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**BDNF Val66Met genotype modulates the effect of childhood adversity on subgenual anterior cingulate cortex volume in healthy subjects**

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## **Abstract**

### **Background**

According to the neurotrophic hypothesis of depression, stress can lead to brain atrophy by modifying brain-derived neurotrophic factor (BDNF) levels. Given that BDNF secretion is affected by a common polymorphism (rs6265, Val66Met), which also is associated with depression, we investigated whether this polymorphism modifies the effect of childhood adversity (CA) on local gray matter volume in depression-relevant brain regions using data from two large cohorts of healthy subjects.

### **Methods**

We included 568 healthy volunteers (aged 18-50 years, 63% female) in our study, for whom complete data were available, with magnetic resonance imaging data at 1.5 Tesla ( $N=275$ ) or 3 Tesla ( $N=293$ ). We used a whole brain optimized voxel-based morphometry (VBM) approach assessing genotype-dependent gray matter differences, with focus on the amygdala, hippocampus and medial prefrontal cortex (including anterior cingulate cortex and orbitomedial prefrontal cortex). CA was assessed using a validated questionnaire.

### **Results**

In both cohorts we found that BDNF Met-allele carriers with a history of CA had significantly less gray matter in subgenual ACC ( $p < 0.05$ ) compared to Met-allele carriers without CA and Val/Val homozygotes with CA. No differences were found in hippocampus, amygdala and orbitomedial prefrontal cortex.

### **Conclusion**

BDNF Met-allele carriers are particularly sensitive to CA. Given the key role of the subgenual ACC in emotion regulation, this finding provides an important mechanistic link between stress and BDNF on one hand and mood impairments on the other hand.

**Key words: Brain-derived neurotrophic factor; Childhood adversities; Voxel-based morphometry; Imaging Genetics; Mood Disorders; Anterior Cingulate Cortex**

## **Introduction**

The experience of childhood adversity (CA) constitutes a major risk factor for psychiatric disorders (1). Early life stress has also frequently been associated with structural brain alterations, particularly in those brain areas that mature late, such as the hippocampus and prefrontal cortex (PFC)(2-4). Such structural brain alterations may be caused by a lack of neurotrophic factors, whose synthesis is reduced in the aftermath of stress (5). For instance, it has been shown in rodents that stress reduces brain-derived neurotrophic factor (BDNF) levels in the hippocampus and PFC (6). Also in humans, hippocampal and prefrontal BDNF levels are lower in depressed persons having committed suicide compared to control subjects (7;8).

There is however a large inter-individual variability in stress susceptibility. The leading explanation for this variability proposes that genetic variance modulates the effect of stress on the brain (9;10). Indeed, numerous studies have shown that persons with a specific genetic make-up are more prone to develop stress-related mental disorders after experiencing major life events like CA (11-13). Moreover, recent studies suggest that gene by stress interactions may be involved in inter-individual differences in local brain volume(14) and may also play a role in the onset of several psychiatric disorders, like bipolar disorder, schizophrenia, attention deficit hyperactivity disorder and depression (for review see (15)). In case of depression it has been suggested that gene by stress interactions may explain up to 60-70 % of the variance in disease susceptibility(16). A common functional polymorphism in the BDNF gene (rs6265, Val66Met) seems to be a relevant candidate for such an effect (17). This polymorphism leads to an amino acid substitution (valine to methionine) at codon 66 in the prodomain

of the protein, which impairs intracellular trafficking and activity-dependent secretion of BDNF (18) and has been implicated in the aetiology of depression (19). Previously, Joffe et al.(20) were the first to show an interaction between the BDNF polymorphism and stress in healthy subjects; they found a significant relation between stress susceptibility and decreased hippocampal volume in Met-allele carriers, but no effect in subjects with the ValVal genotype. Additionally in another study by Gatt and colleagues, who studied the interaction between BDNF Val66Met and CA on local brain volume, differences in amygdala and hippocampal volume were found (17). These authors showed that, compared to ValVal homozygotes, a high number of childhood events was associated with a moderate volume reduction of hippocampus and amygdala in persons carrying the methionine (Met) allele of the BDNF polymorphism, but found no main effects of either BDNF or CA. However, these findings were obtained using volumetry of pre-specified regions and thus, it is unclear whether other brain areas are also affected by an interaction between BDNF and CA.

Therefore, we sought to investigate to which extent BDNF genotype can modify the effect of CA on local gray matter changes using a whole brain optimized voxel-based morphometry (VBM) approach. By doing so we could localize any effects. We used data from two large cohorts of healthy subjects. This way we were able to replicate our findings, reducing the risk of type II errors. We also avoided possible confounding effects of disease, comorbidity, and treatment by investigating healthy subjects.

## **Methods**

### ***Subjects***

This study was part of the Brain Imaging Genetics (BIG) study at the Donders Institute for Brain, Cognition and Behavior of the Radboud University Nijmegen (Medical Centre) (21). All participants were of Caucasian descent and were screened prior to study participation using a self-report questionnaire for the following exclusion criteria: a history of somatic disease potentially affecting the brain, current or past psychiatric or neurological disorder, medication (except hormonal contraceptives) or illicit drug use during the past 6 months, history of substance abuse, current or past alcohol dependence, pregnancy, lactation, menopause and MRI contraindications.

The sample was made up of two independent cohorts based on scanner used. These were analyzed separately, to provide an independent replication of the results.

#### 1.5 Tesla cohort

Two hundred seventy five healthy subjects were scanned using a 1.5 Tesla MRI scanner. The participants were aged 18 – 50 years, with a mean age of 23.7 years (SD= 6.0). The majority was female (63%) and had a university or equivalent degree (61%).

#### 3 Tesla cohort

Two hundred ninety three healthy subjects were scanned using a 3 Tesla MRI scanner. Participants were 18 – 50 years old, with a mean age of 23.1 years (SD= 4.7). The majority was female (64%) and had a university or equivalent degree (73%).

### ***Assessment of childhood adversity***

CA was assessed using an adapted version of the List of Threatening Life Events developed by Brugha and colleagues with the aim of assessing the stress of life events (22). This inventory encompasses life events which are likely to occur relatively frequently and score relatively high on long-term threat. Participants were asked whether they had experienced a list of predefined events before the age of 16. This list comprised events like death of a parent, severe illness of self or spouse, moving house and sexual or physical abuse. Presence of CA was defined as having experienced at least one CA before the age of 16 years. Additionally, we also conducted analyses with CA dichotomized into presence of 2 or more CA versus no or 1 CA, as in an earlier study, which showed that only in persons with a history of 2 or more CA differences in brain volumes were found (17).

### ***Genotyping***

Genetic analyses were carried out at the Department of Human Genetics of the Radboud University Nijmegen Medical Centre, in a laboratory which has a quality certification according to CCKL criteria. High molecular weight DNA was isolated from saliva using Oragene containers (DNA Genotek, Ottawa, Ontario, Canada) according to the protocol supplied by the manufacturer. The BDNF 198-G>A (rs6265) polymorphism (Val66Met) was genotyped using Taqman® analysis (assay ID: C\_11592758\_10; Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Genotyping was carried out in a volume of 10 µl containing 10 ng of genomic DNA, 5 µl of Taqman Mastermix (2×; Applied Biosystems), 0.375 µl of the Taqman assay and 3.625 µl of H<sub>2</sub>O. Genotyping was performed on a 7500 Fast Real-Time PCR System and genotypes were scored using the

algorithm and software supplied by the manufacturer (Applied Biosystems). The genotyping assay had been validated before use and 5% duplicates and blanks were taken along as quality controls during genotyping. Testing for Hardy–Weinberg equilibrium did not show deviations from the expected genotype distribution ( $p > 0.05$ ). Subjects homozygous and heterozygous for the Met-allele were merged into a group of Met-carriers and compared to homozygous Val-allele carriers.

#### *Mood state*

To avoid confounding by current mood state we adjusted the analyses for the negative affect scale of the Positive Affect and Negative Affect Schedule (PANAS) (23).

#### ***MR Image acquisition***

All scans covered the entire brain and had a voxel size of  $1 \times 1 \times 1 \text{ mm}^3$ .

#### 1.5 Tesla cohort

All images were acquired at 1.5T Siemens Sonata or Avanto scanners (Siemens, Erlangen, Germany), using small variations to a standard T1-weighted 3D MPRAGE sequence (TR 2300 ms, TI 1100 ms, TE 3.03 ms, 192 sagittal slices, field of view 256 mm). These variations included a TR/TI/TE/slices of 2730/1000/2.95/176, 2250/850/2.95/176, 2250/850/3.93/176, 2250/850/3.68/176, and the use of GRAPPA parallel imaging with an acceleration factor of 2.

#### 3 Tesla cohort

All images were acquired at 3T Siemens Trio or TimTrio scanners (Siemens, Erlangen, Germany), using small variations to a standard T1-weighted 3D MPRAGE sequence (TR 2300 ms, TI 1100 ms, TE 3.93 ms, 192 sagittal slices, field of view 256 mm). These variations included TR/TI/TE/slices of 2300/1100/3.03/192, 2300/1100/2.92/192,

2300/1100/2.96/192, 2300/1100/2.99/192, 1940/1100/3.93/176, 1960/1100/4.58/176, and the use of GRAPPA parallel imaging with an acceleration factor of 2.

### ***MR Image conversion***

Raw DICOM MR imaging data were converted to NIFTI format using the conversion as implemented in SPM5 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm5/>).

### ***Brain Volume***

Normalizing, bias-correcting and segmenting into gray matter, white matter and cerebrospinal fluid was performed using the VBM5.1 Toolbox version 1.19 (<http://dbm.neuro.uni-jena.de/vbm/>) in SPM using priors (default settings). This method uses an optimized VBM protocol (24) as well as a model based on Hidden Markov Random Fields (HMRF) developed to increase signal-to-noise ratio (25). Total volume of gray matter (GM), white matter (WM) and cerebrospinal fluid was calculated by adding the resulting tissue probabilities. Total brain volume was defined as the sum of white matter and gray matter volume.

### ***Voxel-Based Morphometry (VBM)***

Diffeomorphic Anatomical Registration using Exponentiated Lie algebra (DARTEL) registration toolbox in SPM (26) was applied. First, all images were realigned to templates created from 556 in-house datasets. Second, Jacobian scaled ('modulated') images were calculated and subsequently transformed to MNI space using affine transformation. Finally, all data were smoothed with an 8 mm FWHM Gaussian

smoothing kernel. Analysis was performed in SPM using the GM images. After grouping all datasets according to genotype, images with poor quality or artifacts were identified using the outlier analysis routine implemented in the VBM toolbox. Images that showed a deviation of more than 1.5 times the interquartile range from the median were discarded from further analysis.

A full-factorial ANCOVA was applied on the GM images using genotype and CA as factors and age, gender, total brain volume, scan protocol and PANAS negative scale as covariates. All statistical tests were family-wise error rate (FWE) corrected for multiple comparisons across the entire brain ( $p_{FWE} < 0.05$ ) or across all voxels in a region of interest using a small volume correction (SVC;  $p_{SVC} < 0.05$ ). Regions of interest (ROIs) for the amygdala, hippocampus and medial prefrontal cortex were based on a standardized neuroanatomical atlas (27;28). Here, we defined the medial PFC as encompassing the anterior cingulate cortex (ACC) ( $y > 10$ ; (29)) and the medial part of the inferior, middle and superior frontal gyri, sometimes referred to as 'medial frontal gyrus', that is to say Brodmann areas 24, 25 and 32, as well as the medial part of Brodmann areas 9 and 10 (30). For visualization purposes significant clusters were extracted using the toolbox MarsBar, version 0.42 (31). First, the main effects of CA and BDNF genotype on local GM volume were evaluated. Second, the interaction between CA and BDNF genotype on GM was tested by adding an interaction term to the statistical models. In all analyses age, sex, total brain volume, PANAS negative scale and dummy regressors for the scan protocols were included as covariates. In case of a significant interaction, t-contrasts were created in SPM for post-hoc testing using  $p_{SVC} < 0.05$  for corresponding region of interest.

## Results

There were no significant differences between the two cohorts on any of the sample characteristics (Table 1). Total brain volume measured at 3T appeared significantly smaller than that measured at 1.5T (32).

No significant main effect of BDNF genotype on gray matter volume was found in either cohort, neither within the whole brain analysis ( $p_{FWE} > 0.05$ ), nor within the regional specific ROI analysis (all  $p_{SVC} > 0.05$ ). Within the 1.5T cohort, we found that a history of CA was related to less gray matter in the anterior cingulate cortex (ACC) (BA24) ( $p_{SVC} = 0.02$ ) and the medial orbitofrontal cortex (BA9) ( $p_{SVC} = 0.05$ ). However, these findings could not be replicated at 3T.

The interaction between BDNF genotype and CA did not lead to significant effects observable in the whole brain analysis ( $p_{FWE} > 0.05$ ). In the ROI analysis, however, we found an interaction effect in the subgenual ACC volume in both cohorts (BA 25, on 1.5 T  $p_{SVC} = 0.006$ ; on 3T  $p_{SVC} = 0.019$ ; see Figure 1). Additionally, the interaction affected the volume of the medial orbitofrontal cortex (BA10) (on 1.5 T  $p_{SVC} = 0.02$ ) but this effect could not be replicated in the 3T cohort (on 3T  $p_{SVC} = 0.22$ ). No interaction effects were found in amygdala or hippocampus. For more detailed description of results see Table 2.

Using simple t-tests with contrasts for each comparison Met-carriers were found to be more vulnerable to the effect of CA than homozygous Val-carriers (Figure 2). Within the 1.5T cohort, Met-carriers with a history of CA had less gray matter in the subgenual ACC than Val-homozygotes with a history of CA ( $T=3.33$ ;  $p_{SVC} = 0.007$ ) and Met-carriers without a history of CA ( $T=4.70$ ;  $p_{SVC} < 0.001$ ). Similarly, within the 3T

cohort, the comparison of Met-carriers and Val-homozygotes with a history of CA showed significantly less gray matter in the former ( $T=3.30$ ;  $p_{SVC} = 0.002$ ). The difference between Met-carriers with and without CA did not reach significance within the 3T cohort, though ( $T = 3.46$ ;  $p_{SVC} = 0.12$ ).

In an attempt to replicate findings of a previous study (17) we re-analyzed our data using two CA as the threshold for the presence of CA, however these analyses did not change the associations found in our previous analyses.

## **Discussion**

Within two independent cohorts of healthy subjects we found that carriers of at least one Met-allele of the Val66Met polymorphism in BDNF who had a history of childhood adversity had decreased gray matter volume in the subgenual ACC, compared to Val-homozygotes and Met-carriers without a history of childhood adversity.

Amygdala, hippocampus and medial PFC play a vital role in normal emotional processing and the maturation of these areas continues into adolescence (33). Consequently, we hypothesized that these brain areas might be most vulnerable to environmental adversities during childhood and adolescence. The medial PFC is the last part of the brain to mature (34) and therefore might have been the most sensitive to CA as measured in our study. Within the 1.5T cohort we indeed found that persons with a history of CA showed a reduction of gray matter in the orbital prefrontal cortex as compared to persons without CA. This finding could, however, not be replicated within the 3T cohort and it remains therefore unclear whether this was a false positive finding or whether this non-replication was caused by higher levels of image distortion and signal

dropout at 3T, which particularly affect the orbitofrontal and anterior temporal areas. Consequently these areas are measured with greater reliability at 1.5T than at 3T (32;35).

The subgenual ACC is also part of the medial PFC and plays a role in emotion regulation (36) and negative memory bias (37;38), as shown by functional neuroimaging studies. Reduced gray matter volume in the subgenual ACC has previously been found in persons with a history of CA (39), as well, and volume reduction in this area is a consistent finding in mood disorders (for review see (40)). Furthermore, the subgenual ACC has been shown to play a key role in the autonomic and neuroendocrine stress response and arousal associated with emotional states and behavior (41;42). Taken together, these results suggest that the subgenual ACC is implicated in the pathophysiology of mood and anxiety disorders and that decreased gray matter volume as detected in our study could be an indicator of increased emotional sensitivity as a result of genetic susceptibility interacting with CA.

Contrary to preceding studies among healthy subjects (43-46) we did not find a main effect of the BDNF Val66Met polymorphism on regional brain volume. We had expected to find reduced gray matter volume in Met-allele carriers, irrespective of CA. However, null findings have also been reported before (20;47;48). It thus seems that the effect of the Val66Met polymorphism on regional brain volume is dependent on additional factors that have not been properly controlled for so far. For instance, Szeszko et al. (49) concluded that the effect of the Val66Met polymorphism on regional brain volume is pronounced in patients with schizophrenia. Our findings also suggest that the effect of the BDNF Val66Met polymorphism on regional brain volume is dependent on an additional factor, i.e. history of CA.

Our results fit nicely with the neurotrophic hypothesis of depression (50) and thus suggest that stress (and depression) can lead to structural brain changes in turn leading to increased stress sensitivity. Therefore it is crucial that clinicians are aware of this potentially brain-damaging effect of depression and treat depressed patients as early and effectively as possible (16). Neurotrophic factors play a crucial role in neural plasticity during development and also during adulthood where they modulate axonal and dendritic growth and remodelling, membrane receptor trafficking, neurotransmitter release as well as synapse formation and function (51). Indeed, the transcription and secretion of BDNF can be modified by environmental factors such as stress, which can lead to changes in neuronal plasticity and ultimately will affect the function and structure of certain brain areas. Our results could thus be explained by reduced neuronal plasticity in the subgenual ACC. Neither CA alone nor reduced BDNF secretion alone was sufficient to cause reduced subgenual ACC volume in our samples, the combination of the two factors was critical in causing an effect that we could measure with our approach. It is important to note that this was found in self-reported healthy subjects, suggesting that these structural brain abnormalities indeed may serve as a risk factor for mood disorders and not a consequence of disease or therapy. In line with this, structural brain changes have frequently been identified as endophenotypes for psychiatric disorders (52), because they can represent an elegant link between genes and psychiatric disorders.

We did not replicate the findings of a preceding study reporting smaller hippocampal and amygdala volumes in carriers of the BDNF Met-allele and a history of two or more childhood events (17). The differences in findings may be explained best by the methods applied. In the study by Gatt and colleagues (17), VBM was applied to

calculate volumes of pre-defined regions of interest (namely hippocampus, amygdala and medial PFC), whereas we applied a whole-brain approach and looked into pre-specified regions by limiting the search space for statistical testing, in addition. Another difference is the method for assessing CA. While we used a dichotomized version (one or more CA versus no CA), they looked into total number of CA (ranging from 0 to 5) and found that only in subjects who had experienced two or more CA there was an interactive effect between BDNF genotype and CA on hippocampal and amygdala volume. Possibly, we found no effect of CA in the hippocampus and amygdala, because within our cohorts a relatively small group had experienced two or more events before the age of 16 years (15%), which may have resulted in lack of statistical power.

Strengths of our study include the large number of subjects and the ability to replicate our own findings by using two separate cohorts, which decreases the chance of false positive findings. Also, all of our associations were corrected for family-wise error rate and thus the results appear to be very robust. Furthermore, we used a whole brain optimized DARTEL VBM approach, which is a fully automatic technique that allows an objective analysis of structural differences between groups across the entire brain. By surveying the whole brain, VBM provides a unbiased measure of highly localized regions that may not be investigated in hypothesis-based studies that employ more labor-intensive manual ROI measurement techniques (53). However, the sensitivity of the DARTEL VBM approach for detecting amygdala and hippocampal volume changes is probably less than the sensitivity of manual tracing of these structures. Possibly, we therefore found no changes in the amygdala and hippocampus in relation to CA and BDNF Val66Met polymorphism, as has previously been reported (3;4;54).

History of CA was assessed by means of a questionnaire and it is important to acknowledge the subjectivity of self-reported CA. Retrospective assessment of CA may be subject to recollection bias; persons with higher depressive symptoms might recall more negative events than persons with less depressive symptoms. However, we believe that the influence of negative mood was limited, firstly, because our study was conducted among healthy subjects without psychopathology and secondly, because we adjusted our findings for negative affect and found that current negative mood did not critically affect our results. Also, although the idea that CA in combination with BDNF Val66Met polymorphism is associated with gray matter volume changes in subgenual ACC fits well with numerous preclinical studies, the possibility of reversed causality (e.g. gray matter volume changes leading to less effective coping with stress, which could lead to over-reporting of CA) cannot be excluded by the current findings as our study had a cross-sectional design.

In summary, the gene-environment interaction affecting local gray matter volume suggests that BDNF Met-allele carriers are particularly sensitive to CA. Given the important role of the subgenual ACC in the regulation of emotional behavior, this finding represents an important link in our understanding of how BDNF in combination with CA contributes to increased stress sensitivity. Based on our study it is however not clear whether Met-allele carriers are more prone to the detrimental effects of stress on the brain, or whether CA leads to decreased gray matter volume, irrespective of genotype, and normalization is impaired in Met-allele carriers, resulting in gray matter volume reductions in this group. Future longitudinal studies should therefore aim at unraveling the relation between the susceptibility of BDNF Met-allele carriers to stress on the brain.

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**Conflict of interest:**

All authors have no conflict of interest regarding any financial, personal or other relationships with other people or organizations within three years of beginning the work submitted that could have inappropriately influenced their work.

None of the authors' institutions have contracts relating to this research through which they or any other organization may stand to gain financially now or in the future.

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**Table 1 Characteristics per cohort**

	<b>1.5T cohort</b>	<b>3T cohort</b>
	<b>N=275</b>	<b>N=297</b>
<b>Age (years)</b>		
range	18 – 50	18 – 50
mean (sd)	23.7 (6.0)	23.1 (4.7)
<b>Female %</b>	63	64
<b>Educational level %</b>		
- low	20	15
- intermediate	19	12
- high	61	73
<b>BDNF genotype %</b>		
- Val-Val	62	57
- Met-Val	34	38
- Met-Met	5	5
<b>Childhood adversities</b>		
- range of childhood adversities	0 – 6	0 – 6
- History of childhood adversities (%)	51	56
<b>Mean PANAS negative scale (sd)</b>	13.3 (4.7)	13.4 (4.5)
<b>Mean total brain volume (sd)</b>	1307.4 (125.2)	1258.8 (121.9)

**Table 2. Effects of BDNF Val66Met and childhood adversities on voxel-based morphometry within medial PFC**

	Cohort	Region	Brodmann area	MNI coordinates			Cluster extended (k)	Z-score
				X	Y	Z		
<b>Main effect CA</b>	<b>1.5T</b>	ACC	25	-5	-5	47	112	3.75 <sup>a</sup>
		mOFC	9	32	64	11	547	3.71 <sup>a</sup>
	<b>3 T</b>	ACC	24	10	17	45	50	2.66
		mOFC	10	22	41	40	14	2.50
<b>Interaction BDNF* CA</b>	<b>1.5T</b>	ACC	25	-5	-5	47	300	4.41 <sup>a</sup>
			24	3	-3	51	95	4.17 <sup>a</sup>
				8	-3	51	32	4.07 <sup>a</sup>

		mOFC	10	-23	58	5	162	3.50
				-26	60	6	313	3.16
				23	58	5		3.46
	<b>3T</b>	ACC	25	-2	16	-14	23	3.10 <sup>a</sup>
				2	16	-12	18	3.07
				3	22	-7		2.57
		mOFC	9	32	60	27	45	3.06
				28	69	27		2.97
				28	57	35		2.65

<sup>a</sup>  $p < 0.05$  FWE for small volume correction ( $p_{SVC}$ )

BA: Brodmann Area, BDNF: Brain-Derived Neurotrophic Factor, CA: Childhood Adversities; ACC: Anterior Cingulate Cortex;

mOFC: medial Orbitofrontal Cortex

### **Figure legends**

Figure 1. Reduced gray matter in subgenual anterior cingulate cortex (ACC) as a result of an interaction of BDNF Val66Met genotype with childhood adversities ( $p_{SVC} < 0.05$ ).

Subgenual ACC was defined as Brodmann area 25.

red: 1.5T cohort

blue: 3T cohort

violet: overlap between cohorts

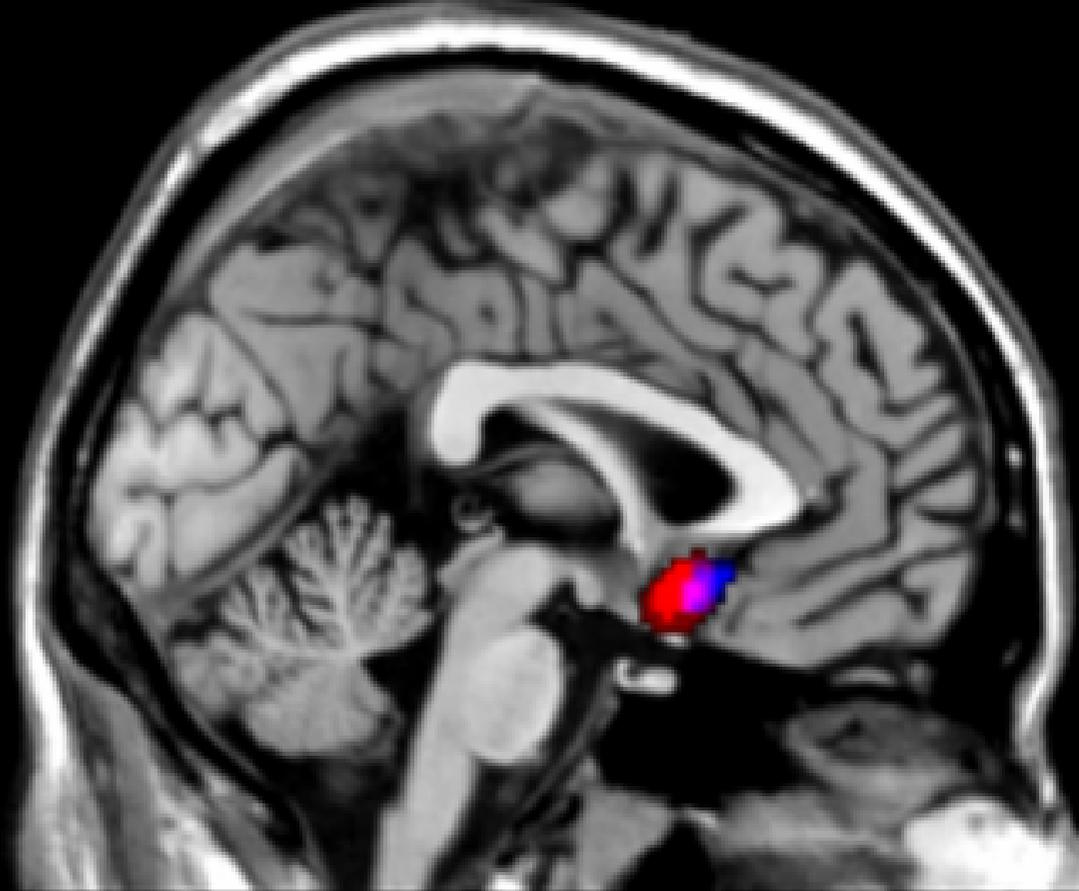
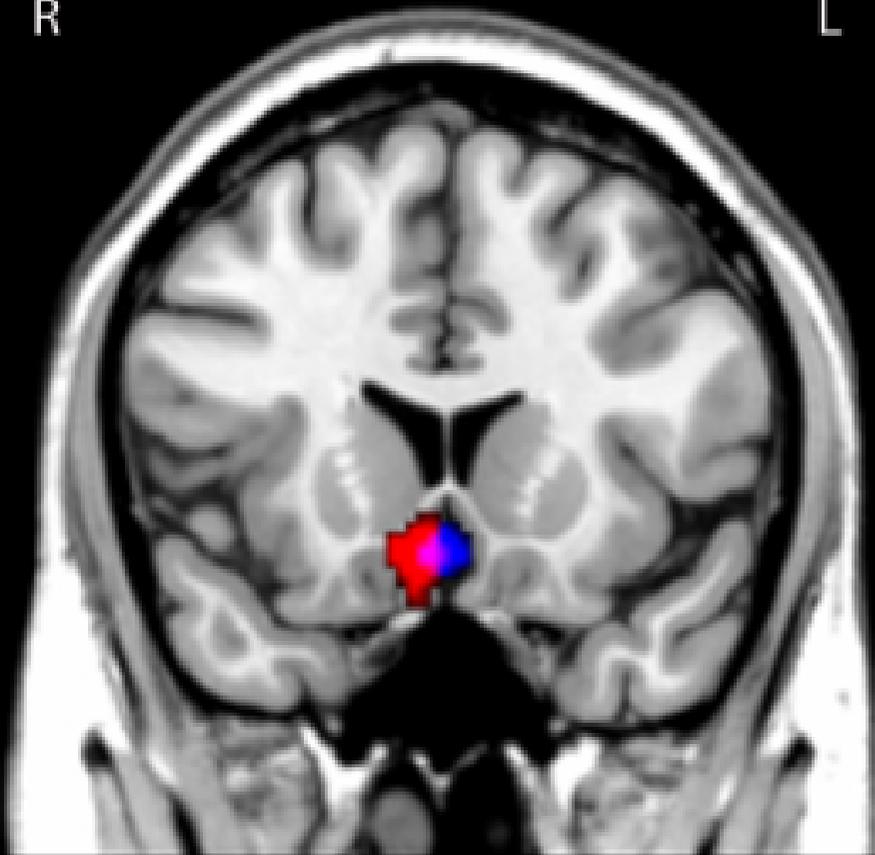
Figure 2. Gray matter volume within subgenual ACC (BA25) in both cohorts, in percentages of total brain volume.

Data were extracted using the toolbox MarsBar in SPM (31)

CA: Childhood Adversities; \*:  $p_{\text{svc}} < 0.05$

R

L



Y: 141

X: 89

