Jere Kiiski

GREY MATTER ALTERATIONS IN SUBCLINICAL DEPRESSION, ANXIETY AND INSECURE ATTACHMENT: A VOXEL-BASED MORPHOMETRY STUDY

Syventävien opintojen kirjallinen työ
Kevätlukukausi 2021

Jere Kiiski

GREY MATTER ALTERATIONS IN SUBCLINICAL DEPRESSION, ANXIETY AND INSECURE ATTACHMENT: A VOXEL-BASED MORPHOMETRY STUDY

Kliininen laitos

Kevätlukukausi 2021

Vastuuhenkilö: Lauri Nummenmaa

UNIVERSITY OF TURKU
Faculty of medicine

KIISKI, JERE:
Grey matter alterations in subclinical depression, anxiety and insecure attachment : A voxel-based morphometry study

Literary work of advanced studies, 20 pages.
Psychiatry
March 2021

The aim of this study was to investigate grey matter alterations in subclinical depression, anxiety and insecure attachment. Attachment is an inborn drive to create and maintain emotional relationships, through which individuals feel safe and protected. It can be categorically divided into secure and insecure attachment styles. Insecure adult romantic attachment has been linked with a range of behavioral problems such as depression, anxiety, aggression and attention deficit hyperactivity disorder. Studying subclinical depressive and anxiety symptoms could help in understanding the pathophysiology of these disorders and it could also improve early diagnostics and treatment.

The subject data used in this study comprised of healthy control groups from previous studies done in Turku PET Centre, University of Turku. Subjects underwent T1-weighted magnetic resonance imaging (MRI) and completed questionnaires measuring depression (BDI-II), anxiety (STAI) and insecure social attachment (ECR-R). ECR-R divides insecure attachment further into avoidant and anxious attachment styles. The correlation between brain GM density and questionnaire scores was measured using voxel-based morphometry, which is a method for automatic quantification of grey (and white) matter densities from T1-weighted magnetic resonance images.

A new common neurological substrate was found for subclinical depression and insecure attachment. Both subclinical depression and avoidant attachment were negatively associated with GM density in the midline of the brain (especially in the anterior cingulate and superior medial prefrontal cortex). This finding suggests that the midline of the brain has a role in the pathophysiology of depression and avoidant attachment as well. Subclinical anxiety was positively associated with GM density in areas of the brain that have previously been linked with fear and anxiety: in the temporal lobe (including fusiform gyrus, amygdala, hippocampus and cerebellum). These findings can be used to improve early diagnostics of depression and anxiety and to improve current neuromodulatory interventions in these disorders.

Key words: depression, anxiety, attachment, grey matter

Contents

Grey matter alterations in subclinical depression, anxiety and insecure attachment: A voxel-based morphometry study

1 Background and aims

The neurobiological basis and pathophysiology of depressive and anxiety disorders remains unclear. Though distinctive patterns of grey matter (GM) alterations have been discovered in major depressive disorder, bipolar disorder and social anxiety disorder [1-3], effects of subclinical depressive and anxious symptoms on GM density (GMD) are still ambiguous. A dimensional approach suggests that a spectrum of disease severeness and psychopathology exists in these disorders. Rather than dividing depressed or anxious subjects categorically in to healthy or sick, this approach looks at the extent of one's symptoms. Studying subclinical symptoms could help to better understand the psychopathology spectrum in depression and anxiety disorders.

Insecure adult romantic attachment has been linked with a range of behavioral problems such as depression, anxiety, aggression and attention deficit hyperactivity disorder [4]. The attachment theory, originally developed by John Bowlby and later elaborated by Mary Ainsworth , states that attachment is an inborn drive to create and maintain emotional relationships, through which individuals feel safe and protected. Links between insecure attachment, depression and anxiety raises a question whether there are common structural correlates in these disorders. In this study we further investigate the link between insecure attachment, depression, anxiety and brain structure with voxel-based morphometry (VBM), which is a method for automatic quantification of grey (and white) matter densities from T1-weighted magnetic resonance images.

According to the Attachment theory, attachment styles and patterns are created already in infants through emotional relationships to their caregivers. These early life emotional relationships create every individual's "internal working model"; an understanding of self-worth and how one should be treated by other relationship partners. Attachment patterns that are molded by this internal working model already in childhood, are thought to be carried out into adulthood as well. Sensitive, consistent and supportive caregiving is likely to promote a secure attachment style in infants. [4] Attachment styles are best reviewed dimensionally as a continuum, which at it’s simplest can be divided into secure and insecure dimensions. Furthermore, insecure attachment can be divided into

avoidant and anxious dimensions.[5] The attachment theory can be applied to romantic relationships in adulthood as well. Both avoidant and anxious attachment in adults is associated with relationship dissatisfaction, distrust of partners, low tendency to forgive, low interpersonal competence and low relationship regulation. Low emotional expressiveness, low self-disclosure, and low investment in family rituals has been specifically linked with avoidant attachment in adults. Anxious attachment in adults is specifically associated with coercive and dominating conflict tactics, maladaptive attributions of negative partner behavior and avoidance motives for sacrificing.[4] Securely attached adults are more satisfied with their romantic relationships compared to insecurely attached adults [4,6]. They are also less likely to suffer from depression, anxiety or excessive aggression [4]. Insecure attachment in adults is associated with depression, anxiety, aggression and attention-deficit disorder as well as inflammation-related conditions across the lifespan [4,7]. Still, it is unclear whether there are common structural correlates between subclinical depression, anxiety and insecure attachment.

To this date, no studies have directly compared the structural basis of attachment styles, depression and anxiety. Insecure adult romantic attachment in adulthood has been associated with brain GM alterations in previous studies, but findings are inconsistent [8-11]. GM alterations have been described in the left insula, pars opercularis of the left inferior frontal gyrus [8], left middle temporal and right parahippocampal gyrus , right ventral anterior cingulate gyrus, right middle occipital gyrus [9], left hippocampus [10], anterior temporal pole and left lateral orbital gyrus [11]. VBM studies on the subclinical aspect of depression and anxiety are currently scarce too. Evidence exists for negative association between subclinical depression and GMD in the anterior cingulate, hippocampus and amygdala [12]. Subclinical anxiety has been associated with GM alterations in the amygdala, parahippocampus, orbitofrontal cortex and anterior cingulate [13]. These findings remain inconsistent, as studies have found both positive and negative correlations between GMD and subclinical anxiety in these areas [13].

There is accumulating neuroimaging evidence for shared brain abnormalities in phenotypically similar diseases such as schizophrenia, depression, anxiety and bipolar disorder [14,15]. According to a recent meta-analysis, bipolar disorder and schizophrenia have partly overlapping GM reductions in the anterior cingulate and bilaterally in the anterior insula [14]. A 2015 meta-analysis comprising of 193 VBM-studies found that GM loss in the dorsal anterior cingulate cortex and in the right and left insula was characteristic for six diverse diagnostics groups (schizophrenia, bipolar disorder, depression, addiction, obsessive-compulsive disorder and anxiety [15]. Major depressive disorder has been associated with GM loss in the anterior cingulate, bilaterally in the insula and in the prefrontal

cortex in multiple studies [1,2]. Social anxiety disorder has been associated with larger GM volume in the left precuneus, right supplementary motor area and middle occipital gyrus and with smaller GM volume in the left putamen [3].

Shared structural and functional alterations in psychiatric diseases could be used as targets for new interventions. It could also be that targets for interventions are harder to find if there are no structural differences. Repetitive transcranial magnetic stimulation (rTMS) , electroconvulsion therapy and other neuromodulatory approaches could benefit from more precise structural correlates in these disorders. Currently there are no strict guidelines for rTMS interventions in mild psychiatric disorders and new evidence for it's use in these milder forms is needed [16]. Finding a common neurobiological substrate for mental illness could help bring new diagnostic applications to the table as well. A substrate existing already in mild and subclinical cases of depression and anxiety would be especially important for early stage detection and intervention in these disorders. Our subclinical approach in this study is important both in an interventional and diagnostical aspect.

We study and compare the effects of subclinical depression, subclinical anxiety and social attachment on brain GMD to reveal any links between the neurobiological basis of these disorders. We hypothesized that subclinical depressive and anxiety symptoms would be negatively correlated with GMD in the limbic system, specifically in the medial prefrontal cortex much in the same way of major depressive disorder, bipolar disorder and social anxiety disorder. We expected that insecure adult attachment style (avoidant and anxious) would produce similar effects in the limbic system to depressive and anxiety symptoms.

2 Methods

The data used in this study comprised of healthy control subjects with no psychiatric conditions from previous studies done in Turku PET Centre, University of Turku. Subjects underwent T1-weighed brain magnetic resonance imaging and completed questionnaires measuring depression, anxiety and insecure social attachment. VBM was then used to analyze the relationship between each questionnaire's points and brain structure alterations. VBM analysis was implemented via matlab r2016b-software. Statistical analysis was done with SPM12-software.

2.1 Participants

The subject data used in this study comprised of healthy control groups from previous studies done in Turku PET Centre, University of Turku. A total of 255 subjects with no previous psychiatric illnesses were included, of which 180 (70.6%) were men and 75 (29.4%) women. Mean age was 29.7 years, standard deviation (s.d) 9.97 years, ranging from 19 to 58. Subjects were screened for anatomical brain anomalies by radiologist. Criteria for control subjects varied slightly across projects, but subjects with psychiatric and neurological diagnoses were always excluded. Table 1 contains complete participant data.

2.2 Questionnaires

We used the Beck depression inventory (BDI-II) as a measure of depressive symptoms. It is a 21-item self-report questionnaire ,which is widely used in healthcare to determine the severity of depressive symptoms. BDI-II contains eight items for affective symptoms and thirteen for somatic symptoms. It is available in many languages and it can be used in both clinical and nonclinical populations [17]. According to the original BDI-II guidelines the cutoff points are as follows: 0-13 for minimal range, 14-19 for mild depression, 20-28 for moderate depression and 29-63 for severe depression [18]. Recently however, different cutoffs have been suggested for different populations. For non-clinical populations, a range of 10-16 has been suggested as a cutoff point to detect major depression [19].

Anxiety symptoms were tested with the State trait anxiety inventory. It is a 20-item self-report questionnaire, which measures current anxiety symptoms and also the tendency to be anxious. Widely used in research and healthcare, it is a reliable and time-effective screening tool for non-clinical populations as well.[20] The Experiences in Close Relationships (ECR-R) was used to measure anxiety and avoidance behavior in social attachment. It is also a 36-item self-report questionnaire which measures anxiety and avoidance in relation to adult romantic attachment style. A Finnish version of all questionnaires mentioned above was used. Table 1 contains complete questionnaire data.

Table 1. Questionnaire and participant data.

Questionnaire	BDI	STAI	ECR-avoidance	ECR-anxiety	Total
Participants	250	221	160	161	255
Males (%)	177 (70.8)	148 (70.0)	99 (61.9)	62 (61.5)	180 (70.6)
Females (%)	73 (29.2)	73 (33.0)	61 (38.1)	62(38.5)	75 (29.4)
Mean age	29.6	30.5	31.5	31.7	29.7
S.d of age	9.99	10.0	10.6	10.6	9.97
Age range	19-58	19-58	19-58	19-58	19-58
Mean score	5.05	37.6	50.3	47.9	-
S.d of scores	5.55	9.22	16.6	14.5	-
Score range	0-30	24-66	19-100	19-96	-

2.3 Voxel-based morphometry

Voxel-based morphometry is a method for automatic quantification of grey (and white) matter densities from T1-weighted MR images. It involves first spatially normalizing the structural MRI data in to a standard stereotactic space, and segmenting grey matter, white matter, cerebrospinal fluid, bone, air and other soft tissues from it based on intensity differences in the MR signal. Second, the target tissue type (here grey matter) is extracted and smoothed for voxel-wise statistical analysis between subjects.[21]

We began by retrieving the control-subject data from the PET Centre PACS server. All MRI-data on the server were in DICOM-format and it had to be converted in to NIFTI-format. Then the NIFTI-format data were normalized and segmented to grey and white matter and cerebrospinal fluid were all separated from the images. 60 mm cut-off of spatial normalization was used, and light affine regularization was set to 0.0001. Next an additional modulation step was performed where volume changes resulting from spatial normalization were taken into account. Correction for differences in total brain size across subjects was also performed. Segmentation and normalization into MNI space was performed via DARTEL-script. DARTEL models the shape of the subject brains by aligning white- and grey matter between the images; the images are aligned iteratively to an average template data created from the sample. The resulting files from DARTEL were then smoothed and normalized to MNI space.

2.4 Statistical analysis

Multiple linear regression analysis was used to predict voxel-wise GM densities separately with scores from each questionnaire. Age and sex were used as nuisance covariates. Cluster level FDR -corrected p-threshold of 0.05 was applied and both negative and positive questionnaire contrasts along with sex and age contrasts were tested. Aal- and AICHA-atlases were used to label the effects.

3 Results

3.1 Questionnaire correlations

Statistically significant correlations were found between all questionnaire scores. BDI and STAI together had a correlation coefficient of 0.694, $p<0.001$. BDI and ECR-anxiety were also strongly correlated; $r=0.587$, $p<0.01$. BDI and ECR-avoidance had a correlation coefficient of 0.338, $p<0,01$. STAI and ECR-anxiety were correlated, $r=0.673$, $p<0.01$. STAI and ECR-avoidance were correlated, $r=0.371$, and $p<0.01$. ECR-anxiety and ECR-avoidance had a correlation coefficient of 0.437 and $p<0.01$. Age was not statistically significantly correlated with any of the questionnaire scores. Sex was statistically significantly correlated with STAI (-0.155) and ECR-anxiety (-0.192), $p<0.001$.Correlations are summed up in table 2.

Table 2. Questionnaire, age and sex correlations.

***. Correlation is significant at the 0.01 level (2-tailed).*

**. Correlation is significant at the 0.05 level (2-tailed).*

	BDI	STAI-score	ECR-anxiety	ECR-avoidance	Age	Sex
BDI	1					
STAI	.694**	1				
ECR-anxiety	.587**	.673**	1			
ECR-avoidance	.338**	.371**	.437**	1		
Age	.039	-.102	.039	.034	1	
Sex	-.117	-.155*	-.192*	-.098	-.393**	1

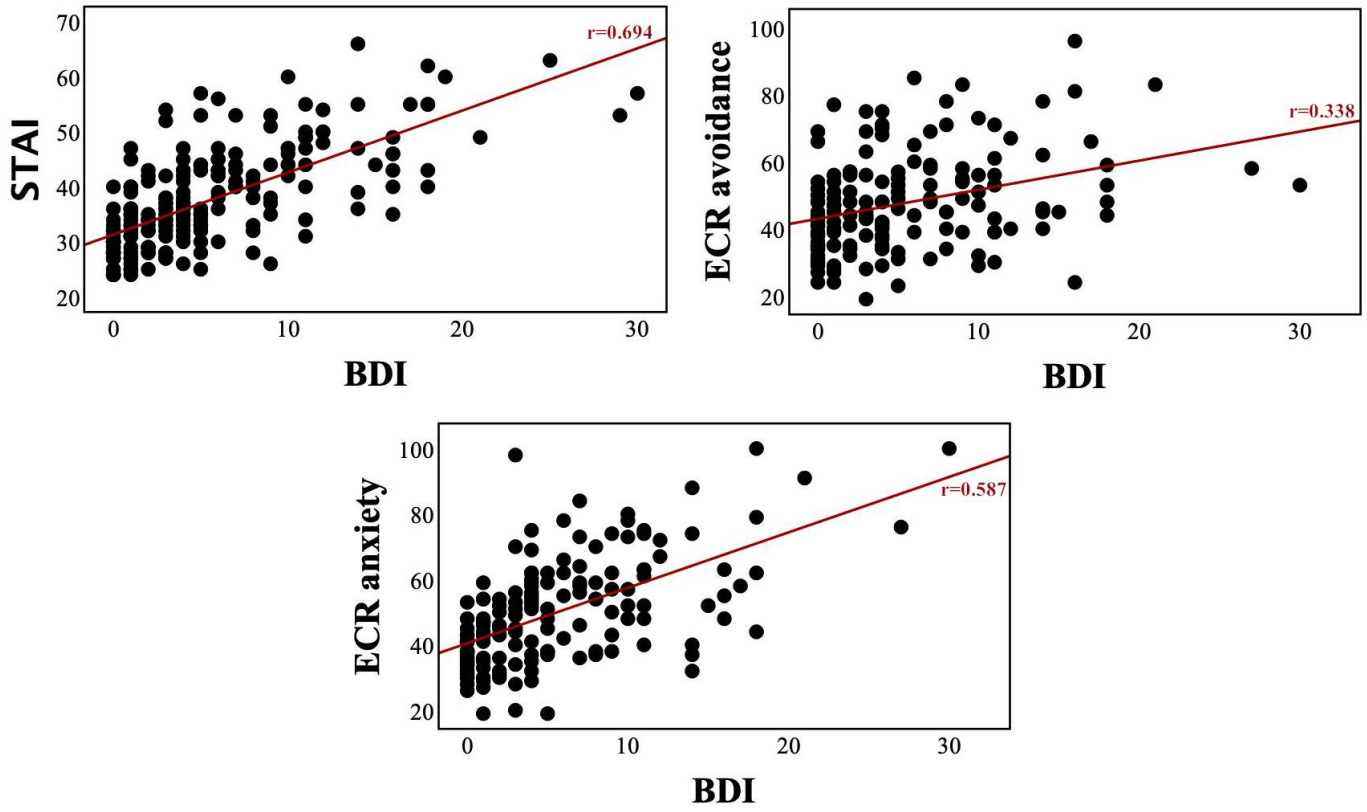

Figure 1. Questionnaire scores scatter plots.

3.2 Effects of depressive scores on grey matter density (GMD)

Depression scores were negatively correlated with GMD. Most of the effects were located medially between the hemispheres. Largest clusters were found in the prefrontal cortex and the cingulum. Distinct clusters were found also in the superomedial parts of the parietal and occipital lobes. Negative association with GMD was found in anterior and middle parts of the cingulum and in the superior medial prefrontal cortex. Inferomedial parts of the supplemental motor area showed negative association too. Negative association with GMD was also seen in the superior parts of precuneus, superior and inferior parietal lobule, paracentral lobule as well as medial parts of the cuneus. Negative association with GMD was quite symmetrical both in the left and right hemispheres. Results are shown in *figure 2*.

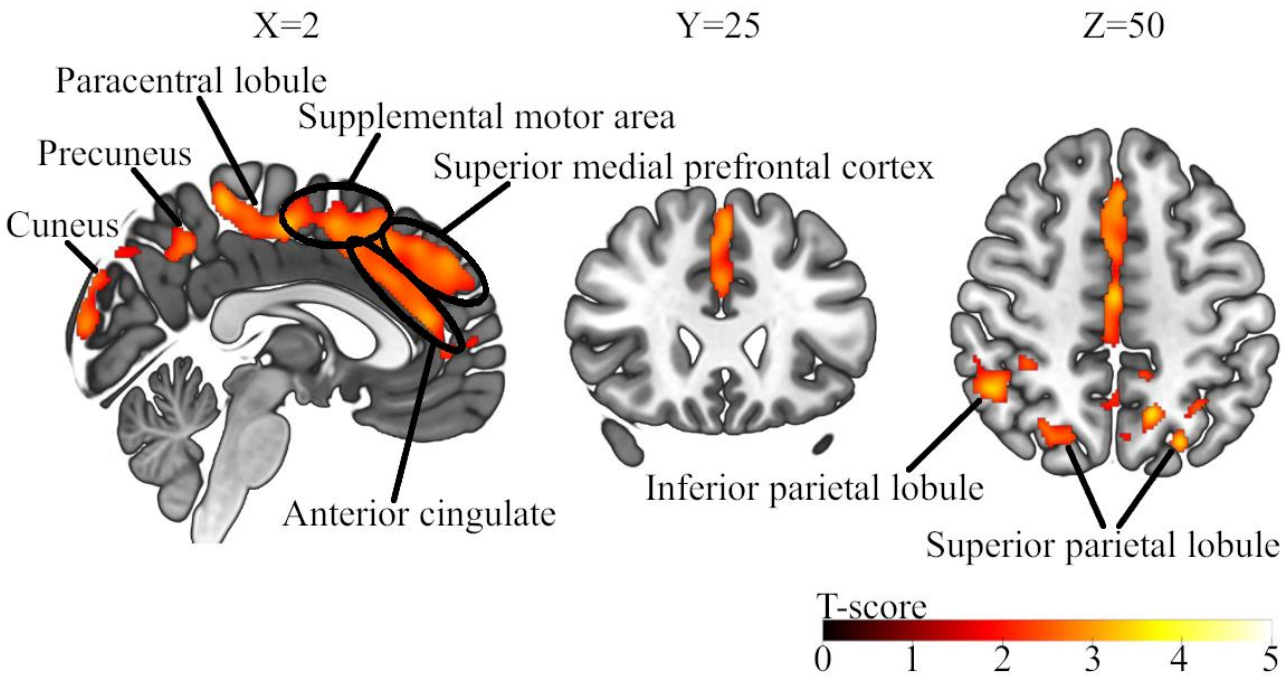

Figure 2. Negative association between GMD and BDI-II-scores. The data are thresholded at p<0.05, FDR-corrected.

3.3 Effects of ECR-avoidance scores on GMD

Attachment avoidance was negatively correlated with GMD. Grey matter alterations saw a similar pattern to the BDI negative contrast; GMD in the cingulum, superior medial prefrontal cortex and

supplemental motor area was negatively associated with avoidant attachment. GMD in the posterior cingulum and the most anteroinferior parts of the precuneus were negatively associated with avoidant attachment too. Negative association was found bilaterally in the lingual gyrus, unilaterally in the right side hippocampal area, superior parts of the temporal pole, in the most inferior parts of the orbital prefrontal cortex and insula. Results are shown in *figure 3*.

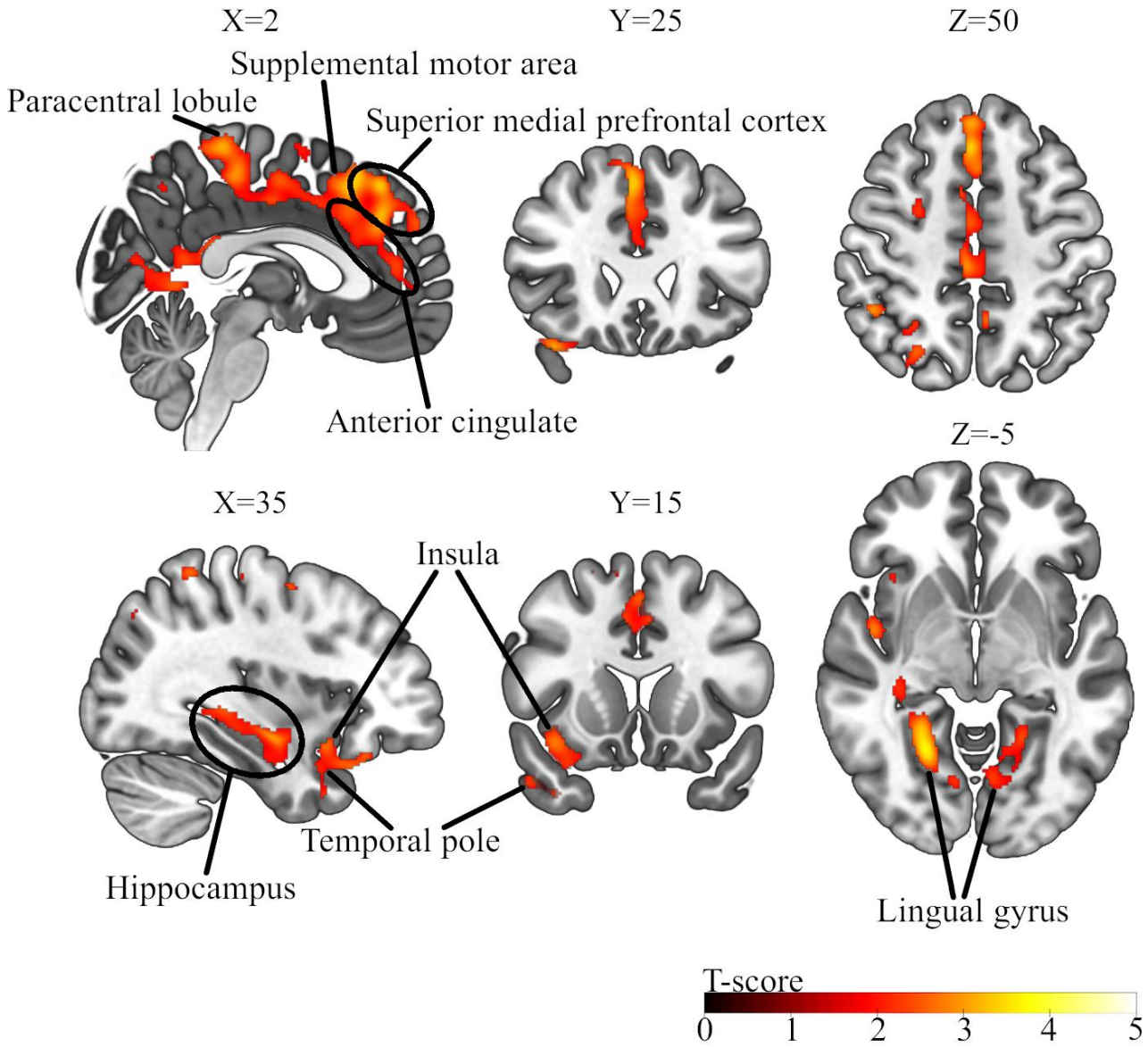

Figure 3. Negative association between GMD and ECR-avoidance scores. The data are thresholded p<0.05, FDR-corrected.

3.4 Overlap between depression and avoidance dependent effects

Both depressive symptoms and avoidant attachment were negatively correlated with GMD in the limbic system. Overlap in the cerebral areas affected by these two questionnaire contrasts was seen especially in the anterior cingulate and superior medial prefrontal cortex. Depression has more effects in the supplemental motor area, cuneus and precuneus. Avoidant attachment has broader effects in the middle cingulate. The right side effects of avoidant attachment in the hippocampus, insula and temporal pole as well as the effects in the lingual gyrus were absent in depression contrast. Overall there is big similarity in the effect patterns with the two contrasts in the frontal and parietal lobe. These similarities are shown in *figure 4.*

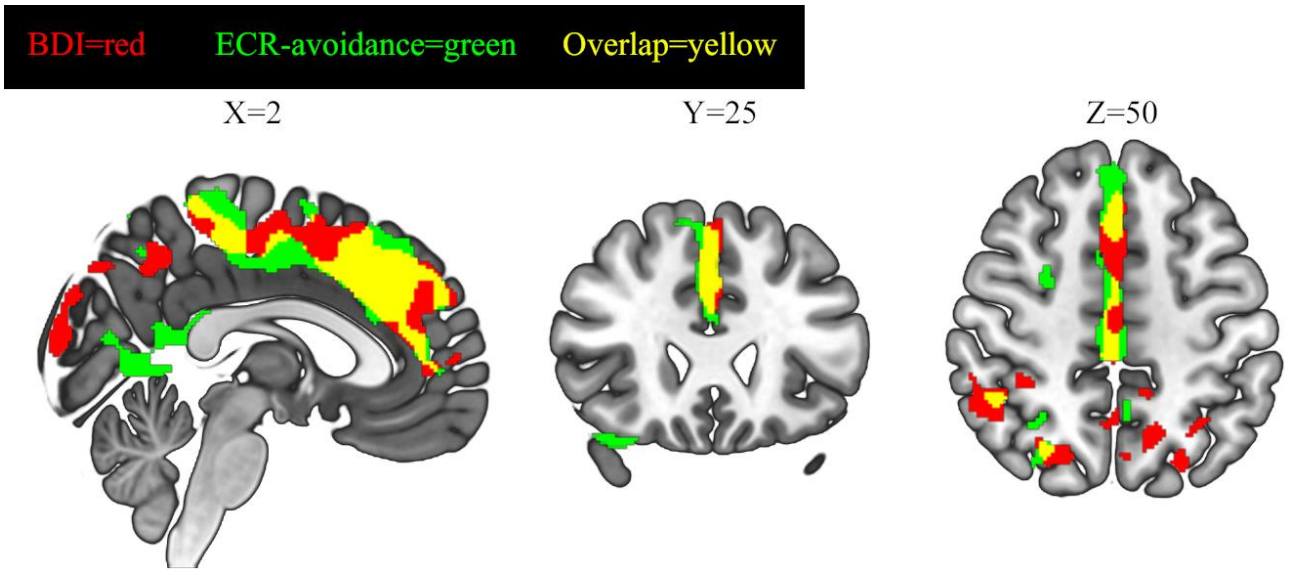

Figure 4. Comparing negative association between GMD and BDI-II- and ECR-avoidance-scores. The data are thresholded p<0.05, FDR-corrected.

3.5 Effects of anxiety scores on GMD

Positive STAI score contrast revealed one effect cluster. GMD in the temporal lobe and in the cerebellum was positively associated with STAI score. Middle parts of the fusiform gyrus and the hippocampal area showed positive association too. Hippocampal effects were located right next to the amygdala bilaterally, some effects could be seen in the amygdala and parahippocampal gyrus as well. Positive association with STAI score was found in the cerebellum, in the inner parts of the

cerebellar cortex around the white matter as well. Inferior and middle temporal and inferior occipital lobes showed positive association too. Results are illustrated in *figure 5.*

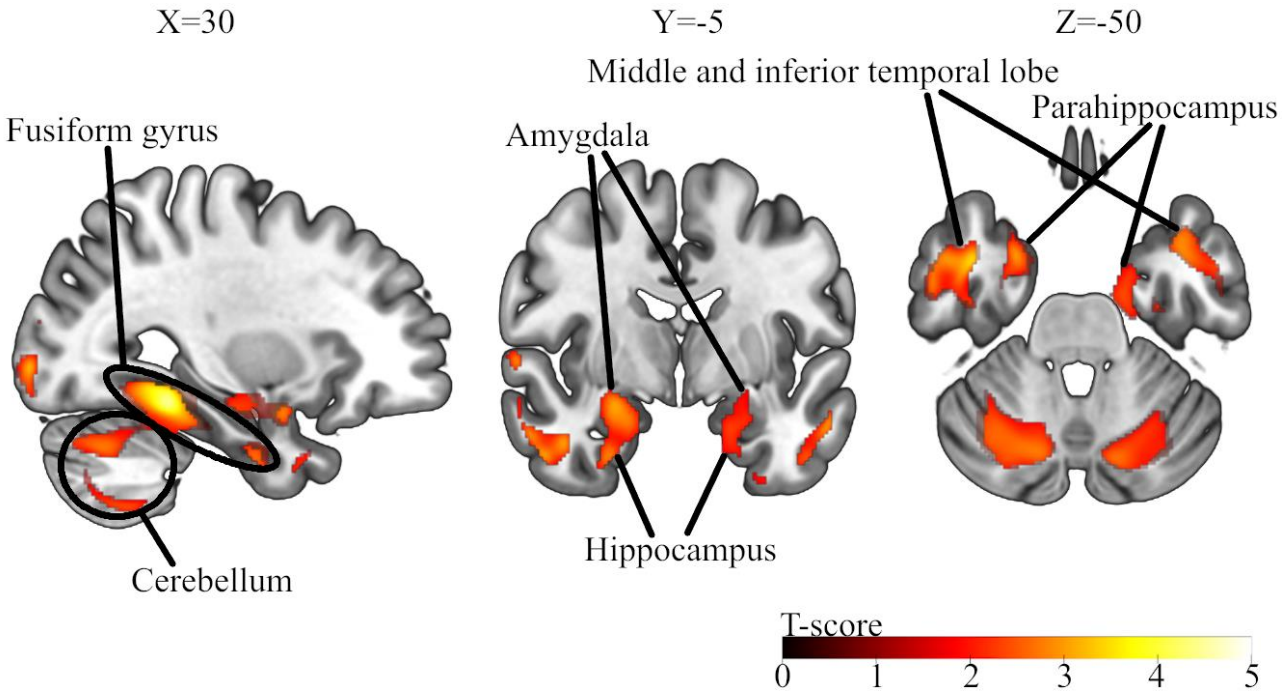

Figure 5. Positive association between GMD and STAI-score, the data are thresholded p<0.05, FDR-corrected.

4 Discussion

Our main finding was that self-reported depression, anxiety and avoidant adult romantic attachment scores were correlated with brain GM alterations. Depressive symptoms as well as avoidant adult romantic attachment trait were negatively correlated with GMD in the midline of the brain. Anxiety symptoms were positively correlated with GMD in the temporal lobes and cerebellum. These findings suggest that subclinical depression, anxiety symptoms and avoidant adult romantic attachment are correlated with widespread brain GM alterations.

4.1 Subclinical depression and GMD

Subclinical depression symptoms were negatively correlated with GMD in the medial prefrontal cortex, anterior and middle cingulum, supplemental motor area, paracentral lobule, superior parts of

precuneus, the superior and inferior parietal lobule, and the medial parts of the cuneus. The anterior cingulate has been negatively associated with subclinical depression in previous studies [12]. GM in the hippocampus and amygdala have also been reported to have negative associations with subclinical depression [12], but we didn't find such effects in our study. Similarly to previous studies, we found no association between subclinical depression and GMD in the insula or thalamus [12]. It is also worth to mention that sex likely moderates the negative association between subclinical depression and GMD in the brain [22]. In contrast to some previous studies [23] we found large effects in the anterior cingulate and other areas as well on a mixed population males and females.

A 2017 with 177 healthy subjects found a positive correlation with the Symptom Checklist-90-Revised (SCL-90-R) depression subscale score and GM volume in the pre- and postcentral gyri and left superior temporal cortex. The same study found negative correlation with the SCL-90-R somatization subscale and GM volume in the cerebellar vermis and right supplementary motor area.[13] Our study did not find positive correlations between BDI-II score and GMD in the brain. Also, we found broad negative correlation effects in the midline of the brain in our study, but not in the cerebellar vermis. Differences between the results of our study to this study could be due to the different structures of the questionnaires used in each study. SCL-90-R differentiates between depression and somatization, BDI-II combines affective and somatic symptoms to produce an overall score for depressive symptoms.

A 2016 longitudinal study with 358 healthy subjects found no significant relationship between current sub-threshold depressive symptoms and GM measures [24]. Again, the definition of subclinical depression symptoms and the inclusion/exclusion criteria need to be considered when comparing studies. In this study, the Centre for Epidemiological Studies Depression (CES-D) questionnaire was used to measure current depressive symptoms. The percentage of subjects with depressive questionnaire scores above the clinical cutoff point in this study (CES-D>10) was similar compared to our study (BDI-II>12); 10% and 9.6% respectively. This study had a subject population of elderly people which could result in different results compared to our study.

Our findings on subclinical depression partly resemble pervious findings in major depressive disorder (MDD) and bipolar disorder populations. Anterior cingulate cortex and medial prefrontal cortex have both been negatively associated with MDD and bipolar in previous studies [1,2]. However other areas affected in MDD such as the insula or the thalamus were not affected in our study nor in previous studies on subclinical depression. Still, our study suggests that GM alterations in

subclinical depression and MDD overlap to some extent with each other. These GM alterations in the anterior cingulate cortex and medial prefrontal cortex could be formed already in subclinically depressed individuals, and as symptoms progress, deeper subcortical structures such as the insula and the thalamus might get involved too. Effects on the superior medial prefrontal cortex, precuneus and the inferior parietal lobule suggest that the default mode network (DMN) has a role in the neurobiological base of subclinical depression. DMN is a resting-state network which is active during remembering, envisioning the future and during social inferences [25]. As stated previously, major depression and bipolar disorder seem to be negatively correlated with some areas of the DMN such as the anterior cingulate and medial prefrontal cortex.

4.2 Insecure adult romantic attachment trait and GMD

We found that attachment avoidance was negatively associated with GMD bilaterally in the ACC, superior medial prefrontal cortex, supplemental motor area, paracentral lobule and lingual gyrus and unilaterally in the right side hippocampus, insula and temporal pole. We didn't find any statistically significant associations between attachment anxiety scores and GMD in the brain. Previous studies on insecure adult romantic attachment are scarce. A 2018 study with 192 healthy subjects found that anxious attachment compared to avoidant attachment was significantly more positively associated with brain GM volume in the left insula and in the pars opercularis of left inferior frontal gyrus [8]. Another 2018 study with 106 healthy subjects found that attachment avoidance was negatively correlated with volume in the left middle temporal and right parahippocampal gyrus. The same study found that attachment anxiety was negatively correlated with the volume of the right ventral anterior cingulate gyrus. Also, in women attachment avoidance was negatively associated with volume in the right middle occipital gyrus, but in men the effect was opposite.[9] A 2010 study with 22 healthy young adults found that both attachment anxiety and avoidance were negatively correlated with left hippocampus GM concentration [10]. Another 2010 study with 32 healthy subjects found that high attachment related-anxiety was associated with decreased gray matter in the anterior temporal pole and increased gray matter in the left lateral orbital gyrus and that attachment avoidance was not associated with any statistically significant GM alterations [11].

Interestingly we found that avoidant attachment yielded similar effects as subclinical depression. GMD in the midline of the brain, including anterior and middle cingulate, superior medial prefrontal cortex and parts of the supplemental motor area was negatively correlated with both subclinical depressive symptoms and avoidant attachment. The anterior cingulate and the superior

medial prefrontal cortex are part of the DMN. The structural involvement of DMN areas in both subclinical depression and avoidant attachment is an important finding. It suggests that the link between depression and insecure attachment has a common neurobiological basis and that DMN is involved in it. We also found that subclinical anxiety is not associated with GM alterations in the same midline area of the brain as subclinical depression and attachment avoidance. Our findings do not support a common structural neurobiological basis between depression or attachment avoidance and anxiety. The fact that attachment anxiety was not associated with any GM alterations, suggests that the link between insecure attachment and behavioral problems stems more from attachment avoidance on a structural level. A common structural correlate between depression symptoms and avoidant attachment could mean that these two disorders share some pathological processes together. It could be that avoidant attachment is a cause of subclinical depression or that it is an effect created by depressive symptoms. Whether this negative correlation with subclinical depression and avoidant attachment on GMD is due to over- or underuse of circuits running through these regions still remains speculative.

4.3 Subclinical anxiety and GMD

We found a positive association between the anxiety measuring STAI score and GMD in the amygdala, hippocampus, fusiform gyrus, cerebellum, parahippocampus and middle and inferior temporal lobe. We didn't find any negative correlations between STAI scores and GMD in the brain. Unlike with BDI-II and ECR-avoidance scores, there were no effects in key areas of the DMN such as the anterior cingulate or precuneus. Majority of the previous studies on subclinical anxiety have found structural alterations in the amygdala, parahippocampus, orbitofrontal cortex and anterior cingulate. In a 2019 review, some studies found higher subclinical anxiety symptoms to correlate with higher GM volume in these areas, others with lower.[12]

A 2015 study with 393 subjects found a positive association between trait anxiety and cortical thickness in amygdala, ACC, insula, OFC and temporal cortex, but found no effects for trait anxiety with GM volume [26]. It is well established that aging is related with GM volume decrease [28,29]. It could be, that subtle GMD changes are masked by age-related atrophy in older populations. A 2017 study found that SCL-90-R anxiety subscale scores were positively associated with GM volume in the middle temporal gyrus, the Rolandic operculum, the middle cingular gyrus and the precuneus bilaterally [11]. These findings differ from ours and this could be due to the differences between SCL-90-R and STAI questionnaires.

Some of our findings on subclinical anxiety and GMD were similar to previous findings on social anxiety disorder (SAD) and GMD. In a 2018 meta-analysis associations were found between SAD and GM volume in amygdala, hippocampus and middle and inferior temporal gyri, but in other areas as well [3]. However these findings were rather inconsistent. Inconsistency could be due to many of these studies being underpowered to find subtle structural changes between healthy- and SAD-subjects.

Our structural findings are partly in line with what is known about fear and anxiety related circuitry in the brain. We found higher anxiety symptoms to be correlated with higher GMD in the amygdala and hippocampus, which are known to play a central role in these circuits. Our findings also suggest cerebellar involvement in subclinical anxiety. Although, the cerebellum has previously been linked mainly with fine-tuning motor functions, recent studies have shown it's connections to fear- and anxiety-related areas in the brain and suggest that it has a role in fear- and anxiety-related circuits. We didn't find effects in other areas that are central in fear and anxiety related circuits such as the anterior cingulate or the prefrontal cortex.[30]

5 Strengths and limitations

Our study had a sufficiently large sample size; a total of 255 subjects, of which 250 completed BDI-II, 221 STAI, 160 ECR-anxiety and 161 ECR-avoidance. We included only healthy subjects with no psychiatric illnesses. Because our subject population was made up of healthy controls from multiple different studies, the inclusion and exclusion criteria were not as strict and controlled as in a subject population tailored specifically for a single study. Also, we included subjects from different age groups and our broad age range of 19-58 might interfere with some of our findings. However age was not correlated with questionnaire scores. Every questionnaire used in our study was correlated $p<0.001$ with each other. This could results in false interpretations between brain structure alterations and questionnaire scores. Image acquisition was done with two different scanners, which means that image quality might vary between subjects. Finally, we used DARTEL in our VBM analysis, which improves inter-subject alignment compared to older VBM approaches.

6 Conclusions

We provide new evidence for a common neural substrate between avoidant attachment and subclinical depression. GMD in the midline of the brain seems to be negatively associated with both subclinical depression and attachment avoidance. Avoidant attachment had a negative association with GMD unilaterally in the right side temporal lobe. Subclinical anxiety was not negatively correlated with GMD in the midline of the brain, but positively bilaterally in the temporal lobes. We also found evidence for cerebellar involvement in subclinical anxiety. These findings could stimulate future research in the subclinical mood disorder field as well as in the attachment style field. In the light of our new findings, there should be more studies done with rTMS and other neuromodulatory approaches focusing on the midline of the brain in depressive disorders. More longitudinal studies should be done on insecure attachment and it's effects on brain structure and depressive symptoms. Whether insecure attachment screening questionnaires should be used more broadly in healthcare, is a question that needs to be answered too.

References

1. Wise T, Radua J, Via E, et al. Common and distinct patterns of grey-matter volume alteration in major depression and bipolar disorder: evidence from voxel-based meta-analysis. *Mol Psychiatry*. 2017;22(10):1455-1463. doi:10.1038/mp.2016.72

2. Hellewell SC, Welton T, Maller JJ, et al. Profound and reproducible patterns of reduced regional gray matter characterize major depressive disorder. *Transl Psychiatry*. 2019;9(1):176. Published 2019 Jul 24. doi:10.1038/s41398-019-0512-8

3. Wang X, Cheng B, Luo Q, Qiu L, Wang S. Gray Matter Structural Alterations in Social Anxiety Disorder: A Voxel-Based Meta-Analysis. *Front Psychiatry*. 2018;9:449. Published 2018 Sep 21. doi:10.3389/fpsyt.2018.00449

4. Cassidy, J., & Shaver, P. R. (Eds.). (2016). Handbook of attachment, third edition : Theory, research, and clinical applications. ProQuest Ebook Central. https://ebookcentral.proquest.com

5. Fraley RC, Hudson NW, Heffernan ME, Segal N. Are adult attachment styles categorical or dimensional? A taxometric analysis of general and relationship-specific attachment orientations. *Journal of Personality and Social Psychology*. 2015;109(2):354-368. doi:10.1037/pspp0000027

6. Gleeson, Gwen & Fitzgerald, Amanda. (2014). Exploring the Association between Adult Attachment Styles in Romantic Relationships, Perceptions of Parents from Childhood and Relationship Satisfaction. Health. 6. 1643-1661. 10.4236/health.2014.613196.

7. Puig J, Englund MM, Simpson JA, Collins WA. Predicting adult physical illness from infant attachment: a prospective longitudinal study. *Health Psychol*. 2013;32(4):409-417. doi:10.1037/a0028889

8. Acosta H, Jansen A, Nuscheler B, Kircher T. A voxel-based morphometry study on adult attachment style and affective loss. *Neuroscience*. 2018;392:219-229. doi:10.1016/j.neuroscience.2018.06.045

9. Zhang X, Deng M, Ran G, et al. Brain correlates of adult attachment style: A voxel-based morphometry study. *Brain Res*. 2018;1699:34-43. doi:10.1016/j.brainres.2018.06.035

10. Quirin M, Gillath O, Pruessner JC, Eggert LD. Adult attachment insecurity and hippocampal cell density. *Soc Cogn Affect Neurosci*. 2010;5(1):39-47. doi:10.1093/scan/nsp042

11. Benetti S, McCrory E, Arulanantham S, De Sanctis T, McGuire P, Mechelli A. Attachment style, affective loss and gray matter volume: a voxel-based morphometry study. *Hum Brain Mapp*. 2010;31(10):1482-1489. doi:10.1002/hbm.20954

12. Besteher B, Gaser C, Nenadić I. Brain Structure and Subclinical Symptoms: A Dimensional Perspective of Psychopathology in the Depression and Anxiety Spectrum. *Neuropsychobiology*. 2020;79(4-5):270-283. doi:10.1159/000501024

13. Besteher B, Gaser C, Langbein K, Dietzek M, Sauer H, Nenadić I. Effects of subclinical depression, anxiety and somatization on brain structure in healthy subjects. *J Affect Disord*. 2017;215:111-117. doi:10.1016/j.jad.2017.03.039

14. Ellison-Wright I, Bullmore E. Anatomy of bipolar disorder and schizophrenia: a meta-analysis. *Schizophr Res*. 2010;117(1):1-12. doi:10.1016/j.schres.2009.12.022

15. Goodkind M, Eickhoff SB, Oathes DJ, et al. Identification of a common neurobiological substrate for mental illness. *JAMA Psychiatry*. 2015;72(4):305-315. doi:10.1001/jamapsychiatry.2014.2206

16. Lefaucheur JP, Aleman A, Baeken C, et al. Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS): An update (2014-2018) [published correction appears in Clin Neurophysiol. 2020 May;131(5):1168-1169]. *Clin Neurophysiol*. 2020;131(2):474-528. doi:10.1016/j.clinph.2019.11.002

17. Jackson-Koku G. Beck Depression Inventory. *Occup Med (Lond)*. 2016;66(2):174-175. doi:10.1093/occmed/kqv087

18. Beck AT, Steer RA, Brown GK. BDI-II: Beck Depression Inventory Manual. 2nd ed. San Antonio: Psychological Corporation; 1996.

19. Wang YP, Gorenstein C. Psychometric properties of the Beck Depression Inventory-II: a comprehensive review. *Braz J Psychiatry*. 2013;35(4):416-431. doi:10.1590/1516-4446-2012-1048

20. Julian LJ. Measures of anxiety: State-Trait Anxiety Inventory (STAI), Beck Anxiety Inventory (BAI), and Hospital Anxiety and Depression Scale-Anxiety (HADS-A). *Arthritis Care Res (Hoboken)*. 2011;63 Suppl 11(0 11):S467-S472. doi:10.1002/acr.20561

21. Ashburner J, Friston KJ. Voxel-based morphometry--the methods. *Neuroimage*. 2000;11(6 Pt 1):805-821. doi:10.1006/nimg.2000.0582

22. Carlson JM, Depetro E, Maxwell J, Harmon-Jones E, Hajcak G. Gender moderates the association between dorsal medial prefrontal cortex volume and depressive symptoms in a subclinical sample. *Psychiatry Res*. 2015;233(2):285-288. doi:10.1016/j.pscychresns.2015.06.005

23. Hayakawa YK, Sasaki H, Takao H, et al. Structural brain abnormalities in women with subclinical depression, as revealed by voxel-based morphometry and diffusion tensor imaging. *J Affect Disord*. 2013;144(3):263-268. doi:10.1016/j.jad.2012.10.023

24. Allan CL, Sexton CE, Filippini N, et al. Sub-threshold depressive symptoms and brain structure: A magnetic resonance imaging study within the Whitehall II cohort. *J Affect Disord*. 2016;204:219-225. doi:10.1016/j.jad.2016.06.049

25. Buckner RL, DiNicola LM. The brain's default network: updated anatomy, physiology and evolving insights. *Nat Rev Neurosci*. 2019;20(10):593-608. doi:10.1038/s41583-019-0212-7

26. Potvin O, Catheline G, Bernard C, et al. Gray matter characteristics associated with trait anxiety in older adults are moderated by depression. *Int Psychogeriatr*. 2015;27(11):1813-1824. doi:10.1017/S1041610215000836

27. Crivello F, Tzourio-Mazoyer N, Tzourio C, Mazoyer B. Longitudinal assessment of global and regional rate of grey matter atrophy in 1,172 healthy older adults: modulation by sex and age. *PLoS One*. 2014;9(12):e114478. Published 2014 Dec 3. doi:10.1371/journal.pone.0114478

28. Lemaître H, Crivello F, Grassiot B, Alpérovitch A, Tzourio C, Mazoyer B. Age- and sex-related effects on the neuroanatomy of healthy elderly. *Neuroimage*. 2005;26(3):900-911. doi:10.1016/j.neuroimage.2005.02.042

29. Moreno-Rius J. The cerebellum in fear and anxiety-related disorders. *Prog Neuropsychopharmacol Biol Psychiatry*. 2018;85:23-32. doi:10.1016/j.pnpbp.2018.04.00