction for the number of comparisons made. This reflects previous findings of abnormalities in resting state connectivity in schizophrenia (1). These results however do not support the view that alterations in connectivity between the amygdala and fusiform gyrus account for the primary differences in amygdala activation in patients with schizophrenia reported in the present paper.

1. Zhou Y, Liang M, Tian L, Wang K, Hao Y, Liu H, et al. (2007): Functional disintegration in paranoid schizophrenia using resting-state fMRI. *Schizophrenia Research*. 97:194.

A)

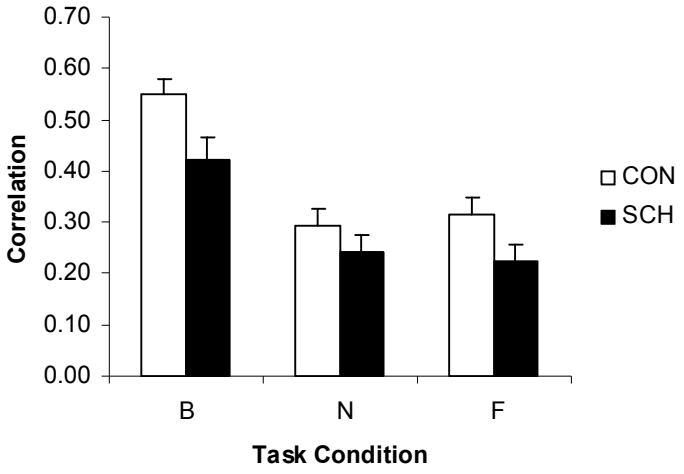


B)



Supplementary Figure 1. Plots of left amygdala activation within a VOI (8mm sphere of centre -22 -1 -12) for both task runs in the contrasts of A) Neutral versus Baseline, B) Fear versus Baseline. CON = control group. SCH = schizophrenia group. Error bars show SEM.

Supplemental Fig 2



Supplementary Figure 2. Functional connectivity between the amygdala (5mm sphere of centre -22 -1 -12) and the fusiform gyrus (5mm sphere of centre 33 -39-11). B = Baseline (rest) condition, N = Neutral condition, F = Fear condition. CON = control group. SCH = schizophrenia group. Error bars show SEM.

*** Manuscript Submission Form**

FOR OFFICE USE ONLY: Manuscript #

MANUSCRIPT SUBMISSION FORM—*BIOLOGICAL PSYCHIATRY*

Section 1: Instructions. A copy of this form, with signatures included from **ALL authors** on the manuscript, must accompany every new manuscript submission before it will be considered for publication. Please fully complete to eliminate delays in submission. Use an additional form if there are more than 10 authors. Please scan this completed form and attach it electronically during the submission process. If you are unable to do so, fax the completed form to the Editorial Office at (214) 648-0881.

Section 2: Manuscript Information.

Manuscript title: OVERACTIVATION OF FEAR SYSTEMS TO NEUTRAL FACES IN SCHIZOPHRENIA.

All author names (in order of appearance on the manuscript title page): HALL J, WHALLEY H, MCKIRDY J, MCGONIGLE D, MCINTOSH A, BALG B, GOUNTOUNA V-E, JOB D, DONALDSON D, SPRENGELMEYER R, YOUNG A, JOHNSTONE E, LAWRIE S.

Corresponding author name: JEREMY HALL

The complete text of the Acknowledgments and Financial Disclosures sections of the manuscript: (or attach as a separate page)

See attached.

Section 3: Acknowledgments. By signing below, I acknowledge my acceptance to and/or certification of the following information.

√ **Approval of the Submitted Work and Acknowledgment of Role of Corresponding Author.** I have personally reviewed and given final approval of the version submitted, and with the exception of previously published work or data which is clearly acknowledged in the manuscript and for which appropriate written permission is included as part of the submission, to my knowledge, neither the manuscript nor its data have been previously published (except in abstract) or are currently under consideration for publication by any other publication. I agree that the corresponding author (named above) shall be the sole correspondent with the Editorial Office on all matters related to this submission. In the event of acceptance, I designate the corresponding author as the responsible party for all communications with the journal's publisher related to this work, including review and correction of the typeset proof. I understand that once a manuscript is submitted, no substantial changes to the content will be allowed.

√ **Authorship Contribution.** I have participated sufficiently in the work to take public responsibility for all or part of the content, AND have made substantive intellectual contributions to the submitted work in the form of: 1) conception and design, and/or acquisition of data, and/or analysis of data; AND 2) drafting the article, and/or revising it critically for important intellectual content.

√ **Conflict of Interest, Disclosures, Financial, and Material Support.** To my knowledge, all of my possible conflicts of interest and those of my coauthors, financial or otherwise, related directly or indirectly to this work are clearly and completely indicated, and all grant or other financial support, as well as any material support, is listed in the appropriate sections of this manuscript.

√ **Transfer of Copyright.** I have read and understand Elsevier Science Inc. Transfer of Copyright Agreement and agree to all of the terms set forth therein. To the extent that this work was not performed by employees of the U.S. Federal Government, I agree to transfer copyright to the Society of Biological Psychiatry upon acceptance of this submission for publication in the Journal. I authorize the corresponding author to sign the Transfer of Copyright Agreement on my behalf.

	Author Name (Printed)	Author Signature	Date
1.	JEREMY HALL		6/9/7
2.	HEATHER C. WHALLEY		10/9/7
3.	JAMES W. MCKIRDY		10/9/7
4.	DAVID MCGONIGLE		10/9/7
5.	ANDREW M. MCINTOSH		10/9/7
6.	BEN J. BALG		10/9/7
7.	VIKTORIA-ELENI GOUNTOUNA		10/9/7
8.	DOMINIC E. JOB		10/9/7
9.	DAVID I. DONALDSON		7/9/7
10.	REINER SPRENGELMEYER		23/8/7

FOR OFFICE USE ONLY: Manuscript #

(9)

MANUSCRIPT SUBMISSION FORM—*BIOLOGICAL PSYCHIATRY*

Section 1: Instructions. A copy of this form, with signatures included from **ALL authors** on the manuscript, must accompany every new manuscript submission before it will be considered for publication. Please fully complete to eliminate delays in submission. Use an additional form if there are more than 10 authors. Please scan this completed form and attach it electronically during the submission process. If you are unable to do so, fax the completed form to the Editorial Office at (214) 648-0881.

Section 2: Manuscript Information.

Manuscript title: OVERACTIVATION OF FEAR SYSTEMS TO NEUTRAL FACES IN SCHIZOPHRENIA

All author names (in order of appearance on the manuscript title page): CONTINUED

Corresponding author name:

The complete text of the Acknowledgments and Financial Disclosures sections of the manuscript: (or attach as a separate page)

CONTINUED.

Section 3: Acknowledgments. By signing below, I acknowledge my acceptance to and/or certification of the following information.

√ **Approval of the Submitted Work and Acknowledgment of Role of Corresponding Author.** I have personally reviewed and given final approval of the version submitted, and with the exception of previously published work or data which is clearly acknowledged in the manuscript and for which appropriate written permission is included as part of the submission, to my knowledge, neither the manuscript nor its data have been previously published (except in abstract) or are currently under consideration for publication by any other publication. I agree that the corresponding author (named above) shall be the sole correspondent with the Editorial Office on all matters related to this submission. In the event of acceptance, I designate the corresponding author as the responsible party for all communications with the journal's publisher related to this work, including review and correction of the typeset proof. I understand that once a manuscript is submitted, no substantial changes to the content will be allowed.

√ **Authorship Contribution.** I have participated sufficiently in the work to take public responsibility for all or part of the content, AND have made substantive intellectual contributions to the submitted work in the form of: 1) conception and design, and/or acquisition of data, and/or analysis of data; AND 2) drafting the article, and/or revising it critically for important intellectual content.

√ **Conflict of Interest, Disclosures, Financial, and Material Support.** To my knowledge, all of my possible conflicts of interest and those of my coauthors, financial or otherwise, related directly or indirectly to this work are clearly and completely indicated, and all grant or other financial support, as well as any material support, is listed in the appropriate sections of this manuscript.

√ **Transfer of Copyright.** I have read and understand Elsevier Science Inc. Transfer of Copyright Agreement and agree to all of the terms set forth therein. To the extent that this work was not performed by employees of the U.S. Federal Government, I agree to transfer copyright to the Society of Biological Psychiatry upon acceptance of this submission for publication in the Journal. I authorize the corresponding author to sign the Transfer of Copyright Agreement on my behalf.

	Author Name (Printed) (CONTINUED:)	Author Signature	Date
1.	ANDREW W. YOUNG	A. Young	10/9/7
2.	EVE C. JOHNSTONE	Eve Johnstone	10/9/7
3.	STEPHEN M. LAWRIE	Stephen Lawrie	10/9/7
4.			
5.			
6.			
7.			
8.			
9.			
10.			

Acknowledgements

The work was funded by the Sir Mortimer and Theresa Sackler Foundation. JH is supported by a MRC Clinical Research Training Fellowship and AMcI is supported by The Health Foundation. We would like to express our thanks to Iona Hamilton, Elaine Sandeman and Andrew Saul for their expert assistance in this work. We would also like to thank all the participants.

Financial Disclosures

The authors have no competing financial interests to declare in relation to the current work.