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Is treatment-resistance in unipolar melancholic depression characterized by decreased serotonin $_{2A}$ receptors in the dorsal prefrontal – anterior cingulate cortex?

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Abstract

Objectives: Quite a number of patients diagnosed with major depression are resistant to several well carried-out psychopharmacological interventions. It remains unclear as to how the serotonergic system is implicated in the phenomenon of treatment-resistance.

Methods: We examined the involvement of post-synaptic 5-HT_{2A} receptors in the pathophysiology of treatment resistance in unipolar melancholic major depression with ^{123}I-5-I-R91150 SPECT. 15 antidepressant-naïve (ADN) first-episode depressed patients, 15 antidepressant-free treatment-resistant depressed (TRD) patients and 15 never-depressed individuals, matched for age and gender were studied.

Results: Compared to ADN patients and healthy controls, TRD patients displayed significantly lower 5-HT_{2A} receptor binding index (BI) in the dorsal regions of the prefrontal and the anterior cingulate cortex. No significant 5-HT_{2A} receptor BI differences between ADN patients and controls were observed.

Conclusions: At the cortical level, 5-HT_{2A} receptor BI does not significantly differ in first-episode melancholic depressed patients compared to healthy controls. This observation might imply a limited short-term impact on the serotonergic system in first episode depression. Our results also suggest that when encountered with treatment resistance, the 5-HT_{2A} receptors in the DPFC-ACC axis are significantly down-regulated. However, whether this assumed underlying pathophysiological mechanism is due solely to abnormalities in the serotonergic system remains to be answered.

1. Introduction

Major depression is a worldwide mental health problem affecting millions (Nemeroff 2007a). Unfortunately, not all depressed patients respond to the available pharmacological treatment algorithms and refractory depression is not uncommon (Fava 2003). It is estimated that treatment-resistance occurs in up to 40% of depressive episodes that are adequately treated with first-line antidepressant therapy (Souery et al. 2007). Ten percent or even more of patients suffering from major depression are resistant to several psychopharmacological interventions, even when adhering to treatment guidelines (Fagiolini & Kupfer 2003; Berlim & Turecki 2007). Not surprisingly, it has been proposed that severe treatment resistance could be a different type of depression (Nemeroff 2007b). Furthermore, when challenged with clinical non-response, treatment options are limited (Shelton et al. 2010; Ward & Irazoqui 2010).

The serotonergic system remains one of the main targets of psychotropic drug intervention to treat depression (Neumeister & Charney 2002). Ascending serotonergic projections arise primarily from the raphe nuclei in the brainstem and 'arborize' widely throughout cortico-subcortical structures, innervating forebrain and limbic areas (Hensler 2006). Prefrontal cortical serotonergic innervations have been found to be reduced in depressed individuals (Larisch et al. 2001; Arango et al. 2002). Being part of the G-protein coupled 5-HT_2 serotonin receptor family, post-synaptic 5-HT_{2A} receptors are implicated in the pathophysiology of many neuropsychiatric disorders including major depression, and they are implicated in several brain functions such as appetite control, thermoregulation, emotion, personality, cognition, ageing and sleep (Aghajanian & Sander-Bush 2002; Celada et al. 2004; Frokjaer et al. 2010). These receptors have widespread distributions throughout the cortex, with high densities in the frontal cortex (Barnes & Sharp 1999). However, it has to be noted that discrepant results in 5-HT_{2A} receptor research in depression have been reported, with some authors demonstrating 5-HT_{2A} receptor increases, others demonstrating decreases, or no differences in receptor ligand binding at all (D'haenen 2004; Bhagwagar et al. 2006). Confounding variables such as sample size, heterogeneity, age, antidepressant drug status and suicide levels could be an explanation for these observed discrepancies. Nevertheless, there seems to be a growing consensus indicating a decreased cortical 5-HT_{2A} receptor binding in depressed patients compared with normal controls (D'haenen 2001).

Few studies have examined the differences in 5-HT_{2A} receptor binding between currently depressed antidepressant naïve (ADN) patients, depressed patients who have been treated with antidepressants, and never-depressed healthy controls (Massau et al. 1997; Messa et al. 2003; Schins et al. 2005). Although negative results are also reported (Schins et al. 2005), Messa et al (2003) found decreased frontal cortical 5-HT_{2A} binding indices in symptomatic ADN patients as compared to their control group. They suggested that 5-HT_{2A} receptors are reduced in depressive patients during symptomatic periods. However, at this point it is not clear whether these serotonergic abnormalities found in unipolar depressed samples are any different when encountered with treatment resistance. No studies examined the possible differences at the level of 5-HT_{2A} receptor BI between untreated depressed patients and AD-free depressed patients who were "unsuccessfully" treated with several psychopharmacological interventions.

In the present study, we focused on the 5-HT_{2A} receptors in well defined gender-controlled homogeneous groups of unipolar depressed patients, using SPECT and the radioligand 4-amino-N-[1-[3-(4-fluorophenoxy)propyl]-4-methyl-4-piperidinyl]-5-iodo-2-methoxybenzamide (^{123}I-5-I-R91150) (Terriere et al. 1995). As age-dependent reductions in 5-HT_{2A} receptor binding indices in the cortex have consistently been reported (Baeken et al. 1998; Meltzer et al. 1998), patients and controls were also matched for age. Because hormonal processes seem to differ between depression subtypes (Porter & Gallagher 2006), our patient group consisted only of depressed patients with melancholic features. Melancholic depression is characterized by anhedonia, i.e., distinct quality of depressed mood, depression symptoms worse in the mornings, early morning awakenings, psychomotor retardation, weight loss, and excessive feelings of guilt (Gold & Chrousos 2002). Treatment-resistant depressed (TRD) patients had had a minimum of two unsuccessful treatment trials with serotonin reuptake inhibitors/ noradrenaline and serotonin reuptake inhibitors (SSRI/NSRI) and one failed clinical trial with a tricyclic antidepressant (TCA). TRD patients were tapered-off their psychotropic drugs, and all were at least medication-free for at least two weeks before SPECT scanning. Only when necessary TRD patients were kept on a steady dose of benzodiazepines.

Firstly, in line with former research, we hypothesized that compared to never depressed-individuals in both the currently depressed ADN patients and the treatment-resistant depressed (TRD) patients, 5-HT_{2A} receptor BI would be significantly lower in all the cortical areas examined. Secondly, we

expected that these reductions in 5-HT_{2A} receptor BI would be even larger in TRD patients compared to ADN patients. Thirdly, because in primary depression clinical symptoms and brain imaging data point to a functional deficit in the frontal cortical regions (Mayberg 2003), we expected that the differences in 5-HT_{2A} receptor BI would be most apparent in these areas.

2. Materials and Methods

2.1. Subjects

The study was approved by the ethics committee of the University Hospital (UZBrussel) and all subjects gave written informed consent. As the focus of this research is treatment resistance, fifteen right-handed unipolar melancholic TRD patients (Female:Male (F:M)= 9:6; age= 38.6±9.5y) were studied. See also Table 1. They were closely matched with fifteen first episode right-handed medication-free unipolar melancholic ADN depressed patients (F:M= 9:6; age= 36.3±9.8y) and fifteen never-depressed medication-free healthy volunteers (F:M= 9:6; age= 37.01± 9.8y), who were included as control group. In other words, for each female or male TRD patient a suitable female or male ADN patient and healthy control was selected, matched for age as close as possible. Parts of the TRD patient and the control SPECT data were also used for another study, examining the treatment effects of high frequency repetitive transcranial magnetic stimulation in these kinds of patients (Baeken et al. 2011). According to the Diagnostic and Statistical Manual of Mental Disorders criteria (Diagnostic and Statistical Manual of Mental Disorders, Fourth edition, text revision. Washington, DC, American Psychiatric association, 2000), all patients were diagnosed with unipolar major depression of the melancholic subtype (ICD-9-CM code 296.23 and 296.33). Current or past psychotic features or a history of bipolarity were considered as exclusion criteria. Patients with substance abuse/dependence were not included in the study. Because in post-mortem studies increased 5-$HT_{2(A)}$ receptor BI are reported in the frontal cortices of suicide victims (van Heeringen et al. 2003), suicidal attempts during the current depressive episode were considered as an exclusion criterion. All participants were in good physical health. Severity of depression was assessed with the 17-item Hamilton Depression scale (HDRS; Hamilton 1967) by a qualified psychiatrist, unrelated to the study. All ADN patients were by definition drug-free in the current episode, but they were also never treated with antidepressants in the past. All TRD patients were considered at least stage III treatment resistant as described by Rush et al (2003): they had had a minimum of two unsuccessful treatment trials with an SSRI/NSRI and one failed clinical trial with a TCA. All unsuccessful treatment trials were carried-out at therapeutic antidepressant dosages for at least eight weeks. After a washout period, all TRD patients were free from antidepressants, neuroleptics and mood stabilizers for at least two weeks. This AD-free time frame is commonly used in PET/SPECT brain imaging studies examining serotonin receptors (D'haenen et

al., 1992; Parsey et al., 2006). All TRD patients were closely monitored by their personal physicians during the washout of their current psychopharmacological treatments. In agreement with the treating physician, when necessary TRD patients were kept on a steady dose of benzodiazepines (n=6).

2.3. Scanning Procedure

All patients received a static baseline SPECT scan. SPECT-imaging was performed with a Siemens MultiSPECT triple-headed gamma camera, equipped with parallel-hole medium-energy collimators. All subjects received oral Lugol's solution containing 400mg of potassium iodide 15 min prior to injection for thyroid blockage. An average of 150MBq ^{123}I-5-I-R91150, was manually injected. SPECT acquisition was performed at a minimum of 120 min after administration of the tracer. Data were collected from 96 angular positions over 360° in a 128x128 matrix, with a total acquisition time of 32 min. Reconstruction of the acquired projection images was performed using an iterative reconstruction algorithm (Ordered Subset Expectation Maximization, OSEM, 8 iterations, 8 subsets) and filtered with a 3D Gaussian using 15mm full-width at half-maximum.

SPECT scans were automatically co-registered to a template image that was placed in a predefined stereotactic (image) space (BRASS; Nuclear Diagnostics Ltd., Sweden). For a more detailed description of the methods for co-registration of SPECT receptor data using transmission images, the reader is referred to the paper of Van Laere and colleagues (2001). The sequential acquisition of transmission and emission images can be used to anatomically standardize the emission image using the same linear parameters as those used for the transmission image (Audenaert et al. 2003). The latter is first reoriented to a template in Talairach space and the same transformation is then given to the emission images. On the Talairach templates, a predefined volume-of-interest (VOI) set can be constructed (originally performed on perfusion SPECT images), which allows a user-independent sampling of the whole brain volume of different individuals without previously available structural information. All images were visually double-checked to ensure correct anatomical positioning of the predefined VOIs (Goethals et al. 2004).

Radioactivity estimates in the cortex were assumed to represent total ligand binding (specific plus nonspecific binding plus free ligand) (Goethals et al. 2004). Because very few 5-HT_{2A} receptors are present in the cerebellum, this region was chosen to represent nonspecific activity (Terriere et al. 1995).

Calculation of relative indices of specific BI was performed by VOI normalization to the activity per volume element in the cerebellum. Under these pseudo-equilibrium circumstances, BI is directly related to the *in vivo* receptor density (B_{max}) and affinity (K_d). BI was defined as (target activity-background activity in the brain)/(background activity) which was operationally estimated as (counts/pixel in VOI- counts/pixel in the cerebellum)/(counts/pixel in the cerebellum) (Audenaert et al. 2003).

Four bilateral cortical VOIs were defined and selected: the frontal cortex (FC), the temporal cortex (TC), the parietal cortex (PC) and the occipital cortex (OC). See also Fig 1 A. Because of our interest in the frontal cortical areas and the observed group differences in FC 5-HT_{2A} receptor BI measurements, we further examined 5-HT_{2A} receptor binding indices in separate frontal areas. The following four bilateral prefrontal cortical VOIs were defined: dorsal prefrontal cortex (DPFC), ventral prefrontal cortex (VPFC), orbitofrontal (OFC) cortex, and the anterior cingulate cortex (ACC). According to the BRASS program the DPFC consisted of the gyrus frontalis superior and the gyrus frontalis superior pars medialis. The VPFC consisted of the gyrus frontalis medius and the gyrus frontalis inferior. The OFC consisted of the gyrus rectus and the gyrus orbitalis. The ACC VOI consisted of the ventral and dorsal ACC. See also Fig 1 B.

2.4. Statistical Methods

All statistical analyses were performed with SPSS 15 (Statistical Package for the Social Sciences). Significance threshold was set at a two-tailed probability of $p<.05$ for all analyses.

To compare group 5-HT_{2A} receptor BI including the four cortical VOIs, in a first set of analyses the 5-HT_{2A} receptor uptake values were analyzed as multiple dependent variables in a MANCOVA analysis with the four regions (VOI) as the dependent variables, group (ADN versus TRD patients versus never-depressed controls) as the fixed between subjects factor, and age as covariate. For the univariate follow-up analyses of the omnibus MAN(C)OVA analyses we used Bonferroni corrected p-values for 4 comparisons. All further post hoc analyses were also Bonferroni corrected.

In a second set of analyses, to compare group 5-HT_{2A} receptor BI in the frontal cortex, these analyses were repeated with the four frontal VOIs.

In a last set of analyses confined to the TRD patients, we evaluated whether illness duration, SSRI/TCA last treatment before washout could influence 5-HT_{2A} receptor BI patterns. These analyses were separately performed for the whole brain cortical VOIs and the frontal VOIs.

3. Results

3.1. Group results.

See Table 2 for an overview of the 5-HT_{2A} receptor BI for the different groups.

Mean HDRS scores for ADN depressed patients were 22.4±6.8 and 26.5±3.3 for TRD patients, indicating moderate to severe depression. Independent t-tests showed no significant differences in depression severity ($t(28)=1.81$, *ns*). A one-way ANOVA, with age as the dependent variable and group as the fixed factor, did not show significant group differences in age.

3.1.1. Whole brain.

The MANCOVA analysis, with the different cortical VOIs (FC, TC, PC, OC) as dependent variables, group (ADN versus TRD patients versus never-depressed controls) as the between group fixed factor, and age as covariate, showed that there were no significant effects implying the covariate age. Further, no significant group by region interaction effect was found. Therefore, age was excluded in further analyses.

MANOVA analysis revealed a significant main effect of group (Wilks' Lambda: $F(8,78)= 2.03$, $p< .05$). The individual univariate tests (Bonferroni corrected) showed that the three groups differed significantly in 5-HT_{2A} receptor BI in the FC ($F(2,42)= 7.20$, $p< .01$), but not for the TC and OC. Bonferroni corrected post hoc analysis revealed that for the FC the 5-HT_{2A} receptor BI in TRD patients was significantly lower (mean= 106.69) when compared to healthy controls (mean= 113.31) ($p< .01$). In TRD patients the 5-HT_{2A} receptor BI in the FC was also significantly lower compared to the ADN patients (mean= 112.52) ($p<.05$). No significant difference in FC 5-HT_{2A} receptor BI was observed between ADN patients and controls. See also Fig 2A.

3.1.2. Frontal cortex.

To evaluate 5-HT_{2A} receptor binding indices in the separate frontal areas (DPFC, VPFC, OFC, ACC), as before, we first performed a MANCOVA analysis, with the different frontal cortical VOIs as the dependent variables, group (ADN versus TRD patients versus never-depressed controls) as the fixed factor,

and age as covariate. Again, this analysis showed that there was no significant contribution of the covariate age. Consequently, age was excluded in further analyses.

MANOVA analysis revealed a significant main effect of group (Wilks' Lambda: $F(8,78)= 2.32$, $p< .05$). The individual univariate tests (Bonferroni corrected) showed that the three groups differed significantly in 5-HT_{2A} receptor BI in the DPFC ($F(2,42)= 8.11$, $p< .01$) and the ACC ($F(2,42)= 5.24$, $p< .05$), but not for the VPFC and the OFC.

Bonferroni corrected post hoc analysis showed that in the DPFC, TRD patients displayed significantly lower 5-HT_{2A} receptor BI (mean= 105.44) compared to controls (mean= 111.96) ($p<.01$). TRD patients also displayed significantly lower 5-HT_{2A} receptor BI compared to ADN patients (mean= 112.53) ($p<.05$). The DPFC 5-HT_{2A} receptor BI did not significantly differ between controls and ADN patients. Bonferroni post hoc analysis for the ACC also showed that the 5-HT_{2A} receptor BI in TRD patients (mean= 106.00) was significantly lower compared to controls (mean= 114.55) ($p< .05$) and a trend-like lower BI compared to ADN patients (mean= 112.56) ($p= .07$). No significant ACC 5-HT_{2A} receptor BI differences were observed between controls and ADN patients. See also Fig 2B.

3.2. 5-HT_{2A} receptor BI results confined to the TRD patients.

3.2.1. Whole brain.

First, to examine whether illness duration of the current depressive episode in TRD patients influences 5-HT_{2A} receptor BI measurements, again we performed a MANCOVA analysis, with the four different cortical VOIs as the dependent variables and duration of illness (in years) as covariate. This analysis showed that there was no significant contribution of the covariate illness duration.

Second, to verify whether the last medication in the TRD patient group before washout could have influenced FC 5-HT_{2A} receptor BI measurements, we compared patients with TCA washout (n=8) and patients without TCA washout (n=7). Independent *t*-tests showed no significant differences in 5-HT_{2A} receptor BI between these two groups ($t(13)= .71$, *ns*). Age ($t(13)=.02$, *ns*), depression severity ($t(13)=.24$, *ns*) and gender (χ^2 (1, $n= 15) = .60$, *ns*) were also not different between these two groups. Furthermore, we evaluated whether benzodiazepine treatment interfered with our measurements. Independent *t*-test showed no differences between those TRD patients under benzodiazepine treatment (n= 6) and those without

benzodiazepines (n= 9) in FC 5-HT_{2A} receptor BI (t(13)=.45, *ns*), age (t(13)=.11, *ns*) or gender (χ^2 (1, n= 15) 2.27, *ns*). Because of the small sample size, we also applied non-parametric Mann-Whitney U tests to evaluate the influence of TCA treatment on 5-HT_{2A} receptor BI measurements. Again no significant group differences were observed for the FC. Mann-Whitney U tests also did not reveal significant benzodiazepine influences in FC 5-HT_{2A} receptor BI.

3.2.2. Frontal cortex.

To evaluate whether illness duration of their current depressive episode might influence 5-HT_{2A} receptor BI measurements in the frontal cortex, we performed a MANCOVA analysis, with the four different frontal cortical VOIs as the dependent variables and duration of illness (in years) as covariate. This analysis again showed no significant contribution of the covariate illness duration.

To verify whether the last medication before washout might have influenced 5-HT_{2A} receptor BI measurements, again we compared TRD patients with TCA washout (n=8) and patients without TCA washout (n=7). Independent *t*-tests showed no significant differences in 5-HT_{2A} receptor BI between these two groups for the DPFC (t(13)=1.10, *ns*) nor for the ACC (t(13)=.97, *ns*). To examine whether the use of benzodiazepines could have been a confounding factor, independent *t*-tests did not demonstrate 5-HT_{2A} receptor BI differences for the DPFC (t(13)=1.25, *ns*) nor for the ACC (t(13)=.85, *ns*). We also applied the non-parametric Mann-Whitney U tests to evaluate the influence of current TCA treatment on 5-HT_{2A} receptor BI measurements. No significant group differences were observed for the DPFC and the ACC. Mann-Whitney U tests confirmed the lack of benzodiazepine confounds on 5-HT_{2A} receptor BI for the DPFC and the ACC.

4. Discussion

In agreement with our initial hypothesis, TRD patients displayed significantly less frontal cortical 5-HT_{2A} receptor binding indices compared to controls. Compared to ADN patients, treatment resistant depression was also characterized by lower 5-HT_{2A} receptor BI. In contrast to our initial hypothesis, no significant differences in cortical 5-HT_{2A} receptor BI between ADN patients and never-depressed healthy controls were observed. Compared to both other groups, TRD patients displayed significantly less 5-HT_{2A} receptor BI specifically in the DPFC and, at a trend level, also in the ACC

Our observations in ADN patients are nevertheless in line with the Schins et al (2005) study, which also found no significant differences in 5-HT_{2A} receptor binding when AD-naïve post-myocardial depressed patients were compared a non-depressed healthy group. However, our results do not support the observations of Messa et al (2003), who reported that symptomatic ADN patients showed decreases in frontal cortical 5-HT_{2A} binding indices compared to their control group. Although not explicitly mentioned, it could be possible that the heterogeneous sampling of depressed patients could have introduced more variability in their data. Further, some ADN patients had experienced one to three depressive episodes in the past whereas our ADN patients were all first episode patients. The lack of differences in 5-HT_{2A} receptor BI between healthy controls and ADN patients could indicate that melancholic depression might not be directly related to alterations in frontal cortical 5-HT_{2A} receptor BI in first episode depressed patients.

Concerning the results in our TRD patient group, one could argue that the observed 5-HT_{2A} receptor BI decreases in the frontal cortex in the TRD patient group are merely the result of long-term AD therapy (Van Oekelen et al. 2003). Given that our first episode ADN individuals, with a similar subtype of depression, showed no differences in 5-HT_{2A} BI compared to healthy controls we cannot exclude the possibility that the differences between ADN and TRD group are due to treatment exposure rather than depression itself. However, Messa et al (2003) studied a group of antidepressant naive depressed patients (thus without treatment exposure) who had from 0 to 3 former depressive episodes and they also showed decreased 5-HT_{2A} BI compared to healthy controls. Moreover, if this would have been the case this decrease in 5-HT_{2A} receptor BI should also have been present in the other examined cortical areas. For instance, Bhagwager et al (2006) found in a sample of 6 month medication-free formerly depressed

euthymic patients that recovery from depression resulted not only in a prefrontal increase of 5-HT_{2A} receptor binding but also in the parietal and occipital cortices. Furthermore, in our study illness duration of the current depressive episode did not influence 5-HT_{2A} receptor BI measurements in none of the VOIs examined. As mentioned before, during the current depressive episode all our TRD patients failed to show response after at least three well carried-out pharmacological trials, including non-response to SSRI/NSRIs and TCAs. Importantly, the influence of the last AD treatment before washout had no significant effect on 5-HT_{2A} receptor BI measurements. Half of our TRD patients were not on TCA treatment before washout and frontal cortical 5-HT_{2A} receptor binding indices were not significantly different from those who had received TCA treatment as last unsuccessful pharmacological intervention. In addition to the 5-HT_{2A} receptor down-regulation associated with successful TCA treatment (Van Oekelen et al. 2003), it has been shown that successful treatment response with SSRI and electroconvulsive shock therapy (ECT) was associated with prefrontal 5-HT_{2A} receptor up-regulation (Massau et al. 1997; Burnet et al. 1999; Zanardi et al. 2001). Another confounding factor could have been the use of benzodiazepines in the TRD patient group. However, our analyses did not suggest such a possible influence on 5-HT_{2A} receptor BI in the cortical areas examined. Indeed, not all studies demonstrated an impact of these psychotropic drugs (Leysen et al. 1987; Messa et al. 2003), and if they did, instead of a down-regulation, an up-regulation of 5-HT_{2A} receptors could be expected (Zanardi et al. 2001; Akin et al. 2004). Since increased 5-$HT_{2(A)}$ receptor BI has been reported post-mortem in the frontal cortices of suicide victims (van Heeringen et al. 2003), it is also important to point-out that no suicide attempt had occurred in our sample at least 6 months prior to this study.

Our results could imply that the reduced cortical 5-HT_{2A} receptor binding found in melancholic TRD patients might be specific for the underlying pathophysiological state of the disease. This assumption is also in line with the prefrontal neurodegenerative effects due to hypercortisolaemia in long-term melancholically depressed states (Porter & Gallagher 2006).

To further elucidate the impact of treatment resistance in melancholic depression, we further examined the 5-HT_{2A} receptor BI within these frontal cortical areas. Compared to controls and ADN patients, significant reductions in the DPFC and ACC 5-HT_{2A} receptor BI in TRD patients were observed. Interestingly, other types of brain imaging studies, not all related to the serotonergic neurotransmitter

system, reported on dysfunctional 'fronto-cingulate networks' frequently found in major depressive disorder (Mayberg 2003; Drevets et al. 2008; Pizzagalli 2011). Besides prefrontal decreases in neuronal activity, decreased ACC activation patterns have frequently been documented (Davidson et al. 2002). In major depression dorsal prefrontal-cingulate cortical abnormalities have been commonly observed, in particular during emotional processing and cognitive control (Drevets et al. 2008). These cognitive processes are in particular problematic for depressed patients (Mayberg 2003). Our observations of a lower DPFC-ACC 5-HT_{2A} receptor BI in TRD patients might indicate a specific cognitive control problem during emotional processing in patients with increasing vulnerability for depression after multiple episodes (De Raedt & Koster 2010). Furthermore, the observation that healthy controls and ADN depressed patients did not show different 5-HT_{2A} receptor BI in these areas suggests that possible cognitive control problems would not be significantly present during a first depressive episode. However, as we did not integrate neurocognitive tasks in our design these assumptions must be interpreted cautiously.

Although our findings could suggest specific neurobiological abnormalities for treatment resistant melancholic depression, at this point these assumptions remain to some extent speculative as this study is limited by some methodological issues. Besides that the sample size is relatively small, the antidepressant-free period of two weeks after washout could be interpreted as relatively short, in spite that this extent of time is in range with other unipolar depressed patient studies examining 5-HT_{2A} receptors (D'haenen et al. 1992; Meyer et al. 1999; Van Oekelen et al. 2003). Although the main goal of this study was to investigate treatment-resistance in melancholic depressed patients by examining the 5-HT_{2A} receptor BI, at this point we cannot predict who in the end of our ADN patients will be a stage III AD non-responder. Albeit statistically this should be around 10-15% of this group, this could have introduced some bias in our 5-HT_{2A} receptor BI measurements. However, the fact that we observed a significant difference between the TRD and the ADN patients despite that a part of the ADN group might reveal to be also treatment resistant is indicative of the magnitude of the effect.

In conclusion, our observations indicate that on the cortical level 5-HT_{2A} receptor binding indices do not significantly differ in first-episode melancholic depressed patients compared to healthy controls, at least indicating a limited short-term impact on the serotonergic system when first-time currently depressed. Further, our results suggest that when challenged with treatment resistance the DPFC-ACC neurocircuitry

is significantly more affected when compared to currently depressed ADN patients or controls. Whether this assumed underlying pathophysiological mechanism is due solely to abnormalities in the serotonergic system remains to be answered. It is possible that the long-term depressive episodes present in our TRD group could have resulted in reduced serotonergic innervations and/or atrophy in the frontal cortical regions, reflected by the lower 5-HT_{2A} receptor BI. Importantly, all interpretations should be limited to depressed patients of the melancholic subtype. Future 5-HT_{2A} receptor BI studies might do well to combine brain imaging with executive functioning tests, BDNF measurements or structural MRI to further unravel the underlying payhophysiology of treatment resistance in well-defined groups of majorly depressed samples. This comparison will be central for differentiating the two groups and for ascertaining the role of prefronto-cingulate 5-HT_{2A} receptor BI in conferring treatment resistant in melancholic unipolar depressives. Importantly, as the hippocampus has been put forward as a target region to investigate in 'serotonin' research (Fujita et al. 2000), future research should also focus on possible hippocampal 5-HT_{2A} receptor abnormalities in first episode depressed patients as well as in patients unresponsive to current pharmacological algorithms.

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Statement of interest

None to declare

References

Aghajanian, G.K. and Sander-Bush, E., 2002. Serotonin, in Kenneth L. Davis, Dennis Charney, Joseph T. Coyle, and Charles Nemeroff (Eds.), Neuropsychopharmacology: The Fifth Generation of Progress, Philadelphia (Penn.): Lippincott Williams & Wilkins, pp. 15-34.

Akin, D., Manier, D.H., Sanders-Bush, E., Shelton, R.C., 2004. Decreased serotonin 5-HT2A receptor-stimulated phosphoinositide signaling in fibroblasts from melancholic depressed patients. Neuropsychopharmacology. 29, 2081-2087.

Arango, V., Underwood, M.D., Mann, J.J., 2002. Serotonin brain circuits involved in major depression and suicide. Prog. Brain. Re.s 136, 443-453.

Attar-Lévy, D., Martinot, J-L., Blin, J., Dao-Castellana, M.H., Crouzel, C., Mazoyer, B., Poirier, M.F., Bourdel, M.C., Aymard, N., Syrota, A., Féline, A., 1999. The cortical serotonin2 receptors studies with positron-emission tomography and [18F]-setoperone during depressive illness and antidepressant treatment with clomipramine. Biol. Psychiatry. 45, 180-186.

Audenaert, K., Van Laere, K., Dumont, F., Slegers, G., Mertens, J., van Heeringen, C., Dierckx, R.A., 2001. Decreased frontal serotonin 5-HT 2a receptor binding index in deliberate self-harm patients. Eur. J. Nucl. Med. 28, 175-182.

Audenaert, K., Van Laere, K., Dumont, F., Vervaet, M., Goethals, I., Slegers, G., Mertens, J., van Heeringen, C., Dierckx, R.A., 2003. Decreased 5-HT2a receptor binding in patients with anorexia nervosa. J. Nucl. Med. 44, 163-169.

Baeken, C., D'haenen, H., Flamen, P., Mertens, J., Terriere, D., Chavatte, K., Boumon, R., Bossuyt, A., 1998. 123I-5-I-R91150, a new single-photon emission tomography ligand for 5-HT2A receptors: influence of age and gender in healthy subjects. Eur. J. Nucl. Med. 25, 1617-1622.

Baeken, C., De Raedt, R., Bossuyt, A., Van Hove, C., Mertens, J., Dobbeleir, A., Blanckaert, P., Goethals, I., 2011. The impact of HF-rTMS treatment on serotonin 2A receptors in unipolar depression. Brain. Stimulation. 4, 104-114.

Barnes, N.M., Sharp, T., 1999. A review of central 5-HT receptors and their function. Neuropharmacology. 38, 1083-1152.

Berlim, M.T., Turecki, G., 2007. Definition, assessment, and staging of treatment-resistant refractory major depression: a review of current concepts and methods. Can. J. Psychiatry. 52, 46-54.

Bhagwagar, Z., Hinz, R., Taylor, M., Fancy, S., Cowen, P., Grasby, P., 2006. Increased 5-HT(2A) receptor binding in euthymic, medication-free patients recovered from depression: a positron emission study with [(11)C]MDL 100,907. Am. J. Psychiatry. 163, 1580-1507.

Biver, F., Wikler, D., Lotstra, F., Damhaut, P., Goldman, S. and Mendlewicz, J., 1997. Serotonin 5-HT2 receptor imaging in major depression: focal changes in orbito-insular cortex. Br. J. Psychiatry. 171, 444-448.

Burnet, P.W., Sharp, T., LeCorre, S.M., Harrison, P.J., 1999. Expression of 5-HT receptors and the 5-HT transporter in rat brain after electroconvulsive shock. Neurosci. Lett. 277, 79-82.

Celada, P., Puig, M., Amargos,-Bosch M., Adell, A., Artigas, F., 2004. The therapeutic role of 5-HT1A and 5-HT2A receptors in depression. J. Psychiatry. Neurosci. 29, 252-265.

Davidson, R.J., Pizzagalli, D., Nitschke, J.B., Putnam, K., 2002. Depression: perspectives from affective neuroscience. Annu. Rev. Psychol. 53, 545-574.

De Raedt, R., Koster, E.H., Joormann, J. 2010. Attentional control in depression: A translational affective neuroscience approach. Cogn. Affect. Behav. Neurosci. 10, 1-7.

De Raedt, R., Koster, E.H., 2010. Understanding vulnerability for depression from a cognitive neuroscience perspective: A reappraisal of attentional factors and a new conceptual framework. Cogn. Affect. Behav. Neurosci. 10, 50-70.

D'haenen, H., Bossuyt, A., Mertens, J., Bossuyt-Piron, C., Gijsemans, M. and Kaufman, L., 1992. SPECT imaging of serotonin2 receptors in depression. Psychiatry. Res. Neuroimaging. 45, 227-237.

D'haenen, H., 2001. Imaging the serotonergic system in depression. Eur. Arch. Psychiatry Clin. Neurosci. 251(S2), II/76-II/80.

D'haenen, H., 2004. Radioligand studies in depression, in: Otte, A., Audenaert, K., Peremans, K., Van Heeringen, K. and Dierckx, R.A. (Eds.), Nuclear Medicine in Psychiatry. Springer-Verlag, Berlin, pp. 350-357.

Drevets, W.C., Price, J.L., Furey, M.L., 2008. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. Brain. Struct. Funct. 213, 93-118.

Fagiolini, A., Kupfer, D.J., 2003. Is treatment-resistant depression a unique subtype of depression? Biol. Psychiatry. 53, 640-648.

Fava, M., 2003. The role of the serotonergic and noradrenergic neurotransmitter systems in the treatment of psychological and physical symptoms of depression. J. Clin. Psychiatry. 64, 26-29.

Frokjaer, V.G., Vinberg, M., Erritzoe, D., Baaré, W., Holst, K.K., Mortensen, E.L., Arfan, H., Madsen, J., Jernigan, T.L., Kessing, L.V., Knudsen, G.M., 2010. Familial risk for mood disorder and the personality risk factor, neuroticism, interact in their association with frontolimbic serotonin 2A receptor binding. Neuropsychopharmacology. 35, 1129-1137.

Fujita, M., Charney, D.S., Innis, R.B., 2000. Imaging serotonergic neurotransmission in depression: hippocampal pathophysiology may mirror global brain alterations. Biol Psychiatry. 48, 801-812.

Goethals, I., Vervaet, M., Audenaert, K., Van de Wiele, C., Ham, H., Vandecapelle, M., Slegers, G., Dierckx, R., van Heeringen, C., 2004. Comparison of cortical 5-HT2A receptor binding in bulimia nervosa patients and healthy volunteers. Am. J. Psychiatry. 161, 1916-1918.

Gold, P.W., Chrousos, G.P., 2002. Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. Mol. Psychiatry. 7, 254-275.

Hamilton, M., 1967. Development of a rating scale for primary depressive illness. British Journal of Social & Clinical Psychology. 6, 278–296.

Hensler, J.G., 2006. Serotonergic modulation of the limbic system. Neurosci. Biobehav. Rev.30:203-214.

Larisch, R, Klimke, A., Mayoral, F., Hamacher, K., Herzog, H.R., Vosberg, H., Tosch, M., Gaebel, W., Rivas, F., Coenen, H.H., Müller-Gärtner, H.W., 2001. Disturbance of serotonin 5HT2 receptors in remitted patients suffering from hereditary depressive disorder. Nuklearmedizin. 40, 129-134.

Leysen, J.E., Van Gompel, P., de Chaffoy de Courcelles, D. and Niemegeers, C.J.E., 1987. Opposite regulation of serotonin-S2 and dopamine-D2 receptors in rat brain following chronic receptor blockade. J. Recept. Res. 7, 223–239.

Massou, J.M., Trichard, C., Attar-Levy, D., Feline, A., Corruble, E., Beaufils, B., Martinot, J.L., 1997. Frontal 5-HT2A receptors studied in depressive patients during chronic treatment by selective serotonin reuptake inhibitors. Psychopharmacology (Berl). 133, 99-101.

Mayberg, H.S., 2003. Modulating dysfunctional limbic-cortical circuits in depression: towards development of brain-based algorithms for diagnosis and optimised treatment. Br. Med.l Bull. 65, 193-207.

Meltzer, C.C., Smith, G., DeKosky, S.T., Pollock, B.G., Mathis, C.A., Moore, R.Y., Kupfer, D.J., Reynolds, C.F. 3rd., 1998. Serotonin in aging, late-life depression, and Alzheimer's disease: the emerging role of functional imaging. Neuropsychopharmacology. 18, 407-430.

Messa, C., Colombo, C., Moresco, R.M., Gobbo, C., Galli, L., Lucignani, G., Gilardi, M.C., Rizzo, G., Smeraldi, E., Zanardi, R., Artigas, F., Fazio, F., 2003. 5-HT(2A) receptor binding is reduced in drug-naive and unchanged in SSRI-responder depressed patients compared to healthy controls: a PET study. Psychopharmacology (Berl). 167, 72-78.

Meyer, J.H., Kapur, S., Houle, S., DaSilva, J., Owczarek, B., Brown, G.M., Wilson, A.A., Kennedy, S.H., 1999. Prefrontal cortex 5-HT2 receptors in depression: an [18F]setoperone PET imaging study. Am. J. Psychiatry. 156, 1029-1034.

Meyer, J.H., Kapur, S., Eisfeld, B., Brown, G.M., Houle, S., DaSilva, J., Wilson, A.A., Rafi-Tari, S., Mayberg, H.S., Kennedy, S.H., 2001. The effect of paroxetine on 5-HT(2A) receptors in depression: an [(18)F]setoperone PET imaging study. Am. J. Psychiatry. 158, 78-85.

Meyer, J.H., McMain, S., Kennedy, S.H., Korman, L., Brown, G.M., DaSilva, J.N., Wilson, A.A., Blak, T., Eynan-Harvey, R., Goulding, V.S., Houle, S., Links, P., 2003. Dysfunctional attitudes and 5-HT2 receptors during depression and self-harm. Am. J. Psychiatry. 160, 90-99.

Moresco, R.M., Colombo, C., Fazio, F., Bonfanti, A., Lucignani, G., Messa, C., Gobbo, C., Galli, L., Del Sole, A., Lucca, A., Smeraldi, E., 2000. Effects of fluvoxamine treatment on the in vivo binding of [F-18]FESP in drug naive depressed patients: a PET study. Neuroimage. 12, 452-465.

Nemeroff, C.B., 2007a. Prevalence and management of treatment-resistant depression. J. Clin. Psychiatry. 68, 17-25.

Nemeroff, C.B., 2007b. The burden of severe depression: a review of diagnostic challenges and treatment alternatives. J. Psychiatr. Res. 41, 189-206.

Neumeister, A. and Charney, D.S., 2002. Monoaminergic transmitter systems, in: D'haenen, H.A.H., den Boer, J.A. and Willner, P. (Eds.), Biological Psychiatry, John Wiley & Sons Ltd, Chichester, pp. 727-738.

Parsey, R.V., Hastings, R.S., Oquendo, M.A., Huang, Y.Y., Simpson, N., Arcement, J., Huang, Y., Ogden, R.T., Van Heertum, R.L., Arango, V., Mann, J.J., 2006. Lower serotonin transporter binding potential in the human brain during major depressive episodes. Am. J. Psychiatry. 163, 52-58.

Pizzagalli, D.A., 2011. Frontocingulate dysfunction in depression: toward biomarkers of treatment response. Neuropsychopharmacology. 36, 183-206.

Porter, R.J., Gallagher, P., 2006. Abnormalities of the HPA axis in affective disorders: clinical subtypes and potential treatments. Acta. Neuropsychiatrica. 18, 193-209.

Rush, A.J., Thase, M.E., Dubé, S., 2003. Research issues in the study of difficult-to-treat depression. Biol. Psychiatry. 53, 743-753.

Schins, A., Van Kroonenburgh, M., Van Laere, K., D'haenen, H., Lousberg, R., Crijns, H., Eersels, J., Honig, A., 2005. Increased cerebral serotonin-2A receptor binding in depressed patients with myocardial infarction. Psychiatry. Res. 139, 155-63.

Shelton, R.C., Osuntokun, O., Heinloth, A.N., Corya, S.A., 2010. Therapeutic options for treatment-resistant depression. CNS Drugs. 24, 131-161.

Souery, D., Papakostas, G.I,. Trivedi, M.H., 2006. Treatment-resistant depression. J. Clin. Psychiatry. 67, 16-22.

Terriere, D., Janssen, P.M.F., Gommeren, W., Gysemans, M., Mertens, J.J.R., Leysen, J.E., 1995. Evaluation of radioiodo-4-amino-N-[1-[3-(4-fluorophenoxy)-propyl]-4-methyl-4-piperidinyl]-5-iodo-2-methoxybenzamide as a potential 5-HT2 receptor tracer for SPE(C)T. Nucl. Med. Biol. 22,1005-1010.

van Heeringen, C., Audenaert, K., Van Laere, K., Dumont, F., Slegers G., Mertens, J., Dierckx, R.A., 2003. Prefrontal 5-HT2a receptor binding index, hopelessness and personality characteristics in attempted suicide. J. Affect. Disord. 74, 149-158.

Van Laere, K., Koole, M., D'Asseler, Y., Versijpt, J., Audenaert, K., Dumont, F., Dierckx, R., 2001. Automated stereotactic standardization of brain SPECT receptor data using single-photon transmission images. J. Nucl. Med. 42, 361-375.

Van Oekelen, D., Luyten, W.H.M.L. and Leysen, J.E., 2003. 5-HT2A and 5-HT2C receptors and their atypical regulation properties. Life. Sci. 72, 2429-2449.

Ward, M.P., Irazoqui, P.P., 2010. Evolving refractory major depressive disorder diagnostic and treatment paradigms: toward closed-loop therapeutics. Front Neuroengineering 31, 3-7.

Yatham, L.N., Liddle, P.F., Dennie, J., Shiah, I-S., Adam, M.J., Lane, C.J., Lam, R.W., Ruth, T.J., 1999. Depression following desipramine treatment: a positron emission tomography study with fluorine-18-labeled setoperone. Arch Gen Psychiatry 56, 705-711.

Yatham, L.N., Liddle, P.F., Shiah, I.S., Scarrow, G., Lam, R.W., Adam, M.J., Zis, A.P., Ruth, T.J., 2000. Brain serotonin2 receptors in major depression: a positron emission tomography study. Arch Gen Psychiatry 57, 850-858.

Zanardi, R., Artigas, F., Moresco, R., Colombo, C., Messa, C., Gobbo, C., Smeraldi, E., Fazio, F., 2001. Increased 5-hydroxytryptamine-2 receptor binding in the frontal cortex of depressed patients responding to paroxetine treatment: a positron emission tomography scan study. J Clin Psychopharmacol 21, 53-58.

TRD Patient	Gender	Age	Thase and Rush stage	Duration current episode (years)	HDRS	Last antidepressant medication before washout	Benzodiazepines (dose/day)
1	F	45	3	3	32	Duloxetine	Clonazepam 1mg
2	F	49	3	3	32	Amytriptiline	Lormetazepam 2 mg
3	F	32	3	2	29	Venlafaxine	Alprazolam 2 mg
4	F	47	3	12	30	Amytriptiline	Bromazepam 24mg
5	F	42	3	4	23	Clomipramine	no
6	F	50	3	4	27	Nortryptiline	no
7	F	52	3	5	23	Amytriptiline	no
8	F	45	3	1.5	25	Dosulepine	Flunitrazepam 1mg
9	F	48	3	5	26	Venlafaxine	no
10	M	25	3	2	22	Clomipramine	no
11	M	40	4	2.5	26	Paroxetine	Alprazolam 1mg
12	M	34	3	11	23	Clomipramine	no
13	M	39	3	1	27	Clomipramine	no
14	M	34	5	7	21	Fenelzine	no
15	M	54	4	4	27	Fenelzine	no
Mean (SD)		38.55 (9.53)		4.47 (3.25)	26.53 (3.29)		

Table 1: Demographic data of the treatment resistant group of melancholic depressed patients (TRD). HDRS: 17- item Hamilton Depression Rating Scale. F: female. M: male. SD: standard deviation.

VOI	Healthy controls	ADN patients	TRD patients
Prefrontal cortex	113.31 (4.39)	112.52 (4.73)	106.39 (6.32)
Temporal cortex	110.16 (4.26)	109.49 (4.44)	107.49 (5.17)
Parietal cortex	109.76 (6.44)	109.88 (5.67)	108.12 (6.67)
Occipital cortex	111.18 (7.49)	110.53 (7.81)	107.52 (4.72)
Dorsoprefrontal cortex	111.96 (4.45)	112.53 (4.64)	105.44 (6.70)
Ventroprefrontal cortex	112.62 (4.88)	112.00 (5.42)	108.06 (4.64)
Orbitofrontal cortex	114.71 (7.28)	113.02 (7.19)	106.94 (11.21)
Anterior cingulate cortex	114.55 (8.55)	112.56 (7.63)	106.00 (6.37)

Table 2. Means and standard deviations of 5-HT_{2A} receptor binding indices in the different volumes of interest (VOI) examined.

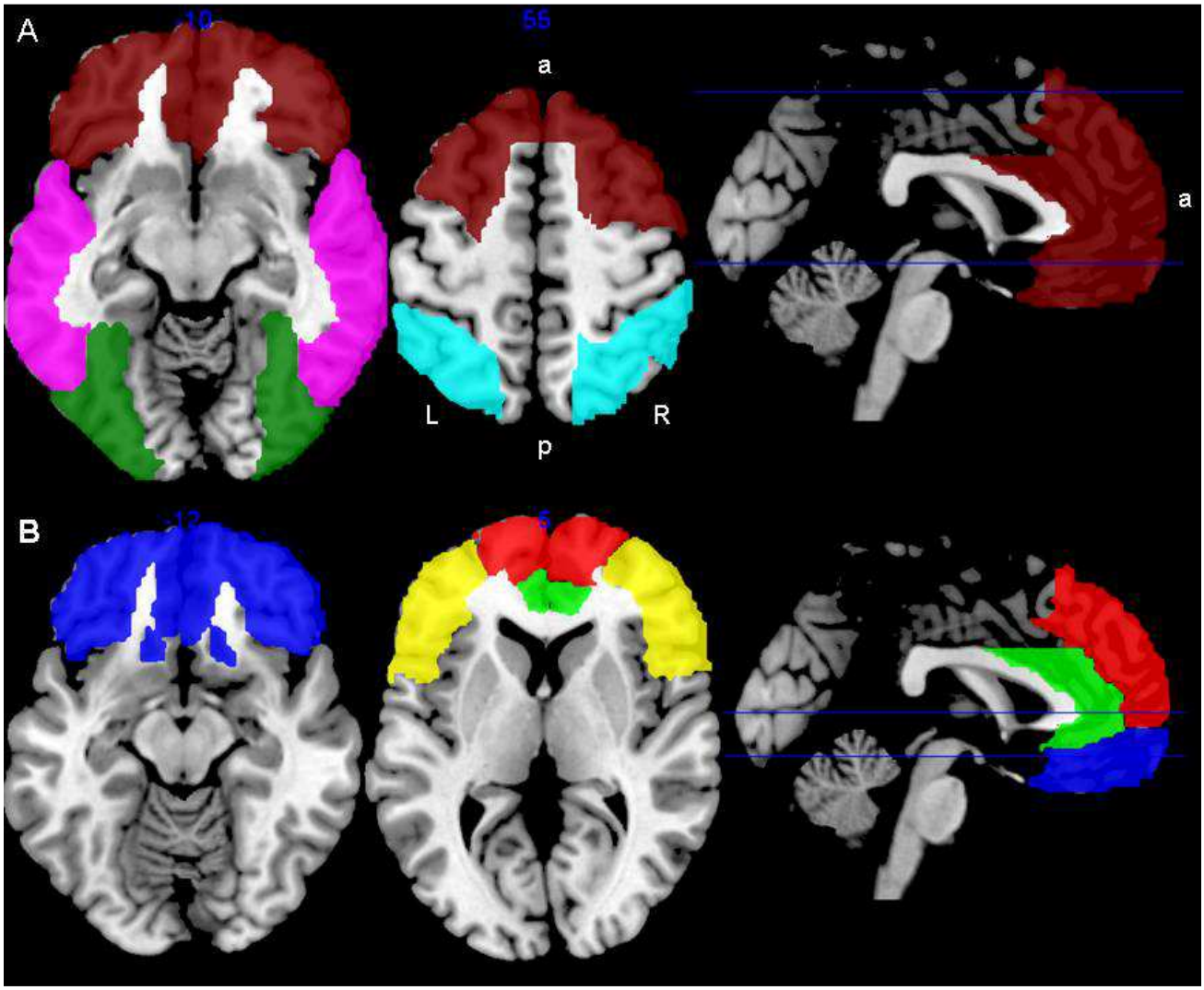

Fig 1: Overview of the different volumes of interest (VOI). For a more detailed description of the VOIs see text.

A) Cortical brain VOIs. Axial views (Z= -10 and 55). Sagittal view (X= 0). Dark red: frontal cortex (FC), Violet: temporal cortex (TC), Cyan: parietal cortex (PC), Dark green: occipital cortex (OC).

B) Prefrontal cortical VOIs. Axial views (Z= -12 and 5). Sagittal view (X= 0). Green: Red: dorsal prefrontal cortex, Green: anterior cingulate cortex, Blue: orbitofrontal cortex, Yellow: ventral prefrontal cortex.

a= anterior, p= posterior, L= left, R= right.

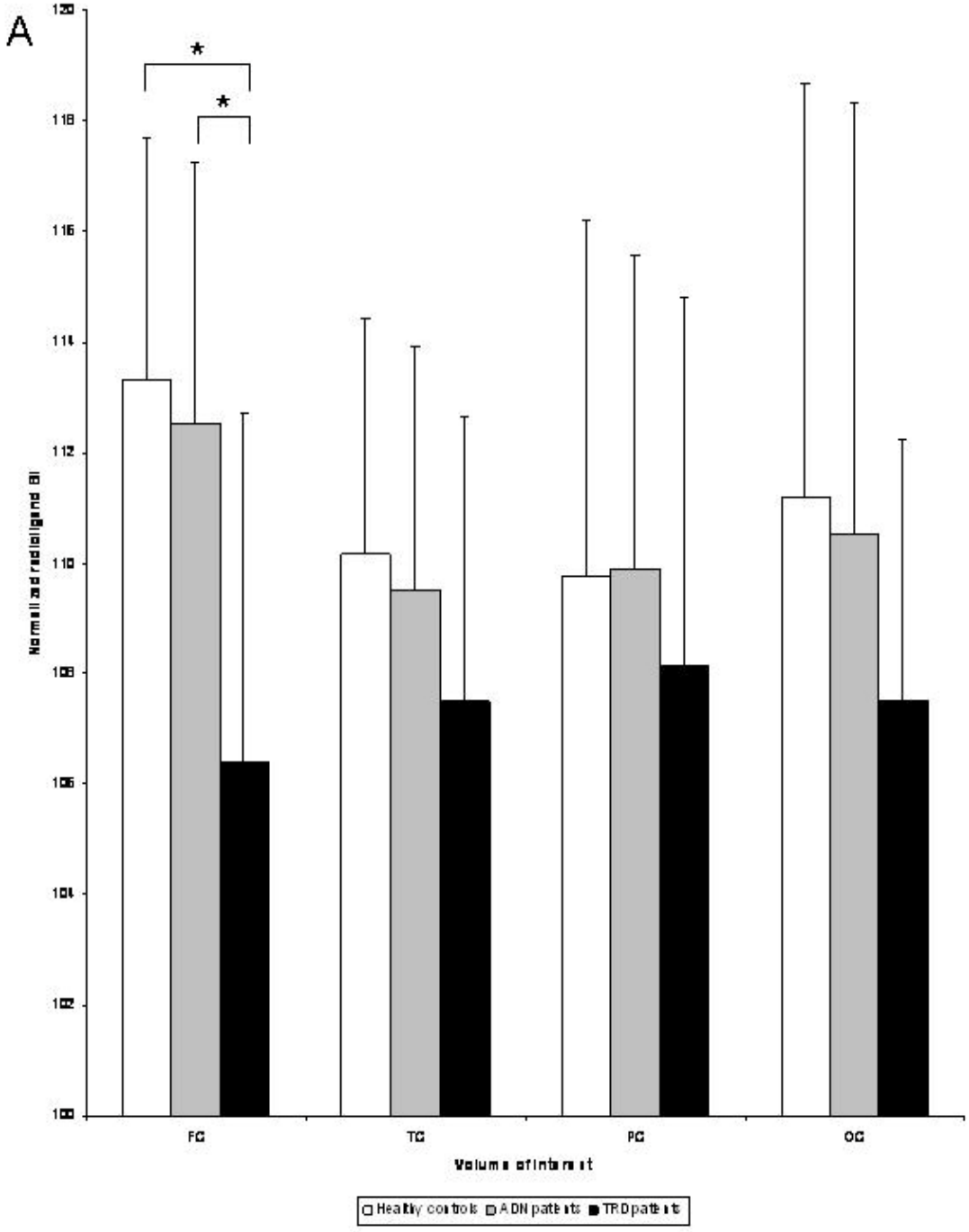

Fig 2 A. Results of the cortical brain VOIs represented in bar graphs. FC= frontalcortex, TC= temporal cortex, PC= parietal cortex ,OC= occipital cortex.

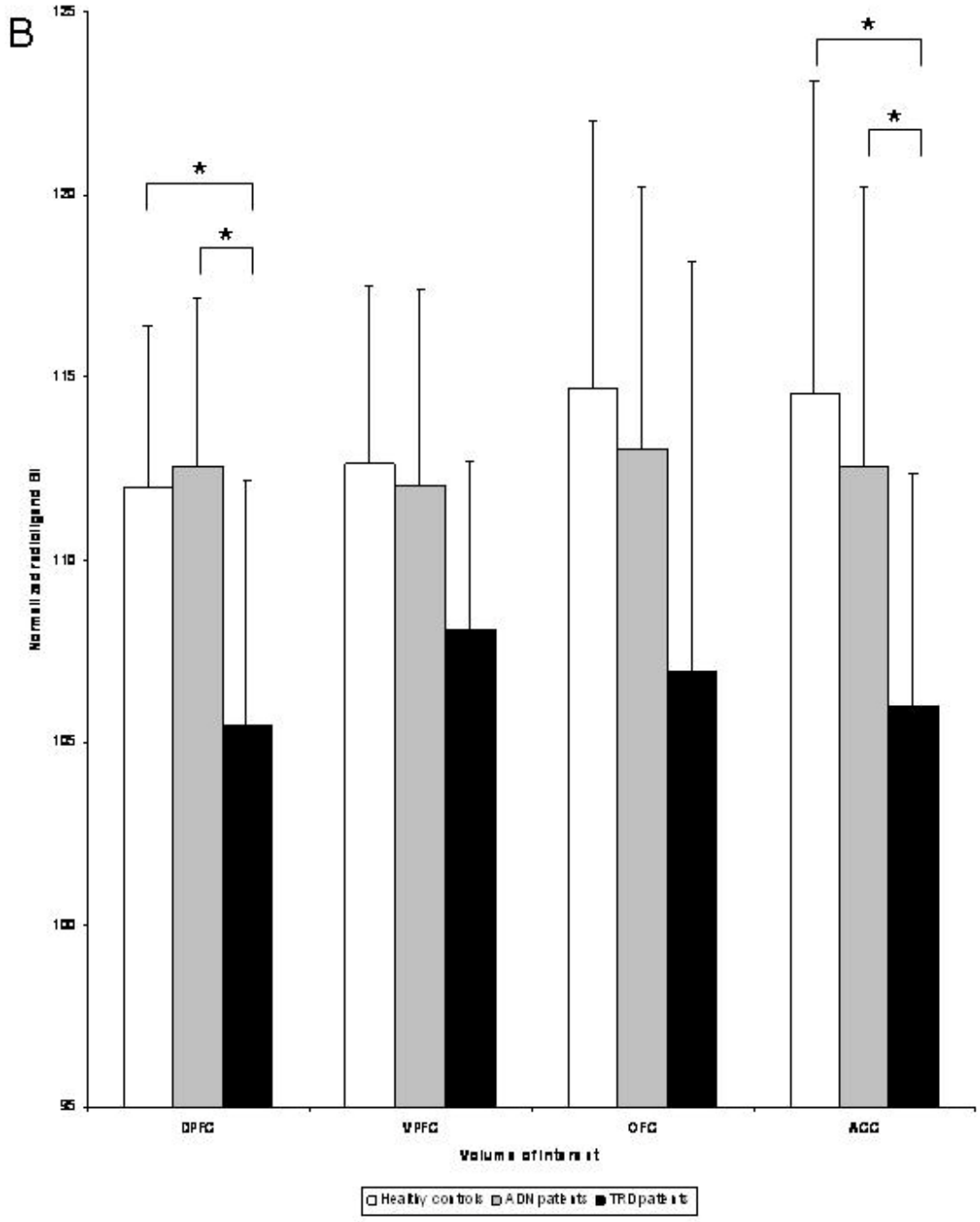

Fig 2 B. Results of the prefrontal cortical VOIs represented in bar graphs. DPFC= dorsal prefrontal cortex, VPFC= ventral prefrontal cortex ,OFC= orbitofrontal cortex, ACC= anterior cingulate cortex.

(*) significant different group effects at a two-tailed probability of p< .05. ADN= antidepressant naïve. TRD= treatment resistant depression. BI= Binding Index.