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

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\textbf{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 (\textsuperscript{1}H) magnetic resonance spectroscopy (\textsuperscript{1}H-MRS), little is known about GABA\textsubscript{A} receptor availability in depression. Here, the aim was to characterize GABA and Glu-levels in dorsal anterior cingulate cortex (dACC), whole-brain GABA\textsubscript{A} availability, and their relationship in patients with depression compared to healthy controls. Forty-two patients and 45 controls underwent \textsuperscript{1}H-MRS using a MEGA-PRESS sequence to quantify dACC GABA\textsubscript{A} and Glu (contrasted against creatinine [Cr]). Immediately preceding the \textsuperscript{1}H-MRS, a subsample of 28 patients and 15 controls underwent positron emission tomography (PET) with \textsuperscript{[\textsuperscript{11}C]}Flumazenil to assess whole-brain GABA\textsubscript{A} receptor availability. There were no differences in dACC GABA\textsubscript{A}/Cr or Glu/Cr ratios between patients and controls. The same was true for whole-brain GABA\textsubscript{A} receptor availability. However, there was a significant negative relationship between GABA\textsubscript{A}/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.

\section{1. Introduction}

\subsection{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\textsubscript{A} and metabotropic GABA\textsubscript{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) (Germer 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 (dPFC) 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 (Czech 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 (\textsuperscript{1}H) magnetic resonance spectroscopy (\textsuperscript{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.
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 uptake in depression. One positron emission tomography (PET) study measured GABA availability in depression using $[^{123}]$Iomazenil 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 $[^{11}C]$Iomazenil as a radiotracer, widespread increase in $[^{123}]$Iomazenil binding following electro-convulsive therapy (ECT) (Mervaala et al., 2001) has been reported. However, using the same method, no difference in GABA uptake density compared to controls (Kogna 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 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 antagonist $[^{11}]$CFlumazenil as a radiotracer, widespread increase in $[^{123}]$Iomazenil binding following electro-convulsive therapy (ECT) (Mervaala et al., 2001) has been reported. However, using the same method, no difference in GABA uptake density compared to controls (Kogna 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 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 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 Coutelier, 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-Muzii 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 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 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 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 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. T1-weighted images were collected using a 3D T1-weighted turbo FFE sequence with TR/TE=8.2/3.8 ms, flip angle = 8°, field of view = 256 × 256 mm², spatial resolution = 1 × 1 mm². 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 mm³ 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 T1-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 (Provencencher, 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 [11C]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. [11C]Flumazenil was synthesized as previously described (Eng et al., 2010).

2.4. PET image processing

All dynamic PET images were corrected for within scan movement utilizing VOlager 4.0.7 (GE Healthcare) software. The T1-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 (BPND) 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. (Klumper 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 cm³. 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 (BPND) images after co-registration to the T1-weighted images, in order to bring them into MNI space, resampling them to 2 × 2 × 2 mm³ voxels. The BPND images were then smoothed with a kernel with full-width at half maximum (FWHM) of 8 × 8 × 8 mm³. Additionally, individual mask images representing the MRS voxel were defined and used to extract average BPND 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 BPND, 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 BPND differed significantly between patients and healthy controls. To assess voxels where BPND was related to dACC GABA levels, BPND 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 pWE < 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
A significant negative relationship between the GABA+ concentration (GABA+/Cr) in the MRS voxel and BPND in bilateral dACC was observed in a whole-brain analysis, F(1,39) = 27.67, pFWE = 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) = 26.37, pFWE = 0.034, cluster extent = 312 voxels) or MADRS-S scores (F(1,38) = 27.81, pFWE = 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 BPND (>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, pFWE = 0.025, cluster extent = 337 voxels, x = 10, y = 52, z = 10).

In addition to a whole-brain voxel-wise analysis, average BPND 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 BPND.

To assess the effect of medication on GABA+, Glu, and BPND, 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).

| Neurontransmitter levels | MDD | HC | t  | p   | Hedge’s g | 95% CI (Hedge’s g) |
|--------------------------|-----|----|----|-----|-----------|--------------------|
| GABA+/Cr (a.u.)          | .101 (0.01) | .099 (0.01) | -.61 | .547 | -.13 | -.56 (.20) |
| Glu/Cr (a.u.)            | .714 (0.07) | .714 (0.07) | -.04 | .965 | .009 | .04 (.42) |
| GABA+/Glu (a.u.)         | .142 (0.02) | .139 (0.02) | -.56 | .578 | -.12 | -.55 (.31) |

| tissue volume fractions in mrs voxel | MDD | HC | t  | p   | Hedge’s g | 95% CI (Hedge’s g) |
|-------------------------------------|-----|----|----|-----|-----------|--------------------|
| gray matter fraction (%)            | 42 (4) | 41 (3) | -1.07 | .287 | .23 | -.66 (.20) |
| White matter fraction (%)           | 49 (4) | 50 (3) | 1.31 | .193 | .28 | .15 (.71) |
| csf fraction (%)                    | 08 (2) | 08 (2) | -0.49 | .627 | .11 | -.53 (.32) |
| gray- to white matter ratio         | 86 (0.12) | 83 (0.12) | -1.24 | .219 | .27 | -.69 (.16) |

### Quality indicators

| GABA+ fitting error (Gannet) (%)   | 4.58 (1.4) | 4.16 (0.9) | -.72 | .089 | .37 | -.80 (.06) |
| Cr fitting error (Gannet) (%)      | 1.47 (0.2) | 1.40 (0.2) | -1.97 | .052 | .42 | -.85 (.008) |
| Glu (CRLB) (%)                     | 7.75 (1.6) | 7.75 (1.3) | 0   | 1    | 0   | -.43 (.43) |
| Cr (CRLB) (%)                      | 1.90 (0.3) | 1.96 (0.2) | -.963 | .338 | .21 | -.22 (.64) |

Fig. 2. Axial slices showing mean non-displaceable binding potential (BPND) for A) healthy controls and B) patients with depression.
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, disease 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, 1H-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 (Prevot 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 white 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 [11C]Flumazenil BPND 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 BPND 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.

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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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