Psychiatry Research: Neuroimaging 315 (2021) 111327

Contents lists available at ScienceDirect

Psychiatry Research: Neuroimaging

journal homepage: www.elsevier.com/locate/psychresns

Inhibitory and excitatory neurotransmitter systems in depressed and healthy: A positron emission tomography and magnetic resonance spectroscopy study

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ARTICLE INFO

Keywords:
GABA
Glutamate
PET
Magnetic resonance spectroscopy
Depression

ABSTRACT

The Gamma-aminobutyric acid (GABA) and glutamate (Glu) neurotransmitter systems are implicated in depression. While previous studies found reduced GABA levels, and a tendency towards reduced Glu, using proton (^{1}H) magnetic resonance spectroscopy (^{1}H-MRS), little is known about $GABA_A$ receptor availability in depression. Here, the aim was to characterize GABA and Glu-levels in dorsal anterior cingulate cortex (dACC), whole-brain $GABA_A$ availability, and their relationship in patients with depression compared to healthy controls. Forty-two patients and 45 controls underwent ^{1}H-MRS using a MEGA-PRESS sequence to quantify dACC GABA+ and Glu (contrasted against creatine [Cr]). Immediately preceding the ^{1}H-MRS, a subsample of 28 patients and 15 controls underwent positron emission tomography (PET) with [^{11}C]Flumazenil to assess whole-brain $GABA_A$ receptor availability. There were no differences in dACC GABA+/Cr or Glu/Cr ratios between patients and controls. The same was true for whole-brain $GABA_A$ receptor availability. However, there was a significant negative relationship between GABA+/Cr ratio and receptor availability in ACC, in a whole-brain voxel-wise analysis across patients and controls, controlling for group or depressive symptoms. This relatively large study did not support the GABA-deficit hypothesis in depression, but shed light on GABA-system functioning, suggesting a balance between neurotransmitter concentration and receptor availability in dACC.

1. Introduction

1.1. Gamma-aminobutyric acid in depression

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system, binding postsynaptically to the ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors. GABA has long been implicated in major depression, with early studies showing a reduction in GABA in plasma (Petty et al., 1990) and cerebrospinal fluid (CSF) (Gerner et al., 1984; Gold et al., 1980; Kasa et al., 1982). Further, post mortem studies found a reduction in calbindin immunoreactive GABA-ergic neurons as well as glutamic acid carboxylase (GAD)−67, a GABA synthesizing enzyme, in the dorsolateral prefrontal cortex (dlPFC) in depression (Karolewicz et al., 2010; Rajkowska et al., 2007). Animal chronic mild stress models of depression have shown impairments in GABA synthesis and uptake, GABA-ergic innervation, GABA neuron density and network functioning in the medial prefrontal cortex (Czéh et al., 2018; Ma et al., 2016). Together, these findings suggest both pre- and postsynaptic GABA alterations in depression.

More recently, methodological advancements in proton (^{1}H) magnetic resonance spectroscopy (^{1}H-MRS) has allowed for more direct assessment of GABA concentrations in the central nervous system (CNS) in vivo (Mullins et al., 2014; Terpstra et al., 2002; van Veenendaal et al., 2018). Several studies to date have used MRS to measure GABA in major depression and a recent meta-analysis reported decreased GABA compared to healthy controls, though findings were heterogeneous

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https://doi.org/10.1016/j.pscychresns.2021.111327
Received 8 December 2020; Received in revised form 8 June 2021; Accepted 29 June 2021
Available online 1 July 2021

(Godfrey et al., 2018). A meta-analysis considering occipital GABA only, however, did not find any difference (Truong et al., 2021). Given the central role of the anterior cingulate cortex (ACC) in depression, with commonly observed hyperactivity that may be attributable to GABA-ergic deficits (Luscher et al., 2011), some MRS studies have focused on GABA in the dorsal ACC (dACC) and found reduced GABA in depression (Godfrey et al., 2018).

In contrast, only a few studies considered $GABA_A$ receptor availability in depression. One positron emission tomography (PET) study measured $GABA_A$ availability in depression using [^{11}C]Flumazenil as a tracer and found reduced binding in bilateral temporal cortices compared to controls (Klumpers et al., 2010). Using single-photon emission tomography (SPECT), with the selective benzodiazepine antagonist [^{123}I]Iomazenil as a radiotracer, widespread increase in [^{123}I]Iomazenil binding following electro-convulsive therapy (ECT) (Mervaala et al., 2001) has been reported. However, using the same method, no difference in $GABA_A$ density compared to controls (Kugaya et al., 2003) was observed. This latter study also considered occipital GABA levels using MRS, and while they found reduced GABA, no relationship with $GABA_A$ availability was observed.

Regardless of the study population, GABA studies reporting findings from MRS and PET in the same subjects are scarce, meaning that the in vivo relationship between GABA and $GABA_A$ receptor availability is elusive. One study performed [^{11}C]Flumazenil PET and GABA MRS in the occipital cortex and frontal eye field (FEF) close in time, in 13 healthy controls and 14 patients with neurofibromatosis. In patients, they found a negative relationship between GABA concentration and receptor availability with no relationship in controls (Violante et al., 2016).

1.2. Glutamate in depression

The observation that ketamine, an N-Methyl-D-Aspartate (NMDA) receptor antagonist, can lead to rapid relief of depressive symptoms, suggests the involvement of glutamate (Glu) in major depression as well (Berman et al., 2000). A meta-analysis considering Glu-concentrations from MRS studies found no overall difference in patients with depression compared to controls, but a trend towards reduced Glu-in the anterior cingulate cortex (ACC) (Godfrey et al., 2018). Similarly, another recent meta-analysis of MRS studies found a decrease in the glutamate + glutamine complex in depression within the medial frontal cortex (Moriguchi et al., 2019). Again, no difference was observed considering the occipital cortex specifically (Truong et al., 2021).

1.3. GABA-glutamate balance in depression

The GABA and Glu-systems are inherently interdependent, given that GABA is synthesized from Glu-by the two isoforms of GAD, GAD-65 and GAD-67. Both GABA and Glu-are taken up by glia cells where they are transformed to glutamine, the precursor of glutamate, thus completing the cycle (Petroff, 2002). Given this interdependence, the ratio between GABA and Glu-estimates may be more sensitive to pathological changes than either measure alone, especially since GAD-67 is decreased in depression (Karolewicz et al., 2010). Indeed, an excitatory-inhibitory imbalance has been suggested as a mechanism of depression (Page and Coutellier, 2019). In bipolar depression a reduced Glu/GABA ratio in the dorsal ACC (dACC) has been observed compared to controls, which was affected by mood stabilizers (Scotti-Muzzi et al., 2020). In healthy controls, dACC Glu/GABA ratio has been related to resting-state connectivity in the salience and default mode networks (Levar et al., 2019) suggesting that this ratio captures also regional brain functioning.

To summarize, both the GABA and Glu-transmitter systems have been implicated in depression, especially within the dACC. While evidence point to reduced GABA, only a handful of studies with small samples have considered $GABA_A$ receptor availability and its relationship to prefrontal GABA levels, in depression and overall. Likewise, the relationship between GABA-ergic measures and Glu-levels in depression is largely unknown.

1.4. Aims

Here, the aim is to assess differences in GABA and Glu-levels, as measured with MRS, and their ratio in the dACC, and differences in whole-brain voxel-wise $GABA_A$ receptor availability, measured with [^{11}C]Flumazenil PET, between patients with depression and healthy controls. Further we aim to assess the relationship between dACC GABA levels and whole-brain voxel-wise $GABA_A$ receptor availability.

2. Materials and methods

2.1. Participants

Forty-two patients with an ongoing depressive episode were recruited from the psychiatric outpatient clinic at Uppsala University Hospital and 45 healthy volunteers were recruited through advertising. The inclusion criteria for the patients comprised: being 18–59 years old, having a uni- or bipolar depression diagnosis with an ongoing depressive episode verified through Mini International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al., 1998), ≤40 points on The Motivation and Pleasure Scale-Self-Report (Llerena et al., 2013), unchanged psychiatric pharmacotherapy the past month. The exclusion criteria were: epilepsy, magnetic and other metal implants, active substance use disorder (except for nicotine and caffeine), benzodiazepine treatment, and pregnancy. To be included in the study, the healthy volunteers were screened negative for psychotic or affective disorders with the M.I.N.I. All participants provided written informed consent for study participation in accordance with the Declaration of Helsinki. The study was approved by the Swedish Ethical Review Authority.

All participants underwent magnetic resonance imaging (MRI), including MRS to quantify GABA and glutamate concentrations. Due to technical issues, the MRS scan was not performed in one patient, leaving 41 patients with MRS scans. Twenty-eight of the patients and 15 of the healthy volunteers additionally underwent [^{11}C]Flumazenil PET just before the MRI visit to assess $GABA_A$ receptor availability. Depressive symptom severity was assessed using the Montgomery Åsberg Depression Rating Scale (MADRS-S) (Montgomery and Åsberg, 1979).

2.2. Magnetic resonance imaging and spectroscopy

Measurements were performed on a 3 Tesla scanner (Achieva dStream, Philips Healthcare, Best, The Netherlands), using a 32-channel head coil. T_1-weighted images were collected using a 3D T_1-weighted turbo FFE sequence with TR/TE=8.2/3.8 ms, flip angle = 8°, field of view = 256 × 256 mm^2, spatial resolution = 1 × 1 × 1 mm^3. MRS was performed using a J-difference Mescher-Garwood spectral editing technique implemented in a point resolved spectroscopy sequence (MEGA-PRESS) (Mescher et al., 1998). The following main acquisition parameters were applied: TR/TE 2000/68 ms, spectral bandwidth 2000 Hz, 1024 time domain complex points, and phase cycling 4. A total of 160 pairs of ON and OFF spectra were collected in 40 groups. Each group contained one unsuppressed water line followed by four pairs of water suppressed ON—OFF spectra. The water lines served for static magnetic field drift correction and for updating the carrier frequency of RF pulses in each group. The spectroscopic volume of interest (voxel) size was 40 × 40 × 20 mm^3 in the left-right, anterior-posterior and feet-head directions, respectively. The voxel was positioned medially over the bilateral cingulate gyrus, with its anterior-posterior axis aligned with the main axis of the cingulate gyrus, its anterior border located in the plane intersecting with the most anterior part of the genu of the corpus callosum, and its inferior border coinciding with the superior border of the corpus callosum. Fig. 1 illustrates the mean voxel placement and distribution in Montreal Neurological Institute (MNI) space.

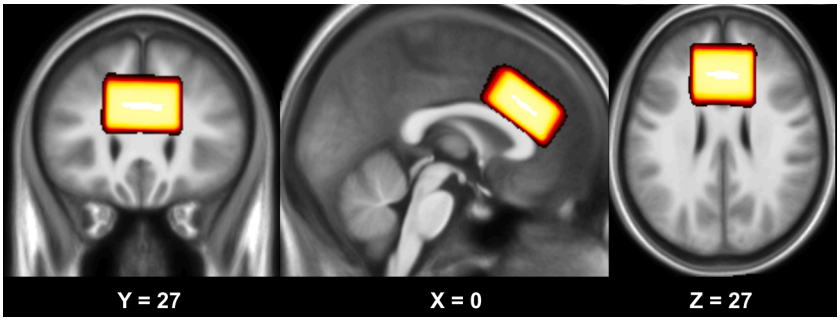

Fig. 1. Average placement of the magnetic resonance spectroscopy (MRS) voxel. The individual voxel masks were normalized to Montreal Neurological Institute (MNI) space and averaged. The resulting image was thresholded at 10% overlap and overlaid on the group average T_1-weighted image.

The MEGA-PRESS spectra were processed using Gannet 3.0 in MATLAB R2020a (Edden et al., 2014). Gannet was used to estimate GABA+ to total creatine (Cr) spectral intensity ratio. It should be noted that signals of various macromolecules (MM) overlap with GABA resonances at 3 ppm (Mikkelsen et al., 2017). The resulting GABA peak is therefore referred to as GABA+ to indicate the summation of MM signals with GABA.

The processing included frequency and phase corrections, 3 Hz Gaussian line broadening, zero-filling, and rejection of distorted spectra in a pairwise (ON—OFF) manner. GABA+ resonances at 3 ppm were fitted by a single Gaussian. The fitting error was defined as the percentage ratio of standard deviation of the residue to the amplitude of the GABA+ peak. Cr was fitted in the OFF spectrum by Lorenzian peak shape. The individual difference spectra after phase and frequency correction were visually inspected (see Supplementary Figure 1).

Since Gannet is unable to quantify Glu, the following approach was used for Glu-estimation. Java-based graphical user interface (jMRUI) software package (Naressi et al., 2001) was used for selection and averaging of the OFF free induction decays (FIDs). The resulting FID was then exported as a text file and converted by an in house program to the format required by the LCModel spectrum processing software (Provencher, 1993). Glu/Cr concentration ratio was then quantified by LCModel. No apodization was used in the spectrum preprocessing. The LCModel algorithm provides the standard errors estimate called Cramér-Rao lower bound (CRLB) expressed in percent of the estimated concentration.

2.3. Positron emission tomography

The PET scans were performed on a Discovery MI PET/computed tomography (CT) system (GE Healthcare, Waukesha, WI). First, a CT attenuation correction scan (120 kV, 10–20 mA, noise index 170) was obtained whereupon [^{11}C]Flumazenil (2–4 MBq/kg body weight) was injected and a PET acquisition (4 × 15 s, 4 × 60 s, 2 × 150 s, 2 × 300 s, 2 × 600 s, totaling 40 min) was started simultaneously with the tracer injection. Images were reconstructed using time-of-flight ordered subsets expectation maximization with 3 iterations and 34 subsets, including resolution recovery, and a 3-mm Gaussian post-processing filter. [^{11}C]Flumazenil was synthetized as previously described (Eng et al., 2010).

2.4. PET image processing

All dynamic PET images were corrected for within scan movement utilizing VOIager 4.0.7 (GE Healthcare) software. The T_1-weighted MRI images were co-registered to a summation over the first 5-min of the PET scan using Statistical Parametric Mapping 8 (SPM8; Wellcome Trust centre for Neuroimaging Institute of Neurology, University College of London, UK) and segmented into gray and white matter. Parametric images of non-displaceable binding potential (BP_{ND}) were generated using a basis function implementation of the simplified reference tissue method (SRTM), (Gunn et al., 1997; Lammertsma and Hume, 1996) with centrum semiovale white matter as reference region. (Klumpers et al., 2008). The reference region was obtained by global erosion of the two outer layers of voxels in subject space from the white matter segmented image including only voxels in the cranial half of the images. The average volume of the resulting reference region across the sample was 4.4 ± 1.4 cm^3. This region was projected over all frames for the dynamic PET scan to obtain the reference tissue time-activity curve.

2.5. Registration to standard space

The T1-weighted images were simultaneously segmented and registered to MNI space using the unified segmentation algorithm in SPM12 in MATLAB R2020a. The resulting deformation fields were applied to the T1-weighted images and non-displaceable binding potential (BP_{ND}) images after co-registration to the T1-weighted images, in order to bring them into MNI space, resampling them to 2 × 2 × 2 mm^3 voxels. The BP_{ND} images were then smoothed with a kernel with full-width at half maximum (FWHM) of 8 × 8 × 8 mm^3. Additionally, individual mask images representing the MRS voxel were defined and used to extract average BP_{ND} from coregistered PET data in subject space as well as normalized to MNI space for visualization of voxel placement.

2.6. Statistical analysis

Group differences in demographical variables and MRS derived measures were assessed using two-sample t-tests in JASP 0.11.1, with a p-value less than 0.05 considered significant. To assess group differences in whole-brain voxel-wise BP_{ND}, the smoothed images were entered into a voxel-wise General Linear Model (GLM) defining groups as separate regressors. T-contrasts were defined to assess voxels where BP_{ND} differed significantly between patients and healthy controls. To assess voxels where BP_{ND} was related to dACC GABA levels, BP_{ND} images were entered into GLMs with individual GABA level as group specific regressors. Contrasts were entered to assess voxels with a significant relationship across the whole sample, as well as voxels exhibiting group differences in this relationship. In all whole-brain voxel-wise analyses, voxels were considered significant at a cluster extent threshold of $p_{FWE} < 0.05$, using a cluster forming threshold of $p < .001$, uncorrected.

3. Results

There was no difference between patients and healthy controls in age or gender distribution (Table 1). Among the patients, 11 were on an SSRI, 19 on an SNRI, 15 on another antidepressant, 7 on lithium, 5 on a mood-stabilizing antiepileptic, and 8 on an antipsychotics, where one patient could have more than one type of medication. Five patients were unmedicated.

MRS results are shown in Table 2. Due to a poor fit, the GABA

Table 1
Demographical variables for patients with depression (MDD) and healthy controls (HC).

	Whole sample MDD (n = 42)	HC (n = 45)	PET sample MDD (n = 28)	HC (n = 15)
Age (yrs), mean (sd)	29.2 (9.4)	29.5 (11.2)	29.2 (9.8)	32.9 (12.8)
Range (yrs)	18–54	18–58	18–54	21–57
Gender, male/female	21/21	18/27	15/13	6/9
MADRS-S, mean (sd)	29.6 (7.7)	3.3 (2.8)	29.5 (8.2)	2.6 (1.9)

estimate from one healthy control was excluded (fitting error > 10%), and the Glu-estimates from one healthy control and one patient were excluded (fitting error > 20%) from analyses. The groups did not differ in the concentration of either GABA+, Glu, or their ratio. There was a tendency towards greater fit error of the GABA+ and Cr estimates from Gannet in patients than controls. However, the GABA+ estimate did not vary systematically with fit error for GABA+ (r=−0.06, p = .589) or tCr (r = 0.15, p = .183). There were no group differences in gray matter, white matter, gray to white matter ratio or cerebrospinal fluid fraction within the MRS voxel. Neither GABA+ nor Glu-estimates correlated with gray to white matter ratio (r = 0.002, p= .989 and r = 0.09, p = .588, respectively).

In the PET subsample, there was no difference between groups in BP_{ND} in a whole-brain voxel-wise analysis, see Fig. 2 for mean BP_{ND} for patients and controls. Considering patients and controls together, a significant negative relationship between the GABA+ concentration (GABA+/Cr) in the MRS voxel and BP_{ND} in bilateral dACC was observed in a whole-brain analysis, F(1,39) = 27.67, p_{FWE} = 0.020, cluster extent = 362 voxels, x = 10, y = 52, z = 10, see Fig. 3. This relationship remained after controlling for group (F[1,38] = 26.57, p_{FWE} = 0.034, cluster extent = 312 voxels) or MADRS-S scores (F[1,38] = 27.81, p_{FWE} = 0.029, cluster extent = 326 voxels) and did not differ between patients and healthy controls. However, no relationship was observed when considering controls or patients separately.

To exclude the possibility that the significant results were influenced by the inclusion of two patients with bipolar depression or one patient with greater BP_{ND} (>100, see Fig. 3), the above analysis was repeated while covarying out these three patients, with the results remaining largely unchanged (F(1,38) = 20.71, p_{FWE} = 0.025, cluster extent = 337 voxels, x = 10, y = 52, z = 10).

In addition to a whole-brain voxel-wise analysis, average BP_{ND} within the MEGA-PRESS voxel was correlated against GABA+. No significant relationship was found for patients (r=−0.24, p = .238), healthy controls (r=−0.32, p=.271) or the whole sample (r=−0.26, p = .100).

Additional analyses were performed in patients, investigating the relationship between the GABA and Glu-measurements and depressive symptoms. No correlation was found between MADRS-S scores and GABA+ (r = 0.04, p = .804), Glu (r=−0.15, p = .361), or GABA+/Glu-ratio (r = 0.16, p = .324). Similarly, no significant clusters were found when regressing MADRS-S scores against voxel-wise BP_{ND}.

To assess the effect of medication on GABA+, Glu, and BP_{ND}, regression analyses were run with dummy variables encoding the presence of different groups of medication within the patient group. The only significant effect was lower GABA+ in patients on antiepileptics.

Table 2
Comparison of magnetic resonance spectroscopy (MRS) measures of patients with depression (MDD) and healthy controls (HC).

	MDD	HC	t	p	Hedge's g	95% CI (Hedge's g) Lower	Upper
Neurotransmitter levels							
GABA+/Cr (a.u.)	.101 (0.01)	.099 (0.01)	−0.61	.547	−0.13	−0.56	.30
Glu/Cr (a.u.)	.714 (0.07)	.714 (0.07)	−0.04	.965	−0.009	−0.44	.42
GABA+/Glu (a.u.)	.142 (0.02)	.139 (0.02)	−0.56	.578	−0.12	−0.55	.31
tissue volume fractions in mrs voxel							
gray matter fraction (%)	42 (4)	41 (3)	−1.07	.287	−0.23	−0.66	.20
White matter fraction (%)	49 (4)	50 (3)	1.31	.193	.28	−0.15	.71
csf fraction (%)	08 (2)	08 (2)	−0.49	.627	−0.11	−0.53	.32
gray- to white matter ratio	.86 (0.12)	.83 (0.12)	−1.24	.219	−27	−0.69	.16
Quality indicators							
GABA+ fitting error (Gannet) (%)	4.58 (1.4)	4.16 (0.9)	−1.72	.089	−0.37	−0.80	.06
Cr fitting error (Gannet) (%)	1.47 (0.2)	1.40 (0.2)	−1.97	.052	−0.42	−0.85	.008
Glu (CRLB) (%)	7.75 (1.6)	7.75 (1.3)	0	1	0	−0.43	.43
Cr (CRLB) (%)	1.90 (0.3)	1.96 (0.2)	.963	.338	.21	−0.22	.64

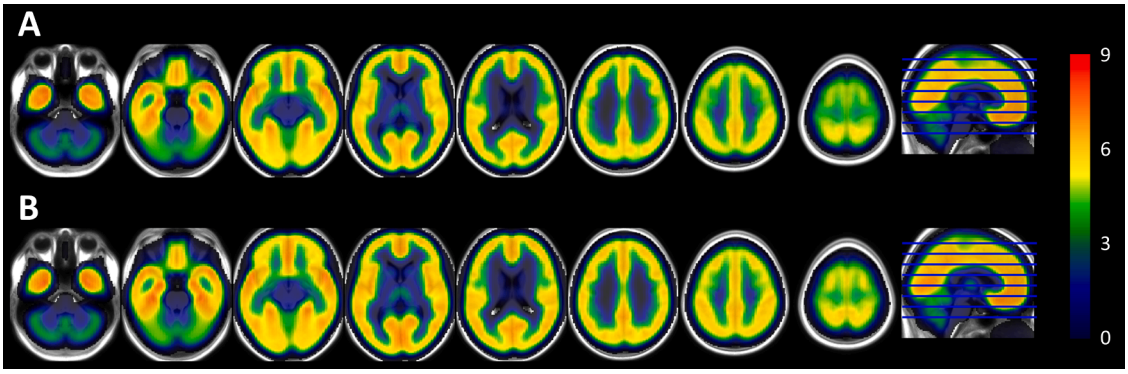

Fig. 2. Axial slices showing mean non-displaceable binding potential (BP_{ND}) for A) healthy controls and B) patients with depression.

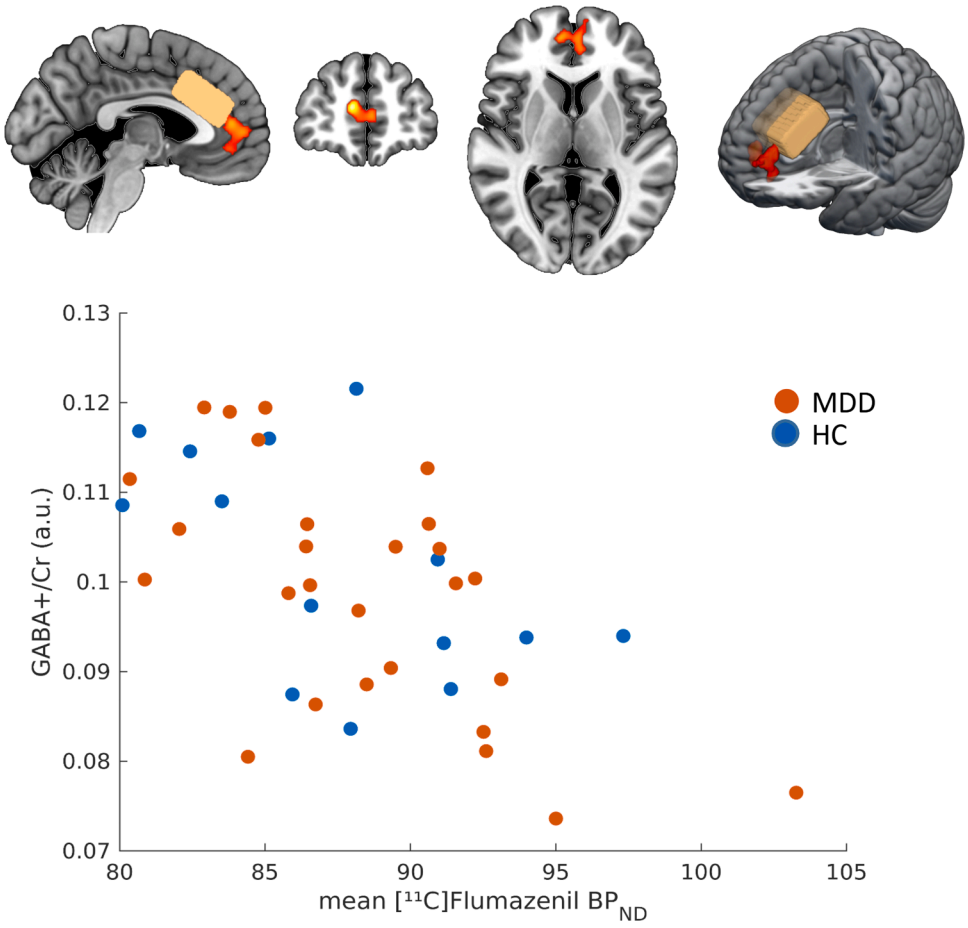

Fig. 3. Graphical representation of the negative relationship between GABA+/Cr spectral intensity ratio in the dorsal anterior cingulate cortex (dACC) and non-displaceable binding potential (BP_{ND}). Top slices show the location of the significant cluster in overlayed on an anatomical image and in relation to the magnetic resonance spectroscopy (MRS) voxel. Results were corrected for multiple comparisons using a cluster-wise threshold of $p_{FWE}<0.05$ with a cluster forming threshold of $p<.001$, uncorrected. Bottom panel shows a scatter plot based on the average BP_{ND} within the significant cluster, for illustrative purposes only. A.u. = arbitrary units.

4. Discussion

In this first study combining GABA+ in the ACC and [^{11}C]Flumazenil binding in patients with depression and healthy controls we show a negative relationship between GABA+/Cr ratio in the dACC and $GABA_A$ receptor availability in a nearby ACC region, across participants. This relationship suggests a regional balance between the transmitter and receptor of the GABA system in the brain, possibly reflecting a homeostatic regulation.

A previous study found a negative relationship between plasma GABA and [^{11}C]Flumazenil binding in the right insula in depression (Klumpers et al., 2010). While plasma GABA and GABA concentration as measured by MRS are not equivalent, their findings similarly suggest that more available GABA is associated with less $GABA_A$ receptor availability in the brain.

We do not replicate earlier findings of decreased GABA+ in the dACC (Godfrey et al., 2018). However, earlier findings are heterogeneous, with only four out of seven studies in the recent meta-analysis reporting a significant finding in the dACC (Godfrey et al., 2018). One possible explanation is that we included patients receiving antidepressant medication, whereas previous positive findings were reported on medication free samples (Gabbay et al., 2013, 2012; Price et al., 2009; Wang et al., 2016). Further, our sample comprise patients with treatment resistant depression (TRD) and pronounced anhedonia. One earlier study found decreased GABA+ specific to patients with TRD (Price et al., 2009), while another failed to find decreased GABA+ in patients with pronounced anhedonia (Walter et al., 2009). It is also worth noting that studies reporting positive findings have assessed specific subgroups, such as adolescents (Gabbay et al., 2013, 2012) or postmenopausal women (Wang et al., 2016), who may not be generalizable to the population studied here.

Similarly, we do not find any group difference in $GABA_A$ receptor availability, which is in line with Kugaya et al., 2003 but at odds with Klumpers et al., 2010 who observed reduced binding potential in the temporal cortices, although they applied a more liberal statistical threshold ($p < .001$, uncorrected) which may account for this difference.

We used an algorithm to delineate the centrum semiovale with erosion of segmented white matter images which is operator independent and minimizes the spillover of signal from tracer in the gray matter. Klumpers et al., 2008 (Klumpers et al., 2008) reported that both pons and centrum semiovale can be used for SRTM, and that pons performed slightly better as reference region in some deep gray matter structures, i, e, thalamus and putamen. However, in our experience, the advantages of an automatic delineation of the reference region is superior to the potential disadvantages.

Again, there are also differences in the population considered. Klumpers et al., studied medication-free patients with a sample that was older and less treatment naïve compared to the present study. The latter may suggest that reduced $GABA_A$-receptor availability is observed only early in the disease progression.

Neither did we find a difference in Glu-levels between patients and controls. This is largely in agreement with the literature (Godfrey et al., 2018), where reductions have mainly been found when considering the glutamate + glutamine complex (Moriguchi et al., 2019).

Overall, our findings lend little support to the GABA deficit hypothesis (Luscher et al., 2011) in a sample of medicated patients with treatment resistant depression, though the neuroimaging techniques employed here may not be sensitive enough to detect more subtle group differences. Further, the effects of patient subgroup, decease progression and medication status need to be disentangled in future studies.

4.1. Limitations

The present sample size is among the larger MRS studies to consider Glu-and GABA in depression and the largest to date to measure GABA+ levels in the ACC, to our best knowledge, making the failure to replicate earlier findings unlikely to be a power issue. However, ^{1}H-MRS is only able to capture total GABA+ concentration within a predefined and relatively large volume of the brain. This signal is influenced by both intra- and extracellular GABA, while some post mortem studies point to somatostatin expressing GABA-ergic interneurons being specifically affected in depression (reviewed in (Prévot and Sibille, 2020)), suggesting that in vivo MRS may have low sensitivity to detect more subtle deviations in the GABA system. Furthermore, the MEGA-PRESS sequence used here does not enable measuring MM-free GABA and recent studies showed that approximately 50% of GABA+ intensity originates from MM (Mikkelsen et al., 2017), further reducing the sensitivity to detect specific changes in GABA concentration in a heterogeneous sample. To our knowledge, there is no study which have quantified MEGA-PRESS MM signal contribution to GABA at 3 ppm for different age groups and psychiatric populations.

While the use of Cr as a reference when estimating GABA+ is well established, this assumes that Cr levels are relatively stable. However, findings of a relationship between prefrontal Cr and depression symptomatology (Faulkner et al., 2021; Kondo et al., 2016) raises the concern that GABA+ differences compared to controls may be canceled out with this reference. The fact that we do not find any difference in Cr levels compared to healthy controls, in line with previous findings (Auer et al., 2000), ameliorates this concern though.

While a difference in gray matter to while matter volume ratios within the MEGA-PRESS voxel could potentially mask a group difference, given that GABA levels are higher in gray matter, this is unlikely, given that there was no group difference in gray to white matter ratio within the voxel and this ratio did not correlate with GABA+ or Glu-estimates.

The relationship between GABA+ and $GABA_A$ reported here only holds for the whole sample, but not in healthy controls or patients separately. This leaves open the concern that the correlation is driven by group differences. However, the groups are largely overlapping as seen in Fig. 3, and the correlation is of similar strength in both groups. Importantly, the results remain when controlling for group (controls vs patients), suggesting that the lack of a significant relationship in the groups separately is likely a power issue.

One possible concern is that increased GABA levels could influence GABA-A receptor affinity and thereby [^{11}C]Flumazenil BP_{ND} as previously shown (Frankle et al., 2015). However, even if this effect could be detected at the level of endogenous fluctuations, this would predict a positive relationship between GABA+ and BP_{ND} rather than the negative relationship reported here.

We aimed to minimize the effect of medication on the results by excluding participants on GABAergic agents. Further we assessed the effect of medication in additional analyses, indicating no effect of type of medication with the possible exception of antiepileptics on GABA+ concentration. However, the fact that most patients received some form of antidepressant makes it difficult to exclude their effect on the findings.

4.2. Conclusion

We report for the first time on a relationship in the dACC between GABA+ concentration and $GABA_A$ receptor availability, in patients with depression and healthy, suggesting a regional balance between transmitter levels and receptor density. In this study comprising one of the largest samples of patients with depression we could not replicate earlier findings of GABA-ergic and glutamatergic deficits observed in small selected subgroups of depression.

Funding

This research was supported by unrestricted grants from the Swedish Research Council (Grant number 2016–02,362), the Swedish Society of Medicine, and Märta and Nicke Nasvell Foundation. RB was supported by a Gullstrand Research Fellow grant from Uppsala University Hospital and JP was supported by a postdoctoral grant from the Swedish Brain Foundation. The sponsors had no involvement in study design, data collection, analysis or interpretation of data.

Declaration of Competing Interest

The authors declare no competing interests.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.pscychresns.2021.111327.

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