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# Oxidative stress and brain morphology in individuals with depression, anxiety and healthy controls

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ABSTRACT

Oxidative stress is a biological process, caused by an imbalance between reactive oxygen species (ROS) and antioxidants, in favour of the ROS. This imbalance leads to oxidative damage to lipids, proteins and DNA and ultimately cell death. Studies in rodents have shown that the brain, particularly the amygdala and hippocampus, is sensitive to oxidative stress, although studies on the association between oxidative stress and brain morphology in humans are lacking. Oxidative stress has also been associated with major depressive disorder (MDD) and may be related to volumetric abnormalities in the amygdala and hippocampus in MDD and anxiety disorders. In this study we aimed to examine the association between two robust measures of oxidative damage in plasma (8-OHdG and F2-isoprostanes) and volume of the hippocampus and amygdala in a large sample of individuals with and without MDD and/or anxiety (N = 297). In secondary analyses, we examine whether this association is similar in patients and controls. 8-OHdG and F2-isoprostanes plasma levels were determined using liquid chromatography tandem mass spectrometry and volume of the hippocampus and amygdala and hippocampal subfields was determined using Freesurfer. We found no association between plasma markers (or interaction with MDD and/or anxiety disorder diagnosis) and subcortical volume, suggesting that peripheral oxidative stress damage is not associated with subcortical brain volume.



## 1. Introduction

Oxidative stress is a complex biological process, which is the result of an imbalance between the production of (or exposure to) reactive oxygen species (ROS) and antioxidants defences, in favour of the ROS. ROS play an important role in many biological processes, such as apoptosis, transcription factor activation and cell signalling pathways (Halliwell, 2000, 2011). However, when present in excessive amounts, ROS can cause damage to cellular structures such as membranes, proteins and DNA (Ďuračková, 2010), and prolonged exposure to oxidative stress can therefore cause cellular dysfunction and cell death (Filomeni and Ciriolo, 2006). Oxidative stress has also been related to various psychiatric disorders, such as major depressive disorder (Hovatta et al., 2010; Black et al., 2015; Bouayed et al., 2009) and psychosis (Martinez-Cengotitabengoa et al., 2012) and may contribute to the onset or a negative course trajectory of psychiatric disorders through changes in brain structure (Moylan et al., 2013).

The brain is particularly susceptible to oxidative stress, due to its high rate of oxygen consumption, large content of polyunsaturated fatty acids, its regional high iron levels, and proportionately low antioxidant capacity (Noseworthy and Bray, 1998). Structures particularly sensitive to oxidative stress are the amygdala and the hippocampus (Wang and Michaelis, 2010). Amygdala and hippocampal neurons are most sensitive to degeneration in neurodegenerative diseases with increased oxidative stress, such as Alzheimer's disease (Braak and Braak, 1991; Terry et al., 1991). Furthermore, a preclinical study in rodents has shown that induced oxidative stress causes lipid peroxidation specifically in the hippocampus and the amygdala of the rat brain (Candelario-Jalil et al., 2001). Hippocampal neurons are particularly susceptible to oxidative stress, because of their high consumption of oxygen, large content of easily oxidizable polyunsaturated fatty acids and

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relatively low antioxidant levels (Evans, 1993). Various studies have shown that oxidative stress in the hippocampus causes reduced neurogenesis and increased neuronal death (Mattson, 2000; Huang et al., 2012). In the only human study to date, Lindqvist et al. (2014) calculated a 'total net antioxidant score', which consisted of oxidants (oxidized glutathione) and antioxidants (reduced glutathione, glutathione peroxidase and vitamin C) and reported an association with total hippocampal volume, and volume of the CA3 & dentate gyrus subfields in particular. However, this study did not correct for lifestyle and medication use, which may influence both oxidative stress markers (Black et al., 2016a,b) and hippocampal volume (Vermetten et al., 2003) and the sample size was limited (N = 35).

Measuring ROS in vivo is also difficult due to the high reactivity and short half-life of ROS. An alternative approach is examining the effect of oxidative damage on lipids or DNA. 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 8-isoprostaglandin F2α (F2-isoprostanes) have been widely used as measure of oxidative DNA and lipid damage respectively and are considered robust markers of oxidative stress (Niki, 2014; Valavanidis et al., 2009).

In order to gain more insight into the association between oxidative stress and brain morphology in humans, we examined the association between these two peripheral plasma markers and amygdala and hippocampal volume in a large sample of participants (N = 297). Furthermore, we explored whether oxidative stress was related to volume of hippocampal subfields. Based on previous findings within a larger sample from the same study, we expected similar oxidative stress levels in patients and controls after controlling for lifestyle and supplement use (Black et al., 2016a,b). We also expected a negative association between plasma markers of oxidative stress and brain morphology in both patients and controls. Therefore in our primary analyses, we examined across groups which regions are associated with oxidative DNA and lipid damage. In secondary analyses we explored whether there was an interaction with psychiatric status in the association between oxidative stress markers and brain morphology.

## 2. Materials and methods

### 2.1. Participants

The Netherlands Study of Depression and Anxiety (NESDA) is a longitudinal cohort study, with the aim to study the psychosocial, biological and genetic determinants of the course of depression and anxiety disorders in 2981 participants. The NESDA study includes individuals with major depressive disorder (MDD) and/or an anxiety disorder and individuals without a psychiatric disorder. Participants were recruited from the community, general practitioners and specialized mental health care institutions (for details, please see Penninx et al., 2008).

A subgroup of 301 NESDA patients and healthy controls participated in the neuroimaging study of NESDA. An inclusion criterion for individuals in the patient group was a 6-month DSM-IV diagnosis of MDD and/or anxiety disorder. Participants in the control group had no history of psychiatric disorders. DSM-IV diagnoses were established using the Composite International Diagnostic Interview (CIDI version 2.1) (Wittchen, 1994). Severity of depression and anxiety was assessed using the Dutch versions of the Beck Anxiety Inventory (BAI; Beck et al., 1988) and the Inventory of Depressive Symptomology (IDS: Rush et al., 1986). Both patients and controls with a history of drug or alcohol abuse were excluded from the imaging study, as well as subjects with general MRI contraindications and presence or history of a severe internal or neurological disorder. Additional exclusion criteria were the use of psychotropic medication with the exception of stable use of selective serotonin reuptake inhibitors (SSRIs) or infrequent benzodiazepine use for patients and use of any psychoactive medication for control subjects. The Ethical Review Boards of the three participating centres (Academic Medical Centre Amsterdam, Leiden University Medical Centre and University Medical Centre Groningen) have approved this study and all individuals have provided a written informed consent.

In this current study we included all healthy controls and all patients with a diagnosis of MDD and/or anxiety from whom structural MRI measures were obtained. Four patients were excluded because of poor image quality, which left a total of 297 subjects (66 patients and 231 controls). 8-OHdG levels were available for 285 subjects, while F2-isoprostane levels were available for 248 participants.

### 2.2. Imaging

3 T Philips scanners (Philips, Best, The Netherlands) were used to perform the imaging study at the three participating centres (Academic Medical Centre Amsterdam, Leiden University Medical Centre and University Medical Centre Groningen). In Amsterdam, a SENSE-6 channel head coil was used, while the scan sites in Leiden and Groningen used a SENSE-8 channel head coil. Anatomical scans were acquired using a sagittal three-dimensional gradient-echo T1-weighted sequence (TR: 9 ms; TE: 3.5 ms; matrix 256 × 256; voxel size: 1 $mm^3$; 170 slices).

Volumetric segmentation was performed using FreeSurfer image analysis suite (version 5.3; Martinos Center for Biomedical Imaging, Harvard-MIT, Boston, MA; http://surfer.nmr.mgh.harvard.edu/). FreeSurfer performs averaging and motion correction, Talairach transformation, removal of non-brain tissue, intensity normalization and cortical reconstruction and segmentation of cortical regions and subcortical structures. For a quality check, a visual inspection of subcortical structures was performed, using a protocol developed by the ENIGMA consortium (http://enigma.ini.usc.edu/protocols/imaging-protocols/). In our current study, we focus on the amygdala and hippocampus volumes due to their specific vulnerability to oxidative stress (Lindqvist et al., 2014; Wang and Michaelis, 2010).

In secondary analyses, we examined the relationship between oxidative stress and the volume of four hippocampal subfields: the subiculum, CA1, CA3 and the molecular layer of granule cells of the dentate gyrus (GCMLDG). These regions were chosen based on the regions included by the study of Lindqvist et al. (2014). However, manual segmentation of hippocampal subfields was used in this study, while we used an automated parcellation protocol. However, our chosen regions are comparable with the regions included in the aforementioned study (in the study by Lindqvist et al., referred to as: subiculum, CA1, CA1/2 and CA3/DG). We used a revised version of the automated subregion parcellation protocol (Van Leemput et al., 2009) to segment the hippocampus into hippocampal subregions. This new module is incorporated in FreeSurfer 6.0 (Iglesias et al., 2015) and uses a probabilistic atlas, built upon manual delineations of the hippocampus from 15 ex-vivo scans (see Iglesias et al., 2015 for a description of this method). A previous study from the ENIGMA consortium has reported high test–retest reliability of subfield segmentation using this method (Whelan et al., 2016).

### 2.3. Oxidative stress markers

Blood was collected in the morning after an overnight fast using a vacutainer blood collection tube and transported to local laboratory sites for processing within 1 h of withdrawal. Plasma samples were stored at −80 °C and transported to the Metabolic Laboratory of the VU University. The measurement of both markers in this sample has previously been described in more detail elsewhere (Black et al., 2016a). F2-isoprostanes (the total, i.e. free and esterified, concentration of 8-iso prostaglandin $F_{2\alpha}$ [iPF2α-III]) were determined by liquid chromatography tandem mass spectrometry (LC–MS/MS), with intra- and inter-assay variations were 4.6% and 8.2%, respectively. Plasma levels of 8-hydroxy-2-deoxyguanosine (8-OHdG) were determined by LC–MS/MS with intra- and inter-assay CVs of 3.1% and 6.3%, respectively.