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Q2 Q1 ^{1}H-magnetic resonance spectroscopy in social anxiety disorder

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ABSTRACT

Background: Social anxiety disorder (SAD) is characterized by excessive anxiety about social interaction or performance situations, leading to avoidance and clinically significant distress. A growing literature on the neurobiology of SAD has suggested that the reward/avoidance basal ganglia circuitry in general and the glutamatergic system in particular may play a role. In the current study, we investigated ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) concentrations in cortical, striatal, and thalamic circuitry, as well as their associations with measures of social anxiety and related symptoms, in patients with primary SAD.
Methodology: Eighteen adult individuals with SAD and 19 age- and sex- matched controls participated in this study. ^{1}H-MRS was used to determine relative metabolite concentrations in the anterior cingulate cortex (ACC) using single voxel spectroscopy (reporting relative N-acetyl-aspartate (NAA), N-acetyl-aspartate with N-acetyl-aspartyl-glutamate (NAA + NAAG), glycerophosphocholine with phosphocholine (GPC + PCh), *myo*-inositol, glutamate (Glu), and glutamate with its precursor glutamine (Glu + Gln)), and the caudate, putamen and thalami bilaterally using two dimensional chemical shift imaging (reporting relative NAA + NAAG and GPC + PCh). Relationships between metabolite concentrations and measures of social anxiety and related symptoms were also determined. Measures of social anxiety included symptom severity, blushing propensity, and gaze anxiety/avoidance.
Results: We found, first, decreased relative glutamate concentration in the ACC of SAD and changes in *myo*-inositol with measures of social anxiety. Second, NAA metabolite concentration was increased in thalamus of SAD, and choline metabolite concentrations were related to measures of social anxiety. Lastly, choline metabolite concentration in the caudate and putamen showed changes in relation to measures of social anxiety.
Conclusion: These findings are consistent with evidence that the reward/avoidance basal ganglia circuitry, as well as the glutamatergic system, play a role in mediating SAD symptoms.

1. Introduction

Social anxiety disorder (SAD) is characterized by excessive anxiety about social interaction or performance situations, leading to avoidance and clinically significant distress (American Psychiatric Association, 2013). SAD is one of the most common anxiety disorders, with lifetime prevalence estimates of 6.1% in developed countries, and 2.1% in developing countries (Kessler et al., 2005a,b; Stein et al., 2010).

The neurobiology of SAD remains to be fully delineated; however, the reward/avoidance circuitry of the basal ganglia and the fear circuitry of the amygdala have been implicated (Argyropoulos et al., 2001; Atmaca, 2013; Caouette and Guyer, 2013). The reward/avoidance basal ganglia circuitry includes the caudate and putamen (collectively known as the striatum), the thalamus and cortical regions, such as the anterior cingulate cortex (ACC) (Caouette and Guyer., 2013). Existing literature indicates dysfunction of the basal ganglia circuitry in SAD. One study in SAD found dysfunction of dopamine in the striatum, specifically lower reuptake dopamine site density (Tiihonen et al., 1997). Neurochemical investigations have also revealed deficiencies in the GABAergic system and over-activity in glutamatergic systems within basal ganglia structures (Atmaca, 2013). Functional imaging studies in SAD have shown decreased activation of the putamen during a task that induced anticipatory anxiety (Boehme et al., 2013), and sustained activation of the dorsal cingulate cortex during video-induced symptom provocation (Boehme et al., 2014). While a recent resting state functional connectivity study in SAD has shown hyper-connectivity of resting state networks from the caudate to the ACC, the putamen to the fronto-parietal regions, and within the thalamus (Anteraper et al., 2014). Previous studies in SAD using ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) are sparse. However, one SAD study found reduced neuronal integrity and viability in basal ganglia circuitry, as indicated by reduced N-acetyl-aspartate (NAA) concentration, reduced phospholipid membrane turnover as indicated by reduced choline (Cho) concentration, reduced available

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energy as indicated by reduced creatine (Cr) concentration, and increased glial activity as indicated by increased *myo*-inositol (mI) concentration (Davidson et al., 1993). Only two ^{1}H-MRS studies have measured concentrations of glutamate (i.e. the major excitatory neurotransmitter in the brain) in SAD patients, and these found increased glutamate relative to creatine in the ACC but not in the occipital cortex (Phan et al., 2005) as well as increased glutamate:creatine for the whole brain (Pollack et al., 2008).

Thus far two ^{1}H-MRS studies in SAD have determined correlations between neurometabolite concentrations with measures of social anxiety and related symptoms. The one study found glutamate:creatine concentration in the right dorsolateral prefrontal cortex to be positively associated with increased illness severity (Yue et al., 2012). The second study found decreased creatine concentrations in the ACC was associated with intensity of fears which co-vary with SAD (Phan et al., 2005). Further investigation is therefore warranted to understand the relationship between ^{1}H-MRS findings and measures of social anxiety and related symptoms.

SAD refers to pathological anxiety and avoidance in specific or generalized social situations. These symptoms are often associated with blushing and anxiety about and/or avoidance of making eye contact. Indeed, blushing is considered one of the key symptoms of SAD (American Psychiatric Association, 2013; Gerlach et al., 2001) and is characterized by involuntary, emotionally triggered bouts of a flushed face, ears and often the neck (Hofmann et al., 2006). The tendency to experience anxiety about making eye contact—also called gaze anxiety—has also been linked to social anxiety (Hietanen et al., 2008; Horley et al., 2004). Thus it may be argued that our understanding of SAD pathophysiology may be increased by including correlational analyses between of ^{1}H-MRS findings and measures of social anxiety and related symptoms such as blushing and gaze anxiety/avoidance (Leary and Meadow, 1991; Schneier et al., 2011).

Here, we aimed to contribute to the emerging field of ^{1}H-MRS research in SAD by performing both single voxel ^{1}H-magnetic resonance spectroscopy (SVS) of the ACC and two-dimensional chemical shift imaging ^{1}H-magnetic resonance spectroscopy (2D CSI ^{1}H-MRS) of the caudate, putamen, and thalami bilaterally. We also determined correlations between ^{1}H-MRS metabolite concentrations and measures of social anxiety and related symptoms.

2. Methodology

2.1. Participants

Participants were required to meet DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th edition) criteria for a primary diagnosis of SAD. Participants who passed the telephonic screening were personally interviewed by a clinical psychologist or psychiatrist to screen for DSM-IV psychiatric disorders using the Structured Clinical Interview for Axis I Disorders (SCID-I/P, (First et al., 2002)) for inclusion. In addition, they were required to have no significant psychiatric comorbidity, and to be free of psychotropic medication. Healthy control research participants were age- and sex-matched to the patients.

2.2. Measures of social anxiety and related symptoms

Social anxiety and related symptoms were measured using the Liebowitz Social Anxiety Scale (LSAS; (Liebowitz, 1987), the Blushing Propensity Scale (BPS; (Leary and Meadow, 1991), and the Gaze Anxiety Rating Scale (GARS; (Schneier et al., 2011). The 48-item clinician-administered LSAS comprises 24 social situations that are each rated for level of anxiety and avoidance (Liebowitz, 1987). In SAD patients, the LSAS has shown good internal consistency, test–retest reliability, convergent and discriminant validity, and sensitivity to treatment (Heimberg et al., 1999). Notably, the LSAS differs from most other social anxiety measures in that it assesses level of anxiety and avoidance in specific social situations (e.g., attending or hosting parties), rather than assessing specific social anxiety symptoms (e.g., blushing in social situations or gaze anxiety). We have therefore included 2 additional measures; in particular, the BPS, a self-rating questionnaire, was administered to assess participants' frequency of blushing on a five point Likert-type rating scale in 14 different social situations (Leary and Meadow, 1991). This questionnaire has good psychometric properties, i.e. an internal consistency of 0.86 and a test–retest reliability of 0.81 over a 4-week interval (Leary and Meadow, 1991). The GARS, also a self-report measure, was administered to assess anxiety about, and avoidance of, eye contact (Schneier et al., 2011). The GARS subscales have also been found psychometrically valid (Langer et al., 2014)."

2.3. Magnetic resonance imaging

Following screening and the above assessments, participants underwent ^{1}H-MRS at the Cape Universities Brain Imaging Centre (CUBIC) at the Faculty of Medicine and Health Sciences at Stellenbosch University. Participants were scanned in a 3 T Allegra Siemens head scanner (VA25 platform) using a four-channel head coil receiver. A high-resolution multi-echo MPRAGE (van der Kouwe et al., 2008) sequence (TR = 2530 ms, graded TEs = 1.53, 3.21, 4.89, 6.57 ms, flip angle = 7°, FOV = 256 mm, slice thickness = 1.33 mm, 128 slices, scan time 8:06) was acquired for positioning of ^{1}H-MRS slice and voxel.

2.3.1. Single voxel magnetic resonance spectroscopy (SVS) of the anterior cingulate cortex (ACC)

A single voxel magnetic resonance spectroscopy (SVS) of the anterior cingulate cortex (ACC) was acquired (PRESS, TE = 30 ms, TR = 1500 ms, 128 averages, delta = −2.7 ppm delta frequency, volume of interest (VOI) 20 x 20 mm with a thickness of 10 mm, scan time 3:18). The ACC voxel was placed anterior and superior to the genu of the corpus callosum, including bilateral ACC (Fig. 1). Careful placement to avoid the anterior cerebral artery was necessary to avoid pulsation artefacts produced by this vessel.

Analysis of the SVS was performed in LCModel (Provencher, 1993), LCModel fits spectra in relation to basis spectra provided by Provencher (PRESS TE30), default parameters (ppmed = 0.2 and pmst = 3.85) were used. The relative ^{1}H-MRS SVS data are reported in relation to creatine containing metabolites (PCr + Cr). Only metabolites with a Cramér-Rao of %SD < 20% are reported. Relative metabolites reported included NAA and its metabolites (NAA, NAA + NAAG), choline metabolites (GPC + PCh), *myo*-inositol, glutamate, and glutamate with its precursor glutamine (Glu, Glu + Gln).

2.3.2. Two dimensional chemical shift imaging magnetic resonance spectroscopy (2D CSI ^{1}H-MRS) of caudate nucleus, putamen, and thalami bilaterally

A 2D CSI ^{1}H-MRS slice was acquired (PRESS, TE = 30 ms, TR = 2000 ms, Hamming filter, 2 averages, delta = −2.7 ppm delta frequency, weighted phase encoding, FOV = 160 × 200 mm, VOI = 90 × 100 mm, voxel size 10 × 8.0 mm, thickness 10 mm, automated CHESS water suppression, scan time 10:52). The ^{1}H-MRS 2D slice was first positioned with standard reconstruction of the sagittal plane. Finer positioning of the slice was achieved with the axial and coronal reconstructions. The slice position included bilateral voxels located in the caudate head, anterior putamen, and anterior thalami. Voxels were extracted for each of the six brain areas (Fig. 2).

Analysis of the CSI spectra was completed in LCModel (Provencher, 1993), settings for the CSI analysis were similar to those used for the SVS. Due to Bayesian learning in LCModel the CSI slice was analyzed by hemisphere, to exclude noise from midline ventricles. The metabolite concentrations are reported in relation to creatine containing metabolites (PCr + Cr). Only metabolites with a Cramér-Rao of %SD < 20% were included in the present study's analysis. We report NAA metabolites (N-acetylaspartate + N-acetylaspartylglutamate (NAA + NAAG))

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