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Insular volume reductions in patients with major depressive disorder

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ABSTRACT

Background: Major Depressive Disorder (MDD) is one of the most common mental disorders. Converging evidence suggests that the insula plays an important role in the pathophysiology of MDD. Little is known regarding in which insula subregion volume alterations occur in patients with MDD.
Methods: We analyzed voxel-based morphometry in T1-weighted MRI scans of unmedicated DSM-IV MDD patients (n = 26) and in age, education, and sex matched healthy controls (HC, n = 26). Furthermore, we performed a quantitative meta-analysis across 14 structural MRI MDD studies by applying the anatomical likelihood estimation technique to identify concordant volume reductions in MDD in the insula cortex.
Results: We found significantly reduced grey matter volumes (GMV) in patients with MDD compared to HCs in the left mid-insula and in the right and left caudate nucleus. The left mid-insular volume reduction in our sample was consistent with the coordinate-based meta-analysis results.
Conclusions: The findings highlight the role of the mid-insula in the psychopathology of MDD. The mid-insula subregion might be associated with reduced interoceptive abilities in patients with MDD that is the ability to process information of "how the body feels". In addition, the caudate nucleus has been described as being part of a network that mediates emotional and motivational processes which seems to be affected in MDD.

1. Introduction

Major depressive disorder (MDD) is among the most prevalent mental disorders worldwide with a lifetime prevalence ranging from 6.6 to 21% across cultures (Kessler & Bromet, 2013). According to DSM-5, MDD is characterized by depressed mood, diminished interests, impaired cognitive function and vegetative symptoms (American Psychiatric Association, 2013). MDD profoundly impacts individuals' lifes (Otte et al., 2016) and has been associated with medical comorbidity such as chronic pain (Kessler & Bromet, 2013) and increased risk for cardiovascular disorders (Whooley & Wong, 2013).

In the last decades, a large number of neuroimaging studies have investigated functional brain characteristics in depression. A meta-analysis of functional neuroimaging studies showed that responses to emotional stimuli in depression involve the insula, prefrontal cortices, hippocampus, amygdala, anterior cingulate cortex, cerebellum, thalamus, caudate nucleus, putamen and globus pallidus (Delaveau et al., 2011). Another meta-analysis focused on the insular cortex and showed that during emotional processing in depressed individuals, insula activation may be topographically shifted toward insula subregions typically associated with the experience of pain (Mutschler, Ball, Wankerl, & Strigo, 2012). More recently, Li and colleagues reported abnormalities in resting-state brain activity in medication-free MDD patients in meta-analyses of ALFF (amplitude of low-frequency fluctuations) studies and rCBF (regional cerebral blood flow) studies in different brain regions including the left insula, anterior cingulate cortex, inferior frontal gyrus, right caudate nucleus, supplementary motor area and hippocampus (Li et al., 2017). Furthermore, a meta-analysis on structural MRI studies found reduced grey matter volume in the insula in MDD samples (Peng, Chen, Yin, Jia, & Gong, 2016). Together these findings provide strong support for the assumption that the insula plays a pivotal role in the pathophysiology of MDD. The insula —

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a brain structure that is situated in the depth of the Sylvian fissure — is anatomically connected to many other brain regions (Nieuwenhuys, 2012). The insula is pyramid-shaped and *anatomically* divided by the central insular sulcus into an anterior and posterior part (Ture, Yasargil, Al-Mefty, & Yasargil, 1999). It is thought that the insular cortex might underlie a wide range of *functions* in humans such as processing information related to the state of the body as well self-awareness, cognitive and emotional functioning, somatomotor control, and language (Christopher, Koshimori, Lang, Criaud, & Strafella, 2014; Craig, 2002; Kelly et al., 2012a; Mutschler et al., 2009; Uyanikgil, Cavusoglu, Celik, & Kilic, 2018).

Little is known, however, regarding in which insula subregion volume alterations occur in individuals with MDD. There is growing evidence that there is a *functional organization* in the insula and that different insula subregions may serve different functions (Deen, Pitskel, & Pelphrey, 2011; Kelly et al., 2012b; Mutschler et al., 2009). The dorsal anterior insula may be involved in perception of vocalizations and language (Mutschler et al., 2009), cognition (Deen et al., 2011) and the ventral anterior insula may be involved in peripheral physiological processing resulting from emotional experiences (Mutschler et al., 2009). The mid insula has been associated with interoception (see Fig. 5 in reference (Kelly et al., 2012a). Hence, knowing in which insula subregion grey matter volume reductions in MDD occur may shed light on functional impairments in MDD. On this background, we performed two studies. In the first study, T1-weighted MRI scans were analyzed in unmedicated DSM-IV MDD patients (n = 26) and in age, education, and sex matched healthy controls (HC, n = 26) using voxel-based morphometry (VBM). We hypothesized that GM volumes within the insula would be significantly lower in depressed patients compared to healthy controls. In the second study, a quantitative meta-analysis was performed across 14 structural MRI MDD studies reporting insular volume reduction in order to identify concordant volume reductions in insula subregions in MDD by applying the anatomical likelihood estimation technique.

2. Methods

2.1. MRI study

2.1.1. Participants

Twenty-six unmedicated patients with MDD and 26 healthy controls without a neurological, or medical illness completed the study. Patients with MDD and HC were matched regarding age, sex, and years of education. 25 patients were Caucasian and one patient was Asian. All HC were Caucasian. Nineteen patients with MDD were medication-naïve, and all other patients stopped treatment with antidepressant medications 6 weeks or more before they participated in the MRI study. Patients were unmedicated because they also participated in a randomized-controlled trial of cognitive-behavioral therapy (Grosse Holtforth et al., 2019). The aim was to not confound the effects of psychotherapy with medication intake. The exclusion criteria for both groups included an age out of the range of 18 or 65 years, current or past psychosis or mania, major medical or neurological illness, current drug or alcohol abuse, MRI contraindications (assessed by an MRI safety questionnaire), and the use of antipsychotics and benzodiazepines. Inclusion in the MDD group based on a diagnosis of current MDD based on a Structured Clinical Interview for DSM-IV Axis-I Disorders (SCID-I) semi-structured interview. Seven individuals with MDD had comorbid anxiety disorders: Four individuals had panic disorder, one subject had specific phobia and one social phobia, and one individual had generalized anxiety disorder. Control subjects did not have a lifetime history of MDD, had no history of MDD in first-degree relatives, and were currently free of all Axis-I disorders based on a SCID-I interview. Demographics and clinical characteristics are shown in Table 1. The study was approved by the University of Zurich`s Institutional Review Board, and all participants gave written informed consent.

Table 1
Demographics, symptom severity and other clinical characteristics for patients with MDD and healthy controls (HC) investigated in the MRI study. Individuals with MDD and HCs completed the Hopelessness Scale (H-Scale) (Krampen, 1994) and the State-Trait Anxiety Inventory (STAI) (Laux et al., 1981). Individuals with MDD were also assessed with the Inventory for Depressive Symptomatology (IDS) (Helmreich et al., 2011; Rush et al., 1996), and the Beck Depression Inventory II (BDI-II) (Beck et al., 1996). Statistics: Last column shows statistical analysis of two-sample *t*-test comparing the MDD group and the HC group ([a] based on n = 25; [b] based on n = 24; SD = Standard deviation).

Subjects	MDD	HCs	Statistics
Number of subjects	n = 26	n = 26	
Sex			
male/female	n = 17/9	n = 17/9	
Age			
mean (range)	40.19 (22-63)	35.92 (20-60)	p = 0.25
Years of education			
mean (SD)	15.64 (3.60)	15.5 (2.44)	p = 0.21
Episodes:			
First	n = 9		
Recurrent	n = 17		
BDI score	25.26 (8.34)		
mean (SD)			
IDS (SD)	34.54 (8.51)		
STAI Trait (SD)	56.0 (9.85)	33.1 (8.01)	p = 2.5E-12
STAI State (SD)	43.62 (5.92)[a]	32.4 (5.29)[b]	p = 8.5E-09
H-Scale (SD)	75.1 (12.90)	45.7 (11.01)	p = 8.5E-12

2.1.2. Psychometric measures

Individuals with MDD and HCs completed the Hopelessness Scale (H-Scale) (Krampen, 1994) and the State-Trait Anxiety Inventory (STAI) (Laux, Glanzmann, Schaffner, & Spielberger, 1981). Individuals with MDD were also assessed with the Inventory for Depressive Symptomatology (IDS) (Helmreich et al., 2011; Rush, Gullion, Basco, Jarrett, & Trivedi, 1996), and the Beck Depression Inventory II (BDI-II) (Beck, Steer, & Brown, 1996). Results are shown in Table 1.

2.1.3. Magnetic resonance imaging data acquisition

Images were acquired on a Philips Achieva 3 T whole body MRI unit equipped with an eight-channel head coil capable for sensitivity encoding. A T1-weighted gradient echo sequence (turbo field echo) with a spatial resolution of $0.94 \times 0.94 \times 1.00$ mm^3 (acquisition matrix: 240×240 pixels; 160 slices), field of view = 240×240 mm^2, echo time TE = 3.7 ms, repetition time TR = 8.06 ms, and a flip angle of 8° was applied.

2.1.4. Voxel-based morphometry

Between-group differences in grey matter volume were evaluated by using voxel-based morphometry (VBM) (Ashburner & Friston, 2000; Good, Ashburner, & Frackowiak, 2001). T1-weighted MRI scans were preprocessed and analyzed with the FSL-VBM tool (Douaud et al., 2007) (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM), an optimized VBM protocol (Good, Johnsrude et al., 2001) that is implemented in the FMRIB software library (FSL) version 5.0.10 (Smith et al., 2004) (www.fmrib.ox.ac.uk/fsl). First, structural images were brain-extracted and GM-segmented before being registered to the Montreal neurological institute (MNI) 152 standard space using non-linear registration (Andersson, Jenkinson, & Smith, 2007). The resulting images were averaged and flipped along the x-axis to create a left-right symmetric, study-specific GM template. Second, all native GM images were non-linearly registered to this study-specific template and "modulated" to correct for local expansion (inflation) and contraction (deflation) due to the non-linear component of the spatial transformation. The modulated GM images were then smoothed with sigma = 2 corresponding to a Gaussian smoothing kernel of about 4.6 mm full width at half maximum. These maps were then subjected to statistical analyses (see

below).

2.1.5. Statistical analysis

The VBM processed data were statistically analyzed using group comparisons. Voxel-wise general linear models across the whole brain were applied using permutation-based non-parametric testing that also corrects for multiple comparisons across space (FSL' s randomise tool, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomise). The threshold free cluster enhancement technique was used in addition (Smith & Nichols, 2009). Error probability was set at $p < 0.05$ corrected for multiple comparisons using 5000 permutations of the group labels. Demographics and clinical characteristics were analyzed using IBM SPSS (Version 25.0. Armonk, NY: IBM Corp.) by calculating two-sample t-tests and comparing the MDD group and the HC group (see Table 1).

2.2. Meta-Analysis

2.2.1. Publication selection

The meta-analysis was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher, Liberati, Tetzlaff, Altman, & Group, 2010). The selection criteria for the meta-analysis consisted of (1) studies investigating individuals with MDD compared to HC, and (2) methodologies including whole-brain VBM. (3) At least one peak with a structural GM difference in the insular cortex had to be reported. Peaks referred to as 'insula/operculum' were not included in the meta-analysis because the insular cortex was our region of interest. (4) Talairach or MNI coordinates had to be provided. (5) The study had to provide information about MDD diagnosing process and (6) investigated subjects had to be at least 18 years old. (7) The article had to be published in a peer-reviewed journal. A comprehensive literature search was conducted using PubMed, Web of Science, PsycINFO, Scopus, Google Scholar in order to identify peer reviewed English articles investigating neuroanatomical differences in MDD using the following keywords: MDD, reduced grey (or gray) matter volume, major depressive disorder, depression, insula, insular cortex, MRI, VBM, voxel-based morphometry, structural change. Additional articles were found by reviewing the references of studies selected for the meta-analysis. Our search criteria yielded a total of 139 peer-reviewed published articles after duplicates were removed. Only coordinates of reduced GM were extracted. Of the 139 articles, 14 studies met the inclusion criteria. Two authors (IM and MF) reviewed those 14 articles and any inconsistencies were discussed to reach a consensus. The PRISMA Flow Diagram (Fig. 1) illustrates the study selection process and studies meeting inclusion criteria are listed in Table 2.

2.2.2. ALE meta-analysis

Descriptive information (authors, journal and publication year, sample size, age, medication, gender) and MNI coordinates of significantly reduced grey matter measures in MDD compared to healthy controls were extracted from each article. Studies that reported their results in Talairach coordinates were converted to MNI space using GingerALE (version 2.3.6; available under http://brainmap.org). Across 14 studies that met inclusion criteria the anatomical likelihood estimate (ALE) technique was applied using GingerALE. The ALE meta-analysis method was originally developed by (Turkeltaub, Eden, Jones, & Zeffiro, 2002) for coordinate-based meta-analysis and the current version of the software that has been used is described in (Eickhoff et al., 2009). The analysis was performed using GingerALE with the following statistical settings: cluster-level at $p < 0.01$ (uncorrected, with no assumptions to correlations within dataset) and 1000 permutations. Anatomical assignments were carried out with FSL viewer from FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) which uses primarily the Harvard-Oxford structural atlas.

3. Results

3.1. MRI (FSLVBM)

Significantly reduced grey matter volumes (GMV) were found in MDD in comparison to HC and left mid-insula ($p = 0.025$, 33 voxels, MNI coordinates x/y/z: -38/-4/-6) as well as in the right caudate nucleus ($p = 0.032$, 190 voxels, MNI coordinates x/y/z: 6/18/4) and left caudate nucleus ($p = 0.034$, 153 voxels, MNI coordinates x/y/z: -10/16/-8). These clusters are illustrated in Fig. 2.

3.2. ALE meta-analysis

In total, 14 studies investigating 662 patients (392 females and 270 males) with MDD und 559 healthy controls (329 females and 230 males), reporting a total of 117 separate neuroanatomical foci met the inclusion criteria and entered the ALE analysis. Age ranged for MDD from 27.75 to 79.4 years, and for HC from 28.61 to 79.5 years. One significant ALE cluster of less grey matter in MDD was found (434 mm^3) in left mid-insula (-38/6/2) extending into the left frontal operculum (-46/14/-2). These findings are illustrated in Fig. 3.

4. Discussion

In the present coordinate-based ALE meta-analysis, we found areas of reproducible grey matter reductions in MDD compared to HC in left mid-insula extending into IFG. The coordinates in the left mid-insula were at a similar location as in our MRI study. These findings together with the results from the MRI study that revealed reduced left mid-insular volumes in MDD compared to HC are in agreement with recent functional MRI studies that showed that interoceptive abnormalities in patients with MDD are associated with the mid-insula (Avery et al., 2014; Simmons et al., 2016). Individuals with depression oftentimes experience disturbances in sensory perceptions of the body such as being less accurate in heart beat perception than a control group (Dunn, Dalgleish, Ogilvie, & Lawrence, 2007; Terhaar, Viola, Bar, & Debener, 2012) and also experience alterations in pain perception (Klauenberg et al., 2008). The mid-insula has been considered to be an integration area, where interoceptive information from the posterior insula is represented (Uyanikgil et al., 2018). Craig postulated a posterior to anterior functional gradient assuming that sensory information about the body`s physiological state is mapped in the posterior insula and re-represented in the mid and anterior insula where it becomes consciously accessible, enabling a general subjective affective experience (2009, Craig, 2003). The mid-insular cortex may be functional distinct from the dorsal and ventral anterior insula. The mid-insula has been described to be involved in sensorimotor processing such as hand- and foot-movement (Mutschler et al., 2009).

As mentioned above, the dorsal anterior insula may be involved in language and perception of vocalizations (Mutschler et al., 2009), cognition (Deen et al., 2011) and the ventral anterior insula may be involved in peripheral physiological processing resulting from emotional experiences (Mutschler et al., 2009).

It is important to note that interoceptive awareness is also affected in other mental disorders than MDD such as in autism spectrum disorders, schizophrenia, anorexia nervosa, and post-traumatic stress disorder (Brooks et al., 2011; Cauda et al., 2011; Herringa, Phillips, Almeida, Insana, & Germain, 2012; Honea, Crow, Passingham, & Mackay, 2005; Kerr et al., 2016) and reduced grey matter in the insula has also been described in autism spectrum disorders, schizophrenia, anorexia nervosa, and post-traumatic stress disorder (Brooks et al., 2011; Cauda et al., 2011; Herringa et al., 2012; Honea et al., 2005) suggesting that volume reduction in the (mid) insula seems not to reveal a trait-related biomarker for MDD specifically, but may be rather associated with dysfunction in interoceptive processing which can be affected in other mental disorders as well.

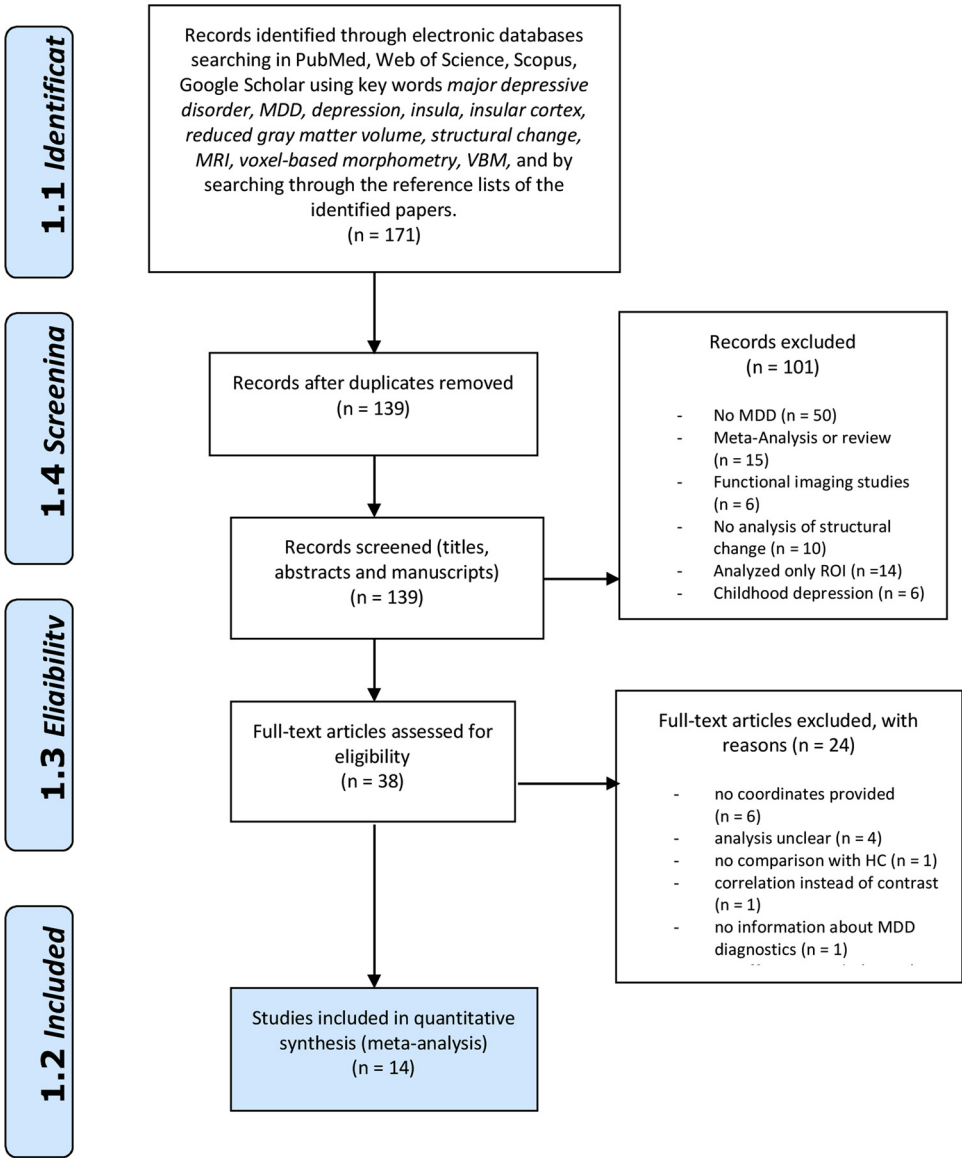

Fig. 1. PRISMA Flow Diagram illustrates study selection process for ALE meta-analysis (Moher et al., 2010).

In the MRI study, we also found a significantly reduced GMV in MDD in comparison to HC in the right and left caudate nucleus. This finding is in agreement with research studies showing a role of the caudate nucleus in patients with depression: Smaller caudate nucleus volumes have been described in depressed individuals in comparison to HC (Kim, Hamilton, & Gotlib, 2008; Krishnan et al., 1992; Pizzagalli et al., 2009). As interpretation of these findings it has been suggested that it may reflect a degeneration of striatal neurons that are involved in the regulation of mood (Krishnan et al., 1992). The caudate nucleus has been described as being part of a network that mediates emotional and motivational processes (Delgado, Stenger, & Fiez, 2004; Haber & Calzavara, 2009). Research studies revealed that caudate nuclei are connected with the insular cortex (Chikama, McFarland, Amaral, & Haber, 1997; Ghaziri et al., 2018). Recent research shows that the insula and caudate activity co-respond differently in depressed individuals versus control subjects when faced with economic decisions (Engelmann, Berns, & Dunlop, 2017). The findings from our MRI study suggest that the insula and the caudate nucleus may represent a specific network which may be affected in patients with MDD.

4.1. Limitations

Both studies, the MRI study and the meta-analysis have some potential limitations. First, the broad age range is a weakness for both analyses (VBM and meta-analysis). Future research preferably with a larger MDD sample should investigate potential age-related changes in the cortex.

Second, the findings of the meta-analysis could be affected by publication biases, as we did not take into account unpublished findings (Sutton, 2005).

Furthermore, MDD has also been reported to be associated with reduced GM in other brain regions such as the amygdala, hippocampus, and the cingulate cortex (Lee et al., 2011; Vasic, Walter, Hose, & Wolf, 2008). In our MRI study, we did not find reduced GM in the amygdala or hippocampus which can be due to the fact that our patients were unmedicated. In the study by Lee et al., 2011 two-thirds of the patients were taking antidepressant medications. In the study by Vasic et al., 2008 all patients with MDD were treated with antidepressant medications. In our meta-analysis, we did not find reduced GM in other brain regions either. The small number of MRI studies investigating heterogeneous MDD samples included in the meta-analysis may have resulted

Table 2

MRI studies included in the meta-analysis. Description of the 14 MRI studies investigating patients with MDD in comparison to HC. In 13 studies patients with MDD were diagnosed according to DSM-IV, in 1 study according to ICD-10; AD = Antidepressant; F = Female; M = Male; NA = not available; with/without Sui = with/without suicide attempt; responder: those who showed a 50% decrease of HDRS-17 score after treatment, compared to their initial score; ° Study did not include individuals with other mental disorders (other than anxiety disorders); * No exclusion of other mental disorders (other than anxiety disorders); + No information given regarding comorbidity. Three studies investigated individuals with MDD that were medication-free, 10 studies investigated individuals with MDD with medication. One study did not provide information (NA).

References	Sample Size	Medication	Age, years (mean/range)	Effects
(Hwang et al., 2010)*	70 MDD (all M)	NA	MDD: 79.4	Contrast: late-onset geriatric MDD < HC
	26 HC (all M)		HC: 79.5	Claustrum Culmen Cuneus Inferior frontal gyrus Inferior parietal lobule Inferior semilunar lobule Insula Lentiform nucleus Medial frontal cortex Midbrain Middle frontal gyrus Postcentral gyrus Posterior cingulate cortex Precentral gyrus Superior frontal gyrus Superior parietal lobule Superior semilunar lobule Superior temporal gyrus
(Igata et al., 2017)°	27 MDD (first-episode; 12 F/ 15 M)	No (medication-naïve)	MDD: 45.8	Contrast: first-episode treatment-naïve MDD < HC
	47 HC (12 F/ 35 M		HC: 41.2	Insula
(Jung et al., 2014)°	26 MDD non-responder (19 F/ 7 M)	Yes (ADs, benzo- diazepines)	MDD non-responder: 40.8	Contrast: non responder MDD < HC
	24 MDD responder (17 F/ 7 M)		MDD responder: 43.0	Insula
	29 HC (21 F/ 8 M)		HC: 43.6	Superior frontal gyrus Contrast: responder MDD < HC Insula
(Lai & Wu, 2014)°	38 MDD (first-episode; 20 F/ 18 M)	No (medication-naïve	MDD: 36.57	Contrast: first-episode medication-naïve MDD < HC
	27 HC (15 F/ 12 M)		HC: 38.29	Insula Middle frontal gyrus Superior frontal gyrus
(Lee et al., 2011)+	47 MDD (42 F/ 5 M)	2/3 with medication (ADs, benzodiazepines)	MDD: 46.0	Contrast: MDD < HC
	51 HC (45 F/ 6 M)		HC: 45.7	Amygdala Central lobule (cerebellum) Fusiform gyrus Hippocampus Lingual gyrus Midbrain encompassing raphe nucleus Middle temporal gyrus Nucleus accumbens Short insular gyrus Subgenual anterior cingulate gyrus Thalamus
(Leung et al., 2009)°	17 MDD (all F)	Yes (ADs, antipsychotics, benzodiazepines, hypnotics)	MDD: 45.5/ 30-61	Contrast: MDD < HC
	17 HC (all F)		HC: 45.8/ 28-58	Angular gyrus Anterior cingulate gyrus Fusiform gyrus Inferior frontal gyrus Insula Median cingulate gyrus Middle frontal gyrus Middle temporal gyrus Precentral gyrus Precuneus Superior frontal gyrus Superior temporal gyrus
(Peng et al., 2011)°	22 MDD (14 F/ 8 M) 30 HC (19 F/ 11 M)	5 patients with AD	MDD: 46.7 HC: 45.9	Contrast: first-episode MDD < HC Anterior Insula Cerebellum Inferior frontal gyrus Middle frontal gyrus Middle temporal gyrus Orbitofrontal gyrus

(*continued on next page*)

Table 2 (*continued*)

References	Sample Size	Medication	Age, years (mean/range)	Effects
				Parahippocampal gyrus Superior temporal gyrus
(Peng et al., 2014)°	20 MDD with Sui (13 F/ 7 M) 18 MDD without Sui (12 F/ 6 M) 28 HC (13 F/ 15 M)	Yes (ADs)	MDD with Sui: 27.75/ 18-45 MDD without Sui: 31.06/ 18-45 HC: 28.61	Contrast: non suicidal MDD < HC Insula Contrast: suicidal MDD < HC Middle temporal gyrus
(Qi et al., 2014)°	18 MDD without anxiety disorder (11 F/ 7 M) 20 MDD with anxiety disorder (9 F/ 11 M) 28 HC (13 F/ 15 M)	Yes (ADs)	MDD without anxiety disorder: 31.06/ 18-45 MDD with anxiety disorder: 28.65: 18-45 HC: 28.61	Contrast: MDD without anxiety < HC Insula
(Serra-Blasco et al., 2013)°	22 MDD (first-episode; 15 F/ 7 M) 22 MDD (remitted-recurrent; 20 F/ 2 M) 22 MDD (chronic; 18 F/ 4 M) 32 HC (23 F/ 9 M)	Yes (ADs, mood-stabilizer, antipsychotics, benzodiazepines	MDD first: 44 MDD remitted-recurrent: 48 MDD chronic: 49 HC: 46	Contrast: chronic MDD < HC Cingulate gyrus Inferior frontal gyrus Insula Medial frontal gyrus Parahippocampal gyrus Post-central gyrus Superior frontal gyrus Transverse-temporal gyrus
(Soriano-Mas et al., 2011)°	70 MDD (41 F/ 29 M) 40 HC (23 F/ 17 M)	Yes (ADs)	MDD: 61.56/ 37-82 HC: 59.23/ 49-76	Contrast: melancholic MDD < HC Posterior insula
(Sprengelmeyer et al., 2011)°	27 MDD (16 F/ 11 M) 51 HC (33 F/ 18 M)	Yes (ADs, lithium)	Study 1: MDD: 49.7 HC: 49.9 Study 2: MDD: 45.6 HC: 42.0	Contrast: MDD < HC Amygdala Fusiform gyrus Insula Middle temporal gyrus Parahippocampal gyrus Superior frontal gyrus
(Stratmann et al., 2014)°	35 MDD (first; 21 F/ 14 M)) 97 MDD (recurrent; 55 F/ 42 M) 132 HC (74 F/ 58 M)	Yes (ADs, antipsychotics)	MDD first: 34.86 MDD recurrent: 38.94 HC: 37.82	Contrast: MDD < HC Anterior insula Parahippocampal gyrus Superior parietal lobule Superior temporal gyrus
(Tae, 2015)°	20 MDD (all F) 21 HC (all F)	No (no medication at least three months before MRI scanning)	MDD: 42.3/ 17-56 HC: 42.45/ 24-58	Contrast: MDD < HC Amygdala Anterior insula Cingulate gyrus Fusiform gyrus Gyrus ambient Hippocampus Inferior frontal gyrus Lingual gyrus Parahippocampal gyrus Thalamus

in low statistical power and might explain why we did not find reduced GM in other brain regions than the insula too.

Finally, in our MRI study, 7 out of 26 patients with MDD also fulfilled diagnostic criteria for an anxiety disorder and our group of patients with MDD scored significantly higher in the questionnaire that assesses trait anxiety (STAI-Trait) (Laux et al., 1981). In our meta-analysis of previously published MRI research several studies did investigate MDD patients with comorbid anxiety disorders (Stratmann et al., 2014; Lai & Wu, 2014; Qi et al., 2014; Sprengelmeyer et al., 2011; Tae, 2015). A recent study reported reduced insular volume in patients with social anxiety disorder (Kawaguchi et al., 2016). However, the authors did not specify in which subregions they found insular volume reductions and also reported that 4 out of 13 patients did also fulfill the diagnostic criteria for MDD. It has also been discussed whether regressing out anxiety in depressed patients is appropriate at all and whether an analysis of covariance is the best statistical model to achieve that aim. “If we compare a sample of depressed patients with non-patient controls and co-vary out anxiety, which happens to be higher in the patients, it is not necessarily the case that the residual group difference is a clear, clean representation of depression as it would exist without the comorbid anxiety (Miller & Chapman, 2001). What we should believe about that depends on our model of the relationship between depression and anxiety. If they happen to co-occur because of nonspecific severity factors that themselves are not specifically related to depression, our ANCOVA might be effective in removing such variance, leaving "pure" depression. If, however, we believe that the negative affect that depression and anxiety share is central to the concept of depression, then removing negative affect (by removing anxiety) will mean that the group variance that remains has very poor construct validity for depression (Miller & Chapman, 2001).” On this background, future research is needed to investigate how specific insular volume reductions are for patients with MDD.

4.2. Conclusions

In summary, the data in our current MDD patients confirm the findings or our coordinate-based meta-analysis of previous MRI studies in other MDD patients that the mid-insula grey matter volumes is

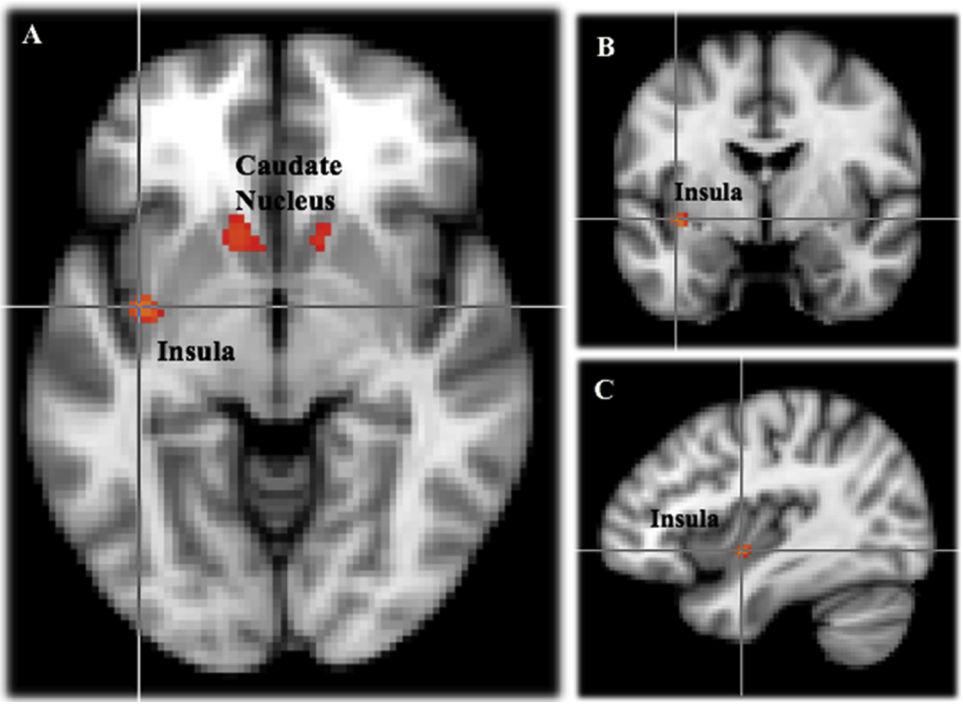

Fig. 2. MRI Study: Significantly decreased grey matter volumes (GMV) were found in MDD in comparison to HC and left mid-insula (p = 0.025, 33 voxels, MNI coordinates x/y/z: -38/-4/-6) as well as in the right caudate nucleus (p = 0.032, 190 voxels, MNI coordinates x/y/z: 6/18/4) and left caudate nucleus (p = 0.034, 153 voxels, MNI coordinates x/y/z: -10/16/-8). A) Shows axial, B) coronal, and C) sagittal view.

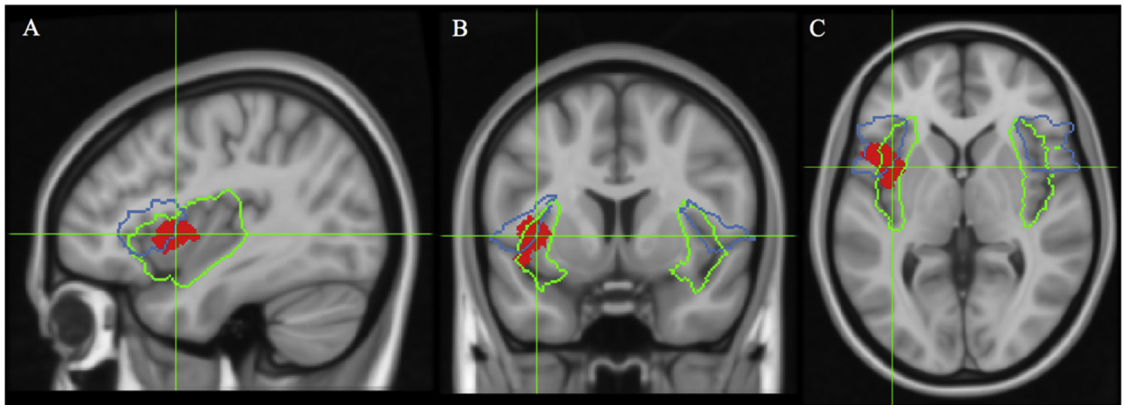

Fig. 3. ALE Meta-analysis: One significant ALE cluster (illustrated in red; 4.34 mm^3 in size) of less grey matter in MDD compared to HC (healthy controls) was found in left mid-insula (-38/6/2) extending into the left frontal operculum (-46/14/-2). The insula is outlined in a green contour and the frontal operculum is outlined in a blue contour. Note that the two regions of interest overlap in their extension because both regions of interest are derived from unthresholded probability maps. The findings in the left mid-insula where at a similar location as the findings in the insula in the MRI study (see Fig. 2). The results are shown at -38/6/2 (MNI x/y/z coordinate). A) Shows sagittal (at x = -38), B) coronal (at y = 6), and C) axial (at z = 2) view (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

reduced. Thus, this insula subregion might be associated with the pathophysiology of MDD. It has been shown to be related with reduced interoceptive abilities in patients with MDD. In addition, our MRI data revealed reduced caudate nucleus volumes. The caudate nucleus has been described as being part of a network that mediates emotional and motivational processes that is connected with the insula. Because our meta-analysis did not yield alterations in these specific regions, this finding needs to be further validated.

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Declaration of competing interest

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CRediT authorship contribution statement

Isabella Mutschler: Conceptualization, Formal analysis, Fund acquisition, Methodology, Project administration, Software, Validation, Visualization, Writing original – draft, Writing review & editing. **Jürgen Hänggi:** Conceptualization, Formal analysis, Methodology, Project administration, Software, Validation, Visualization, Writing review & editing. **Manuela Frei:** Formal analysis, Validation, Visualization. **Roselind Lieb:** Resources, Supervision, Validation, Writing review & editing. **Martin grosse Holforth:** Conceptualization, Validation, Resources, Writing review & editing. **Erich Seifritz:** Funding acquisition, Resources, Supervision, Validation. **Simona Spinelli:** Conceptualization, Formal analysis, Fund acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing original – draft, Writing review &

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