Resting brain perfusion in social anxiety disorder: A voxel-wise whole brain comparison with healthy control subjects

J.M. Warwick a,⁎, P. Carey a, G.P. Jordaan b, P. Dupont c, D.J. Stein a

a MRC Unit for Stress and Anxiety Disorders, Faculty of Health Sciences, Stellenbosch University, Cape Town, South Africa
b Psychiatry, Faculty of Health Sciences, Stellenbosch University, Cape Town, South Africa
c Laboratory for Cognitive Neurology and Medical Imaging Center, KU Leuven, Leuven, Belgium

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Introduction: Social anxiety disorder (SAD) is a condition characterised by fears of social interaction and performance situations. SAD may be related to a dysregulation or hyperactivity of cortico-limbic circuitry. This is the first voxel-based whole brain study comparing resting function in SAD to a normal control group.

Methods: Resting perfusion in adult subjects with generalised SAD was compared with healthy adult volunteers using Statistical Parametric Mapping (SPM). In subjects with SAD, correlations were also sought between resting perfusion and clinical severity measured using the total Liebowitz Social Anxiety Scale (LSAS).

Results: Twenty-eight subjects with SAD were compared with 19 healthy volunteers. SAD subjects had increased resting perfusion in the frontal cortex and right cerebellum, and decreased perfusion in the pons, left cerebellum, and right precuneus. Total LSAS correlated positively with left frontal cortex resting perfusion, and negatively with right fusiform and right lingual perfusion.

Conclusion: This study demonstrated increased resting frontal function in social anxiety disorder that is consistent with its hypothesised role in the modulation of excessive limbic activity in anxiety disorders. The correlation of posterior cortical resting function with the severity of SAD symptoms may point to defective perception of self and others.

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1. Introduction

Social anxiety disorder (SAD) is recognized as a discrete anxiety disorder in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) (American Psychiatric Association, 2000). It is a condition characterised by fears of social interaction and performance situations. These fears cause sufferers to avoid these situations or endure them with extreme distress. Unlike normal social anxiety, SAD results in significant distress and impairment (Wittchen and Beloch, 1996). Indeed SAD, the most prevalent of the major anxiety disorders with a lifetime prevalence of 10–15%, is associated with significant comorbidity and morbidity (Ballenger, 1998, Magee et al., 1996, Weiller et al., 1996).

Abbreviations: arMPFC, anterior-rostral medial frontal cortex; DSM, Diagnostic and Statistical Manual of Mental Disorders; FLAIR, fluid attenuated inversion recovery; HMPAO, hexamethylpropylene amine oxime; LSAS, Liebowitz Social Anxiety Scale; MNI, Montreal Neurological Institute; MRI, magnetic resonance imaging; OCD, obsessive compulsive disorder; SAD, social anxiety disorder; SD, standard deviation; SPECT, single photon emission computed tomography; SPM, Statistical Parametric Mapping; TTM, trichotillomania

⁎ Corresponding author. Nuclear Medicine, Tygerberg Hospital, Francie van Zijl Drive, Tygerberg, 7505 Cape Town, South Africa. Tel.: +27 21 9384265; fax: +27 21 9384694.
E-mail address: jw@sun.ac.za (J.M. Warwick).

While no clear neuroanatomical model yet exists for SAD, there is a growing neuroscience literature devoted to social functioning. A previous review (Adolphs, 2003) proposes that social cognition occurs at three levels. Firstly there is the representation of sensory stimuli by higher order sensory cortices. Secondly these sensory representations are linked to social judgments by a neural system involving the ventral striatum, amygdala, and orbito-frontal cortex. Thirdly, other cortical areas such as the left prefrontal, right parietal, and anterior and posterior cingulate, are involved in the construction of an internal representation of the social environment. A review of the role of the medial frontal cortex in social cognition by Amodio and Frith (2006) suggests that the anterior-rostral medial frontal cortex (arMPFC) plays a role in the social cognition by integrating afferents from posterior rostral MFC (involved in monitoring of actions), and from orbito-frontal cortex (involved in monitoring of reward or punishment). The arMPFC appears to play a role in understanding the mental states of others, the perception of the long term dispositions and attitudes of both others and self, and the perception of own current feelings (Frith, 2007). This second order representation of others’ mental states is required for thinking about their communicative intentions.

Conditioned fear provides one experimental model (LeDoux, 1998, 2000) that provides a mechanism to explain fear and anxiety in general, and may at least in part be applicable in SAD. This is
supported by a recent meta-analysis demonstrating amygdala and insular hyperactivity being common to both SAD and healthy subjects undergoing fear conditioning (Etkin and Wager, 2007). The model for conditioned fear (LeDoux, 1998, 2000) can be summarised as follows: Fear conditioning results from the coupling of a neutral stimulus to an unpleasant event. Consequently the neutral stimulus evokes anxiety and fear, manifest by endocrine, autonomic, and unconscious behavioural responses. Sensory afferents are sent to the thalamus and then relayed to the amygdala. The amygdala are believed to associate a value with perceived objects (Frith, 2007), and therefore play a central role in the assessment of these thalamic inputs, and in determining the triggering of responses via the hypothalamo-pituitary axis, the autonomic nervous system, and behavioural responses via cortico-limbic connections. The hippocampi, anatomically intimately related to the amygdala, are believed to be responsible for processing the context of fear conditioning, and as such modulate the more “automatic” amygdala responses. The prefrontal cortex has also been shown to be crucial to the extinction of conditioned responses, and appears to have a modulatory effect on amygdala function (LeDoux, 1998, 2000; Phelps et al., 2004; Milad et al., 2007).

It can be hypothesised that the outcome of this evaluative process by the amygdala (whether there is a fear response or not), may be dependant on amygdala (1) input, (2) function, (3) context processing, and (4) prefrontal cortex modulation. SAD may be due to an over responsiveness to social stimuli (Adolphs, 2003). A variety of paradigms point to SAD being related, at least in part, to a dysregulation or hyperactivity of the amygdala (Tillfors et al., 2001; Birbaumer et al., 1998; Schneider et al., 1999). If humans have developed a network to evaluate social signals for threatening content, this hypothesised system may well be dysfunctional in SAD (Rauch et al., 2003).

Neuroimaging studies of SAD have demonstrated increased activity in cortico-limbic circuits during anticipation (Lorderbaum et al., 2004; Tillfors et al., 2002), public speaking (Tillfors et al., 2001), and conditioned response (Veit et al., 2002; Birbaumer et al., 1998; Schneider et al., 1999) paradigms. Similarly these regions are hyperactive when these subjects are exposed to harsh faces, and the degree of this hyperactivity has been shown to correlate with the severity of SAD symptoms (Stein et al., 2002; Phan et al., 2006). Simultaneously activity in many cortical areas appears to decrease during anxiety provocation, perhaps reflecting impaired cognitive processing at these times (Lorderbaum et al., 2004; Tillfors et al., 2001; Tillfors et al., 2002; Van Ameringen et al., 2004).

While the literature contains numerous imaging studies investigating resting cerebral blood flow and metabolism in other psychiatric conditions, it can be argued that because resting studies do not elucidate a response to relevant stimuli, they may be limited in their ability to delineate functional abnormalities in SAD. However an improved understanding of SAD may still be achieved by the detection of findings at rest that may not be present or may be masked during an activation paradigm.

Little data is available on brain function in SAD in the resting state. A previous region of interest based study SAD patients were found to have no differences in resting rCBF in a number of regions implicated in obsessive compulsive disorder (OCD), when compared with normal controls (Stein and Leslie, 1996). We are not aware of any published work comparing resting rCBF in SAD patients with a normal control group in the remainder of the brain. In addition this is the first voxel-based study performing an analysis such as this.

2. Methods

2.1. Subjects

Group 1 was comprised of adult subjects with a primary diagnosis of generalised SAD recruited from the Anxiety Disorders Clinic of our tertiary hospital. All subjects were interviewed with the Structured Clinical Interview for the Diagnosis of Axis-I Disorders to ascertain the diagnosis according to DSM-IV criteria (DSM-IV) (First et al., 1996). Subjects with other primary psychiatric disorders, significant medical illness or a neurological condition were excluded. Co-morbidity anxiety spectrum disorders were uncommon in our sample and in all cases were considered secondary in terms of temporal course, symptom severity, and associated distress (2 — panic disorder, 1 — trichotillomania (TTM), 1 — body dysmorphic disorder). The subjects have therefore been included in the analysis. All but one subject (receiving alprazolam 0.25 mg/day) were not taking any medication during the study. The highest level of education was recorded for all subjects.

Group 2 was made up of healthy adult volunteers with a similar age and gender distribution who initially underwent a psychiatric screening interview, a physical examination, and a magnetic resonance imaging (MRI) scan. Only individuals without abnormalities on these screening tests were included in this group. All healthy volunteers were interviewed with the Mini International Neuropsychiatric Interview (Version 4.4) (Sheehan et al., 1998). Individuals who met criteria for an active DSM-IV Axis-I Disorder were excluded from the study. A general physical examination was performed. Blood chemistry, including urea and electrolytes, liver function tests, random glucose, full blood count and serology for syphilis were performed. Individuals with clinically significant medical or neurological disorders were excluded. None of the healthy volunteers was taking any medication at the time of the study. All healthy volunteers were required to undergo MRI scanning of the brain to exclude major structural brain lesions using axial T2-weighted and fluid attenuated inversion recovery (FLAIR) sequences. Individuals with significant structural lesions were excluded. The highest level of education was recorded for all healthy volunteers.

The subjects were initially recruited for each of the two groups as part of separate studies, resulting in the use of different screening procedures. In particular it is noted that some differences exist between the psychiatric screening interviews, the Structured Clinical Interview for the Diagnosis of Axis-I Disorders used for group 1, and the Mini International Neuropsychiatric Interview used for group 2. Some of the subjects in the present study formed part of data from two previous studies involving different analyses (Carey et al., 2004 and Warwick et al., 2006).

2.2. Measures

For group 1 social anxiety symptom severity was rated using the Liebowitz Social Anxiety Scale (LSAS) (Liebowitz 1987).

2.3. SPECT imaging

Single photon emission computed tomography (SPECT) was conducted in the resting state for all subjects in both groups. Subjects lay supine in a quiet dimly lit room for 30 min prior to injection of the radiopharmaceutical. Apart from administration of the injection by a physician, they remained alone in the room during this period. Subjects were asked to remain at rest for 10 min after the injection of the radiopharmaceutical to allow uptake of the radiopharmaceutical in the brain.

An injection of 555 MBq (15 mCi) of technetium-99 m hexamethylpropylene amine oxime (Tc-99 m HMPAO) was given into an arm vein through a previously placed intravenous cannula. After completion of the 10 minute rest period described above, SPECT imaging of the brain was performed, with the subject's head supported by a headrest, using a dual detector gamma camera (Elscint Helix, GE Medical Systems, USA) equipped with fanbeam collimators. Data were acquired in the step-and-shoot mode, using a 360 degree circular orbit, with the detectors of the gamma camera as close as...
possible to the subject's head. Data were acquired using a 128×128 image matrix in 3 degree steps of 15 s per step. Data were reconstructed by filtered backprojection, using a Metz filter (power=5, FWHM=14 mm). The Chang method (μ=0.11/cm) was used for attenuation correction (Chang, 1978). The final reconstructed voxel size was 1.7×1.7×3.9 mm³. Image files were converted from interfile to analyze format using conversion software (Medcon, Erik Nolf, UZ Ghent, Belgium).

2.4. Spatial preprocessing

Statistical analyses were conducted on a voxel-by-voxel basis using Statistical Parametric Mapping (SPM2, Wellcome Department of Cognitive Neurology, UK (http://www.fil.ion.ucl.ac.uk/spm). The images of each subject were normalised to the Montreal Neurological Institute (MNI) standard anatomical space with 4×4×4 mm³ voxels, and to a value of 50 using proportional scaling. This was achieved using a 12 parameter affine transformation followed by non-linear warping using 7×8×7 non-linear basis functions. The normalised images were then smoothed using a 3D Gaussian kernel with a FWHM of 12 mm.

2.5. Analysis

A multi-group study design was first performed using 2 groups (SAD and control group). Contrasts were applied to detect: (i) areas of significantly increased resting perfusion in SAD subjects compared to control subjects, and (ii) areas of significantly decreased resting perfusion in SAD subjects compared to control subjects. A second analysis was performed on the scans of subjects in group 1. Correlations were sought between total LSAS scores, and resting perfusion in the brain. Areas of significant (i) positive and (ii) negative correlation were sought. For the above analyses, an uncorrected p-value of p<0.001 was chosen as a threshold for statistical significance. This relatively liberal threshold was used to maximise our sensitivity for the detection of any differences and/or correlations. Clusters were located to anatomical regions using MRicro software (Rorden and Brett, 2000).

3. Results

Group 1 consisted of 28 subjects (21 male, 7 female) with a mean (SD) age of 34 (10) years. Six of the subjects had not completed formal schooling, 12 subjects had completed formal schooling, and 10 subjects had completed diplomas or degrees following their schooling. Group 2 consisted of 19 subjects (14 male, 5 female) with a mean (SD) age of 37 (8) years. Four of the subjects had not completed formal schooling, 7 subjects had completed formal schooling, and 8 subjects

Table 1

Differences in rCBF in the group of subjects with SAD compared to a control group, and correlations between total LSAS score and rCBF in SAD subjects (p<0.001 uncorrected)

<table>
<thead>
<tr>
<th>Region</th>
<th>MNI coordinates (x, y, z (mm))</th>
<th>Cluster size (mm³)</th>
<th>t</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Increased for SAD relative to controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left frontal</td>
<td>−36, 36, 40</td>
<td>5184</td>
<td>4.53</td>
<td>4.09</td>
</tr>
<tr>
<td>Right anterior frontal</td>
<td>20, 52, 36</td>
<td>2176</td>
<td>4.34</td>
<td>3.95</td>
</tr>
<tr>
<td>Right lateral frontal</td>
<td>52, 28, 8</td>
<td>1216</td>
<td>3.74</td>
<td>3.47</td>
</tr>
<tr>
<td>Right cerebellum</td>
<td>40, −76, −28</td>
<td>704</td>
<td>3.52</td>
<td>3.29</td>
</tr>
<tr>
<td><strong>Decreased for SAD relative to controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pons</td>
<td>−12, −28, −32</td>
<td>1408</td>
<td>4.72</td>
<td>4.23</td>
</tr>
<tr>
<td>Left cerebellum</td>
<td>−8, −36, −8</td>
<td>1088</td>
<td>4.39</td>
<td>3.98</td>
</tr>
<tr>
<td>Right precuneus</td>
<td>16, −48, 36</td>
<td>1216</td>
<td>4.18</td>
<td>3.82</td>
</tr>
<tr>
<td><strong>Positive correlation between total LSAS and perfusion in SAD subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left frontal</td>
<td>−40, 40, 40</td>
<td>256</td>
<td>3.57</td>
<td>3.19</td>
</tr>
<tr>
<td><strong>Negative correlation between total LSAS and perfusion in SAD subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right fusiform</td>
<td>28, −72, −12</td>
<td>3392</td>
<td>4.94</td>
<td>4.11</td>
</tr>
<tr>
<td>Right lingual</td>
<td>12, −48, 0</td>
<td>1600</td>
<td>4.68</td>
<td>3.95</td>
</tr>
</tbody>
</table>

Fig. 1. Four clusters of significantly increased rCBF in the left frontal cortex (A), right frontal cortex (B and C), and right cerebellum (D) in the group of SAD subjects compared to the group of controls. Two clusters of significantly decreased rCBF in the pons (E), left cerebellum (F) and right precuneus (G) in the group of SAD subjects compared to the group of controls. The clusters are superimposed onto a representative T1-weighted MRI study in MNI space (Courtesy of MRicro, Chris Rorden).
had completed diplomas or degrees following their schooling. All but one subject on alprazolam 0.25 mg/day, were free of any other medication during the study. SAD subjects had a LSAS score ranging from 50 to 141, with a mean (SD) score of 99.6 (21.8).

The results of the first SPM analysis are summarised in Table 1 and Fig. 1. Comparison of resting scans showed that SAD subjects had significantly increased resting perfusion in 4 clusters compared to the control group. These clusters were located in the left anterior frontal, and the right anterior frontal cortex superiorly, in the right lateral frontal cortex on middle cuts, and in the superior postero-lateral cerebellum on the right. The position of the left frontal cluster was found to overlap with a cluster showing significant positive correlation (p<0.001) between total LSAS and resting perfusion, with a distance between the cluster maxima of 4 mm.

Significantly decreased resting perfusion was located in clusters involving the posterior pons just left of the midline, the antero-medial aspect of the superior left cerebellum, and the right precuneus.

The second analysis of the SAD subjects revealed a significant positive correlation (R²=0.29) between resting perfusion and total LSAS score at the site of the left frontal cortex cluster detected above (Table 1, Fig. 2). No significant positive correlation was detected elsewhere in the brain at this level of significance.

An analysis of brain perfusion for negative correlations between resting perfusion and total LSAS score, revealed significant clusters in the right fusiform (R²=0.48), and the right lingual cortex (R²=0.46) (Table 1, Fig. 2).

Scrutiny of the results of these analyses, even at the uncorrected threshold of p<0.05, did not reveal significant differences in resting perfusion of the amygdala or hippocampi of SAD subjects compared to the control group. Similarly no correlation was found between total LSAS and resting perfusion in these regions at this level of significance.

4. Discussion

The findings of this study were that subjects with SAD had: 1) increased resting perfusion in the left and right anterior frontal cortex, the right lateral frontal cortex, and in the right cerebellum, 2) decreased resting perfusion in the pons, the left cerebellum, and the right precuneus, 3) a positive correlation between resting perfusion and the total LSAS in the left frontal cortex, 4) a negative correlation between resting perfusion and the total LSAS score in the right fusiform, and the right lingual cortex, 5) no differences in resting perfusion of the amygdala or hippocampi.

In this study resting rCBF in subjects with social anxiety disorder was found to be significantly increased in frontal cortex bilaterally compared to healthy controls, a finding that is strengthened by the independent finding of a positive correlation between the total LSAS score and resting perfusion at one of these sites in the SAD group. A recent SAD study (Kilts et al., 2006) showed several regions of altered rCBF using social performance and evaluation, and social anxiety provocation paradigms. Changing rCBF was also noted following pharmacotherapy. There is a relatively close spatial concordance between all of the frontal findings in this study and regions demonstrated by Kilts et al. Medial prefrontal cortex is hypothesised to play a role in the inhibition or extinction of excessive cortico-limbic activity in anxiety disorders (LeDoux, 1998).

Fig. 2. A cluster in the left frontal region with a significant positive correlation between regional cerebral blood flow (rCBF) and total LSAS score (A). Two clusters in the right fusiform and right lingual region with a significant negative correlation between rCBF and total LSAS score (B and C). The clusters are superimposed onto a representative T1-weighted MRI study in MNI space (Courtesy of MRicro, Chris Rorden).
The results of this study did not show any evidence for excessive amygdalo–hippocampal activity in the SAD group. These findings are perhaps not surprising as unlike a number of previous neuroimaging studies in SAD, these studies were performed in the resting state with no potentially threatening social cues. It is likely that in the resting state, frontal compensation, seen by increases in frontal perfusion, is effective in preventing limbic overactivity. A second possibility that cannot be ruled out is that rather than reflecting frontal inhibition of excessive cortico–limbic function, the frontal changes are due to inherent differences in frontal function in SAD. Prefrontal cortex is believed to be involved in the internal representation of the social environment (Adolphs, 2003), and it may play a role in our ability to read other peoples mental states (Frith, 2007). If this is the case frontal findings from this study may reflect dysfunction of cortical regions which contribute directly to the aetiology of SAD when exposed to social cues.

We recently reported that effective pharmacotherapy decreases resting insular perfusion bilaterally in subjects with SAD (Warwick et al., 2006). A number of the subjects in the present study formed part of that cohort. In that study we speculated that resting perfusion may be increased in the insulae in SAD patients, with normalisation following successful pharmacotherapy, although no experimental data existed at that stage to substantiate or refute this. This study did not demonstrate increased insular perfusion in SAD.

Resting cerebellar perfusion also demonstrated significant differences between the two groups of subjects. SAD subjects had a cluster of significantly increased resting perfusion in the right posterior cerebellum, and a cluster of significantly decreased perfusion in the left medial anterior cerebellum as well as in the pons. There is growing evidence of cognitive functions being mediated by the cerebellum, although the inconsistent findings here make it difficult to provide a simple hypothesis. While there is little literature on the role of the cerebellum in anxiety disorders, previous work has suggested an association between anxiety disorders and cerebellar–vestibular dysfunction (Levinson, 1989a,b). With specific respect to SAD, a more recent PET study demonstrated that anticipatory anxiety in SAD subjects was associated with decreased cerebellar perfusion bilaterally (Tillfors et al., 2002). These also correspond with changes demonstrated by Kilts et al. (2006).

The right precuneus was found to have decreased resting perfusion in SAD patients compared to healthy controls. SAD subjects have previously been shown to have enhanced right precuneus activation in response to a social performance and evaluation task following pharmacotherapy (Kilts et al., 2006). It can be argued that abnormal precuneus function may be related to the pathophysiology underlying SAD. The precuneus is believed to be involved in a network involved in the perception of novel faces, along with the right parietal and secondary visual cortices (Kuskowski and Pardo 1999; Kim et al., 1999). Some workers have suggested that prosopagnosia or face blindness may be related to defective fusiform function (Kleinschmidt and Cohen, 2006). It has previously been suggested that SAD may be the result of a defective system for the assessment or assignment of threat to human faces (Rauch et al., 2003). Again, it is possible that this network is defective in SAD, resulting in abnormal processing of self–other schemas.

A number of limitations of the current study should be noted. First, one subject was receiving a regular dose of alprazolam 0.25 mg/day. Combined with evidence suggesting that rCBF is unaffected by the chronic administration of alprazolam (Roy-Byrne et al., 1993), we believe that the effect on our findings is likely to be small. Second, while the two groups of subjects were comparable with respect to age, gender, and level of education, the comparability of the two groups for other potentially confounding factors such as socioeconomic status and treatment history were not considered. Comorbidities in 4 subjects in group 1 may have also had a confounding effect on the results of this study, although as explained in the methodology, these conditions were considered secondary to the patients’ SAD. Third, as stated above, the subjects were initially recruited for each of the two groups as part of separate studies, resulting in the use of different screening procedures. In particular it is noted that some differences exist between the psychiatric screening interviews, the Structured Clinical Interview for the Diagnosis of Axis–I Disorders used for group 1, and the Mini International Neuropsychiatric Interview used for group 2. While this situation is not ideal, we believe we were still able to perform a comparison of two reasonably well defined groups. Fourth, there are significant intrinsic limitations to SPECT. Although SPECT has a high sensitivity, it has relatively poor spatial resolution compared to other neuroimaging techniques. In addition, activity from deeper brain structures is attenuated by overlying tissue, although this can be corrected to some extent by attenuation correction. Finally, it needs to be noted that this study used a low significance threshold for a whole brain, voxel–wise analysis (p < 0.001, uncorrected). The emphasis in this analysis was to maximise sensitivity and to avoid missing significant findings with the use of a more strict threshold. Once made however, these findings were looked at critically in the light of existing knowledge.

Despite these limitations, we believe that our findings contribute to a growing literature on the functional imaging of anxiety disorders.

5. Conclusion

This study demonstrated bilateral increased resting frontal function in social anxiety disorder compared to controls and left frontal perfusion correlating with disease severity. These findings are consistent with its hypothesised role in the modulation of excessive limbic activity in anxiety disorders. The right fusiform and right lingual gyri showed a negative correlation between the total LSAS score and resting perfusion in SAD subjects which may point to defective perception of self and others in these patients.

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References

LeDoux J. Fear and the brain: where have we been and where are we going? Biol Psychiatry 2001;158:1220–38.