Low GABA concentrations in occipital cortex and anterior cingulate cortex in medication-free, recovered depressed patients

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Abstract

Studies using proton magnetic resonance spectroscopy (1H-MRS) indicate that unmedicated, acutely depressed patients have decreased levels of γ-aminobutyric acid (GABA) in the occipital cortex. The aim of this study was to use 1H-MRS to determine if changes in occipital and frontal cortical GABA levels were present in patients with a history of depression who had recovered and were no longer taking medication. We used 1H-MRS to measure levels of GABA in both occipital cortex and anterior cingulate cortex/prefrontal cortex in medication-free, fully recovered subjects with a history of recurrent unipolar depression. Levels of GABA in both occipital and anterior cingulate cortex were significantly lower in recovered depressed subjects than healthy controls. Our data provide preliminary evidence that a history of recurrent depression is associated with decreased GABA levels in anterior cingulate cortex and occipital cortex. These changes could represent part of the neurobiological vulnerability to recurrent depressive episodes.

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Introduction

Investigations using proton magnetic resonance spectroscopy (1H-MRS) have shown decreases in γ-aminobutyric acid (GABA) concentration in the occipital cortex in unmedicated depressed patients (Sanacora et al., 1999, 2004). Treatment with selective serotonin re-uptake inhibitors and electroconvulsive therapy, but not cognitive behavioural therapy, increases cortical GABA levels in depressed subjects (Sanacora et al., 2002, 2003, 2006). It is therefore important to establish whether low cortical GABA levels in depression may be a trait marker of depression, i.e., still detectable following clinical recovery and withdrawal of antidepressant treatment.

For technical reasons MRS studies of acutely depressed patients have usually measured GABA concentration in the occipital cortex; however, it is also necessary to determine whether similar GABAergic abnormalities are present in the frontal areas more usually associated with the pathophysiology of depression (Drevets, 1998). Abnormal activity of the anterior cingulate cortex (ACC) has been implicated in functional imaging studies of depression (Drevets, 1998) and there is also evidence that this brain region is affected by neuropathological processes that could be associated with lowered GABA availability (Harrison, 2002).

The present study was a pilot investigation designed to assess the feasibility of measuring GABA levels in the occipital cortex and ACC in the same scanning session. Our hypothesis was that GABA concentrations would be decreased in both brain regions in recovered depressed subjects in comparison to healthy controls.
Methods

Subjects

The 23 subjects studied were a subgroup of a larger MRS investigation of the occipital cortex in patients with recurrent mood disorder (Bhagwagar et al., 2007) in whom it proved possible to measure GABA concentrations in both the occipital cortex and ACC in the same scanning session. Participants included 11 healthy controls (7 males, 4 females) aged 34.3 ± 4.1 yr with no psychiatric history and 12 patients (4 males, 8 females) aged 40.6 ± 4.2 yr who had experienced at least two episodes of unipolar major depression in the past (‘recovered depressed’). All patients had been medication-free and euthymic for a minimum of 6 months. Subjects were screened using the Structured Clinical Interview for DSM-IV Disorders (SCID; First et al., 1997). Subjects were rated to be recovered from depression on four criteria: self-reported recovery, the absence of clinically relevant symptoms during a clinical interview with an experienced psychiatrist, absence of criterion for a major depressive episode judged by the SCID, and a score of <7 on the 17-item Hamilton Rating Scale for Depression. All female subjects were scanned in the first half of the menstrual cycle. The study was approved by the Oxfordshire Psychiatric Research Ethics Committee. All subjects gave written informed consent for participation in the study.

MRS methodology

All subjects were scanned between 13:00 and 17:00 hours, using a 3 T Varian INOVA system (Varian Inc., Palo Alto, CA, USA), with dedicated head gradient coil (Magnex Scientific, Oxford, UK), and head-only transmit/receive quadrature birdcage RF coil. MRS data were acquired from two 30 x 30 x 20 mm voxels; one in the occipital-parietal region of the brain, and the other in the anterior cingulate region. The voxels were positioned on a T1-weighted axial image, located slightly above the corpus callosum (Figure 1). The occipital voxel was positioned to include parts of the posterior cingulate gyrus, parietal lobe and occipital lobe. This brain region offers clear landmarks for placement of the spectroscopic voxel, and is removed from major sources of static magnetic-field inhomogeneities greatly facilitating measurement of cortical GABA levels. The ACC voxel was centred on the anterior part of the cingulate sulcus, such that the voxel included parts of the anterior cingulate and the pre-frontal cortex.

Figure 1. T1-weighted image showing typical locations of (a) the frontal voxel, (b) the occipital-parietal voxel.

1H point-resolved spectroscopy sequence (PRESS; Bottomley, 1984) spectra with water suppression were acquired with the following parameters; repetition time (TR) = 3 s, echo time (TE) = 68 ms, 32 averages, 1024 complex acquisition points. The total acquisition time for each PRESS spectrum was ~1.5 min. GABA measurements were made using a MEGA-PRESS editing sequence (Mescher et al., 1998). Briefly, unwanted resonances in the PRESS spectrum are edited using the J-coupling between the GABA resonances at 3.0 ppm and 1.9 ppm. By applying a frequency-selective 180° pulse to the 1.9 ppm GABA resonance in alternate acquisitions, a modulation is introduced to the phase of the 3.0 ppm resonance. Uncoupled resonances, such as the creatine resonance at 3.0 ppm that overlap the GABA peak at the same chemical shift, are left unaffected by the frequency-selective 180° pulse, as are coupled resonances, such as glutathione, that do not have a coupling partner within the frequency range of the MEGA-PRESS pulse. Thus, subtraction of the alternate scans reveals only the components with a coupling partner within the bandwidth of the frequency-selective 180°; all other components are cancelled by the subtraction. Specifically, resonances from glutamate, glutamine, homocarnosine and the mobile brain macromolecules (MM) are present in the edited sequence (see Figure 2 for sample spectra).
The parameters used during the acquisition of edited spectra were: TR = 3 s, TE = 68 ms, 128 averages, 1024 complex data-points. Total acquisition time for each edited spectrum was \( \approx 6.5 \) min. For both PRESS and MEGA-PRESS acquisitions a 2 kHz spectral width was used, centred on 2.5 ppm. Spatial localization used Shinnar–Leroux-optimized 90° (pulse duration 2 ms, 5-lobe, 6085 ms-Hz time-bandwidth product) and 180° (3 ms pulse duration, 5-lobe, 3850 ms-Hz time-bandwidth product) pulses. The MEGA-PRESS sequence incorporated a double-banded pulse with a 20 ms Gaussian envelope for spectral editing. The GABA concentrations quoted here (total GABA) contain GABA plus homocarnosine, which also has a 3 ppm resonance edited by this MEGA-PRESS sequence. Values in the literature show the homocarnosine contribution to be quite small, of the order of 0.46 mM (Petroff et al., 2001).

Analysis was performed using the LC model (LCM; Provencher, 1993). The linewidth, signal-to-noise ratio and baseline of each spectrum were checked to ensure the robustness of the data. Eddy current correction was applied using an unsuppressed water spectrum. The PRESS LCM basis functions (NAA, NAAG, glutamate, glutamine, GABA, choline, creatine, myo-inositol, taurine, alanine, lactate, aspartate, glycerophosphocholine (GPC), phosphocholine (PCh), glycine) were derived from phantom spectra at TE = 68 ms and simulated basis spectra of macromolecules and lipids. The analysis of edited spectra used LCM basis functions (NAA, NAAG, glutamate, glutamine, GABA) that were generated from phantom measurements using the MEGA-PRESS sequence with the appropriate acquisition parameters. To ensure consistency in the basis functions, all spectra were analysed using the same spectral range (0.2–4.0 ppm), and were unfiltered. Spectra in which GABA had a Cramer–Rao lower bound greater than 20% were rejected.

GABA detection methods that rely on J-difference editing of the 3.0 ppm GABA resonance are likely to include contamination from the nearby MM resonance. To minimize this effect, basis functions were generated for the MM components visible in the edited spectra, which were then introduced into the LCM analysis. The MM basis functions were derived from in-vivo measurements in the occipital and frontal voxels of a subset of subjects in the study using the MEGA-PRESS editing sequence with an inversion-recovery pre-pulse (14 ms hyperbolic inversion pulse, TI = 800 ms, TR = 3 s with dummy scan preparation). These spectra were analysed for all visible MM peaks, resulting in a group-averaged ratio being derived for the relative intensity of the MM peaks at 3.0 and 0.9 ppm. This group-averaged value was then incorporated, with a soft constraint, as a parameter in the LCM fit. Applying an inversion-recovery at the beginning of the editing sequence can null the metabolite signal, revealing the contribution of the macromolecules to this edited spectrum due to their substantially shorter \( T_1 \) values. All metabolite concentrations were evaluated as ratios to creatine (Cr). Glutamate and glutamine were measured together as the ratio of Glx:Cr.

For subjects who were willing to remain in the scanner (controls 6, recovered depressed 9), \( T_1 \)-weighted structural images were acquired with

![Figure 2](image-url)
The ANCOVA of the Glx:Cr ratio showed no significant main effect of group or region; however, there was a significant interaction between group and region ($F=6.95$, $d.f.=1, 19$, $p=0.016$). In the occipital cortex the Glx:Cr ratios in recovered patients were significantly higher than those of controls (mean $\pm$ s.e.m.) (1.70 $\pm$ 0.07 vs. 1.44 $\pm$ 0.06, $p=0.01$). In the ACC, however, Glx:Cr ratios were non-significantly lower in patients (1.44 $\pm$ 0.10 vs. 1.62 $\pm$ 0.06, $p=0.16$). Similarly NAA:Cr ratios showed no significant main effect of group or region but there was a significant interaction ($F=6.88$, $d.f.=1, 19$, $p=0.017$). In the ACC there was no difference in NAA:Cr ratios between patients and controls (1.39 $\pm$ 0.05 vs. 1.36 $\pm$ 0.03, $p=0.53$) while in the occipital cortex there was a trend to reduction in patients (1.54 $\pm$ 0.67 vs. 1.70 $\pm$ 0.04, $p=0.068$). Glx:Cr ratios correlated negatively with age in the ACC ($r=-0.54, p=0.009$) but not the occipital cortex (0.12, $p=0.57$). There was no significant correlation between ACC and occipital values of Glx:Cr or NAA:Cr ratios. In the ACC there was a significant positive correlation between GABA:Cr and Glx:Cr ratios ($r=0.71, p=0.001$) but this was not apparent in the occipital cortex ($r=-0.37, p=0.075$).

Discussion

We provide preliminary evidence that GABA:Cr ratios are decreased in both occipital cortex and ACC in recovered unmedicated subjects with a history of recurrent depression. The reductions we found ($\sim 10\%$) were substantially less than those described by Sanacora and colleagues who, using $^1$H-MRS, found decreases in occipital GABA levels of $\sim 50\%$, in acutely ill unmedicated patients with melancholic depression (Sanacora et al., 1999). Depressed patients not meeting criteria for melancholic depression had smaller reductions of $\sim 20\%$ in occipital GABA (Sanacora et al., 2004). This raises the possibility that clinical recovery from depression is associated with some restoration of occipital GABA levels towards normal, although a detectable deficiency apparently remains.

Why GABA levels should be decreased in recovered depressed subjects requires further investigation. However, there is increasing evidence of neuropathological changes in cortical tissue in patients with recurrent affective illness and reports of decreased glial cell numbers in ACC might be relevant to our findings (Harrison, 2002; Ongur et al., 1998). Glial cells, notably

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**Figure 3.** Mean (s.e.m.) GABA:Cr ratios in the anterior cingulate cortex (ACC) and occipital cortex (OC) in recovered depressed subjects (■, $n=12$) and healthy controls (□, $n=11$). Main effect of group: $F=4.46, d.f.=1, 20$, $p=0.048$. Resolutions 1$\times$1$\times$1 mm and segmented with FSL FAST to calculate the grey matter fraction within the PRESS volume (Zhang et al., 2001). In this subset of subjects grey matter partial volumes did not differ between patients and controls.

**Statistical methods**

The GABA:Cr data were analysed with a two-way repeated measures analysis of covariance (ANCOVA) with ‘region’ (ACC vs. occipital cortex) as the main within-subject factor while ‘group’ (recovered depressed vs. control) was the main between-group factor (SPSS version 12; SPSS Inc., Chicago, IL, USA). The Glx:Cr and NAA:Cr ratios were similarly analysed. Unpaired $t$ tests were used as post-hoc tests. Age was a significant correlate of the GABA:Cr and Glx:Cr ratios and was added to the analyses as a covariate. There were no main or interactive effects of gender, which was therefore omitted from the analyses. Correlations were carried out using Pearson’s correlation coefficient.

**Results**

There was no significant difference between the groups in age ($t=1.07, p=0.29$), or gender ($\chi^2 p=0.3$). The ANCOVA of the GABA:Cr ratio showed a main effect of group ($F=4.46, d.f.=1, 20$, $p=0.048$). There was no main effect of brain region ($F=1.01, d.f.=1, 20$, $p=0.33$) or group $\times$ region interaction ($F=0.09, d.f.=1, 20$, $p=0.77$). The reduction in GABA:Cr values in the occipital cortex (10%) and ACC (11%) were very similar (Figure 3). GABA:Cr ratios in the occipital cortex correlated with those in the ACC ($r=0.46, p=0.027$). Age correlated inversely with the GABA:Cr ratio in both the occipital cortex ($r=-0.55, p=0.007$) and ACC ($r=-0.58, p=0.004$).
astrocytes, are an important source of the GABA precursor, glutamine, to GABA nerve terminals (Shulman et al., 2004) and it is therefore possible that impairment of glial cell activity could lead to diminished GABA synthesis and low tissue GABA levels (Sanacora et al., 2004).

Another purpose of our study was to assess whether changes in GABA levels in recovered depressed subjects might also be present in frontal cortical areas. Previous MRS work in acute depression has focused on the occipital cortex because placing the spectroscopic voxel in this brain region decreases interference from static magnetic field inhomogeneities, thereby facilitating GABA measures. Our findings show that recovered depressed patients appear to show similar decreases in GABA levels in both the ACC and occipital cortex. We also found a significant correlation between GABA levels in the two brain regions.

Against this, Hasler and colleagues (2005) have recently reported normal GABA levels in two regions of pre-frontal cortex in a larger sample of subjects remitted from major depression. The reason for this discrepancy requires further study but it could reflect differences in patient population. For example, some of the patients in the latter study had experienced only a single episode of depression, albeit in association with a positive family history. In addition the location of the frontal voxel in our study was centred on the ACC rather than prefrontal cortex. However, because of the size of the MRS voxel there would have been substantial overlap between the tissue we sampled and that reported by Hasler et al. (2005).

Interestingly in a subsequent MRS study in acutely depressed patients, Hasler et al. (2007) found decreased levels of both GABA and Glx in the prefrontal cortex. In both the present study and the larger sample reported elsewhere (Bhagwagar et al., 2007) we found increased levels of Glx in occipital cortex in recovered depressed patients; however, this change is apparently not present in the ACC. Furthermore, Glx and GABA levels showed a positive correlation in the ACC but not occipital cortex. The former finding, which was also reported by Hasler et al. (2007), suggests that the coupling between GABA and Glx synthesis can show striking regional variation. Despite this, our findings suggest that GABA levels are decreased in both ACC and occipital cortex. In depressed patients GABA levels are also reportedly decreased in plasma and cerebrospinal fluid (see Brambilla et al., 2003). This raises the possibility that low cortical levels of GABA could be related to a more general defect in GABA synthesis.

Methodological limitations of our study must also be acknowledged. The number of patients and controls studied was small, because it was difficult for the majority of subjects to remain in the scanner for sufficient time to allow acquisition of adequate spectra for both brain regions. In addition, our GABA measures have been referenced to creatine. While this is a common methodology in MRS studies (Coupland et al., 2005), it leaves open the possibility that changes in creatine concentrations between patient and control groups could account for our findings. Finally, particularly in the ACC, there is a relatively low signal-to-noise ratio of GABA spectra which is likely to affect the reliability of our data.

Further work is needed to establish whether decreased cortical GABA levels may be present in recovered patients with a history of recurrent depression and if so what such a deficit may mean in terms of future risk of illness and response to maintenance treatments.

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Statement of Interest

None.

References


