An Investigation of Amygdala and Hippocampal Subregions and their Relation to Ageing in Anxiety and Related Disorders

Ziphozihle Ntwatwa

NTWZIP001

Neuroscience

February 2024

Dissertation presented for the Degree of

DOCTOR OF PHILOSOPHY

in the Department of Psychiatry and Mental Health,

Faculty of Health Sciences,

University Of Cape Town

Supervisors

Dr. Jonathan C. Ipser

Department of Psychiatry and Mental Health

University of Cape Town

Co-supervisors

Dr. Nynke A. Groenewold

Professor Dan J. Stein

Professor Jack van Honk

Department of Psychiatry and Mental Health

University of Cape Town

Table of Contents

DECLARATION

I, Ziphozihle Ntwatwa, present this dissertation in fulfilment of the requirements for the degree of Doctor of Philosophy in the Department of Psychiatry and Mental Health, Faculty of Health Sciences, University of Cape Town (UCT). I confirm that this dissertation was written by myself (Ziphozihle Ntwatwa), is my original work, and will not be submitted, in part or full, for another degree at UCT or any other university.

This dissertation includes a retrospective analysis of secondary data that was obtained from the European and South African Research Network in Anxiety Disorders and Obsessive-Compulsive Disorder Brain Imaging Consortium. Data usage agreements from each consortium and the study protocol were approved by UCT's Faculty of Health Sciences Human Research Ethics Committee (HREC REF:038/2020).

I, Ziphozihle Ntwatwa, confirm that this dissertation begins with an introduction (Chapter 1) and ends with a discussion and conclusion (Chapter 7). For the two chapters that have been published (Chapters 3 and 4), the referencing style and use of terms have been formatted for the dissertation, however, the content remains unchanged.

Chapter 1:

Chapter one forms the introduction including the rationale and objectives of my dissertation. This chapter is in line with my Ph.D. proposal and provides an overview of the prevalence and characteristics of SAD and OCD, the nature of subcortical volume involvement in these disorders, and their relation to brain aging. Dr. Jonathan Ipser and Prof. Dan Stein guided this process and provided feedback.

Chapter 2

A systematic review of Indicators of biological aging in anxiety and related disorders I performed the database search using a string of search terms that Dr. Jonathan Ipser and I curated. Dr. Sheri Koopowitz and I complied the updated version of the review, including re-evaluating all studies for eligibility (abstract and full-text review), compiling data extraction tables and quality assessment (using the 22 items Strengthening the reporting of observational studies in epidemiology guideline) under the guidance of Dr Jonathan Ipser.

Chapter 3

Hippocampal and amygdala subfield volumes in OCD. The data was sourced using data agreements through the OCD Brain Imaging Consortium. I worked with Dr. Jonathan Ipser to segment the hippocampus and amygdala using Freesurfer 6.0 and produced the data files for qualitative and quantitative quality control. Dr. Annerine Roos, Tatum Sevenoaks and I completed quality control (QC) under the guidance of Dr. Nynke Groenewold. I completed the statistical analysis by adapting a mixed effects model R script, provided the interpretation of the results, and compiled tables for the description of study characteristics and mixed effect size estimates, under the guidance of Dr. Jonathan Ipser and Dr. Nynke Groenewold. All the named co-authors provided feedback on the manuscript. Prof. Dan Stein, Prof. Odile van den Heuvel, Dr Jonathan Ipser, and Dr Nynke Groenewold provided further guidance by supporting the revision of the manuscript, as well as providing additional commentary.

Chapter 4

Hippocampal and amygdala subfield volumes in SAD. The data was sourced using data agreements through MEGA-SAD. Dr. Jonathan Ipser and I segmented the hippocampus and amygdala using Freesurfer 6.0, Dr. Jonathan Ipser produced the datafiles for qualitative and quantitative quality control. Jule Spreckelmeyer and I completed QC under the guidance of Dr. Nynke Groenewold. I completed the statistical analysis by adapting a mixed effects model R script, provided the interpretation of the results, and compiled tables for the description of study characteristics and mixed effect size estimates, under the guidance of Dr. Jonathan Ipser and Dr. Nynke Groenewold. In addition, all the named co-authors provided feedback on the manuscript. Prof. Janna Marie Bas-Hoogendam, Prof. Dan Stein, Dr. Jonathan Ipser, and Dr. Nynke Groenewold provided further guidance by supporting the revision of the manuscript, as well as providing additional commentary.

Chapter 5

Brain aging in OCD: whole and regional brain-PADs. I worked with Dr. Jonathan Ipser to run the brain age model and analysis using the Kaufman brain age estimate model for whole brain and regional brain-PADs. Prof. Dan Stein and Dr. Jonathan Ipser provided further guidance and additional commentary on the completion of the manuscript.

Chapter 6

Brain aging in SAD: whole and regional brain-PADs. I worked with Dr. Jonathan Ipser to run the brain age model and analysis using the Kaufman brain age estimate model for whole brain and regional brain-PADs. Prof. Dan Stein, Dr. Jonathan Ipser, and Dr. Nynke Groenewold provided further guidance and additional commentary on the completion of the manuscript.

Chapter 7

Chapter seven forms the discussion and conclusion of chapters 2 to 6. I compiled the chapter with the guidance of Prof. Dan Stein, Dr. Jonathan Ipser, and Dr Nynke Groenewold.

Signed,

Ziphozihle Ntwatwa

2023

The study protocol was approved by UCT's Faculty of Health Sciences Human Research Ethics Committee (HREC 038/2020).

I confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publication(s) in my PhD thesis, and where co-authorships are involved, my co-authors have agreed that I may include the publication(s):

1. Ntwatwa, Z., Lochner, C., Roos, A., Sevenoaks, T., van Honk, J., Alonso, P., Batistuzzo, M.C., Choi, S., Hoexter, M.Q., Kim, M. and Kwon, J.S., 2023. Hippocampal and amygdala subfield volumes in obsessive-compulsive disorder differ according to medication status. *bioRxiv*, pp.2023-03. https://doi.org/10.1101/2023.03.28.534348. Submitted to Journal of Psychiatry and Neuroscience, 18th August 2023.
2. Ntwatwa, Z., Sprecklemeyer, J., Bas-Hoogendam, J.M., van Honk, J., Mufford, M., Boraxbekk, C.J., remers, H.R., Fouche, J.P., Frick, A., Furmark, T. and Klumpp, H., 2024. Specific amygdala and hippocampal subfield volumes in social anxiety disorder and their relation to clinical characteristics; an international mega-analysis. *bioRxiv*, pp.2024-01. doi: https://doi.org/10.1101/2024.01.29.576056. Submitted to Translational Psychiatry, 6th February 2024.

Signed,

Signed by candidate

Ziphozihle Ntwatwa

2024

ACKNOWLEDGEMENTS

To all the women who have come before me, those who were previously denied education, and those who sacrificed for our existence, thank you!

To my grandmother who used to work as a ‘tea girl’ in the city, in this moment I think of you.

To my mother and brother, thank you for your unwavering belief and support. Thank you for your nurture and generosity, and for allowing me the space and opportunity to pursue this work. The PhD journey had many challenging moments, but through your support I made it to the end. I love you.

To my husband, Bart, always kind. Your broad shoulders have carried us through the difficulties of research. Thank you for your refreshing take on science, and for being my sounding board. To many more years with you.

Artuur, my son, your presence is a joy. You fill the room and demand to be seen, you have shown me a life worth living. I love being your mother and I pray for your success.

To all of my friends, you inspire me. Thank you for the decades of friendships and walking this path with me.

Dr Sheri Koopowitz, your tenacity is infectious. Thank you for your contribution to this dissertation, I have learned tremendously from you.

Dr Jonathan Ipser, my dear supervisor, thank you for your gentle spirit, your mentorship and guidance have been invaluable in the completion of this dissertation. Thank you for the time that you set aside for me, thank you for the knowledge transfer- I am in awe of your skills and expertise.

Dr Nynke Groenewold, my co-supervisor, your insightful feedback, constructive criticism, and work-ethic have greatly contributed to the development and refinement of this work.

Professor Dan Stein, thank you for your support of this work. I am grateful for the countless opportunities for growth and development, especially in a research field that was previously unknown to me. I am forever grateful for your support.

Lastly, thank you to all the individuals who spent their time participating in the research, your efforts are truly appreciated.

FUNDING

Ziphozihle Ntwatwa was funded *(in part) by grant G-21-58838 from Carnegie Corporation of New York*. Additional funding was obtained from the *KW Johnston Bequest* and the *Marcus Rubin Scholarship.*

LIST OF FIGURES

cell layer of dentate gyrus (DG), molecular layer (ML), hippocampus–amygdala transition area (HATA), corticoamygdaloid transition area (CAT), anterior amygdaloid area (AAA).

Figure 4.2: Scatterplot depicting the relationship between chronological age and (A) Basal nucleus, (B) Accessory basal, (C) CA3, and (D) CA4 volume.

Figure 4.3: Scatterplot depicting the relationship between chronological age and (A) Molecular layer, (B) DG, (C) CAT volume.

Figure 5.1: violin plot showing whole and regional brain-PADs in OCD (n=376) with (n=158) and without (n=218) psychotropic medication use (n=335), compared to HCs (n=335).

Figure 6.1: violin plot showing whole and regional brain-PADs for individuals with SAD (n=107) with (n=15) and without (n=86) psychotropic medication use (n=137), compared to HCs (n=137).

LIST OF TABLES

LIST OF SUPPLEMENTARY TABLES

Supplemental Table S3.1a: Mega analysis linear regression results for **bilateral** amygdala and hippocampal subnuclei volumes for individuals with OCD(n=381) compared with adult HCs (n=338), adjusting for age, sex and scan site and whole volumes for amygdala and hippocampus.

Supplemental Table S3.1b: Mega analysis linear regression results for **separate left and right** amygdala and hippocampal subnuclei volumes for individuals with OCD(n=381) compared with adult HCs (n=338), adjusting for age, sex and scan site and whole volumes for amygdala and hippocampus.

Supplemental Table S3.2a: Mega analysis linear regression results for **bilateral** amygdala and hippocampal subnuclei volumes for individuals with OCD(n=369) **without PTSD** compared with adult HCs (n=338), adjusting for age, sex and scan site and whole volumes for amygdala and hippocampus.

Supplemental Table S3.2b: Mega analysis linear regression results **for left and right** amygdala and hippocampal subnuclei volumes for individuals with OCD(n=369) **without PTSD** compared with adult HCs (n=338), adjusting for age, sex and scan site and whole volumes for amygdala and hippocampus.

Supplemental Table S3.3a: Mega analysis linear regression results for **bilateral** amygdala and hippocampal subnuclei volumes for individuals with OCD(n=74) **with anxiety disorder comorbidity** compared with adult HCs (n=338), adjusting for age, sex and scan site and whole volumes for amygdala and hippocampus.

Supplemental Table S3.3b: Mega analysis linear regression results for **left and right** amygdala and hippocampal subnuclei volumes for individuals with OCD(n=74) **with anxiety disorder comorbidity** compared with adult HCs (n=338), adjusting for age, sex and scan site and whole volumes for amygdala and hippocampus.

Supplemental Table S3.4a: Mega analysis linear regression results for **bilateral** amygdala and hippocampal subnuclei volumes for individuals with OCD(n=307) **without anxiety disorder comorbidity** compared with adult HCs (n=338), adjusting for age, sex and scan site and whole volumes for amygdala and hippocampus.

Supplemental Table S3.4b: Mega analysis linear regression results for **left and right** amygdala and hippocampal subnuclei volumes for individuals with OCD(n=307) **without anxiety disorder comorbidity** compared with adult HCs (n=338), adjusting for age, sex and scan site and whole volumes for amygdala and hippocampus.

Supplemental Table S3.5a: Mega analysis linear regression results for **bilateral** amygdala and hippocampal subnuclei volumes for individuals with OCD(n=95) **with MDD comorbidity** compared with adult HCs (n=338), adjusting for age, sex and scan site and whole volumes for amygdala and hippocampus.

Supplemental Table S3.5b: Mega analysis linear regression results for **left and right** amygdala and hippocampal subnuclei volumes for individuals with OCD(n=95) **with MDD comorbidity** compared with adult HCs (n=338), adjusting for age, sex and scan site and whole volumes for amygdala and hippocampus.

Supplemental Table S3.6a: Mega analysis linear regression results for **bilateral** amygdala and hippocampal subnuclei volumes for individuals with OCD(n=286) **without MDD comorbidity** compared with adult HCs (n=338), adjusting for age, sex and scan site and whole volumes for amygdala and hippocampus.

Supplemental Table S3.6b: Mega analysis linear regression results for **left and right** amygdala and hippocampal subnuclei volumes for individuals with OCD(n=286) **without MDD comorbidity** compared with adult HCs (n=338), adjusting for age, sex and scan site and whole volumes for amygdala and hippocampus.

Supplemental Table S3.7a: Mega analysis linear regression results for **bilateral** amygdala and hippocampal subnuclei volumes for individuals with OCD(n=161) **with medication use** compared with adult HCs (n=291), adjusting for age, sex and scan site and whole volumes for amygdala and hippocampus.

Supplemental Table S3.7b: Mega analysis linear regression results for **left and right** amygdala and hippocampal subnuclei volumes for individuals with OCD(n=161) **with medication use** compared with adult HCs (n=291), adjusting for age, sex and scan site and whole volumes for amygdala and hippocampus.

Supplemental Table S3.8a: Mega analysis linear regression results for **bilateral** amygdala and hippocampal subnuclei volumes for individuals with OCD(n=220) **without medication use** compared with adult HCs (n=338), adjusting for age, sex and scan site and whole volumes for amygdala and hippocampus.

Supplemental Table S3.8b: Mega analysis linear regression results for **left and right amygdala** and hippocampal subnuclei volumes for individuals with OCD(n=220) **without medication use** compared with adult HCs (n=338), adjusting for age, sex and scan site and whole volumes for amygdala and hippocampus.

Supplemental Table 3.9: Mega analysis linear regression results for the association between amygdala and hippocampal subnuclei volumes in individuals with OCD(n=351) and **YBOCS score**.

Supplementary Table S4.1: Characteristics of T1-weighted scans collected at 3T MRI scanners.

Supplementary Table S4.2: Clinical characteristics for sub-group mega-analysis (reported for individuals with SAD).

Supplementary Table S4.3a: Mega analysis linear regression results for **bilateral** amygdala and hippocampal subfield volumes for **patients with SAD** (n=107) compared with healthy controls (HCs) (n=140), adjusting for age, sex, scan site and whole AH volumes.

Supplementary Table S4.3b: Mega analysis linear regression results for **separate left and right** amygdala and hippocampal subfield volumes for **patients with SAD** (n=107) compared with healthy controls (HCs) (n=140), adjusting for age, sex, scan site and whole AH volumes.

Supplementary Table S4.3c: SAD (n=107, full aggregate sample) left and right mean values for amygdala and hippocampal subfields (unadjusted).

Supplementary Table S4.3d: HCs (n=140, full aggregate sample) left and right mean values for amygdala and hippocampal subfields (unadjusted).

Supplementary Table S4.4: Mega analysis linear regression results for **bilateral** amygdala and hippocampal subfield volumes for **patients with SAD without comorbid anxiety disorder** (n=86) compared with adult healthy controls (HCs) (n=140), adjusting for age, sex, scan site and whole AH volumes.

Supplementary Table S4.5: Mega analysis linear regression results for **bilateral** amygdala and hippocampal subfield volumes for **patients with SAD without MDD** (n=83) compared with adult healthy controls (HCs) (n=140), adjusting for age, sex, scan site and whole AH volumes.

Supplementary Table S4.6: Mega analysis linear regression results for **bilateral** amygdala and hippocampal subfield volumes for **patients with SAD without medication** (n=89) compared with adult healthy controls (HCs) (n=140), adjusting for age, sex, scan site and whole AH volumes.

Supplementary Table S4.7: Mega analysis linear regression results for **bilateral** amygdala and hippocampal subfield volumes and **symptom severity** (n=76) adjusting for age, sex, and scan site whole AH volumes.

LIST OF ABBREVIATIONS

AAA	Anterior Amygdaloid Area
AB	Accessory Basal
AD	Alzheimer's disease
APA	American Psychiatric Association
BA	Basal
BARACUS	Brain Age and Brain-Age Regression Analysis and Computation Utility Software
BLA	Basolateral Division
BMI	Body mass index
Brain-PAD	Brain predicted age difference
CA	Cornus Ammonis
CAT	Cortico-Amygdaloid Transition
CBT	Cognitive-behavioural therapy
Ce	Central
CMA	Centromedial Division
Co	Cortical
CSTC	Cortico-striato-thalamo-cortical
DG	Dentate Gyrus
DSM	Diagnostic and Statistical Manual
EURSANAD	European and South African Research Network in Anxiety Disorders
FDR	False discovery rate
fMRI	Functional magnetic resonance imaging
HATA	Hippocampal-amygdala transition area
HCs	Healthy controls
HPA	Hypothalamic-pituitary-adrenal
ICD	International Classification of Diseases

LA	Lateral
LSAS	Liebowitz Social Anxiety Scale
MAE	Mean absolute error
MDD	Major depressive disorder
Me	Medial
ML	Molecular Layer
MLA	Machine learning algorithm
MRI	Magnetic resonance imaging
NESDA	Netherlands Study of Depression and Anxiety
OBIC	Obsessive-Compulsive Disorder Brain Imaging Consortium
OCD	Obsessive-compulsive disorder
PL	Paralaminar Nucleus
PRISMA	Preferred reporting items for systematic reviews and meta-analyses
PTSD	Posttraumatic stress disorder
QC	Quality control
qPCR	Quantitative polymerase chain reaction
SAD	Social anxiety disorder
SFA	Superficial Division
SSRI	Selective serotonin reuptake Inhibitors
UCT	University of Cape Town
YBOCS	Yale-Brown Obsessive-Compulsive Scale

ABSTRACT

Background

Obsessive-compulsive disorder (OCD) and social anxiety disorder (SAD) are debilitating disorders that are associated with (inconsistent) evidence of hippocampal and amygdala volumetric abnormalities. In addition, both OCD and SAD are associated with accentuated biological aging, as indexed by cellular and molecular markers. Nevertheless, little is known about brain aging in OCD and SAD, or the extent to which inconsistencies in hippocampal and amygdala volume findings in these disorders may be due to the differential effect of age on the subfields from which these structures are composed. Accordingly, this dissertation set out to characterise differences in hippocampal and amygdala subfield volumes between healthy controls (HCs) and participants with OCD and SAD in large-scale MRI datasets and relate these to whole and regional brain aging.

Methods

Hippocampal and amygdala subfield volumes and brain age estimates were derived from T1-weighted MRI images from the OCD Brain Imaging Consortium (De Wit et al., 2014) and the European and South African Research Network in Anxiety Disorders (Bas-Hoogendam et al., 2017). Subfield volumes were segmented using an automated segmentation algorithm from Freesurfer (v6.0). The brain age analysis was performed by using a previously trained machine learning algorithm that provides brain age estimates for the whole brain, as well as for regions of interest (occipital, frontal, temporal, parietal, cingulate, insula, or cerebellar–subcortical features) (Kaufmann et al., 2019). Differences in relative brain age (brain predicted age difference; brain-PAD) were calculated by subtracting chronological age from the predicted brain age. Between-group differences (diagnosis vs HCs) in volumetric and brain-PAD estimates were assessed using a mixed-effects (*d*) model adjusted for several covariates. Subgroup analyses were performed to determine the association of the main findings with clinical characteristics. Finally, unique associations between subfield volumes and whole brain age were estimated using partial correlation analysis.

Results

There was no evidence for a difference in subfield volumes between individuals with OCD and HCs. However, we found that psychotropic medication use was associated with significantly smaller hippocampal dentate gyrus (*d*=-0.26, *pFDR*=0.042), molecular layer (d=-0.29, *pFDR*=0.042) and larger lateral (*d*=0.23, *pFDR*=0.049) and basal (*d*=0.25, *pFDR*=0.049) amygdala subfields than HCs. Individuals with OCD without psychotropic medication use had significantly smaller hippocampal CA1 (d=-0.28, pFDR=0.016) compared to HCs. No association was found for symptom severity.

In contrast to the findings for OCD, individuals with SAD demonstrated significantly smaller basal ($d=-0.32$, $pFDR=0.022$), accessory basal ($d=-0.42$, $pFDR=0.005$) and corticoamygdaloid transition area ($d=0.37$, $pFDR=0.014$) amygdala subfields overall compared to HCs, and larger hippocampal CA3 ($d=0.34$, $pFDR=0.024$), CA4 ($d=0.44$, $pFDR= 0.007$), dentate gyrus ($d=0.35$, $pFDR= 0.022$) and molecular layer ($d=0.28$, $pFDR=0.033$). In addition, individuals with SAD without comorbid anxiety disorder had smaller lateral amygdala and hippocampal amygdala transition area, compared to HCs. No association was found for psychotropic medication use and symptom severity.

Individuals with OCD (n=375) had significantly higher whole brain-PAD (+1.6 years, $p_{FDR}=0.006$, $d=0.20$) compared to HCs (n=335), but no differences were observed in the regional models. The effect on whole brain brain-PAD estimates was largely driven by psychotropic medication use as higher relative brain age was evident in individuals with OCD with psychotropic medication use (+2.98 years, d=0.38, $p <0.001$) compared to HCs, but not in individuals without psychotropic medication use (+0.57 years, d=0.07, $p =0.374$) compared to HCs. No association was found for symptom severity. Partial correlation analysis found a significant negative association between hippocampal and amygdala volume and whole brain PAD in the OCD group (R=-0.224, p=0.00001), but not in the HC group (R=0.081, p=0.138), specifically the lateral nucleus (R=-0.18), CAT(R=-0.13), hippocampal fimbria (R=0.17), and hippocampal fissure (R=0.17) were significant in OCD.

Individuals with SAD (n=107) had significantly higher whole brain-PAD (+2.5 years, d=0.33, $p_{FDR}=0.010$) compared to HCs (n=137), and significantly higher regional brain-PAD in the temporal (+3.80 years, d=0.37, $p_{FDR}=0.008$,), parietal (+3.57 years, d=0.38, $p_{FDR}=0.008$), occipital (+3.26 years, d = 0.33, $p_{FDR}=0.010$), and frontal regions (+2.97 years, d=0.33, $p_{FDR}=0.010$,) compared to HCs. Brain-PAD was higher in SAD without comorbid anxiety disorder, without MDD, and without psychotropic medication use. No association was found for symptom severity. There was no partial correlation between subfields and brain age.

Discussion & Conclusion

The evidence presented in the thesis suggests that 1) differences in subfield volumes between OCD and HCs were influenced by psychotropic medication use, which is consistent with previous studies that suggest that psychotropic medication status is a strong confounder for subcortical brain volumes observed in OCD, 2) differences in subfield volumes between SAD and HCs were observed in the areas associated with sensory information processing and these differences were partially influenced by psychiatric comorbidity, 3) both OCD and SAD were associated with accentuated brain aging with differential patterns in the whole and regional brain, dependent on clinical characteristics, and 4) only OCD relative brain age was associated with subfield volumes. It is unclear whether our findings in OCD and SAD reflect an adaptive response or are a pre-existing risk factor to these disorders, or both. Future longitudinal analysis is required to investigate whether the observed differences in subfield volume and brain age remain over time.

CHAPTER 1: INTRODUCTION

Structural abnormalities in subcortical regions and aging indicators in obsessive-compulsive disorder and social anxiety disorder

Obsessive-compulsive disorder (OCD) and social anxiety disorder (SAD) are prevalent and debilitating anxiety and related disorders. Despite their separation into different chapters in the recent diagnostic manual (DSM5, APA, 2013), it is important to note that this does not mean that OCD is not accompanied by anxiety, but rather that OCD may involve certain neurobiological pathways. Specifically, as detailed further below, a substantial body of work indicates that the cortico-striato-thalamo-cortical (CTSC) loop is the predominant neurobiological model of OCD, although there has been the recent addition of limbic structures including the hippocampus and amygdala. The hippocampus and amygdala are also significant in SAD as the integral structures involved in the neurocircuitry of fear model implicated in SAD. There is some evidence of hippocampus and amygdala activation in SAD; however, structural findings have been mixed for both disorders. This dissertation aims to investigate hippocampus and amygdala subfield volumes and their relation to brain aging in anxiety and related disorders.

In this chapter (chapter 1), I outline the characteristics, prevalence, and comorbidity of OCD and SAD. I also present evidence of structural and functional abnormalities in both disorders. Lastly, I introduce the topic of brain aging, how it is measured, and its relevance in anxiety disorders. This background chapter provides the context for the aims of the work undertaken in this dissertation, which are outlined at the close of the chapter.

1.1. Characteristics of OCD and SAD

OCD is characterized by obsessions and/or compulsions. In obsessions, individuals with OCD experience unwanted, persistent, and intrusive thoughts, urges, or impulses that elicit excessive anxiety. In compulsions, the individual performs repetitive, ritualistic, and recurring behaviours, or mental acts, to alleviate the anxiety caused by the obsessions (APA, 2013). While the compulsion may ease the anxiety induced by the obsession, the compulsion is not intrinsically pleasurable. In most cases, individuals diagnosed with OCD present with both obsessions and compulsions, however, a diagnosis can be reached even if one of these components is absent (Goodman et al., 2014). Studies suggest that a key component of OCD is difficulty inhibiting irrelevant information (Bannon et al., 2002). Typically, individuals with OCD experience obsessions and compulsions concerning contamination (washing or cleaning), symmetry (ordering or arranging), and intrusive aggressive thoughts (involving mental rituals). In some cases, avoidance behaviours are observed as individuals may refrain from certain activities to prevent triggering the obsessions (Stein et al., 2019).

Studies suggest that there are two main age of onset peaks when individuals may start experiencing OCD symptoms (Geller et al., 2021). Most individuals with OCD experience symptoms before puberty, between the ages of 7 and 12 years old (Dell'Osso et al., 2016), or during their early 20's (Ruscio et al., 2010). Following onset, longitudinal data suggest that OCD has a long duration as symptoms can persist for years (Sharma et al., 2014). Only less than 10% of individuals with OCD experience spontaneous remission, whilst another 5-10 % report worsening symptoms (Eisen et al., 2010).

SAD, previously known as social phobia, is characterized by heightened and persistent anxiety in social or performance-based situations. Individuals with SAD fear social settings that involve exposure to unfamiliar people where they may face possible scrutiny or perform in a manner that may cause embarrassment to themselves (APA, 2013). Typically, these fears lead to the avoidance of social settings or the endurance of such environments with great distress. Studies of SAD suggest that these individuals have heightened sensitivity to perceived social threats, underlined by biased perception of social settings. This leads to innocuous social cues being interpreted as potential threats, which elicits anxiety (Pantazatos et al., 2014). In the majority of cases, SAD is characterized by early age of onset, with symptoms emerging in adolescence, and in some cases, symptoms emerge in childhood (Keller, 2003; Marazziti et al., 2015). Following its onset, typically SAD is a lifetime condition with a chronic trajectory involving frequent and recurrent episodes (Keller, 2003). Like many psychiatric disorders, including OCD, the aetiology of SAD is not fully understood and likely involves a complex relationship between environmental, genetic, and neurobiological factors (Bandelow et al., 2016).

1.2. Prevalence of OCD and SAD

Epidemiological studies suggest that OCD has a 12-month and lifetime prevalence of 1.2% (SE=0.3) and 2.3% (SE=0.3), respectively (Ruscio et al., 2010). On the other hand, SAD is the third most common psychiatric disorder (Pollack, 2001) with a 12-month and lifetime prevalence of 7.1% (SE=0.3) and ± 12.1% (SE=0.4) respectively (Stein, 2008). Both OCD and SAD are debilitating conditions, affecting social and professional relationships, as well as education and socioeconomic status (Hollander et al., 2010). In individuals with 12-month OCD, Ruscio et al. (2010) reported that the percentage of role impairment in relationships was 47.1% (SE=±12.4%) and 33.6% (SE=±9.6%) in the social sphere, whilst role impairment in home management and work-life was 35.3% (±12.7%) and 20.9% (±7.7%), respectively (Ruscio et al., 2010). Similarly, SAD has been associated with school absenteeism and drop-out, as well as functional disability in adults (Mack et al., 2005).

1.3. Comorbidity and overlap between OCD and SAD

In many cases, OCD is comorbid with mood disorders, impulse-control disorders, and SAD (Ruscio et al., 2010). OCD and SAD are highly comorbid, which is suggested to lead to greater impairment and worsened long-term outcomes (Keller, 2003). In the general population, the comorbidity between OCD and SAD ranges from 15% and 43.5%, whereas in clinical samples comorbidity overlap ranges from 12% and 42 % (Baldwin et al., 2008). Individuals with a primary diagnosis of OCD are more prone to lifetime SAD comorbidity (range between 33.3% and 43.5%) than individuals with a primary SAD diagnosis are prone to lifetime OCD comorbidity (lifetime comorbidity rates 2.3 and 11%) (Assunção et al., 2012; Rudy et al., 2014). The factors that contribute to the variation in comorbidity patterns observed in OCD and SAD have not yet been fully understood but may involve genetic and cognitive moderators (Rudy et al., 2014).

The fifth edition of the Diagnostic and Statistical Manual (DSM-5) has differentiated OCD from anxiety disorders (APA, 2013). In the new revision, OCD is categorized with other disorders that involve repetitive behaviours including trichotillomania, and skin-picking disorder as "Obsessive-compulsive related disorders" (APA, 2013). Similar implementations have been made in the International Classification of Diseases, 11th Revision (ICD-11) (Carpita et al., 2019). These changes do not mean that OCD is not accompanied by anxiety (Goodman et al., 2014), but rather that OCD may have distinct neurobiological pathways that may not be as prominent in other types of anxiety disorders (Cisler et al., 2009; Fiddick, 2011; Goodman et al., 2014).

Despite the separation in recent diagnostic systems between these disorders, OCD and SAD have some commonalities such as; 1) avoidance behaviours as individuals with SAD are likely to avoid social situations and individuals with OCD may avoid triggers that lead to compulsions and obsession (APA, 2013), 2) symptom severity as studies show that individuals with OCD and those with SAD tend to cluster according to the severity of their symptoms rather than their diagnostic category (Vigne et al., 2019)) and 3) response to selective serotonin reuptake Inhibitors (SSRIs) treatment (López-Solà et al., 2014, 2016). That being said, anxiety and related disorders are highly complex and polygenic (Caldiroli et al., 2023), and further evidence is required to elucidate how OCD and SAD overlap.

1.4. Psychotropic and psychological treatment of OCD and SAD.

Studies suggest that both OCD and SAD have a moderate response to SSRIs and exposure therapies. Data from randomized, double-blind trials involving placebos and comparator medications show that more than 60% of individuals respond to treatment (Bandelow, 2008; Williams et al., 2020). SSRIs are more effective than noradrenergic reuptake inhibitors (Fineberg & Gale, 2005). In some anxiety and related disorders, SSRIs are the first line of treatment (Stein et al., 2019), have good tolerability, and have been shown to reduce symptoms (Soomro et al., 2008). Hoexter et al. 2013 found symptom improvement in OCD after fluoxetine treatment, which was correlated with smaller pre-treatment gray matter volume within the right middle lateral orbitofrontal cortex. On the other hand, symptom improvement following cognitive-behavioural therapy (CBT) was associated with larger pre-treatment gray matter volume within the right medial prefrontal cortex. These findings suggest that certain brain regions may be involved in responding to specific treatment modalities in OCD (Hoexter et al., 2013). A systematic review and meta-analysis of randomized clinical trials conducted in SAD indicated mainly positive outcomes for several psychotropic medications including SSRIs. (Williams et al., 2020). Specifically, Paroxetine was found to be the most effective in the reducing symptom severity compared to placebo (Williams et al., 2020).

1.5. Structural differences in hippocampal and amygdala volumes in OCD.

Converging neuroimaging evidence supports a CSTC model as a prevailing neurobiological model of OCD (van den Heuvel et al., 2016). The CSTC model of OCD suggests that there is dysregulation between direct and indirect pathways from cortical brain regions (such as the orbitofrontal cortex and the anterior cingulate cortex) to the thalamus via the striatum, resulting in reduced inhibition of the thalamus and increased excitatory feedback to frontal brain regions (Mahjani et al., 2021). In recent years, studies suggest that OCD likely involves additional brain circuits including cerebellar, fronto-parietal, and fronto-limbic circuits (Stein et al., 2019). Of note, the current CSTC model in OCD may have overlooked the involvement of the hippocampus and amygdala and their interaction with the frontal cortex in mediating fear and anxiety (Milad & Rauch, 2012; van den Heuvel et al., 2022). There is evidence of differences in hippocampal-based fear extinction and adaptive goal-directed behaviour in individuals with OCD (n=21), compared to healthy controls (HCs) (n=21) (Milad et al., 2013). Moreover, a meta-analysis of emotional processing in OCD showed hyperactivation in the right amygdala in individuals with OCD compared to HCs, and these findings were linked to clinical characteristics such as medication status and psychiatric comorbidity (Thorsen et al., 2018).

Structural magnetic resonance imaging (MRI) studies in OCD have heterogeneous findings, with both increases and decreases in hippocampal and amygdala volume reported in individuals with OCD compared to HCs (Pujol, 2004: OCD n=72, HCs n=72; Atmaca et al., 2008: OCD n=14, HCs n=14; Kwon et al., 2003: OCD n=22, HCs n=22; Rao et al., 2018: OCD n=26, HCs n=20). Possible explanations for these inconsistent findings include methodological differences and small sample sizes. In a multisite meta and mega-analysis, Boedhoe et al. (2017) found smaller hippocampal volumes in individuals with OCD (n=1,495) compared to HCs (n=1,472)(d=−0.13; p<0.001, 2.8 % difference), although no differences were observed for the amygdala (Boedhoe et al., 2017). In other work, Fouche et al. (2017) found smaller hippocampal volumes in individuals with OCD (n=412) compared to HCs (n=368) (left hemisphere: F=8.630, p=0.0002; right hemisphere: F=6.727, p=0.0007), which was also dependent on psychiatric comorbidity and medication status (Fouche et al., 2017). In recent work, Fouche et al. (2022) did not find a significant between-group difference (OCD vs HCs) in shape thickness or surface area of the hippocampus in the main analysis. However, in a subgroup analysis, the thickness of the hippocampus was found to be lower (d=−0.111, 2.4% difference) in individuals with OCD with comorbid anxiety (n=83), compared to HCs (n=368) (Fouche et al., 2022). In addition, there was a lower surface area in the right hippocampus (d=−0.153, 5.6% difference) and right amygdala (d=−0.137, 6.8% difference), and lower thickness in the bilateral hippocampus (left: d=−0.161, 5.8% difference, right: d=−0.146, 4.6% difference) in individuals with OCD with psychotropic medication use (Fouche et al., 2022). In another study, Ivanov et al. (2022) found smaller hippocampal volumes in individuals with OCD (n=1611, aged 20 years and older) with psychotropic medication use (tricyclics and/or benzodiazepines), compared to HCs (Ivanov et al., 2022). Taken together, these findings suggest that OCD may involve differences in hippocampus and amygdala volume and that these differences may be associated with clinical characteristics (van den Heuvel et al., 2022).

1.6. Structural differences in hippocampal and amygdala volumes in SAD.

The hippocampus, amygdala, insular, and medial prefrontal cortex have been considered important regions of interest in SAD (Shin & Liberzon, 2010). Neurobiological models of SAD have highlighted the involvement of some of these structures in the context of threat perception, and social avoidance-approach conflicts in the neurocircuitry of fear of SAD (Brühl et al., 2014; Shin & Liberzon, 2010).

Structural MRI studies have yielded inconsistent results (Brühl et al., 2014; Syal et al., 2012). Studies found smaller hippocampal and amygdala volumes (Irle et al., 2010: SAD n=67, HCs n=64; Liao et al., 2011: SAD n=18, HCs n=18; Meng et al., 2013: SAD n=20, HCs n=19), larger left hippocampal and amygdala volume (Machado-de-Sousa et al., 2014: SAD n=12, HCs n=14) and no differences in the amygdala volumes (Syal et al., 2012: SAD n=13, HCs n=13) in individuals with SAD compared to HCs. Of note, these studies were conducted in small sample sizes, which reduces the statistical power to detect between-group differences (Bas-Hoogendam et al., 2022). In a multisite large-scale study, Bas-Hoogendam et al. (2017) did not find a significant difference in the amygdala-hippocampal complex in individuals with SAD (n=174), compared to HCs (n=213) (Bas-Hoogendam et al., 2017). The study concluded that the previous inconsistent findings were likely due to methodological differences and a higher likelihood of smaller sampled studies (generally less than 30 individuals) producing false-positive results (Bas-Hoogendam et al., 2017). Additionally, it is possible that potential confounders (i.e., psychiatric comorbidity, medication use, and symptom severity) differ amongst individuals and thus may influence study findings (Bas-Hoogendam et al., 2022). In a recent mega-analysis (including 37 international sites), Groenewold et al. (2023) found smaller left amygdala volume (d=-0.145,

$p_{FWE}=0.017$) in individuals with SAD with comorbid anxiety disorders (n=404) compared to HCs (*n*=2538), however, these findings did not reach significance when selectively including adults with SAD with comorbid anxiety disorder, despite a similar effect size (d=−0.174, $p_{FWE}=0.077$); and neither in adolescents with SAD with comorbid anxiety disorder(d=−0.110, $p_{FWE}=1.00$) (Groenewold et al., 2023).

1.7. Hippocampal and amygdala divisions (subfields) and their functions

The hippocampus can be divided into the cornus ammonis (CA) 1-4, molecular layer (ML), alveus, dentate gyrus (DG), subiculum, presubiculum, parasubiculum, fimbria, hippocampal tail, and fissure (Iglesias et al., 2015). The CA1 is thought to be the major output area of the hippocampus, and projects to the subiculum, entorhinal cortex, parahippocampal, and pre-frontal area including the orbitofrontal cortex (Yang & Wang, 2017). These pathways are suggested to be involved in retrieving information from the hippocampus to the neocortex (Rolls, 2013) and thereby assist in hippocampal-dependent cognition and behaviour (Knierim & Neunuebel, 2016).

The CA3/DG are suggested to be involved in pattern separation and completion which is a process that allows the hippocampus to form new episodic memories without disturbing those that already exist (Besnard & Sahay, 2016). The DG is suggested to be involved in pattern separation which is the distinguishing of similar cortical inputs during memory storage. On the other hand, the CA3 is linked to pattern completion, a process whereby a sequence is completed based on partial features. Dysregulation in pattern separation is suggested to underly fear generalization which leads to the inability to differentiate between safe and unsafe stimuli, thereby causing exaggerated fear and anxiety responses to innocuous social stimuli (Besnard & Sahay, 2016; Knierim & Neunuebel, 2016; Rolls, 2013).

There is ongoing debate about the names and boundaries of the amygdala, but it is typically divided into three groups (1) the basolateral division (BLA) which is the ventromedial branch of the claustrum anlage, (2) the centromedial division (CMA) which is considered a ventromedial extension of the striatum, and (3) a superficial division (SFA) which is a cortical-like group found on the medial surface of the amygdala (Figure 2)(Amunts et al., 2005; Schmitz-Koep et al., 2021; Wang et al., 2021). The BLA is associated with fear learning and contains the lateral nucleus (LA), which is suggested to be the primary sensory input area of the amygdala and receives information from the thalamus and other sensory areas. The BLA also contains the basal (BA) and basomedial (BM) nuclei, which receive projections from the LA (Janak & Tye, 2015; Yang & Wang, 2017). The CMA is thought to be the primary output area, contains the central and medial nucleus (LeDoux., 2007), and is associated with emotive responses and surprise in fear learning (Christian et al., 2013). Lastly is the SFA, which contains the nucleus in the lateral olfactory tract and the cortical nuclei (Wang et al., 2021). There are additional nuclei that do not easily fall into the three categories, namely the intercalated masses and the hippocampal-amygdala transition area (HATA) (which joins the neighbouring hippocampus to the amygdala, as a part of the uncal hippocampal formation) (Amunts et al., 2005; Sah et al., 2003).

1.8. Hippocampal and amygdala subfields in anxiety related behaviours

The extensive connectivity within and between the hippocampal and amygdala subfields is suggested to modulate anxiety and social behaviours (Qin et al., 2014). For example, anxious behaviours are heightened when BLA projections to the centrolateral nucleus are inhibited (Tye et al., 2011); the central amygdala pathway regulates defensive behaviours like passive freezing or active escape (Terburg et al., 2018). There is also evidence that both the BLA and the ventral hippocampus are responsible for the expression of anxiety-related behaviours (Yang & Wang, 2017). In a resting-state static and dynamic functional connectivity study, Wang et al. (2021) found that individuals with high trait anxiety (n=257) had lower static functional connectivity between the CMA, BLA, and SFA and the executive control network (Wang et al., 2021). In a study of functional connectivity between amygdala subfields in individuals with OCD, Cao et al. (2022) found that in HCs (n=90) the BLA had strong connectivity with the insula, supplementary motor area, midcingulate cortex, superior temporal gyrus, and postcentral gyrus, compared to CMA (Cao et al., 2022). However, in individuals with OCD (n=92), the CMA had stronger connectivity than the BLA to these regions (Cao et al., 2022). While the study also found smaller BLA and CMA volumes in individuals with OCD compared to HCs, the amygdala was segmented using k-means clustering using connectivity-based parcellation techniques (Eickhoff et al., 2015; Reuter et al., 2020), resulting in subfields that are not identical to those produced using more commonly employed probabilistic-based segmentation techniques (Avecillas-Chasin et al., 2023; Saygin et al., 2017).

1.9. Hippocampal and amygdala subfield volumes in anxiety and related disorders

There are increasing investigations of hippocampal and amygdala subfield volume differences in various psychiatric disorders (over 1700 articles between 2015 and 2022 (Canada et al., 2023)).

In posttraumatic stress disorder (PTSD), the lateral and paralaminar amygdala subfields were found to be smaller, while the central, medial, and cortical subfields were found to be larger in individuals (military veterans with PTSD n=149), compared to HCs (n=206) (Morey et al., 2020). In a study comparing hippocampal subfields in PTSD to SAD, Ahmed-Leitao et al. (2019) found that only the parasubiculum and HATA were smaller in PTSD (n=17)(Ahmed-Leitao et al., 2019). However, in this study, the individuals with SAD (n=26) were not compared to HCs (n=25) (Ahmed-Leitao et al., 2019).

A recent meta-analysis (n=2876 individuals) found smaller CA1, CA3, DG, and subiculum, and altered lateral, basal, and central subfield volumes in PTSD (Ben-zion et al., 2023).

In OCD, smaller subfield volumes in the hippocampal subiculum, presibiculum, CA2/3, and hippocampal tail, and larger hippocampal fimbria, were observed in individuals with OCD (n=81) compared to HCs (n=95) (Zhang et al., 2019). In another study, smaller central subfield volumes were observed in individuals with OCD (n=81) compared to HCs (n=95) (Zhang et al., 2020). Additionally, the volume of the central nucleus was associated with illness duration (Zhang et al., 2020). While these findings have provided the first insight into subfield differences in OCD, they were conducted in small sample sizes (n=81, for both papers) and excluded potential confounders like psychiatric comorbidity and medication use.

In a larger study of hippocampal subfields across four major psychiatric disorders including schizophrenia (n=171), bipolar disorder (n=197), OCD (n=205), and major depressive disorder (MDD)(n=136), OCD was associated with larger volumes of the hippocampal subiculum, DG, presubiculum and ML (Jiang et al., 2022). These findings were similar to reports in paediatric OCD, where larger volumes in the left hippocampal CA4, DG, ML, and parasubiculum were observed in OCD (n=29) compared to HCs (n=28) (Vattimo et al., 2021). However, both Jiang et al., 2022 and Vattimo et al., 2021 did not control for medication use, which may have some influence on hippocampal and amygdala volumes in OCD (Bruin et al., 2020; van den Heuvel et al., 2022).

Summary

In addition to the structural and functional differences observed in certain brain regions in OCD and SAD, both disorders have also been associated with indicators of advanced biological aging and early mortality suggesting that individuals with these disorders may be vulnerable to accentuated aging. In the following section, I introduce the concept of aging (chronological and biological) and the markers that are used to indicate biological aging in healthy conditions and psychiatric disorders. I also introduce the use of neuroimaging data to predict brain age, and I highlight the latest findings in predicted brain age differences in psychiatric conditions.

1.10. Defining aging and its importance.

Healthy successful aging is necessary to maintain the well-being of a growing older population, however, currently, we seldom find extremely aged individuals (+90 years) who remain in good health until the end of life (Dong et al., 2016). In the global population, it is estimated that dementia will double every 20 years, to 65.7 million by 2030 (Abbott, 2010). Given that aging is a prevailing risk factor for chronic diseases including neurodegenerative diseases like Alzheimer's disease (AD) and Parkinson's disease (Cole & Franke, 2017), it is becoming increasingly important to improve our understanding of the biological process of aging, how individuals deviate from "normal healthy aging", and identify individuals with underlying conditions who may be particularly vulnerable to age-related diseases.

While there is no uniform definition of aging, it is considered a natural decline in physiological function over time, driven by changes in the internal molecular pathways of a living organism (López-Otín et al., 2013; Xia et al., 2017). Chronological age is determined by the number of calendar years that have passed since birth, and as such it is considered to be a purely quantitative measure of time (Han et al., 2019). While chronological age may provide a straightforward measure of the number of years lived, it does not account for factors that influence an individual's overall well-being, nor biological age (Khan et al., 2017).

The definition of biological age is not fixed, however, it is proposed as "a quantity expressing the 'true global state' of an aging organism better than corresponding chronological age", although this definition is problematic given the different rates of biological aging between tissue types and methods of assessment (Han et al., 2019). Biological age is characterized as corresponding better to "true life expectancy" than what is reflected by the chronological age (Klemera & Doubal, 2006). Moreover, unlike chronological age, biological age is dynamic and it indicates the biological and functional state of an individual, under the influence of internal (i.e., genetics) and external (adversity, diet) factors (De Magalhães, 2012).

1.11. Aging in the developing brain

During the early stages of healthy brain development, the aging process involves a series of highly synchronized events of progression (e.g., cell growth) and regression (e.g., synaptic pruning) (Franke et al., 2020), which facilitate the maturation of sensorimotor, cognitive, social and emotional functions (Hou et al., 2019; Niu et al., 2022; Sone & Beheshti, 2022). A study using four-dimensional maps and time-lapse sequences in children (aged between 8 years and 10 years old, n=13) revealed that brain maturation first occurs in the lower-order somatosensory and visual cortices followed by the higher-order association cortices and that older phylogenetic regions mature earlier than newer regions

(Gogtay et al., 2004). This pattern is also found in the resting state-fMRI infant literature (first 2 years of life, n=143) where neonates develop local connectivity and bilateral symmetric functional connections, resembling adult-like primary visual and motor-sensory networks (Gao et al., 2015). In a large-scale MRI study (scans n=123,984) from 101,457 humans between 115 days post-conception to 100 years of age, Bethlehem et al. (2022) demonstrated lifespan curves showing pronounced increase in global gray matter volume from mid-gestation, peaking at 5.9 years, followed by a linear decrease in volume (Bethlehem et al., 2022). This pattern was similar in white matter volume where a rapid increase was observed from mid-gestation to early childhood, peaking at 28.7 years and subsequently decreasing after 50 years old (Bethlehem et al., 2022). Other structures like the hippocampus are suggested to have distinct subfield-specific developmental trajectories during normal human development. For example, Gogtay et al. (2006) found that the posterior portion of the hippocampus increased in volume over time whereas the anterior portion decreased in volume (100 scans in 31 children and adolescents scanned every 2 years for 6–10 years between ages 4 and 25) (Gogtay et al., 2006).

1.12. Aging in the mature brain

In middle to late adulthood, brain aging may have a different connotation, as age-related brain changes are associated with the risk of neurodegenerative diseases (Cole & Franke, 2017). Structurally, cross-sectional and longitudinal studies, derived from postmortem, histological, and neuroimaging data, indicate that the processes of aging are related to brain atrophy, enlargement of the ventricles, deepening and widening of the sulcus, and increased cerebrospinal fluid to white and gray matter ratio (Armstrong et al., 2019: male n=295, and female n=328, aged 55 years to 92 years; Hugenschmidt et al., 2008: n=66, aged 18years to 80 years old). On a cellular level, it is suggested that because the brain contains postmitotic neurons and oligodendrocytes, which are shown to be more susceptible to DNA damage, it is particularly vulnerable to the process of aging (Madabhushi et al., 2014).

The brain is also suggested to have regional and inter-individual variability during aging (Dinsdale et al., 2021). Indeed, studies suggest that aging in the prefrontal lobe is associated with axonal degeneration and myelin disruption (which is thought to be linked to age-related slowing in cognitive processing speed), while no such disruptions are found in the corpus callosum (aged between 55 years and 80 years old, n=152) (Lu et al., 2011). A longitudinal study investigating differences in 12 regional brain volumes across 2.5 years in individuals (>= 49 years of age, n=40) found a significant reduction in the volume of the hippocampus, orbital–frontal and entorhinal cortices as well as in the cerebellum at each time point (two follow-ups, 15 months apart)(Raz et al., 2010). Moreover, in this study, in addition to significant individual differences in reduction rates, the reduction of the hippocampal volume accelerated with time. On the other hand, the reduction of the caudate nucleus, prefrontal subcortical white matter, and corpus callosum were only evident at the second follow-up, whilst no changes were observed for the lateral prefrontal and primary visual cortices, putamen, and the pons (Raz et al., 2010).

An anticipated ramification of aging on brain function is cognitive decline (i.e., verbal memory, working memory, and processing speed) (Cole et al., 2018). Cognitive function is also proposed to be dependent on synaptic density, and studies suggest that synaptic density decreases due to increased synaptic pruning and decreased synaptic plasticity in older adults (Masliah et al., 2006). While the exact connection between cognitive aging, functioning, and overall neurophysiological changes in the brain remains elusive (Cole et al., 2018), studies suggest that aging of the brain increases the likelihood of developing AD and dementia (Cole & Franke, 2017; Madabhushi et al., 2014). Additionally, even in the absence of age-related risk, advanced aging is likely to exacerbate pre-existing disease symptoms (Cole et al., 2019).

1.13. Aging in the hippocampus and amygdala

There are a plethora of studies that suggest that aging is associated with a decrease in the volume of the hippocampus and amygdala (Fjell et al., 2013a; Pfefferbaum et al., 2013; Walhovd et al., 2005, 2011). A study investigating 17 neuroanatomic structures in 1100 healthy adults (aged between 18 and 94 years) found that 1) cross-sectionally the areas with the steepest volume loss were the cerebral white matter, followed by the hippocampus, cerebellum white matter and thalamus, and 2) longitudinally, the most notable decrease in subcortical volumes was in the hippocampus (-0.83% annually) followed by the amygdala (-0.81% annually) and thalamus (-0.69% annually)(Fjell et al., 2013b).

Volume loss in both the hippocampus and amygdala has been demonstrated in neurodegenerative disorders such as AD (Prestia et al., 2011; Topkan et al., 2022) and is thought to partially contribute to the cognitive and behavioural changes associated with these disorders. Meta analytic findings show that the smaller the volume of the hippocampus the greater the severity in cognitive impairment or AD suggesting that hippocampal volume loss may potentially underly mechanisms of AD pathology, although further evidence is still required (Rao et al., 2023).

1.14. Aging in hippocampal and amygdala subfields

Some of the first studies to measure hippocampal subfield volume and normal aging were conducted in postmortem samples. In one study, West et al. (1994) found a linear association between the hippocampal DG and subiculum and chronological age (37% and 43 % volume loss, respectively, between the ages of 13 years and 101 years old, healthy participants=45) (West et al., 1994). Another study, by Simic et al., 1997 found a linear association in neuronal loss in the hippocampal CA1 and subiculum with chronological age (67% and 32% loss, respectively)(Šimić et al., 1997). Using manual tracing, Mueller et al. (2007) found a linear association between the CA1 and increasing chronological age in HCs (n=22) (Mueller et al., 2007). In a follow-up study, a linear effect of chronological age on the CA3/DG of the hippocampus was found in HCs (Mueller & Weiner, 2009).

Kurth et al. (2017) divided the whole hippocampus into three subfields (CA, fascia dentata and subiculum) as well as two hippocampus-adjacent areas (entorhinal cortex and HATA) and found that all of the subfields, except for the entorhinal cortex, were smaller with increasing chronological age (aged between 18 years and 69 years old, healthy participants n=96) (Kurth et al., 2017). In a following study, Kurth et al. (2019) found that the amygdala subfields were smaller with increasing chronological age in both a linear and nonlinear relationship (aged between 18 years and 69 years old, healthy participants n=100) (Kurth et al., 2019). While these studies provide evidence of differential aging amongst the subfields, the impact of aging on subfield volumes in normal and psychiatric conditions is still unknown (de Flores et al., 2015).

1.15. How does the aging process influence structural and functional changes in the brain in psychiatric conditions?

In a cross-sectional study, Sacchet et al. (2017) found a negative association between increasing chronological age and the volume of the putamen in individuals with MDD (n=116) compared to HCs (n=116) (Sacchet et al., 2017). This is consistent with other findings of age-related brain volume differences in MDD (Sachs-Ericsson et al., 2018). Previous studies in elderly MDD found caudate and ventricle enlargement (Beats et al., 1991; Skoog, 2011) and cortical atrophy (Schweitzer et al., 2001) with increasing chronological age. In a longitudinal study of elderly adults (n=1328; age between 65 and 80 years) symptoms of depression, as well as a history of major depression, were associated with greater grey matter loss in the hippocampus (Elbejjani et al., 2015).

In a mega-analysis of subcortical volumes in SAD (n=1115), Groenewold et al. (2023) found a significant interaction between SAD and chronological age. The study found smaller bilateral putamen and amygdala volumes with increasing chronological age in SAD compared to HCs (Groenewold et al., 2023), suggesting that there may be age-related volumes loss in these structures in SAD although further investigation is needed.

1.16. Machine learning models: what are they and how are they built?

Whilst there is some indication that brain structure and function are influenced by the process of aging (Dinsdale et al., 2021), until recently there have been very few techniques to measure aging in the brain. While some research suggests that individuals with psychiatric disorders may be prone to accentuated aging as indexed by molecular and cellular indicators of biological aging (see chapter 2 for review)(Darrow et al., 2016; Malouff & Schutte, 2017; Ridout et al., 2016), recent developments have allowed for the prediction of brain aging using neuroimaging data and machine learning- which has been an important step in increasing our understanding of the aging brain.

Machine learning algorithms (MLAs) in the context of brain age models refer to the use of complex computational methods to predict or estimate an individual's brain age based on various brain features extracted from neuroimaging data (Cole et al., 2018: Franke et al., 2020). MLA-based brain age models have become increasingly popular due to their ability to capitalize on multivariate data (Bray et al., 2009), utilizing comprehensive neuroimaging data to identify brain aging patterns and highlight patterns that deviate from healthy aging trajectories (Nielsen et al., 2020). Unlike traditional statistical models that relate overall population averages to chronological age, MLA-based brain age models are considered to have potential clinical relevance as their predictions are based on the unique brain features of the individual (Baecker et al., 2021; Dinsdale et al., 2021).

There are myriad techniques, with varying degrees of complexity, that can be used to build brain age models. Similarly, different MRI modalities (including T2 FLAIR, functional MRI, and diffusion MRI) can be used as input data for machine learning, although the most commonly used data is from T1-weighted images (Miller et al., 2016). Most brain age models are built using supervised machine learning, which is a technique where the algorithm is trained using labelled data (Baecker et al., 2021). In supervised learning, neuroimaging data is divided into three subsets; 1) training, 2) validation, and 3) testing (Zou et al., 2019). Depending on the exact MLA used, the MRI data can be pre-processed to extract a rich set of brain features like cortical thickness and surface area (Baecker et al., 2021).

In the training dataset, the MLA learns to identify a relationship between multivariate features and participant labels, which depending on the research design may include diagnosis (e.g., diagnosis vs HCs) or patient states (e.g., depressed vs remitted)(Davatzikos, 2019; Nielsen et al., 2020). This step may rely on different types of algorithms like support vector machines (which are effective for binary classification when there is a clear separation between different data classes), or random forest classifiers (which bring together numerous decision trees to make a prediction) (Nielsen et al., 2020). Each technique has its own set of advantages and disadvantages e.g., sensitivity to noise, scalability, and versatility (for review see Angermueller et al., 2016; Ching et al., 2018).

After training, the resultant model is applied to a validation dataset for model optimization and tuning (by adjusting certain parameters), and cross-validation (dataset divided into multiple subsets or "k-folds" for an iterative process of training and validation) (Angermueller et al., 2016). After validation, the best model is selected based on its prediction accuracy (Baecker et al., 2021; Zou et al., 2019) and applied to an independent test sample where it is expected to make predictions without the use of a label (Baecker et al., 2021). In contrast to supervised machine learning, unsupervised machine learning is used to discover a relationship between multivariate features without the use of a label from the beginning. This technique often relies on principal components analysis, clustering, and outlier detection to make predictions from the unlabelled data (Angermueller et al., 2016; Maceachern & Forkert, 2021; Zou et al., 2019).

Predicted brain age calculation

Brain-PAD (brain predicted age difference) is typically calculated by subtracting an individual's chronological age from the estimated predicted brain age (Cole et al., 2018). A positive brain-PAD refers to predictions that are higher than an individual's chronological age, suggesting accentuated brain aging. On the other hand, a negative brain-PAD refers to predictions that are lower than an individual's chronological age, which is related to "delay" in brain aging (Baecker et al., 2021). Further evidence is required to elucidate the neurobiological mechanism of brain aging and how it relates to "accelerated" and "slowed" aging (Baecker et al., 2021; Han et al., 2019). For this dissertation, we will use the term "accentuated" brain aging when describing positive brain-PAD, as the term "accelerated" is only appropriate when used for longitudinal studies of brain aging.

Brain-PAD: what has been shown in psychiatric disorders?

Accentuated aging has been demonstrated in a variety of psychiatric disorders. In mood disorders, specifically MDD, multiple studies indicate positive brain-PAD ranging between 2 years to 4 years (Sone & Beheshti, 2022). For example, some studies found higher brain-PAD (+2.78 years, d=0.25, p_{FDR}=0.048) only when correcting for antidepressant use (Han et al., 2021), whilst other studies suggest that brain-PAD was associated with body mass index (BMI) but not with treatment response (Ballester et al., 2021). A recent meta-analysis found brain-PAD of +3.12 years in adults with psychotic disorders, +2.04 years in bipolar disorder, and +0.90 years in MDD (Blake et al., 2023). Similarly, other meta-analyses show brain-PAD of +3.08 years in psychotic disorders, +1.93 years in bipolar disorder, and +1.12 years in MDD (Ballester et al., 2022). In the largest and most comprehensive study of brain-PAD across psychiatric and neurodegenerative disorders, Kauffman et al., 2019 used T1-weighted images from 10,141 individuals and 35,474 HCs aged 3 to 96 years. This study found a brain-PAD of +1.03 years in dementia, +0.41 years in MCI, +0.10 years in MDD, +0.74 years in multiple sclerosis, +0.29 years in bipolar disorder, +0.51 years in schizophrenia, +0.06 years in attention-deficit hyperactivity disorder, and +0.07 years in autism spectrum disorder (Kaufmann et al., 2019).

While the evidence presented above suggests that there is accentuated brain aging in anxiety disorders, the research is still limited (see chapter 2 for review). Additionally, while the hippocampal and amygdala subfields are associated with aging, very little is known about how subfield volumes contribute to brain aging.

Rationale and objectives

OCD and SAD are debilitating psychiatric disorders that have been previously associated with structural differences in the hippocampus and amygdala. Both increases and decreases have been found in the volumes of these structures, but few studies have investigated subfield volume differences in OCD or SAD. Moreover, while it is known that aging imparts structural and functional changes in the brain, very few studies have correlated hippocampal and amygdala subfield volumes and predicted brain aging in anxiety disorders.

The present dissertation begins with a systematic review focusing on biological indicators of aging in anxiety and related disorders. This sets the stage for a series of secondary analyses of neuroimaging data from large multi-site datasets obtained from the European and South African Research Network in Anxiety Disorders (EURSANAD) and the Obsessive-Compulsive Disorder Brain Imaging Consortium (OBIC), to achieve the following aims:

> **Chapter 3:** Investigate the association between OCD and hippocampal and amygdala subfield volumes, and then assess diagnosis-by-age interactions to test whether chronological age moderates the subfield volumes.
> *Research question:* Are there volumetric differences in hippocampal and amygdala subfields in individuals with OCD compared to HCs? Is there an association between chronological age and subfield volumes, and does this relationship differ in individuals with OCD compared to controls?
>
> *Hypothesis:* It is anticipated that individuals with OCD will have differences in both hippocampal and amygdala subfield volumes and that these effects may be particularly apparent in subfields previously implicated in OCD, including the BLA and central nucleus of the amygdala, as well as hippocampal subiculum and presubiculum (Zhang et al., 2019, Zhang et al., 2020).
>
> **Chapter 4:** Investigate the association between SAD and hippocampal and amygdala subfield volumes, and then assess diagnosis-by-age interactions to test whether chronological age moderates the subfield volumes.
> *Research question:* Are there volumetric differences in hippocampal and amygdala subfields in individuals with SAD compared to HCs? Is there an association between chronological age and subfield volumes, and does this relationship differ in individuals with SAD compared to controls?
> *Hypothesis:* it is anticipated that individuals with SAD will have differences in the volume of the BLA amygdala given its involvement in other anxiety disorders (Asami et al., 2018) and its

role in threat evaluation (Besnard & Sahay, 2016; Etkin et al., 2004; Hortensius et al., 2016). With regards to the hippocampus, multiple hippocampal subfields have been implicated in anxiety and related disorders therefore we refrained from making subfield-specific predictions.

Chapter 5: Investigate evidence for greater brain-PADs, in individuals with OCD compared to HCs, and assess the extent to which brain-PAD predicts hippocampal and amygdala subfield volumes.

Research question: Are individuals with OCD more likely to have accentuated brain aging as indexed by brain-PADs, compared to HCs?

Hypothesis: Building upon the recent evidence of brain aging in OCD (Liu et al., 2022), it is anticipated that there will be accentuated brain aging in OCD, as shown by higher brain-PAD in individuals compared to HCs.

Chapter 6: Investigate evidence for greater brain-PADs, in individuals with SAD compared to HCs, and assess the extent to which brain-PAD predicts hippocampal and amygdala subfield volumes.

Research question: Are individuals with SAD more likely to have accentuated brain aging as indexed by brain-PADs, compared to HCs?

Hypothesis: While recent evidence suggests that brain aging in anxiety disorders is modulated by medication status (Han et al., 2021), it is nevertheless, anticipated that there will be evidence for greater brain aging in SAD versus HCs, irrespective of medication status, using a large sample of participants.

References

Abbott, B. Y. A. (2010). *Artigo Revista - Alzheimer'S Disease Outlook*.

Ahmed-Leitao, F., Rosenstein, D., Marx, M., Young, S., Korte, K., & Seedat, S. (2019). Posttraumatic stress disorder, social anxiety disorder and childhood trauma: Differences in hippocampal subfield volume. *Psychiatry Research - Neuroimaging*, *284*, 45–52. https://doi.org/10.1016/j.pscychresns.2018.12.015

Amunts, K., Kedo, O., Kindler, M., Pieperhoff, P., Mohlberg, H., Shah, N. J., Habel, U., Schneider, F., & Zilles, K. (2005). Cytoarchitectonic mapping of the human amygdala, hippocampal region and entorhinal cortex: Intersubject variability and probability maps. *Anatomy and Embryology*, *210*(5–6), 343–352. https://doi.org/10.1007/s00429-005-0025-5

Angermueller, C., Pärnamaa, T., Parts, L., & Stegle, O. (2016). Deep learning for computational biology. *Molecular Systems Biology*, *12*(7), 878. https://doi.org/10.15252/msb.20156651

APA. (2013). Diagnostic and statistical manual of mental disorders: DSM-5^{TM}, 5th ed. In *Diagnostic and statistical manual of mental disorders: DSM-5*^{TM}*, 5th ed.* American Psychiatric Publishing, Inc. https://doi.org/10.1176/appi.books.9780890425596

Armstrong, N. M., An, Y., Beason-Held, L., Doshi, J., Erus, G., Ferrucci, L., Davatzikos, C., & Resnick, S. M. (2019). Sex differences in brain aging and predictors of neurodegeneration in cognitively healthy older adults. *Neurobiology of Aging*, *81*, 146–156. https://doi.org/10.1016/j.neurobiolaging.2019.05.020

Article, O. (2004). *Mapping Structural Brain Alterations in Obsessive-Compulsive Disorder*. *61*(July).

Asami, T., Nakamura, R., Takaishi, M., Yoshida, H., Yoshimi, A., Whitford, T. J., & Hirayasu, Y. (2018). Smaller volumes in the lateral and basal nuclei of the amygdala in patients with panic disorder. *PLoS ONE*, *13*(11), 1–8. https://doi.org/10.1371/journal.pone.0207163

Assunção, M. C., Da Conceição Costa, D. L., Mathis, M. A. De, Gedanke Shavitt, R., Arzeno Ferrão, Y., Rosário, M. C. Do, Constantino Miguel, E., & Rodrigues Torres, A. (2012). Social phobia in obsessive-compulsive disorder: Prevalence and correlates. *Journal of Affective Disorders*, *143*(1–3), 138–147. https://doi.org/10.1016/j.jad.2012.05.044

Atmaca, M., Ozdemir, H., Ozler, S., Kara, B., Ozler, Z., Kanmaz, E., Mermi, O., & Tezcan, E. (2008). *Progress in Neuro-Psychopharmacology & Biological Psychiatry Hippocampus and amygdalar volumes in patients with refractory obsessive – compulsive disorder*. *32*, 1283–1286. https://doi.org/10.1016/j.pnpbp.2008.04.002

Avecillas-Chasin, J. M., Levinson, S., Kuhn, T., Omidbeigi, M., Langevin, J. P., Pouratian, N., & Bari, A. (2023). Connectivity-based parcellation of the amygdala and identification of its main white matter connections. *Scientific Reports*, *13*(1), 1–10. https://doi.org/10.1038/s41598-023-28100-6

Baecker, L., Garcia-Dias, R., Vieira, S., Scarpazza, C., & Mechelli, A. (2021). Machine learning for brain age prediction: Introduction to methods and clinical applications. *EBioMedicine*, *72*, 103600. https://doi.org/10.1016/j.ebiom.2021.103600

Baldwin, D. S., Brandish, E. K., & Meron, D. (2008). The Overlap of Obsessive-Compulsive Disorder and Social Phobia and its Treatment. *CNS Spectrums*, *13*(S14), 47–53. https://doi.org/10.1017/S1092852900026936

Ballester, P. L., Romano, M. T., de Azevedo Cardoso, T., Hassel, S., Strother, S. C., Kennedy, S. H., & Frey, B. N. (2022). Brain age in mood and psychotic disorders: a systematic review and meta-analysis. *Acta Psychiatrica Scandinavica*, *145*(1), 42–55. https://doi.org/10.1111/acps.13371

Ballester, P. L., Suh, J. S., Nogovitsyn, N., Hassel, S., Strother, S. C., Arnott, S. R., Minuzzi, L., Sassi, R. B., Lam, R. W., Milev, R., Müller, D. J., Taylor, V. H., Kennedy, S. H., & Frey, B. N. (2021). Accelerated brain aging in major depressive disorder and antidepressant treatment response: A CAN-BIND report. *NeuroImage: Clinical*, *32*. https://doi.org/10.1016/j.nicl.2021.102864

Bandelow, B. (2008). Funding for this academic supplement has been provided by an educational grant from Jazz Pharmaceuticals . Faculty Affiliation and Disclosures Nonpharmacologic Treatments. *CNS Spectr*, *9*(Suppl 14), 37–46.

Bandelow, B., Baldwin, D., Abelli, M., Altamura, C., Dell'Osso, B., Domschke, K., Fineberg, N. A., Grünblatt, E., Jarema, M., Maron, E., Nutt, D., Pini, S., Vaghi, M. M., Wichniak, A., Zai, G., & Riederer, P. (2016). Biological markers for anxiety disorders, OCD and PTSD – a consensus statement. Part I: Neuroimaging and genetics. *World Journal of Biological Psychiatry*, *17*(5), 321–365. https://doi.org/10.1080/15622975.2016.1181783

Bannon, S., Gonsalvez, C. J., Croft, R. J., & Boyce, P. M. (2002). Response inhibition deficits in obsessive-compulsive disorder. *Psychiatry Research*, *110*(2), 165–174. https://doi.org/10.1016/S0165-1781(02)00104-X

Bas-Hoogendam, J. M., Groenewold, N. A., Aghajani, M., Freitag, G. F., Harrewijn, A., Hilbert, K., Jahanshad, N., Thomopoulos, S. I., Thompson, P. M., Veltman, D. J., Winkler, A. M., Lueken, U., Pine, D. S., van der Wee, N. J. A., Stein, D. J., Agosta, F., Åhs, F., An, I., Alberton, B. A. V., … Zwiebel, H. L. (2022). ENIGMA-anxiety working group: Rationale for and organization of large-scale neuroimaging studies of anxiety disorders. *Human Brain Mapping*, *43*(1), 83–112. https://doi.org/10.1002/hbm.25100

Bas-Hoogendam, J. M., van Steenbergen, H., Nienke Pannekoek, J., Fouche, J. P., Lochner, C., Hattingh, C. J., Cremers, H. R., Furmark, T., Månsson, K. N. T., Frick, A., Engman, J., Boraxbekk, C. J., Carlbring, P., Andersson, G., Fredrikson, M., Straube, T., Peterburs, J., Klumpp, H., Phan, K. L., … van der Wee, N. J. A. (2017). Voxel-based morphometry multi-center mega-analysis of brain structure in social anxiety disorder. *NeuroImage: Clinical*, *16*(May), 678–688. https://doi.org/10.1016/j.nicl.2017.08.001

Beats, B., Levy, R., & Förstl, H. (1991). Ventricular enlargement and caudate hyperdensity in elderly depressives. *Biological Psychiatry*, *30*(5), 452–458. https://doi.org/10.1016/0006-3223(91)90306-7

Ben-zion, Z., Korem, N., Fine, N., Katz, S., Siddhanta, M., Funaro, C., Duek, O., Spiller, T. R., Danbock, S., Levy, I., & Harpaz-rotem, I. (2023). of. *Biological Psychiatry Global Open Science*. https://doi.org/10.1016/j.bpsgos.2023.07.001

Besnard, A., & Sahay, A. (2016). Adult Hippocampal Neurogenesis, Fear Generalization, and Stress. *Neuropsychopharmacology*, *41*(1), 24–44. https://doi.org/10.1038/npp.2015.167

Bethlehem, R. A. I., Seidlitz, J., White, S. R., Vogel, J. W., Anderson, K. M., Adamson, C., Adler, S., Alexopoulos, G. S., Anagnostou, E., Areces-Gonzalez, A., Astle, D. E., Auyeung, B., Ayub, M., Bae, J., Ball, G., Baron-Cohen, S., Beare, R., Bedford, S. A., Benegal, V., … Alexander-Bloch, A. F. (2022). Brain charts for the human lifespan. *Nature*, *604*(7906), 525–533. https://doi.org/10.1038/s41586-022-04554-y

Blake, K. V., Ntwatwa, Z., Kaufmann, T., Stein, D. J., Ipser, J. C., & Groenewold, N. A. (2023). Advanced brain ageing in adult psychopathology: A systematic review and meta-analysis of structural MRI studies. *Journal of Psychiatric Research*, *157*, 180–191. https://doi.org/10.1016/j.jpsychires.2022.11.011

Boedhoe, P. S. W., Schmaal, L., Abe, Y., Ameis, S. H., Arnold, P. D., Batistuzzo, M. C., Benedetti, F., Beucke, J. C., Bollettini, I., Bose, A., Brem, S., Calvo, A., Cheng, Y., Cho, K. I. K., Dallaspezia, S., Denys, D., Fitzgerald, K. D., Fouche, J.-P., Giménez, M., … Zhao, Q. (2017). Distinct Subcortical Volume Alterations in Pediatric and Adult OCD: A Worldwide Meta- and Mega-Analysis. *American Journal of Psychiatry*, *174*(1), 60–69. https://doi.org/10.1176/appi.ajp.2016.16020201

Bray, S., Chang, C., & Hoeft, F. (2009). Applications of multivariate pattern classification analyses in developmental neuroimaging of healthy and clinical populations. *Frontiers in Human Neuroscience*, *3*(OCT), 1–12. https://doi.org/10.3389/neuro.09.032.2009

Brühl, A. B., Delsignore, A., Komossa, K., & Weidt, S. (2014). Neuroimaging in social anxiety disorder-A meta-analytic review resulting in a new neurofunctional model. *Neuroscience and Biobehavioral Reviews*, *47*, 260–280. https://doi.org/10.1016/j.neubiorev.2014.08.003

Bruin, W. B., Taylor, L., Thomas, R. M., Shock, J. P., Zhutovsky, P., Abe, Y., Alonso, P., Ameis, S. H., Anticevic, A., Arnold, P. D., Assogna, F., Benedetti, F., Beucke, J. C., Boedhoe, P. S. W., Bollettini, I., Bose, A., Brem, S., Brennan, B. P., Buitelaar, J. K., … van Wingen, G. A. (2020). Structural neuroimaging biomarkers for obsessive-compulsive disorder in the ENIGMA-OCD consortium: medication matters. *Translational Psychiatry*, *10*(1). https://doi.org/10.1038/s41398-020-01013-y

Caldiroli, A., Capuzzi, E., Affaticati, L. M., Surace, T., Forti, C. L. Di, Dakanalis, A., Clerici, M., & Buoli, M. (2023). *Candidate Biological Markers for Social Anxiety Disorder : A Systematic Review*.

Canada, K. L., Saifullah, S., Gardner, J. C., Sutton, B. P., Fabiani, M., Gratton, G., Raz, N., & Daugherty, A. M. (2023). Development and validation of a quality control procedure for automatic segmentation of hippocampal subfields. *Hippocampus*, *May*. https://doi.org/10.1002/hipo.23552

Cao, L., Li, H., Liu, J., Jiang, J., Li, B., Li, X., Zhang, S., Gao, Y., Liang, K., Hu, X., Bao, W., Qiu, H., Lu, L., Zhang, L., Hu, X., Gong, Q., & Huang, X. (2022). Disorganized functional architecture of amygdala subregional networks in obsessive-compulsive disorder. *Communications Biology*, *5*(1), 1–10. https://doi.org/10.1038/s42003-022-04115-z

Carpita, B., Muti, D., Petrucci, A., Romeo, F., Gesi, C., Marazziti, D., Carmassi, C., & Dell'Osso, L. (2019). Overlapping features between social anxiety and obsessive-compulsive spectrum in a clinical sample and in healthy controls: Toward an integrative model. *CNS Spectrums*. https://doi.org/10.1017/S109285291900138X

Ching, T., Himmelstein, D. S., Beaulieu-Jones, B. K., Kalinin, A. A., Do, B. T., Way, G. P., Ferrero, E., Agapow, P. M., Zietz, M., Hoffman, M. M., Xie, W., Rosen, G. L., Lengerich, B. J., Israeli, J., Lanchantin, J., Woloszynek, S., Carpenter, A. E., Shrikumar, A., Xu, J., … Greene, C. S. (2018). Opportunities and obstacles for deep learning in biology and medicine. In *Journal of the Royal Society Interface* (Vol. 15, Issue 141). https://doi.org/10.1098/rsif.2017.0387

Christian, B., Boll, S., Gamer, M., & Gluth, S. (2013). *Separate amygdala subregions signal surprise and predictiveness during associative fear learning in humans*. *37*(November 2012), 758–767. https://doi.org/10.1111/ejn.12094

Cisler, J. M., Olatunji, B. O., & Lohr, J. M. (2009). Disgust, fear, and the anxiety disorders: A critical review. *Clinical Psychology Review*, *29*(1), 34–46. https://doi.org/10.1016/j.cpr.2008.09.007

Cole, J. H., & Franke, K. (2017a). Predicting Age Using Neuroimaging: Innovative Brain Ageing Biomarkers. *Trends in Neurosciences*, *40*(12), 681–690. https://doi.org/10.1016/j.tins.2017.10.001

Cole, J. H., & Franke, K. (2017b). Predicting Age Using Neuroimaging: Innovative Brain Ageing Biomarkers. *Trends in Neurosciences*, *40*(12), 681–690. https://doi.org/10.1016/j.tins.2017.10.001

Cole, J. H., Marioni, R. E., Harris, S. E., & Deary, I. J. (2019). Brain age and other bodily 'ages': implications for neuropsychiatry. *Molecular Psychiatry*, *24*(2), 266–281. https://doi.org/10.1038/s41380-018-0098-1

Cole, J. H., Ritchie, S. J., Bastin, M. E., Valdés Hernández, M. C., Muñoz Maniega, S., Royle, N., Corley, J., Pattie, A., Harris, S. E., Zhang, Q., Wray, N. R., Redmond, P., Marioni, R. E., Starr, J. M., Cox, S. R., Wardlaw, J. M., Sharp, D. J., & Deary, I. J. (2018). Brain age predicts mortality. *Molecular Psychiatry*, *23*(5), 1385–1392. https://doi.org/10.1038/mp.2017.62

Darrow, S. M., Verhoeven, J. E., Révész, D., Lindqvist, D., Penninx, B. W. J. H., Delucchi, K. L., Wolkowitz, O. M., & Mathews, C. A. (2016). The Association between Psychiatric Disorders and Telomere Length: A Meta-Analysis Involving 14,827 Persons. *Psychosomatic Medicine*, *78*(7), 776–787. https://doi.org/10.1097/PSY.0000000000000356

Davatzikos, C. (2019). Machine learning in neuroimaging: Progress and challenges. *NeuroImage*, *197*, 652–656. https://doi.org/10.1016/j.neuroimage.2018.10.003

de Flores, R., La Joie, R., & Chételat, G. (2015). Structural imaging of hippocampal subfields in healthy aging and Alzheimer's disease. *Neuroscience*, *309*(August), 29–50. https://doi.org/10.1016/j.neuroscience.2015.08.033

De Magalhães, J. P. (2012). Programmatic features of aging originating in development: Aging mechanisms beyond molecular damage? *FASEB Journal*, *26*(12), 4821–4826. https://doi.org/10.1096/fj.12-210872

Dell'Osso, B., Benatti, B., Hollander, E., Fineberg, N., Stein, D. J., Lochner, C., Nicolini, H., Lanzagorta, N., Palazzo, C., Altamura, A. C., Marazziti, D., Pallanti, S., Van Ameringen, M., Karamustafalioglu, O., Drummond, L. M., Hranov, L., Figee, M., Grant, J. E., Zohar, J., … Menchon, J. M. (2016). Childhood, adolescent and adult age at onset and related clinical correlates in obsessive–compulsive disorder: a report from the International College of Obsessive–Compulsive Spectrum Disorders (ICOCS). *International Journal of Psychiatry in Clinical Practice*, *20*(4), 210–217. https://doi.org/10.1080/13651501.2016.1207087

Dinsdale, N. K., Bluemke, E., Smith, S. M., Arya, Z., Vidaurre, D., Jenkinson, M., & Namburete, A. I. L. (2021). Learning patterns of the ageing brain in MRI using deep convolutional networks. *NeuroImage*, *224*(September 2020), 117401. https://doi.org/10.1016/j.neuroimage.2020.117401

Dong, X., Milholland, B., & Vijg, J. (2016). Evidence for a limit to human lifespan. *Nature*, *538*(7624), 257–259. https://doi.org/10.1038/nature19793

Eickhoff, S. B., Thirion, B., Varoquaux, G., & Bzdok, D. (2015). Connectivity-based parcellation: Critique and implications. *Human Brain Mapping*, *36*(12), 4771–4792. https://doi.org/10.1002/hbm.22933

Eisen, J. L., Pinto, A., Mancebo, M. C., Dyck, I. R., Orlando, M. E., & Rasmussen, S. A. (2010). A 2-year prospective follow-up study of the course of obsessive-compulsive disorder. *Journal of Clinical Psychiatry*, *71*(8), 1033–1039. https://doi.org/10.4088/JCP.08m04806blu

Elbejjani, M., Fuhrer, R., Abrahamowicz, M., Mazoyer, B., Crivello, F., Tzourio, C., & Dufouil, C. (2015). Depression, depressive symptoms, and rate of hippocampal atrophy in a longitudinal cohort of older men and women. *Psychological Medicine*, *45*(9), 1931–1944.

https://doi.org/10.1017/S0033291714003055

Emerit, J., Edeas, M., & Bricaire, F. (2004). Neurodegenerative diseases and oxidative stress. *Biomedicine and Pharmacotherapy*, *58*(1), 39–46. https://doi.org/10.1016/j.biopha.2003.11.004

Fiddick, L. (2011). There is more than the amygdala: Potential threat assessment in the cingulate cortex. *Neuroscience and Biobehavioral Reviews*, *35*(4), 1007–1018. https://doi.org/10.1016/j.neubiorev.2010.09.014

Fineberg, N. A., & Gale, T. M. (2005). *Evidence-based pharmacotherapy of obsessive – compulsive disorder*. 107–129.

Fjell, A. M., Westlye, L. T., Grydeland, H., Amlien, I., Espeseth, T., Reinvang, I., Raz, N., Holland, D., Dale, A. M., & Walhovd, K. B. (2013a). Critical ages in the life course of the adult brain: Nonlinear subcortical aging. *Neurobiology of Aging*, *34*(10), 2239–2247. https://doi.org/10.1016/j.neurobiolaging.2013.04.006

Fjell, A. M., Westlye, L. T., Grydeland, H., Amlien, I., Espeseth, T., Reinvang, I., Raz, N., Holland, D., Dale, A. M., & Walhovd, K. B. (2013b). Critical ages in the life course of the adult brain: Nonlinear subcortical aging. *Neurobiology of Aging*, *34*(10), 2239–2247. https://doi.org/10.1016/j.neurobiolaging.2013.04.006

Fouche, J. P., Du Plessis, S., Hattingh, C., Roos, A., Lochner, C., Soriano-Mas, C., Sato, J. R., Nakamae, T., Nishida, S., Kwon, J. S., Jung, W. H., Mataix-Cols, D., Hoexter, M. Q., Alonso, P., De Wit, S. J., Veltman, D. J., Stein, D. J., & Van Den Heuvel, O. A. (2017). Cortical thickness in obsessive-compulsive disorder: Multisite mega-analysis of 780 brain scans from six centres. *British Journal of Psychiatry*, *210*(1), 67–74. https://doi.org/10.1192/bjp.bp.115.164020

Fouche, J. P., Groenewold, N. A., Sevenoaks, T., Heany, S., Lochner, C., Alonso, P., Batistuzzo, M. C., Cardoner, N., Ching, C. R. K., de Wit, S. J., Gutman, B., Hoexter, M. Q., Jahanshad, N., Kim, M., Kwon, J. S., Mataix-Cols, D., Menchon, J. M., Miguel, E. C., Nakamae, T., … Stein, D. J. (2022). Shape analysis of subcortical structures in obsessive-compulsive disorder and the relationship with comorbid anxiety, depression, and medication use: A meta-analysis by the OCD Brain Imaging Consortium. *Brain and Behavior*, *12*(10), 1–12. https://doi.org/10.1002/brb3.2755

Franke, K., Bublak, P., Hoyer, D., Billiet, T., Gaser, C., Witte, O. W., & Schwab, M. (2020). In vivo biomarkers of structural and functional brain development and aging in humans. *Neuroscience and Biobehavioral Reviews*, *117*(November 2017), 142–164. https://doi.org/10.1016/j.neubiorev.2017.11.002

Gao, W., Alcauter, S., Smith, J. K., Gilmore, J. H., & Lin, W. (2015). Development of human brain cortical network architecture during infancy. *Brain Structure and Function*, *220*(2), 1173–1186. https://doi.org/10.1007/s00429-014-0710-3

Geller, D. A., Homayoun, S., & Johnson, G. (2021). Developmental Considerations in Obsessive Compulsive Disorder: Comparing Pediatric and Adult-Onset Cases. *Frontiers in Psychiatry*, *12*(June), 1–15. https://doi.org/10.3389/fpsyt.2021.678538

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., Nugent, T. F., Herman, D. H., Clasen, L. S., Toga, A. W., Rapoport, J. L., & Thompson, P. M. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(21), 8174–8179. https://doi.org/10.1073/pnas.0402680101

Gogtay, N., Nugent, T. F., Herman, D. H., Ordonez, A., Greenstein, D., Hayashi, K. M., Clasen, L., Toga, A. W., Giedd, J. N., Rapoport, J. L., & Thompson, P. M. (2006). Dynamic mapping of normal human hippocampal development. *Hippocampus*, *16*(8), 664–672. https://doi.org/10.1002/hipo.20193

Goodman, W. K., Grice, D. E., Lapidus, K. A. B., & Coffey, B. J. (2014). Obsessive-compulsive disorder. *Psychiatric Clinics of North America*, *37*(3), 257–267. https://doi.org/10.1016/j.psc.2014.06.004

Groenewold, N. A., Bas-Hoogendam, J. M., Amod, A. R., Laansma, M. A., Van Velzen, L. S., Aghajani, M., Hilbert, K., Oh, H., Salas, R., Jackowski, A. P., Pan, P. M., Salum, G. A., Blair, J. R., Blair, K. S., Hirsch, J., Pantazatos, S. P., Schneier, F. R., Talati, A., Roelofs, K., … Van der Wee, N. J. A. (2023). Volume of subcortical brain regions in social anxiety disorder: mega-analytic results from 37 samples in the ENIGMA-Anxiety Working Group. *Molecular Psychiatry*, *December 2022*. https://doi.org/10.1038/s41380-022-01933-9

Han, L. K. M., Schnack, H. G., Brouwer, R. M., Veltman, D. J., van der Wee, N. J. A., van Tol, M. J., Aghajani, M., & Penninx, B. W. J. H. (2021). Contributing factors to advanced brain aging in depression and anxiety disorders. *Translational Psychiatry*, *11*(1). https://doi.org/10.1038/s41398-021-01524-2

Han, L. K. M., Verhoeven, J. E., Tyrka, A. R., Penninx, B. W. J. H., Wolkowitz, O. M., Månsson, K. N. T., Lindqvist, D., Boks, M. P., Révész, D., Mellon, S. H., & Picard, M. (2019a). Accelerating research on biological aging and mental health: Current challenges and future directions. *Psychoneuroendocrinology*, *106*(January), 293–311. https://doi.org/10.1016/j.psyneuen.2019.04.004

Han, L. K. M., Verhoeven, J. E., Tyrka, A. R., Penninx, B. W. J. H., Wolkowitz, O. M., Månsson, K. N. T., Lindqvist, D., Boks, M. P., Révész, D., Mellon, S. H., & Picard, M. (2019b). Accelerating research on biological aging and mental health: Current challenges and future directions. *Psychoneuroendocrinology*, *106*(September 2018), 293–311. https://doi.org/10.1016/j.psyneuen.2019.04.004

Hoexter, M. Q., Dougherty, D. D., Shavitt, R. G., D'Alcante, C. C., Duran, F. L. S., Lopes, A. C., Diniz, J. B., Batistuzzo, M. C., Evans, K. C., Bressan, R. A., Busatto, G. F., & Miguel, E. C. (2013). Differential prefrontal gray matter correlates of treatment response to fluoxetine or cognitive-behavioral therapy in obsessive-compulsive disorder. *European Neuropsychopharmacology*, *23*(7), 569–580. https://doi.org/10.1016/j.euroneuro.2012.06.014

Hou, Y., Dan, X., Babbar, M., Wei, Y., Hasselbalch, S. G., Croteau, D. L., & Bohr, V. A. (2019). Ageing as a risk factor for neurodegenerative disease. *Nature Reviews Neurology*, *15*(10), 565–581. https://doi.org/10.1038/s41582-019-0244-7

Hugenschmidt, C. E., Peiffer, A. M., Kraft, R. A., Casanova, R., Deibler, A. R., Burdette, J. H., Maldjian, J. A., & Laurienti, P. J. (2008). Relating imaging indices of white matter integrity and volume in healthy older adults. *Cerebral Cortex*, *18*(2), 433–442. https://doi.org/10.1093/cercor/bhm080

Iglesias, J. E., Augustinack, J. C., Nguyen, K., Player, C. M., Player, A., Wright, M., Roy, N., & Frosch, M. P. (2015). A computational atlas of the hippocampal formation using ex vivo , ultra-high resolution MRI. *NeuroImage*, 115–137.

Irle, E., Ruhleder, M., Lange, C., Seidler-Brandler, U., Salzer, S., Dechent, P., Weniger, G., Leibing, E., & Leichsenring, F. (2010). Reduced amygdalar and hippocampal size in adults with generalized social phobia. *Journal of Psychiatry and Neuroscience*, *35*(2), 126–131. https://doi.org/10.1503/jpn.090041

Ivanov, I., Boedhoe, P. S. W., Abe, Y., Alonso, P., Ameis, S. H., Arnold, P. D., Balachander, S., Baker, J. T., Banaj, N., Bargalló, N., Batistuzzo, M. C., Benedetti, F., Beucke, J. C., Bollettini, I., Brem, S., Brennan, B. P., Buitelaar, J., Calvo, R., Cheng, Y., … Fouche, J.-P. (2022). Associations of medication with subcortical morphology across the lifespan in OCD: Results from the international ENIGMA Consortium. *Journal of Affective Disorders*, *318*(August), 204–216. https://doi.org/10.1016/j.jad.2022.08.084

J S Kwon, Y-W Shin, C-W Kim, Y I Kim, T Youn, M H Han, K-H Chang, J.-J. K. (2003). *Similarity and disparity of obsessive-compulsive disorder and schizophrenia in MR volumetric abnormalities of the hippocampus-amygdala complex*. 962–964.

Janak, P. H., & Tye, K. M. (2015). From circuits to behaviour in the amygdala. *Nature*, *517*(7534), 284–292. https://doi.org/10.1038/nature14188

Jiang, L., Qiao, K., Li, Q., Hu, Y., Zhang, X., Wang, J., Peng, D., Fan, Q., Zhao, M., Sheng, J., Wang, J., Li, C., Fang, Y., Wang, Z., & Yang, Z. (2022). Categorical and dimensional deficits in hippocampal subfields among schizophrenia, obsessive-compulsive disorder, bipolar disorder, and major depressive disorder. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*. https://doi.org/10.1016/j.bpsc.2022.06.011

Kaufmann, T., van der Meer, D., Doan, N. T., Schwarz, E., Lund, M. J., Agartz, I., Alnæs, D., Barch, D. M., Baur-Streubel, R., Bertolino, A., Bettella, F., Beyer, M. K., Bøen, E., Borgwardt, S., Brandt, C. L., Buitelaar, J., Celius, E. G., Cervenka, S., Conzelmann, A., … Westlye, L. T. (2019). Common brain disorders are associated with heritable patterns of apparent aging of the brain. *Nature Neuroscience*, *22*(10), 1617–1623. https://doi.org/10.1038/s41593-019-0471-7

Keller, M. B. (2003). The lifelong course of social anxiety disorder: A clinical perspective. *Acta Psychiatrica Scandinavica, Supplement*, *108*(417), 85–94. https://doi.org/10.1034/j.1600-0447.108.s417.6.x

Khan, S. S., Singer, B. D., & Vaughan, D. E. (2017). Molecular and physiological manifestations and measurement of aging in humans. *Aging Cell*, *16*(4), 624–633. https://doi.org/10.1111/acel.12601

Klemera, P., & Doubal, S. (2006). A new approach to the concept and computation of biological age. *Mechanisms of Ageing and Development*, *127*(3), 240–248. https://doi.org/10.1016/j.mad.2005.10.004

Knierim, J. J., & Neunuebel, J. P. (2016). Tracking the flow of hippocampal computation: Pattern separation, pattern completion, and attractor dynamics. *Neurobiology of Learning and Memory*, *129*, 38–49. https://doi.org/10.1016/j.nlm.2015.10.008

Kurth, F., Cherbuin, N., & Luders, E. (2017). The impact of aging on subregions of the hippocampal complex in healthy adults. *NeuroImage*, *163*, 296–300. https://doi.org/10.1016/j.neuroimage.2017.09.016

Kurth, F., Cherbuin, N., & Luders, E. (2019). Age but no sex effects on subareas of the amygdala. *Human Brain Mapping*, *40*(6), 1697–1704. https://doi.org/10.1002/hbm.24481

Liao, W., Xu, Q., Mantini, D., Ding, J., MacHado-De-Sousa, J. P., Hallak, J. E. C., Trzesniak, C., Qiu, C., Zeng, L., Zhang, W., Crippa, J. A. S., Gong, Q., & Chen, H. (2011). Altered gray matter morphometry and resting-state functional and structural connectivity in social anxiety disorder. *Brain Research*, *1388*, 167–177. https://doi.org/10.1016/j.brainres.2011.03.018

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2013). The hallmarks of aging. *Cell*, *153*(6), 1194. https://doi.org/10.1016/j.cell.2013.05.039

López-Solà, C., Fontenelle, L. F., Alonso, P., Cuadras, D., Foley, D. L., Pantelis, C., Pujol, J., Yücel, M., Cardoner, N., Soriano-Mas, C., Menchón, J. M., & Harrison, B. J. (2014). Prevalence and heritability of obsessive-compulsive spectrum and anxiety disorder symptoms: A survey of the Australian twin registry. *American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics*, *165*(4), 314–325. https://doi.org/10.1002/ajmg.b.32233

López-Solà, C., Fontenelle, L. F., Bui, M., Hopper, J. L., Pantelis, C., Yücel, M., Menchón, J. M., Alonso, P., & Harrison, B. J. (2016). Aetiological overlapbetweenobsessive-Compulsive related and anxiety disorder symptoms: Multivariate twin study. *British Journal of Psychiatry*, *208*(1), 26–33. https://doi.org/10.1192/bjp.bp.114.156281

Lu, P. H., Lee, G. J., Raven, E. P., Tingus, K., Khoo, T., Thompson, P. M., & Bartzokis, G. (2011). Age-related slowing in cognitive processing speed is associated with myelin integrity in a very healthy elderly sample. *Journal of Clinical and Experimental Neuropsychology*, *33*(10), 1059–1068. https://doi.org/10.1080/13803395.2011.595397

Maceachern, S. J., & Forkert, N. D. (2021). Machine learning for precision medicine. *Genome*, *64*(4), 416–425. https://doi.org/10.1139/gen-2020-0131

Madabhushi, R., Pan, L., & Tsai, L. H. (2014). DNA damage and its links to neurodegeneration. *Neuron*, *83*(2), 266–282. https://doi.org/10.1016/j.neuron.2014.06.034

Mahjani, B., Bey, K., Boberg, J., & Burton, C. (2021). *Genetics of obsessive-compulsive disorder*.

Malouff, J. M., & Schutte, N. S. (2017). A meta-analysis of the relationship between anxiety and telomere length. *Anxiety, Stress and Coping*, *30*(3), 264–272. https://doi.org/10.1080/10615806.2016.1261286

Marazziti, D., Abelli, M., Baroni, S., Carpita, B., Ramacciotti, C. E., & Osso, L. D. (2015). *Neurobiological correlates of social anxiety disorder : an update*. 100–111. https://doi.org/10.1017/S109285291400008X

Masliah, E., Crews, L., & Hansen, L. (2006). Synaptic remodeling during aging and in Alzheimer's disease. *Journal of Alzheimer's Disease*, *9*(SUPPL. 3), 91–99. https://doi.org/10.3233/jad-2006-9s311

Meng, Y., Lui, S., Qiu, C., Qiu, L., Lama, S., Huang, X., Feng, Y., Zhu, C., Gong, Q., & Zhang, W. (2013). Neuroanatomical deficits in drug-naïve adult patients with generalized social anxiety disorder: A voxel-based morphometry study. *Psychiatry Research: Neuroimaging*, *214*(1), 9–15. https://doi.org/10.1016/J.PSCYCHRESNS.2013.06.002

Milad, M. R., Furtak, S. C., Greenberg, J. L., Keshaviah, A., Im, J. J., Falkenstein, M. J., Jenike, M., Rauch, S. L., & Wilhelm, S. (2013). Deficits in conditioned fear extinction in obsessive-compulsive disorder and neurobiological changes in the fear circuit. *JAMA Psychiatry*, *70*(6), 608–618. https://doi.org/10.1001/jamapsychiatry.2013.914

Milad, M. R., & Rauch, S. L. (2012). Obsessive-compulsive disorder: Beyond segregated cortico-striatal pathways. *Trends in Cognitive Sciences*, *16*(1), 43–51. https://doi.org/10.1016/j.tics.2011.11.003

Miller, K. L., Alfaro-Almagro, F., Bangerter, N. K., Thomas, D. L., Yacoub, E., Xu, J., Bartsch, A. J., Jbabdi, S., Sotiropoulos, S. N., Andersson, J. L. R., Griffanti, L., Douaud, G., Okell, T. W., Weale, P., Dragonu, I., Garratt, S., Hudson, S., Collins, R., Jenkinson, M., … Smith, S. M. (2016). Multimodal population brain imaging in the UK Biobank prospective epidemiological study. *Nature Neuroscience*, *19*(11), 1523–1536. https://doi.org/10.1038/nn.4393

Morey, R. A., Clarke, E. K., Haswell, C. C., Phillips, R. D., Clausen, A. N., Mufford, M. S., Saygin, Z., Brancu, M., Beckham, J. C., Calhoun, P. S., Dedert, E., Elbogen, E. B., Fairbank, J. A., Hurley, R. A., Kilts, J. D., Kimbrel, N. A., Kirby, A., Marx, C. E., McDonald, S. D., … LaBar, K. S. (2020). Amygdala Nuclei Volume and Shape in Military Veterans With Posttraumatic Stress Disorder. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *5*(3), 281–290. https://doi.org/10.1016/j.bpsc.2019.11.016

Mueller, S. G., Stables, L., Du, A. T., Schuff, N., Truran, D., Cashdollar, N., & Weiner, M. W. (2007). Measurement of hippocampal subfields and age-related changes with high resolution MRI at 4 T. *Neurobiology of Aging*, *28*(5), 719–726. https://doi.org/10.1016/j.neurobiolaging.2006.03.007

Mueller, S. G., & Weiner, M. W. (2009). Selective effect of age, Apo e4, and Alzheimer's disease on hippocampal subfields. *Hippocampus*, *19*(6), 558–564. https://doi.org/10.1002/hipo.20614

Nielsen, A. N., Barch, D. M., Petersen, S. E., Schlaggar, B. L., & Greene, D. J. (2020). Machine Learning With Neuroimaging: Evaluating Its Applications in Psychiatry. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *5*(8), 791–798. https://doi.org/10.1016/j.bpsc.2019.11.007

Niu, X., Taylor, A., Shinohara, R. T., Kounios, J., & Zhang, F. (2022). Multidimensional brain-age prediction reveals altered brain developmental trajectory in psychiatric disorders. *Cerebral Cortex (New York, N.Y. : 1991)*, *32*(22), 5036–5049. https://doi.org/10.1093/cercor/bhab530

Pantazatos, S. P., Talati, A., Schneier, F. R., & Hirsch, J. (2014). Reduced anterior temporal and hippocampal functional connectivity during face processing discriminates individuals with social anxiety disorder from healthy controls and panic disorder, and increases following treatment. *Neuropsychopharmacology*, *39*(2), 425–434. https://doi.org/10.1038/npp.2013.211

Pfefferbaum, A., Rohlfing, T., Rosenbloom, M. J., Chu, W., Colrain, I. M., & Sullivan, E. V. (2013). Variation in longitudinal trajectories of regional brain volumes of healthy men and women (ages 10 to 85years) measured with atlas-based parcellation of MRI. *NeuroImage*, *65*, 176–193. https://doi.org/10.1016/j.neuroimage.2012.10.008

Pollack, M. H. (2001). Comorbidity, neurobiology, and pharmacotherapy of social anxiety disorder. *Journal of Clinical Psychiatry*, *62*(SUPPL. 12), 24–29.

Prestia, A., Boccardi, M., Galluzzi, S., Cavedo, E., Adorni, A., Soricelli, A., Bonetti, M., Geroldi, C., Giannakopoulos, P., Thompson, P., & Frisoni, G. (2011). Hippocampal and amygdalar volume changes in elderly patients with Alzheimer's disease and schizophrenia. *Psychiatry Research - Neuroimaging*, *192*(2), 77–83. https://doi.org/10.1016/j.pscychresns.2010.12.015

Qin, S., Young, C. B., Duan, X., Chen, T., Supekar, K., & Menon, V. (2014). Amygdala subregional structure and intrinsic functional connectivity predicts individual differences in anxiety during early childhood. *Biological Psychiatry*, *75*(11), 892–900. https://doi.org/10.1016/j.biopsych.2013.10.006

Rao, G., Gao, H., Wang, X., Zhang, J., Ye, M., & Rao, L. (2023). MRI measurements of brain hippocampus volume in relation to mild cognitive impairment and Alzheimer disease: A systematic review and meta-analysis. *Medicine (United States)*, *102*(36), E34997. https://doi.org/10.1097/MD.0000000000034997

Rao, S., Raveendranathan, D., Shivakumar, V., Narayanaswamy, J. C., Venkatasubramanian, G., & Reddy, Y. C. J. (2018). Hippocampus volume alterations and the clinical correlates in medication naïve obsessive compulsive disorder. *Journal of Affective Disorders*, *236*, 1–5. https://doi.org/10.1016/j.jad.2018.04.048

Raz, N., Ghisletta, P., Rodrigue, K. M., Kennedy, K. M., & Lindenberger, U. (2010). Trajectories of brain aging in middle-aged and older adults: Regional and individual differences. *NeuroImage*, *51*(2), 501–511. https://doi.org/10.1016/j.neuroimage.2010.03.020

Reuter, N., Genon, S., Kharabian Masouleh, S., Hoffstaedter, F., Liu, X., Kalenscher, T., Eickhoff, S. B., & Patil, K. R. (2020). CBPtools: a Python package for regional connectivity-based parcellation. *Brain Structure and Function*, *225*(4), 1261–1275. https://doi.org/10.1007/s00429-020-02046-1

Ridout, K. K., Ridout, S. J., Price, L. H., Sen, S., & Tyrka, A. R. (2016). Depression and telomere length:

A meta-analysis. *Journal of Affective Disorders*, *191*, 237–247. https://doi.org/10.1016/j.jad.2015.11.052

Rolls, E. T. (2013). The mechanisms for pattern completion and pattern separation in the hippocampus. *Frontiers in Systems Neuroscience*, *7*(OCT), 1–21. https://doi.org/10.3389/fnsys.2013.00074

Rudy, B. M., May, A. C., Whiting, S. E., Davis, T. E., Jenkins, W. S., & Reuther, E. T. (2014). Differentiating Among Singular and Comorbid Obsessive-Compulsive Disorder and Social Phobia Symptomology. *Cognitive Behaviour Therapy*, *43*(2), 111–121. https://doi.org/10.1080/16506073.2013.859170

Ruscio, A. M., Stein, D. J., Chiu, W. T., & Kessler, R. C. (2010). The epidemiology of obsessive-compulsive disorder in the National Comorbidity Survey Replication. *Molecular Psychiatry*, *15*(1), 53–63. https://doi.org/10.1038/mp.2008.94

Sacchet, M. D., Camacho, M. C., Livermore, E. E., Thomas, E. A. C., & Gotlib, I. H. (2017). Accelerated aging of the putamen in patients with major depressive disorder. *Journal of Psychiatry and Neuroscience*, *42*(3), 164–171. https://doi.org/10.1503/jpn.160010

Sachs-Ericsson, N. J., Hajcak, G., Sheffler, J. L., Stanley, I. H., Selby, E. A., Potter, G. G., & Steffens, D. C. (2018). Putamen Volume Differences Among Older Adults: Depression Status, Melancholia, and Age. *Journal of Geriatric Psychiatry and Neurology*, *31*(1), 39–49. https://doi.org/10.1177/0891988717747049

Sah, P., Faber, E. S. L., De Armentia, M. L., & Power, J. (2003). The amygdaloid complex: Anatomy and physiology. *Physiological Reviews*, *83*(3), 803–834. https://doi.org/10.1152/physrev.00002.2003

Saygin, Z. M., Kliemann, D., Iglesias, J. E., van der Kouwe, A. J. W., Boyd, E., Reuter, M., Stevens, A., Van Leemput, K., McKee, A., Frosch, M. P., Fischl, B., & Augustinack, J. C. (2017). High-resolution magnetic resonance imaging reveals nuclei of the human amygdala: manual segmentation to automatic atlas. *NeuroImage*, *155*(April), 370–382. https://doi.org/10.1016/j.neuroimage.2017.04.046

Schmitz-Koep, B., Zimmermann, J., Menegaux, A., Nuttall, R., Bäuml, J. G., Schneider, S. C., Daamen, M., Boecker, H., Zimmer, C., Wolke, D., Bartmann, P., Hedderich, D. M., & Sorg, C. (2021). Within amygdala: Basolateral parts are selectively impaired in premature-born adults. *NeuroImage: Clinical*, *31*(April). https://doi.org/10.1016/j.nicl.2021.102780

Schweitzer, I., Tuckwell, V., Ames, D., & O'Brien, J. (2001). Structural neuroimaging studies in late-life depression: A review. *World Journal of Biological Psychiatry*, *2*(2), 83–88. https://doi.org/10.3109/15622970109027497

Shin, L. M., & Liberzon, I. (2010). The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology*, *35*(1), 169–191. https://doi.org/10.1038/npp.2009.83

Šimić, G., Kostović, I., Winblad, B., & Bogdanović, N. (1997). Volume and number of neurons of the human hippocampal formation in normal aging and Alzheimer's disease. *Journal of Comparative Neurology*, *379*(4), 482–494. https://doi.org/10.1002/(SICI)1096-9861(19970324)379:4<482::AID-CNE2>3.0.CO;2-Z

Skoog, I. (2011). Psychiatric disorders in the elderly. *Canadian Journal of Psychiatry*, *56*(7), 387–397. https://doi.org/10.1177/070674371105600702

Sone, D., & Beheshti, I. (2022). Neuroimaging-Based Brain Age Estimation: A Promising Personalized Biomarker in Neuropsychiatry. *Journal of Personalized Medicine*, *12*(11). https://doi.org/10.3390/jpm12111850

Soomro, G. M., Altman, D., Rajagopal, S., & Oakley-Browne, M. (2008). Selective serotonin re-uptake inhibitors (SSRIs) versus placebo for obsessive compulsive disorder (OCD). *Cochrane Database of Systematic Reviews*, *1*. https://doi.org/10.1002/14651858.CD001765.pub3

Stein. (2008). Socail Anxiety Disorder.Pdf. *Elsevier*, *371*(9618), 1115–1125. https://doi.org/10.1016/S0140-6736(08)60488-2

Stein, D. J., Costa, D. L. C., Lochner, C., Miguel, E. C., Reddy, Y. C. J., Shavitt, R. G., van den Heuvel, O. A., & Simpson, H. B. (2019). Obsessive–compulsive disorder. *Nature Reviews Disease Primers*, *5*(1), 1–21. https://doi.org/10.1038/s41572-019-0102-3

Syal, S., Hattingh, C. J., Fouché, J. P., Spottiswoode, B., Carey, P. D., Lochner, C., & Stein, D. J. (2012). Grey matter abnormalities in social anxiety disorder: A pilot study. *Metabolic Brain Disease*, *27*(3), 299–309. https://doi.org/10.1007/s11011-012-9299-5

Terburg, D., Scheggia, D., Triana del Rio, R., Klumpers, F., Ciobanu, A. C., Morgan, B., Montoya, E. R., Bos, P. A., Giobellina, G., van den Burg, E. H., de Gelder, B., Stein, D. J., Stoop, R., & van Honk, J. (2018). The Basolateral Amygdala Is Essential for Rapid Escape: A Human and Rodent Study. *Cell*, *175*(3), 723-735.e16. https://doi.org/10.1016/j.cell.2018.09.028

Thorsen, A. L., Hagland, P., Radua, J., Mataix-Cols, D., Kvale, G., Hansen, B., & van den Heuvel, O. A. (2018). Emotional Processing in Obsessive-Compulsive Disorder: A Systematic Review and Meta-analysis of 25 Functional Neuroimaging Studies. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *3*(6), 563–571. https://doi.org/10.1016/j.bpsc.2018.01.009

Topkan, T. A., Erdogan, N., Barutcu, B., Cindil, E., Tali, E. T., & Karaman, Y. (2022). Volumetric Assessment of Hippocampus and Subcortical Gray Matter Regions in Alzheimer Disease and Amnestic Mild Cognitive Impairment. *Cognitive and Behavioral Neurology : Official Journal of the Society for Behavioral and Cognitive Neurology*, *35*(2), 95–103. https://doi.org/10.1097/WNN.0000000000000296

Tye, K. M., Prakash, R., Kim, S. Y., Fenno, L. E., Grosenick, L., Zarabi, H., Thompson, K. R., Gradinaru, V., Ramakrishnan, C., & Deisseroth, K. (2011). Amygdala circuitry mediating reversible and bidirectional control of anxiety. *Nature*, *471*(7338), 358–362. https://doi.org/10.1038/nature09820

van den Heuvel, O. A., Boedhoe, P. S. W., Bertolin, S., Bruin, W. B., Francks, C., Ivanov, I., Jahanshad, N., Kong, X. Z., Kwon, J. S., O'Neill, J., Paus, T., Patel, Y., Piras, F., Schmaal, L., Soriano-Mas, C., Spalletta, G., van Wingen, G. A., Yun, J. Y., Vriend, C., … Yamada, K. (2022). An overview of the first 5 years of the ENIGMA obsessive–compulsive disorder working group: The power of worldwide collaboration. *Human Brain Mapping*, *43*(1), 23–36. https://doi.org/10.1002/hbm.24972

van den Heuvel, O. A., van Wingen, G., Soriano-Mas, C., Alonso, P., Chamberlain, S. R., Nakamae, T., Denys, D., Goudriaan, A. E., & Veltman, D. J. (2016). Brain circuitry of compulsivity. *European Neuropsychopharmacology*, *26*(5), 810–827. https://doi.org/10.1016/j.euroneuro.2015.12.005

Vattimo, E. F. Q., dos Santos, A. C., Hoexter, M. Q., Frudit, P., Miguel, E. C., Shavitt, R. G., & Batistuzzo, M. C. (2021). Higher volumes of hippocampal subfields in pediatric obsessive-compulsive disorder. *Psychiatry Research - Neuroimaging*, *307*(September), 111200. https://doi.org/10.1016/j.pscychresns.2020.111200

Vigne, P., Simões, B. F. T., de Menezes, G. B., Fortes, P. P., Dias, R. V., Laurito, L. D., Loureiro, C. P., Moreira-de-Oliveira, M. E., Albertella, L., Lee, R. S. C., Stangier, U., & Fontenelle, L. F. (2019). The relationship between obsessive-compulsive disorder and anxiety disorders: A question of diagnostic boundaries or simply severity of symptoms? *Comprehensive Psychiatry*, *94*, 152116. https://doi.org/10.1016/j.comppsych.2019.152116

Walhovd, K. B., Fjell, A. M., Reinvang, I., Lundervold, A., Dale, A. M., Eilertsen, D. E., Quinn, B. T., Salat, D., Makris, N., & Fischl, B. (2005). Effects of age on volumes of cortex, white matter and subcortical structures. *Neurobiology of Aging*, *26*(9), 1261–1270. https://doi.org/10.1016/j.neurobiolaging.2005.05.020

Walhovd, K. B., Westlye, L. T., Amlien, I., Espeseth, T., Reinvang, I., Raz, N., Agartz, I., Salat, D. H., Greve, D. N., Fischl, B., Dale, A. M., & Fjell, A. M. (2011). Consistent neuroanatomical age-related volume differences across multiple samples. *Neurobiology of Aging*, *32*(5), 916–932. https://doi.org/10.1016/j.neurobiolaging.2009.05.013

Wang, C., Wang, Y., Lau, W. K. W., Wei, X., Feng, X., Zhang, C., Liu, Y., Huang, R., & Zhang, R. (2021). Anomalous static and dynamic functional connectivity of amygdala subregions in individuals with high trait anxiety. *Depression and Anxiety*, *38*(8), 860–873. https://doi.org/10.1002/da.23195

West, J., Coleman, P. D., Flood, G., & Troncoso, C. (1994). Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer ' s disease. *The Lancet*, *344*(8925), 769–772.

Williams, T., McCaul, M., Schwarzer, G., Cipriani, A., Stein, D. J., & Ipser, J. (2020). Pharmacological treatments for social anxiety disorder in adults: A systematic review and network meta-analysis. *Acta Neuropsychiatrica*. https://doi.org/10.1017/neu.2020.6

Xia, X., Chen, W., McDermott, J., & Han, J.-D. J. (2017). Molecular and phenotypic biomarkers of aging. *F1000Research*, *6*(0), 860. https://doi.org/10.12688/f1000research.10692.1

Yang, Y., & Wang, J. Z. (2017). From structure to behavior in basolateral amygdala-hippocampus circuits. *Frontiers in Neural Circuits*, *11*(October), 1–8. https://doi.org/10.3389/fncir.2017.00086

Zhang, L., Hu, X., Lu, L., Li, B., Hu, X., Bu, X., Li, H., Tang, S., Gao, Y., Yang, Y., Sweeney, J. A., Gong, Q., & Huang, X. (2020). Anatomic alterations across amygdala subnuclei in medication-free patients with obsessive–compulsive disorder. *Journal of Psychiatry and Neuroscience*, *45*(5), 334–343. https://doi.org/10.1503/jpn.190114

Zhang, L., Hu, X., Lu, L., Li, B., Hu, X., Bu, X., Li, H., Tang, S., Yang, Y., Roberts, N., Sweeney, J. A., Gong, Q., & Huang, X. (2019). Abnormalities of hippocampal shape and subfield volumes in medication-free patients with obsessive–compulsive disorder. *Human Brain Mapping*, *40*(14), 4105–4113. https://doi.org/10.1002/hbm.24688

Zou, J., Huss, M., Abid, A., Mohammadi, P., Torkamani, A., & Telenti, A. (2019). A primer on deep learning in genomics. *Nature Genetics*, *51*(1), 12–18. https://doi.org/10.1038/s41588-018-0295-5

CHAPTER 2: Systematic review of Indicators of biological aging in anxiety and related disorders

In chapter 1, I introduced OCD and SAD in terms of their prevalence, clinical characteristics, and the brain structures implicated in the neurobiological models of these disorders. I also introduced the concept of biological aging and detailed the recent neuroimaging techniques that are utilized to predict brain aging in anxiety and related disorders. In this chapter, I conduct a systematic review that focuses on molecular, cellular, and neuroimaging-based tools used to index biological aging in healthy conditions and anxiety and related disorders. In this review, we (SK, ZN, and JI) performed an iterative search of electronic databases to identify publications that met our inclusion criteria. The STROBE guidelines were used to assess good reporting and transparency of all the publications presented in the results of this review. In the discussion, I present a synthesis of the findings from the most common and up-to-date indicators of biological aging. Moreover, I identify gaps in the literature concerning biological aging, anxiety disorders, and the brain, and pinpoint areas where further research is required.

2.1. Introduction

Anxiety disorders; evidence of age-related diseases

Anxiety disorders are persistent and stressful conditions that are commonly comorbid with age-related diseases, as well as an increased risk of mortality (Meier, Mattheisen, Mors, Mortensen, et al., 2016). As such, anxiety disorders have been linked to unhealthy and possibly ‘accelerated’ aging due to the high incidence of cardiovascular diseases, stroke, dementia, immune diseases, and metabolic disorders diagnosed with these disorders. A recent meta-analysis consisting of 46 cohort studies including 2,017,276 participants and 222,253 individuals with anxiety and related disorders (i.e., PTSD, phobic anxiety, and panic disorder) found that individuals with these disorders had significantly increased risk of cardiovascular mortality, coronary heart disease, stroke, and heart failure. Additionally, phobic anxiety was related to a higher risk of coronary heart disease, and PTSD was associated with a higher risk of stroke (Emdin et al., 2016). Other studies suggest an association between panic disorder and increased risk for cardiovascular morbidity and mortality, even after adjusting for risk factors like major depression (Machado et al., 2017).

While certain lifestyle factors including smoking, alcohol use, poor eating habits, and low physical activity may partially account for the increased risk of medical comorbidity and mortality found in individuals with psychiatric disorders (Oude Voshaar et al., 2021), studies suggest that medical comorbidity is still significant even after adjusting for these factors (Penninx et al., 2013). This suggests that there are additional factors that may account for the increased risk associated with these disorders. Anxiety disorders may be associated with dysregulation in the immune system, as men with current anxiety disorders are found to have increased inflammatory markers compared to HCs (Vogelzangs et al., 2013). Other studies suggest that anxiety disorders are accompanied by disruptions in the major stress systems including the hypothalamic-pituitary-adrenal (HPA) axis, as individuals with panic disorder (with agoraphobia) and anxious individuals with comorbid depressive disorder have been found to have higher awakening cortisol levels (Vreeburg et al., 2010), although these findings are considered to be inconsistent (Penninx et al., 2013).

It is hypothesized that HPA dysregulations may lead to accentuated aging (Wolkowitz et al., 2010), perhaps through allostatic load (McEwen, 2000). Allostatic load refers to the cost of chronic exposure to fluctuating or heightened neural and neuroendocrine responses resulting from repeated or chronic environmental challenges that an individual reacts to as being particularly stressful (Guidi et al., 2021). McEwen first introduced the concept of allostatic load and allostasis, allostasis refers to “maintaining homeostasis through change”, which describes how the body adapts to stress (McEwen, 2000). A meta-analysis found that traumatic events that occur as adverse childhood experiences are associated with higher allostatic load scores and poorer health outcomes in adulthood suggesting that stress can have long-term consequences on the health of the body (Finlay et al., 2022). Additionally, longitudinal data shows that an increase in an individual's allostatic load score is associated with an increased risk of all-cause mortality (Karlamangla et al., 2006).

In response to environmental stress, typically the stress system is initiated through the activation of amygdala nuclei, which communicate with hypothalamic neurons (through multiple pathways), leading to the activation of the HPA axis. In the presence of chronic stress, the negative feedback mechanism involved in regulating the HPA axis is compromised, leading to the chronic release of cortisol (Danese & McEwen, 2012; Faravelli et al., 2012; Wolkowitz et al., 2010). In normal circumstances the HPA axis regulates inflammatory responses, however, persistent activation of the HPA axis possibly leads to impaired reactivity of the immune system to glucocorticoids, thereby increasing inflammation (Miller et al., 2002; Wirtz et al., 2003). Although plausible, the exact mechanisms underlying accentuated biological aging in anxiety and related disorders still remain unclear (Wolkowitz et al., 2010). However, in the past decades, substantial efforts have been made in developing biomarkers that index accentuated aging in human and animal models (Johnson, 2006).

The challenges of 'biomarkers'

The American Federation for Aging Research stipulates that a biomarker must, (a) predict the rate of aging whilst monitoring basic processes, (b) withstand repeated testing without harm to the individual, and (c) function in both human and animal laboratory studies (Johnson, 2006; Jylhävä et al., 2017). While some markers have proved to be sensitive to chronological age, not all have convincingly proven their specificity (for review see Han et al., 2019), and most markers are yet to be used in clinical settings (Colloca et al., 2020). This is perhaps not surprising, as aging is a biologically complex process occurring at various rates in different cell/organ types per individual (López-Otín et al., 2013). In a longitudinal study investigating the relationship between age and multiple 'omics' measures, including transcriptomics, and proteomics, Ahadi et al. (2020) found unique patterns of aging that were specific to each individual (referred to as 'ageotypes'), and that whilst some individual displayed accentuated aging patterns in the kidneys, other individuals had accentuated aging patterns in the kidneys and metabolic pathways (in 106 healthy individuals aged between 29 and 75 years old)(Ahadi et al., 2020). Given the challenges in identifying reliable biomarkers of aging, it has been proposed that rather than 'biomarker', the phrase 'indicator of biological aging' is more appropriate especially when referring to measures that are correlated with chronological age (Han et al., 2019).

Molecular and cellular indicators of biological aging

There are several indicators of biological aging including DNA and chromosomes (Telomeres and DNA methylation), metabolism (lipid and proteomic), cellular senescence, oxidative stress, mitochondrial DNA, and recently brain age (Franke et al., 2020). Other indicators measure phenotypic functions such as walking speed, grip strength, and standing balance (Cole et al., 2019; Jylhävä et al., 2017; López-Otín et al., 2013; Xia et al., 2017). For the purposes and scope of the present systematic review, I focused on molecular, cellular, and neuroimaging-based indicators of aging that have been associated with anxiety disorders across the adult lifespan (age 18-65 years old).

Telomere length: what are telomeres?

To date, the most commonly utilized indicator of biological aging is telomere length (TL). There are over 6000 papers investigating TL in various conditions (Jylhävä et al., 2017), including reviews on TL in basic function (Blackburn et al., 2015), aging (Sanders & Newman, 2013), and psychiatric disorders (Monroy-Jaramillo et al., 2018). Telomeres are non-coding DNA protein complexes with tandem repeats of TTAGGG nucleotide sequences at the end of every chromosome (Darrow et al., 2016; Demanelis et al., 2020). During each cell division, the DNA content of the telomeres is not fully replicated because the DNA polymerase enzyme can only synthesize new DNA in the 5′−3′ direction (Saldanha et al., 2003). When the telomeres reach a critically short length, (also known as the ‘Hayflick limit’, the critical point when the cell’s TL is excessively short) the cell loses the ability to divide which induces cellular senescence (Allaire et al., 2023; Darrow et al., 2016; Demanelis et al., 2020; Franke et al., 2020). While there is some debate, studies suggest that TL is a suitable indicator of biological aging as it can be associated with basic aging mechanisms, correlates with age-related diseases, is heritable, and is influenced by environmental factors. However, the clinical application of TL remains elusive (Franke et al., 2020).

Cross-sectional data suggest TL shortening by approximately 14 to 103 base pairs per year, whereas longitudinal data suggest TL shortening of 32.2 to 45.5 base pairs per year (Müezzinler et al., 2013). Shorter TL has been associated with older age, race, and gender (Sanders & Newman, 2013). However, findings of the association between TL and both age-related diseases and mortality have been inconsistent. While there was an initial report that shorter TL was associated with an increased risk of death (Cawthon et al., 2003) subsequent studies have either confirmed (Bakaysa et al., 2007; Ehrlenbach et al., 2009; Kimura et al., 2008) or contradicted these findings (Fitzpatrick et al., 2007; Harris et al., 2006). These differences may be explained by various factors including differences in methodology, sample demographics, and sizes (Darrow et al., 2016). Some studies suggest that TL may not reflect all age-related diseases as no association has been observed between TL and other age-related outcomes like bone mineral density and pulmonary function (Sanders & Newman, 2013). In the largest population-based TL study to date (n = 105,539), Lapham et al. (2015) showed that TL was significantly longer in women after the age of 50 years compared to men, suggesting that TL shortening is influenced by sex and age differences (Lapham et al., 2015). Other studies suggest that both lifestyle factors and somatic health status influence TL including BMI, exercise, smoking, alcohol consumption, and oxidative and inflammatory stress (Allaire et al., 2023; Bojesen, 2013).

Shorter TL has also been associated with various psychiatric disorders. Meta-analysis suggests shorter TL in depression (Ridout et al., 2016; n=38 studies), and higher anxiety states (Malouff & Schutte, 2017; n=17). Darrow et al. (2016) performed a meta-analysis including 14827 individuals with MDD, bipolar disorders, anxiety disorders, psychotic disorders, and PTSD. Overall TL was found to be shorter across the psychiatric disorders. Longitudinal data found that individuals with anxiety disorders had shorter TL at 2 years follow-up even after adjusting for lifestyle factors, educational level, and antidepressant use (Hoen et al., 2013). However, in this study, no association was found for depressive disorders at baseline and follow-up (Hoen et al., 2013).

DNA methylation is used to estimate epigenetic aging.

The expression of genetic information is governed by both the intrinsic instructions encoded within the DNA sequence as well as the information contained outside of the DNA (Galkin et al., 2020). Epigenetic studies focus on heritable changes in gene expression that occur without changing the DNA sequence (Bollati & Baccarelli, 2010). The epigenome is suggested to be a dynamic area where gene expression can be influenced by endogenous processes such as epigenetic drift, and exogenous processes including physical activity, diet, smoking, alcohol consumption, and stress (Bollati & Baccarelli, 2010; Pagiatakis et al., 2021). Aging cells undergo an accumulation of epigenetic changes which may disturb the cell’s normal functioning. The increasing recognition of epigenetics in biological aging has contributed to the development of methods that index epigenetic aging in different types of human cells (Duan et al., 2022).

DNA methylation is the most commonly investigated epigenetic factor that is known to modulate genes (Marino et al., 2023). Briefly, DNA methylation is the covalent modification of DNA bases that induces heritable silencing of DNA sequences, thereby maintaining genomic integrity (Bollati & Baccarelli, 2010; Kiefer et al., 2007). This process primarily occurs on the cytosine residues located within CG-dinucleotide-rich genomic regions. These genomic regions are known as CpG islands and are ubiquitous in the human genome. The methylation of cytosine can directly impede the binding of transcription factors to recognition sites on the DNA. Alternatively, methylation can lead to methyl-CpG binding domain proteins, to induce the formation of silent chromatin (Pagiatakis et al., 2021).

DNA methylation of CpG sequences in the genome has been used in the development of indicators of cellular aging. Seminal papers have focused on the development of DNA methylation microarrays that capture the methylation occurring at numerous CpG sites using a single bead-chip and a small DNA sample, thus creating methylation-based estimations of biological age. In a twin-pair study, Bocklandt et al. (2011) found that 88 CpG sites in or near 80 genes were significantly associated with chronological age (Bocklandt et al., 2011). Building on this, across 7844 samples, Horvath, 2013 identified 353 CpG sites where methylation status predicted chronological age across multiple tissues, with a median error rate of 2.9 years. This data was used in the development of a DNA methylation age algorithm, or epigenetic 'clock', that can be used to predict epigenetic aging (Horvath, 2013). In the same year, in an independent study, Hannum et al. (2013) used 656 samples and identified 71 CpG sites that were correlated with chronological age from blood samples. This DNA methylation clock had a root mean square error of 3.9 years. A substantial proportion of the loci featured in this algorithm are proximal to genes that are associated with the development of age-related diseases, oxidative stress, and DNA damage (Hannum et al., 2013).

Several studies have investigated the association between DNA methylation and psychiatric disorders. In two different studies, Wolf et al. (2018) found that PTSD symptoms were associated with advanced DNA methylation age based on the Hannum clock, however, no association was found by Wolf et al. (2016) when using the Horvath clock. In a longitudinal study, Boks et al. (2015) found a negative association between PTSD and age over time, and a positive association between combat trauma and age, using the Horvath clock (Boks et al., 2015). While studies point to an association between DNA methylation and accentuated epigenetic aging in anxiety and related disorders, the findings reported are inconsistent (Boks et al., 2015; Han et al., 2018; Wolf, et al., 2018) (Boks et al., 2015; Han et al., 2018; Wolf et al., 2018).

Brain-based indicators of aging: can molecular and cellular indicators of biological aging predict brain aging?

The use of molecular and cellular markers to estimate brain age is limited by the inherent challenges of obtaining a tissue sample. Both postmortem and neurosurgical sampling can lead to the formation of artifacts in the tissue, introduce mortality bias, and are generally performed in small sample sizes (Higgins-Chen et al., 2021). Some studies suggest that since there may be some inter-correlation between the rate of TL shortening across different tissue types including the cerebral cortex, myocardium, liver, and renal cortex within an individual, it is possible to generalize TL shortening across different cell types (Takubo et al., 2002). Indeed, an association has been observed between leukocyte TL, brain atrophy, and white matter hyperintensities, in non-demented individuals aged 64–75 years

(Wikgren et al., 2014). In a recent study, Hillary et al. (2021) found an association between DNA methylation (using GrimAge) and poorer cognitive ability, a decrease in grey matter and white matter volumes, and an increase in white matter hyperintensity in the frontal and temporal regions of the brain (Hillary et al., 2021).

While the evidence presented above suggests that there may be some correlation between molecular and cellular indicators of biological aging and brain aging, it is important to note that neurons are generally post-mitotic cells and therefore some brain regions like the occipital lobe may be less influenced by mitotic-related age predictors than other areas that contain neural stem cells and progenitor cells like the hippocampus (Lindqvist et al., 2015; Thanseem et al., 2017). Moreover, post-mortem studies of aging and AD suggest that there are divergent cell-type-specific methylation signatures in neurons and glial cells (Gasparoni et al., 2018). Postmortem studies of TLs in the cortex of individuals with MDD found no significant difference between MDD (n=24) and HCs (n=12) (Teyssier et al., 2010). These findings suggest that further evidence is required to determine if molecular and cellular indicators of biological aging can be used to measure brain aging and the factors that contribute to differences between brain and blood-based predictions.

As discussed in Chapter 1, recent advances in machine learning algorithms have allowed for the prediction of brain age using a rich set of brain features derived from neuroimaging data. The most precise brain age models have demonstrated a mean absolute error below 5 years and have been proven to have high test-retest reliability (Cole & Franke, 2017). Based on these developments, brain age has been assessed in various neurodegenerative disorders, especially those that are associated with aging such as AD and mild cognitive impairment (Gaser et al., 2013; Sone & Beheshti, 2022). Studies suggest that AD is associated with accentuated brain age ranging from +2.88 to 9.29 years compared to HCs (Beheshti et al., 2018, 2020; Millar et al., 2022). For a review of brain-PAD in neurodegenerative diseases please see Sone & Beheshti, 2022.

Other markers of biological aging

There are several other indicators of biological aging, however, these are beyond the scope of the present systematic review (see Cole & Franke, 2017; López-Otín et al., 2013; Mattson & Arumugam, 2018; Wagner et al., 2016). Briefly, transcriptomics is shown to predict chronological age and is suggested to be elevated in bipolar disorder, and schizophrenia but not MDD (Lin et al., 2021), however, omics strategies are known to be susceptible to noise (Eling et al., 2019). Serum, urine, and fatty metabolism have been shown to predict chronological age, all-cause mortality, and AD (Deelen et al., 2019; Johnson et al., 2019; Wang et al., 2020). Markers of neuroinflammation may provide another possible indicator of brain aging. In the aged brain, glial cells are found to be in an activated state associated with the over-production of pro-inflammatory markers such as interleukin 1β, 6, and tumor necrosis factor-alpha (Cribbs et al., 2012; Norden & Godbout, 2013).

This review aims to investigate the association between anxiety and related disorders and biological indicators of aging indexed at a molecular, cellular, and brain level. We aim to critically evaluate the existing literature and set the stage for the subsequent chapters presented in this dissertation.

2.2. Methods

2.2.1. Search strategy and selection criteria

The preferred reporting items for systematic reviews and meta-analyses (PRISMA) were followed (Shamseer et al., 2015). The search process and study eligibility determination for this review were conducted by a postdoctoral research fellow (S.K) and PhD candidate (ZN), under the supervision of Dr Jonathan Ipser. We performed an electronic database search of PubMed (www.ncbi.nlm.nih.gov/pubmed) and SCOPUS (https://www.scopus.com/search/), from inception date to 06 October 2020. The searches were an iterative process resulting in the following final search query.

PubMed; ("ageing" OR "aging") AND ("anxiety disorders"[Mesh] OR "social phobia" OR "social anxiety disorder" OR "generalized anxiety disorder" OR "panic disorder" OR "obsessive-compulsive disorder" OR "OCD" OR "post-traumatic stress disorder" OR "PTSD") AND ("biomarkers" OR "brain ag*" OR "telomere*" OR "epigenetic" OR "methylation").

Scopus; TITLE-ABS-KEY ("ageing” OR “aging”) AND TITLE-ABS-KEY ("Anxiety Disorders" OR "social phobia" OR "social anxiety disorder" OR "generalized anxiety disorder" OR "panic disorder" OR "obsessive compulsive disorder" OR "OCD" OR "post-traumatic stress disorder" OR "PTSD") AND TITLE-ABS-KEY ("biomarkers" OR "brain ag*" OR "telomere*" OR "epigenetic" OR "methylation").

The search query resulted in 115 publications from PubMed and 151 from SCOPUS (Figure 2.1). This search was repeated on 02 November 2021, using the same search query in PubMed and SCOPUS with an additional filter for publication year; 2020-2021 (duplicates were excluded, and new publications were included for title and abstract review). The reference lists of highly-cited publications (Malouff & Schutte, 2017; Solomon et al., 2017; Verhoeven et al., 2015) were searched for additional citations to include in the abstract review.

2.2.2. Inclusion and exclusion criteria

We selected original publications written in English, all reviews, conference abstracts, and editorials were excluded. Publications were considered for inclusion if they were studies of human adults (no post-mortem studies), aged between 18 and older (no upper age limit). The participants of eligible studies were required to have an anxiety disorder as the primary diagnosis (diagnosed using the DSMIV/ DSM-5/ICD-10). We included studies on the condition that the anxiety disorder group was compared to a non-psychiatric control group. Psychiatric comorbidity was allowed secondary to the primary diagnosis. The publications had to focus primarily on biological indicators of aging including brain age (based on structural neuroimaging data), DNA and chromosome (TL, DNA methylation), RNA and transcriptome (transcriptome profiles) (Xia et al., 2017). Exclusion criteria for our study included phenotypic biomarkers of aging i.e., walking speed, chair stand, standing balance, and grip strength, as studies suggest that it is difficult to relate phenotypic biomarkers to underlying molecular processes (Xia et al., 2017). We also excluded diagnoses of neurodevelopmental disorders, disruptive disorders, impulse-control disorders, conduct disorders, neurocognitive disorders, and neurodegenerative disorders.

2.2.3. Study selection

The two independent raters (SK and ZN) screened each abstract using the inclusion criteria. After the abstract review, 86 eligible publications were identified, and all discrepancies were resolved by a third reviewer (Dr Jonathan Ipser). Next, we reviewed the full text of the eligible publications using the same inclusion criteria. The supplementary material was searched for those articles that were unclear. For those publications with missing information, the corresponding authors were contacted for feedback. If the information was not provided, then the publication was excluded from present systematic review.

2.2.4. Data extraction

We extracted information about the primary anxiety diagnosis, the diagnostic tool used to access anxiety disorder, the study sample size, age, age range, the biomarker of aging, and the method used to access the biomarker of aging, statistical covariates, secondary outcomes, and the main findings. Since lifestyle factors are shown to have an impact on markers of aging (Allaire et al., 2023; Demanelis et al., 2020) we also extracted information about smoking, alcohol use, and any other covariate that was significantly associated with the biological indicator of aging.

2.2.5. Quality assessment of studies

The STROBE 22-item checklist was used to assess good reporting and transparency of each publication by evaluating key aspects of study design, conduct, and reporting (Strengthening the reporting of observational studies in epidemiology; https://www.strobe-statement.org/checklists/). Each rater independently assessed the reporting quality of each publication by answering the checklist items, assigning a “yes”, or “no” based on the information provided by the publication (for example also see Vandenbroucke et al., 2007; von Elm et al., 2014).

2.3. Results

2.3.1. Study selection and characteristics

The search strategy conducted in 2020 identified a total of 194 abstracts from PUBMED and SCOPUS, after the removal of duplicates (n=74). After applying the inclusion criteria, 86 abstracts passed the initial screening for full-text review. After a full-text review, 10 papers were found to be eligible for inclusion. For some papers we contacted authors for additional information; in 3 papers the anxiety disorder group was combined with MDD (Chagnon et al., 2015; Révész et al., 2016; Verhoeven et al., 2015), 1 paper included subthreshold cases of the anxiety disorders and did not report data separately for threshold cases (Kananen et al., 2010), and 1 paper mentioned PTSD comparison to HC but did not provide descriptive statistics for the HC group (Solomon et al., 2017). Of these, we received feedback from 1 author, who sent a paper where the anxiety group was analyzed separately from MDD (Verhoeven et al., 2015; included in the review).

The search strategy was repeated in 2021 (filtered publication year; 2020-2021), and we found a total of 53 additional abstracts for screening. After the abstract and full-text screening, we identified 3 papers eligible to be included in the final review (Han et al., 2021; Kang et al., 2021; Kuan et al., 2021). Lastly, one eligible paper was identified by manually searching the references (Clausen et al., 2021). Taken together, 14 eligible papers were identified for inclusion in the final systematic review (Figure 2.1).

2.3.2. Excluded studies

A total of 234 studies were excluded from the review. Briefly, the most common reason for exclusion was that the papers were not original, but rather systematic or meta-analytic reviews (n= 60). Secondly, some studies did not include a diagnosis of an anxiety disorder, or they measured aspects of anxiety or worry (n=35). Several studies were animal research and therefore not eligible for inclusion in the review (n=15).

2.3.3. Summary of studies

The 14 studies included in our systematic review were published between the year 2011 and 2021, including 13 cross-sectional studies and one longitudinal analysis (Yang et al., 2020) (Table 2.1). Of these, the most commonly assessed disorder was PTSD (10 studies), two studies examined a single disorder (panic disorder (Prelog et al., 2016); OCD (Kang et al., 2021)), and two studies included a combination of social anxiety disorder, generalized anxiety disorder and panic disorder (Han et al., 2021; Verhoeven et al., 2015). The majority of the studies recruited participants from high-income countries, except for those studies that recruited participants from Croatia (Jergović et al., 2014), and

Korea (Kang et al., 2021; Kim et al., 2017). Moreover, Katrinli et al. (2020) assessed participants recruited from low-income communities in the USA (Katrinli et al., 2020). Two of the studies used data from the Netherlands Study of Depression and Anxiety (NESDA); one measured leukocyte TL (Verhoeven et al., 2015), and the other measured brain aging (Han et al., 2021). Two of the studies investigated PTSD in male veterans from Operation Enduring Freedom and/or Operation Iraqi Freedom, with participants recruited from the Manhattan, Bronx, and Brooklyn Veterans Affairs Medical Centres. Of these two studies, one study measured TL, telomerase activity, and DNA methylation using the Horvath epigenetic clock (Verhoeven et al., 2018) and the other study measured DNA methylation using various epigenetic clocks, cross-sectionally and longitudinally (Yang et al., 2020).

Concerning the measured outcome, the majority of studies focused on the association between anxiety disorders and TL (Jergovic et al., 2014; Kang et al., 2020, 2021; Kim et al., 2017; O'Donovan et al., 2011; Prelog et al., 2016; Roberts et al., 2017; Verhoeven et al., 2015, 2018), some included an additional analysis of telomerase activity (Jergović et al., 2014; Verhoeven et al., 2018) and one study measured TL and mitochondrial DNA content (Kang et al., 2021). In most of the studies that described TL, TL was measured using quantitative polymerase chain reaction(qPCR) (n=9), taken from blood draws from the study participants. Lastly, 2 studies used machine learning algorithms to predict brain age (Clausen et al., 2021; Han et al., 2021). Table 2.1 shows the main characteristics of the studies that are included in the present systematic review.

2.3.4. Quality of study reports

The majority of studies included in our analysis provided an adequate description of the study background, methods, and findings in the abstract of the analysis, however, none of the studies used a common term in the title or the abstract to easily identify the design of the study (e.g., "case-control" or "cross-sectional study"). For the introduction, all studies appropriately described their scientific background, provided a clear rationale, and stated specific study objectives or hypotheses. Most studies, except for Yang et al., 2020, were cross-sectional and provided adequate information on eligibility criteria for inclusion in the analysis.

The study methodology was generally poorly reported, particularly (item 5) describing the setting, locations, and relevant dates specifically including periods of recruitment, exposure, follow-up, and data collection, and (item 9) describing any efforts to address potential sources of bias. Clausen et al. (2021) and Han et al. 2021 were a few of the studies that explicitly described dealing with bias; bias in this study was identified as regression dilution bias a phenomenon that occurs in brain age estimation where the Brain-PAD is typically overestimated in younger individuals and overestimated in older individuals. The studies overcome this regression bias by including chronological age in the regression model employed. Very few studies adequately reported how they arrived at the sample size (item 10) and explained how missing data was addressed (item 12).

For the discussion, studies provided a clear and short summary of the main findings concerning the study objectives at the beginning of the discussion section. Additionally, the overall interpretation of the study findings was cautious with consideration of the generalizability and limitations of the study. Lastly, all but one of the studies (Roberts et al., 2017) declared sources of funding and/or role of funders in the present study. Overall, the studies were clear and transparent in their introduction and discussion, however, the results section could have been improved with more details (Vandenbroucke et al., 2007).

Telomere Length (nine studies)

2.3.5. Telomere length in PTSD (six studies)

The majority of papers focusing on TL were on PTSD (six studies), four of the studies reported shorter TL in PTSD compared to HCs (Jergović et al., 2014; Kang et al., 2020; O'Donovan et al., 2011; Roberts et al., 2017), and two studies had null findings (T. Y. Kim et al., 2017; Verhoeven et al., 2018).

O'Donovan et al. (2011) investigated leukocyte TL in a community-based sample of male and female adult individuals with current chronic PTSD (n=43, n=18 with multiple categories of childhood trauma) and HCs (n=47, all without childhood trauma). The results showed that individuals with PTSD had significantly shorter TL, compared to HCs. Additionally, individuals with PTSD with multiple categories of childhood trauma had significantly shorter TL than HCs. No association was found between TL and PTSD symptom severity (O'Donovan et al., 2011).

Jergovic et al. (2014) investigated the relative TL and telomerase activity in peripheral blood mononuclear cells in a sample of 30 Croatian male combat veterans with PTSD, 17 HCs, and 15 elderly control group volunteers aged 80 years or older. Additionally, this study measured Immunosenescence. The study found that individuals with PTSD individuals had shorter TL compared to HCS, whilst the elderly volunteers had the shortest telomeres. Telomerase activity did not differ between individuals with PTSD and HCs, however, the elderly volunteers had lower activity than the PTSD group and HCs. No association was found between TL and immune factors (Jergović et al., 2014).

In an all-female sample of individuals with PTSD (n=25) enrolled from a substudy of the Nurses' Health Study II, Roberts et al. (2017) found shorter TL in females with PTSD, after adjusting for age at blood draw. However, the type of trauma exposure did not affect TL (trauma grouped into 5 categories; rape;

interpersonal violence; other event to self; sudden death of a loved one; other event to a loved one) (Roberts et al., 2017).

When investigating TL in male veterans with PTSD who were exposed to combat trauma in the Vietnam War (recruited through the Veterans Health Service), Kim et al. (2017) found no significant difference between individuals with PTSD (n=122) and HCs (n=120). Additionally, no significant difference in relative TL was found in individuals exposed to severe trauma and those exposed to light-to-moderate trauma, even when adjusting for socio-demographic variables (Kim et al., 2017).

Verhoeven et al. (2018) did not find a significant difference in TL in male combat-exposed war veterans with PTSD (n=79) compared to male combat-exposed war veterans without PTSD (n=81) (adjusted for chronological age). The study also assessed DNA methylation levels, using the Horvath epigenetic clock, in PTSD veterans compared to HCs. The study found that epigenetic age was lower in veterans with PTSD compared to HCs, even after adjusting for ethnicity, lifestyle factors, and childhood trauma. However, when adjusting for antidepressants, the finding that PTSD was associated with lower epigenetic age became non-significant which suggests that antidepressants partially explained this association. In addition, higher telomerase activity was associated with less epigenetic aging in veterans with PTSD compared to HCs (Verhoeven et al., 2018).

In a sample exposed to high levels of combat trauma, Kang et al. (2020) found shorter TL and larger amygdala volume in individuals with PTSD (n=53) compared to a non-PTSD group (n=24), after adjusting for ethnicity, smoking, alcohol use, substance use, antidepressant use, and psychotropic medication use. On the other hand, in a subset exposed to low levels of combat trauma, no association was found in individuals with PTSD (n=12) compared to a non-PTSD group (n=59). In addition, amygdala volume was positively associated with PTSD symptom severity and faster heart rate, and TL was inversely associated with diastolic blood pressure and norepinephrine levels (Kang et al., 2020).

2.3.6. Telomere length in other anxiety disorders (three studies)

In a large-scale study of TL in anxiety disorders, Verhoeven et al., 2015 found shorter leukocyte TL in individuals with a current anxiety disorder (n=1283) compared to HCs (n=582) and compared to the individuals with remitted anxiety disorder (n=459). In comparing the mean leukocyte TL in different anxiety disorders, the authors of this study reported that panic disorder with agoraphobia (n=414), social phobia (n=652), and generalized anxiety disorder (n=454) had significantly shorter TL than HCs (n=582), after adjusting for age, gender, and education. These results remained statistically significant even when including sociodemographic, health, and lifestyle variables. No association was found for agoraphobia (n=187) and panic disorder without agoraphobia (n=224). Additionally, shorter TL was associated with several symptom severity, specifically social phobic symptoms, arousal, and worrying in the total sample. No association was found for comorbid MDD or psychotropic medication use (Verhoeven et al., 2015). The null findings for panic disorder without agoraphobia were replicated in a smaller study of relative TL in cases (n=131) compared to HCs (n=131) (Prelog 2016). However, female individuals with panic disorder (n=85) had shorter TL compared to males (n=44). In addition, significantly higher levels of methylation were observed across 5 specific CpG sites in females with panic disorders compared to HCs, but not in males with panic disorder (Prelog et al., 2016). Finally, Kang et al. (2021) found that females with OCD (n=94) had significantly shorter TL compared to HC (n=234, 41.5% female), this study also found lower mitochondrial DNA in the same female sample. Additionally, males with OCD (n=149) also had lower mitochondrial DNA, however, no difference in TL was found between individuals and HCs (adjusted for age, body mass index, and childhood trauma) (Kang et al., 2021).

2.3.7. DNA methylation in anxiety disorders (four studies)

There is some evidence that anxiety disorders are associated with advanced epigenetic aging (Katrinli et al., 2020; Kuan et al., 2021; Yang et al., 2020). Verhoeven et al. (2018) investigated DNA methylation, TL, and telomerase activity in veterans with PTSD (n=79) and veterans without PTSD (n=81), using the Horvath epigenetic age calculator. This study found less epigenetic aging in the veterans with PTSD compared to controls, suggesting that the PTSD group had a 'younger' epigenetic age profile, even after adjusting for race, BMI, alcohol, and smoking (Verhoeven et al., 2018).

Katrinli et al. (2020) used a sample of 218 individuals with current PTSD, a separate 209 with lifetime PTSD, and 427 trauma-exposed controls (without a history of PTSD) to investigate accentuated aging in PTSD using Horvath's GrimAge calculator. Additionally, T1-weighted structural MRI images of 69 female individuals (26 from the current PTSD group and 43 from the control group) were used to examine the association between epigenetic age and cortical thickness. This study found a significant difference in 'GrimAge acceleration' (age-adjusted 'GrimAge') between PTSD (current or lifetime) and controls. When examining cortical thickness, GrimAge acceleration in current PTSD was negatively associated with thickness of the right lateral orbitofrontal cortex in the current PTSD group, but not in the control group. While this study suggests that PTSD was associated with accentuated epigenetic aging (Katrinli et al., 2020), studies suggest that epigenetic predictions may vary according to the training and sampling data used (Lu et al., 2019; Marioni et al.,2015), and therefore findings need to be replicated using the different types of epigenetic age calculators.

Yang et al. (2020) investigated age acceleration in combat trauma-exposed male veterans with and without PTSD using cross-sectional and longitudinal data from two independent samples. Briefly, the discovery cohort included 80 combat trauma-exposed individuals with PTSD and 82 combat trauma-exposed controls, the longitudinal PTSD group included individuals who were diagnosed with PTSD at baseline (T1) and then returned for long follow-up three years later (T2) (n=26 with initial diagnoses of PTSD) (see Table S9 in supplemental material). The age acceleration results were then compared to those obtained using other DNA methylation clocks including Horvath, Hannum, PhenoAge, and GrimAge. This study found that PTSD was associated with higher age acceleration compared to controls using GrimAge and Horvath, but not Hannum. PTSD symptom severity was positively correlated to age acceleration. In the longitudinal group, longitudinal changes in PTSD symptom severity were positively correlated to age accelerations (increases or decreases in PTSD severity related to increases or decreases in age respectively), additionally, the longitudinal changes in age acceleration were correlated with changes in the hyperarousal symptom complex (see Table S3 in supplemental material) (Yang et al., 2020). Lastly, this study did not find an association between epigenetic age and inflammatory factors, interferon-gamma, interleukin 6, 8, and 10, C-Reactive Protein (CRP), and tumor necrosis factor (TNF) alpha across all subjects (Yang et al., 2020).

Kuan et al. (2021) investigated the association between males with PTSD (current vs past; World Trade Centre responders) and accentuated aging using a multi-tissue transcriptional age calculator (RNAAgeCalc). The results were then compared to different DNA methylation age calculators including Horvath, Hannum, PhenoAge, and GrimAge. The study found that males with current PTSD (n=81) had higher transcriptional age than those individuals without PTSD (n=201), after controlling for age and race. The study also found that the transcriptional age was positively associated with the avoidance dimension, however, no association was found for the total PCL, reexperiencing, hyperarousal dimensions, and numbing.

When comparing the four DNA methylation age calculators, only GrimAge was found to be significantly higher in the current PTSD compared to individuals without PTSD, but no significant differences were observed between current and past PTSD. GrimAge was positively associated with total PCL, avoidance, and numbing dimensions ($\beta = 0.186$, $p = 0.012$), no association was found for reexperiencing and hyperarousal dimensions. Additionally, after further adjusting for cell type proportions (CD8 and CD4 T cells, natural killer cells, B cells, and monocytes), all of the DNA methylation ages (besides Horvath) showed a significant association with PTSD (Kuan et al., 2021). The association between DNA methylation and immune cell type proportions observed in this study was consistent with a previous meta-analysis that indicated a link between epigenetic age and Immunosenescence (Chen et al., 2016).

2.3.8. Brain age in anxiety disorders (2 studies)

While we only identified 2 studies concerning brain age and anxiety disorders, one study used large-sample sizes (more than 2000 individuals) (Clausen et al., 2021), and both studies were measured across a wide adult age range; 18–69 years (Clausen et al., 2021; Han et al., 2021). Clausen et al. (2021) used 3 pre-trained machine learning brain age models; brainageR, PHOTON-BA, and BARACUS (Brain Age and Brain-Age Regression Analysis and Computation Utility Software). The BrainageR model, which was trained using a Gaussian process regression algorithm, was found to be the most accurate in predicting brain age (tested in a subset of 386 HCs, from 3 international sites).

The model was applied to T1-weighted structural MRI from a total of 2229 subjects, split into individuals with and without PTSD (n = 882 vs 1347, respectively). The results revealed that brain age predictions differed significantly between males and females. The sample was divided by sex and the relationship between chronological age and predicted brain age by PTSD status was plotted. Males with PTSD had higher brain age at a younger age, and lower brain-PAD at an older age compared to males HCs (of all ages). On the other hand, females with PTSD had higher brain age predictions compared to females without PTSD, except for young HCs. No association was found between brain age prediction and symptom severity (Clausen et al., 2021). In another study, Han et al. (2021) used an open-source ENIGMA brain age model (Photon-AI), trained using a ridge regression algorithm. This model was applied to 3T MRI data to predict brain age in 67 individuals with anxiety disorder compared to 65 HCs: anxiety disorder including generalized anxiety disorder, panic disorder, and social anxiety disorder. This study found that anxiety disorders had significantly higher brain age predictions (+2.91 years, d=0.27, 95% CI, 0.08–0.61, p_{FDR}=0.048) compared to HCs, only when correcting for antidepressant use. No association was found between brain-PAD and biological stress variables such as inflammatory markers and HPA axis, although the association with cortisol awakening response trended towards significance (b=−0.23 years per nmol/l, p =0.06). There were no significant associations between brain-PAD and exposure to childhood trauma. In an exploratory analysis, the study found no significant correlation between brain age, epigenetic clocks, and TL (Han et al., 2021).

2.3.9. The influence of clinical characteristics, lifestyle factors, and somatic health status on brain aging

2.3.9.1. Clinical comorbidity

Because PTSD is frequently comorbid with MDD, a few studies analyzed the impact of comorbid MDD in PTSD on biological aging indicators. Jergovic et al. (2014) found no association between the diagnosis of comorbid disorders and TL in individuals with PTSD (comorbid with MDD n=24; panic disorder n=13; OCD n=9; SAD n=7) (Jergović et al., 2014). Roberts et al. (2017) found that shorter TL was not significantly associated with comorbid depression in women with PTSD (Roberts et al., 2017). Yang et al. (2020) did not find a significant difference in epigenetic age between individuals with PTSD with comorbid MDD (n=63) and those without comorbid MDD (n=30) (Yang et al., 2020). Han et al. (2021), found higher brain age estimates in comorbid MDD and anxiety group (+2.23 years, d=0.21, p_{FDR}=0.08), with small to moderate effect size (Han, et al., 2021).

2.3.9.2. Medication use

In an exploratory analysis, Jergovic et al. (2014) found that the use of mood stabilizers in individuals with PTSD (n=21) was a significant predictor of shorter TL, even after adjusting for age, BMI, comorbidity, and psychometric test scores (e.g., Clinician-Administered PTSD Scale, State-Trait Anxiety Inventory) (Jergović et al., 2014). In a post-hoc analysis, Kim et al. (2017) found that the use of antidepressants was associated with longer TL in veterans with PTSD with severe trauma, than in non-users with severe trauma (Kim et al., 2017). In a sensitivity analysis, Verhoeven et al. (2018) found that when adjusting for current antidepressants, the association between PTSD and epigenetic age (lower epigenetic age in those with PTSD) that was observed in the main analysis became non-significant. A subgroup analysis found that individuals with PTSD with antidepressant use had significantly lower epigenetic age than those not using antidepressants. However, a post-hoc analysis showed that while current antidepressant use lowered the association between PTSD and epigenetic age, it was not a statistically significant mediator (Verhoeven et al., 2018). In a post hoc analysis, Han et al. (2021) only found a significant association between brain-PAD and the diagnosis of anxiety disorders when correcting for the use of antidepressants. Indeed, the authors reported that the variables that explained the most variability in brain age were somatic depression symptoms and antidepressant use (Han et al., 2021). Kang et al. (2021), found no significant difference in TL and mitochondrial DNA concentration between individuals with OCD with medication use and non-users, in males or females (Kang et al., 2021).

2.3.9.3. Lifestyle factors and somatic health status

Studies suggest that TL is associated with health and lifestyle factors (Patel et al., 2017; Rehkopf et al., 2016). Jergovic et al. (2014) found that smoking, sex, and age predicted TL in PTSD, but depression and antidepressants were not as important. The study found that individuals with PTSD who were current smokers (n=15) had shorter TL than non-smokers (n=12). Prelog et al. (2016) did not find an association between panic disorder and TL but found significantly shorter TL in smokers compared to non-smokers across individuals. Roberts et al. (2017) found that women with PTSD had higher BMI, however, lifestyle factors did not affect the association between PTSD and TL. In Yang et al. (2020), the PTSD group had a higher BMI and percentage of smokers compared to HCs, with both BMI and smoking associated with accentuated epigenetic age, even after controlling for DNAmPACKYRS (surrogate variable used in the development of the epigenetic clock, built on smoking data). Han et al. (2021) found that greater brain age was associated with higher BMI and the number of current somatic diseases under medical treatment in all individuals (heart disease, epilepsy, diabetes, osteoarthritis, cancer, stroke, intestinal disorders, ulcers, and lung-, liver-, and thyroid disease). No significant association was found between brain age and smoking or alcohol use in individuals with anxiety disorder or MDD, compared to HCs. No significant association was found in biological stress variables (inflammatory markers, ANS, and HPA axis) in all individuals (Han et al., 2021).

2.4. Discussion

In this systematic review, we investigated the association between anxiety disorders and a wide range of biological indicators of accentuated aging. We identified 14 studies reporting an association between anxiety disorder and TL, DNA methylation, and brain age. The majority of our studies investigated TL in PTSD, with fewer findings on other anxiety disorders and other indicators of biological aging. We observed partial evidence for an association between PTSD and TL, as mixed findings were observed in the literature. Both DNA methylation and brain age metrics were sensitive to diagnosis with anxiety disorders, though the extent of the association observed depended on the type of epigenetic clock and machine learning algorithm used, and whether or not the study adjusted for confounders. In some reports, the biological indicator of aging was significantly influenced by medication use and lifestyle factors like smoking, whilst some studies did not find an association. Based on these studies, the general impression is that anxiety disorders are associated with an increased risk of accentuated aging, depending on confounders, however, we note that most of these publications were cross-sectional, and these results need further longitudinal investigation to help establish causality.

Telomere length in anxiety disorders

A majority of the studies focused on the relationship between PTSD and TL. The predominant findings support the idea of accentuating cellular aging in PTSD, although this association is not unanimous across all the studies. While there are neurobiological theories to support accentuating cellular aging in PTSD (Wolf et al., 2018), the small number of identified studies, and the varying methodological approaches used (e.g., trauma indices, statistical modelling) by each study limits our ability to draw a conclusion on the nature of the association.

A meta-analysis investigating PTSD and accentuated aging (included two of the studies from the present review; Jergović et al., 2014; O’Donovan et al., 2011) indicated shorter leukocyte TL in PTSD (medium effect sizes; pooled Cohen’s d=0.76) (Lohr et al., 2015). Another meta-analysis on TL in psychiatric disorders (included Jergović et al., 2014; O’Donovan et al., 2011) found significantly shorter leukocyte TL across all psychiatric disorders including PTSD. Subgroup analysis found that PTSD effect sizes were statistically significant, and that PTSD had a large effect size (adjusted Hedge $g=-1.27$) whereas depressive and anxiety disorders had medium effect sizes (adjusted Hedge $g=-0.55$ and -0.53, respectively) (Darrow et al., 2016). A study of TL in PTSD found no association between childhood trauma and TL; however, PTSD symptoms were associated with longer TL (Boks et al., 2015), which is similar to some cross-sectional findings (Küffer et al., 2016). Despite the findings of longer TL in PTSD, we note that in Küffer et al. (2016) TL was assessed from buccal cells, unlike Boks et al. (2015)

(and other studies in Darrow et al., 2016) who extracted TL from whole blood cells, and studies suggest that TL may differ by cell type (Lin et al., 2016).

Some possible explanations for the mixed findings in TL in PTSD are that 1) while there is some evidence that suggests that females have a higher lifetime prevalence of PTSD than males (Tolin & Foa, 2006; Zoladz & Diamond, 2013), only two of publications identified in present systematic review included females (O'Donovan et al., 2011; Roberts et al., 2017), 2) the majority of the studies used combat trauma-related PTSD and it is unclear whether the findings are relevant to civilian PTSD, 3) some of the studies indexed trauma exposure (high vs low trauma), and only found an association between TL and PTSD in exposure to high trauma (Kang et al., 2020). Given these mixed findings, it remains unclear whether the suggested biological aging observed in PTSD stems from the trauma itself or is a consequence of the disease (Roberts et al., 2017). Some studies suggest that TL shortening is a predisposing risk factor for the development of PTSD (Malan et al., 2011), whilst others suggest that TL shortening is a consequence of trauma or the onset of PTSD (Ladwig et al., 2013; O'Donovan et al., 2011). The mixed findings may also reflect the heterogeneity in the stress response and the interplay between stress, behavioural, and lifestyle factors (Higgins-Chen et al., 2021). Moreover, although most of the studies investigating TL identified in this present review used the same technique (qPCR) to measure TL, studies suggest that TL may vary according to the type of DNA extraction method used (Cunningham et al., 2013). For example, Cunningham et al. (2013) found that TL differed according to Phenol/chloroform extraction (mean TL=0.78) compared with PureGene-extracted DNA (mean TL=0.75). Additionally, the range of TL measured by qPCR from QIAamp-extracted DNA (0.17–0.58) was less than PureGene or phenol/chloroform (ranges, 0.04–2.67 and 0.32–2.81, respectively) (Cunningham et al., 2013). Therefore, the inconsistent findings reported for TL in PTSD may be partly attributable to differences in methodological protocols between studies (Turner et al., 2019).

Human studies found that shorter leukocyte TL was related to age-related diseases, and cardiovascular diseases in particular (Haycock et al., 2014). Additionally, a longitudinal study of leukocyte TL in the adult population (3259 individuals with a median age of 69 years and age range of 50 to 98 years at the time of blood collection) found that leukocyte TL was associated with increased risk of mortality from noncancer causes (Arbeev et al., 2020). On the contrary, longer leukocyte TL has been shown to increase the risk of developing site-specific cancers such as gliomas and melanomas (Haycock et al., 2017) as well as lung adenocarcinoma (Zhang et al., 2015). Given the above implications of TL attrition, further investigation is required to fully elucidate the role of telomere biology in the pathogenesis of aging and anxiety.

DNA methylation in anxiety disorders

Except for Verhoeven et al. (2018), all of the studies identified in the present systematic review found accelerated DNA methylation profiles in PTSD compared to controls (Katrinli et al., 2020; Kuan et al., 2021; Yang et al., 2020). Using the Horvath method, Verhoeven et al. (2018) contradicted these findings as significantly less epigenetic aging was found in male combat-exposed war veterans with PTSD than those without PTSD. These differences remained significant even after adjusting for possible confounders (race, BMI, smoking, alcohol use, and cell type proportion), and only became non-significant when adjusting for antidepressant use suggesting that medication may have likely influenced this relationship. These results were difficult to interpret because they were contradictory to the literature and it was unclear which biological mechanism was associated with DNA methylation (Verhoeven et al., 2018).

In the 3 cross-sectional studies, the DNA methylation age was highly correlated with chronological age, suggesting that the prediction model fit the data well (Katrinli et al., 2023: R=0.82, $p<2.2^{-16}$; Kuan et al., 2021: R>0.8, p<0.01; Verhoeven et al., 2018: R=0.81, p<0.001). In Kuan et al. (2021) all of the 4 DNA methylation clocks were highly correlated with each other (R>0.8, p<0.01) suggesting that the models yielded similar results (Kuan et al., 2021). However, whilst Katrinli et al. (2020) found that GrimAge estimates correlated with the DNA methylation clocks, the degree of correlation between the residuals was lower with Horvath (R=0.07, p=0.04) and Hannum (R=0.18, $p=1.66^{-9}$), suggesting that GrimAge may have been more sensitive than the other models (Katrinli et al., 2020).

Despite the high correlation between the prediction models and the chronological age, due to the small number of studies identified by the present systematic review and the discrepancies among the study findings, it is difficult to conclude whether anxiety and related disorders are associated with increased epigenetic aging. The inconsistent findings may underscore the nuanced relationship between PTSD and biological aging and the significance of the type of epigenetic clock used in each study. Meta-analysis focusing on PTSD and DNA methylation age (including 9 cohorts, combined n= 2186) found that PTSD severity, childhood trauma, sex, and white blood cell counts were associated with increased epigenetic age suggesting that trauma and the immune system may contribute to biological aging in PTSD (Wolf et al., 2018). Of note, associations between traumatic stress and increased epigenetic age were observed using the Hannum epigenetic age algorithm, but not the Horvath epigenetic calculator indicating the epigenetic clocks may be capturing differential aging patterns (Wolf et al., 2018).

Katrinli et al. (2020) found an association between PTSD and epigenetic age using the Horvath method, Yang et al. (2020) found an association using the GrimAge and Horvath but not Hannum, and Kuan et al. (2021) found an association with GrimAge but not Horvath or Hannum. Differences between epigenetic age estimates produced by these algorithms should perhaps be expected, given how methodological divergent they are; for instance, the Hannum algorithm was developed using 71 CpGs in leukocytes from whole blood cells)(Hannum et al., 2013), the Horvath algorithm used 353 CpGs from multiple tissues)(Horvath, 2015), and GrimAge was built from seven DNA methylation surrogates as well as CpGs associated with smoking and plasma proteins (Lu et al., 2019). It is unclear which mechanism of cellular aging, amongst HPA axis reactivity, oxidative stress (Lohr et al., 2015), or inflammation (Miller & Sadeh, 2014), is associated with the reported findings of epigenetic aging in PTSD in the present review. Wolf et al. (2018) found differing age residuals (not correlated with chronological age) between the Hannum and Horvath algorithms indicating differential patterns of association with the white blood cells in this study. Taken together, these findings imply that though epigenetic clocks are powerful predictors of accelerated aging (Jylhävä et al., 2017), it is likely that epigenetic clocks are predicting a wide spectrum of aging outcomes (Hannum et al., 2013; Horvath, 2015; Wolf et al., 2018).

For biomarker purposes (Johnson, 2006), it may not be feasible to estimate the epigenetic age of an individual in a way that generalizes across tissue types, given that the rate of epigenetic aging may be tissue-specific (Jylhävä et al., 2017). Additionally, it remains unclear if DNA methylation is the primary driver of age-related changes in the body or if it is an indicator of other occurring genomic mechanisms. Given the inconsistent findings in the present review, further cross-sectional and longitudinal studies that contrast the different epigenetic clocks in a head-to-head fashion are required to compare the predictability of the clocks in anxiety disorders and related disorders. Additionally, further investigation is needed to ascertain whether DNA methylation contributes to the pathology of anxiety disorder, or if the disorder drives DNA methylation or a combination of both.

Brain age in anxiety disorders

Few studies have evaluated the relationship between brain age and anxiety disorders, compared to other psychiatric disorders, like mood disorders, schizophrenia, and psychosis (Sone & Beheshti, 2022). Our search identified single studies that investigated brain aging in PTSD (Clausen et al., 2021) and combined anxiety disorders (Han et al., 2021). Clausen et al. (2021) found higher brain-PAD in young males with PTSD compared to age-matched male HCs and lower brain age in old males with PTSD compared to male HCs of all ages. These findings suggest that the association between PTSD and brain age may be mediated by an interaction between chronological age, sex, and diagnosis. On the other hand, Han et al. (2021), examined the association between brain age and combined anxiety disorders (not including PTSD) and found that individuals with anxiety disorders had significantly higher brain-

PAD compared to HCs across the entire age range of the sample, but only after correcting for antidepressant use. Taken together, these studies suggest that the relationship between anxiety disorders and brain age is modulated by demographic and clinical characteristics.

In both of the brain age studies, the correlation between the predicted age and chronological age was high, and the mean absolute error (MAE) was moderate, suggesting that the brain age model was a good fit for data that it had not been trained on (Clausen et al., 2021: R=0.72, MAE=5.68 years; Han et al., 2021: R=0.72, MAE=6.73 in the patient group). Han et al. (2021) suggested that brain-PAD was not specific to depression or anxiety, but rather an overall indicator of the impact of psychiatric disorders on the brain, under the influence of confounding variables. Similarly, the findings presented by Clausen et al. (2021) of higher brain-PAD in younger individuals with PTSD than older individuals with PTSD indicate that brain-PAD may not be a specific marker of brain aging in the context of PTSD (Clausen et al., 2021). In previous research, PTSD is associated with higher brain-PAD in children and adolescents (aged between 8 years and 21 years old), albeit in relatively small samples (n=70), and without correction for medication use (Liang et al., 2019). The evidence of brain-PADs in younger individuals than older individuals may be suggestive of a critical period where brain aging may occur in PTSD (Clausen et al., 2021). In anxiety disorders including specific phobia (n=70), social phobia(n=77), PTSD (n=44), and depression(n=70)) brain-PAD was associated with differential regional brain age patterns (brain regions grouped in clusters) in children mean age 14.23 years old (Niu et al., 2022).

The influence of clinical characteristics, lifestyle factors, and somatic health status on biological aging

In the present systematic review, mixed findings were observed in the association between psychiatric comorbidity (i.e., comorbid MDD) and biological aging. Most studies reported negative findings (Jergović et al., 2014; Weiner et al., 2014; Yang et al., 2020) whilst Han et al. (2021) found higher brain age in MDD/anxiety comorbidity (the group was combined), however, these results were marginally significant (p_{FDR}=0.08) (Han et al., 2021). It is unclear how psychiatric comorbidity may influence biological aging. While one may speculate that comorbidity would have a cumulative effect on brain age (Sone et al., 2019), there is very little evidence in the literature to substantiate this speculation. Of note, the lack of an association between brain age and variables including childhood trauma history, age of onset of illness, and duration of symptoms reported by Han et al. (2021) implies that brain age may be more associated with the current disease state than the combination of the different variables (Han et al., 2021).

The influence of medication status on biological aging

Han et al. (2021) found lower brain age with antidepressant use in anxiety disorders, suggesting that antidepressants may have had a protective effect on the brain. Similarly, Verhoeven et al. (2018) found that lower epigenetic aging was influenced by antidepressant use in PTSD. Some possible explanations for these findings are 1) antidepressant treatment has been shown to increase serum levels of brain-derived neurotrophic factor (Molendijk et al., 2011), which is essential in maintaining synaptic plasticity, and mediates the relationship between inflammation and neuroplasticity (Gao et al., 2022), 2) in animal models, antidepressants (i.e., tricyclics, imipramine, and SSRI paroxetine) have been shown to reduce DNA methylation (Zimmermann et al., 2012), and 3) in human cells, mood stabilizers have also been shown to modulate DNA methylation levels (Detich et al., 2003). However, while antidepressants are suggested to have some epigenetic effects (Menke & Binder, 2014), it is unlikely that this relationship is casual. It is more likely that the relationship is complex and may be modulated by other factors including symptom severity, comorbidity, and lifestyle factors (Han et al., 2021; Verhoeven et al., 2018).

Both Prelog et al. (2016) and Jergovic et al. (2014) found shorter TL in individuals with PTSD who smoked. This is perhaps to be expected, given that TL is influenced by a variety of factors including physical activity (Arsenis et al., 2017), obesity, chronic diseases, sex, and ethnicity (see Table 2 in Turner et al., 2019). Although both studies had small sample sizes (n>39, n=15, respectively), their findings are consistent with a meta-analysis reporting shorter TL in smokers compared to non-smokers, and in current smokers compared to never or former smokers (Astuti et al., 2017). Whilst smoking is one of the markers that is included in the DNA methylation GrimAge calculator (represented by DNAmPACKYRS to estimate smoking history, and the cumulative effect of smoking as "packs per year" (Horvath, 2015). DNAmPACKYRS also provides additional information about aging outside of smoking-related factors since the DNA methylation sites that are associated with smoking are also related to various aspects of aging (Lu et al., 2019). For example, methylation of cg05575921 (codes for aryl hydrocarbon receptor repressor (AHRR)) is not only related to smoking but to inflammation and cell differentiation (Stejskalova et al., 2011). It is plausible that epigenetic aging reflects a variety of age-related factors that are associated with overall health, however since aging is a complex biological process further investigation is required.

Han et al. (2021) did not find an association between smoking and brain age in anxiety disorders, these findings were contradictory to Cole 2020 who found an association between brain-PAD (+2.1 years brain-PAD) and current smoking in the UK BIOBANK dataset (n>14,000 individuals) (Cole, 2020). Cole et al. (2020) reported that the effect sizes were generally small, suggesting that the failure to detect the effect of smoking in Han 2021 could have been due to insufficient power. In Cole et al. (2020), brain-PADs in smokers were smaller than those associated with disorders such as ADs (+10 years) or multiple sclerosis (+11 years) (Cole et al., 2019; Franke et al., 2010), suggesting that the association between smoking and brain age should not be overstated (Cole, 2020). Given that brain age studies are still in their infancy, more studies are required to investigate the influence of lifestyle factors on biological aging in the brain.

General discussion

Taken together the overall findings presented in this systematic review suggest that anxiety disorders are associated with various levels of biological aging as indexed by TL, epigenetic aging, and brain aging. Of note, while not all of the studies reported effect sizes, Han et al. (2021) reported effect sizes in brain age in anxiety disorders (d=0.34) that were slightly higher or equal to indicators of biological aging including TL in MDD (Verhoeven et al., 2014; d=0.12) and current anxiety disorders (Verhoeven et al., 2015; d=0.12), epigenetic aging in MDD (Han et al., 2018; d=0.14), but not epigenetic aging in PTSD (Verhoeven et al., 2018; d=0.42). This suggests that compared to other indicators of biological aging, there was a stronger association between brain age and anxiety disorders. The degree to which the indicators of biological aging are correlated with one another still needs to be determined. Cole et al. (2018) found that when coupled together (DNA methylation estimates and brain age), the models' prediction of risk of mortality was improved (Cole et al., 2018), suggesting that each method explains unique variability in the outcome. However, brain age prediction and DNA methylation were not correlated in this study, and brain age was a better predictor of mortality than DNA methylation-based age prediction (Cole et al., 2018). Indeed, a multimodal approach may be more informative as there is some evidence, though limited, that as TL becomes shorter, chromosome looping, and gene expression patterns change indicating that there is a connection between TL and DNA methylation in certain cell types (Buxton et al., 2014). In DNA from EBV-transformed cell lines, Buxton et al. (2014), found 65 promoter genes concentrated with CpG sites (Cytosine-phosphate-Guanine) where methylation level was associated with leukocyte TL and 36 promoter genes concentrated with CpG sites where methylation levels were associated with TL in DNA (EBV-transformed cell lines) (Buxton et al., 2014). Han et al. (2021) did not find a significant correlation between brain-PAD, epigenetic, transcriptomic, metabolomic clocks, and TL (R range between 0.03–0.15) (Han et al., 2021). This is in line with findings from Katrinli et al. (2023) where no association was found between GrimAge and brain age in individuals with lifetime PTSD (Katrinli et al., 2023). Of note, while studies show that aging in the brain is associated with several factors in addition to cellular changes, including increased DNA damage, a build-up of reactive oxygen species, mitochondrial dysregulation, inflammation (Yankner et al., 2008), these processes are cell-specific (Erraji-Benchekroun et al., 2005), and it is unclear if these processes are reflected throughout the body (Jylhävä et al., 2017). Even when measuring biological aging in the peripheral body (i.e., using blood markers), studies demonstrate a weak association (R<0.2) between TL, epigenetic, transcriptional, proteomic, and metabolomic aging (Jansen et al., 2021), suggesting that the current methods of tracking biological aging are measuring distinctive processes of aging, even if they may be associated with the same lifestyle, demographics, and somatic health factors.

2.5. Limitations

One limitation of the studies presented in this review is that the studies differed in clinical characteristics, especially in PTSD (i.e., diagnosis vs HCs or diagnosis vs trauma-exposed controls). In PTSD, the level of trauma/trauma index varied amongst studies, making it difficult to account for the potential influence of trauma on the findings. This is of importance as studies suggest that biological changes that occur in military war veterans may be associated with trauma regardless of PTSD diagnosis (De Kloet et al., 2007). Besides PTSD, there were very few findings of aging in other anxiety disorders including SAD (Han, et al., 2021; Verhoeven et al., 2015) and panic disorder (Prelog et al., 2016), and those studies that did focus on these disorders often combined the groups. Whilst this may have been done to overcome small sample sizes, it was difficult to ascertain which disorder contributed to the biological aging, if any. Due to the prevalence of studies on the association between PTSD and TL, the findings reported in the present systematic review were skewed towards one disorder and one measure of biological aging. Lastly, only a few studies included additional covariates that would be informative for biological mechanisms, such as immune markers (i.e., CRP, IL-6, IL-1b, and TNF) (Jergović et al., 2014; Prelog et al., 2016), which would have helped determine the extent to which inflammatory pathways mediated biological aging (Cribbs et al., 2012; Norden & Godbout, 2013; Xia et al., 2017).

2.6. Conclusion

The present review summarized findings in the current literature on indicators of biological aging in individuals diagnosed with anxiety disorders. Only a few studies were identified, with very little research on biological aging in OCD, while SAD was combined with other anxiety disorders in most studies. Overall, the findings of epigenetic aging or TL presented in this review were inconsistent, specifically for PTSD. These inconsistencies, reflect what is observed in the larger literature (Wolf et al. 2018) and may be due to differences between studies in methodology, sample size, and clinical characteristics (types and level of trauma), as well as the fact that epigenetic clocks may represent biological aging from various cellular levels. Both of the brain age studies identified in the present review suggest accentuated brain aging in anxiety disorders (Han et al., 2021) and PTSD (Clausen et al., 2021), however these findings seem to be modulated by medication status, sex, and chronological age. Taken together the findings suggest that there is an association between anxiety disorders and different types of biological aging. Further longitudinal and large-scale evidence is required to elucidate whether brain aging is correlated to somatic aging and whether biological aging is a response to or a pre-existing factor of anxiety disorders.

2.7. Tables and figures

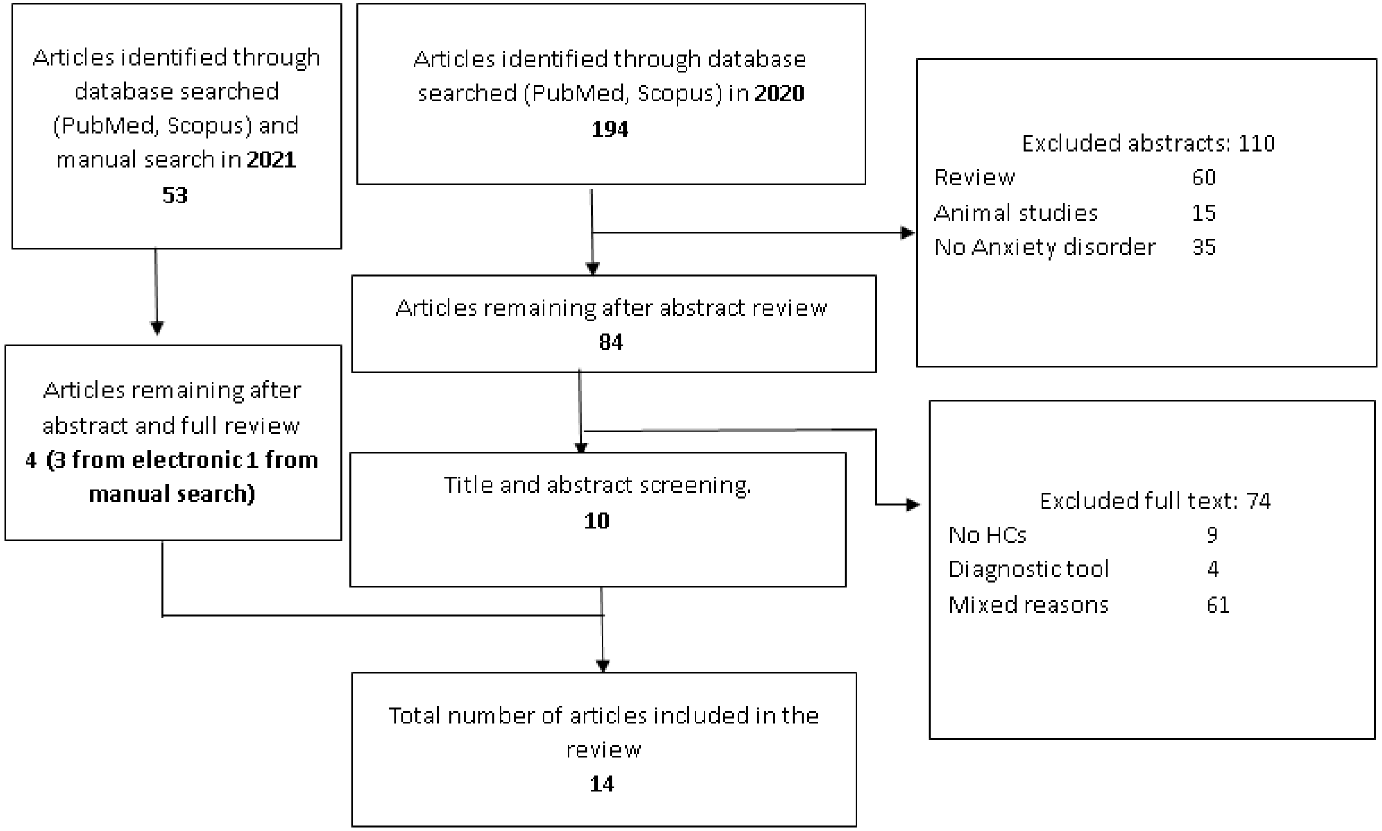

Figure 2.1: PRISMA flow diagram for studies included in the systematic review

Table 2.1: Study characteristics for systematic review

First Author and Year	Study design	Sample description	Pychiatric disorder	Biomarker of aging	Main methods/assays	n	Mean age (SD): control	Mean age (SD): patients	Main findings
Clausen et al. (2021)	Cross sectional	ENIGMA-PGC	PTSD	BrainPAD	BrainAgeR	2229	35,4 (11,0); 1347	35,9 (10,9); 884	young males with PTSD exhibited higher brain-PAD relative to male HCs in young and old age groups; old males with PTSD exhibited lower brain PAD compared to male HCs of all ages.
Han et al. (2021)	Cross sectional	NESDA	MDD and Anxiety disorder	BrainPAD	Photon-ai	285	40,81 (9,78)	37,37 (10,2)	brain-PAD: MDD (+2.78 years, d=0.25) and anxiety patients (d=0.27), compared with HCs, adjusting for antidepressants
Jergovic et al. (2014)	Cross sectional	Outpatients; Dr. Josip Benčević, General Hospital, Slavonski Brod, Croatia	PTSD	TL; telomerase activity	qPCR	47	47.2 (1.71); 17; no data provided for elderly HCs	45.9 (1.12); 30	PTSD shorter TL (0.86 ± 0.031, N = 28) than HCs (1.03 ± 0.041, N = 17)
Kang et al. (2021)	Cross sectional	OCD-specialized outpatient clinic; Severance Hospital, Yonsei University Health System	OCD	TL; mitochondrial DNA content	qPCR	469	27,23 (7,72); n = 234	27,7 (7,55); n = 235	female with OCD shorter TL compared to HCs. fe(male) reduced mtDNAcn compared to HCs.
Kang et al. (2020)	Cross sectional	Department of Defense– funded Systems Biology of PTSD Study	PTSD	TL	qPCR	213	33,17 (8,69); 111	33,66 (8,17); 102	PTSD shorter TL and larger amygdala volume in individuals with non-PTSD
Katrinli et al. (2020)	Cross sectional	GTP	PTSD	DNA methylation	GrimAge; Hovarth	854	42,8 (12,7); n = 427	Current PTSD: 40,93 (11,43); n = 218; Lifetime PTSD: 43,3 (11,8); n = 209	GrimAge acceleration differed significantly between current PTSD and HCs (t=2.25, p 0.02).

Kim et al. (2017)	Cross sectional	VHS, Medical Center, South Korea	PTSD	TL	qPCR	242	62,94 (4,41); n = 120	62,99 (3,4); n = 122	PTSD no difference in TL between PTSD and non-PTSD groups
Kuan et al. (2021)	Cross sectional	Stony Brook WTC Health program	PTSD	DNA methylation; transcritional age	Horvath; Hannum; DNAm age; PhenoAge	324	n = 201	current PTSD n = 81; Past PTSD n = 42	Among the four DNAm age predictions, only GrimAge age acceleration was significantly higher in the current PTSD group than the never PTSD group (β=0.335, p=0.0097)
Odonovan et al. (2011)	Cross sectional	Community/clinics	PTSD	TL	qPCR	88	Men: 30.7 (8.7); 21; Women: 30.6 (8.1); 25; N = 46	Men: 30.9 (6.4); 22; Women: 29.4 (6.1); 20; N = 42	PTSD shorter TL than HCs.
Prelog et al. (2016)	Cross sectional	Department of Psychiatry and Psychotherapy, University of Muenster, Germany	Panic disorder	TL	qPCR	258	W 36.8 ± 10.9, n = 85; Men 34.1 ± 10.8, n = 44	w36.9 ± 10.8, n = 85; Men 34.1 ± 11.7, n = 44	Panic disorder no difference in TL compared to HCs
Roberts et al. (2017)	Cross sectional	Nurses' Health Study II	PTSD	TL	qPCR	116	45.5 (3.6), n = 25	PTSD: 46.6 (3.9), n = 25; subclinical PTSD: 46.6 (3.7), n = 66	civiclian women with PTSD shorter TL (*β* = −.112, 95% confidence interval (CI) = −0.196, −0.028) compared to non-PTSD
Verhoeven et al . (2015)	Cross sectional	NESDA	Anxiety disorder	TL	qPCR	2324	41,7 (14,8); n = 582	Remitted: 43,6 (12,7); n = 459; current: 41,3 (12,4); n= 1283	Patients in the current anxiety group (bp=5431) had shorter LTL compared to HCs (bp=5506, P=0.01) and the remitted anxiety group (bp=5499, P=0.03)
Verhoeven et al. (2018)	Cross sectional	Mental Health Services of the Manhattan, Bronx and Brooklyn Veterans Affairs Medical Centers	PTSD	TL; telomerase activity; DNA methylation	PCR; Horvath	160	32.6 (8.0); n = 81	33.0 (7.8); n = 79	PTSD had less epigenetic age (age=3.2) compared to those without PTSD (age=5.0; p=0.02; d=0.42)

Yang et al. (2020)	Longitudinal	Veterans from Operation Enduring Freedom (OEF) and/or Operation Iraqi Freedom (OIF) conflicts	PTSD	DNA methylation	GrimAge	268	Discovery PTSD-; 32.52 (8.03); n = 82; Validation PTSD-: 33.96 (9.35); n = 27; Recall PTSD-: 35.13 (7.47); n = 30	Discovery PTSD+:32.67 (7.35); n = 80; Validation PTSD+; 36.85 (10.24); n = 26; Recall PTSD+: 33.00 (7.47); n = 14;	In both cohorts, AgeAccelGrim was significantly higher in the PTSD compared HCs (n=162, 1.26 vs −0.57, p=0.001 and n=53, 0.93 vs −1.60 Years, p = 0.008)

2.8. References

Abbott, B. Y. A. (2010). *Artigo Revista - Alzheimer’S Disease Outlook*.

Ahmed-Leitao, F., Rosenstein, D., Marx, M., Young, S., Korte, K., & Seedat, S. (2019a). Posttraumatic stress disorder, social anxiety disorder and childhood trauma: Differences in hippocampal subfield volume. *Psychiatry Research - Neuroimaging*, *284*, 45–52. https://doi.org/10.1016/j.pscychresns.2018.12.015

Ahmed-Leitao, F., Rosenstein, D., Marx, M., Young, S., Korte, K., & Seedat, S. (2019b). Posttraumatic stress disorder, social anxiety disorder and childhood trauma: Differences in hippocampal subfield volume. *Psychiatry Research - Neuroimaging*, *284*(December 2018), 45–52. https://doi.org/10.1016/j.pscychresns.2018.12.015

Amunts, K., Kedo, O., Kindler, M., Pieperhoff, P., Mohlberg, H., Shah, N. J., Habel, U., Schneider, F., & Zilles, K. (2005). Cytoarchitectonic mapping of the human amygdala, hippocampal region and entorhinal cortex: Intersubject variability and probability maps. *Anatomy and Embryology*, *210*(5–6), 343–352. https://doi.org/10.1007/s00429-005-0025-5

Andersen, S. L., & Teicher, M. H. (2004). Delayed effects of early stress on hippocampal development. *Neuropsychopharmacology*, *29*(11), 1988–1993. https://doi.org/10.1038/sj.npp.1300528

Andersen, S. L., Tomada, A., Vincow, E. S., Valente, E., Polcari, A., & Teicher, M. H. (2008). Preliminary Evidence for Sensitive Periods in the Effect of Childhood Sexual Abuse on Regional Brain Development. *Journal of Neuropsychiatry*, *20*(3), 292–301. https://doi.org/10.1176/appi.neuropsych.20.3.292

Angermueller, C., Pärnamaa, T., Parts, L., & Stegle, O. (2016). Deep learning for computational biology. *Molecular Systems Biology*, *12*(7), 878. https://doi.org/10.15252/msb.20156651

APA. (2013). Diagnostic and statistical manual of mental disorders: DSM-5^{TM}, 5th ed. In *Diagnostic and statistical manual of mental disorders: DSM-5^{TM}, 5th ed.* American Psychiatric Publishing, Inc. https://doi.org/10.1176/appi.books.9780890425596

Armstrong, N. M., An, Y., Beason-Held, L., Doshi, J., Erus, G., Ferrucci, L., Davatzikos, C., & Resnick, S. M. (2019). Sex differences in brain aging and predictors of neurodegeneration in cognitively healthy older adults. *Neurobiology of Aging*, *81*, 146–156. https://doi.org/10.1016/j.neurobiolaging.2019.05.020

Article, O. (2004). *Mapping Structural Brain Alterations in Obsessive-Compulsive Disorder*. *61*(July).

Asami, T., Nakamura, R., Takaishi, M., Yoshida, H., Yoshimi, A., Whitford, T. J., & Hirayasu, Y. (2018). Smaller volumes in the lateral and basal nuclei of the amygdala in patients with panic disorder. *PLoS ONE*, *13*(11), 1–8. https://doi.org/10.1371/journal.pone.0207163

Assunção, M. C., Da Conceição Costa, D. L., Mathis, M. A. De, Gedanke Shavitt, R., Arzeno Ferrão, Y., Rosário, M. C. Do, Constantino Miguel, E., & Rodrigues Torres, A. (2012). Social phobia in obsessive-compulsive disorder: Prevalence and correlates. *Journal of Affective Disorders*, *143*(1–3), 138–147. https://doi.org/10.1016/j.jad.2012.05.044

Atmaca, M., Ozdemir, H., Ozler, S., Kara, B., Ozler, Z., Kanmaz, E., Mermi, O., & Tezcan, E. (2008). *Progress in Neuro-Psychopharmacology & Biological Psychiatry Hippocampus and amygdalar volumes in patients with refractory obsessive – compulsive disorder*. *32*, 1283–1286. https://doi.org/10.1016/j.pnpbp.2008.04.002

Avecillas-Chasin, J. M., Levinson, S., Kuhn, T., Omidbeigi, M., Langevin, J. P., Pouratian, N., & Bari, A. (2023). Connectivity-based parcellation of the amygdala and identification of its main white matter connections. *Scientific Reports*, *13*(1), 1–10. https://doi.org/10.1038/s41598-023-28100-6

Baecker, L., Garcia-Dias, R., Vieira, S., Scarpazza, C., & Mechelli, A. (2021). Machine learning for brain age prediction: Introduction to methods and clinical applications. *EBioMedicine*, *72*, 103600. https://doi.org/10.1016/j.ebiom.2021.103600

Baksh, R. A., Ritchie, C. W., Terrera, G. M., Norton, J., Raymont, V., & Ritchie, K. (2021). The association between anxiety disorders and hippocampal volume in older adults. *Psychology and Aging*, *36*(2), 288–297. https://doi.org/10.1037/pag0000597

Baldwin, D. S., Brandish, E. K., & Meron, D. (2008). The Overlap of Obsessive-Compulsive Disorder and Social Phobia and its Treatment. *CNS Spectrums*, *13*(S14), 47–53. https://doi.org/10.1017/S1092852900026936

Ballester, P. L., Romano, M. T., de Azevedo Cardoso, T., Hassel, S., Strother, S. C., Kennedy, S. H., & Frey, B. N. (2022). Brain age in mood and psychotic disorders: a systematic review and meta-analysis. *Acta Psychiatrica Scandinavica*, *145*(1), 42–55. https://doi.org/10.1111/acps.13371

Ballester, P. L., Suh, J. S., Nogovitsyn, N., Hassel, S., Strother, S. C., Arnott, S. R., Minuzzi, L., Sassi, R. B., Lam, R. W., Milev, R., Müller, D. J., Taylor, V. H., Kennedy, S. H., & Frey, B. N. (2021). Accelerated brain aging in major depressive disorder and antidepressant treatment response: A CAN-BIND report. *NeuroImage: Clinical*, *32*. https://doi.org/10.1016/j.nicl.2021.102864

Bandelow, B. (2008). Funding for this academic supplement has been provided by an educational grant from Jazz Pharmaceuticals . Faculty Affiliation and Disclosures Nonpharmacologic Treatments. *CNS Spectr*, *9*(Suppl 14), 37–46.

Bandelow, B., Baldwin, D., Abelli, M., Altamura, C., Dell'Osso, B., Domschke, K., Fineberg, N. A., Grünblatt, E., Jarema, M., Maron, E., Nutt, D., Pini, S., Vaghi, M. M., Wichniak, A., Zai, G., & Riederer, P. (2016). Biological markers for anxiety disorders, OCD and PTSD – a consensus statement. Part I: Neuroimaging and genetics. *World Journal of Biological Psychiatry*, *17*(5), 321–365. https://doi.org/10.1080/15622975.2016.1181783

Bannon, S., Gonsalvez, C. J., Croft, R. J., & Boyce, P. M. (2002). Response inhibition deficits in obsessive-compulsive disorder. *Psychiatry Research*, *110*(2), 165–174. https://doi.org/10.1016/S0165-1781(02)00104-X

Bas-Hoogendam, J. M., Groenewold, N. A., Aghajani, M., Freitag, G. F., Harrewijn, A., Hilbert, K., Jahanshad, N., Thomopoulos, S. I., Thompson, P. M., Veltman, D. J., Winkler, A. M., Lueken, U., Pine, D. S., van der Wee, N. J. A., Stein, D. J., Agosta, F., Åhs, F., An, I., Alberton, B. A. V., … Zwiebel, H. L. (2022). ENIGMA-anxiety working group: Rationale for and organization of large-scale neuroimaging studies of anxiety disorders. *Human Brain Mapping*, *43*(1), 83–112. https://doi.org/10.1002/hbm.25100

Bas-Hoogendam, J. M., van Steenbergen, H., Nienke Pannekoek, J., Fouche, J. P., Lochner, C., Hattingh, C. J., Cremers, H. R., Furmark, T., Månsson, K. N. T., Frick, A., Engman, J., Boraxbekk, C. J., Carlbring, P., Andersson, G., Fredrikson, M., Straube, T., Peterburs, J., Klumpp, H., Phan, K. L., … van der Wee, N. J. A. (2017). Voxel-based morphometry multi-center mega-analysis of brain structure in social anxiety disorder. *NeuroImage: Clinical*, *16*(May), 678–688. https://doi.org/10.1016/j.nicl.2017.08.001

Bayer, J. M. M., Thompson, P. M., Ching, C. R. K., Liu, M., Chen, A., Panzenhagen, A. C., Jahanshad, N., Marquand, A., Schmaal, L., & Sämann, P. G. (2022). Site effects how-to and when: An overview of retrospective techniques to accommodate site effects in multi-site neuroimaging analyses. *Frontiers in Neurology*, *13*. https://doi.org/10.3389/fneur.2022.923988

Beats, B., Levy, R., & Förstl, H. (1991). Ventricular enlargement and caudate hyperdensity in elderly depressives. *Biological Psychiatry*, *30*(5), 452–458. https://doi.org/10.1016/0006-3223(91)90306-7

Ben-zion, Z., Korem, N., Fine, N., Katz, S., Siddhanta, M., Funaro, C., Duek, O., Spiller, T. R., Danbock, S., Levy, I., & Harpaz-rotem, I. (2023). of. *Biological Psychiatry Global Open Science*. https://doi.org/10.1016/j.bpsgos.2023.07.001

Besnard, A., & Sahay, A. (2016). Adult Hippocampal Neurogenesis, Fear Generalization, and Stress. *Neuropsychopharmacology*, *41*(1), 24–44. https://doi.org/10.1038/npp.2015.167

Bethlehem, R. A. I., Seidlitz, J., White, S. R., Vogel, J. W., Anderson, K. M., Adamson, C., Adler, S., Alexopoulos, G. S., Anagnostou, E., Areces-Gonzalez, A., Astle, D. E., Auyeung, B., Ayub, M., Bae, J., Ball, G., Baron-Cohen, S., Beare, R., Bedford, S. A., Benegal, V., … Alexander-Bloch, A. F. (2022). Brain charts for the human lifespan. *Nature*, *604*(7906), 525–533. https://doi.org/10.1038/s41586-022-04554-y

Blake, K. V., Ntwatwa, Z., Kaufmann, T., Stein, D. J., Ipser, J. C., & Groenewold, N. A. (2023). Advanced brain ageing in adult psychopathology: A systematic review and meta-analysis of structural MRI studies. *Journal of Psychiatric Research*, *157*, 180–191. https://doi.org/10.1016/j.jpsychires.2022.11.011

Boedhoe, P. S. W., Schmaal, L., Abe, Y., Ameis, S. H., Arnold, P. D., Batistuzzo, M. C., Benedetti, F., Beucke, J. C., Bollettini, I., Bose, A., Brem, S., Calvo, A., Cheng, Y., Cho, K. I. K., Dallaspezia, S., Denys, D., Fitzgerald, K. D., Fouche, J.-P., Giménez, M., … Zhao, Q. (2017). Distinct Subcortical Volume Alterations in Pediatric and Adult OCD: A Worldwide Meta- and Mega-Analysis. *American Journal of Psychiatry*, *174*(1), 60–69. https://doi.org/10.1176/appi.ajp.2016.16020201

Bray, S., Chang, C., & Hoeft, F. (2009). Applications of multivariate pattern classification analyses in developmental neuroimaging of healthy and clinical populations. *Frontiers in Human Neuroscience*, *3*(OCT), 1–12. https://doi.org/10.3389/neuro.09.032.2009

Brühl, A. B., Delsignore, A., Komossa, K., & Weidt, S. (2014). Neuroimaging in social anxiety disorder-A meta-analytic review resulting in a new neurofunctional model. *Neuroscience and Biobehavioral Reviews*, *47*, 260–280. https://doi.org/10.1016/j.neubiorev.2014.08.003

Bruin, W. B., Taylor, L., Thomas, R. M., Shock, J. P., Zhutovsky, P., Abe, Y., Alonso, P., Ameis, S. H., Anticevic, A., Arnold, P. D., Assogna, F., Benedetti, F., Beucke, J. C., Boedhoe, P. S. W., Bollettini, I., Bose, A., Brem, S., Brennan, B. P., Buitelaar, J. K., … van Wingen, G. A. (2020). Structural neuroimaging biomarkers for obsessive-compulsive disorder in the ENIGMA-OCD consortium: medication matters. *Translational Psychiatry*, *10*(1). https://doi.org/10.1038/s41398-020-01013-y

Caldiroli, A., Capuzzi, E., Affaticati, L. M., Surace, T., Forti, C. L. Di, Dakanalis, A., Clerici, M., & Buoli, M. (2023). *Candidate Biological Markers for Social Anxiety Disorder : A Systematic Review*.

Canada, K. L., Saifullah, S., Gardner, J. C., Sutton, B. P., Fabiani, M., Gratton, G., Raz, N., & Daugherty,

A. M. (2023). Development and validation of a quality control procedure for automatic segmentation of hippocampal subfields. *Hippocampus*, *May*. https://doi.org/10.1002/hipo.23552

Cano, M., & Takeuchi, D. T. (2020). Recent/Childhood Adversities and Mental Disorders Among US Immigrants. *Frontiers in Psychiatry*, *11*(November), 1–19. https://doi.org/10.3389/fpsyt.2020.573410

Cao, L., Li, H., Liu, J., Jiang, J., Li, B., Li, X., Zhang, S., Gao, Y., Liang, K., Hu, X., Bao, W., Qiu, H., Lu, L., Zhang, L., Hu, X., Gong, Q., & Huang, X. (2022). Disorganized functional architecture of amygdala subregional networks in obsessive-compulsive disorder. *Communications Biology*, *5*(1), 1–10. https://doi.org/10.1038/s42003-022-04115-z

Carpita, B., Muti, D., Petrucci, A., Romeo, F., Gesi, C., Marazziti, D., Carmassi, C., & Dell'Osso, L. (2019). Overlapping features between social anxiety and obsessive-compulsive spectrum in a clinical sample and in healthy controls: Toward an integrative model. *CNS Spectrums*. https://doi.org/10.1017/S109285291900138X

Ching, T., Himmelstein, D. S., Beaulieu-Jones, B. K., Kalinin, A. A., Do, B. T., Way, G. P., Ferrero, E., Agapow, P. M., Zietz, M., Hoffman, M. M., Xie, W., Rosen, G. L., Lengerich, B. J., Israeli, J., Lanchantin, J., Woloszynek, S., Carpenter, A. E., Shrikumar, A., Xu, J., … Greene, C. S. (2018). Opportunities and obstacles for deep learning in biology and medicine. In *Journal of the Royal Society Interface* (Vol. 15, Issue 141). https://doi.org/10.1098/rsif.2017.0387

Christian, B., Boll, S., Gamer, M., & Gluth, S. (2013). *Separate amygdala subregions signal surprise and predictiveness during associative fear learning in humans*. *37*(November 2012), 758–767. https://doi.org/10.1111/ejn.12094

Cisler, J. M., Olatunji, B. O., & Lohr, J. M. (2009). Disgust, fear, and the anxiety disorders: A critical review. *Clinical Psychology Review*, *29*(1), 34–46. https://doi.org/10.1016/j.cpr.2008.09.007

Clausen, A. N., Fercho, K. A., Monsour, M., Disner, S., Salminen, L., Haswell, C. C., Rubright, E. C., Watts, A. A., Buckley, M. N., Maron-Katz, A., Sierk, A., Manthey, A., Suarez-Jimenez, B., Olatunji, B. O., Averill, C. L., Hofmann, D., Veltman, D. J., Olson, E. A., Li, G., … Morey, R. A. (2022). Assessment of brain age in posttraumatic stress disorder: Findings from the ENIGMA PTSD and brain age working groups. *Brain and Behavior*, *12*(1), 1–14. https://doi.org/10.1002/brb3.2413

Cole, J. H., & Franke, K. (2017a). Predicting Age Using Neuroimaging: Innovative Brain Ageing Biomarkers. *Trends in Neurosciences*, *40*(12), 681–690. https://doi.org/10.1016/j.tins.2017.10.001

Cole, J. H., & Franke, K. (2017b). Predicting Age Using Neuroimaging: Innovative Brain Ageing Biomarkers. *Trends in Neurosciences*, *40*(12), 681–690. https://doi.org/10.1016/j.tins.2017.10.001

Cole, J. H., Marioni, R. E., Harris, S. E., & Deary, I. J. (2019). Brain age and other bodily 'ages': implications for neuropsychiatry. *Molecular Psychiatry*, *24*(2), 266–281. https://doi.org/10.1038/s41380-018-0098-1

Cole, J. H., Ritchie, S. J., Bastin, M. E., Valdés Hernández, M. C., Muñoz Maniega, S., Royle, N., Corley, J., Pattie, A., Harris, S. E., Zhang, Q., Wray, N. R., Redmond, P., Marioni, R. E., Starr, J. M., Cox, S. R., Wardlaw, J. M., Sharp, D. J., & Deary, I. J. (2018). Brain age predicts mortality. *Molecular Psychiatry*, *23*(5), 1385–1392. https://doi.org/10.1038/mp.2017.62

Darrow, S. M., Verhoeven, J. E., Révész, D., Lindqvist, D., Penninx, B. W. J. H., Delucchi, K. L., Wolkowitz, O. M., & Mathews, C. A. (2016). The Association between Psychiatric Disorders and Telomere Length: A Meta-Analysis Involving 14,827 Persons. *Psychosomatic Medicine*, *78*(7), 776–787. https://doi.org/10.1097/PSY.0000000000000356

Davatzikos, C. (2019). Machine learning in neuroimaging: Progress and challenges. *NeuroImage*, *197*, 652–656. https://doi.org/10.1016/j.neuroimage.2018.10.003

de Flores, R., La Joie, R., & Chételat, G. (2015). Structural imaging of hippocampal subfields in healthy aging and Alzheimer's disease. *Neuroscience*, *309*(August), 29–50. https://doi.org/10.1016/j.neuroscience.2015.08.033

De Magalhães, J. P. (2012). Programmatic features of aging originating in development: Aging mechanisms beyond molecular damage? *FASEB Journal*, *26*(12), 4821–4826. https://doi.org/10.1096/fj.12-210872

Dell'Osso, B., Benatti, B., Hollander, E., Fineberg, N., Stein, D. J., Lochner, C., Nicolini, H., Lanzagorta, N., Palazzo, C., Altamura, A. C., Marazziti, D., Pallanti, S., Van Ameringen, M., Karamustafalioglu, O., Drummond, L. M., Hranov, L., Figee, M., Grant, J. E., Zohar, J., … Menchon, J. M. (2016). Childhood, adolescent and adult age at onset and related clinical correlates in obsessive–compulsive disorder: a report from the International College of Obsessive–Compulsive Spectrum Disorders (ICOCS). *International Journal of Psychiatry in Clinical Practice*, *20*(4), 210–217. https://doi.org/10.1080/13651501.2016.1207087

Dinsdale, N. K., Bluemke, E., Smith, S. M., Arya, Z., Vidaurre, D., Jenkinson, M., & Namburete, A. I. L. (2021). Learning patterns of the ageing brain in MRI using deep convolutional networks. *NeuroImage*, *224*(September 2020), 117401.

https://doi.org/10.1016/j.neuroimage.2020.117401

Dong, X., Milholland, B., & Vijg, J. (2016). Evidence for a limit to human lifespan. *Nature*, *538*(7624), 257–259. https://doi.org/10.1038/nature19793

Eickhoff, S. B., Thirion, B., Varoquaux, G., & Bzdok, D. (2015). Connectivity-based parcellation: Critique and implications. *Human Brain Mapping*, *36*(12), 4771–4792. https://doi.org/10.1002/hbm.22933

Eisen, J. L., Pinto, A., Mancebo, M. C., Dyck, I. R., Orlando, M. E., & Rasmussen, S. A. (2010). A 2-year prospective follow-up study of the course of obsessive-compulsive disorder. *Journal of Clinical Psychiatry*, *71*(8), 1033–1039. https://doi.org/10.4088/JCP.08m04806blu

Elbejjani, M., Fuhrer, R., Abrahamowicz, M., Mazoyer, B., Crivello, F., Tzourio, C., & Dufouil, C. (2015). Depression, depressive symptoms, and rate of hippocampal atrophy in a longitudinal cohort of older men and women. *Psychological Medicine*, *45*(9), 1931–1944. https://doi.org/10.1017/S0033291714003055

Emerit, J., Edeas, M., & Bricaire, F. (2004). Neurodegenerative diseases and oxidative stress. *Biomedicine and Pharmacotherapy*, *58*(1), 39–46. https://doi.org/10.1016/j.biopha.2003.11.004

Farah, M. J. (2017). The Neuroscience of Socioeconomic Status: Correlates, Causes, and Consequences. *Neuron*, *96*(1), 56–71. https://doi.org/10.1016/j.neuron.2017.08.034

Fiddick, L. (2011). There is more than the amygdala: Potential threat assessment in the cingulate cortex. *Neuroscience and Biobehavioral Reviews*, *35*(4), 1007–1018. https://doi.org/10.1016/j.neubiorev.2010.09.014

Fineberg, N. A., & Gale, T. M. (2005). *Evidence-based pharmacotherapy of obsessive – compulsive disorder*. 107–129.

Fjell, A. M., Westlye, L. T., Grydeland, H., Amlien, I., Espeseth, T., Reinvang, I., Raz, N., Holland, D., Dale, A. M., & Walhovd, K. B. (2013a). Critical ages in the life course of the adult brain: Nonlinear subcortical aging. *Neurobiology of Aging*, *34*(10), 2239–2247. https://doi.org/10.1016/j.neurobiolaging.2013.04.006

Fjell, A. M., Westlye, L. T., Grydeland, H., Amlien, I., Espeseth, T., Reinvang, I., Raz, N., Holland, D., Dale, A. M., & Walhovd, K. B. (2013b). Critical ages in the life course of the adult brain: Nonlinear subcortical aging. *Neurobiology of Aging*, *34*(10), 2239–2247. https://doi.org/10.1016/j.neurobiolaging.2013.04.006

Fouche, J. P., Du Plessis, S., Hattingh, C., Roos, A., Lochner, C., Soriano-Mas, C., Sato, J. R., Nakamae, T., Nishida, S., Kwon, J. S., Jung, W. H., Mataix-Cols, D., Hoexter, M. Q., Alonso, P., De Wit, S. J., Veltman, D. J., Stein, D. J., & Van Den Heuvel, O. A. (2017). Cortical thickness in obsessive-compulsive disorder: Multisite mega-analysis of 780 brain scans from six centres. *British Journal of Psychiatry*, *210*(1), 67–74. https://doi.org/10.1192/bjp.bp.115.164020

Fouche, J. P., Groenewold, N. A., Sevenoaks, T., Heany, S., Lochner, C., Alonso, P., Batistuzzo, M. C., Cardoner, N., Ching, C. R. K., de Wit, S. J., Gutman, B., Hoexter, M. Q., Jahanshad, N., Kim, M., Kwon, J. S., Mataix-Cols, D., Menchon, J. M., Miguel, E. C., Nakamae, T., … Stein, D. J. (2022). Shape analysis of subcortical structures in obsessive-compulsive disorder and the relationship with comorbid anxiety, depression, and medication use: A meta-analysis by the OCD Brain Imaging Consortium. *Brain and Behavior*, *12*(10), 1–12. https://doi.org/10.1002/brb3.2755

Franke, K., Bublak, P., Hoyer, D., Billiet, T., Gaser, C., Witte, O. W., & Schwab, M. (2020). In vivo biomarkers of structural and functional brain development and aging in humans. *Neuroscience and Biobehavioral Reviews*, *117*(November 2017), 142–164. https://doi.org/10.1016/j.neubiorev.2017.11.002

Frodl, T., Reinhold, E., Koutsouleris, N., Reiser, M., & Meisenzahl, E. M. (2010). Interaction of childhood stress with hippocampus and prefrontal cortex volume reduction in major depression. *Journal of Psychiatric Research*, *44*(13), 799–807. https://doi.org/10.1016/j.jpsychires.2010.01.006

Gao, W., Alcauter, S., Smith, J. K., Gilmore, J. H., & Lin, W. (2015). Development of human brain cortical network architecture during infancy. *Brain Structure and Function*, *220*(2), 1173–1186. https://doi.org/10.1007/s00429-014-0710-3

Geller, D. A., Homayoun, S., & Johnson, G. (2021). Developmental Considerations in Obsessive Compulsive Disorder: Comparing Pediatric and Adult-Onset Cases. *Frontiers in Psychiatry*, *12*(June), 1–15. https://doi.org/10.3389/fpsyt.2021.678538

Glasser, M. F., Coalson, T. S., Robinson, E. C., Hacker, C. D., Harwell, J., Yacoub, E., Ugurbil, K., Andersson, J., Beckmann, C. F., Jenkinson, M., Smith, S. M., & Van Essen, D. C. (2016). A multi-modal parcellation of human cerebral cortex. *Nature*, *536*(7615), 171–178. https://doi.org/10.1038/nature18933

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., Nugent, T. F., Herman, D. H., Clasen, L. S., Toga, A. W., Rapoport, J. L., & Thompson, P. M. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proceedings of the*

National Academy of Sciences of the United States of America, *101*(21), 8174–8179. https://doi.org/10.1073/pnas.0402680101

Gogtay, N., Nugent, T. F., Herman, D. H., Ordonez, A., Greenstein, D., Hayashi, K. M., Clasen, L., Toga, A. W., Giedd, J. N., Rapoport, J. L., & Thompson, P. M. (2006). Dynamic mapping of normal human hippocampal development. *Hippocampus*, *16*(8), 664–672. https://doi.org/10.1002/hipo.20193

Gold, A. L., Steuber, E. R., White, L. K., Pacheco, J., Sachs, J. F., Pagliaccio, D., Berman, E., Leibenluft, E., & Pine, D. S. (2017). Cortical thickness and subcortical gray matter volume in pediatric anxiety disorders. *Neuropsychopharmacology*, *42*(12), 2423–2433. https://doi.org/10.1038/npp.2017.83

Goodman, W. K., Grice, D. E., Lapidus, K. A. B., & Coffey, B. J. (2014). Obsessive-compulsive disorder. *Psychiatric Clinics of North America*, *37*(3), 257–267. https://doi.org/10.1016/j.psc.2014.06.004

Green, J. G., McLaughlin, K. A., Berglund, P. A., Gruber, M. J., Sampson, N. A., Zaslavsky, A. M., & Kessler, R. C. (2010). Childhood adversities and adult psychiatric disorders in the national comorbidity survey replication I: Associations with first onset of DSM-IV disorders. *Archives of General Psychiatry*, *67*(2), 113–123. https://doi.org/10.1001/archgenpsychiatry.2009.186

Groenewold, N. A., Bas-Hoogendam, J. M., Amod, A. R., Laansma, M. A., Van Velzen, L. S., Aghajani, M., Hilbert, K., Oh, H., Salas, R., Jackowski, A. P., Pan, P. M., Salum, G. A., Blair, J. R., Blair, K. S., Hirsch, J., Pantazatos, S. P., Schneier, F. R., Talati, A., Roelofs, K., … Van der Wee, N. J. A. (2023). Volume of subcortical brain regions in social anxiety disorder: mega-analytic results from 37 samples in the ENIGMA-Anxiety Working Group. *Molecular Psychiatry*, *December 2022*. https://doi.org/10.1038/s41380-022-01933-9

Han, L. K. M., Schnack, H. G., Brouwer, R. M., Veltman, D. J., van der Wee, N. J. A., van Tol, M. J., Aghajani, M., & Penninx, B. W. J. H. (2021). Contributing factors to advanced brain aging in depression and anxiety disorders. *Translational Psychiatry*, *11*(1). https://doi.org/10.1038/s41398-021-01524-2

Han, L. K. M., Verhoeven, J. E., Tyrka, A. R., Penninx, B. W. J. H., Wolkowitz, O. M., Månsson, K. N. T., Lindqvist, D., Boks, M. P., Révész, D., Mellon, S. H., & Picard, M. (2019a). Accelerating research on biological aging and mental health: Current challenges and future directions. *Psychoneuroendocrinology*, *106*(January), 293–311. https://doi.org/10.1016/j.psyneuen.2019.04.004

Han, L. K. M., Verhoeven, J. E., Tyrka, A. R., Penninx, B. W. J. H., Wolkowitz, O. M., Månsson, K. N. T., Lindqvist, D., Boks, M. P., Révész, D., Mellon, S. H., & Picard, M. (2019b). Accelerating research on biological aging and mental health: Current challenges and future directions. *Psychoneuroendocrinology*, *106*(September 2018), 293–311. https://doi.org/10.1016/j.psyneuen.2019.04.004

Hoexter, M. Q., Dougherty, D. D., Shavitt, R. G., D'Alcante, C. C., Duran, F. L. S., Lopes, A. C., Diniz, J. B., Batistuzzo, M. C., Evans, K. C., Bressan, R. A., Busatto, G. F., & Miguel, E. C. (2013). Differential prefrontal gray matter correlates of treatment response to fluoxetine or cognitive-behavioral therapy in obsessive-compulsive disorder. *European Neuropsychopharmacology*, *23*(7), 569–580. https://doi.org/10.1016/j.euroneuro.2012.06.014

Hou, Y., Dan, X., Babbar, M., Wei, Y., Hasselbalch, S. G., Croteau, D. L., & Bohr, V. A. (2019). Ageing as a risk factor for neurodegenerative disease. *Nature Reviews Neurology*, *15*(10), 565–581. https://doi.org/10.1038/s41582-019-0244-7

Hugenschmidt, C. E., Peiffer, A. M., Kraft, R. A., Casanova, R., Deibler, A. R., Burdette, J. H., Maldjian, J. A., & Laurienti, P. J. (2008). Relating imaging indices of white matter integrity and volume in healthy older adults. *Cerebral Cortex*, *18*(2), 433–442. https://doi.org/10.1093/cercor/bhm080

Iglesias, J. E., Augustinack, J. C., Nguyen, K., Player, C. M., Player, A., Wright, M., Roy, N., & Frosch, M. P. (2015). A computational atlas of the hippocampal formation using ex vivo , ultra-high resolution MRI. *NeuroImage*, 115–137.

Irle, E., Ruhleder, M., Lange, C., Seidler-Brandler, U., Salzer, S., Dechent, P., Weniger, G., Leibing, E., & Leichsenring, F. (2010). Reduced amygdalar and hippocampal size in adults with generalized social phobia. *Journal of Psychiatry and Neuroscience*, *35*(2), 126–131. https://doi.org/10.1503/jpn.090041

Ivanov, I., Boedhoe, P. S. W., Abe, Y., Alonso, P., Ameis, S. H., Arnold, P. D., Balachander, S., Baker, J. T., Banaj, N., Bargalló, N., Batistuzzo, M. C., Benedetti, F., Beucke, J. C., Bollettini, I., Brem, S., Brennan, B. P., Buitelaar, J., Calvo, R., Cheng, Y., … Fouche, J.-P. (2022). Associations of medication with subcortical morphology across the lifespan in OCD: Results from the international ENIGMA Consortium. *Journal of Affective Disorders*, *318*(August), 204–216. https://doi.org/10.1016/j.jad.2022.08.084

J S Kwon, Y-W Shin, C-W Kim, Y I Kim, T Youn, M H Han, K-H Chang, J.-J. K. (2003). *Similarity and disparity of obsessive-compulsive disorder and schizophrenia in MR volumetric abnormalities of the hippocampus-amygdala complex*. 962–964.

Janak, P. H., & Tye, K. M. (2015). From circuits to behaviour in the amygdala. *Nature*, *517*(7534), 284–292. https://doi.org/10.1038/nature14188

Jiang, L., Qiao, K., Li, Q., Hu, Y., Zhang, X., Wang, J., Peng, D., Fan, Q., Zhao, M., Sheng, J., Wang, J., Li, C., Fang, Y., Wang, Z., & Yang, Z. (2022). Categorical and dimensional deficits in hippocampal subfields among schizophrenia, obsessive-compulsive disorder, bipolar disorder, and major depressive disorder. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*. https://doi.org/10.1016/j.bpsc.2022.06.011

Kalisch, R., Schubert, M., Jacob, W., Keßler, M. S., Hemauer, R., Wigger, A., Landgraf, R., & Auer, D. P. (2006). Anxiety and hippocampus volume in the rat. *Neuropsychopharmacology*, *31*(5), 925–932. https://doi.org/10.1038/sj.npp.1300910

Kaufmann, T., van der Meer, D., Doan, N. T., Schwarz, E., Lund, M. J., Agartz, I., Alnæs, D., Barch, D. M., Baur-Streubel, R., Bertolino, A., Bettella, F., Beyer, M. K., Bøen, E., Borgwardt, S., Brandt, C. L., Buitelaar, J., Celius, E. G., Cervenka, S., Conzelmann, A., … Westlye, L. T. (2019). Common brain disorders are associated with heritable patterns of apparent aging of the brain. *Nature Neuroscience*, *22*(10), 1617–1623. https://doi.org/10.1038/s41593-019-0471-7

Keller, M. B. (2003). The lifelong course of social anxiety disorder: A clinical perspective. *Acta Psychiatrica Scandinavica, Supplement*, *108*(417), 85–94. https://doi.org/10.1034/j.1600-0447.108.s417.6.x

Khan, S. S., Singer, B. D., & Vaughan, D. E. (2017). Molecular and physiological manifestations and measurement of aging in humans. *Aging Cell*, *16*(4), 624–633. https://doi.org/10.1111/acel.12601

Kier, E. L., Kim, J. H., Fulbright, R. K., & Bronen, R. A. (1997). Embryology of the human fetal hippocampus: Mr imaging, anatomy, and histology. *American Journal of Neuroradiology*, *18*(3), 525–532.

Klemera, P., & Doubal, S. (2006). A new approach to the concept and computation of biological age. *Mechanisms of Ageing and Development*, *127*(3), 240–248. https://doi.org/10.1016/j.mad.2005.10.004

Knierim, J. J., & Neunuebel, J. P. (2016). Tracking the flow of hippocampal computation: Pattern separation, pattern completion, and attractor dynamics. *Neurobiology of Learning and Memory*, *129*, 38–49. https://doi.org/10.1016/j.nlm.2015.10.008

Koç, M., Kuloğlu, Ö., Yıldırım, H., & Atmaca, M. (2017). The investigation of hippocampus and amygdala volume changes with MRI in patients with social anxiety disorder. *Anatolian Journal of Psychiatry*, *19*(2), 1. https://doi.org/10.5455/apd.268817

Kurth, F., Cherbuin, N., & Luders, E. (2017). The impact of aging on subregions of the hippocampal complex in healthy adults. *NeuroImage*, *163*, 296–300. https://doi.org/10.1016/j.neuroimage.2017.09.016

Kurth, F., Cherbuin, N., & Luders, E. (2019). Age but no sex effects on subareas of the amygdala. *Human Brain Mapping*, *40*(6), 1697–1704. https://doi.org/10.1002/hbm.24481

Lee, T., Jarome, T., Li, S. J., Kim, J. J., & Helmstetter, F. J. (2009). Chronic stress selectively reduces hippocampal volume in rats: A longitudinal magnetic resonance imaging study. *NeuroReport*, *20*(17), 1554–1558. https://doi.org/10.1097/WNR.0b013e328332bb09

Li, Y., Li, J., Segal, S., Wegiel, J., De Santi, S., Zhan, J., & De Leon, M. J. (2006). Hippocampal cerebrospinal fluid spaces on MR imaging: Relationship to aging and Alzheimer disease. *American Journal of Neuroradiology*, *27*(4), 912–918.

Liao, W., Xu, Q., Mantini, D., Ding, J., MacHado-De-Sousa, J. P., Hallak, J. E. C., Trzesniak, C., Qiu, C., Zeng, L., Zhang, W., Crippa, J. A. S., Gong, Q., & Chen, H. (2011). Altered gray matter morphometry and resting-state functional and structural connectivity in social anxiety disorder. *Brain Research*, *1388*, 167–177. https://doi.org/10.1016/j.brainres.2011.03.018

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2013). The hallmarks of aging. *Cell*, *153*(6), 1194. https://doi.org/10.1016/j.cell.2013.05.039

López-Solà, C., Fontenelle, L. F., Alonso, P., Cuadras, D., Foley, D. L., Pantelis, C., Pujol, J., Yücel, M., Cardoner, N., Soriano-Mas, C., Menchón, J. M., & Harrison, B. J. (2014). Prevalence and heritability of obsessive-compulsive spectrum and anxiety disorder symptoms: A survey of the Australian twin registry. *American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics*, *165*(4), 314–325. https://doi.org/10.1002/ajmg.b.32233

López-Solà, C., Fontenelle, L. F., Bui, M., Hopper, J. L., Pantelis, C., Yücel, M., Menchón, J. M., Alonso, P., & Harrison, B. J. (2016). Aetiological overlapbetweenobsessive-Compulsive related and anxiety disorder symptoms: Multivariate twin study. *British Journal of Psychiatry*, *208*(1), 26–33. https://doi.org/10.1192/bjp.bp.114.156281

Lu, P. H., Lee, G. J., Raven, E. P., Tingus, K., Khoo, T., Thompson, P. M., & Bartzokis, G. (2011). Age-related slowing in cognitive processing speed is associated with myelin integrity in a very healthy elderly sample. *Journal of Clinical and Experimental Neuropsychology*, *33*(10), 1059–1068. https://doi.org/10.1080/13803395.2011.595397

Maceachern, S. J., & Forkert, N. D. (2021). Machine learning for precision medicine. *Genome*, *64*(4), 416–425. https://doi.org/10.1139/gen-2020-0131

Machado-de-Sousa, J. P., De Lima Osório, F., Jackowski, A. P., Bressan, R. A., Chagas, M. H. N., Torro-Alves, N., DePaula, A. L. D., Crippa, J. A. S., & Hallak, J. E. C. (2014). Increased amygdalar and hippocampal volumes in young adults with social anxiety. *PLoS ONE*, *9*(2), 1–5. https://doi.org/10.1371/journal.pone.0088523

Madabhushi, R., Pan, L., & Tsai, L. H. (2014). DNA damage and its links to neurodegeneration. *Neuron*, *83*(2), 266–282. https://doi.org/10.1016/j.neuron.2014.06.034

Mahjani, B., Bey, K., Boberg, J., & Burton, C. (2021). *Genetics of obsessive-compulsive disorder*.

Malouff, J. M., & Schutte, N. S. (2017). A meta-analysis of the relationship between anxiety and telomere length. *Anxiety, Stress and Coping*, *30*(3), 264–272. https://doi.org/10.1080/10615806.2016.1261286

Marazziti, D., Abelli, M., Baroni, S., Carpita, B., Ramacciotti, C. E., & Osso, L. D. (2015). *Neurobiological correlates of social anxiety disorder : an update*. 100–111. https://doi.org/10.1017/S109285291400008X

Masliah, E., Crews, L., & Hansen, L. (2006). Synaptic remodeling during aging and in Alzheimer's disease. *Journal of Alzheimer's Disease*, *9*(SUPPL. 3), 91–99. https://doi.org/10.3233/jad-2006-9s311

McEwen, B. S. (2000). The neurobiology of stress: From serendipity to clinical relevance. *Brain Research*, *886*(1–2), 172–189. https://doi.org/10.1016/S0006-8993(00)02950-4

McEwen, B. S. (2002). Sex, stress and the hippocampus: Allostasis, allostatic load and the aging process. *Neurobiology of Aging*, *23*(5), 921–939. https://doi.org/10.1016/S0197-4580(02)00027-1

Meng, Y., Lui, S., Qiu, C., Qiu, L., Lama, S., Huang, X., Feng, Y., Zhu, C., Gong, Q., & Zhang, W. (2013). Neuroanatomical deficits in drug-naïve adult patients with generalized social anxiety disorder: A voxel-based morphometry study. *Psychiatry Research: Neuroimaging*, *214*(1), 9–15. https://doi.org/10.1016/J.PSCYCHRESNS.2013.06.002

Milad, M. R., Furtak, S. C., Greenberg, J. L., Keshaviah, A., Im, J. J., Falkenstein, M. J., Jenike, M., Rauch, S. L., & Wilhelm, S. (2013). Deficits in conditioned fear extinction in obsessive-compulsive disorder and neurobiological changes in the fear circuit. *JAMA Psychiatry*, *70*(6), 608–618. https://doi.org/10.1001/jamapsychiatry.2013.914

Milad, M. R., & Rauch, S. L. (2012). Obsessive-compulsive disorder: Beyond segregated cortico-striatal pathways. *Trends in Cognitive Sciences*, *16*(1), 43–51.

https://doi.org/10.1016/j.tics.2011.11.003

Miller, K. L., Alfaro-Almagro, F., Bangerter, N. K., Thomas, D. L., Yacoub, E., Xu, J., Bartsch, A. J., Jbabdi, S., Sotiropoulos, S. N., Andersson, J. L. R., Griffanti, L., Douaud, G., Okell, T. W., Weale, P., Dragonu, I., Garratt, S., Hudson, S., Collins, R., Jenkinson, M., … Smith, S. M. (2016). Multimodal population brain imaging in the UK Biobank prospective epidemiological study. *Nature Neuroscience*, *19*(11), 1523–1536. https://doi.org/10.1038/nn.4393

Morey, R. A., Clarke, E. K., Haswell, C. C., Phillips, R. D., Clausen, A. N., Mufford, M. S., Saygin, Z., Brancu, M., Beckham, J. C., Calhoun, P. S., Dedert, E., Elbogen, E. B., Fairbank, J. A., Hurley, R. A., Kilts, J. D., Kimbrel, N. A., Kirby, A., Marx, C. E., McDonald, S. D., … LaBar, K. S. (2020). Amygdala Nuclei Volume and Shape in Military Veterans With Posttraumatic Stress Disorder. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *5*(3), 281–290. https://doi.org/10.1016/j.bpsc.2019.11.016

Mueller, S. G., Stables, L., Du, A. T., Schuff, N., Truran, D., Cashdollar, N., & Weiner, M. W. (2007). Measurement of hippocampal subfields and age-related changes with high resolution MRI at 4 T. *Neurobiology of Aging*, *28*(5), 719–726. https://doi.org/10.1016/j.neurobiolaging.2006.03.007

Mueller, S. G., & Weiner, M. W. (2009). Selective effect of age, Apo e4, and Alzheimer's disease on hippocampal subfields. *Hippocampus*, *19*(6), 558–564. https://doi.org/10.1002/hipo.20614

Nelson, C. A., Scott, R. D., Bhutta, Z. A., Harris, N. B., Danese, A., & Samara, M. (2020). Adversity in childhood is linked to mental and physical health throughout life. *The BMJ*, *371*, 1–9. https://doi.org/10.1136/bmj.m3048

Nielsen, A. N., Barch, D. M., Petersen, S. E., Schlaggar, B. L., & Greene, D. J. (2020). Machine Learning With Neuroimaging: Evaluating Its Applications in Psychiatry. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *5*(8), 791–798. https://doi.org/10.1016/j.bpsc.2019.11.007

Niu, X., Taylor, A., Shinohara, R. T., Kounios, J., & Zhang, F. (2022). Multidimensional brain-age prediction reveals altered brain developmental trajectory in psychiatric disorders. *Cerebral Cortex (New York, N.Y. : 1991)*, *32*(22), 5036–5049. https://doi.org/10.1093/cercor/bhab530

Oliveira, J., Paixão, V., Cardoso, G., Xavier, M., Caldas de Almeida, J. M., & Oliveira-Maia, A. J. (2021). Childhood adversities and the comorbidity between mood and general medical disorders in adults: Results from the WHO World Mental Health Survey Portugal. *Brain, Behavior, and Immunity - Health*, *17*(January). https://doi.org/10.1016/j.bbih.2021.100329

Pantazatos, S. P., Talati, A., Schneier, F. R., & Hirsch, J. (2014). Reduced anterior temporal and hippocampal functional connectivity during face processing discriminates individuals with social anxiety disorder from healthy controls and panic disorder, and increases following treatment. *Neuropsychopharmacology*, *39*(2), 425–434. https://doi.org/10.1038/npp.2013.211

Pfefferbaum, A., Rohlfing, T., Rosenbloom, M. J., Chu, W., Colrain, I. M., & Sullivan, E. V. (2013). Variation in longitudinal trajectories of regional brain volumes of healthy men and women (ages 10 to 85years) measured with atlas-based parcellation of MRI. *NeuroImage*, *65*, 176–193. https://doi.org/10.1016/j.neuroimage.2012.10.008

Pollack, M. H. (2001). Comorbidity, neurobiology, and pharmacotherapy of social anxiety disorder. *Journal of Clinical Psychiatry*, *62*(SUPPL. 12), 24–29.

Prestia, A., Boccardi, M., Galluzzi, S., Cavedo, E., Adorni, A., Soricelli, A., Bonetti, M., Geroldi, C., Giannakopoulos, P., Thompson, P., & Frisoni, G. (2011). Hippocampal and amygdalar volume changes in elderly patients with Alzheimer's disease and schizophrenia. *Psychiatry Research - Neuroimaging*, *192*(2), 77–83. https://doi.org/10.1016/j.pscychresns.2010.12.015

Qin, S., Young, C. B., Duan, X., Chen, T., Supekar, K., & Menon, V. (2014). Amygdala subregional structure and intrinsic functional connectivity predicts individual differences in anxiety during early childhood. *Biological Psychiatry*, *75*(11), 892–900. https://doi.org/10.1016/j.biopsych.2013.10.006

Rao, G., Gao, H., Wang, X., Zhang, J., Ye, M., & Rao, L. (2023). MRI measurements of brain hippocampus volume in relation to mild cognitive impairment and Alzheimer disease: A systematic review and meta-analysis. *Medicine (United States)*, *102*(36), E34997. https://doi.org/10.1097/MD.0000000000034997

Rao, S., Raveendranathan, D., Shivakumar, V., Narayanaswamy, J. C., Venkatasubramanian, G., & Reddy, Y. C. J. (2018). Hippocampus volume alterations and the clinical correlates in medication naïve obsessive compulsive disorder. *Journal of Affective Disorders*, *236*, 1–5. https://doi.org/10.1016/j.jad.2018.04.048

Raz, N., Ghisletta, P., Rodrigue, K. M., Kennedy, K. M., & Lindenberger, U. (2010). Trajectories of brain aging in middle-aged and older adults: Regional and individual differences. *NeuroImage*, *51*(2), 501–511. https://doi.org/10.1016/j.neuroimage.2010.03.020

Reuter, N., Genon, S., Kharabian Masouleh, S., Hoffstaedter, F., Liu, X., Kalenscher, T., Eickhoff, S. B., & Patil, K. R. (2020). CBPtools: a Python package for regional connectivity-based parcellation. *Brain Structure and Function*, *225*(4), 1261–1275. https://doi.org/10.1007/s00429-020-02046-1

Ridout, K. K., Ridout, S. J., Price, L. H., Sen, S., & Tyrka, A. R. (2016). Depression and telomere length:

A meta-analysis. *Journal of Affective Disorders*, *191*, 237–247. https://doi.org/10.1016/j.jad.2015.11.052

Rolls, E. T. (2013). The mechanisms for pattern completion and pattern separation in the hippocampus. *Frontiers in Systems Neuroscience*, *7*(OCT), 1–21. https://doi.org/10.3389/fnsys.2013.00074

Rudy, B. M., May, A. C., Whiting, S. E., Davis, T. E., Jenkins, W. S., & Reuther, E. T. (2014). Differentiating Among Singular and Comorbid Obsessive-Compulsive Disorder and Social Phobia Symptomology. *Cognitive Behaviour Therapy*, *43*(2), 111–121. https://doi.org/10.1080/16506073.2013.859170

Ruscio, A. M., Stein, D. J., Chiu, W. T., & Kessler, R. C. (2010). The epidemiology of obsessive-compulsive disorder in the National Comorbidity Survey Replication. *Molecular Psychiatry*, *15*(1), 53–63. https://doi.org/10.1038/mp.2008.94

Sacchet, M. D., Camacho, M. C., Livermore, E. E., Thomas, E. A. C., & Gotlib, I. H. (2017). Accelerated aging of the putamen in patients with major depressive disorder. *Journal of Psychiatry and Neuroscience*, *42*(3), 164–171. https://doi.org/10.1503/jpn.160010

Sachs-Ericsson, N. J., Hajcak, G., Sheffler, J. L., Stanley, I. H., Selby, E. A., Potter, G. G., & Steffens, D. C. (2018). Putamen Volume Differences Among Older Adults: Depression Status, Melancholia, and Age. *Journal of Geriatric Psychiatry and Neurology*, *31*(1), 39–49. https://doi.org/10.1177/0891988717747049

Sah, P., Faber, E. S. L., De Armentia, M. L., & Power, J. (2003). The amygdaloid complex: Anatomy and physiology. *Physiological Reviews*, *83*(3), 803–834. https://doi.org/10.1152/physrev.00002.2003

Saygin, Z. M., Kliemann, D., Iglesias, J. E., van der Kouwe, A. J. W., Boyd, E., Reuter, M., Stevens, A., Van Leemput, K., McKee, A., Frosch, M. P., Fischl, B., & Augustinack, J. C. (2017). High-resolution magnetic resonance imaging reveals nuclei of the human amygdala: manual segmentation to automatic atlas. *NeuroImage*, *155*(April), 370–382. https://doi.org/10.1016/j.neuroimage.2017.04.046

Schmitz-Koep, B., Zimmermann, J., Menegaux, A., Nuttall, R., Bäuml, J. G., Schneider, S. C., Daamen, M., Boecker, H., Zimmer, C., Wolke, D., Bartmann, P., Hedderich, D. M., & Sorg, C. (2021). Within amygdala: Basolateral parts are selectively impaired in premature-born adults. *NeuroImage: Clinical*, *31*(April). https://doi.org/10.1016/j.nicl.2021.102780

Schweitzer, I., Tuckwell, V., Ames, D., & O'Brien, J. (2001). Structural neuroimaging studies in late-life depression: A review. *World Journal of Biological Psychiatry*, *2*(2), 83–88. https://doi.org/10.3109/15622970109027497

Shin, L. M., & Liberzon, I. (2010). The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology*, *35*(1), 169–191. https://doi.org/10.1038/npp.2009.83

Šimić, G., Kostović, I., Winblad, B., & Bogdanović, N. (1997). Volume and number of neurons of the human hippocampal formation in normal aging and Alzheimer's disease. *Journal of Comparative Neurology*, *379*(4), 482–494. https://doi.org/10.1002/(SICI)1096-9861(19970324)379:4<482::AID-CNE2>3.0.CO;2-Z

Skoog, I. (2011). Psychiatric disorders in the elderly. *Canadian Journal of Psychiatry*, *56*(7), 387–397. https://doi.org/10.1177/070674371105600702

Sone, D., & Beheshti, I. (2022). Neuroimaging-Based Brain Age Estimation: A Promising Personalized Biomarker in Neuropsychiatry. *Journal of Personalized Medicine*, *12*(11). https://doi.org/10.3390/jpm12111850

Soomro, G. M., Altman, D., Rajagopal, S., & Oakley-Browne, M. (2008). Selective serotonin re-uptake inhibitors (SSRIs) versus placebo for obsessive compulsive disorder (OCD). *Cochrane Database of Systematic Reviews*, *1*. https://doi.org/10.1002/14651858.CD001765.pub3

Stein. (2008). Socail Anxiety Disorder.Pdf. *Elsevier*, *371*(9618), 1115–1125. https://doi.org/10.1016/S0140-6736(08)60488-2

Stein, D. J., Costa, D. L. C., Lochner, C., Miguel, E. C., Reddy, Y. C. J., Shavitt, R. G., van den Heuvel, O. A., & Simpson, H. B. (2019). Obsessive–compulsive disorder. *Nature Reviews Disease Primers*, *5*(1), 1–21. https://doi.org/10.1038/s41572-019-0102-3

Syal, S., Hattingh, C. J., Fouché, J. P., Spottiswoode, B., Carey, P. D., Lochner, C., & Stein, D. J. (2012). Grey matter abnormalities in social anxiety disorder: A pilot study. *Metabolic Brain Disease*, *27*(3), 299–309. https://doi.org/10.1007/s11011-012-9299-5

Teicher, M. H., Anderson, C. M., & Polcari, A. (2012). Childhood maltreatment is associated with reduced volume in the hippocampal subfields CA3, dentate gyrus,and subiculum. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(9). https://doi.org/10.1073/pnas.1115396109

Terburg, D., Scheggia, D., Triana del Rio, R., Klumpers, F., Ciobanu, A. C., Morgan, B., Montoya, E. R., Bos, P. A., Giobellina, G., van den Burg, E. H., de Gelder, B., Stein, D. J., Stoop, R., & van Honk, J. (2018). The Basolateral Amygdala Is Essential for Rapid Escape: A Human and Rodent Study.

Cell, *175*(3), 723-735.e16. https://doi.org/10.1016/j.cell.2018.09.028

Thorsen, A. L., Hagland, P., Radua, J., Mataix-Cols, D., Kvale, G., Hansen, B., & van den Heuvel, O. A. (2018). Emotional Processing in Obsessive-Compulsive Disorder: A Systematic Review and Meta-analysis of 25 Functional Neuroimaging Studies. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *3*(6), 563–571. https://doi.org/10.1016/j.bpsc.2018.01.009

Topkan, T. A., Erdogan, N., Barutcu, B., Cindil, E., Tali, E. T., & Karaman, Y. (2022). Volumetric Assessment of Hippocampus and Subcortical Gray Matter Regions in Alzheimer Disease and Amnestic Mild Cognitive Impairment. *Cognitive and Behavioral Neurology : Official Journal of the Society for Behavioral and Cognitive Neurology*, *35*(2), 95–103. https://doi.org/10.1097/WNN.0000000000000296

Tye, K. M., Prakash, R., Kim, S. Y., Fenno, L. E., Grosenick, L., Zarabi, H., Thompson, K. R., Gradinaru, V., Ramakrishnan, C., & Deisseroth, K. (2011). Amygdala circuitry mediating reversible and bidirectional control of anxiety. *Nature*, *471*(7338), 358–362. https://doi.org/10.1038/nature09820

van den Heuvel, O. A., Boedhoe, P. S. W., Bertolin, S., Bruin, W. B., Francks, C., Ivanov, I., Jahanshad, N., Kong, X. Z., Kwon, J. S., O'Neill, J., Paus, T., Patel, Y., Piras, F., Schmaal, L., Soriano-Mas, C., Spalletta, G., van Wingen, G. A., Yun, J. Y., Vriend, C., … Yamada, K. (2022). An overview of the first 5 years of the ENIGMA obsessive–compulsive disorder working group: The power of worldwide collaboration. *Human Brain Mapping*, *43*(1), 23–36. https://doi.org/10.1002/hbm.24972

van den Heuvel, O. A., van Wingen, G., Soriano-Mas, C., Alonso, P., Chamberlain, S. R., Nakamae, T., Denys, D., Goudriaan, A. E., & Veltman, D. J. (2016). Brain circuitry of compulsivity. *European Neuropsychopharmacology*, *26*(5), 810–827. https://doi.org/10.1016/j.euroneuro.2015.12.005

Vattimo, E. F. Q., dos Santos, A. C., Hoexter, M. Q., Frudit, P., Miguel, E. C., Shavitt, R. G., & Batistuzzo, M. C. (2021). Higher volumes of hippocampal subfields in pediatric obsessive-compulsive disorder. *Psychiatry Research - Neuroimaging*, *307*(September), 111200. https://doi.org/10.1016/j.pscychresns.2020.111200

Vigne, P., Simões, B. F. T., de Menezes, G. B., Fortes, P. P., Dias, R. V., Laurito, L. D., Loureiro, C. P., Moreira-de-Oliveira, M. E., Albertella, L., Lee, R. S. C., Stangier, U., & Fontenelle, L. F. (2019). The relationship between obsessive-compulsive disorder and anxiety disorders: A question of diagnostic boundaries or simply severity of symptoms? *Comprehensive Psychiatry*, *94*, 152116. https://doi.org/10.1016/j.comppsych.2019.152116

Walhovd, K. B., Fjell, A. M., Reinvang, I., Lundervold, A., Dale, A. M., Eilertsen, D. E., Quinn, B. T., Salat, D., Makris, N., & Fischl, B. (2005). Effects of age on volumes of cortex, white matter and subcortical structures. *Neurobiology of Aging*, *26*(9), 1261–1270. https://doi.org/10.1016/j.neurobiolaging.2005.05.020

Walhovd, K. B., Westlye, L. T., Amlien, I., Espeseth, T., Reinvang, I., Raz, N., Agartz, I., Salat, D. H., Greve, D. N., Fischl, B., Dale, A. M., & Fjell, A. M. (2011). Consistent neuroanatomical age-related volume differences across multiple samples. *Neurobiology of Aging*, *32*(5), 916–932. https://doi.org/10.1016/j.neurobiolaging.2009.05.013

Wang, C., Wang, Y., Lau, W. K. W., Wei, X., Feng, X., Zhang, C., Liu, Y., Huang, R., & Zhang, R. (2021). Anomalous static and dynamic functional connectivity of amygdala subregions in individuals with high trait anxiety. *Depression and Anxiety*, *38*(8), 860–873. https://doi.org/10.1002/da.23195

Weniger, G., Lange, C., Sachsse, U., Irle, E., & Irle, E. (2009). Asjdgbjkadsbgl. *De J Psychiatry Neurosci*, *34*(5), 383–391.

West, J., Coleman, P. D., Flood, G., & Troncoso, C. (1994). Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer ' s disease. *The Lancet*, *344*(8925), 769–772.

Williams, T., McCaul, M., Schwarzer, G., Cipriani, A., Stein, D. J., & Ipser, J. (2020). Pharmacological treatments for social anxiety disorder in adults: A systematic review and network meta-analysis. *Acta Neuropsychiatrica*. https://doi.org/10.1017/neu.2020.6

Xia, X., Chen, W., McDermott, J., & Han, J.-D. J. (2017). Molecular and phenotypic biomarkers of aging. *F1000Research*, *6*(0), 860. https://doi.org/10.12688/f1000research.10692.1

Yang, Y., & Wang, J. Z. (2017). From structure to behavior in basolateral amygdala-hippocampus circuits. *Frontiers in Neural Circuits*, *11*(October), 1–8. https://doi.org/10.3389/fncir.2017.00086

Zhang, L., Hu, X., Lu, L., Li, B., Hu, X., Bu, X., Li, H., Tang, S., Gao, Y., Yang, Y., Sweeney, J. A., Gong, Q., & Huang, X. (2020). Anatomic alterations across amygdala subnuclei in medication-free patients with obsessive–compulsive disorder. *Journal of Psychiatry and Neuroscience*, *45*(5), 334–343. https://doi.org/10.1503/jpn.190114

Zhang, L., Hu, X., Lu, L., Li, B., Hu, X., Bu, X., Li, H., Tang, S., Yang, Y., Roberts, N., Sweeney, J. A., Gong, Q., & Huang, X. (2019). Abnormalities of hippocampal shape and subfield volumes in medication-free patients with obsessive–compulsive disorder. *Human Brain Mapping*, *40*(14), 4105–4113. https://doi.org/10.1002/hbm.24688

Zimmerman, M. E., Ezzati, A., Katz, M. J., Lipton, M. L., Brickman, A. M., Sliwinski, M. J., & Lipton, R. B. (2016). Perceived stress is differentially related to hippocampal subfield volumes among older adults. *PLoS ONE*, *11*(5), 1–10. https://doi.org/10.1371/journal.pone.0154530

Zou, J., Huss, M., Abid, A., Mohammadi, P., Torkamani, A., & Telenti, A. (2019). A primer on deep learning in genomics. *Nature Genetics*, *51*(1), 12–18. https://doi.org/10.1038/s41588-018-0295-5

CHAPTER 3: Hippocampal and amygdala subfield volumes in obsessive-compulsive disorder differ according to medication status.

In chapter 2, I conducted a systematic review of molecular, cellular, and neuroimaging-based tools that are used to index biological aging in anxiety and related disorders. The findings presented in the review were mixed concerning whether anxiety disorders are associated with accentuated biological aging. In one study, anxiety and related disorders were associated with biological aging as indexed by shorter TL, which was correlated with larger amygdala volume (Kang et al., 2020). These findings suggest a possible link between amygdala volume, cellular aging, and anxiety. However, it remains unclear whether altered amygdala volume is a predisposing risk factor or a consequence of anxiety, warranting further investigation to pinpoint the underlying mechanisms of biological aging in anxiety disorders.

While the dominant neurobiological model in OCD has been suggested to be the CSTC (van den Heuvel et al., 2016), recent evidence suggests that the hippocampus and amygdala may also be involved (Milad & Rauch, 2012; van den Heuvel et al., 2022). Both the hippocampus and amygdala are of particular importance in anxiety due to their roles in fear learning, social behaviour, and goal-directed behaviour (González-García & Visser, 2023). However, findings on whole hippocampal and amygdala volumes in the OCD literature have been inconsistent. Recent evidence suggests that the hippocampus and amygdala can be segmented to reveal subfield-specific volume differences in OCD, although this evidence is only presented in medication-naïve individuals (Zhang et al., 2019, 2020: n=90). Building upon this evidence, in chapter 3, I investigate hippocampal and amygdala subfield volumes in individuals with OCD (n=381). I use an automated segmentation algorithm on T1-weighted images obtained from OBIC to segment the hippocampus and amygdala into their respective subfields. In a subgroup analysis, I assess the potential influence of clinical characteristics on subfield volumes. Through a diagnosis-by-age interaction analysis, I explore whether chronological age influences the volume of specific subfields in individuals with OCD. The results of the diagnosis-by-age interaction are presented as an additional analysis in the postscript of chapter 3 (end of chapter, see section 3.9 on page 126 and Table 3.4 on page 127). In chapter 3, it is hypothesized that individuals with OCD will have differences in both hippocampal and amygdala subfield volumes and that these effects may be particularly apparent in subfields previously implicated in OCD, including the BLA and central nucleus of the amygdala, as well as hippocampal subiculum and presubiculum (Zhang et al., 2019, Zhang et al., 2020).

Abstract

Intro: Although it has been suggested that the hippocampus and amygdala (HA) are involved in the neurobiology of obsessive-compulsive disorder (OCD), volumetric findings have been inconsistent. Furthermore, the HA consists of heterogeneous anatomic units with specific functions and cytoarchitecture, and little work has been undertaken on the volumetry of these subfields in OCD. **Methods:** T1-weighted images from 381 individuals with OCD and 338 healthy controls (HCs) from the OCD Brain Imaging Consortium were segmented to produce twelve hippocampal subfields and nine amygdala subfields using FreeSurfer 6.0. We assessed between-group differences in subfield volume using a mixed-effects model adjusted for age, quadratic effects of age, sex, site, and whole HA volume. We also performed subgroup analyses to examine subfield volume in relation to comorbid anxiety and depression, medication status, and symptom severity. **Results:** Individuals with OCD and HCs did not significantly differ in HA subfield volume. However, medicated individuals with OCD had significantly smaller hippocampal dentate gyrus ($d=-0.26$, $p_{FDR}=0.042$) and molecular layer ($d=-0.29$, $p_{FDR}=0.042$) and larger lateral ($d=0.23$, $p_{FDR}=0.049$) and basal ($d=0.25$, $p_{FDR}=0.049$) amygdala subfields than HCs. Unmedicated individuals had significantly smaller hippocampal CA1 ($d=-0.28$, $p_{FDR}=0.016$) than HCs. No association was detected between any subfield volume and OCD severity. **Limitations**: We used cross-sectional data which caveats the interpretation of our analysis. **Conclusion**: Differences in HA subfields between OCD and HCs are dependent on medication status, in line with previous work on other brain volumetric alterations in OCD. This emphasizes the importance of considering psychotropic medication in neuroimaging studies of OCD.

Keywords: Obsessive-Compulsive Disorder, Magnetic Resonance Imaging, hippocampal subfields, amygdala subfields, medication status.

3.1. Introduction

Obsessive-compulsive disorder (OCD) is a common psychiatric disorder characterized by persistent intrusive thoughts (obsessions) or repetitive ritualistic overt or covert behaviours (compulsions), or both (APA, 2013). Typical obsessive thoughts concerning, contamination, harm, sexual or religious ideas, and exactness are accompanied by anxiety or distress, which may, in turn, incite compulsions such as excessive cleaning, checking, ordering, arranging, and counting (Goodman et al., 2014). OCD has a lifetime prevalence of 1.9% to 2.5% in the adult population with a strong negative impact on occupational and social functioning (Ruscio et al., 2010). In many cases, OCD is comorbid with other disorders, including major depressive disorder (MDD) and anxiety disorders (Nazeer et al., 2020; Ruscio et al., 2010). Additionally, differences in symptom severity are likely to contribute to OCD heterogeneity (Mataix-Cols et al., 2005; Stein et al., 2019; van Oudheusden et al., 2020).

Neuroimaging studies suggest that OCD is associated with structural and/or functional changes in the cortico-striato-thalamo-cortical loops (CSTC) (Pauls et al., 2014; van den Heuvel et al., 2016). However, emerging evidence suggests that OCD involves additional brain circuits including the cerebellar, fronto-parietal, and fronto-limbic circuits (van den Heuvel et al., 2016). There has also been interest in investigating the hippocampal formation and amygdala in OCD, given the established roles of these brain structures in anxiety (Brühl et al., 2014; González-García & Visser, 2023; Shi et al., 2023) and fear conditioning (Cheng et al., 2003). Indeed, an fMRI study suggested that during fear conditioning, the hippocampus has reduced activation in individuals with OCD compared to healthy controls (HCs) (Milad et al., 2013), and a meta-analysis indicated increased amygdala activation during emotional processing in individuals with OCD versus HCs (Thorsen et al., 2018).

However, structural MRI studies in OCD have yielded inconsistent findings, reporting both increases and decreases in HA volumes (Kwon et al., 2004; Pujol et al., 2004; Atmaca et al., 2008; Rao et al., 2018). Such inconsistency could be attributable to small sample sizes, the presence of comorbidities, or medication use. Additionally, previous studies investigated whole HA volumes rather than investigating subfield volumes, which may not reveal subtle OCD-related differences in volume when these vary between the individual subfields. Work from the Enhancing Neuroimaging Genetics through Meta-Analysis (ENIGMA) OCD Consortium (ENIGMA-OCD), a large brain imaging consortium, found smaller hippocampal volumes in medicated individuals with OCD compared to HCs, which was also related to adult-onset OCD and comorbid depression (Boedhoe et al., 2017). These findings were corroborated by work from the multisite OCD Brain Imaging Consortium (OBIC) which demonstrated that smaller hippocampi were associated with medication use (Fouche et al., 2017; Fouche et al., 2022).

The HA are anatomically complex structures, consisting of multiple interconnected nuclei that can be segmented according to their cytoarchitecture, histochemistry, and connectivity profile (Sah et al., 2003), -however little is known about hippocampal and amygdala subfield volumes in OCD. Recent developments in structural MRI segmentation techniques have allowed for the robust delineation of HA subfields using a Bayesian algorithm that is based on the transformation of manual segmentation to an automated atlas (Saygin et al., 2017). Indeed, a previous study showed that paediatric individuals with OCD have larger hippocampal subfields, i.e., the left subiculum body, left cornu ammonis (CA) 4, left granule cell layer of the dentate gyrus, left molecular layer, and right parasubiculum, compared to HCs (Vattimo et al., 2021). Recent reports indicate that medication-free individuals with OCD have smaller volumes in the hippocampal subiculum, presubiculum, CA2/3, and tail (Zhang et al., 2019) and smaller basolateral and central amygdala subfield volumes (Zhang et al., 2020). However, these studies were conducted in smaller sample sizes and did not include individuals with psychotropic medication use.

To the best of our knowledge, this is the first study to investigate HA subfield volume in a large international multi-site sample of adult individuals with OCD (n=381) and HCs (n=338). We utilized the automated segmentation algorithm on T1-weighted MRI scans to segment the HA into 12 and 9 subfields, respectively. Given the evidence of the potential confounding effects of clinical characteristics on brain volumes, we performed separate analyses for individuals with and without psychotropic medication use and studied the effect of comorbid anxiety and depression. We also studied the association of subfield volumes with OCD symptom severity.

3.2. Methods

Participants and MRI acquisition

Sociodemographic and neuroimaging data were obtained from six research sites as part of OBIC. Collaborating sites and participant details have been described in-depth in a previous publication (De Wit et al., 2014). Briefly, individuals with OCD were recruited through local outpatient clinics, whereas HCs were sourced through local advertisements. All participants were screened for DSM-IV Axis I disorder. For the patient group, the primary diagnosis had to be OCD, but comorbidity with mood and anxiety disorders was allowed. To be included, healthy participants were required to be without current Axis I psychiatric disorders. Participants were excluded if they were younger than 18 or older than 65 years, had a current psychotic disorder, history of substance use disorder, intellectual disability, and severe organic or neurological pathology. Additional data was collected on age of OCD onset, medication status, and symptom severity (assessed with the Yale-Brown Obsessive Compulsive Scale (Y-BOCS)) (Goodman et al., 1989) (see Table 1A). Approval was obtained per site from all local ethical review boards and written informed consent was provided by each participant. In addition, for multi-site pooling of data, approval was obtained from the medical ethical committee Amsterdam, University Medical Center (UMC).

MRI image analysis and segmentation

All participants underwent 1.5T structural T1-weighted MRI scanning.[27] MRI image analysis was performed on the high-performance computing (HPC) cluster at the University of Cape Town, South Africa. First, we applied the standard FreeSurfer v5.3 analysis pipeline using *recon-all* to initiate all cortical reconstruction processes http://surfer.nmr.mgh.harvard.edu/). *Recon-all* initiates bias-field correction to the T1-weighted images, as well as registration to Talairach space, intensity normalisation, and skull stripping (Fischl et al., 2002).

Next, subfield segmentation was performed using the *segmentHA_T1.sh* script in FreeSurfer v6.0. This script simultaneously segments the HA, thereby preventing structural overlap (Iglesias, et al., 2015). The probability atlas applied by the script is based on the transformation of *ex vivo* manual segmentation to an automated algorithm that segments *in vivo* MRI data in target space. The atlas was built using Bayesian inference based on a tetrahedral mesh spanning the amygdala and neighbouring structures (Saygin et al., 2017). For each participant, the model produces left and right volumes for the HA subfields as well as whole HA volume and intracranial volume (ICV).

The hippocampus was segmented as follows: parasubiculum, presubiculum, subiculum, cornu ammonis (CA) sectors, CA1, CA2-3, CA4, dentate gyrus (DG), molecular layer (ML), hippocampus–amygdala transition area (HATA), fimbria, hippocampal tail, and hippocampal fissure (Iglesias, Augustinack, Nguyen, Player, Player, Wright, Roy, Frosch, et al., 2015). The hippocampal subfields were grouped according to the FreeSurfer 6.0 hippocampal module without head/body subdivision and the ML was not absorbed to the nearest DG layer (see https://surfer.nmr.mgh.harvard.edu/fswiki/HippocampalSubfieldsAndNucleiOfAmygdala). The amygdala was segmented into 7 nuclei (lateral (LA), basal (BA), accessory basal (AB), central (Ce), medial (Me), cortical (Co), paralaminar nucleus (PL)), and 2 transition areas (anterior amygdaloid area (AAA) and cortico-amygdaloid transition (CAT)). Studies suggest that the amygdala can be grouped into the following three regions: basolateral ((BLA) lateral, basal, accessory basal, paralaminar nucleus), centro-medial (central and medial), and superficial area ((SFA) cortical, AAA and CAT) (Schmitz-Koep et al., 2021). Shown in Figure 1 is an example of HA Freesurfer segmentation.

Quality control: visual inspection

We used a combination of visual inspection and quantitative measures to identify inaccurate subfield segmentation. To visually identify segmentation failures, we used an adaptation of the ENIGMA Consortium Quality Control protocol for subcortical and hippocampal subfields (https://enigma.ini.usc.edu/protocols/imaging-protocols/). In brief, three independent raters (ZN, AR, TS) examined each scan, slice-by-slice, within an html-based image gallery for partial or atypical segmentation. A list of questionable cases was generated for 3D inspection, using the Freeview utility included with FreeSurfer (Sämann et al., 2022). Additional cases were identified as follows: 1) we z-standardized each subfield and excluded participants whose score exceeded ± 5 SD from the mean (van der Meer et al., 2018, supplemental data): and 2) we generated automated outliers using an *R* script provided by ENIGMA-MDD working group (https://enigma.ini.usc.edu/ongoing/enigma-hippocampal-subfields). For the latter, participants flagged as outliers for 5+ subfields were added to the list for 3D inspection.

As shown in Table 2, 55 participants were excluded from the main analysis, i.e., 40 participants from visual QC, an additional 9 participants based on both visual QC after outlier flags and 6 participants as their z-scores exceeded ±5SD from the mean of any subfield. The final sample consisted of 381 individuals with OCD and 338 HCs.

Statistical analysis

Covariate selection

In addition to adjustment for age, quadratic effects of age, sex, and scanner site across all analyses (Barnes et al., 2010; L. W. Chen et al., 2018; Nordenskjöld et al., 2013; Sargolzaei et al., 2015), we also included those covariates that demonstrated an association with HA volumes in specific models (see supplemental data).

Linear mixed-effects models

All statistical analyses were conducted in R (https://www.r-project.org/). We used the R package *lme4* to perform our analysis and used mixed-effects *d* effect sizes, as calculated using the t values from linear mixed effects models (Nakagawa & Cuthill, 2007) that included a random intercept for the scan site. We averaged the left and right hemisphere volumes to produce a single value per participant in total, tests were performed for 21 separate subfields. All analyses were corrected for multiple comparisons across 21 subfields using the false discovery rate (FDR). We corrected all models for the total subfield volume using the combined HA volume (as recommended in the FreeSurfer manual; https://surfer.nmr.mgh.harvard.edu/fswiki/HippocampalSubfieldsAndNucleiOfAmygdala). We performed separate analyses where we compared subgroups of interest to HCs, including individuals with OCD with anxiety comorbidity (n=74), those without anxiety comorbidity (n=356), those with MDD (n=95), and those without MDD (n=286). We also included those with a history of psychotropic medication use (n=161), and those without medication use (n=220).

3.3. Results

Sample characteristics

In the full sample (OCD: n=381; HC: n=338, see Table 1A), individuals with OCD were significantly older (OCD: 32.0 years (SD = 9.4); HCs: 30.2 years (SD =9.3), t(708.7)=-2.5, p = 0.012) and on average, had lower education level (OCD: 13.7 years (SD=2.8); HCs (14.6 years (SD =3.4), t(653)= 3.6, p < 0.001) than HCs. There were no significant differences in sex and whole HA volume between individuals with OCD and HCs. The mean Y-BOCS score for individuals with OCD was 24.9 (SD=6.2). See Table 1B for details on demographic and clinical characteristics of subgroup individuals with OCD.

Group difference in subfield volumes

Between-group comparisons were conducted on 381 individuals with OCD and 338 HCs. There were no significant differences in HA subfield volumes ($p < 0.05$, FDR-corrected), after adjusting for age, quadratic effects of age, sex, site, and whole HA volume, between individuals with OCD and HCs (Figure 2A/Table 3). There were no group-by-age interaction effects for all subfield volumes.

Subgroup analysis: association of subfield volume and clinical factors

Psychotropic medication

Medicated individuals with OCD (n=161) had significantly smaller hippocampal DG (d=-0.26, p_{FDR}=0.042) and ML (d=-0.29, p_{FDR}=0.042), and larger LA (d=0.23, p_{FDR}=0.049) and BA (d=0.25, p_{FDR}=0.049) amygdala, compared to HCs (n=291) (Figure 2B). Unmedicated individuals with OCD (n=220) had significantly smaller hippocampal CA1 subfield volumes (d=-0.28, p_{FDR}=0.016) than HCs (n=338).

Anxiety and MDD comorbidity

There were no significant differences in HA subfield volumes between HCs (n=338) and either individuals with OCD with (n=95) or without (n=286) MDD, nor individuals with OCD with (n=74) or without anxiety comorbidity (n=356).

Symptom severity

We also tested whether subfield volumes were influenced by OCD symptom severity. We found no significant association between the volume of subfields and Y-BOCS scores (see supplemental data).

3.4. Discussion

In this paper, we report findings from the largest neuroimaging study of HA subfield volumes in OCD conducted to date. While we did not detect any significant differences between individuals with OCD and HCs in HA subfield volumes after multiple comparison corrections, one key finding emerged from our analysis. We found that, compared to HCs, medicated individuals with OCD had both smaller volumes in the DG and ML of the hippocampal formation and larger volumes in the LA and BA amygdala. Our findings affirm previous work demonstrating medication effects on subcortical brain volumes in OCD, suggesting that medication status is a robust confounding factor that may influence the ability to detect neuroanatomical abnormalities in OCD (Boedhoe et al., 2017; van den Heuvel et al., 2022; Weeland et al., 2022).

Our finding, that the hippocampal subfield DG and ML were significantly smaller in medicated individuals with OCD than in HCs, is consistent with previous ENIGMA-OCD studies showing smaller hippocampi in medicated individuals with OCD (Boedhoe et al., 2017; Fouche et al., 2017; Ivanov et al., 2022). However, our findings contradict studies showing smaller volumes in the hippocampal subiculum, presubiculum, and tail in individuals with OCD compared to HCs, although these studies were performed in smaller sample sizes and did not include clinical characteristics (Zhang et al., 2019). In rodent studies, the DG supports hippocampal-based neurogenesis which in turn influences hippocampal plasticity (Malberg et al., 2000; Mandyam, 2013; Perera et al., 2007; Wang et al., 2008), and there is evidence that antidepressants increase proliferation in hippocampal-based neurogenesis (McEwen, 1999; Santarelli et al., 2003). These findings are in contrast to our observation of smaller DG and ML hippocampal volumes in medicated individuals with OCD.

Although the hippocampus has been commonly studied in relation to adult neurogenesis (Shapiro et al., 2009), some evidence shows that the human amygdala may be involved in postnatal neurogenesis with cell turnover rates that are comparable to the hippocampus (Roeder et al., 2022). Rodent work indicates that the LA and BA contain immunoreactive neural cell adhesion molecules that could allow for the amygdala to participate in neuronal plasticity (Nacher et al., 2002). Additional work in rodent and non-human primates demonstrates that antidepressant-modulated neurogenesis enhances neuronal and glial cell growth in the amygdala (Bernier et al., 2002; Fowler et al., 2002). Although these findings may explain the observation of the larger amygdala BA and LA subfields in our medicated individuals with OCD, it is not known whether the subfield volumetric differences observed in medicated individuals in the present study reflect an innate response to medication use or neurotoxic effect of medication. Further investigation is required to elucidate the effects of medication in subcortical volumes in OCD.

Another finding was that unmedicated individuals with OCD had smaller hippocampal CA1 volume, compared to HCs. Although the CA1 is shown to be susceptible to stress-induced atrophy (Bartsch et al., 2015; Kassem et al., 2013), it is unclear whether unmedicated individuals experience greater stress than medicated individuals with OCD, in our study. A rodent quinpirole sensitization model of OCD showed a downregulation of Arc (a marker of plasticity-related neuronal activity) expressing neurons in the CA1 during stereotypical checking behaviour suggesting that the hippocampus may be involved in OCD more than is currently thought (Brožka, 2020).

3.5. Limitations

There are some limitations to consider. First, even with automated segmentation, the small size of the amygdala poses challenges in accurately identifying its borders (Saygin et al., 2011). We also note that there is some evidence of poor test-retest reliability in the segmentation of some hippocampal structures, including the medial, paralaminar nucleus, hippocampal fissure, and fimbria (Quattrini et al., 2020). Secondly, the cross-sectional nature of our study limits our interpretation of the effects of medication on subcortical volumes in OCD as these findings require validation using longitudinal studies. Third, due to a lack of detailed information on medication history, we were unable to further investigate the effects of medication type, dosage, and duration on subfield volumes in medicated individuals with OCD. The fourth limitation is the inability of our study to account for heterogeneity in the clinical presentation of OCD in our models, particularly in light of published evidence that suggests an association between OCD symptom profile and hippocampal volume (Reess et al., 2018). Lastly, since our study used secondary data, we only had access to 1.5T MRI data which is lower in resolution than 3T MRI data.

3.6. Conclusion

In summary, the association of medication status with volumetric alterations in OCD is consistent with previous work and emphasizes the importance of considering medication use as an important confounder in neuroimaging findings. Further investigation is required to elucidate the association between medication type, dosage, duration, and brain volumes in OCD over time.

3.7. Tables and figures

Table 3.1a: Demographic and clinical characteristics of individuals with OCD (n=381) and HCs (n=338)

	OCD (n=381)		**HCs (n=338)**		**Statistical analysis**		
Characteristics	**mean**	**SD**	**mean**	**SD**	**t**	**df**	**p**
Age (years)	32	9.4	30.2	9.3	-2.5	708.7	0.001
Education level (years)	13.7	2.8	14.6	3.4	3.6	653	< 0.001
Y-BOCS score mean	25	6.2	--	--			
Age at onset of OCD[b]	20.3	8.8	--	--			
Total hippocampal volume	3544.4	340.4	3587.5	361.6	1.6	694.4	0.101
Total amygdala volume	1770.3	187.9	1783	198.1	0.8	696.3	0.377
	N	**%**	**N**	**%**			
Male	186	48.8	179	52.9	-1.1	707	0.268
Right-handed	327	85.8	303	89.6			
Medication use at the time of scan	161	43.6	--	--			

[a] Measured by the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) total score. [b]As measured by the Y-BOCS symptom checklist.

Table 3.1b: Demographic and clinical characteristics of subgroup individuals with OCD

	A: OCD on medication (n=161)	**B: OCD not on medication (n=220)**	**C: OCD with anxiety (n=74)**	**D: OCD without anxiety (n=356)**	**E: OCD with MDD**	**F: OCD without MDD (n=286)**	**G: HCs**	**Statistics**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**		
Age (years)	32.89 (9.13)	31.33 (9.54)	32.27 (8.67)	31.81 (9.39)	34.99 (9.56)	30.99 (9.14)	30.2 (9.3)	A>G C>G D>G
Education level (years)	12.90 (2.88)	14.33 (2.62)	13.40 (2.97)	13.63 (2.81)	13.48 (2.92)	13.78 (2.79)	14.6 (3.4)	A>G D>G E>G F>G
	N (%)	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**		
Male	83 (51.55)	103 (46.81)	32 (43.24)	179 (50.28)	32 (33.68)	154 (53.84)	179 (52.9)	C>G E>G

Note: The mean age of OCD on medication (A), OCD with anxiety (C), and OCD without anxiety (D) significantly larger than HCs (G), respectively. Years of education significantly greater in OCD on medication (A), OCD without anxiety (D), OCD with MDD (E), and without MDD (F) significantly larger than HCs (G), respectively. Significantly larger number of men in OCD with anxiety (C), and OCD with MDD (E) than HCs (G), respectively.

Table 3.2: Number of scans provided and excluded for individuals with OCD and HCs after quality checking

	Initial number of scans[a]			Excluded for missing data			Excluded after visual QC			Reasons for exclusion		
Site	**OCD**	**HC**	**Total**	**OCD**	**HC**	**Total**	**OCD**	**HC**	**Total**	**Segmentation[b]**	**QC + outlier[c]**	***Z-score*[d]**
Amsterdam	53	49	102	1	1	2	4	1	5	4	1	0
Barcelona	86	102	188	0	0	0	9	6	15	9	4	2
Brazil	58	40	98	1	1	2	6	2	8	13	2	1
Japan	88	48	136	2	5	7	5	3	8	12	0	2
Korea	87	97	184	0	0	0	5	9	14	0	0	0
London	44	33	77	0	0	0	2	3	5	2	2	1
Total	416	369	785	4	7	11	31	24	55	40	9	6

[a] Reported in a previous publication (De Wit et al., 2014). [b] Participants excluded based on visual screening for partial or atypical segmentation using an adaptation of the ENIGMA Consortium Quality Control protocol for subcortical and hippocampal subfields (see https://enigma.ini.usc.edu/ongoing/enigma-hippocampal-subfields/). [c] Participants excluded based on *R script* flag for abnormalities on more than 5 subfields and flagged for visual QC. [d] Participants excluded based on exceeding ±5SD away from the z-standardized mean of any of the subfields.

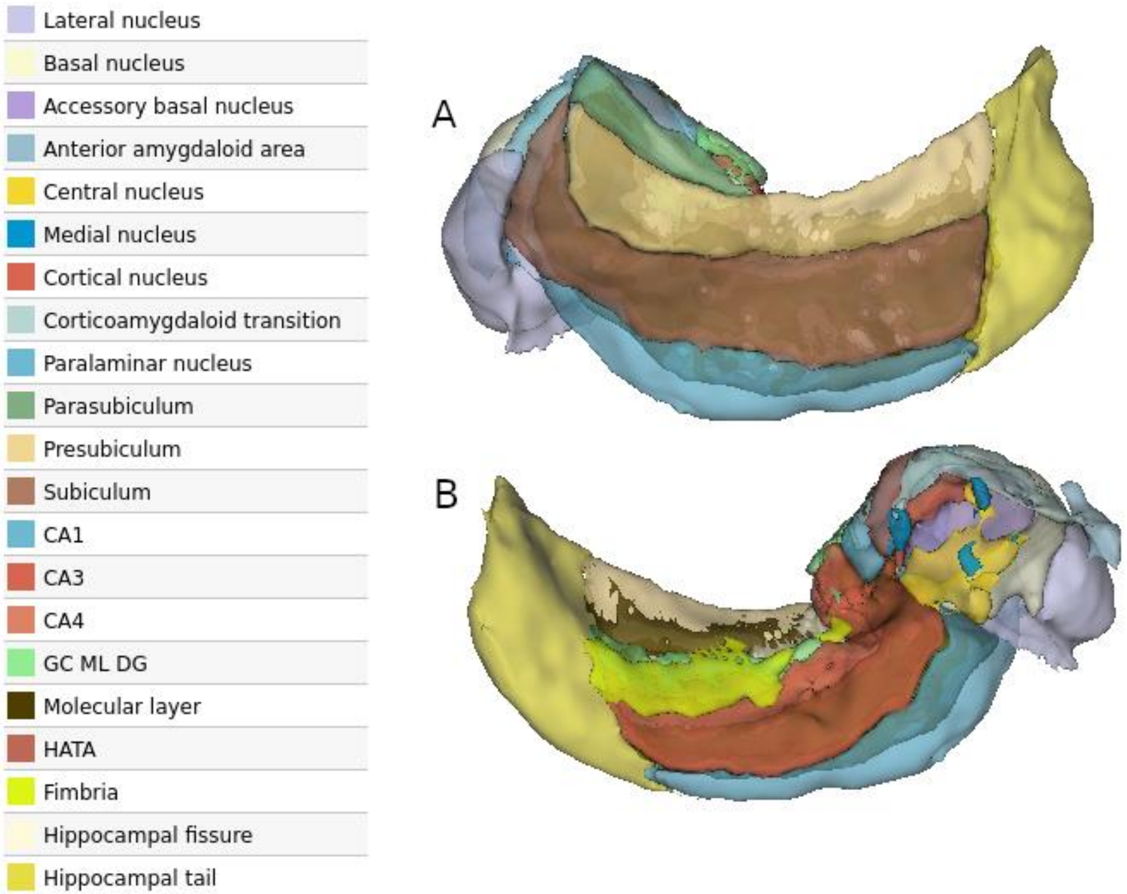

Figure 3.3: Visualisation of amygdala/hippocampal Freesurfer subfield segmentation from the right hemisphere of single representative (HC), using 3DSlicer (https://www.slicer.org/). A: Lateral view, B: Medial view. The hippocampal fimbria was not included in our analysis (Brown et al., 2020). Abbreviations: cornu ammonis (CA) sectors, CA1, CA2-3, CA4, granule cell layer of dentate gyrus (DG), molecular layer (ML), hippocampus–amygdala transition area (HATA), corticoamygdaloid transition area (CAT), anterior amygdaloid area (AAA).

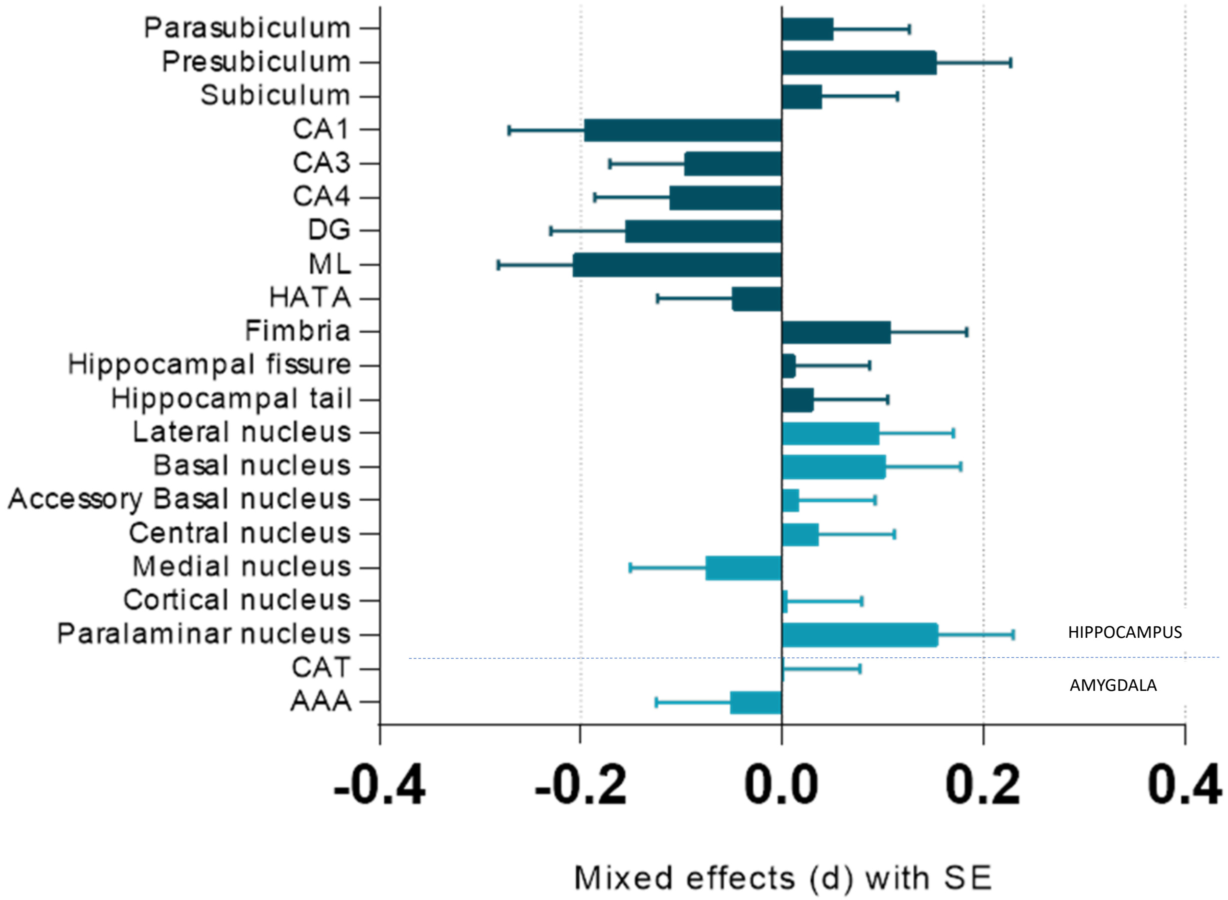

Figure 3.2a: Mixed effect size estimates (d) for hippocampal and amygdala subfield volumes between individuals with OCD (n=381) and HCs(n=338). Data presented with SE. Hippocampal subfields are presented in dark blue, and amygdala subfields presented in light blue. Abbreviations: cornu ammonis (CA) sectors, CA1, CA2-3, CA4, granule cell layer of dentate gyrus (DG), molecular layer (ML), hippocampus–amygdala transition area (HATA), corticoamygdaloid transition area (CAT), anterior amygdaloid area (AAA).

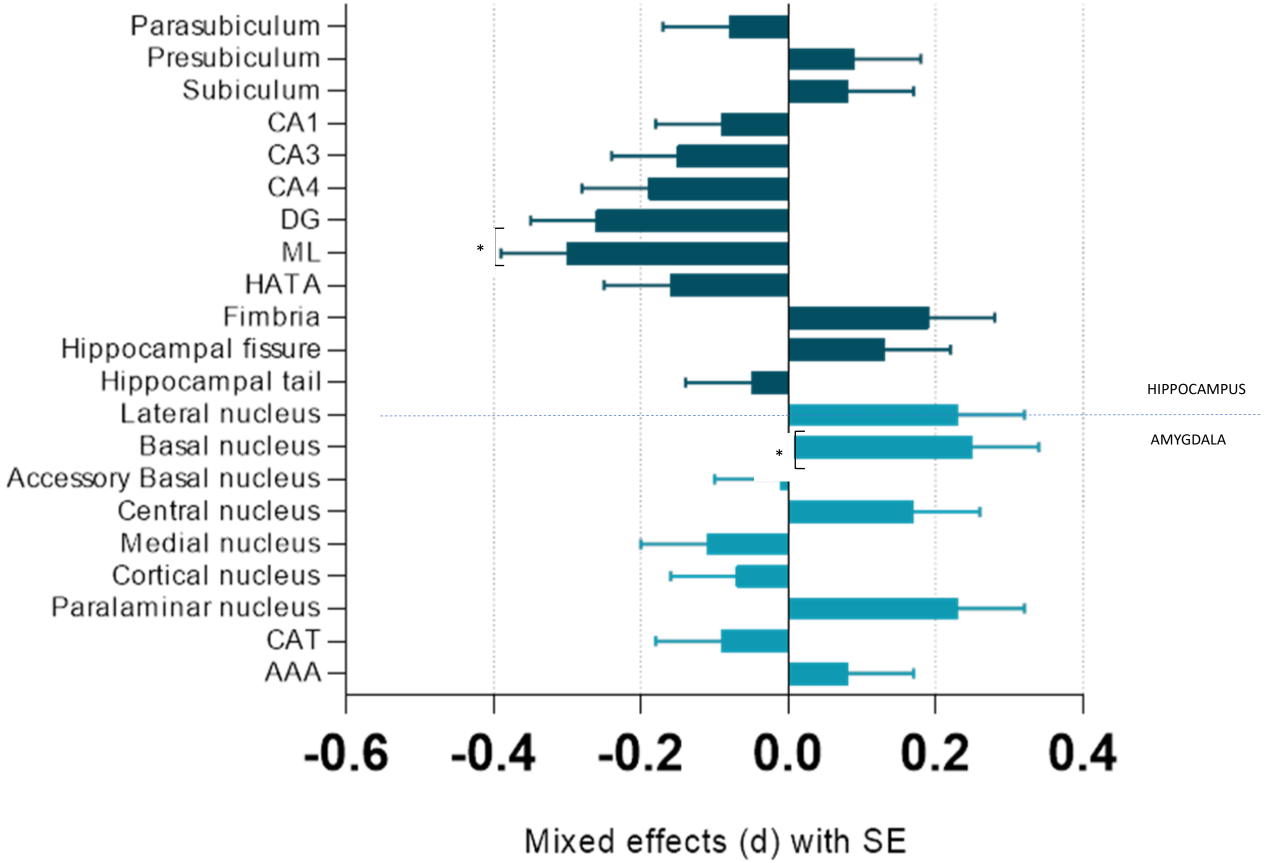

Figure 3.2b: Mixed effect size estimates (d) for hippocampal and amygdala subfield volumes between **medicated individuals with OCD** (n=161) and HCs (n=291). Data presented with SE, (*) Denotes a significant difference *p*<0.05. Hippocampal subfields are presented in dark blue, and amygdala subfields presented in light blue. Abbreviations: cornu ammonis (CA) sectors, CA1, CA2-3, CA4, granule cell layer of dentate gyrus (DG), molecular layer (ML), hippocampus–amygdala transition area (HATA), corticoamygdaloid transition area (CAT), anterior amygdaloid area (AAA).

Table 3.3: Mixed effect size estimates (d), SE, uncorrected and corrected (FDR) p-value for hippocampal and amygdala subfield volumes between individuals with OCD (n=381) and HCs (n=338).

	Effect size (mixed-effects *d*)	**Standard Error (SE)**	**P value (uncorrected)**	**P value (corrected (p_{FDR}))**
Hippocampal subfields				
Parasubiculum	0.051	0.075	0.443	0.664
Presubiculum	0.152	0.075	0.037	0.156
Subiculum	0.039	0.075	0.540	0.756
CA1	-0.196	0.075	0.006	0.063
CA3	-0.096	0.075	0.164	0.353
CA4	-0.111	0.075	0.104	0.353
DG	-0.155	0.075	0.023	0.150
ML	-0.207	0.075	0.003	0.060
HATA	-0.049	0.075	0.363	0.636
fimbria	0.108	0.075	0.122	0.353
Hippocampal fissure	0.012	0.075	0.864	0.955
Hippocampal tail	0.030	0.075	0.681	0.841
Amygdala subfields				
Lateral nucleus	0.095	0.075	0.168	0.353
Basal nucleus	0.102	0.075	0.149	0.353
Accessory basal nucleus	0.017	0.075	0.802	0.935
Central nucleus	0.036	0.075	0.631	0.828
Medial nucleus	-0.076	0.075	0.296	0.564
Cortical nucleus	0.004	0.075	0.956	0.979
Paralaminar nucleus	0.154	0.075	0.029	0.150
CAT	0.002	0.075	0.979	0.979
AAA	-0.050	0.075	0.419	0.664

Abbreviations: cornu ammonis (CA) sectors, CA1, CA2-3, CA4, granule cell layer of dentate gyrus (DG), molecular layer (ML), hippocampus–amygdala transition area (HATA), corticoamygdaloid transition area (CAT), anterior amygdaloid area (AAA). P-values are presented before and after correction for multiple comparisons using the false discovery rate (FDR).

3.8. References

APA. 2013. *Diagnostic and Statistical Manual of Mental Disorders: DSM-5^{TM}, 5th Ed.* Arlington, VA, US: American Psychiatric Publishing, Inc.

Atmaca, Murad, Huseyin Ozdemir, Sinan Ozler, Bilge Kara, Zuhal Ozler, Ebru Kanmaz, Osman Mermi, and Ertan Tezcan. 2008. "Progress in Neuro-Psychopharmacology & Biological Psychiatry Hippocampus and Amygdalar Volumes in Patients with Refractory Obsessive – Compulsive Disorder." 32:1283–86. doi: 10.1016/j.pnpbp.2008.04.002.

Barnes, Josephine, Gerard R. Ridgway, Jonathan Bartlett, Susie M. D. Henley, Manja Lehmann, Nicola Hobbs, Matthew J. Clarkson, David G. MacManus, Sebastien Ourselin, and Nick C. Fox. 2010. "Head Size, Age and Gender Adjustment in MRI Studies: A Necessary Nuisance?" *NeuroImage* 53(4):1244–55. doi: 10.1016/j.neuroimage.2010.06.025.

Bartsch, Thorsten, Juliane Döhring, Sigrid Reuter, Carsten Finke, Axel Rohr, Henriette Brauer, Günther Deuschl, and Olav Jansen. 2015. "Selective Neuronal Vulnerability of Human Hippocampal CA1 Neurons: Lesion Evolution, Temporal Course, and Pattern of Hippocampal Damage in Diffusion-Weighted MR Imaging." *Journal of Cerebral Blood Flow and Metabolism* 35(11):1836–45. doi: 10.1038/jcbfm.2015.137.

Bernier, Patrick J., Andréanne Bédard, Jonathan Vinet, Martin Lévesque, and André Parent. 2002. "Newly Generated Neurons in the Amygdala and Adjoining Cortex of Adult Primates." *Proceedings of the National Academy of Sciences of the United States of America* 99(17):11464–69. doi: 10.1073/pnas.172403999.

Brown, Emma M., Meghan E. Pierce, Dustin C. Clark, Bruce R. Fischl, Juan E. Iglesias, William P. Milberg, Regina E. McGlinchey, and David H. Salat. 2020. "Test-Retest Reliability of FreeSurfer Automated Hippocampal Subfield Segmentation within and across Scanners." *NeuroImage* 210(August 2019):116563. doi: 10.1016/j.neuroimage.2020.116563.

Brožka, Hana. 2020. "Hippocampus Dysfunction in Quinpirole Sensitization Model of Obsessive-Compulsive Disorder."

Brühl, Annette Beatrix, Aba Delsignore, Katja Komossa, and Steffi Weidt. 2014. "Neuroimaging in Social Anxiety Disorder-A Meta-Analytic Review Resulting in a New Neurofunctional Model." *Neuroscience and Biobehavioral Reviews* 47:260–80. doi: 10.1016/j.neubiorev.2014.08.003.

Chen, Lyon W., Delin Sun, Sarah L. Davis, Courtney C. Haswell, Emily L. Dennis, Chelsea A. Swanson, Christopher D. Whelan, Boris Gutman, Neda Jahanshad, Juan Eugenio Iglesias, Paul Thompson,

H. Ryan Wagner, Philipp Saemann, Kevin S. LaBar, and Rajendra A. Morey. 2018. “Smaller Hippocampal CA1 Subfield Volume in Posttraumatic Stress Disorder.” *Depression and Anxiety* 35(11):1018–29. doi: 10.1002/da.22833.

Cheng, Dominic T., David C. Knight, Christine N. Smith, Elliot A. Stein, and Fred J. Helmstetter. 2003. “Functional MRI of Human Amygdala Activity during Pavlovian Fear Conditioning: Stimulus Processing versus Response Expression.” *Behavioral Neuroscience* 117(1):3–10. doi: 10.1037/0735-7044.117.1.3.

De Wit, Stella J., Pino Alonso, Lizanne Schweren, David Mataix-Cols, Christine Lochner, José M. Menchón, Dan J. Stein, Jean Paul Fouche, Carles Soriano-Mas, Joao R. Sato, Marcelo Q. Hoexter, Damiaan Denys, Takashi Nakamae, Seiji Nishida, Jun Soo Kwon, Joon Hwan Jang, Geraldo F. Busatto, Narcís Cardoner, Danielle C. Cath, Kenji Fukui, Wi Hoon Jung, Sung Nyun Kim, Euripides C. Miguel, Jin Narumoto, Mary L. Phillips, Jesus Pujol, Peter L. Remijnse, Yuki Sakai, Na Young Shin, Kei Yamada, Dick J. Veltman, and Odile A. Van Den Heuvel. 2014. “Multicenter Voxel-Based Morphometry Mega-Analysis of Structural Brain Scans in Obsessive-Compulsive Disorder.” *American Journal of Psychiatry* 171(3):340–49. doi: 10.1176/appi.ajp.2013.13040574.

Fischl, Bruce, David H. Salat, Evelina Busa, Marilyn Albert, Megan Dieterich, Christian Haselgrove, Andre Van Der Kouwe, Ron Killiany, David Kennedy, Shuna Klaveness, Albert Montillo, Nikos Makris, Bruce Rosen, and Anders M. Dale. 2002. “Whole Brain Segmentation: Automated Labeling of Neuroanatomical Structures in the Human Brain.” *Neuron* 33(3):341–55. doi: 10.1016/S0896-6273(02)00569-X.

Fouche, Jean Paul, Stefan Du Plessis, Coenie Hattingh, Annerine Roos, Christine Lochner, Carles Soriano-Mas, Joao R. Sato, Takashi Nakamae, Seiji Nishida, Jun Soo Kwon, Wi Hoon Jung, David Mataix-Cols, Marcelo Q. Hoexter, Pino Alonso, Stella J. De Wit, Dick J. Veltman, Dan J. Stein, and Odile A. Van Den Heuvel. 2017. “Cortical Thickness in Obsessive-Compulsive Disorder: Multisite Mega-Analysis of 780 Brain Scans from Six Centres.” *British Journal of Psychiatry* 210(1):67–74. doi: 10.1192/bjp.bp.115.164020.

Fowler, Christie D., Yan Liu, Charles Ouimet, and Zuoxin Wang. 2002. “The Effects of Social Environment on Adult Neurogenesis in the Female Prairie Vole.” *Journal of Neurobiology* 51(2):115–28. doi: 10.1002/neu.10042.

Goodman, Wayne K., Dorothy E. Grice, Kyle A. B. Lapidus, and Barbara J. Coffey. 2014. “Obsessive-Compulsive Disorder.” *Psychiatric Clinics of North America* 37(3):257–67. doi: 10.1016/j.psc.2014.06.004.

Iglesias, Juan Eugenio, Jean C. Augustinack, Khoa Nguyen, Christopher M. Player, Allison Player, Michelle Wright, Nicole Roy, Matthew P. Frosch, Ann C. McKee, Lawrence L. Wald, Bruce Fischl, and Koen Van Leemput. 2015. "A Computational Atlas of the Hippocampal Formation Using Ex Vivo, Ultra-High Resolution MRI: Application to Adaptive Segmentation of in Vivo MRI." *NeuroImage* 115:117–37. doi: 10.1016/j.neuroimage.2015.04.042.

Ivanov, Iliyan, Premika S. W. Boedhoe, Yoshinari Abe, Pino Alonso, Stephanie H. Ameis, Paul D. Arnold, Srinivas Balachander, Justin T. Baker, Nerisa Banaj, Nuria Bargalló, Marcelo C. Batistuzzo, Francesco Benedetti, Jan C. Beucke, Irene Bollettini, Silvia Brem, Brian P. Brennan, Jan Buitelaar, Rosa Calvo, Yuqi Cheng, Kang Ik K. Cho, Sara Dallaspezia, Damiaan Denys, Juliana B. Diniz, Benjamin A. Ely, Jamie D. Feusner, Sónia Ferreira, Kate D. Fitzgerald, Patricia Gruner, Gregory L. Hanna, Yoshiyuki Hirano, Marcelo Q. Hoexter, Chaim Huyser, Keisuke Ikari, Anthony James, Fern Jaspers-Fayer, Hongyan Jiang, Norbert Kathmann, Christian Kaufmann, Minah Kim, Kathrin Koch, Jun Soo Kwon, Luisa Lázaro, Yanni Liu, Christine Lochner, Rachel Marsh, Ignacio Martínez-Zalacaín, David Mataix-Cols, José M. Menchón, Luciano Minuzzi, Astrid Morer, Pedro Morgado, Akiko Nakagawa, Takashi Nakamae, Tomohiro Nakao, Janardhanan C. Narayanaswamy, Erika L. Nurmi, Sanghoon Oh, Chris Perriello, John C. Piacentini, Maria Picó-Pérez, Fabrizio Piras, Federica Piras, Y. C. Janardhan Reddy, Daniela Rodriguez Manrique, Oana Yuki Sakai, Eiji Shimizu, H. Blair Simpson, Noam Soreni, Carles Soriano-Mas, Gianfranco Spalletta, Emily R. Stern, Michael C. Stevens, S. Evelyn Stewart, Philip R. Szeszko, David F. Tolin, Daan van Rooij, Dick J. Veltman, Ysbrand D. van der Werf, Guido A. van Wingen, Ganesan Venkatasubramanian, Susanne Walitza, Zhen Wang, Anri Watanabe, Lidewij H. Wolters, Xiufeng Xu, Je-Yeon Yun, Mojtaba Zarei, Fengrui Zhang, Qing Zhao, Paul M. Thompson, Dan J. Stein, Odile A. van den Heuvel, Joseph O'Neill, Sara Poletti, Egill Axfjord Fridgeirsson, Toshikazu Ikuta, Stella J. de Wit, Chris Vriend, Selina Kasprzak, Masaru Kuno, Jumpei Takahashi, Euripedes C. Miguel, Roseli G. Shavitt, Morgan Hough, Jose C. Pariente, Ana E. Ortiz, Sara Bertolín, Eva Real, Cinto Segalàs, Pedro Silva Moreira, Nuno Sousa, Jin Narumoto, Kei Yamada, Jinsong Tang, and Jean-Paul Fouche. 2022. "Associations of Medication with Subcortical Morphology across the Lifespan in OCD: Results from the International ENIGMA Consortium." *Journal of Affective Disorders* 318(August):204–16. doi: 10.1016/j.jad.2022.08.084.

Kassem, Mustafa S., Jim Lagopoulos, Tim Stait-Gardner, William S. Price, Tariq W. Chohan, Jonathon C. Arnold, Sean N. Hatton, and Maxwell R. Bennett. 2013. "Stress-Induced Grey Matter Loss Determined by MRI Is Primarily Due to Loss of Dendrites and Their Synapses." *Molecular Neurobiology* 47(2):645–61. doi: 10.1007/s12035-012-8365-7.

Malberg, Jessica E., Amelia J. Eisch, Eric J. Nestler, and Ronald S. Duman. 2000. "Chronic Antidepressant

Treatment Increases Neurogenesis in Adult Rat Hippocampus." *Journal of Neuroscience* 20(24):9104–10. doi: 10.1523/jneurosci.20-24-09104.2000.

Mataix-Cols, David, Maria Conceição Do Rosario-Campos, and James F. Leckman. 2005. "A Multidimensional Model of Obsessive-Compulsive Disorder." *American Journal of Psychiatry* 162(2):228–38. doi: 10.1176/appi.ajp.162.2.228.

McEwen, Bruce S. 1999. "Stress and Hippocampal Plasticity." *Annual Review of Neuroscience* 22:105–22. doi: 10.1146/annurev.neuro.22.1.105.

Milad, Mohammed R., Sharon C. Furtak, Jennifer L. Greenberg, Aparna Keshaviah, Jooyeon J. Im, Martha J. Falkenstein, Michael Jenike, Scott L. Rauch, and Sabine Wilhelm. 2013. "Deficits in Conditioned Fear Extinction in Obsessive-Compulsive Disorder and Neurobiological Changes in the Fear Circuit." *JAMA Psychiatry* 70(6):608–18. doi: 10.1001/jamapsychiatry.2013.914.

Nakagawa, Shinichi, and Innes C. Cuthill. 2007. "Effect Size, Confidence Interval and Statistical Significance: A Practical Guide for Biologists." *Biological Reviews* 82(4):591–605. doi: 10.1111/j.1469-185X.2007.00027.x.

Nazeer, Ahsan, Finza Latif, Aisha Mondal, Muhammad Waqar Azeem, and Donald E. Greydanus. 2020. "Obsessive-Compulsive Disorder in Children and Adolescents: Epidemiology, Diagnosis and Management." *Translational Pediatrics* 9:S76–93. doi: 10.21037/TP.2019.10.02.

Nordenskjöld, Richard, Filip Malmberg, Elna Marie Larsson, Andrew Simmons, Samantha J. Brooks, Lars Lind, Håkan Ahlström, Lars Johansson, and Joel Kullberg. 2013. "Intracranial Volume Estimated with Commonly Used Methods Could Introduce Bias in Studies Including Brain Volume Measurements." *NeuroImage* 83:355–60. doi: 10.1016/j.neuroimage.2013.06.068.

Pauls, David L., Amitai Abramovitch, Scott L. Rauch, and Daniel A. Geller. 2014. "Obsessive-Compulsive Disorder: An Integrative Genetic and Neurobiological Perspective." *Nature Reviews Neuroscience* 15(6):410–24. doi: 10.1038/nrn3746.

Perera, Tarique D., Jeremy D. Coplan, Sarah H. Lisanby, Cecilia M. Lipira, Mohamed Arif, Cristina Carpio, Gila Spitzer, Luca Santarelli, Bruce Scharf, Rene Hen, Gorazd Rosoklija, Harold A. Sackeim, and Andrew J. Dwork. 2007. "Antidepressant-Induced Neurogenesis in the Hippocampus of Adult Nonhuman Primates." *Journal of Neuroscience* 27(18):4894–4901. doi: 10.1523/JNEUROSCI.0237-07.2007.

Quattrini, Giulia, Michela Pievani, Jorge Jovicich, Marco Aiello, Núria Bargalló, Frederik Barkhof, David Bartres-Faz, Alberto Beltramello, Francesca B. Pizzini, Olivier Blin, Regis Bordet, Massimo Caulo, Manos Constantinides, Mira Didic, Antonios Drevelegas, Antonio Ferretti, Ute Fiedler, Piero Floridi, Hélène Gros-Dagnac, Tilman Hensch, Karl Titus Hoffmann, Joost P. Kuijer, Renaud Lopes, Camillo Marra, Bernhard W. Müller, Flavio Nobili, Lucilla Parnetti, Pierre Payoux, Agnese Picco, Jean Philippe Ranjeva, Luca Roccatagliata, Paolo M. Rossini, Marco Salvatore, Peter Schonknecht, Björn H. Schott, Julien Sein, Andrea Soricelli, Roberto Tarducci, Magda Tsolaki, Pieter J. Visser, Jens Wiltfang, Jill C. Richardson, Giovanni B. Frisoni, and Moira Marizzoni. 2020. "Amygdalar Nuclei and Hippocampal Subfields on MRI: Test-Retest Reliability of Automated Volumetry across Different MRI Sites and Vendors." *NeuroImage* 218(May). doi: 10.1016/j.neuroimage.2020.116932.

Rauch, Scott L., Lisa M. Shin, and Christopher I. Wright. 2003. "Neuroimaging Studies of Amygdala Function in Anxiety Disorders." *Annals of the New York Academy of Sciences* 985:389–410. doi: 10.1111/j.1749-6632.2003.tb07096.x.

Reess, Tim Jonas, Oana Georgiana Rus, Deniz A. Gürsel, Benita Schmitz-Koep, Gerd Wagner, Götz Berberich, and Kathrin Koch. 2018. "Association between Hippocampus Volume and Symptom Profiles in Obsessive–Compulsive Disorder." *NeuroImage: Clinical* 17(November 2017):474–80. doi: 10.1016/j.nicl.2017.11.006.

Roeder, Sebastian S., Petra Burkardt, Fabian Rost, Julian Rode, Lutz Brusch, Roland Coras, Elisabet Englund, Karl Håkansson, Göran Possnert, Mehran Salehpour, Daniel Primetzhofer, László Csiba, Sarolta Molnár, Gábor Méhes, Anton B. Tonchev, Stefan Schwab, Olaf Bergmann, and Hagen B. Huttner. 2022. "Evidence for Postnatal Neurogenesis in the Human Amygdala." *Communications Biology* 5(1):1–8. doi: 10.1038/s42003-022-03299-8.

Ruscio, A. M., D. J. Stein, W. T. Chiu, and R. C. Kessler. 2010. "The Epidemiology of Obsessive-Compulsive Disorder in the National Comorbidity Survey Replication." *Molecular Psychiatry* 15(1):53–63. doi: 10.1038/mp.2008.94.

Sah, P., E. S. L. Faber, M. Lopez De Armentia, and J. Power. 2003. "The Amygdaloid Complex: Anatomy and Physiology." *Physiological Reviews* 83(3):803–34. doi: 10.1152/physrev.00002.2003.

Sämann, Philipp G., Juan Eugenio Iglesias, Boris Gutman, Dominik Grotegerd, Ramona Leenings, Claas Flint, Udo Dannlowski, Emily K. Clarke-Rubright, Rajendra A. Morey, Theo G. M. van Erp, Christopher D. Whelan, Laura K. M. Han, Laura S. van Velzen, Bo Cao, Jean C. Augustinack, Paul M. Thompson, Neda Jahanshad, and Lianne Schmaal. 2022. "FreeSurfer-Based Segmentation of Hippocampal Subfields: A Review of Methods and Applications, with a Novel Quality Control Procedure for ENIGMA Studies and Other Collaborative Efforts." *Human Brain Mapping*

43(1):207–33. doi: 10.1002/hbm.25326.

Santarelli, Luca, Michael Saxe, Cornelius Gross, Alexandre Surget, Fortunato Battaglia, Stephanie Dulawa, Noelia Weisstaub, James Lee, Ronald Duman, Ottavio Arancio, Catherine Belzung, and René Hen. 2003. "Requirement of Hippocampal Neurogenesis for the Behavioral Effects of Antidepressants." *Science* 301(5634):805–9. doi: 10.1126/science.1083328.

Sargolzaei, Saman, Arman Sargolzaei, Mercedes Cabrerizo, Gang Chen, Mohammed Goryawala, Shirin Noei, Qi Zhou, Ranjan Duara, Warren Barker, and Malek Adjouadi. 2015. "A Practical Guideline for Intracranial Volume Estimation in Patients with Alzheimer's Disease." *BMC Bioinformatics* 16(7):1–10. doi: 10.1186/1471-2105-16-S7-S8.

Saygin, Z. M., D. Kliemann, J. E. Iglesias, A. J. W. van der Kouwe, E. Boyd, M. Reuter, A. Stevens, K. Van Leemput, A. McKee, M. P. Frosch, B. Fischl, and J. C. Augustinack. 2017. "High-Resolution Magnetic Resonance Imaging Reveals Nuclei of the Human Amygdala: Manual Segmentation to Automatic Atlas." *NeuroImage* 155(April):370–82. doi: 10.1016/j.neuroimage.2017.04.046.

Saygin, Zeynep M., David E. Osher, Jean Augustinack, Bruce Fischl, and John D. E. Gabrieli. 2011. "NeuroImage Connectivity-Based Segmentation of Human Amygdala Nuclei Using Probabilistic Tractography." *NeuroImage* 56(3):1353–61. doi: 10.1016/j.neuroimage.2011.03.006.

Schmitz-Koep, Benita, Juliana Zimmermann, Aurore Menegaux, Rachel Nuttall, Josef G. Bäuml, Sebastian C. Schneider, Marcel Daamen, Henning Boecker, Claus Zimmer, Dieter Wolke, Peter Bartmann, Dennis M. Hedderich, and Christian Sorg. 2021. "Within Amygdala: Basolateral Parts Are Selectively Impaired in Premature-Born Adults." *NeuroImage: Clinical* 31(April). doi: 10.1016/j.nicl.2021.102780.

Thorsen, Anders Lillevik, Pernille Hagland, Joaquim Radua, David Mataix-Cols, Gerd Kvale, Bjarne Hansen, and Odile A. van den Heuvel. 2018. "Emotional Processing in Obsessive-Compulsive Disorder: A Systematic Review and Meta-Analysis of 25 Functional Neuroimaging Studies." *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging* 3(6):563–71. doi: 10.1016/j.bpsc.2018.01.009.

van den Heuvel, Odile A., Guido van Wingen, Carles Soriano-Mas, Pino Alonso, Samuel R. Chamberlain, Takashi Nakamae, Damiaan Denys, Anna E. Goudriaan, and Dick J. Veltman. 2016. "Brain Circuitry of Compulsivity." *European Neuropsychopharmacology* 26(5):810–27. doi: 10.1016/j.euroneuro.2015.12.005.

van den Heuvel, Odile A., Premika S. W. Boedhoe, Sara Bertolin, Willem B. Bruin, Clyde Francks, Iliyan

Ivanov, Neda Jahanshad, Xiang Zhen Kong, Jun Soo Kwon, Joseph O'Neill, Tomas Paus, Yash Patel, Fabrizio Piras, Lianne Schmaal, Carles Soriano-Mas, Gianfranco Spalletta, Guido A. van Wingen, Je Yeon Yun, Chris Vriend, H. Blair Simpson, Daan van Rooij, Marcelo Q. Hoexter, Martine Hoogman, Jan K. Buitelaar, Paul Arnold, Jan C. Beucke, Francesco Benedetti, Irene Bollettini, Anushree Bose, Brian P. Brennan, Alessandro S. De Nadai, Kate Fitzgerald, Patricia Gruner, Edna Grünblatt, Yoshiyuki Hirano, Chaim Huyser, Anthony James, Kathrin Koch, Gerd Kvale, Luisa Lazaro, Christine Lochner, Rachel Marsh, David Mataix-Cols, Pedro Morgado, Takashi Nakamae, Tomohiro Nakao, Janardhanan C. Narayanaswamy, Erika Nurmi, Christopher Pittenger, Y. C. Janardha. Reddy, João R. Sato, Noam Soreni, S. Evelyn Stewart, Stephan F. Taylor, David Tolin, Sophia I. Thomopoulos, Dick J. Veltman, Ganesan Venkatasubramanian, Susanne Walitza, Zhen Wang, Paul M. Thompson, Dan J. Stein, Yoshinari Abe, Pino Alonso, Francesca Assogna, Nerisa Banaj, Marcelo C. Batistuzzo, Silvia Brem, Valentina Ciullo, Jamie Feusner, Ignacio Martínez-Zalacaín, José M. Menchón, Euripedes C. Miguel, John Piacentini, Federica Piras, Yuki Sakai, Lidewij Wolters, and Kei Yamada. 2022. "An Overview of the First 5 Years of the ENIGMA Obsessive–Compulsive Disorder Working Group: The Power of Worldwide Collaboration." *Human Brain Mapping* 43(1):23–36. doi: 10.1002/hbm.24972.

van der Meer, Dennis, Jaroslav Rokicki, Tobias Kaufmann, Aldo Córdova-Palomera, Torgeir Moberget, Dag Alnæs, Francesco Bettella, Oleksandr Frei, Nhat Trung Doan, Ida E. Sønderby, Olav B. Smeland, Ingrid Agartz, Alessandro Bertolino, Janita Bralten, Christine L. Brandt, Jan K. Buitelaar, Srdjan Djurovic, Marjolein van Donkelaar, Erlend S. Dørum, Thomas Espeseth, Stephen V. Faraone, Guillén Fernández, Simon E. Fisher, Barbara Franke, Beathe Haatveit, Catharina A. Hartman, Pieter J. Hoekstra, Asta K. Håberg, Erik G. Jönsson, Knut K. Kolskår, Stephanie Le Hellard, Martina J. Lund, Astri J. Lundervold, Arvid Lundervold, Ingrid Melle, Jennifer Monereo Sánchez, Linn C. Norbom, Jan E. Nordvik, Lars Nyberg, Jaap Oosterlaan, Marco Papalino, Andreas Papassotiropoulos, Giulio Pergola, Dominique J. F. de Quervain, Geneviève Richard, Anne Marthe Sanders, Pierluigi Selvaggi, Elena Shumskaya, Vidar M. Steen, Siren Tønnesen, Kristine M. Ulrichsen, Marcel P. Zwiers, Ole A. Andreassen, and Lars T. Westlye. 2018. "Brain Scans from 21,297 Individuals Reveal the Genetic Architecture of Hippocampal Subfield Volumes." *Molecular Psychiatry*. doi: 10.1038/s41380-018-0262-7.

van Oudheusden, Lucas J. B., Rens van de Schoot, Adriaan Hoogendoorn, Patricia van Oppen, Maarten Kaarsemaker, Gerben Meynen, and Anton J. L. M. van Balkom. 2020. "Classification of Comorbidity in Obsessive–Compulsive Disorder: A Latent Class Analysis." *Brain and Behavior* 10(7):1–10. doi: 10.1002/brb3.1641.

Vattimo, Edoardo F. Q., Allan Christiano dos Santos, Marcelo Q. Hoexter, Paula Frudit, Euripedes C. Miguel, Roseli G. Shavitt, and Marcelo C. Batistuzzo. 2021. “Higher Volumes of Hippocampal Subfields in Pediatric Obsessive-Compulsive Disorder.” *Psychiatry Research - Neuroimaging* 307(September):111200. doi: 10.1016/j.pscychresns.2020.111200.

Wang, Jing Wen, Denis J. David, James E. Monckton, Fortunato Battaglia, and René Hen. 2008. “Chronic Fluoxetine Stimulates Maturation and Synaptic Plasticity of Adult-Born Hippocampal Granule Cells.” *Journal of Neuroscience* 28(6):1374–84. doi: 10.1523/JNEUROSCI.3632-07.2008.

Weeland, Cees J., Chris Vriend, Ysbrand van der Werf, Chaim Huyser, Manon Hillegers, Henning Tiemeier, Tonya White, and Odile A. van den Heuvel. 2022. “Thalamic Subregions and Obsessive-Compulsive Symptoms in 2,500 Children From the General Population.” *Journal of the American Academy of Child and Adolescent Psychiatry* 61(2):321–30. doi: 10.1016/j.jaac.2021.05.024.

Zhang, Lianqing, Xinyu Hu, Lu Lu, Bin Li, Xiaoxiao Hu, Xuan Bu, Hailong Li, Shi Tang, Yingxue Gao, Yanchun Yang, John A. Sweeney, Qiyong Gong, and Xiaoqi Huang. 2020. “Anatomic Alterations across Amygdala Subnuclei in Medication-Free Patients with Obsessive–Compulsive Disorder.” *Journal of Psychiatry and Neuroscience* 45(5):334–43. doi: 10.1503/jpn.190114.

Zhang, Lianqing, Xinyu Hu, Lu Lu, Bin Li, Xiaoxiao Hu, Xuan Bu, Hailong Li, Shi Tang, Yanchun Yang, Neil Roberts, John A. Sweeney, Qiyong Gong, and Xiaoqi Huang. 2019. “Abnormalities of Hippocampal Shape and Subfield Volumes in Medication-Free Patients with Obsessive–Compulsive Disorder.” *Human Brain Mapping* 40(14):4105–13. doi: 10.1002/hbm.24688.

3.9. Additional analysis: diagnosis-by-age interaction in OCD

To assess the influence of chronological age on the association between hippocampal and amygdala subfield volumes and OCD, a diagnosis-by-age interaction term was included in the mixed effects model. Given the literature suggesting smaller hippocampal volumes with increasing chronological age (Nobis et al., 2019), we expected to find that chronological age would influence the volume of the subfields, especially for the hippocampus. While there was a trend for smaller CA1 (rME=-0.80, $p_{uncorrected}$=0.023 P_{FDR}=0.352) and HATA (rME=-0.50, $p_{uncorrected}$=0.049, P_{FDR}=0.352) subfield volumes in individuals compared to HCs (Table 3.4, page 127), perhaps suggesting that there is a differential association with between subfield volumes and chronological age, this trend did not survive correction for multiple comparison. Studies of hippocampal subfield volumes in healthy cognitive aging indicate non-linear and differential effects of chronological age on subfield volume. For instance, in Malykhin et al. (2017), the total subiculum and DG volumes were associated with chronological age whilst findings for the CA1-3 were only present in the hippocampal body (n=129 HCs, age between 18 and 85 years old) (Malykhin et al., 2017). Differential aging effects in the hippocampus have also been shown in functional connectivity studies. Stark et al. (2020) found greater connectivity in younger adults (n=31, mean age= 16.1 years) than older adults (n=31, mean age= 76 years) between the hippocampus and parahippocampal cortex and perirhinal cortex. The reduction in functional connectivity with greater chronological age was more pronounced in the anterior subfields of the hippocampus than the posterior subfields (Stark et al., 2021). Systematic reviews reveal that most studies find smaller CA1 volumes in age-related diseases and can be used as a diagnostic marker for mild cognitive impairment (de Flores et al., 2015). There are also reports indicating that a smaller CA1 is related to memory score and delayed recall sub-scores (Mak et al., 2016) and poor memory collection in the verbal episodic memory task (Chi et al., 2022). The absence of a significant diagnosis-by-age interaction in our analysis suggests that the relationship between the subfield volumes and age was not substantially different in individuals with OCD and HCs. Given the mixed findings, specifically on amygdala volume with age (Kurth et al., 2019), further analysis is warranted on the neurobiological underpinnings of subfield-specific differences in the context of anxiety disorders and aging.

Table 3.4: Additional analysis, diagnosis-by-age in OCD

OCD (n=381), HC (n=338)	**rME**	**P value**	**P value corrected (p_{FDR})**
Amygdala subfields			
Lateral	-0.002	0.951	0.984
Basal	-0.027	0.445	0.779
Accessory Basal	0.001	0.984	0.984
AAA	-0.009	0.774	0.977
Central	0.035	0.351	0.670
Medial	0.010	0.791	0.977
Cortical	0.004	0.906	0.984
CAT	0.015	0.631	0.940
Paralaminar	-0.015	0.671	0.940
Hippocampal subfields			
Parasubiculum	0.051	0.123	0.369
Presubiculum	0.060	0.101	0.354
Subiculum	0.014	0.669	0.940
CA 1	-0.081	0.023	0.352
CA 3	-0.063	0.068	0.352
CA 4	-0.036	0.293	0.615
DG	-0.044	0.197	0.461
ML	-0.066	0.057	0.352
HATA	-0.053	0.049	0.352
Fimbria	0.047	0.179	0.461
Hippocampal fissure	0.004	0.902	0.984
Hippocampal tail	0.063	0.084	0.352

3.10. References

Chi, Chia Hsing, Fu Chi Yang, and Yu Ling Chang. 2022. "Age-Related Volumetric Alterations in Hippocampal Subiculum Region Are Associated with Reduced Retention of the 'When' Memory Component." *Brain and Cognition* 160(1):105877. doi: 10.1016/j.bandc.2022.105877.

de Flores, Robin, Renaud La Joie, and Gaël Chételat. 2015. "Structural Imaging of Hippocampal Subfields in Healthy Aging and Alzheimer's Disease." *Neuroscience* 309(August):29–50. doi: 10.1016/j.neuroscience.2015.08.033.

Kurth, Florian, Nicolas Cherbuin, and Eileen Luders. 2019. "Age but No Sex Effects on Subareas of the Amygdala." *Human Brain Mapping* 40(6):1697–1704. doi: 10.1002/hbm.24481.

Mak, Elijah, Li Su, Guy B. Williams, Rosie Watson, Michael Firbank, Andrew Blamire, and John O'Brien. 2016. "Differential Atrophy of Hippocampal Subfields: A Comparative Study of Dementia with Lewy Bodies and Alzheimer Disease." *American Journal of Geriatric Psychiatry* 24(2):136–43. doi: 10.1016/j.jagp.2015.06.006.

Malykhin, Nikolai V., Yushan Huang, Stanislau Hrybouski, and Fraser Olsen. 2017. "Differential Vulnerability of Hippocampal Subfields and Anteroposterior Hippocampal Subregions in Healthy Cognitive Aging." *Neurobiology of Aging* 59:121–34. doi: 10.1016/j.neurobiolaging.2017.08.001.

Nobis, Lisa, Sanjay G. Manohar, Stephen M. Smith, Fidel Alfaro-almagro, Mark Jenkinson, Clare E. Mackay, Masud Husain, Oxford Centre, Brain Activity, and Wellcome Centre. 2019. "NeuroImage : Clinical Hippocampal Volume across Age : Nomograms Derived from over 19 , 700 People in UK Biobank." *NeuroImage: Clinical* 23(March):101904. doi: 10.1016/j.nicl.2019.101904.

Stark, Shauna M., Amy Frithsen, and Craig E. L. Stark. 2021. "Age-Related Alterations in Functional Connectivity along the Longitudinal Axis of the Hippocampus and Its Subfields." *Hippocampus* 31(1):11–27. doi: 10.1002/hipo.23259.

CHAPTER 4: Specific amygdala and hippocampal subfield volumes in social anxiety disorder and their relation to clinical characteristics – an international mega-analysis

In chapter 3, I investigated hippocampal and amygdala volumes in OCD, and their association to chronological age. In results presented in this chapter indicated that the association between hippocampal and amygdala subfield volumes was dependent on medication status suggesting that medication is a potential confounder of subcortical volume differences in OCD. With respect age age-related effects on subfield volumes, the CA1 and HATA trended towards significance, however, these findings did not reach significance after correction for multiple comparisons (FDR). These findings indicated that chronological age did not influence subfield volumes in OCD.

In chapter 4, I perform the same analysis as in chapter 3 but with a focus on SAD. The neurobiological models of SAD highlight the importance of the subcortical fear circuitry in processes related to social approach-avoidance conflicts and threat perception, which involves regions such as the hippocampus and amygdala (Groenewold et al., 2023). It is suggested that the amygdala, under top-down control from the prefrontal or anterior cingulate cortices, may be involved in heightened fear responses and hypervigilance to threat cues observed in SAD (Furmark, 2009), although the neurobiology of SAD is not yet fully understood (Groenewold et al., 2023). As in OCD literature, the findings on whole hippocampal and amygdala volume in SAD are inconsistent. It is suggested that these inconsistent findings are due to the heterogeneous nature of both the hippocampus and amygdala (Hortensius et al., 2016). As in chapter 3, I use an automated segmentation algorithm on T1-weighted images obtained from EUSARNAD to segment the hippocampus and amygdala into their respective subfields. In a subgroup analysis, I assess the potential influence of clinical characteristics on subfield volumes in SAD. Through a diagnosis-by-age interaction analysis, I explore whether chronological age influences the volume of specific subfields in individuals with SAD. The results of the diagnosis-by-age interaction are presented as an additional analysis in the postscript of chapter 4 (end of chapter, see section 4.9 on page 161 and Table 4.4 on page 162). In chapter 4, it is hypothesized that individuals with SAD will have differences in the volume of the BLA amygdala given its involvement in other anxiety disorders (Asami et al., 2018) and its role in threat evaluation (Besnard & Sahay, 2016; Etkin et al., 2004; Hortensius et al., 2016).

Abstract

Social anxiety disorder (SAD) has been associated with alterations in amygdala and hippocampal volume but there is mixed evidence for the direction of volumetric alterations. Additionally, little is known about the involvement of the distinct subfields in the pathophysiology of SAD. Volumetric data from a large multi-centre sample of 107 adult individuals with SAD and 140 healthy controls (HCs) was segmented using FreeSurfer to produce 9 amygdala and 12 hippocampal subfield volumes. Volumes were compared between groups using linear mixed-effects models adjusted for age, age-squared, sex, site and whole amygdala and hippocampal volumes. Subgroup analyses examined subfield volumes in relation to comorbid anxiety disorder, and comorbid major depressive disorder (MDD), psychotropic medication status, and symptom severity. In the full sample, SAD was associated with smaller amygdala volumes in the basal (d=-0.32, p_{FDR}=0.022), accessory basal (d=-0.42, p_{FDR}=0.005) and corticoamygdaloid transition area (d=-0.37, p_{FDR}=0.014), and larger hippocampal volume in the CA3 (d=0.34, p_{FDR}=0.024), CA4 (d=0.44, p_{FDR}=0.007), dentate gyrus (d=0.35, p_{FDR}=0.022) and molecular layer (d=0.28, p_{FDR}= 0.033), compared to HCs. SAD without comorbid anxiety, in addition, demonstrated smaller lateral amygdala (d=-0.30, p_{FDR}=0.037) and hippocampal amygdala transition area (d=-0.33, p_{FDR}=0.027) relative to HCs. In SAD without comorbid MDD, only the smaller accessory basal amygdala remained significant (d=-0.41, p_{FDR}=0.017). No association was found between subfield volume and medication status or symptom severity. In conclusion, we observed distinct patterns of volumetric differences across specific amygdala and hippocampal subfields, regions that are associated with sensory information processing, threat evaluation and fear generalization. These findings suggest a possible disruption in information flow between the amygdala and hippocampal formation for fear processing in SAD.

Word count: 267

4.1. Introduction

Social anxiety disorder (SAD), previously referred to as social phobia, is a debilitating anxiety disorder characterized by excessive fear and avoidance of social situations. Typically, individuals with SAD exhibit traits of social fearfulness or experience heightened anxiety towards and during social and performance-based tasks (APA, 2013). The social fears may lead to avoidance behaviours in daily and professional life (Stein and Stein, 2008), which in its more severe forms increases the risk of school drop-out, work absenteeism, unemployment (Lecrubier et al., 2000), and functional disability (Mack et al., 2015). SAD has a 12-month and lifetime prevalence of 7.1% (SE=0.3) and ± 12.1% (SE=0.4) respectively (Stein and Stein, 2008). Clinical studies suggest that SAD is often comorbid with major depressive disorder (MDD) and other anxiety-related disorders, including panic disorder and other phobias, with MDD most likely to develop within the first two years after the onset of SAD (Koyuncu et al., 2019).

Neuroimaging studies suggest that SAD is associated with dysregulation in the brain's 'fear circuit' consisting of the amygdala, hippocampus, insula, anterior cingulate, and prefrontal cortex (PFC). While there is evidence of abnormalities in the volume of the amygdala and hippocampus, findings in often small samples have proved inconsistent as both smaller (Irle. et al., 2010; Liao et al., 2011), larger (Machado-de-Sousa et al., 2014) and no differences in volumes (Syal et al., 2012) have been reported in individuals with SAD compared to HCs. Large-scale studies, however, using pooled structural MRI data from various international research centres, suggest that there is no difference in the volume of amygdala and hippocampus between individuals with SAD and HCs (Bas-Hoogendam et al., 2017; Wang et al., 2021).

One possible explanation for the inconsistent findings is the influence of clinical characteristics on volumetric differences observed in subcortical regions in SAD (Bas-Hoogendam et al., 2022). A recent mega-analytic study using 37 samples consisting of 1115 individuals with SAD and 2775 HCs was conducted by ENIGMA-Anxiety. In a subgroup analysis, this study found smaller amygdala volumes in SAD individuals with comorbid anxiety disorder and comorbid MDD (Groenewold et al., 2023). Additionally, given that the amygdala and hippocampus consist of structurally heterogenous units of specialized subfields that can be identified by their cytoarchitecture, histochemistry and connectivity profile (LeDoux, 2007), it is likely that investigating the volume of the whole amygdala and hippocampal might not reveal subfield specific differences that may occur in SAD.

There are multiple lines of evidence that suggest the distinct roles of the amygdala and hippocampal subfields in fear, threat, and anxiety. In the human amygdala, the centromedial (CM) amygdala is suggested to be reactive to aversive outcomes but not to predictive aversive cues (Michely et al., 2020).

On the other hand, the basolateral (BLA) amygdala is associated with threat evaluation (Etkin et al., 2004; Hortensius et al., 2016) and responsive to aversive cues in high trait-anxiety (Michely et al., 2020). Rodent studies suggest that anxiety-like behaviours in social interactions are mediated by the activation of specific subfields, for example, activation of the BLA to central amygdala projections temporarily reduced anxiety behaviours, whilst the inhibition of this pathway had the opposing effect (Tye et al., 2011). Similarly, another rodent study found that activation of the BLA to ventral hippocampal projections reduced social behaviours, whereas inhibition of this pathway increased social behaviours (Faria et al., 2012; Felix-Ortiz & Tye, 2014).

Given the roles of the amygdala and hippocampus in fear and anxiety (González-García & Visser, 2023; Shi et al., 2023), and developments in amygdala and hippocampal segmentation techniques (Saygin et al., 2017), there have been increasing reports of subfield volume alterations in anxiety-related disorders (Canada et al., 2023). Reviews of the hippocampal subfields literature suggest that individuals with MDD have smaller volumes of the CA3/4 and larger volume of the hippocampus–amygdala transition area (HATA) compared to HCs (Sun et al., 2023), while individuals with PTSD have smaller volumes of the CA1/3 and dentate gyrus (DG) compared to HCs (Ben-Zion et al., 2023). Moreover, Koch and colleagues (2021) found that a smaller dentate gyrus volume prospectively predicted PTSD vulnerability. With respect to the amygdala, Morey et al. (2020) found smaller lateral and paralaminar subfields, but larger central, medical, and cortical amygdala subfields in PTSD compared (n=149) to HCs. Zhang et al. (2021) found larger medial amygdala subfields, whereas all the other of the subfields were found to be smaller in PTSD (n=69) compared to HCs. Taken together, these findings suggest that subfields volumes may be differentially altered depending on the disorder.

Through collaborative international efforts we leveraged multi-site data to examine amygdala and hippocampal subfield volumes in a unique large sample of 107 adult individuals with SAD and 140 HCs. We used an *in vivo* automated segmentation algorithm on T1-weighted MRI scans, allowing for the analysis of 9 amygdala and 12 hippocampal subfields. In line with the evidence of the influence of clinical characteristics on subcortical volumes in SAD, we performed subgroup analyses that were restricted to SAD without lifetime 1) comorbid anxiety disorder, 2) comorbid MDD, and 3) psychotropic medication use. Lastly, in a post-hoc analysis, we explored whether volumetric differences in individuals with SAD are greater with higher symptom severity.

4.2. Methods

Participants and MRI acquisition

Sociodemographic and neuroimaging data were obtained from a previous voxel-based morphometry multi-centre mega-analysis, which was part of the European and South African Research Network in Anxiety Disorders (EUSARNAD)(Baldwin & Stein, 2012; Bas-Hoogendam et al., 2017). Data had been aggregated from multiple completed studies, conducted in five different countries (Germany, Sweden, United States of America, The Netherlands, and South Africa). The sample description has been detailed in a previous publication (Bas-Hoogendam et al., 2017). Briefly, ethical approval was obtained per site from local ethical review boards and written informed consent was obtained from each participant. Participants were sourced through clinics and public announcements. All participants were assessed using either the structured clinical interview (SCID) (First et al., 1997), mini-international neuropsychiatric interview (MINI) (Lecrubier et al., 1997) or composite international diagnostic interview (CIDI) (Robins et al., 1988). Individuals were required to have SAD as the primary diagnosis, and HCs were participants without lifetime psychiatric disorders. The exclusion criteria were age younger than 18 or older than 65 years, and presence of general MRI contraindications (ferromagnetic implants, claustrophobia, pregnancy). The data that was collected also included information on clinical characteristics such as psychiatric comorbidity (comorbid anxiety disorder and comorbid MDD), psychotropic medication use, age of onset of SAD, and SAD illness severity (as measured by the Liebowitz Social Anxiety Scale (LSAS) (Mennin et al., 2002). Structural T1-weighted 3T MRI scans were collected from all participants (see Supplementary Table S1 for characteristics of T1-weighted MRI scans).

MRI image analysis and segmentation

Image analysis was performed on the University of Cape Town's High-performance computing (HPC) cluster, Cape Town, South Africa. First, we applied the standard Freesurfer (FS) v5.3 analysis pipeline using *recon-all* to initiate all cortical reconstruction processes (http://surfer.nmr.mgh.harvard.edu/). *Recon-all* initiates bias-field correction to the T1-weighted images, as well as registration to Talairach space, intensity normalisation, and skull stripping (Fischl et al., 2002).

Next, subfield segmentation was performed using the *segmentHA_T1.sh* script bundled with FS v6.0. This script simultaneously segments the amygdala and hippocampus (AH), thereby preventing structural overlap. The probability atlas applied by the script is based on the transformation of *ex vivo* manual segmentation to an automated algorithm that segments *in vivo* MRI data in target space. The atlas was built using Bayesian inference based on a tetrahedral mesh spanning the amygdala and neighbouring structures (Saygin et al., 2017). Subfields are illustrated in Figure 1. The following amygdala subfields were extracted: lateral (LA), basal (BA), accessory basal (AB), central (Ce), medial (Me), cortical (Co), paralaminar nucleus (PL) and 2 transition areas (anterior amygdaloid area (AAA) and cortico-amygdaloid transition (CAT)). These amygdala subfields can be grouped into amygdala's three main structures; 1) the BLA complex containing the LA, BA, AB, and PL, 2) the centro-medial containing the Ce and Me nucleus, and 3) the superficial area containing the AAA, Co, and CAT (Schmitz-Koep et al., 2021). The hippocampus was segmented into the following subfields: parasubiculum, presubiculum, subiculum, three cornu ammonis (CA) sectors (CA1, CA2-3, CA4), dentate gyrus (DG), molecular layer (ML), hippocampus–amygdala transition area (HATA), fimbria, hippocampal tail, and hippocampal fissure (Iglesias, Augustinack, Nguyen, Player, Player, Wright, Roy, & Frosch, 2015). In addition, we also extracted whole volumes of the AH as well as total intracranial volume (ICV).

Quality control

We used a combination of visual inspection and quantitative measures to identify inaccurate subfield segmentation. To this end, we used an adaptation of the Enhancing Neuroimaging Genetic through Meta-Analysis (ENIGMA) (Thompson et al., 2020) quality control protocols for subcortical brain regions and hippocampal subfields (see https://enigma.ini.usc.edu/protocols/imaging-protocols/). In brief, each T1-weighted scan was examined by two independent raters, ZN and JMS, who were blind to the diagnostic status of the participant. Scans were examined for poor quality including motion and other scan artefacts, insufficient contrast, or presence of anatomical deviations, and segmentations were examined for incorrect assignment of the subfields (Sämann et al., 2022). The Freeview utility included with FreeSurfer was used for 3D inspection to confirm that poor quality scan data did not meet quality standards for inclusion. Any subjects for whom the same subfield was identified as problematic through visual QC and outlier detection procedures were excluded from subsequent analyses.

Statistical analysis

Covariate selection

Covariates were selected based on their established association with amygdala and hippocampal volume. We corrected for age, age-squared, sex, and scanner site (Barnes et al., 2010; Chen et al., 2018; Nordenskjöld et al., 2013; Sargolzaei et al., 2015; and consistent with Groenewold et al., 2023). We also corrected for total subfield volume using the combined AH rather than ICV, as recommended by the developers of FreeSurfer (https://surfer.nmr.mgh.harvard.edu/fswiki/HippocampalSubfields). Although SAD can contribute to lower educational achievement (Stein and Stein 2008), we did not find a significant difference in education between individuals with SAD and HCs, and we thus did not use education as a covariate in our analysis.

General Linear Models

All statistical analyses were conducted in R (https://www.r-project.org/). As we did not have an a-priori hypothesis regarding effects of laterality on subfield volumes, we combined the volumes of the left and right hemisphere to produce a single bilateral value per subfield per participant (Hayes et al., 2017). However, we performed a post-hoc analysis to assess possible hemispheric differences in AH subfield volumes (Ocklenburg et al., 2022) between individuals with SAD and HCs (Supplementary Materials). In our main analysis, the bilateral AH subfield volumes were used as dependent variables in separate models, with diagnostic group (HC, SAD) as the main independent variable and scanner site included as random intercept. In total, tests were performed for 21 separate subfields. Analyses were corrected for multiple comparisons across all 21 subfields using the false discovery rate (FDR)((Benjamini & Hochberg, 1995)). We used the R package *lme4* with restricted maximum likelihood (ReML) (Kenward & Roger, 1997) and outputted mixed effect (*d*) sizes, as calculated using the t values from linear mixed effects models (equation 22, Nakagawa & Cuthill, 2007). We performed separate subgroup analyses where we investigated the association between AH subfield volumes and clinical characteristics. Here, we selected participants with SAD, leaving out those with lifetime 1) comorbid anxiety disorder (SAD=86), 2) comorbid MDD (SAD=83), and 3) medication use (SAD=89), compared to HC (n=140). Participants with SAD that had unknown comorbidities were retained in this analysis. Due to small sample sizes and collinearity between comorbidities and scan sites we did not perform subgroup analyses for individuals with SAD with 1) comorbid anxiety disorder (SAD=21), 2) comorbid MDD (SAD=24), and 3) medication use (SAD=18) (for sample description see Supplementary Table S2). Last, we assessed the association between AH subfield volumes and SAD symptom severity (as measured by the LSAS).

4.3. Results

Sample characteristics

In the full sample (SAD: n=107; HC: n=140), we found no significant difference in age (SAD: 32.21 years; HC: 34.72 years; t(229.18)=1.86, p-value=0.06), sex (SAD: 42% male; HC: 62%; t(230.05)=0.79, p-value=0.42) and mean years of education (SAD: 14.64 years; HC: 15.12 years; t(133.42)=0.95, p-value=0.34), between individuals with SAD and HCs (see Table 1). Additionally, we found no significant difference in the whole hippocampal volume between SAD and HCs (SAD: 3504.92; HC: 3472.43; t(222.85)=-0.74, p-value=0.45). However, the whole amygdala volume was found to be significantly smaller in individuals with SAD compared to HCs (SAD: 1814.73; HC: 1873.26; t(229.98)=2.23, p-value=0.02). For demographic and clinical characteristics of subgroups see Table 2b.

Group differences

Between group comparisons were conducted on 107 individuals with SAD and 140 HCs (full aggregated sample). With regard to amygdala subfields, individuals with SAD had significantly smaller BA (d= -0.32, p_{FDR}= 0.022), AB (d= -0.42, p_{FDR}= 0.005) and CAT nucleus (d= -0.37, p_{FDR}= 0.014) compared to HCs (after adjustment for sex, age, age-squared, and total volume of AH, in this and subsequent analyses). In contrast, the hippocampal subfields were significantly larger for the CA3 (d= 0.34, p_{FDR}= 0.024), CA4 (d= 0.44, p_{FDR}= 0.007), DG (d= 0.35, p_{FDR}= 0.022) and ML (d= 0.28, p_{FDR}= 0.033) in individuals with SAD in comparison to HCs (Figure 2A/Table 3/ Supplementary Table S3a). A trend was observed for smaller HATA between individuals with SAD and HCs; however, this did not reach the threshold for significance after correction for multiple comparison (d=0.27, p_{FDR}= 0.052). In follow-up exploratory tests of laterality effects for the bilateral subfields between SAD and HCs, trends for a laterality effect (p_{FDR} =0.05-0.1) were detected for some of the subfields across the AH, however none reached significance after correction for multiple comparison (Supplementary Table S3b, and Table S3c and S3d for unadjusted left and right mean volumes).

Subgroup analyses: subfields in relation to clinical characteristics

SAD without comorbid anxiety disorder

Individuals with SAD without comorbid anxiety disorder (n=86) were found to have significantly smaller LA and HATA subfields, in addition to those subfields that were significant in the main analysis (Figure 2B, also see Supplementary Table S4). We observed significantly smaller LA (d= -0.30, p_{FDR}= 0.037), BA (d= -0.34, p_{FDR}= 0.020), AB (d= -0.45, p_{FDR}= 0.005) and CAT nucleus (d= -0.42, p_{FDR}= 0.007) in individuals compared to HCs (n=140). For the hippocampus, with exception of the HATA (d= -0.33, p_{FDR}= 0.027), we observed significantly larger CA3 (d= 0.38, p_{FDR}= 0.020), CA4 (d= 0.47, p_{FDR}= 0.007), DG (d=

0.35, p_{FDR}= 0.023), and ML (*d*= 0.34, p_{FDR}= 0.018) in individuals compared to the HCs. All nuclei had generally medium effect sizes with the CA4 having the largest effect (*d*= 0.47).

SAD without comorbid MDD

When selecting individuals with SAD without comorbid MDD (n=83), we observed that only the volume of AB nucleus of the amygdala remained significantly smaller (*d*= -0.41, p_{FDR}= 0.017) in individuals compared to HCs (Figure 2C, also see Supplementary Table S5).

SAD without psychotropic medication

There was no significant difference between individuals with SAD without medication use (n=89) and HCs (n=140) following adjustment for multiple comparisons (Supplementary Table S6).

SAD symptom severity

We did not observe any associations between symptom severity (measured with the LSAS) and amygdala nor hippocampal subfield volumes (Supplementary Table S7).

4.4. Discussion

The present study is the first to explore neuroanatomical differences in amygdala and hippocampal subfields related to SAD, in a large sample of adult individuals with SAD (n=107) and HC (n=140). In our primary analysis we demonstrated that individuals with SAD have significantly smaller amygdala subfields (BA, AB, and CAT) and significantly larger hippocampal subfields (CA3, CA4, DG, and ML) than HCs. In our subgroup analyses, individuals with SAD without comorbid anxiety disorder had significantly smaller LA and HATA subfields than HCs, in addition to all of the significant subfields in our main analysis. In individuals with SAD without comorbid MDD, only the AB amygdala remained significantly smaller compared to HCs. We did not find an association between subfield volume and psychotropic medication use or symptom severity. While previous, small scale, studies of amygdala and hippocampal volumes in SAD have yielded inconsistent results, we suggest that SAD is associated with distinct volumetric differences in specific subfields that are implicated in sensory information, threat evaluation, and pattern separation and completion. Additionally, the results suggest that subfield volume alterations are influenced by clinical characteristics, possibly contributing to the previous inconsistent findings.

Main analysis (SAD vs HCs): The potential involvement of individual subfields in SAD

Our main findings indicate that individuals with SAD have a smaller bilateral BLA amygdala, specifically in BA and AB subfields, compared to HCs. Post-hoc unilateral analyses showed attenuated differences, with slightly stronger effect sizes for the left compared to right amygdala. Smaller BA and AB volumes have been reported in other anxiety-related disorders with similar or smaller sample sizes as the present study (panic disorder: Asami et al., 2018 (n=38); and PTSD: Morey et al., 2020 (n=149); Zhang et al., 2021 (n=69)). In rodent studies, smaller BLA volume is associated with increased fear response to auditory tones and contextual stimuli (Yang et al., 2008). Considering that the BA, together with the LA and AB nucleus, forms part of the primary sensory input area of the amygdala (LeDoux., 2007), and that the BA is associated with threat evaluation of sensory stimuli (Davis & Whalen, 2001), it is possible that BA and AB structure could impact amygdala-dependent responses to sensory information, resulting in heightened fear and anxiety in SAD. Here, elaborate and sustained anxious appraisals as subserved by the left amygdala could be of particular interest (as discussed in Ocklenburg et al. 2022, Groenewold et al., 2023). Rodent studies suggest that the BLA dually modulates social interactions and anxiety-related behaviours through its projections to downstream targets, thereby controlling an anxiogenic phenotype (Felix-Ortiz et al., 2016; Felix-Ortiz & Tye, 2014; Tye et al., 2011b). In humans, a study investigating amygdala reactivity during social-evaluative learning found hyperactivity in the basolateral amygdala in socially anxious families, suggesting a possible neurobiological endophenotype of SAD (Bas-Hoogendam et al., 2020).

The BLA also projects to various structures of the neighbouring hippocampus including the trisynaptic circuitry made of the CA1, CA3 and DG (Mandyam, 2013). BLA-hippocampal projections have been shown to influence social interactions in rodent studies (Felix-Ortiz & Tye, 2014). In our study we observed significantly larger hippocampal subfield volume in the CA3, CA4, DG and ML, in SAD compared to HCs. These findings are contrary to what has been reported in other anxiety disorders, as smaller CA3/4 and DG were found in PTSD compared to HCs (Hayes et al., 2017; Postel et al., 2021; Wang et al., 2010). A study comparing hippocampal subfields in PTSD to SAD, in the context of childhood trauma, found that only the parasubiculum and HATA were smaller in PTSD (Ahmed-Leitao et al., 2019b). However, this study used smaller sample sizes (n<30) than the present study, and individuals with SAD were not compared to HCs. While our results indicate a trend towards smaller HATA in SAD compared to HCs, this trend did not reach significance in the main analysis.

A possible explanation for hippocampal volume differences (CA3, CA4, DG, and ML) observed in our study may be neuroplasticity and neurogenesis, which are processes that are supported by the CA4 and DG (Malberg et al., 2000; Mandyam, 2013). Studies suggest that adult hippocampal neurogenesis modulates DG-associated pattern separation, which in turn influences the overgeneralization of fear (Aimone et al., 2010; Besnard & Sahay, 2016). Deficits in pattern separation and irregular fear generalization may lead to the inability to differentiate between safe and unsafe stimuli, thereby producing exaggerated fear and anxiety responses to innocuous social stimuli (Besnard & Sahay, 2016). However, it is unclear whether hippocampal subfield volume differences reflect an adaptive response to heightened anxiety through neuroplasticity, or if it is a pre-existing risk factor for SAD, or both. Further investigation ideally using prospective longitudinal designs is required to fully elucidate this relationship.

Subgroup analyses (clinical characteristics)

SAD without comorbid anxiety disorder

In subsequent analyses on subgroups of individuals with SAD without comorbid anxiety disorder (n=86), we observe that in addition to the subfields observed in our main analysis (BA, AB, CAT, CA3, CA4 DG and ML), the LA and HATA were significantly smaller in individuals with SAD without comorbid anxiety disorder, compared to HCs. This suggests that comorbid anxiety disorders may have masked volumetric differences resulting in non-significant findings for the LA (main analysis *d*=-0.18; without comorbid anxiety disorder *d*=-0.30) and HATA (marginally significant) in the main analysis. Our findings are contrary to Groenewold et al. (2023) who found smaller amygdala volumes in SAD individuals with comorbid anxiety disorder, but no differences in individuals without comorbid anxiety disorder (Groenewold et al., 2023). Methodological differences may partly account for the discrepancy, as the present study investigated subfield volumes, after adjusting for differences in whole amygdala volume, whereas the whole amygdala volume was the region of interest in Groenewold et al. (2023).

Of note, the subfields where the differences remained significant (BA, AB, CAT, CA3, CA4 DG and ML) in individuals with SAD without comorbid anxiety displayed similar effect sizes (range: 0.34-0.47) as in the main analysis (range: 0.27-0.43), which suggests that the volumes of these subfields may not be as affected by comorbid anxiety disorder. Our findings highlight the influence of clinical characteristics on select subfield volumes observed in SAD. The HATA is suggested to be a connection for communication between the amygdala and hippocampus (Foo et al.,2017), and other work has indicated that the HATA may influence amygdala responses to memories stored in the hippocampus (Fudge et al., 2012). Furthermore, the LA is thought to play a role in processing sensory information and fear conditioning (Nader et al., 2001). We speculate that LA and HATA dysregulation could disrupt information flow between the hippocampus and adjoining amygdala, thus altering emotional and social processing (Bickart et al., 2014; Foo et al., 2016).

SAD without comorbid MDD

In individuals with SAD without comorbid MDD (n=83), only the AB subfield remained significantly smaller compared to HCs. This might partly be explained by loss of power, since the effect sizes of the relevant subfields were only marginally attenuated (range: 0.20-0.41) and overlapped with those from the main analysis (range: 0.27-0.43). Of note, the applied FDR correction uses an adaptive threshold for significance that varies across sub-analyses. Studies of amygdala subfield volumes in MDD suggest that the lateral and anterior amygdaloid areas are smaller in individuals with MDD compared to HCs (individuals=147; HCs=144)(H. Kim et al., 2021), whilst other studies report null findings (patient=76; HC=77) (Tesen et al., 2022). The present findings of larger hippocampal subfield volumes further contrast with recent meta-analyses which suggest that MDD is associated with smaller hippocampal CA3, CA4 and larger HATA volumes, compared to HCs (Sun et al., 2023). The fact that only the AB remained significant suggests that major depression comorbidity may have an influence on subfield volumes in SAD. It may be possible that SAD-related differences in subfield volumes are more pronounced in individuals with SAD and comorbid MDD, though this subgroup in the present study was too small (N = 24) to test this hypothesis.

SAD without psychotropic medication use

While we did not find a significant difference between individuals with SAD without medication use (n = 89) and HCs, we observed comparable effect sizes (range 0.25-0.36), but in a smaller sample size than the main analysis. This suggests that the difference in the findings were mainly attributable to loss of power in this sub-group analysis. A voxel-based morphometry meta-analysis on grey matter volume alterations in SAD found that the data on clinical characteristics like medication use is inconclusive as mixed effects on grey matter volume are observed in individuals with SAD (Wang et al., 2021). In our study, only a small number of individuals with SAD were taking medication at the time of scan (n= 18), thus restricting our ability to investigate this further.

Symptom severity

Consistent with Groenewold et al. (2023) and Jayakar et al. (2020) investigating total amygdala volumes in SAD, we did not find an association between symptom severity and amygdala subfield volumes. We do note that the symptom severity data was not available for a sizeable proportion of the total sample of cases, and we therefore had less statistical power compared to the main analysis. Future studies with robust SAD symptom severity data, including multiple dimensions of anxious symptomatology, might be able to shed light on the possible association between severity and amygdala and hippocampal subfield volumes.

4.5. Strengths and limitations

Our study has multiple strengths but also limitations. We report on a large, aggregated sample and performed detailed subgroup analyses by investigating individuals with SAD without comorbid anxiety, without MDD, and without medication use. This allowed for observations across different clinical subgroups. However, because we used inherited data (original study Bas-Hoogendam et al., 2017) with small sample sizes for individuals with SAD with comorbid anxiety (n=21), comorbid MDD (n=24), and with medication use (n=18), we were unable to explore subfield differences in these groups. These low rates of comorbidity are the consequence of the inclusion and exclusion criteria of the original studies (Bas-Hoogendam et al., 2017). Additionally, due to limited information available from the original studies, we were unable to explore other possible relevant influences of hippocampal subfield volumes, e.g., childhood adversity (as shown in other studies (Chalavi et al., 2015). Lastly, the caveat for automated segmentation is that studies show relatively low test-retest reliability for certain amygdala and hippocampal subfields, particularly the paralaminar nucleus and hippocampal fissure (compared to other subfields), both of which were included in our analysis (Brown et al., 2020; Quattrini et al., 2020). Further prospective longitudinal studies are required to investigate subfield volumes in SAD over time, including in distinct clinical subgroups.

4.6. Conclusion

Our study demonstrates a distinct profile of subtle volumetric differences subfield volumes in individuals with SAD compared to HCs. Specifically, we found differences in the volumes of subfields that are associated with sensory input and fear/threat evaluation (BLA/AB) in line with previous clinical and preclinical work, as well as in pattern separation (DG) and completion (CA-3), and neurogenesis (DG and ML). Our findings further indicate that clinical characteristics are possible predictors of the volumetric characteristics of certain subfields, specifically those associated with the flow of sensory information between the amygdala and adjoining hippocampus, as noted by smaller LA and HATA in individuals with SAD without comorbid anxiety compared to HCs. The evidence of varying subfield volumes observed in SAD in the present study suggests that the heterogeneous nature of the amygdala and hippocampus may have contributed to the inconsistent volumetric findings reported for these structures in the SAD literature until now, in which the amygdala and hippocampus were considered as a whole. More research, and in particular longitudinal analysis, is required to investigate how amygdala and hippocampal subfield volumes are associated with SAD comorbid with MDD, and whether SAD-related differences in subfield volumes may be influenced by other clinical characteristics such as medication status.

Acknowledgments: Ziphozihle Ntwatwa was supported by the South African National Research Foundation. Janna Marie Bas-Hoogendam received grants from the Dutch Research Council (NWO; Rubicon grant 019.201SG.022), Medical Delta (Talent acceleration grant) and the Dutch Research Agenda (NWA-NeuroLabNL: Small Projects for NWA routes; NWA.1418.22.025). Henk van Steenbergen was supported by a grant from Dutch Research Council (NWO) to Bernhard Hommel. Henk van Steenbergen, J. Nienke Pannekoek and Jean-Paul Fouche were partially supported by the EU 7th Framework Marie Curie Actions International Staff Exchange Scheme grant ‘European and South African Research Network in Anxiety Disorders’ (EUSARNAD). Münster (Jena) collaborators were partially supported by the Collaborative Research Center “Fear, Anxiety, and Anxiety disorders” in Münster, funded by the German Research Society (SFB/TRR-58, project C07 awarded to Thomas Straube) and by the Research Group “Person Perception” in Jena, funded by the German Research Society (grant number STR 987/6-1 to Thomas Straube). The infrastructure for the Netherlands Study of Depression and Anxiety (NESDA) was funded through the Geestkracht programme of the Netherlands Organization for Health Research and Development (ZonMw, grant number 10-000-1002) and is supported by participating universities and mental health care organizations (VU University Medical Center, GGZ inGeest, Arkin, Leiden University Medical Center, GGZ Rivierduinen, University Medical Center Groningen, Lentis, GGZ Friesland, GGZ Drenthe, IQ Healthcare, Netherlands Institute for Health Services Research (NIVEL) and Netherlands Institute of Mental Health and Addiction (Trimbos Institute)). Studies in Umea and Uppsala were supported by the Swedish Research Council and the Swedish Research Council for Health, Working Life and Welfare and the Swedish Brain Foundation. This work was made possible in part by a grant from Carnegie Corporation of New York. The funding sources had no involvement in writing this paper nor in the decision to submit this work for publication. We gratefully acknowledge the contribution of Henk Cremers in collecting data for the LUMC sample and making this available for analysis. We would also like to acknowledge the High-performance computing (HPC) cluster at the University of Cape Town, South Africa.

Declarations: No conflicts of interest

Contributions: Z. Ntwatwa, J. van Honk, J. Ipser, and N. Groenewold designed this study of subfield volumes in social anxiety disorder. The data was acquired by the MEGASAD collaborators and curated by J.M. Bas-Hoogendam, H. van Steenbergen and J.N. Pannekoek. The MEGASAD collaboration was funded by D. Stein and N. van der Wee. Data analysis was performed by Z. Ntwatwa, J. Ipser and N. Groenewold. Data quality control was performed by Z. Ntwatwa, J. Spreckelmeyer, and N. Groenewold. The data was interpreted by Z. Ntwatwa, J. Spreckelmeyer, M. Mufford, J. Ipser, and N. Groenewold. The draft article was written by Z. Ntwatwa, J. Spreckelmeyer, J.M. Bas-Hoogendam, D.J. Stein, J. Ipser, and N. Groenewold and all authors critically reviewed and revised this draft. All authors approved the final version of the manuscript to be published, and no other individuals have made substantial contributions that are not listed as authors. We have disclosed all conflicts of interest.

Data availability: The sample described in this work was aggregated prior to the initiation of the ENIGMA-Anxiety Working Group, and has been contributing to ENIGMA-Anxiety. ENIGMA-Anxiety is open to sharing data from their participating samples to researchers for secondary data analysis. To request access to volumetric, clinical, and demographic data, an analysis plan can be submitted to the ENIGMA-Anxiety Working Group (http://enigma.ini.usc.edu/ongoing/enigma-anxiety/). Data access is contingent on approval by PIs from contributing samples.

4.7. Tables and figures

Table 4.1: SAD and HC sample composition as included in the main analysis

		Initial number of scans [a]			**Excluded after visual QC**		**Included number of scans**		
Country	**Sample ID**	**SAD**	**HC**	**Total**	**Segmentation Fail**	**Visual and Outlier QC**	**SAD**	**HC**	**Total**
Germany	University of Muenster	19	22	41	0	0	19	22	41
Netherlands	NESDA Amsterdam	6	27	33	0	3	6	24	30
	NESDA Groningen	8	11	19	0	0	8	11	19
	NESDA Leiden	8	26	34	0	4	7	23	30
	LUMC Social Anxiety	20	20	40	1	2	20	17	37
South Africa	MRC University of Cape Town	12	11	23	0	5	9	9	18
Sweden	Umeå University	26	23	49	0	0	26	23	49
United States of America	University of Illinois	12	11	23	0	0	12	11	23
Total		111	151	262	1	14	107	140	247

[a] Reported in previous publication (Bas-Hoogendam et al., 2017). [b] Participants excluded based on visual screening for partial or atypical segmentation using an adaptation of the ENIGMA Consortium Quality Control protocol for subcortical and hippocampal subfields (see https://enigma.ini.usc.edu/ongoing/enigma-hippocampal-subfields/).

Table 4.2a: Demographic and clinical characteristics of individuals with SAD (n=107) and HCs (n=140)

	SAD (n=107)		**HCs (n=140)**		**Statistical analysis**		
Demographics	**mean**	**SD**	**mean**	**SD**	**t**	**df**	**p**
Age (years)	32.2	10.43	34.3	10.5	1.86	229.18	0.06
Education level (years)	14.7	3.0	15.2	3.0	0.95	133.42	0.34
	N	**%**	**N**	**%**			
Male	42	39.25	62	44.3	-0.79	230.05	0.43
Clinical characteristics	**mean**	**SD**	**mean**	**SD**			
LSAS score [a]	78.1	19.5	--	--			
Age at onset (years) [b]	14.8	7.1	--	--			
Total amygdala volume	1817.73	202.80	1873.26	205.92	2.23	229.98	0.02
Total hippocampal volume	3504.92	347.11	3472.43	331.78	-0.74	222.85	0.45

[a] Liebowitz Social Anxiety Scale (LSAS). [b] measured using LSAS.

Table 4.2b: Demographic and clinical characteristics of subgroup individuals with SAD

	A: SAD without comorbid anxiety disorder (n=86)	**B: SAD without comorbid MDD (n=83)**	**C: SAD without psychotropic medication (n=89)**	**D: HCs (n=140)**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Age (years)	31.14 (9.81)	31.04 (10.27)	31.34 (10.28)	34.72 (10.51)
Education level (years)	14.85 (2.77)	15.02 (2.65)	14.71 (3.02)	15.12(3.01)
	N (%)	**N (%)**	**N (%)**	**N (%)**
Male	35 (40.69)	34 (40.96)	34 (38.20)	62 (44.28)

Note: mean age of SAD without; (A) comorbid anxiety disorder (n=86), (B) comorbid MDD (n=83), (C) psychotropic medication (n=89), and (D) HCs (n=140).

Table 4.3: Mixed effect size estimates (d), SE, uncorrected and corrected (FDR) p-value for amygdala and hippocampal subfield volumes between individuals with SAD (n=107) and HC (n=140)

	Effect size (mixed-effects *d*)	**Standard Error (SE)**	**P value (corrected (p_{FDR}))**	**P value (uncorrected)**
Amygdala subfields				
Lateral	-0.188	0.13	0.193	0.11
Basal	-0.324	0.13	0.022*	0.01
Accessory basal	-0.419	0.13	0.005*	<0.01
Anterior amygdaloid area	-0.081	0.13	0.592	0.48
Central	-0.224	0.13	0.091	0.04
Medial	-0.168	0.13	0.193	0.11
Cortical	-0.198	0.13	0.106	0.05
Cortico-amygdaloid transition	-0.374	0.13	0.014*	<0.01
Paralaminar	-0.136	0.13	0.381	0.29

Table 4.3 *continued*

Hippocampal subfields	**Effect size (mixed-effects *d*)**	**Standard Error (SE)**	**P value (corrected (p_{FDR}))**	**P value (uncorrected)**
Parasubiculum	0.042	0.13	0.773	0.74
Presubiculum	-0.072	0.13	0.626	0.54
Subiculum	0.170	0.13	0.249	0.16
Cornu ammonis 1	0.026	0.13	0.824	0.82
Cornu ammonis 3	0.345	0.13	0.024*	0.01
Cornu ammonis 4	0.438	0.13	0.007*	<0.01
Dentate gyrus	0.347	0.13	0.022*	0.01
Molecular layer	0.275	0.13	0.033*	0.01
Hippocampus–amygdala transition area	-0.277	0.13	0.052	0.02
Fimbria	-0.060	0.13	0.695	0.63
Hippocampal fissure	0.144	0.13	0.334	0.24
Hippocampal tail	0.150	0.13	0.249	0.17

(*) Denotes significant difference $p<0.05$. Mixed effects sizes (d) with SE. P-values before and after correction for multiple comparison (false discovery rate (FDR).

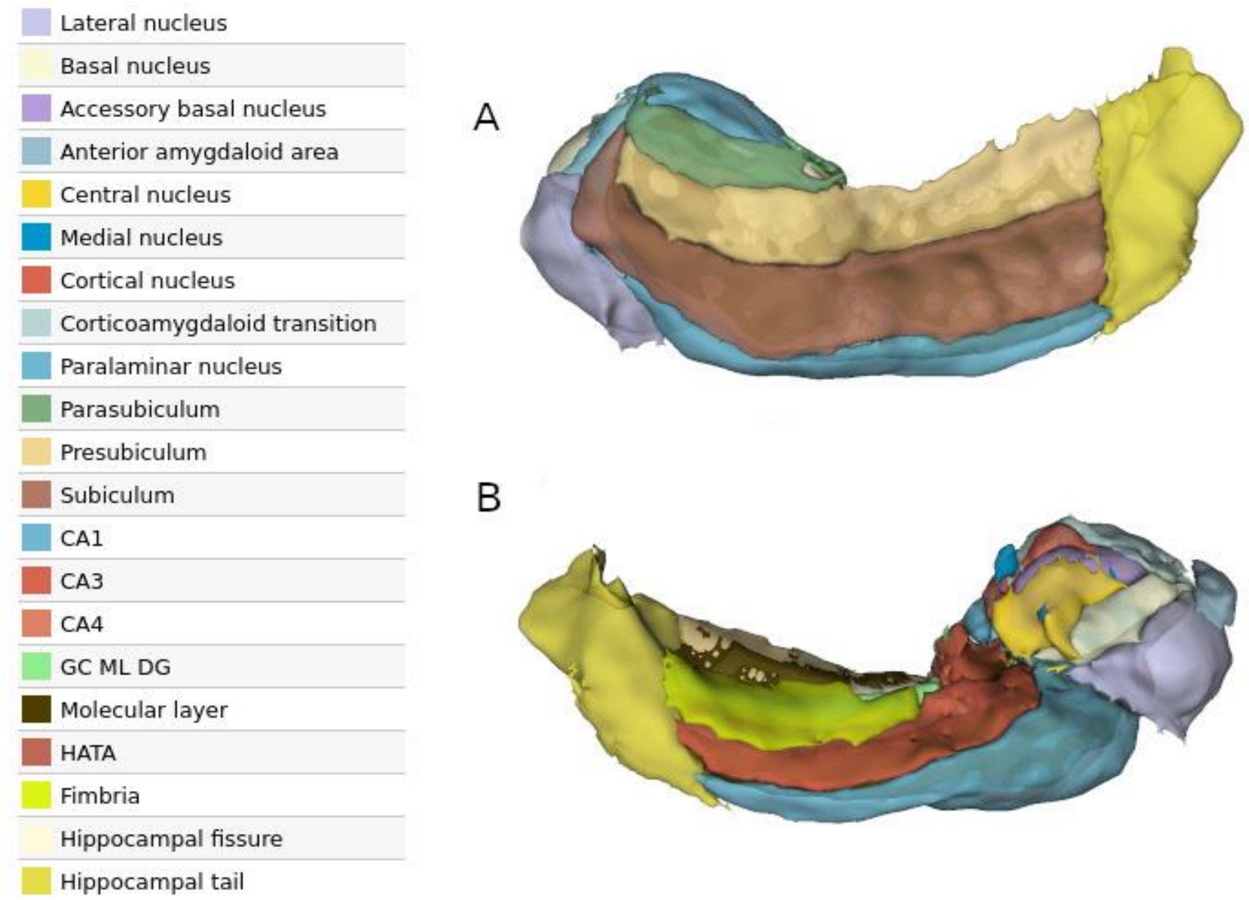

Figure 4.4: Visualisation of amygdala hippocampal Freesurfer subfield segmentation from right hemisphere of single representative (HC). The image was generated using 3DSlicer (https://www.slicer.org/). **A**: Lateral view, **B**: Medial view. Abbreviations: cornu ammonis (CA) sectors, CA1, CA2-3, CA4, granule cell layer of dentate gyrus (DG), molecular layer (ML), hippocampus–amygdala transition area (HATA), corticoamygdaloid transition area (CAT), anterior amygdaloid area (AAA).

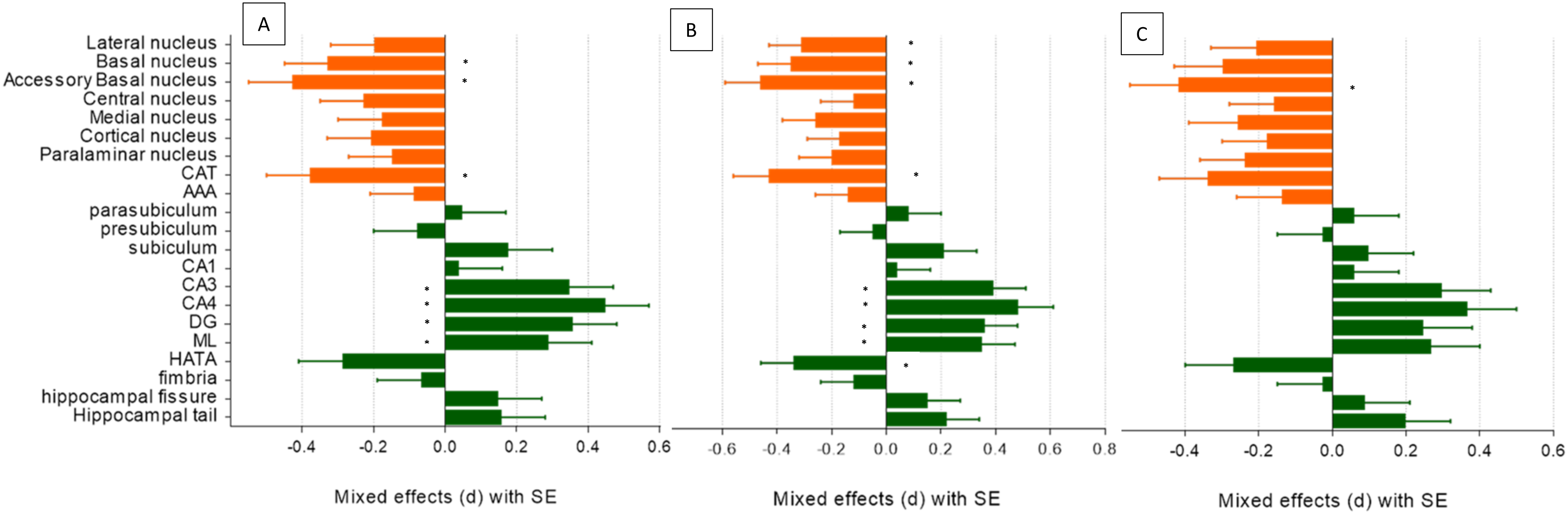

Figure 4.2: **(A)** Mixed effect size estimates (d) for amygdala and hippocampus subfields between **individuals with SAD (n=107) and HCs (n=140)**. **(B)** Mixed effect size estimates (d) for amygdala and hippocampus subfields between **individuals with SAD without comorbid anxiety (n=86) and HCs (n=140)**. **(C)** Mixed effect size estimates (d) for amygdala and hippocampus subfields between **individuals with SAD without comorbid MDD (n=83) and HCs (n=140)**. Data presented with SE. (*) Denotes significant after FDR correction. Amygdala subfields presented in orange; hippocampal subfields presented in green. Abbreviations: cornu ammonis (CA) sectors, CA1, CA2-3, CA4, granule cell layer of dentate gyrus (DG), molecular layer (ML), hippocampus–amygdala transition area (HATA), corticoamygdaloid transition area (CAT), anterior amygdaloid area (AAA).

4.8. References

Ahmed-Leitao, Fatima, David Rosenstein, Melanie Marx, Susanne Young, Kristina Korte, and Soraya Seedat. 2019. "Posttraumatic Stress Disorder, Social Anxiety Disorder and Childhood Trauma: Differences in Hippocampal Subfield Volume." *Psychiatry Research - Neuroimaging* 284(June 2018):45–52. doi: 10.1016/j.pscychresns.2018.12.015.

Aimone, James B., Wei Deng, and Fred H. Gage. 2010. "Adult Neurogenesis: Integrating Theories and Separating Functions The Challenge of New Neurons." *Trends Cogn Sci* 14(7):325–37. doi: 10.1016/j.tics.2010.04.003.Adult.

APA. 2013. *Diagnostic and Statistical Manual of Mental Disorders: DSM-5^{TM}, 5th Ed.* Arlington, VA, US: American Psychiatric Publishing, Inc.

Asami, Takeshi, Ryota Nakamura, Masao Takaishi, Haruhisa Yoshida, Asuka Yoshimi, Thomas J. Whitford, and Yoshio Hirayasu. 2018. "Smaller Volumes in the Lateral and Basal Nuclei of the Amygdala in Patients with Panic Disorder." *PLoS ONE* 13(11):1–8. doi: 10.1371/journal.pone.0207163.

Baldwin, David S., and Dan J. Stein. 2012. "A Joint European and South African Research Network in Anxiety Disorders." *Human Psychopharmacology* 27(1):4–5.

Barnes, Josephine, Gerard R. Ridgway, Jonathan Bartlett, Susie M. D. Henley, Manja Lehmann, Nicola Hobbs, Matthew J. Clarkson, David G. MacManus, Sebastien Ourselin, and Nick C. Fox. 2010. "Head Size, Age and Gender Adjustment in MRI Studies: A Necessary Nuisance?" *NeuroImage* 53(4):1244–55. doi: 10.1016/j.neuroimage.2010.06.025.

Bas-Hoogendam, Janna Marie, Nynke A. Groenewold, Moji Aghajani, Gabrielle F. Freitag, Anita Harrewijn, Kevin Hilbert, Neda Jahanshad, Sophia I. Thomopoulos, Paul M. Thompson, Dick J. Veltman, Anderson M. Winkler, Ulrike Lueken, Daniel S. Pine, Nic J. A. van der Wee, Dan J. Stein, Federica Agosta, Fredrik Åhs, Iseul An, Bianca A. V. Alberton, Carmen Andreescu, Takeshi Asami, Michal Assaf, Suzanne N. Avery, L. Nicholas, Balderston, Jacques P. Barber, Marco Battaglia, Ali Bayram, Katja Beesdo-Baum, Francesco Benedetti, Rachel Berta, Johannes Björkstrand, Jennifer Urbano Blackford, James R. Blair, S. Karina, Blair, Stephanie Boehme, Paolo Brambilla, Katie Burkhouse, Marta Cano, Elisa Canu, Elise M. Cardinale, Narcis Cardoner, Jacqueline A. Clauss, Camilla Cividini, Hugo D. Critchley, Udo, Dannlowski, Jurgen Deckert, Tamer Demiralp, Gretchen J. Diefenbach, Katharina Domschke, Alex Doruyter, Thomas Dresler, Angelika Erhardt, Andreas J. Fallgatter, Lourdes Fañanás, Brandee, Feola, Courtney A. Filippi, Massimo Filippi, Gregory A. Fonzo, Erika E. Forbes, Nathan A. Fox, Mats Fredrikson, Tomas Furmark, Tian Ge, Andrew J. Gerber, Savannah N. Gosnell, Hans J. Grabe, Dominik Grotegerd, Raquel E. Gur, Ruben C. Gur, Catherine J. Harmer, Jennifer Harper, Alexandre Heeren, John Hettema, David Hofmann, Stefan G. Hofmann, Andrea P. Jackowski, Andreas, Jansen, Antonia N. Kaczkurkin, Ellen Kingsley, Tilo Kircher, Milutin Kosti c, Benjamin Kreifelts, Axel Krug, Bart Larsen, Sang Hyuk Lee, Elisabeth J. Leehr, Ellen Leibenluft, Christine Lochner, Eleonora Maggioni, Elena Makovac, Matteo Mancini, Gisele G. Manfro, Kristoffer N. T. Månsson, Frances Meeten, Jarosław Michałowski, Barbara L. Milrod, Andreas Mühlberger, R. Lilianne, Mujica-Parodi, Ana Munjiza, Benson Mwangi, Michael Myers, C. Igor Nenadi, Susanne Neufang, Jared A. Nielsen, Hyuntaek Oh, Cristina Ottaviani, Pedro M. Pan, Spiro P. Pantazatos, P. Martin, Paulus, Koraly Perez-Edgar, Wenceslao Peñate, Michael T. Perino, Jutta Peterburs, Bettina Pfleiderer, K. Luan Phan, Sara Poletti, Daniel Porta-Casteràs, Rebecca B. Price, Jesus Pujol, Andrea, Reinecke, Francisco Rivero, Karin Roelofs, Isabelle Rosso, Philipp Saemann, Ramiro Salas, Giovanni A. Salum, Theodore D. Satterthwaite, Franklin Schneier, Koen R. J. Schruers, Stefan M. Schulz, Hanna Schwarzmeier, Fabian R. Seeger, Jordan W. Smoller, Jair C. Soares, Rudolf Stark, Murray B. Stein, Benjamin Straube, Thomas Straube, Jeffrey R. Strawn, Benjamin Suarez-Jimenez, Boris, Suchan, Chad M. Sylvester, Ardesheer Talati, Erica Tamburo, Rasit Tükel, Odile A. van den Heuvel, Sandra Van der Auwera, Helena van Nieuwenhuizen, Marie José van Tol, Laura S. van Velzen, Carlos Ventura Bort, Robert R. J. M. Vermeiren, Renee M. Visser, Inge Volman, Andre Wannemüller, Julia Wendt, Kathryn E. Werwath, P. Michiel Westenberg, Julian Wiemer, Katharina, Wittfeld, Mon Ju Wu, Yunbo Yang, Anna Zilverstand, Andre Zugman, and Hannah L. Zwiebel. 2022. "ENIGMA-Anxiety Working Group: Rationale for and Organization of Large-Scale Neuroimaging Studies of Anxiety Disorders." *Human Brain Mapping* 43(1):83–112. doi: 10.1002/hbm.25100.

Bas-Hoogendam, Janna Marie, Henk van Steenbergen, J. Nienke Pannekoek, Jean Paul Fouche, Christine Lochner, Coenraad J. Hattingh, Henk R. Cremers, Tomas Furmark, Kristoffer N. T. Månsson, Andreas Frick, Jonas Engman, Carl Johan Boraxbekk, Per Carlbring, Gerhard Andersson, Mats Fredrikson, Thomas Straube, Jutta Peterburs, Heide Klumpp, K. Luan Phan, Karin Roelofs, Dick J. Veltman, Marie José van Tol, Dan J. Stein, and Nic J. A. van der Wee. 2017. "Voxel-Based Morphometry Multi-Center Mega-Analysis of Brain Structure in Social Anxiety Disorder." *NeuroImage: Clinical* 16(May):678–88. doi: 10.1016/j.nicl.2017.08.001.

Bas-Hoogendam, Janna Marie, Henk van Steenbergen, Nic J. A. van der Wee, and P. Michiel Westenberg. 2020. "Amygdala Hyperreactivity to Faces Conditioned with a Social-Evaluative Meaning– a Multiplex, Multigenerational FMRI Study on Social Anxiety Endophenotypes." *NeuroImage: Clinical* 26(October 2019):102247. doi: 10.1016/j.nicl.2020.102247.

Besnard, Antoine, and Amar Sahay. 2016. "Adult Hippocampal Neurogenesis, Fear Generalization, and Stress." *Neuropsychopharmacology* 41(1):24–44. doi: 10.1038/npp.2015.167.

Bickart, Kevin C., Bradford C. Dickerson, and Lisa F. Barrett. 2014. "The Amygdala as a Hub in Brain Networks That Support Social Life." *Neuropsychologia* 63:235–48. doi: 10.1016/j.neuropsychologia.2014.08.013.

Brown, Emma M., Meghan E. Pierce, Dustin C. Clark, Bruce R. Fischl, Juan E. Iglesias, William P. Milberg, Regina E. McGlinchey, and David H. Salat. 2020. "Test-Retest Reliability of FreeSurfer Automated Hippocampal Subfield Segmentation within and across Scanners." *NeuroImage* 210(August 2019):116563. doi: 10.1016/j.neuroimage.2020.116563.

Canada, Kelsey L., Samaah Saifullah, Jennie C. Gardner, Bradley P. Sutton, Monica Fabiani, Gabriele Gratton, Naftali Raz, and Ana M. Daugherty. 2023. "Development and Validation of a Quality Control Procedure for Automatic Segmentation of Hippocampal Subfields." *Hippocampus* (May). doi: 10.1002/hipo.23552.

Chalavi, Sima, Eline M. Vissia, Mechteld E. Giesen, Ellert R. S. Nijenhuis, Nel Draijer, James H. Cole, Paola Dazzan, Carmine M. Pariante, Sarah K. Madsen, Priya Rajagopalan, Paul M. Thompson, Arthur W. Toga, Dick J. Veltman, and Antje A. T. S. Reinders. 2015. "Abnormal Hippocampal Morphology in Dissociative Identity Disorder and Post-Traumatic Stress Disorder Correlates with Childhood Trauma and Dissociative Symptoms." *Human Brain Mapping* 36(5):1692–1704. doi: 10.1002/hbm.22730.

Chen, Lyon W., Delin Sun, Sarah L. Davis, Courtney C. Haswell, Emily L. Dennis, Chelsea A. Swanson, Christopher D. Whelan, Boris Gutman, Neda Jahanshad, Juan Eugenio Iglesias, Paul Thompson,

H. Ryan Wagner, Philipp Saemann, Kevin S. LaBar, and Rajendra A. Morey. 2018. “Smaller Hippocampal CA1 Subfield Volume in Posttraumatic Stress Disorder.” *Depression and Anxiety* 35(11):1018–29. doi: 10.1002/da.22833.

Davis, M., and P. J. Whalen. 2001. “The Amygdala: Vigilance and Emotion.” *Molecular Psychiatry* 6(1):13–34. doi: 10.1038/sj.mp.4000812.

E., Irle, Ruhleder M., Lange C., Seidler-Brandler U., Salzer S., Dechent P., Weniger G., Leibing E., and Leichsenring F. 2010. “Reduced Amygdalar and Hippocampal Size in Adults with Generalized Social Phobia.” *Journal of Psychiatry and Neuroscience* 35(2):126–31. doi: 10.1503/jpn.090041.

Etkin, Amit, Kristen C. Klemenhagen, Joshua T. Dudman, Michael T. Rogan, René Hen, Eric R. Kandel, and Joy Hirsch. 2004. “Individual Differences in Trait Anxiety Predict the Response of the Basolateral Amygdala to Unconsciously Processed Fearful Faces.” *Neuron* 44(6):1043–55. doi: 10.1016/j.neuron.2004.12.006.

Faria, Vanda, Lieuwe Appel, Fredrik Åhs, Clas Linnman, Anna Pissiota, Örjan Frans, Massimo Bani, Paolo Bettica, Emilio M. Pich, Eva Jacobsson, Kurt Wahlstedt, Mats Fredrikson, and Tomas Furmark. 2012. “Amygdala Subregions Tied to SSRI and Placebo Response in with Social Anxiety Disorder.” *Neuropsychopharmacology* 37(10):2222–32. doi: 10.1038/npp.2012.72.

Felix-Ortiz, A. C., A. Burgos-Robles, N. D. Bhagat, C. A. Leppla, and K. M. Tye. 2016. “Bidirectional Modulation of Anxiety-Related and Social Behaviors by Amygdala Projections to the Medial Prefrontal Cortex.” *Neuroscience* 321:197–209. doi: 10.1016/j.neuroscience.2015.07.041.

Felix-Ortiz, Ada C., and Kay M. Tye. 2014. “Amygdala Inputs to the Ventral Hippocampus Bidirectionally Modulate Social Behavior.” *Journal of Neuroscience* 34(2):586–95. doi: 10.1523/JNEUROSCI.4257-13.2014.

Fischl, Bruce, David H. Salat, Evelina Busa, Marilyn Albert, Megan Dieterich, Christian Haselgrove, Andre Van Der Kouwe, Ron Killiany, David Kennedy, Shuna Klaveness, Albert Montillo, Nikos Makris, Bruce Rosen, and Anders M. Dale. 2002. “Whole Brain Segmentation: Automated Labeling of Neuroanatomical Structures in the Human Brain.” *Neuron* 33(3):341–55. doi: 10.1016/S0896-6273(02)00569-X.

Foo, Heidi, Elijah Mak, Russell Jude Chander, Aloysius Ng, Wing Lok Au, Yih Yian Sitoh, Louis C. S. Tan, and Nagaendran Kandiah. 2016. “Associations of Hippocampal Subfields in the Progression of Cognitive Decline Related to Parkinson’s Disease.” *NeuroImage: Clinical* 14:37–42. doi: 10.1016/j.nicl.2016.12.008.

Fudge, J. L., D. M. deCampo, and K. T. Becoats. 2012. “Revisiting the Hippocampal-Amygdala Pathway in Primates: Association with Immature-Appearing Neurons.” *Neuroscience* 212:104–19. doi: 10.1016/j.neuroscience.2012.03.040.

Furmark, Tomas. 2009. “Neurobiological Aspects of Social Anxiety Disorder.” 46(1):5–12.

González-García, Iván, and Maya Visser. 2023. “A Semantic Cognition Contribution to Mood and Anxiety Disorder Pathophysiology.” *Healthcare* 11(6):821. doi: 10.3390/healthcare11060821.

Groenewold, Nynke A., Janna Marie Bas-Hoogendam, Alyssa R. Amod, Max A. Laansma, Laura S. Van Velzen, Moji Aghajani, Kevin Hilbert, Hyuntaek Oh, Ramiro Salas, Andrea P. Jackowski, Pedro M. Pan, Giovanni A. Salum, James R. Blair, Karina S. Blair, Joy Hirsch, Spiro P. Pantazatos, Franklin R. Schneier, Ardesheer Talati, Karin Roelofs, Inge Volman, Laura Blanco-Hinojo, Narcís Cardoner, Jesus Pujol, Katja Beesdo-Baum, Christopher R. K. Ching, Sophia I. Thomopoulos, Andreas Jansen, Tilo Kircher, Axel Krug, Igor Nenadić, Frederike Stein, Udo Dannlowski, Dominik Grotegerd, Hannah Lemke, Susanne Meinert, Alexandra Winter, Michael Erb, Benjamin Kreifelts, Qiyong Gong, Su Lui, Fei Zhu, Benson Mwangi, Jair C. Soares, Mon Ju Wu, Ali Bayram, Mesut Canli, Raşit Tükel, P. Michiel Westenberg, Alexandre Heeren, Henk R. Cremers, David Hofmann, Thomas Straube, Alexander G. G. Doruyter, Christine Lochner, Jutta Peterburs, Marie José Van Tol, Raquel E. Gur, Antonia N. Kaczkurkin, Bart Larsen, Theodore D. Satterthwaite, Courtney A. Filippi, Andrea L. Gold, Anita Harrewijn, André Zugman, Robin Bülow, Hans J. Grabe, Henry Völzke, Katharina Wittfeld, Joscha Böhnlein, Katharina Dohm, Harald Kugel, Elisabeth Schrammen, Peter Zwanzger, Elisabeth J. Leehr, Lisa Sindermann, Tali M. Ball, Gregory A. Fonzo, Martin P. Paulus, Alan Simmons, Murray B. Stein, Heide Klumpp, K. Luan Phan, Tomas Furmark, Kristoffer N. T. Månsson, Amirhossein Manzouri, Suzanne N. Avery, Jennifer Urbano Blackford, Jacqueline A. Clauss, Brandee Feola, Jennifer C. Harper, Chad M. Sylvester, Ulrike Lueken, Dick J. Veltman, Anderson M. Winkler, Neda Jahanshad, Daniel S. Pine, Paul M. Thompson, Dan J. Stein, and Nic J. A. Van der Wee. 2023. "Volume of Subcortical Brain Regions in Social Anxiety Disorder: Mega-Analytic Results from 37 Samples in the ENIGMA-Anxiety Working Group." *Molecular Psychiatry* (December 2022). doi: 10.1038/s41380-022-01933-9.

Hayes, Jasmeet P., Scott Hayes, Danielle R. Miller, Ginette Lafleche, Mark W. Logue, and Mieke Verfaellie. 2017. "Automated Measurement of Hippocampal Subfields in PTSD: Evidence for Smaller Dentate Gyrus Volume." *Journal of Psychiatric Research* 95:247–52. doi: 10.1016/j.jpsychires.2017.09.007.

Hortensius, Ruud, David Terburg, Barak Morgan, Dan J. Stein, Jack van Honk, and Beatrice de Gelder. 2016. "The Role of the Basolateral Amygdala in the Perception of Faces in Natural Contexts." *Philosophical Transactions of the Royal Society B: Biological Sciences* 371(1693). doi: 10.1098/rstb.2015.0376.

Irle, E., Ruhleder, M., Lange, C., Seidler-Brandler, U., Salzer, S., Dechent, P., ... & Leichsenring, F. (2010). Reduced amygdalar and hippocampal size in adults with generalized social phobia. *Journal of psychiatry and neuroscience*, *35*(2), 126-131.

Iglesias, Juan Eugenio, Jean C. Augustinack, Khoa Nguyen, Christopher M. Player, Allison Player, Michelle Wright, Nicole Roy, and Matthew P. Frosch. 2015. "A Computational Atlas of the Hippocampal Formation Using Ex Vivo , Ultra-High Resolution MRI." *NeuroImage* 115–37.

Kenward, Michael G., and James H. Roger. 1997. "Small Sample Inference for Fixed Effects from Restricted Maximum Likelihood." *Biometrics* 53(3):983. doi: 10.2307/2533558.

Kim, Hyeyoung, Kyu Man Han, Kwan Woo Choi, Woo Suk Tae, Wooyoung Kang, Youbin Kang, Aram Kim, and Byung Joo Ham. 2021. "Volumetric Alterations in Subregions of the Amygdala in Adults with Major Depressive Disorder." *Journal of Affective Disorders* 295(June):108–15. doi: 10.1016/j.jad.2021.08.012.

Koyuncu, Ahmet, Ezgi İnce, Erhan Ertekin, and Raşit Tükel. 2019. "Comorbidity in Social Anxiety Disorder: Diagnostic and Therapeutic Challenges." *Drugs in Context* 8:1–13. doi: 10.7573/dic.212573.

Lecrubier, Y., D. V. Sheehan, E. Weiller, P. Amorim, I. Bonora, K. Harnett Sheehan, J. Janavs, and G. C. Dunbar. 1997. "The Mini International Neuropsychiatric Interview (MINI). A Short Diagnostic Structured Interview: Reliability and Validity According to the CIDI." *European Psychiatry*

12(5):224–31. doi: 10.1016/S0924-9338(97)83296-8.

Lecrubier, Y., H. U. Wittchen, C. Faravelli, J. Bobes, A. Patel, and M. Knapp. 2000. "A European Perspective on Social Anxiety Disorder." *European Psychiatry* 15(1):5–16. doi: 10.1016/S0924-9338(00)00216-9.

Liao, Wei, Qiang Xu, Dante Mantini, Jurong Ding, João Paulo MacHado-De-Sousa, Jaime E. C. Hallak, Clarissa Trzesniak, Changjian Qiu, Ling Zeng, Wei Zhang, José Alexandre S. Crippa, Qiyong Gong, and Huafu Chen. 2011. "Altered Gray Matter Morphometry and Resting-State Functional and Structural Connectivity in Social Anxiety Disorder." *Brain Research* 1388:167–77. doi: 10.1016/j.brainres.2011.03.018.

Machado-de-Sousa, João Paulo, Flávia De Lima Osório, Andrea P. Jackowski, Rodrigo A. Bressan, Marcos H. N. Chagas, Nelson Torro-Alves, André L. D. DePaula, José A. S. Crippa, and Jaime E. C. Hallak. 2014. "Increased Amygdalar and Hippocampal Volumes in Young Adults with Social Anxiety." *PLoS ONE* 9(2):1–5. doi: 10.1371/journal.pone.0088523.

Mack, Simon, Frank Jacobi, Katja Beesdo-Baum, Anja Gerschler, Jens Strehle, Michael Höfler, Markus A. Busch, Ulrike Maske, Ulfert Hapke, Wolfgang Gaebel, Jürgen Zielasek, Wolfgang Maier, and Hans Ulrich Wittchen. 2015. "Functional Disability and Quality of Life Decrements in Mental Disorders: Results from the Mental Health Module of the German Health Interview and Examination Survey for Adults (DEGS1-MH)." *European Psychiatry* 30(6):793–800. doi: 10.1016/j.eurpsy.2015.06.003.

Malberg, Jessica E., Amelia J. Eisch, Eric J. Nestler, and Ronald S. Duman. 2000. "Chronic Antidepressant Treatment Increases Neurogenesis in Adult Rat Hippocampus." *Journal of Neuroscience* 20(24):9104–10. doi: 10.1523/jneurosci.20-24-09104.2000.

Mandyam, Chitra D. 2013. "The Interplay between the Hippocampus and Amygdala in Regulating Aberrant Hippocampal Neurogenesis during Protracted Abstinence from Alcohol Dependence." *Frontiers in Psychiatry* 4(JUN). doi: 10.3389/fpsyt.2013.00061.

Mennin, Douglas S., David M. Fresco, Richard G. Heimberg, Franklin R. Schneier, Sharon O. Davies, and Michael R. Liebowitz. 2002. "Screening for Social Anxiety Disorder in the Clinical Setting: Using the Liebowitz Social Anxiety Scale." *Journal of Anxiety Disorders* 16(6):661–73. doi: 10.1016/S0887-6185(02)00134-2.

Michely, Jochen, Francesco Rigoli, Robb B. Rutledge, Tobias U. Hauser, and Raymond J. Dolan. 2020. "Distinct Processing of Aversive Experience in Amygdala Subregions." *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging* 5(3):291–300. doi: 10.1016/j.bpsc.2019.07.008.

Morey, Rajendra A., Emily K. Clarke, Courtney C. Haswell, Rachel D. Phillips, Ashley N. Clausen, Mary S. Mufford, Zeynep Saygin, Mira Brancu, Jean C. Beckham, Patrick S. Calhoun, Eric Dedert, Eric B. Elbogen, John A. Fairbank, Robin A. Hurley, Jason D. Kilts, Nathan A. Kimbrel, Angela Kirby, Christine E. Marx, Scott D. McDonald, Scott D. Moore, Jennifer C. Naylor, Jared Rowland, Cindy Swinkels, Steven T. Szabo, Katherine H. Taber, Larry A. Tupler, Elizabeth E. van Voorhees, Ruth E. Yoash-Gantz, H. Ryan Wagner, and Kevin S. LaBar. 2020. "Amygdala Nuclei Volume and Shape in Military Veterans With Posttraumatic Stress Disorder." *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging* 5(3):281–90. doi: 10.1016/j.bpsc.2019.11.016.

Nader, K., P. Majidishad, P. Amorapanth, and J. E. LeDoux. 2001. "Damage to the Lateral and Central, but Not Other, Amygdaloid Nuclei Prevents the Acquisition of Auditory Fear Conditioning."

Learning and Memory 8(3):156–63. doi: 10.1101/lm.38101.

Nakagawa, Shinichi, and Innes C. Cuthill. 2007. "Effect Size, Confidence Interval and Statistical Significance: A Practical Guide for Biologists." *Biological Reviews* 82(4):591–605. doi: 10.1111/j.1469-185X.2007.00027.x.

Nordenskjöld, Richard, Filip Malmberg, Elna Marie Larsson, Andrew Simmons, Samantha J. Brooks, Lars Lind, Håkan Ahlström, Lars Johansson, and Joel Kullberg. 2013. "Intracranial Volume Estimated with Commonly Used Methods Could Introduce Bias in Studies Including Brain Volume Measurements." *NeuroImage* 83:355–60. doi: 10.1016/j.neuroimage.2013.06.068.

Ocklenburg, Sebastian, Jutta Peterburs, and Annakarina Mundorf. 2022. "Hemispheric Asymmetries in the Amygdala: A Comparative Primer." *Progress in Neurobiology* 214(May):102283. doi: 10.1016/j.pneurobio.2022.102283.

Postel, Charlotte, Alison Mary, Jacques Dayan, Florence Fraisse, Thomas Vallée, Bérengère Guillery-Girard, Fausto Viader, Vincent de la Sayette, Denis Peschanski, Francis Eustache, and Pierre Gagnepain. 2021. "Variations in Response to Trauma and Hippocampal Subfield Changes." *Neurobiology of Stress* 15. doi: 10.1016/j.ynstr.2021.100346.

Quattrini, Giulia, Michela Pievani, Jorge Jovicich, Marco Aiello, Núria Bargalló, Frederik Barkhof, David Bartres-Faz, Alberto Beltramello, Francesca B. Pizzini, Olivier Blin, Regis Bordet, Massimo Caulo, Manos Constantinides, Mira Didic, Antonios Drevelegas, Antonio Ferretti, Ute Fiedler, Piero Floridi, Hélène Gros-Dagnac, Tilman Hensch, Karl Titus Hoffmann, Joost P. Kuijer, Renaud Lopes, Camillo Marra, Bernhard W. Müller, Flavio Nobili, Lucilla Parnetti, Pierre Payoux, Agnese Picco, Jean Philippe Ranjeva, Luca Roccatagliata, Paolo M. Rossini, Marco Salvatore, Peter Schonknecht, Björn H. Schott, Julien Sein, Andrea Soricelli, Roberto Tarducci, Magda Tsolaki, Pieter J. Visser, Jens Wiltfang, Jill C. Richardson, Giovanni B. Frisoni, and Moira Marizzoni. 2020. "Amygdalar Nuclei and Hippocampal Subfields on MRI: Test-Retest Reliability of Automated Volumetry across Different MRI Sites and Vendors." *NeuroImage* 218(May). doi: 10.1016/j.neuroimage.2020.116932.

Robins, Lee N., John Wing, Hans Ulrich Wittchen, John E. Helzer, Thomas F. Babor, Jay Burke, Anne Farmer, Assen Jablenski, Roy Pickens, Darrel A. Regier, Norman Sartorius, and Leland H. Towle. 1988. "The Composite International Diagnostic Interview: An Epidemiologic Instrument Suitable for Use in Conjunction With Different Diagnostic Systems and in Different Cultures." *Archives of General Psychiatry* 45(12):1069–77. doi: 10.1001/archpsyc.1988.01800360017003.

Sämann, Philipp G., Juan Eugenio Iglesias, Boris Gutman, Dominik Grotegerd, Ramona Leenings, Claas Flint, Udo Dannlowski, Emily K. Clarke-Rubright, Rajendra A. Morey, Theo G. M. van Erp, Christopher D. Whelan, Laura K. M. Han, Laura S. van Velzen, Bo Cao, Jean C. Augustinack, Paul M. Thompson, Neda Jahanshad, and Lianne Schmaal. 2022. "FreeSurfer-Based Segmentation of Hippocampal Subfields: A Review of Methods and Applications, with a Novel Quality Control Procedure for ENIGMA Studies and Other Collaborative Efforts." *Human Brain Mapping* 43(1):207–33. doi: 10.1002/hbm.25326.

Sargolzaei, Saman, Arman Sargolzaei, Mercedes Cabrerizo, Gang Chen, Mohammed Goryawala, Shirin Noei, Qi Zhou, Ranjan Duara, Warren Barker, and Malek Adjouadi. 2015. "A Practical Guideline for Intracranial Volume Estimation in Patients with Alzheimer's Disease." *BMC Bioinformatics* 16(7):1–10. doi: 10.1186/1471-2105-16-S7-S8.

Saygin, Z. M., D. Kliemann, J. E. Iglesias, A. J. W. van der Kouwe, E. Boyd, M. Reuter, A. Stevens, K. Van

Leemput, A. McKee, M. P. Frosch, B. Fischl, and J. C. Augustinack. 2017. “High-Resolution Magnetic Resonance Imaging Reveals Nuclei of the Human Amygdala: Manual Segmentation to Automatic Atlas.” *NeuroImage* 155(April):370–82. doi: 10.1016/j.neuroimage.2017.04.046.

Schmitz-Koep, Benita, Juliana Zimmermann, Aurore Menegaux, Rachel Nuttall, Josef G. Bäuml, Sebastian C. Schneider, Marcel Daamen, Henning Boecker, Claus Zimmer, Dieter Wolke, Peter Bartmann, Dennis M. Hedderich, and Christian Sorg. 2021. “Within Amygdala: Basolateral Parts Are Selectively Impaired in Premature-Born Adults.” *NeuroImage: Clinical* 31(April). doi: 10.1016/j.nicl.2021.102780.

Shi, Hu-Jiang, Shuang Wang, Xin-Ping Wang, Rui-Xin Zhang, and Li-Juan Zhu. 2023. “Hippocampus: Molecular, Cellular, and Circuit Features in Anxiety.” *Neuroscience Bulletin* 39(6):1009–26. doi: 10.1007/s12264-023-01020-1.

Stein. 2008. “Socail Anxiety Disorder.Pdf.” *Elsevier* 371(9618):1115–25. doi: 10.1016/S0140-6736(08)60488-2.

Sun, Yuan, Na Hu, Mingqi Wang, Lu Lu, Chunyan Luo, Biqiu Tang, Chenyang Yao, John A. Sweeney, Qiyong Gong, Changjian Qiu, and Su Lui. 2023. “Hippocampal Subfield Alterations in Schizophrenia and Major Depressive Disorder: A Systematic Review and Network Meta-Analysis of Anatomic MRI Studies.” *Journal of Psychiatry & Neuroscience : JPN* 48(1):E34–49. doi: 10.1503/jpn.220086.

Syal, Supriya, Coenraad J. Hattingh, Jean Paul Fouché, Bruce Spottiswoode, Paul D. Carey, Christine Lochner, and Dan J. Stein. 2012. “Grey Matter Abnormalities in Social Anxiety Disorder: A Pilot Study.” *Metabolic Brain Disease* 27(3):299–309. doi: 10.1007/s11011-012-9299-5.

Tesen, Hirofumi, Keita Watanabe, Naomichi Okamoto, Atsuko Ikenouchi, Ryohei Igata, Yuki Konishi, Shingo Kakeda, and Reiji Yoshimura. 2022. “Volume of Amygdala Subregions and Clinical Manifestations in Patients With First-Episode, Drug-Naïve Major Depression.” *Frontiers in Human Neuroscience* 15(January):1–11. doi: 10.3389/fnhum.2021.780884.

Thompson, Paul M., Neda Jahanshad, Christopher R. K. Ching, Lauren E. Salminen, Sophia I. Thomopoulos, Joanna Bright, Bernhard T. Baune, Sara Bertolín, Janita Bralten, Willem B. Bruin, Robin Bülow, Jian Chen, Yann Chye, Udo Dannlowski, Carolien G. F. de Kovel, Gary Donohoe, Lisa T. Eyler, Stephen V. Faraone, Pauline Favre, Courtney A. Filippi, Thomas Frodl, Daniel Garijo, Yolanda Gil, Hans J. Grabe, Katrina L. Grasby, Tomas Hajek, Laura K. M. Han, Sean N. Hatton, Kevin Hilbert, Tiffany C. Ho, Laurena Holleran, Georg Homuth, Norbert Hosten, Josselin Houenou, Iliyan Ivanov, Tianye Jia, Sinead Kelly, Marieke Klein, Jun Soo Kwon, Max A. Laansma, Jeanne Leerssen, Ulrike Lueken, Abraham Nunes, Joseph O’ Neill, Nils Opel, Fabrizio Piras, Federica Piras, Merel C. Postema, Elena Pozzi, Natalia Shatokhina, Carles Soriano-Mas, Gianfranco Spalletta, Daqiang Sun, Alexander Teumer, Amanda K. Tilot, Leonardo Tozzi, Celia van der Merwe, Eus J. W. Van Someren, Guido A. van Wingen, Henry Völzke, Esther Walton, Lei Wang, Anderson M. Winkler, Katharina Wittfeld, Margaret J. Wright, Je Yeon Yun, Guohao Zhang, Yanli Zhang-James, Bhim M. Adhikari, Ingrid Agartz, Moji Aghajani, André Aleman, Robert R. Althoff, Andre Altmann, Ole A. Andreassen, David A. Baron, Brenda L. Bartnik-Olson, Janna Marie Bas-Hoogendam, Arielle R. Baskin-Sommers, Carrie E. Bearden, Laura A. Berner, Premika S. W. Boedhoe, Rachel M. Brouwer, Jan K. Buitelaar, Karen Caeyenberghs, Charlotte A. M. Cecil, Ronald A. Cohen, James H. Cole, Patricia J. Conrod, Stephane A. De Brito, Sonja M. C. de Zwarte, Emily L. Dennis, Sylvane Desrivieres, Danai Dima, Stefan Ehrlich, Carrie Esopenko, Graeme Fairchild, Simon E. Fisher, Jean Paul Fouche, Clyde Francks, Sophia Frangou, Barbara Franke, Hugh P. Garavan, David C. Glahn, Nynke A. Groenewold, Tiril P. Gurholt, Boris A. Gutman, Tim Hahn, Ian H. Harding, Dennis Hernaus, Derrek P. Hibar, Frank G. Hillary, Martine Hoogman, Hilleke E. Hulshoff Pol, Maria Jalbrzikowski, George A. Karkashadze, Eduard T. Klapwijk, Rebecca C. Knickmeyer, Peter Kochunov, Inga K. Koerte, Xiang Zhen Kong, Sook Lei Liew, Alexander P. Lin, Mark W. Logue, Eileen Luders, Fabio Macciardi, Scott Mackey, Andrew R. Mayer, Carrie R. McDonald, Agnes B. McMahon, Sarah E. Medland, Gemma Modinos, Rajendra A. Morey, Sven C. Mueller, Pratik Mukherjee, Leyla Namazova-Baranova, Talia M. Nir, Alexander Olsen, Peristera Paschou, Daniel S. Pine, Fabrizio Pizzagalli, Miguel E. Rentería, Jonathan D. Rohrer, Philipp G. Sämann, Lianne Schmaal, Gunter Schumann, Mark S. Shiroishi, Sanjay M. Sisodiya, Dirk J. A. Smit, Ida E. Sønderby, Dan J. Stein, Jason L. Stein, Masoud Tahmasian, David F. Tate, Jessica A. Turner, Odile A. van den Heuvel, Nic J. A. van der Wee, Ysbrand D. van der Werf, Theo G. M. van Erp, Neeltje E. M. van Haren, Daan van Rooij, Laura S. van Velzen, Ilya M. Veer, Dick J. Veltman, Julio E. Villalon-Reina, Henrik Walter, Christopher D. Whelan, Elisabeth A. Wilde, Mojtaba Zarei, and Vladimir Zelman. 2020. "ENIGMA and Global Neuroscience: A Decade of Large-Scale Studies of the Brain in Health and Disease across More than 40 Countries." *Translational Psychiatry* 10(1):1–28. doi: 10.1038/s41398-020-0705-1.

Tye, Kay M., Rohit Prakash, Sung Yon Kim, Lief E. Fenno, Logan Grosenick, Hosniya Zarabi, Kimberly R. Thompson, Viviana Gradinaru, Charu Ramakrishnan, and Karl Deisseroth. 2011. "Amygdala Circuitry Mediating Reversible and Bidirectional Control of Anxiety." *Nature* 471(7338):358–62. doi: 10.1038/nature09820.

Wang, Xiuli, Bochao Cheng, Song Wang, Fengmei Lu, Ya Luo, Xipeng Long, and Di Kong. 2021. "Distinct Grey Matter Volume Alterations in Adult Patients with Panic Disorder and Social Anxiety Disorder: A Systematic Review and Voxel-Based Morphometry Meta-Analysis." *Journal of Affective Disorders* 281(8):805–23. doi: 10.1016/j.jad.2020.11.057.

Wang, Zhen, Thomas C. Neylan, Susanne G. Mueller, Maryann Lenoci, Diana Truran, Charles R. Marmar, Michael W. Weiner, and Norbert Schuff. 2010. "Magnetic Resonance Imaging of Hippocampal Subfields in Posttraumatic Stress Disorder." *Archives of General Psychiatry* 67(3):296–303. doi: 10.1001/archgenpsychiatry.2009.205.

De Wit, Stella J., Pino Alonso, Lizanne Schweren, David Mataix-Cols, Christine Lochner, José M. Menchón, Dan J. Stein, Jean Paul Fouche, Carles Soriano-Mas, Joao R. Sato, Marcelo Q. Hoexter, Damiaan Denys, Takashi Nakamae, Seiji Nishida, Jun Soo Kwon, Joon Hwan Jang, Geraldo F. Busatto, Narcís Cardoner, Danielle C. Cath, Kenji Fukui, Wi Hoon Jung, Sung Nyun Kim, Euripides C. Miguel, Jin Narumoto, Mary L. Phillips, Jesus Pujol, Peter L. Remijnse, Yuki Sakai, Na Young Shin, Kei Yamada, Dick J. Veltman, and Odile A. Van Den Heuvel. 2014. "Multicenter Voxel-Based Morphometry Mega-Analysis of Structural Brain Scans in Obsessive-Compulsive Disorder." *American Journal of Psychiatry* 171(3):340–49. doi: 10.1176/appi.ajp.2013.13040574.

Yang, Rebecca J., Khyobeni Mozhui, Rose Marie Karlsson, Heather A. Cameron, Robert W. Williams, and Andrew Holmes. 2008. "Variation in Mouse Basolateral Amygdala Volume Is Associated with Differences in Stress Reactivity and Fear Learning." *Neuropsychopharmacology* 33(11):2595–2604. doi: 10.1038/sj.npp.1301665.

Yoav Benjamini, and Yosef Hochberg. 1995. "Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing." *Journal of the Royal Statistical Society. Series B (Methodological)* 57(1):289–300.

Zhang, Lianqing, Lu Lu, Xuan Bu, Hailong Li, Shi Tang, Yingxue Gao, Kaili Liang, Suming Zhang, Xinyue Hu, Yanlin Wang, Lei Li, Xinyu Hu, Kelvin O. Lim, Qiyong Gong, and Xiaoqi Huang. 2021.

"Alterations in Hippocampal Subfield and Amygdala Subregion Volumes in Posttraumatic Subjects with and without Posttraumatic Stress Disorder." *Human Brain Mapping* 42(7):2147–58. doi: 10.1002/hbm.25356.

4.9. Additional analysis: diagnosis by age interaction in SAD

To assess the influence of chronological age on the association between hippocampal and amygdala subfield volumes and SAD, a diagnosis-by-age interaction term was included in the mixed effects model. There was no significant nor trend interaction between chronological age and subfield volumes in individuals with SAD (n=107) Table 4.4, page 162. Additionally, the relationship between chronological age and the subfields that had significant group differences (see Result section 4.3) was nonlinear (Figure 4.3-4.4). Although findings in the literature concerning age effects on hippocampal and amygdala subfield volumes are mixed (Kurth et al., 2017, 2019), our null findings suggest that chronological age did not have an influence on subfield volumes in our sample of SAD. Further large-scale analysis is required to replicate our findings.

Table 4.4: Additional analysis, diagnosis-by-age in SAD

SAD (n=107), HC (n=140)	**rME***	**P value**	**P value corrected (p_{FDR})**
Amygdala subfields			
Lateral	0.063	0.277	0.797
Basal	0.018	0.754	0.797
Accessory Basal	-0.063	0.257	0.797
AAA	0.075	0.190	0.797
Central	-0.023	0.662	0.797
Medial	-0.052	0.321	0.797
Cortical	-0.082	0.100	0.797
CAT	-0.045	0.452	0.797
Hippocampal subfields			
Paralaminar	-0.011	0.869	0.869
Parasubiculum	0.085	0.165	0.797
Presubiculum	0.045	0.440	0.797
Subiculum	-0.071	0.241	0.797
CA 1	0.036	0.530	0.797
CA 3	-0.034	0.588	0.797
CA 4	-0.028	0.664	0.797
DG	-0.021	0.727	0.797
ML	-0.042	0.428	0.797
HATA	0.102	0.081	0.797
Fimbria	0.032	0.596	0.797
Hippocampal fissure	-0.041	0.501	0.797
Hippocampal tail	-0.016	0.759	0.797

*rME = root mean square error

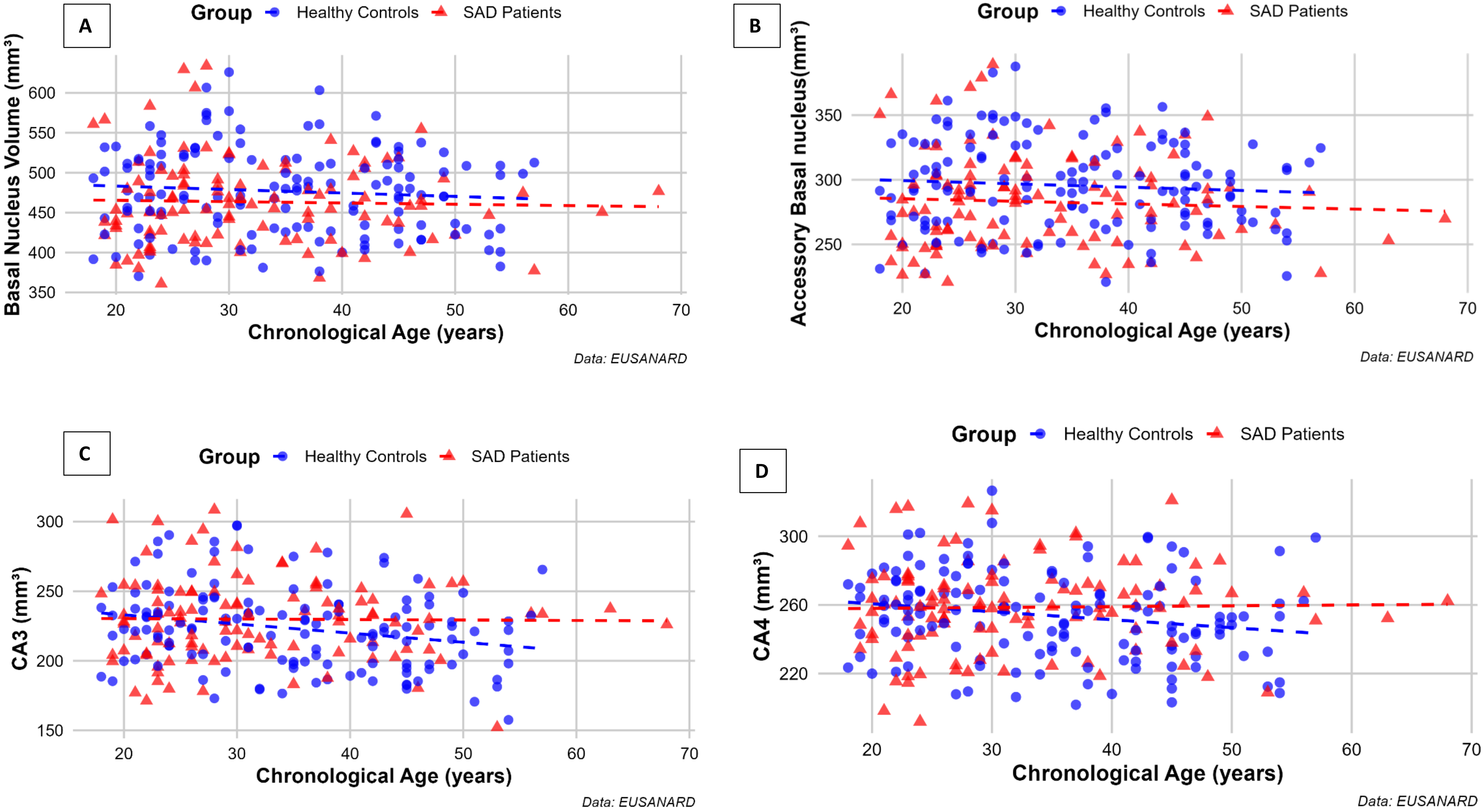

Figure 4.2: Scatterplot depicting the relationship between chronological age and (A) Basal nucleus, (B) Accessory basal, (C) CA3, and (D) CA4 volume.

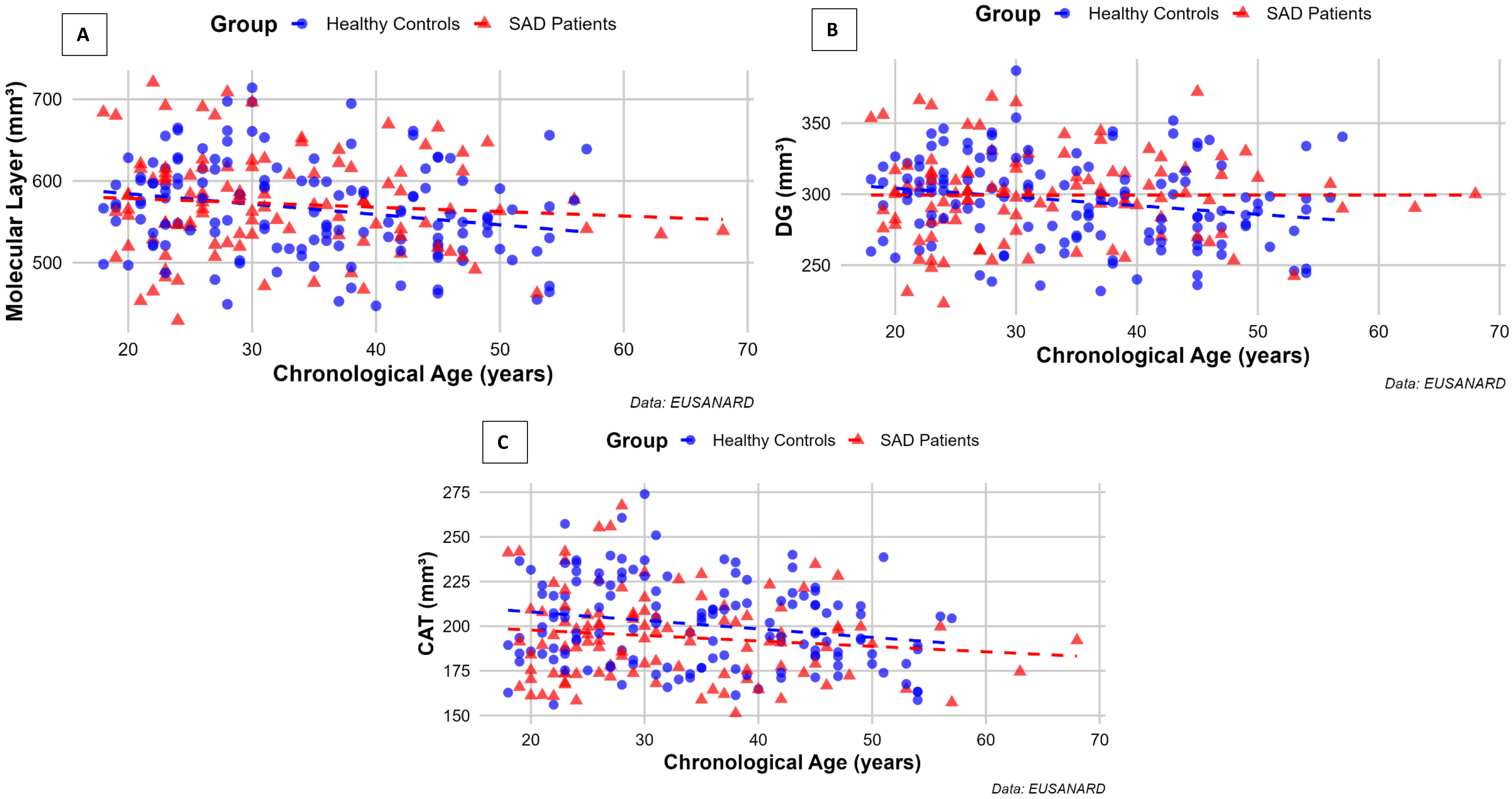

Figure 4.3: Scatterplot depicting the relationship between chronological age and (A) Molecular layer, (B) DG, (C) CAT volume.

4.10. References

Kurth, F., Cherbuin, N., & Luders, E. (2017). The impact of aging on subregions of the hippocampal complex in healthy adults. *NeuroImage*, *163*, 296–300. https://doi.org/10.1016/j.neuroimage.2017.09.016

Kurth, F., Cherbuin, N., & Luders, E. (2019). Age but no sex effects on subareas of the amygdala. *Human Brain Mapping*, *40*(6), 1697–1704. https://doi.org/10.1002/hbm.24481

CHAPTER 5: Investigation of whole and regional brain aging in obsessive-compulsive disorder and its association with clinical characteristics.

In chapter 3, I investigated hippocampal and amygdala subfield volumes in OCD as both of these structures have been implicated in anxiety and related disorders. Moreover, there is some evidence to suggest that these structures are particularly vulnerable to accentuated aging in healthy and neurodegenerative conditions (Fjell et al., 2013; Pfefferbaum et al., 2013; Walhovd et al., 2005, 2011). In chapter 3, in a separate analysis, I used interaction terms (particularly diagnosis-by-age) to test whether chronological age moderated the relationship between OCD diagnosis and subfield volume. Based on the literature, it was expected that chronological age would impact subfield volumes, especially for the hippocampus (Kurth et al., 2017, 2019; Pini et al., 2016).

The age interaction analysis conducted in chapter 3 found no evidence to support the moderation of chronological age on subfield volumes in individuals with OCD. However, as discussed in the postscript of chapter 3, there was a trend observed for the hippocampal CA1 and the HATA, although, after correction for multiple comparisons, both of these subfields did not reach significance. Building upon this analysis, in the present chapter 5, I use a previously trained machine learning algorithm to investigate brain-PAD in individuals with OCD compared to HCs. I also conducted a partial correlation analysis to investigate whether subfield volumes are correlated to brain-PADs within the groups (OCD and HCs).

5.1. Introduction

OCD, a common psychiatric disorder characterized by persistent intrusive thoughts (obsessions) and/or repetitive ritualistic behaviours (compulsions) (APA, 2013) has been associated with increased risk of age-related diseases and premature mortality. In a nationwide longitudinal study, Meier et al. (2016) found that individuals with OCD had a significantly higher incidence of natural or unnatural deaths in the general population, even after excluding individuals with comorbid anxiety disorders, depression, or substance use disorders (Meier et al., 2016). A population and sibling comparison study with long-term follow-up (n=12,497,002) found that individuals with OCD had an increased risk of metabolic (e.g., obesity, type 2 diabetes mellitus) or cardiovascular disorders compared with the general population and their full siblings (without OCD) (Isomura et al., 2018).

Very few studies have investigated accentuated cellular and molecular biological aging in OCD. Kang et al. (2021) found shorter TL and altered mitochondrial DNA in individuals with OCD (n=235) and HCs (n=234), which was also associated with a significant increase in systemic inflammation (Kang et al., 2021). In adolescents, OCD is associated with an increase in oxidative status and a decrease in antioxidant profile (OCD n=28; HCs n=36) (Kandemir et al., 2013; Selek et al., 2008). Despite the initial evidence of cellular and molecular accentuated biological aging in OCD, studies suggest that indicators such as TL have limited predictive and deterministic value when used as surrogate indicators for organ-specific aging, including organs like the brain (Franke et al., 2020; Han et al., 2019). In a large population-based study in healthy aging adults (n=1960), King et al. (2014) demonstrated that the association between shorter TL and hippocampal and amygdala volume was small, suggesting a weak or limited correlation between TL and these brain regions within a certain age range (median age of 50 years; age range between 42 years and 58 years old) (King et al., 2014).

Although limited, there is some structural neuroimaging evidence of age-related differences in brain structure in OCD. Hawco et al. (2017) found white matter thinning with increasing chronological age, specifically in the left superior longitudinal fasciculus in individuals with OCD (within the group) (Hawco et al., 2017). Furthermore, Fouche et al. 2017 found a group-by-age interaction for the parietal cortex, showing increased thinning with increasing chronological age in the individuals with OCD (n=412) compared to HCs (n=368) (Fouche et al., 2017).

With respect to healthy adult aging, despite the extensive evidence pointing towards hippocampal volume loss with increasing chronological age (Dennison *et al.,* 2013), it remains unclear which subfields are associated with age-related changes (Kurth et al., 2017; Mueller et al., 2007; Mueller & Weiner, 2009; Šimić et al., 1997; West et al., 1994). For the amygdala, some subfields, have been negatively associated with increasing (aged between 18 years and 85 years old, n=126)

(Aghamohammadi-Sereshki et al., 2019), which is partially consistent with other reports of a negative correlation between amygdala subfields volume and chronological age (aged between 18 years and 69 years old, n=100) (Kurth et al., 2019).

Recent advancements have suggested that MLAs using structural and/or functional MRI data coupled with statistical modelling can robustly predict chronological age using features of an individual's brain (Cole et al., 2019; Cole & Franke, 2017). Briefly, an MLA is built by recognizing patterns, relationships, and correlations, within complex neuroimaging data (Cole & Franke, 2017). The neuroimaging data may include structural brain features such as cortical thickness and surface curvature (Wang et al., 2014), as well as gray matter, white matter, and cerebrospinal fluid volume (Jonsson et al., 2019). The MLA is typically constructed in an independent dataset called a training model consisting of healthy individuals. The resultant model is then cross-validated before it is applied to an independent test sample to predict brain age (Zou et al., 2019). The subsequent outcome is brain-PAD (brain predicted age difference) which is typically derived by subtracting chronological age from ML predicted brain age (Cole & Franke, 2017; Sone et al., 2019). Higher brain-PADs, indicative of accentuated aging, have been associated with negative health outcomes, negative fateful life events (Hatton et al., 2018), cognitive impairment (Liem et al., 2017), and increased mortality (Cole et al., 2018).

To date, only one study has investigated brain aging in OCD. In Liu et al. (2022), a positive whole brain-PAD (+0.826 years) was observed in individuals with OCD (n=90) compared to HCs (n=106) (Liu et al., 2022). Additionally, brain-PADs were found to be negatively associated with the duration of illness. However, this study was performed in a relatively small sample, and only used a single set of brain features (gray matter volumes only) when training their prediction model. Nevertheless, this initial report supports the importance of identifying brain aging patterns in OCD and establishing if and how they may differ from the typical aging patterns observed in healthy individuals.

Against this background of limited brain age research in OCD, the objective of this chapter is to 1) examine whole and regional brain age in OCD, and 2) correlate whole brain age to amygdala and hippocampal subfield volumes in OCD. We used the Kaufmann et. al. (2019) pre-trained 5-fold cross-validated brain-age prediction model to estimate whole and regional brain age in a large sample of 376 individuals with OCD and 335 HCs. Brain-PAD was calculated for each individual by subtracting chronological age from the predicted brain age. Given the evidence of confounding effects of clinical characteristics on subcortical volumes in OCD (van den Heuvel et al., 2022), we performed subgroup analyses that were restricted to individuals with OCD without lifetime 1) comorbid anxiety disorder, 2) comorbid MDD, and 3) psychotropic medication use. Lastly, we assessed the association between whole-and-regional brain age estimates and OCD symptom severity. Based on the evidence in the literature on accentuated brain aging in OCD (Kaufmann et al., 2019; Liu et al., 2022), it is expected that individuals with OCD will present with accentuated whole and regional brain-PADs relative to HCs.

5.2. Methods

Participant selection and MRI acquisition

Structural T1-weighted MRI scans and sociodemographic data were obtained from a collaboration of six international research sites as part of OBIC. The sample composition has been published in detail elsewhere (De Wit et al., 2014). Briefly, participants who were sourced through local outpatient clinics and advertisements were screened for DSM-IV Axis I disorder. To be included in the patient group, the participant's primary diagnosis had to be OCD; psychiatric comorbidity was permitted secondary to OCD. All participants, including HCs (no current axis I psychiatric disorder), were excluded if they were younger than 18 years or older than 65 years of age, had a current psychotic disorder, history of a substance use disorder, were intellectually disabled, or had severe organic or neurological pathology. Additional information on OCD symptom severity, age of onset, and medication use was collected from the patient group. All of the research sites obtained written informed consent from the participants to participate in the study. In addition, each site obtained approval from their local ethical review board. For multicentre data sharing, additional approval was obtained from the medical ethical committee Amsterdam, University Medical Center (AMC).

Freesurfer segmentation

The neuroimaging data was processed on the high-performance computing (HPC) cluster at the University of Cape Town, South Africa. FreeSurfer 5.3 software (http://surfer.nmr.mgh.harvard.edu/). The processing pipeline was initiated using the *recon-all* function. The Desikan-Killiany anatomical brain atlas was used for cortical parcellation to divide the cerebral cortex into regions based on anatomical landmarks (Fischl et al., 2002).

Quality control

Visual inspection was performed by using an adaptation of the ENIGMA Consortium Quality Control protocol for subcortical and hippocampal subfields (https://enigma.ini.usc.edu/protocols/imaging-protocols/). In addition, the data was statistically examined for outliers, operationalized as lying >= +-3 standard deviations of the mean for the Euler number for each site (Ipser et al., 2022). The Euler statistic is derived from the number of holes in a spherical reconstruction performed by FreeSurfer of the cortical sheath and has been demonstrated as a useful tool for measuring T1 weighted MRI data quality (Dale et al., 1999; Monereo-Sánchez et al., 2021).

Brain age estimation

We used a pre-trained MLA to predict the individual Brain-PAD determined from T1 weighted MRI scans. Model construction and validation have been described in detail in Kaufmann et al., 2019.

Briefly, the MLA was developed using the eXtreme Gradient Boosting (xgboost) package in R (Chen & He, 2014). The MLA was trained on a large sample of HCs consisting of *n* = 26,535; and trained separately for females (n=14,182) and males (n=12,353), aged 3-89 years. Brain-age predictions were based on 1,118 features of brain thickness, area, and volume, including 211 cerebellar/subcortical volume features, that were extracted using a multi-modal parcellation of the cerebral cortex (Glasser et al., 2016).

Regional brain age prediction models were trained in the same fashion as the full brain model except that the feature set was reduced to cover only those features that overlapped more than 50% with a given lobe (Kaufmann et al., 2019). Regions were defined following the Freesurfer *lobesStrict* segmentation as respectively only the occipital (n=96), frontal (n=384), temporal (n=222), parietal (n=258), cingulate (n=60), insula (n=48). In addition, given the limited number of cerebellar features available in the Freesurfer summary statistics, cerebellar and subcortical features were grouped into a *cerebellar/subcortical* region (n=211) (Kaufmann et al., 2019).

The Pearson correlation between predicted brain age and chronological age was estimated using 5-fold cross-validation within the training set (n=35,474) for the full and regional brain age models in males and females. For the full brain age model, high correlations were found between the predicted brain age and chronological age for females (R=0.94) and males (R=0.95) (for regional brain age model validation across sexes and see supplementary Fig. 2 in (Kaufmann et al. 2019)).

For the present study, shell scripts to extract relevant brain features, as well as the R script to estimate brain age using the pre-trained model were provided by Dr. Kaufmann (Kaufmann et al., 2019). Of note, the OBIC dataset was not part of the development of the Kaufmann model, therefore OBIC was independent to the dataset used in the Kaufmann training and testing model. These shell scripts were applied to Freesurfer 5.3 segmented T1 weighted scans consisting of 375 individuals with OCD, and 335 HCs, no additional calibration steps were performed.

To assess the model generalization performance, the following metrics were calculated separately for the OCD group and HCs: (1) the correlation between predicted and chronological age, (2) the R^2 value representing the explained variance of chronological age, and (3) the mean absolute error (MAE) between predicted brain age and chronological age.

Statistical analysis approach

Covariates

Both whole and regional brain-PADs were calculated as the difference between the predicted brain age chronological age (Kaufmann et al., 2019) and were used as the primary outcome measure in the statistical analysis. The covariates of interest included age and age squared (which were both centered to facilitate interpretation of the results), sex, scanner site (to control for scanner differences across sites), and Euler number. Chronological age was included as a covariate in the regression model to reduce ‘regression dilution’. This is a well-known phenomenon often observed in brain age estimates where the predicted brain age is overestimated in younger brains and underestimated in older brains (Zhang et al., 2023). Although there are alternative bias-correction techniques for adjusting brain-PAD predictions including regressing chronological age onto brain-PAD to produce residualized brain-PAD estimates (Le et al., 2018), incorporating chronological age as a covariate is a commonly employed method in other studies that investigate brain-PAD in anxiety and related disorders (Clausen et al., 2021; Han, et al., 2021; Liu et al., 2022).

Linear mixed-effects models

All statistical analyses were conducted in *R* (version 4.2.1; freely available at https://www.r-project.org/). T-tests were conducted to assess between group (OCD n=375; HC n=335) differences in age, sex, education, and Euler number. To assess model fit (performance of the prediction model) per site, Pearsons’s correlation coefficients (R) and proportion of the variance explained by the model (R^2) were calculated between the predicted brain age estimate and chronological age in the OCD group, HCs, and the whole sample (combined OCD group, and HCs). Additionally, the MAE was calculated for all the groups. To examine the main effect of OCD diagnosis, the *R* package *lme4* was used to test group differences in brain-PAD between individuals with OCD and HCs using a linear mixed effects model that included a random intercept for scan site (main analysis: OCD n=375; HC n=335).

A separate subgroup analysis was performed for individuals with OCD with (n=158) and without medication use (n=218), those with (n=93) and without MDD (n=283), and those with (n=72), and without comorbid anxiety disorder (n=351). A within-group analysis (OCD n=84) was performed to assess the association between brain-PAD and OCD symptom severity (assessed using the Y-BOCS total score). Age-by-group and sex-by-group interactions were also tested. To assess the association between hippocampal and amygdala subfield volume and whole brain-PAD for the main group (OCD n=375; HC n=335), a partial correlation analysis was performed (adjusted for ICV) using the *ppcor* R library. All analyses were corrected for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) and the p-value was considered statistically significant at p <0.05.

5.3. Results

Model fit (how well the prediction model fits the dataset) is shown in Table 5.1a including Pearson correlation (R), MAE, and R^2 between the predicted brain age and chronological age. According to these statistics, in the OCD group, there was a moderate positive correlation (R=0.64) suggesting that the predicted brain age tends to increase with chronological age, with a degree of variability (R^2=0.4169; 41.69% of the variability in the predicted brain age can be explained by the chronological age) and MAE of 10.08 years. In the HCs group, there was also a moderate positive correlation (R=0.71) suggesting that the predicted brain age tends to increase with chronological age, with a degree of variability (R^2=0.5087; 50.87% of the variability in the predicted brain age can be explained by the chronological age) and MAE of 8.37 years. The mean brain-PAD of 7.330 years in the HC group was significantly larger than zero (t = 18.510, p < 0.001), suggesting that the model fit to these data is sub-optimal. A statistically significant negative association was observed between chronological age and brain-PAD in the controls (R = -0.322, p < 0.001), as well as in the OCD group (R = -0.263, p < 0.001) (Table 5b). Regional MAEs were high in the main analysis and subgroup analysis (Table 5.1a); main analysis: subcortex MAE=9.367, R=0.562, R2=0.316 to cingulate MAE=20.093, R^2=0.338, R^2=0.114. In the HCs group; subcortex MAE=7.693, R=0.640, R^2=0.41 to cingulate MAE=20.184, R=0.297, R^2=0.088.

Sample characteristics

Individuals with OCD had, on average, a lower education level compared to HCs (OCD: 13.7 years (SD=2.8); HCs (14.5 years (SD =3.4), t = 3.51, p < 0.0004), and were significantly older compared to HCs (OCD: 32.0 years (SD=9.4); HCs: 30.2 years (SD=9.3), t=-2.47, p=0.013). The mean Y-BOCS score for individuals with OCD was 24.9 (SD=6.2) (see Table 5.2).

Main analysis: whole and regional brain analysis

Individuals with OCD (n=375) had significantly higher brain-PAD (+1.6 years, p_{FDR}=0.006, *d*=0.20) compared to HCs (n=335), after adjusting for age, quadratic effects of age, sex, Euler number and scan site (Figure 5.1). There was no significant difference between individuals with OCD and HCs in the 7 regional brain age models: frontal cortex, subcortex, cingulate, insula, occipital, temporal, and parietal lobes.

Subgroup analysis: association between whole and regional brain age and clinical characteristics

OCD with psychotropic medication use

Individuals with OCD with psychotropic medication use (n=158) had significantly higher whole brain-PAD (+2.98 years, d=0.38, p_{FDR}<0.001) compared to HCs (n=289). Additionally, regional brain-PAD was significantly higher in the frontal cortex (+2.90 years, d=0.35, p_{FDR}=0.001), followed by the subcortex

(+2.77 years, d=0.20, p_{FDR}=0.015), the cingulate (+2.06 years, d=0.24, p_{FDR}=0.015), and the insula (+2.03 years, d=0.22, p_{FDR}=0.028) in individuals compared to HCs. No significant differences were found for the occipital, temporal, and parietal regions.

OCD without psychotropic medication use

Individuals with OCD without psychotropic medication use (n=218) did not significantly differ in whole brain-PAD (+0.57 years, d=0.07, p_{FDR}=0.374), nor all of the regional models compared to HCs (n=335).

OCD with comorbid MDD

Individuals with OCD with comorbid MDD (n=93) had significantly higher whole brain-PAD (+2.64 years, d=0.336, p_{FDR}=0.004) compared to HCs (n=335). In addition, individuals with comorbid MDD presented with significantly higher whole brain-PAD exclusively in the subcortex (+3.17 years, d=0.316, p_{FDR}=0.049) compared to HCs.

OCD without comorbid MDD

Individuals with OCD without comorbid MDD (n=283) had significantly higher whole brain-PAD (+1.27 years, d=0.17, p_{FDR}=0.031) compared to HCs (n=335). There were no significant differences in regional brain-PAD in individuals compared to HCs.

OCD with comorbid anxiety disorder

Individuals with OCD with comorbid anxiety disorder (n=72) did not significantly differ in whole brain-PAD, nor regional brain-PAD compared to HCs (n=157).

OCD without a comorbid anxiety disorder

Individuals with OCD without comorbid anxiety disorder (n=351) had significantly higher whole brain-PAD (+1.60 years, d=0.20, p_{FDR}=0.006) compared to HCs (n=335). There were no significant differences in regional brain-PAD in individuals compared to HCs.

OCD Symptom severity

OCD symptom severity (assessed using Y-BOCS) was not associated with whole brain-PAD, nor regional brain-PAD in individuals with OCD (n=346).

Interactions

There was no significant evidence for a group difference in the progression of whole brain-PAD as a function of age (t=0.082, p=0.934), or sex (t=1.557, p_{FDR}=0.120) between individuals with OCD (n=375) and HCs (n=335).

Partial correlation analysis

Partial correlation analysis was conducted between the individual hippocampus and amygdala subfield volumes and whole brain-PAD, to identify unique associations between subfields and brain aging. Partial correlation analysis found a significant negative association between hippocampal and amygdala volume and whole brain PAD in the OCD group (r=-0.224, p=0.00001), but not in the HC group (r=0.081, p=0.138). In the OCD group, after applying Benjamini-Hochberg FDR correction, 4 subfields remained significant namely the lateral nucleus (r=-0.18), CAT(r=-0.13), hippocampal fimbria (r=0.17), and hippocampal fissure (r=0.17)). Interestingly, in the OCD participants, for the amygdala subfields, larger volumes were associated with smaller brain PAD, whereas the opposite was observed for the hippocampal subfields (Table 5.3 a-b).

5.4. Discussion

In this chapter, I investigated the difference between MRI-predicted brain age and chronological age in a large sample of individuals with OCD (n=376) and HCs (n=335). The results demonstrated that whole brain-PAD was significantly higher in individuals with OCD, compared to HCs. However, no associations were found between regional brain-PAD and OCD diagnosis. Individuals with OCD with psychotropic medication use had distinct patterns of regional brain aging shown by higher brain-PAD in the frontal cortex, subcortex, cingulate, and insula, but not in the occipital, temporal, and parietal regions. There were no significant differences observed for whole or regional brain-PAD between HCs and individuals with OCD without psychotropic medication use. Our findings of higher whole brain-PAD in individuals with OCD are consistent with previous work demonstrating accentuated brain aging in OCD (Liu et al., 2022), and that clinical characteristics like medication status may be of importance in OCD brain morphometry (van den Heuvel et al., 2022; Weeland et al., 2022).

Main findings

Higher whole brain aging in OCD.

The main finding presented in this chapter is consistent with recent literature that reported significantly higher brain-PAD in individuals with OCD compared to HCs (+0.826 years, d=0.325 p=0.025) (Liu et al., 2022). Some possible explanations for the differences in the magnitude of brain-PAD between this study (+1.6 years) and that of Liu et al. (2022) are that Liu et al., 2022 used smaller sample sizes (OCD n=90, HCs n=106) than the present chapter (OCD n=376, HCs n=335), which may impact the reliability of the brain age estimate. Additionally, the MLA (gaussian processes) used in Liu et al., 2022 was trained using gray matter volumes of 246 defined brain regions, whereas the present chapter used gray matter volumes and additional features including brain thickness and area (1,118 features in total) (Kaufmann et al., 2019), although the MAE (5.36 years) in Liu et al., 2022 was less than the present chapter MAE (8.37 years).

Another possible explanation for the differences in brain-PADs between Liu et al. (2022) and the present chapter is the clinical and neuroanatomical heterogeneity of OCD. Indeed, in a study using a normative model and non-negative matrix factorization to quantitatively estimate the neuroanatomical heterogeneity of OCD, Han et al. (2023) found that OCD was associated with multiple disease factors instead of single disease factors (with high interindividual differences), suggesting that the differences in brain morphology observed in OCD are due to unique combination of specific neuroanatomical patterns (Han et al., 2023). Given this evidence, it is plausible that OCD heterogeneity between Liu et al., 2022 and the present chapter, as well as other methodological differences, could account for the differences in brain age estimations. The effect sizes observed in Liu et al., 2022 (d=0.32) were comparable to the present chapter (d=0.20) which suggests that the association between brain age and OCD in both studies was moderate.

The finding of positive brain-PAD in OCD is consistent with the theme of accentuated brain aging in anxiety disorders (Han et al., 2021) and other common mental disorders such as MDD (Han et al., 2020; Luo et al., 2022) and PTSD (Clausen et al., 2022). Notably, the effect sizes observed in brain age estimates in OCD in the present chapter are comparable to other indicators of biological aging including TL (Verhoeven et al., 2014; d=0.12), and epigenetic aging (Han et al., 2018; d=0.14) in other major mental disorders that are frequently comorbid with OCD (such as MDD). There are a multitude of possible explanations for accentuated brain age in OCD including neuroinflammation, dysregulation of the HPA-axis (Penninx et al., 2013; Vreeburg et al., 2010), genetic predisposition, and lifestyle factors (Jansen et al., 2021). However, due to limited data, the present chapter was unable to explore the association between brain age and these potential variables. Further evidence is required to elucidate the mechanisms behind accentuated brain aging, as indexed by higher brain-PAD, in OCD, and whether there is a correlation between brain aging and somatic aging.

Given that studies suggest that the incidence of dementia increases by threefold in clinical anxiety compared to non-cases (Santabárbara et al., 2019), and that brain aging often coincides with neurodegenerative diseases such as dementia and Alzheimer's diseases (Cole et al., 2018; Kaufmann et al., 2019), our findings of accentuated aging in OCD suggest a possible vulnerability to developing age-related diseases, although further investigation is required to elucidate the underlying mechanisms in brain aging.

Higher regional brain aging in OCD.

There were no significant differences observed in regional brain-PAD in individuals with OCD compared to HCs. This finding may point to a more distributed than region-specific pattern of aging across the brain in OCD. However, considering the high MAEs observed for most of the 7 regional models both in the OCD group (range; subcortex MAE=9.367, R=0.562, R2=0.316 -cingulate MAE=20.093, R^2=0.338, R^2=0.114) and HCs (range; subcortex MAE=7.693, R=0.640, R^2=0.41-cingulate MAE=20.184, R=0.297, R^2=0.088).

Subgroup analysis: association of whole and regional brain aging and clinical characteristics.

OCD with and without psychotropic medication use

Individuals with OCD with psychotropic medication use had significantly higher brain-PAD compared to HCs. However, no differences were observed in individuals with OCD without psychotropic medication. These findings are consistent with Han et al. (2021) where group differences (anxiety vs

HCs) were found only after correcting for medication use (d=0.27). In addition, Luo et al. (2022) found significantly higher (+2.09 years; d=0.13, p<0.05) brain-PAD in medication users compared to medication-free individuals in MDD (Luo et al., 2022). Notably, Luo et al. (2022) had smaller effect sizes than Han et al. (2021) (d=0.27) and the present study (d=0.38). Moreover, compared to the strong effect sizes observed in the main analysis of the present chapter (full sample, n=375; d=0.20), smaller effect sizes were observed in individuals with OCD with medication use (n=158; d=0.38), which likely reflect a loss of statistical power due to a decrease in sample size (from n=375 to n=158, respectively). The differences in the effect sizes between the main analysis and the medication use sensitivity analysis could also reflect sample heterogeneity, in which clinical characteristics like medication use may have moderating effects. Indeed, in OCD, medication status is a strong confounder of subcortical brain volume alterations, with previous large sample multi-site studies finding smaller brain volumes, particularly in the hippocampi with medication use (Boedhoe et al., 2019; Fouche et al., 2017b).

Ivanov et al. (2022) found a significant chronological age by group by medication association in which the volume of certain brain regions differed in certain age groups in individuals with OCD with medication use, compared to individuals with OCD without medication use (Ivanov et al., 2022). To date, there is limited evidence of an association between brain-PAD and psychotropic medication use in anxiety and related disorders, therefore it remains unclear how brain-PAD responds to pharmacotherapy. It is possible that individuals with psychographic medication use may be experiencing more severe forms of OCD (Han et al., 2021; Luo et al., 2022), although we found no association between brain age and OCD symptom severity scores. We speculate a possible medication-modulated influence on brain PAD as medication has been shown to influence neuronal plasticity and neurogenesis (Bernier et al., 2002; Fowler et al., 2002; Hamilton et al., 2008). Comprehensive, randomized controlled studies are required to investigate the potential impact of medication status on brain-PAD in individuals diagnosed with OCD.

OCD with and without comorbidity (MDD and anxiety disorder)

Individuals with OCD with comorbid MDD had significantly higher whole brain-PAD, compared to HCs. Compared to the main analysis (n=375), the sample size in the subgroup analysis for individuals with OCD with comorbid MDD was considerably smaller (n=93). However, the brain-PADs and effect sizes in this subgroup (+2.64 years, d=0.336, p_{FDR}=0.004) were substantially higher than what was observed in the main analysis (+1.6 years, d=1.56, p_{FDR}=0.006). These findings suggest that the accentuated brain aging observed in individuals with OCD was enhanced in individuals with OCD with comorbid MDD. In other words, the presence of MDD comorbid resulted in more pronounced brain aging in individuals with OCD. In MDD literature, the association between brain-PAD and MDD diagnosis yields mixed findings; while there is more evidence for an association (Koutsouleris et al., 2014), some studies do not find an association (Besteher et al., 2019; Kuo et al., 2020).

The finding that whole brain-PAD was higher in individuals with OCD with comorbid MDD (+2.64 years, d=0.336, p_{FDR}=0.004) than individuals without comorbid MDD PAD (+1.27 years, d=0.17, p_{FDR}=0.031) suggests that MDD comorbidity may have an impact on brain aging in OCD. Han et al. (2021) found that MDD comorbidity in participants diagnosed with anxiety disorders (n=91) was associated with higher brain-PAD, although this association fell just short of statistical significance (+2.23 years, d=0.21, p_{FDR}=0.08) and occurred only after correcting for medication use (Han et al., 2021).

In the present chapter, individuals with OCD without comorbid anxiety disorder (n=351) had significantly higher whole brain-PAD (+1.60 years, d=0.20, p_{FDR}=0.006) compared to HCs (n=335). In individuals with OCD who were free of comorbidity and medication-naïve, Liu et al. (2022) found significantly higher brain-PAD in individuals compared to HCs. There is very little evidence of how the presence or absence of comorbidity influences brain aging, however, it seems reasonable to expect that psychiatric comorbidity would increase brain-PAD by some additional years (Sone et al., 2019), given the potential cumulative effect of overlap in the pathogenesis of different psychiatric disorders (McTeague et al., 2017). However, further evidence is required to determine the influence of comorbid anxiety disorders on brain age.

OCD symptom severity

There was no association between whole or regional brain-PAD and OCD symptom severity (Y-BOCS) (n=84) which is consistent with Liu et al. (2022) who found no significant association between brain-PAD and symptom severity scores in medication-naïve individuals with OCD (n=90). In a meta-analysis of brain aging in psychiatric disorders, Blake et al. (2023) found that higher brain-PAD was associated with greater symptom severity scores, but this was more pronounced in psychotic disorders (Blake et al., 2023). Furthermore, Han et al. (2021) found an association between higher brain-PAD and anxiety symptom severity, however, the inclusion of multiple anxiety disorders in their sample means that their findings are not disorder-specific (Han et al., 2021). When considering OCD symptom severity across brain features, Fouche et al. (2022) found no association between shape, thickness, or surface area in subcortical regions and OCD symptom severity (Fouche et al., 2022) which is consistent with other reports in regional brain volumes in OCD (De Wit et al., 2014). Ivanov et al. (2022) found that certain brain volumes increased or decreased with OCD severity depending on chronological age and medication status (Ivanov et al., 2022). Taken together, these findings highlight that the association between OCD symptom severity and brain neuroanatomy is complex (Han et al., 2023).

Partial correlation analysis

A partial correlation analysis was performed to investigate the association between hippocampal and amygdala subfield volumes and whole brain-PAD within the groups (OCD and HCs). The analysis revealed unique associations between the lateral nucleus (r=-0.18), CAT (r=-0.13), hippocampal fimbria (r=0.17), and fissure (r=0.17) and brain-PAD in the OCD group. No significant association was found in the HCs group.

The findings for the amygdala subfields were consistent with the evidence of age-related effects on the BLA amygdala including the lateral, basal, and accessory basal subfields showing a significant nonlinear negative association with age (Aghamohammadi-Sereshki et al., 2019). Additionally, age-related effects have been reported for specific amygdala subfield volumes with the strongest effects observed for subfields belonging to the SFA (CAT, anterior amygdaloid area, and the cortical nucleus of the amygdala (Schmitz-Koep et al., 2021)) (Kurth et al., 2019). Kurth et al. (2019) speculated that since the SFA functions in olfactory processing (Bzdok et al., 2013), which is shown to decline with age (Attems & Jellinger, 2015; Doty & Kamath, 2014) and since the amygdala is shown to be less responsive to olfactory stimuli in older individuals compared to younger individuals (Cerf-ducastel & Murphy, 2003; Rolls et al., 2015), there may be an interplay between amygdala subfields, olfactory processing and age (Kurth et al., 2019).

The hippocampal fimbria and fissure were the only hippocampal subfields found to be associated with brain-PADs. There is some evidence of a non-linear association between hippocampal subfield volumes and aging, although findings in the individual subfields are inconsistent (Malykhin et al., 2017; Zheng et al., 2018). There is very little evidence of aging of the hippocampal fissure and fimbria. Anatomically, the hippocampal fissure is derived from the telencephalic indentation on the external surface between the primordial DG and the CA, which deepened as the DG developed its granular layer (Humphrey, 1967; Zhang et al., 2014). However, it is not the only fissure that is associated with the hippocampus (Dekeyzer et al., 2017; Duvernoy, 2013). A study of the peri-hippocampal fissure in healthy individuals (n=130) and individuals with Alzheimer's disease (n=27) aged between 20 years and 90 years old found that the collective hippocampal fissures (hippocampal fissure, choroidal fissure, transverse fissure of Bichat) were strongly positively correlated with chronological age in healthy and Alzheimer's disease group (with a steep rise after the age of 55 years, in healthy individuals) (Li et al., 2006). Interestingly, a large volume of the hippocampal fissure is suggested to be an indicator of hippocampal volume atrophy in the rat brain (Li et al., 2018) and a shared feature between OCD and bipolar disorder in a transdiagnostic study of hippocampal subfield volumes in psychiatric disorders (Jiang et al., 2022).

However, given the poor test-retest reliability of the hippocampal fissure segmentation (Sämann et al., 2022), our results should be interpreted with caution (Brown et al., 2020; Quattrini et al., 2020).

Overall, the findings in the present chapter imply that greater relative brain aging, as indexed by brain-PAD, may be more strongly predictive of hippocampal and amygdala subfield volumes in individuals with OCD than those without the disorder (HCs). However, further analysis is required to determine 1) whether these associations are unique to OCD, compared to other anxiety and related disorders (i.e., are these significant associations suggestive of unique subfield-specific aging indicators in OCD), 2) determine the extent to which clinical characteristics such as symptom severity and comorbid psychopathology moderate the magnitude of the observed associations between brain-PAD and subfield volumes, and 3) is the association between subfield volume and brain-PAD associated with cognitive decline.

5.5. Limitations

One notable strength of the present chapter is the use of a large multisite sample (OCD: n=375; HC: n=335) which allowed for subgroup analyses to investigate the association between brain-PAD and clinical characteristics. While we found an association between brain-PAD and individuals with OCD with medication use, there were insufficient data available as part of this secondary data analysis (original data De Wit et al., 2014), to further investigate this association as a function of different types of medication (Ivanov et al., 2022). Additionally, due to missing Y-BOCS data from certain research sites utilized in the present chapter, there was insufficient data to further explore the association between brain-PAD and symptom dimension. Future analysis using larger samples are required to assess whether OCD symptom severity is associated with brain-PAD, and ideally stratifying participants by medication status and symptom dimension. While the brain age model used in this study was trained on a large independent sample (n=35474) using a variety of brain features (thickness, volume surface area) (Kaufmann et al., 2019), a noteworthy limitation was the high MAE (reflecting the performance of the prediction model) observed for the brain age estimates for the diagnosis and HCs group, which are larger than those observed in many other brain age studies (Clausen et al., 2021; Han et al., 2021; Liu et al., 2022). Large MAEs suggest that the brain age model was a poor fit for the data used in the present chapter. However, studies suggest that models with tighter fits (MAE=3 years) tend to prioritize age-related characteristics minimizing disease-related characteristics, whilst less restrictive/looser fits (MAE=7 years) may overlook subtle disease patterns (Bashyam et al., 2020). By including regional brain-PAD, the goal was to enhance the ability to detect subtle region-specific differences that may be specific to OCD, given that the aging process is not spatially nor temporally heterogeneous in the brain

(Dinsdale et al., 2021). Nevertheless, the relatively large MAEs for the regional models presented in this study indicate that findings for the regional models should be interpreted cautiously.

5.6. Conclusion

The findings in the present chapter provide evidence of accentuated brain aging in a large sample of individuals with OCD (n=375), compared to HCs (n=335). There was no evidence of regional brain aging in the main analysis in individuals with OCD and HCs. While this might suggest a more distributed than region-specific pattern of aging across the brain in OCD, the MAEs for the regional models were high and thus future evidence using more sensitive regional brain age models is required to replicate these findings. The present chapter also found a greater brain-PAD in OCD dependent on clinical characteristics such as psychiatric comorbidity and medication use.

5.7. Tables and figures

Table 5.1a: Correlation matrix for HC regional brain-PAD estimates, after regressing out site and chronological age

	Cingulate	Frontal	Insula	Occipital	Parietal	Subcortex	Temporal
Cingulate	1	0.454	0.414	0.282	0.379	0.045	0.244
Frontal	0.454	1	0.483	0.256	0.471	0.043	0.38
Insula	0.414	0.483	1	0.253	0.384	0.086	0.33
Occipital	0.282	0.256	0.253	1	0.304	0.155	0.364
Parietal	0.379	0.471	0.384	0.304	1	0.16	0.42
Sub-cortical	0.045	0.043	0.086	0.155	0.16	1	0.187
Temporal	0.244	0.38	0.33	0.364	0.42	0.187	1

Table 5.1b: Model fit (MAE, R, R^2) for whole and regional brain age model in individuals with OCD (n=376) and HCs (n=335)

	OCD (n=376)			**HCs (n=335)**			
	MAE	**R**	**R^2**		**MAE**	**R**	**R^2**
Whole brain age	10.0816	0.6457	0.4169		8.3787	0.7132	0.5087
Cingulate	20.093	0.338	0.114		20.184	0.297	0.088
Insula	17.173	0.288	0.083		17.245	0.334	0.112
Temporal	15.629	0.340	0.116		16.688	0.316	0.100
Subcortex	9.367	0.562	0.316		7.693	0.640	0.410
Occipital	14.216	0.276	0.076		14.923	0.302	0.091
Frontal	12.657	0.576	0.332		11.149	0.645	0.416
Parietal	14.927	0.474	0.225		14.919	0.464	0.215

Table 5.2: Demographic and clinical characteristics of individuals with OCD (n=346) and HCs (n=335)

	OCD (n=376)		**HCs (n=335)**		**Statistical analysis**		
Characteristics	**mean**	**SD**	**mean**	**SD**	**t**		**p**
Age (years)	32.02	9.41	30.28	9.31	-2.47		0.001
Education level (years)	13.72	2.83	14.56	3.40	3.51		< 0.001
Y-BOCS score mean[a]	24.94	6.19	--	--	--		--
Age of onset[b]	20.36	8.85	--	--	--		--
Euler	-238.03	117.24	-223.57	118.35	1.63		0.10
	N	**%**	**N**	**%**			
Female	195	52.0	179	52.9	X2= 1.2		0.27
Medication use at time of scan	158	42.6	--	--	--		--

[a] Measured by the Yale-Brown Obsessive Compulsive Scale (YBOCS) total score. [b]As measured by the Y-BOCS symptom checklist.

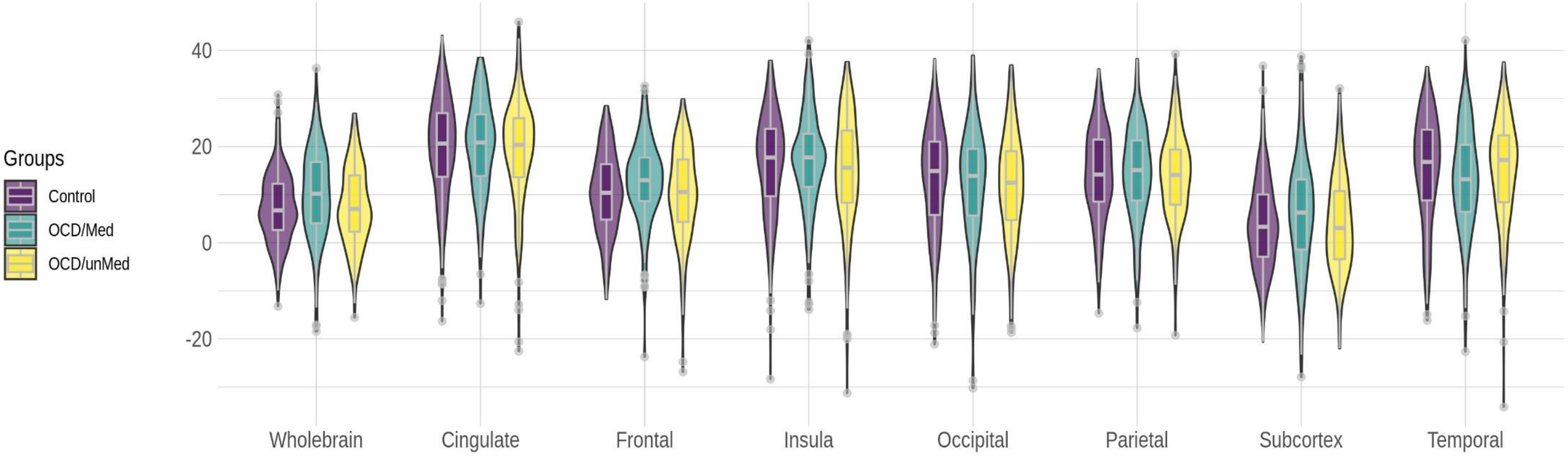

Figure 5.1: violin plot showing whole and regional brain-PADs in OCD (n=376) with (n=158) and without (n=218) psychotropic medication use (n=335), compared to HCs (n=335).

Table 5.3a: Partial correlation between hippocampal and amygdala subfield volumes and brain-PADs in individuals with OCD (n=376)

OCD group partial correlation	R	P value	P value corrected (p_{FDR})	Statistics
Amygdala subfields				
Lateral	-0.182	0.001	0.009*	-3.482
Basal	0.087	0.099	0.188	1.652
Accessory Basal	-0.132	0.012	0.052	-2.515
AAA	-0.058	0.276	0.315	-1.090
Central	0.093	0.081	0.170	1.750
Medial	-0.054	0.306	0.321	-1.026
Cortical	0.109	0.040	0.130	2.058
CAT	-0.138	0.009	0.048*	-2.620
Paralaminar	0.074	0.164	0.230	1.393
Hippocampal subfields				
Parasubiculum	-0.028	0.598	0.598	-0.527
Presubiculum	-0.095	0.073	0.170	-1.797
Subiculum	0.095	0.075	0.170	1.788
CA 1	0.057	0.285	0.315	1.070
CA 3	0.107	0.043	0.130	2.029
CA 4	-0.064	0.228	0.299	-1.208
DG	0.079	0.138	0.223	1.486
ML	-0.074	0.162	0.230	-1.403
HATA	-0.062	0.242	0.299	-1.173
Fimbria	0.175	0.001	0.009*	3.340
Hippocampal fissure	0.171	0.001	0.009*	3.260
Hippocampal tail	-0.085	0.108	0.188	-1.613

Table 5.3b: Partial correlation between hippocampal and amygdala subfield volumes and brain-PADs in HCs (n=335)

HCs group partial correlation.	**R**	**P value**	**P value corrected (p_{FDR})**	**Statistics**
Amygdala subfields				
Lateral	-0.040	0.482	0.482	0.482
Basal	-0.017	0.763	0.763	0.763
Accessory Basal	-0.108	0.055	0.055	0.055
AAA	-0.020	0.718	0.718	0.718
Central	0.122	0.030	0.030	0.030
Medial	-0.083	0.141	0.141	0.141
Cortical	0.079	0.163	0.163	0.163
CAT	0.037	0.514	0.514	0.514
Paralaminar	0.114	0.044	0.230	2.025
Hippocampal subfields				
Parasubiculum	-0.090	0.111	0.311	-1.600
Presubiculum	0.038	0.498	0.674	0.678
Subiculum	0.042	0.454	0.674	0.750
CA 1	0.013	0.821	0.862	0.227
CA 3	0.048	0.398	0.674	0.847
CA 4	0.005	0.926	0.926	0.093
DG	0.031	0.578	0.714	0.557
ML	-0.084	0.139	0.311	-1.484
HATA	-0.122	0.031	0.230	-2.166
Fimbria	0.116	0.040	0.230	2.058
Hippocampal fissure	0.090	0.110	0.311	1.601
Hippocampal tail	0.079	0.162	0.311	1.403

5.8. References

Aghamohammadi-Sereshki, A., Hrybouski, S., Travis, S., Huang, Y., Olsen, F., Carter, R., Camicioli, R., & Malykhin, N. V. (2019). Amygdala subnuclei and healthy cognitive aging. *Human Brain Mapping*, *40*(1), 34–52. https://doi.org/10.1002/hbm.24353

APA. (2013). Diagnostic and statistical manual of mental disorders: DSM-5^{TM}, 5th ed. In *Diagnostic and statistical manual of mental disorders: DSM-5^{TM}, 5th ed.* American Psychiatric Publishing, Inc. https://doi.org/10.1176/appi.books.9780890425596

Bashyam, V. M., Erus, G., Doshi, J., Habes, M., Nasralah, I., Truelove-Hill, M., Srinivasan, D., Mamourian, L., Pomponio, R., Fan, Y., Launer, L. J., Masters, C. L., Maruff, P., Zhuo, C., Völzke, H., Johnson, S. C., Fripp, J., Koutsouleris, N., Satterthwaite, T. D., … Davatzikos, C. (2020). MRI signatures of brain age and disease over the lifespan based on a deep brain network and 14 468 individuals worldwide. *Brain*, *143*(7), 2312–2324. https://doi.org/10.1093/brain/awaa160

Bernier, P. J., Bédard, A., Vinet, J., Lévesque, M., & Parent, A. (2002). Newly generated neurons in the amygdala and adjoining cortex of adult primates. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(17), 11464–11469. https://doi.org/10.1073/pnas.172403999

Besteher, B., Gaser, C., & Nenadić, I. (2019). Machine-learning based brain age estimation in major depression showing no evidence of accelerated aging. *Psychiatry Research - Neuroimaging*, *290*(June), 1–4. https://doi.org/10.1016/j.pscychresns.2019.06.001

Blake, K. V., Ntwatwa, Z., Kaufmann, T., Stein, D. J., Ipser, J. C., & Groenewold, N. A. (2023). Advanced brain ageing in adult psychopathology: A systematic review and meta-analysis of structural MRI studies. *Journal of Psychiatric Research*, *157*, 180–191. https://doi.org/10.1016/j.jpsychires.2022.11.011

Boedhoe, P. S. W., Rooij, D. Van, Hoogman, M., Abe, Y., Alonso, P., Ameis, S. H., Anticevic, A., Aherson, P., Arango, C., Arnold, P. D., Assogna, F., Auzias, G., Banaschewski, T., Batistuzzo, M. C., Baumeister, S., Baur-streubel, R., Bellgrove, M. A., Benedetti, F., Beucke, J. C., … Kwon, J. S. (2019). *Subcortical brain volume , regional cortical thickness and cortical surface area across attention-deficit / hyperactivity disorder (ADHD), autism spectrum disorder (ASD), and obsessive-compulsive disorder (OCD).*

Chen, T., & He, T. (2014). xgboost: Extreme Gradient Boosting. *R Lecture*, *2016*, 1–84.

Clausen, A. N., Fercho, K. A., Monsour, M., Disner, S., Salminen, L., Haswell, C. C., Rubright, E. C., Watts, A. A., Buckley, M. N., Maron-Katz, A., Sierk, A., Manthey, A., Suarez-Jimenez, B., Olatunji, B. O., Averill, C. L., Hofmann, D., Veltman, D. J., Olson, E. A., Li, G., … Morey, R. A. (2022). Assessment of brain age in posttraumatic stress disorder: Findings from the ENIGMA PTSD and brain age working groups. *Brain and Behavior*, *12*(1), 1–14. https://doi.org/10.1002/brb3.2413

Cole, J. H., & Franke, K. (2017). Predicting Age Using Neuroimaging: Innovative Brain Ageing Biomarkers. *Trends in Neurosciences*, *40*(12), 681–690. https://doi.org/10.1016/j.tins.2017.10.001

Cole, J. H., Marioni, R. E., Harris, S. E., & Deary, I. J. (2019). Brain age and other bodily 'ages': implications for neuropsychiatry. *Molecular Psychiatry*, *24*(2), 266–281. https://doi.org/10.1038/s41380-018-0098-1

Cole, J. H., Ritchie, S. J., Bastin, M. E., Valdés Hernández, M. C., Muñoz Maniega, S., Royle, N., Corley, J., Pattie, A., Harris, S. E., Zhang, Q., Wray, N. R., Redmond, P., Marioni, R. E., Starr, J. M., Cox, S. R., Wardlaw, J. M., Sharp, D. J., & Deary, I. J. (2018). Brain age predicts mortality. *Molecular Psychiatry*, *23*(5), 1385–1392. https://doi.org/10.1038/mp.2017.62

Dale, A. M., Fischl, B., & Sereno, M. I. (1999). Cortical Surface-Based Analysis. *NeuroImage*, *9*(2), 179–194. https://doi.org/10.1006/nimg.1998.0395

de Lange, A. M. G., & Cole, J. H. (2020). Commentary: Correction procedures in brain-age prediction. *NeuroImage: Clinical*, *26*(February), 24–26. https://doi.org/10.1016/j.nicl.2020.102229

De Wit, S. J., Alonso, P., Schweren, L., Mataix-Cols, D., Lochner, C., Menchón, J. M., Stein, D. J., Fouche, J. P., Soriano-Mas, C., Sato, J. R., Hoexter, M. Q., Denys, D., Nakamae, T., Nishida, S., Kwon, J. S., Jang, J. H., Busatto, G. F., Cardoner, N., Cath, D. C., … Van Den Heuvel, O. A. (2014). Multicenter voxel-based morphometry mega-analysis of structural brain scans in obsessive-compulsive disorder. *American Journal of Psychiatry*, *171*(3), 340–349. https://doi.org/10.1176/appi.ajp.2013.13040574

Dinsdale, N. K., Bluemke, E., Smith, S. M., Arya, Z., Vidaurre, D., Jenkinson, M., & Namburete, A. I. L. (2021). Learning patterns of the ageing brain in MRI using deep convolutional networks. *NeuroImage*, *224*(September 2020), 117401. https://doi.org/10.1016/j.neuroimage.2020.117401

Fischl, B., Salat, D. H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., Van Der Kouwe, A., Killiany, R., Kennedy, D., Klaveness, S., Montillo, A., Makris, N., Rosen, B., & Dale, A. M. (2002). Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. *Neuron*, *33*(3), 341–355. https://doi.org/10.1016/S0896-6273(02)00569-X

Fouche, J. P., Du Plessis, S., Hattingh, C., Roos, A., Lochner, C., Soriano-Mas, C., Sato, J. R., Nakamae, T., Nishida, S., Kwon, J. S., Jung, W. H., Mataix-Cols, D., Hoexter, M. Q., Alonso, P., De Wit, S. J., Veltman, D. J., Stein, D. J., & Van Den Heuvel, O. A. (2017). Cortical thickness in obsessive-compulsive disorder: Multisite mega-analysis of 780 brain scans from six centres. *British Journal of Psychiatry*, *210*(1), 67–74. https://doi.org/10.1192/bjp.bp.115.164020

Fouche, J. P., Groenewold, N. A., Sevenoaks, T., Heany, S., Lochner, C., Alonso, P., Batistuzzo, M. C., Cardoner, N., Ching, C. R. K., de Wit, S. J., Gutman, B., Hoexter, M. Q., Jahanshad, N., Kim, M., Kwon, J. S., Mataix-Cols, D., Menchon, J. M., Miguel, E. C., Nakamae, T., … Stein, D. J. (2022). Shape analysis of subcortical structures in obsessive-compulsive disorder and the relationship with comorbid anxiety, depression, and medication use: A meta-analysis by the OCD Brain Imaging Consortium. *Brain and Behavior*, *12*(10), 1–12. https://doi.org/10.1002/brb3.2755

Fowler, C. D., Liu, Y., Ouimet, C., & Wang, Z. (2002). The effects of social environment on adult neurogenesis in the female prairie vole. *Journal of Neurobiology*, *51*(2), 115–128. https://doi.org/10.1002/neu.10042

Franke, K., Bublak, P., Hoyer, D., Billiet, T., Gaser, C., Witte, O. W., & Schwab, M. (2020). In vivo biomarkers of structural and functional brain development and aging in humans. *Neuroscience and Biobehavioral Reviews*, *117*(November 2017), 142–164. https://doi.org/10.1016/j.neubiorev.2017.11.002

Franke, K., Ziegler, G., Klöppel, S., & Gaser, C. (2010). Estimating the age of healthy subjects from T1-weighted MRI scans using kernel methods: Exploring the influence of various parameters. *NeuroImage*, *50*(3), 883–892. https://doi.org/10.1016/j.neuroimage.2010.01.005

Glasser, M. F., Coalson, T. S., Robinson, E. C., Hacker, C. D., Harwell, J., Yacoub, E., Ugurbil, K., Andersson, J., Beckmann, C. F., Jenkinson, M., Smith, S. M., & Van Essen, D. C. (2016). A multi-modal parcellation of human cerebral cortex. *Nature*, *536*(7615), 171–178.

https://doi.org/10.1038/nature18933

Hamilton, J. P., Siemer, M., & Gotlib, I. H. (2008). Amygdala volume in major depressive disorder: A meta-analysis of magnetic resonance imaging studies. *Molecular Psychiatry*, *13*(11), 993–1000. https://doi.org/10.1038/mp.2008.57

Han, L. K. M., Aghajani, M., Clark, S. L., Chan, R. F., Hattab, M. W., Shabalin, A. A., Zhao, M., Kumar, G., Xie, L. Y., Jansen, R., Milaneschi, Y., Dean, B., Aberg, K. A., Van Den Oord, E. J. C. G., & Penninx, B. W. J. H. (2018). Epigenetic aging in major depressive disorder. *American Journal of Psychiatry*, *175*(8), 774–782. https://doi.org/10.1176/appi.ajp.2018.17060595

Han, L. K. M., Dinga, R., Hahn, T., Ching, C. R. K., Eyler, L. T., Aftanas, L., Aghajani, M., Aleman, A., Baune, B. T., Berger, K., Brak, I., Filho, G. B., Carballedo, A., Connolly, C. G., Couvy-Duchesne, B., Cullen, K. R., Dannlowski, U., Davey, C. G., Dima, D., … Schmaal, L. (2020). Brain aging in major depressive disorder: results from the ENIGMA major depressive disorder working group. *Molecular Psychiatry*. https://doi.org/10.1038/s41380-020-0754-0

Han, L. K. M., Schnack, H. G., Brouwer, R. M., Veltman, D. J., van der Wee, N. J. A., van Tol, M. J., Aghajani, M., & Penninx, B. W. J. H. (2021). Contributing factors to advanced brain aging in depression and anxiety disorders. *Translational Psychiatry*, *11*(1). https://doi.org/10.1038/s41398-021-01524-2

Han, L. K. M., Verhoeven, J. E., Tyrka, A. R., Penninx, B. W. J. H., Wolkowitz, O. M., Månsson, K. N. T., Lindqvist, D., Boks, M. P., Révész, D., Mellon, S. H., & Picard, M. (2019). Accelerating research on biological aging and mental health: Current challenges and future directions. *Psychoneuroendocrinology*, *106*(January), 293–311. https://doi.org/10.1016/j.psyneuen.2019.04.004

Han, S., Chen, Y., Zheng, R., Li, S., Jiang, Y., Wang, C., Fang, K., Yang, Z., Liu, L., Zhou, B., Wei, Y., Pang, J., Li, H., Zhang, Y., & Cheng, J. (2021). The stage-specifically accelerated brain aging in never-treated first-episode patients with depression. *Human Brain Mapping*, *42*(11), 3656–3666. https://doi.org/10.1002/hbm.25460

Han, S., Xu, Y., Fang, K., Guo, H.-R., Wei, Y., Liu, L., Wen, B., Liu, H., Zhang, Y., & Cheng, J. (2023). Mapping the neuroanatomical heterogeneity of OCD using a framework integrating normative model and non-negative matrix factorization. *Cerebral Cortex*, *May*, 8667–8678. https://doi.org/10.1093/cercor/bhad149

Hawco, C., Voineskos, A. N., Radhu, N., Rotenberg, D., Ameis, S., Backhouse, F. A., Semeralul, M., & Daskalakis, Z. J. (2017). Age and gender interactions in white matter of schizophrenia and obsessive compulsive disorder compared to non-psychiatric controls: commonalities across disorders. *Brain Imaging and Behavior*, *11*(6), 1836–1848. https://doi.org/10.1007/s11682-016-9657-8

Ipser, J. C., Joska, J., Sevenoaks, T., Gouse, H., Freeman, C., Kaufmann, T., Andreassen, O. A., Shoptaw, S., & Stein, D. J. (2022). Limited evidence for a moderating effect of HIV status on brain age in heavy episodic drinkers. *Journal of NeuroVirology*, *28*(3), 383–391. https://doi.org/10.1007/s13365-022-01072-5

Isomura, K., Brander, G., Chang, Z., Kuja-Halkola, R., Rück, C., Hellner, C., Lichtenstein, P., Larsson, H., Mataix-Cols, D., & Fernández de la Cruz, L. (2018). Metabolic and Cardiovascular Complications in Obsessive-Compulsive Disorder: A Total Population, Sibling Comparison Study With Long-Term Follow-up. *Biological Psychiatry*, *84*(5), 324–331. https://doi.org/10.1016/j.biopsych.2017.12.003

Ivanov, I., Boedhoe, P. S. W., Abe, Y., Alonso, P., Ameis, S. H., Arnold, P. D., Balachander, S., Baker, J. T., Banaj, N., Bargalló, N., Batistuzzo, M. C., Benedetti, F., Beucke, J. C., Bollettini, I., Brem, S., Brennan, B. P., Buitelaar, J., Calvo, R., Cheng, Y., … Fouche, J.-P. (2022). Associations of medication with subcortical morphology across the lifespan in OCD: Results from the international ENIGMA Consortium. *Journal of Affective Disorders*, *318*(August), 204–216. https://doi.org/10.1016/j.jad.2022.08.084

Jansen, R., Han, L. K. M., Verhoeven, J. E., Aberg, K. A., van den Oord, E. C. G. J., Milaneschi, Y., & Penninx, B. W. J. H. (2021). An integrative study of five biological clocks in somatic and mental health. *ELife*, *10*, 1–20. https://doi.org/10.7554/eLife.59479

Jonsson, B. A., Bjornsdottir, G., Thorgeirsson, T. E., Ellingsen, L. M., Walters, G. B., Gudbjartsson, D. F., Stefansson, H., Stefansson, K., & Ulfarsson, M. O. (2019). Brain age prediction using deep learning uncovers associated sequence variants. *Nature Communications*, *10*(1), 1–10. https://doi.org/10.1038/s41467-019-13163-9

Kandemir, H., Abuhandan, M., Aksoy, N., Savik, E., & Kaya, C. (2013). Oxidative imbalance in child and adolescent patients with obsessive compulsive disorder. *Journal of Psychiatric Research*, *47*(11), 1831–1834. https://doi.org/10.1016/j.jpsychires.2013.08.010

Kang, J. I., Park, C. Il, Lin, J., Kim, S. T., Kim, H. W., & Kim, S. J. (2021). Alterations of cellular aging markers in obsessive– compulsive disorder: Mitochondrial dna copy number and telomere length. *Journal of Psychiatry and Neuroscience*, *46*(4), E451–E458. https://doi.org/10.1503/jpn.200238

Kaufmann, T., van der Meer, D., Doan, N. T., Schwarz, E., Lund, M. J., Agartz, I., Alnæs, D., Barch, D. M., Baur-Streubel, R., Bertolino, A., Bettella, F., Beyer, M. K., Bøen, E., Borgwardt, S., Brandt, C. L., Buitelaar, J., Celius, E. G., Cervenka, S., Conzelmann, A., … Westlye, L. T. (2019). Common brain disorders are associated with heritable patterns of apparent aging of the brain. *Nature Neuroscience*, *22*(10), 1617–1623. https://doi.org/10.1038/s41593-019-0471-7

King, K. S., Kozlitina, J., Rosenberg, R. N., Peshock, R. M., McColl, R. W., & Garcia, C. K. (2014). Effect of leukocyte telomere length on total and regional brain volumes in a large population-based cohort. *JAMA Neurology*, *71*(10), 1247–1254. https://doi.org/10.1001/jamaneurol.2014.1926

Koutsouleris, N., Davatzikos, C., Borgwardt, S., Gaser, C., Bottlender, R., Frodl, T., Falkai, P., Riecher-Rössler, A., Möller, H. J., Reiser, M., Pantelis, C., & Meisenzahl, E. (2014). Accelerated brain aging in schizophrenia and beyond: A neuroanatomical marker of psychiatric disorders. *Schizophrenia Bulletin*, *40*(5), 1140–1153. https://doi.org/10.1093/schbul/sbt142

Kuo, C. Y., Lee, P. L., Hung, S. C., Liu, L. K., Lee, W. J., Chung, C. P., Yang, A. C., Tsai, S. J., Wang, P. N., Chen, L. K., Chou, K. H., & Lin, C. P. (2020). Large-Scale Structural Covariance Networks Predict Age in Middle-to-Late Adulthood: A Novel Brain Aging Biomarker. *Cerebral Cortex*, *30*(11), 5844–5862. https://doi.org/10.1093/cercor/bhaa161

Kurth, F., Cherbuin, N., & Luders, E. (2017). The impact of aging on subregions of the hippocampal complex in healthy adults. *NeuroImage*, *163*, 296–300. https://doi.org/10.1016/j.neuroimage.2017.09.016

Kurth, F., Cherbuin, N., & Luders, E. (2019). Age but no sex effects on subareas of the amygdala. *Human Brain Mapping*, *40*(6), 1697–1704. https://doi.org/10.1002/hbm.24481

Le, T. T., Kuplicki, R. T., McKinney, B. A., Yeh, H.-W., Thompson, W. K., & Paulus, M. P. (2018). A Nonlinear Simulation Framework Supports Adjusting for Age When Analyzing BrainAGE. *Frontiers in Aging Neuroscience*, *10*(October), 1–11. https://doi.org/10.3389/fnagi.2018.00317

Liem, F., Varoquaux, G., Kynast, J., Beyer, F., Kharabian Masouleh, S., Huntenburg, J. M., Lampe, L., Rahim, M., Abraham, A., Craddock, R. C., Riedel-Heller, S., Luck, T., Loeffler, M., Schroeter, M. L., Witte, A. V., Villringer, A., & Margulies, D. S. (2017). Predicting brain-age from multimodal imaging data captures cognitive impairment. *NeuroImage*, *148*(November 2016), 179–188. https://doi.org/10.1016/j.neuroimage.2016.11.005

Liu, L., Liu, J., Yang, L., Wen, B., Zhang, X., Cheng, J., Han, S., Zhang, Y., & Cheng, J. (2022). Accelerated Brain Aging in Patients With Obsessive-Compulsive Disorder. *Frontiers in Psychiatry*, *13*(May), 1–9. https://doi.org/10.3389/fpsyt.2022.852479

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2013). The hallmarks of aging. *Cell*, *153*(6), 1194. https://doi.org/10.1016/j.cell.2013.05.039

Luo, Y., Chen, W., Qiu, J., & Jia, T. (2022). Accelerated functional brain aging in major depressive disorder: evidence from a large scale fMRI analysis of Chinese participants. *Translational Psychiatry*, *12*(1), 1–9. https://doi.org/10.1038/s41398-022-02162-y

Mamdani, F., Rollins, B., Morgan, L., Myers, R. M., Barchas, J. D., Schatzberg, A. F., Watson, S. J., Akil, H., Potkin, S. G., Bunney, W. E., Vawter, M. P., & Sequeira, P. A. (2015). Variable telomere length across post-mortem human brain regions and specific reduction in the hippocampus of major depressive disorder. *Translational Psychiatry*, *5*(9). https://doi.org/10.1038/tp.2015.134

McTeague, L. M., Huemer, J., Carreon, D. M., Jiang, Y., Eickhoff, S. B., & Etkin, A. (2017). Identification of common neural circuit disruptions in cognitive control across psychiatric disorders. *American Journal of Psychiatry*, *174*(7), 676–685. https://doi.org/10.1176/appi.ajp.2017.16040400

Meier, S. M., Mattheisen, M., Mors, O., Schendel, D. E., Mortensen, P. B., & Plessen, K. J. (2016). Mortality among persons with obsessive-compulsive disorder in Denmark. *JAMA Psychiatry*, *73*(3), 268–274. https://doi.org/10.1001/jamapsychiatry.2015.3105

Monereo-Sánchez, J., de Jong, J. J. A., Drenthen, G. S., Beran, M., Backes, W. H., Stehouwer, C. D. A., Schram, M. T., Linden, D. E. J., & Jansen, J. F. A. (2021). Quality control strategies for brain MRI segmentation and parcellation: Practical approaches and recommendations - insights from the Maastricht study. *NeuroImage*, *237*(January). https://doi.org/10.1016/j.neuroimage.2021.118174

Mueller, S. G., Stables, L., Du, A. T., Schuff, N., Truran, D., Cashdollar, N., & Weiner, M. W. (2007). Measurement of hippocampal subfields and age-related changes with high resolution MRI at 4 T. *Neurobiology of Aging*, *28*(5), 719–726. https://doi.org/10.1016/j.neurobiolaging.2006.03.007

Mueller, S. G., & Weiner, M. W. (2009). Selective effect of age, Apo e4, and Alzheimer's disease on hippocampal subfields. *Hippocampus*, *19*(6), 558–564. https://doi.org/10.1002/hipo.20614

Penninx, B. W. J. H., Milaneschi, Y., Lamers, F., & Vogelzangs, N. (2013). Understanding the somatic consequences of depression: Biological mechanisms and the role of depression symptom profile. *BMC Medicine*, *11*(1), 1. https://doi.org/10.1186/1741-7015-11-129

Santabárbara, J., Lopez-Anton, R., de la Cámara, C., Lobo, E., Gracia-García, P., Villagrasa, B., Bueno-Notivol, J., Marcos, G., & Lobo, A. (2019). Clinically significant anxiety as a risk factor for dementia in the elderly community. *Acta Psychiatrica Scandinavica*, *139*(1), 6–14. https://doi.org/10.1111/acps.12966

Selek, S., Herken, H., Bulut, M., Ceylan, M. F., Celik, H., Savas, H. A., & Erel, O. (2008). Oxidative imbalance in obsessive compulsive disorder patients: A total evaluation of oxidant-antioxidant status. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *32*(2), 487–491. https://doi.org/10.1016/j.pnpbp.2007.10.002

Šimić, G., Kostović, I., Winblad, B., & Bogdanović, N. (1997). Volume and number of neurons of the human hippocampal formation in normal aging and Alzheimer's disease. *Journal of Comparative*

Neurology, *379*(4), 482–494. https://doi.org/10.1002/(SICI)1096-9861(19970324)379:4<482::AID-CNE2>3.0.CO;2-Z

Sone, D., & Beheshti, I. (2022). Neuroimaging-Based Brain Age Estimation: A Promising Personalized Biomarker in Neuropsychiatry. *Journal of Personalized Medicine*, *12*(11). https://doi.org/10.3390/jpm12111850

Sone, D., Beheshti, I., Maikusa, N., Ota, M., Kimura, Y., Sato, N., Koepp, M., & Matsuda, H. (2019). Neuroimaging-based brain-age prediction in diverse forms of epilepsy: a signature of psychosis and beyond. *Molecular Psychiatry*. https://doi.org/10.1038/s41380-019-0446-9

van den Heuvel, O. A., Boedhoe, P. S. W., Bertolin, S., Bruin, W. B., Francks, C., Ivanov, I., Jahanshad, N., Kong, X. Z., Kwon, J. S., O'Neill, J., Paus, T., Patel, Y., Piras, F., Schmaal, L., Soriano-Mas, C., Spalletta, G., van Wingen, G. A., Yun, J. Y., Vriend, C., … Yamada, K. (2022). An overview of the first 5 years of the ENIGMA obsessive–compulsive disorder working group: The power of worldwide collaboration. *Human Brain Mapping*, *43*(1), 23–36. https://doi.org/10.1002/hbm.24972

Verhoeven, J. E., Révész, D., Epel, E. S., Lin, J., Wolkowitz, O. M., & Penninx, B. W. J. H. (2014). Major depressive disorder and accelerated cellular aging: Results from a large psychiatric cohort study. *Molecular Psychiatry*, *19*(8), 895–901. https://doi.org/10.1038/mp.2013.151

Vreeburg, S. A., Zitman, F. G., Van Pelt, J., Derijk, R. H., Verhagen, J. C. M., Van Dyck, R., Hoogendijk, W. J. G., Smit, J. H., & Penninx, B. W. J. H. (2010). Salivary cortisol levels in persons with and without different anxiety disorders. *Psychosomatic Medicine*, *72*(4), 340–347. https://doi.org/10.1097/PSY.0b013e3181d2f0c8

Wang, J., Li, W., Miao, W., Dai, D., Hua, J., & He, H. (2014). Age estimation using cortical surface pattern combining thickness with curvatures. *Medical and Biological Engineering and Computing*, *52*(4), 331–341. https://doi.org/10.1007/s11517-013-1131-9

Weeland, C. J., Vriend, C., van der Werf, Y., Huyser, C., Hillegers, M., Tiemeier, H., White, T., & van den Heuvel, O. A. (2022). Thalamic Subregions and Obsessive-Compulsive Symptoms in 2,500 Children From the General Population. *Journal of the American Academy of Child and Adolescent Psychiatry*, *61*(2), 321–330. https://doi.org/10.1016/j.jaac.2021.05.024

West, J., Coleman, P. D., Flood, G., & Troncoso, C. (1994). Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer ' s disease. *The Lancet*, *344*(8925), 769–772.

Zhang, B., Zhang, S., Feng, J., & Zhang, S. (2023). NeuroImage : Clinical Age-level bias correction in brain age prediction. *NeuroImage: Clinical*, *37*(January), 103319. https://doi.org/10.1016/j.nicl.2023.103319

Zou, J., Huss, M., Abid, A., Mohammadi, P., Torkamani, A., & Telenti, A. (2019). A primer on deep learning in genomics. *Nature Genetics*, *51*(1), 12–18. https://doi.org/10.1038/s41588-018-0295-5

CHAPTER 6: Investigation of whole and regional brain aging in social anxiety disorder, and its association with clinical characteristics

In chapter 4, I investigated hippocampal and amygdala subfield volumes in SAD and their association with chronological aging. While SAD was associated with volumetric differences in both the hippocampus and amygdala, chronological age did not moderate the relationship between SAD and subfield volumes (as discussed in the postscript of chapter 4). In chapter 5, I investigated brain aging in OCD and found significantly higher brain-PADs in individuals with OCD compared to HCs. This finding is consistent with the literature on brain aging in OCD (Liu et al., 2022), and suggests that individuals with anxiety and related disorders may be vulnerable to accentuated aging. In the present chapter 6, I expand on chapters 4 and 5 by investigating whole and regional brain age differences in individuals with SAD compared to HCs. Using the same methods as in chapter 5, I use a previously trained MLA to investigate brain age differences in individuals with SAD compared to HCs. I also conducted a partial correlation analysis to investigate whether hippocampal and amygdala subfield volumes are correlated to brain-PADs within the groups (SAD and HCs). While there is evidence to support biological aging in SAD as indexed by shorter TL (Hoen et al., 2013; Malouff & Schutte, 2017), an association between brain aging and anxiety disorders (SAD combined with other disorders) has only been found when controlling for antidepressant, suggesting that brain-PAD differences may be modulated by clinical characteristics (Han et al., 2021). Similar to chapters 3-5, chapter 6 also includes a subgroup analysis to assess the influence of clinical characteristics such as psychiatric comorbidity and medication status on brain-PADs. In chapter 6, it is anticipated that there will be greater brain aging in SAD.

6.1. Introduction

SAD is a debilitating anxiety disorder characterized by heightened and persistent fear of social settings, or performance activities, whereby one may have to interact with unfamiliar people and possibly be subjected to scrutiny or evaluation by others. Typically, individuals with SAD experience fear of embarrassing themselves, resulting in negative judgment by others, which may lead to the avoidance of social settings (APA, 2013). Studies suggest that individuals with anxiety disorders have significantly higher risk of natural and unnatural causes of death compared to the general population (Meier et al., 2016), and higher risk of cardiovascular mortality (Emdin et al., 2016). While it is speculated that the increased risk of mortality and age-related diseases observed in anxiety disorders is in part due to accentuated biological aging, the mechanisms underlying these processes are still unclear (Danese & McEwen, 2012; Han et al., 2019; Johnson, 2006; López-Otín et al., 2013; McEwen, 2000; Wolkowitz et al., 2010).

Findings in neuroimaging have demonstrated that in anxiety disorders, chronological age is associated with smaller hippocampal volumes and white matter in the anterior cingulate cortex (Hilbert et al., 2014), and smaller gray matter in the amygdala and the hippocampus (Pannekoek et al., 2013). Wang et al. (2018) found that in SAD, increasing chronological age was associated with larger gray matter volumes in the dorsolateral prefrontal cortex, angular gyrus, putamen, and occipital and medial gyrus (Wang et al., 2018). However, this association was more apparent in younger individuals and not in adults with SAD (older than 18 years), which may be more suggestive of neurodevelopment processes than the detrimental effects of aging (Wang et al., 2018).

Machine learning has allowed for the prediction of brain aging using a rich set of brain features derived from neuroimaging data (Cole & Franke, 2017). Briefly, brain age predictions are made using MLA (e.g., gaussian process regression, relevance vector regression, support vector regression) trained to identify patterns and correlations between brain features and chronological age (Baecker et al., 2021; Cole et al., 2019; Franke & Gaser, 2019; Zou et al., 2019). Using various computational models, studies have indicated that psychiatric disorders are associated with accentuated brain aging, although this has been dependent on clinical characteristics (Clausen et al., 2021; Han et al., 2021).

In a comprehensive study, Kaufmann et al., 2019, found distinct regional patterns of brain aging in individuals compared to HCs. Specifically, in schizophrenia, the strongest effect of brain-PAD was observed in the frontal lobe (d=0.71), while MDD was associated with brain-PAD in the temporal lobe (d=0.26) (Kaufmann et al., 2019). These findings were amongst the first reports of disorder-dependent regional specificity in brain aging. To date, regional patterns of brain aging have been reported in only one study that included a group of psychiatric disorders including specific phobia (n=70, mean age=12.57 years), social phobia (n=77, mean age=14.05 years), PTSD (n=44, mean age=15.16 years), depression (n=70, mean age=16 years) (Niu et al., 2022). The study found smaller brain age in specific and social phobia in the gray matter volume of subcortical regions (model fit statistics; R^2=0.798 for males and R^2=0.59 for females). However, because this study was performed in adolescents, who may still be undergoing neurodevelopmental changes (Cole & Franke, 2017; Gogtay et al., 2004; Niu et al., 2022), the findings are difficult to extrapolate to adult SAD.

Given that little is known about brain aging in adult SAD, the objective of this chapter is to 1) examine the relationship between whole and regional brain-PADs in adult individuals with SAD (aged between 18 years and 68 years old), and 2) correlate whole brain-PAD to hippocampal and amygdala subfield volumes in SAD. A pre-trained 5-fold cross-validated brain-age prediction model is used to estimate whole and regional brain age in a large sample of 107 individuals with SAD and 137 HCs. Brain-PAD is calculated for each individual by subtracting chronological age from the predicted brain age. In line with the evidence of the influence of clinical characteristics on subcortical volumes in SAD (Groenewold et al., 2023), subgroup analyses will be performed where the sample is restricted to individuals with SAD without lifetime 1) comorbid anxiety disorder, 2) comorbid MDD, and 3) psychotropic medication use. In a separate analysis, the association between whole-and-regional brain-PADs with SAD symptom severity is tested. Lastly, a partial correlation analysis is conducted to examine the association between subfield volumes and the predicted whole brain age within SAD.

6.2. Methods

Participant selection

Structural T1-weighted 3T MRI scans and sociodemographic data were obtained from a previously published meta-analysis, which was part of EUSARNAD. The sample composition has been published in detail elsewhere (Bas-Hoogendam et al., 2017). Briefly, participants were recruited through clinical and public notices. Participant evaluation was conducted using a structured clinical interview (SCID) (First et al., 1997), mini-international neuropsychiatric interview (MINI) (Lecrubier et al., 1997), or composite international diagnostic interview (CIDI) (Robins et al.,1988). The requirement for the patient group was a primary diagnosis of SAD; psychiatric comorbidity was allowed if it was secondary to SAD. HCs were required not to have any psychiatric diagnosis. Participants were excluded from the study if they were younger than 18 and older than 65 years of age, and if they had general MRI contraindications (for example, claustrophobia or pregnancy). Clinical information on comorbidity, medication status, age of onset, and SAD symptom severity (as measured by the LSAS) (Mennin et al., 2002) was collected. All of the research sites obtained ethical approval from local ethical review boards and written informed consent was obtained from each participant.

Freesurfer segmentation, quality control, and brain age estimation

The methods employed in the present chapter were identical to those used in chapter 5 (Brain Aging in OCD). Briefly, FreeSurfer 5.3 (http://surfer.nmr.mgh.harvard.edu/) was used to process the T1-weighted structural MRI data (Fischl et al., 2002). Visual inspection was performed by using an adaptation of the ENIGMA Consortium Quality Control protocol for subcortical and hippocampal subfields (https://enigma.ini.usc.edu/protocols/imaging-protocols/). In addition, participants were excluded if their Euler number was within 3 standard deviations from the global mean, as calculated for each site (Ipser et al., 2022). For the brain age estimation, model construction and validation have been described in detail by Kaufman et al., 2019. Briefly, the model was built using the eXtreme Gradient Boosting (xgboost) package in R (Chen & He, 2014). For the present study, shell scripts to extract relevant brain features, as well as the R script to estimate brain age using the pre-trained model were provided by Dr. Kaufmann (Kaufmann et al., 2019). These scripts were applied to Freesurfer 5.3 segmented T1 weighted MRI scans consisting of 107 individuals with SAD, and 137 HCs.

Statistical analysis: linear mixed effects model

Covariates

Brain-PAD (predicted brain age − chronological age) was calculated for each participant using the Kaufmann brain age model (Kaufmann et al., 2019). This was used as the primary outcome variable in the statistical analysis. The covariates of interest were age and age squared (which were both centered to facilitate interpretation of the results), sex, scanner site (to control for scanner differences across sites), and Euler number. The inclusion of chronological age as a covariate in our analysis was also to reduce 'regression dilution', as described in chapter 5.

Linear mixed-effects models

All statistical analyses were conducted in *R* (version 4.2.1; freely available at https://www.r-project.org/). T-tests were conducted to assess between group (SAD n=107; HC n=137) differences in age, sex, education, and Euler number. To assess model fit (performance of the prediction model), Pearson's correlation coefficient (R) and the proportion of the variance explained by the model (R^2) were calculated between the predicted brain age estimate and chronological age in the SAD group and HCs. Additionally, the MAE for brain-PAD was calculated for both groups. To examine the main effect of SAD diagnosis, the *R* package *lme4* was used to perform the analysis which used mixed-effects (Cohens d) effect sizes, as calculated using the t values from linear mixed-effects models (Nakagawa & Cuthill, 2007). A linear mixed effects model that included a random intercept for the scan site was used to analyze group differences in brain-PADs between individuals with SAD and HCs (main analysis: SAD n=107; HC n=137).

Separate subgroup analyses were performed for individuals with SAD without lifetime 1) comorbid anxiety disorder (SAD n=86), 2) comorbid MDD (SAD n=83), and 3) psychotropic medication use (SAD n=89) versus HC (n=137). Due to small sample sizes, we were unable to analyze brain-PAD in individuals with SAD with 1) comorbid anxiety disorder (SAD n=21), 2) comorbid MDD (SAD n=24), and 3) psychotropic medication use (SAD n=19). Within-group analysis (SAD n=84) was performed to assess the association between brain-PAD and SAD symptom severity (measured using the LSAS). Separate LME models that included diagnosis-by-age interaction terms were conducted. Partial correlation coefficients were computed to assess the association between whole brain-PAD and hippocampal and amygdala subfield volumes, using the ppcor R library. All analyses were corrected for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) and the p-value was considered statistically significant at pFDR <0.05.

6.3. Results

Model fit (performance of the prediction model) is shown in Table 6.1b. There was a moderate positive correlation (R=0.76) between brain age and chronological age in the SAD group, suggesting that the predicted brain age increased with chronological age. In this group, more than half (58.39%) of the variability in the predicted brain age could be explained by the chronological age. The MAE in the SAD group was relatively high, at 7.04 years. In the HCs group, there was also a moderate positive correlation (R=0.79) suggesting that the predicted brain age increased with chronological age, with a degree of variability (R^2=0.6293; 62.93% of the variability in the predicted brain age can be explained by the chronological age) and MAE of 5.85 years. The mean brain-PAD of 2.056 years in the HC group was significantly larger than zero (t = 3.146, p = 0.002), suggesting that the model fit to these data is sub-optimal. No association was observed between chronological age and brain-PAD in the controls (R = -0.074, p = 0.390), with a significant negative association observed in the SAD group (R = -0.282, p = 0.003) (Table 6.1a). Concerning regional MAEs (Table 6.1b). Briefly, the model fit between the brain age and chronological estimates was particularly poor for the cingulate and insula, in both the SAD (MAE=14.302, R=0.502, R^2=0.252; MAE=13.071, R=0.415, R^2=0.172, respectively) and HCs group (MAE=13.122, R=0.642, R^2=0.412; MAE=11.126, R=0.634, R2 = 0.402, respectively). Of note, model fit was particularly poor in the occipital and parietal models in the SAD group (MAE=14.852, R=0.187, R2=0.035; MAE=13.927, R=0.504, R2=0.254).

Sample characteristics

In the full sample (SAD: n=107; HC: n=137), there was no significant difference in the mean years of education (SAD: 14.65 years; HC: 15.07 years; t=0.85, p-value=0.4), age (SAD: 32.21 years; HC: 34.75 years; t=1.88, p-value=0.06), and sex (SAD: 61% female; HC: 56%; X2=0.34, p-value=0.56) between individuals with SAD and HCs (see Table 6.2).

Main analysis: whole and regional brain age analysis

Individuals with SAD (n=107) had significantly higher whole brain-PAD (+2.5 years, d=0.33, p_{FDR}=0.010) compared to HCs (n=137), after adjusting for sex, age, age squared, site, and Euler number (Figure 6.1). Additionally, regional brain-PAD was significantly higher in the temporal (+3.80 years, d=0.37, p_{FDR}=0.008,), parietal (+3.57 years, d=0.38, p_{FDR}=0.008), occipital (+3.26 years, d = 0.33, p_{FDR}=0.010), and frontal regions (+2.97 years, d=0.33, p_{FDR}=0.010,), in individuals with SAD compared to HCs. No group differences were observed for the insula or the subcortex.

Subgroup analysis: association between whole and regional brain age and clinical characteristics

SAD without a comorbid anxiety disorder

Individuals with SAD without comorbid anxiety (n=86) had significantly higher whole brain-PAD (+2.39 years, d=0.31, p_{FDR}=0.024) compared to HCs (n=137). Additionally, regional brain-PAD was significantly higher in the parietal (+3.84 years, d=0.39, p_{FDR}=0.012) followed by the temporal (+3.55 years, d=0.35, p_{FDR}=0.015), the cingulate (+3.38 years, d=0.30, p_{FDR}=0.015), the occipital cortex (+2.94 years, d=0.29, p_{FDR}=0.040), and the frontal cortex (+2.54 years, d=0.27, p_{FDR}=0.049) in individuals with SAD compared to HCs. No group differences were observed for the insula or the subcortex.

SAD without comorbid MDD

Individuals with SAD without comorbid MDD (n=83) had significantly higher whole brain-PAD (+2.34 years, d=0.30, p_{FDR}=0.031) compared to HCS (n=137). Regional brain-PAD was significantly higher in the temporal (+3.75 years, d=0.35, p_{FDR}=0.022), followed by the parietal (+3.12 years, d=0.32, p_{FDR}=0.031), and the cingulate regions (+3.50 years, d=0.30, p_{FDR}=0.022) in individuals with SAD compared to HCs. No group differences were observed for the insula or the subcortex.

SAD without psychotropic medication use

Individuals with SAD without psychotropic medication use (n=89) had significantly higher whole brain-PAD (+3.15 years, d=0.41, p_{FDR} =0.003) compared to HCs (n=137). Additionally, regional brain-PAD was significantly higher in the temporal (+4.70 years, d=0.45, p_{FDR}=0.004), parietal (+3.88 years, d=0.40, p_{FDR}=0.005), followed by the frontal (+3.80 years, d=0.38, p_{FDR} =0.009), occipital (+3.40 years, d=0.33, p_{FDR} =0.013), and cingulate regions (+3.39 years, d=0.30, p_{FDR} =0.009) in individuals with SAD compared to HCs. No group differences were observed for the insula or the subcortex.

SAD symptom severity

There was no association between SAD symptom severity (assessed using LSAS, n=84) with whole brain-PAD in individuals with SAD (rME=0.198, p=0.056).

Interactions

There was no evidence of a group difference in the progression of whole brain-PAD as a function of age (t=-1.555, p=0.1212) nor sex (t=-0.059, p=0.953) between individuals with SAD (n=107) and HCs (n=137).

Partial correlation analysis

Partial correlation analysis was used to investigate unique associations between hippocampal and amygdala subfield volumes and whole brain-PADs. Unadjusted associations were observed in the SAD group for the lateral nucleus (r=-0.277, p=0.010), and the CA3 (r=0.269, p=0.012), and for the control group in the corticoamygdaloid transition area (r=-0.229, p=0.013) and the CA3 (r=0.228, p=0.014) and whole brain-PAD. However, these associations were not significant after correction for multiple comparisons using Benjamini Hochberg FDR (Table 6.3 a-b).

6.4. Discussion

The findings revealed higher whole and regional brain PAD in the SAD than the HC group, as expected. A similar pattern of findings was observed in individuals with SAD without comorbid anxiety disorder, as well as individuals not on medication. In contrast, individuals with SAD without MDD had higher regional but not whole brain-PAD compared to HCs. No association was found between brain-PAD and SAD symptom severity.

Main findings

Higher brain aging in SAD

The main findings presented in this chapter are consistent with recent reports of significantly higher brain-PADs in individuals with anxiety disorders compared to HCs. Han et al., 2021 found that anxiety disorders (n=67) were associated with significantly higher brain-PAD (+2.9 years), compared to HC, only after controlling for antidepressant use. In another study, Clausen et al. (2021) found an association between brain-PAD and PTSD compared (n=884) to HCs, however the effect of PTSD diagnosis was dependent on sex and age; higher in younger individuals with PTSD than in older individuals with PTSD, compared to HCs of all ages. Of note, both Han, et al. (2021) and Clausen et al. (2021) had similar MAE and R in the patient's groups (R=0.72, MAE=6.73; R=0.72, MAE =5.68, respectively) to those observed in the present chapter (R=0.76; MAE=7.04) suggesting that brain age prediction models used in Han, et al. (2021) and Clausen et al. (2021) (photon-ai; ridge regression model and brainageR; gaussian process regression model, respectively) and the present chapter (xgboost) display similar predictive model fit to the respective data. The effect sizes observed in Han et al., 2021 (d=0.27) are comparable to those observed in the present chapter (d=0.33) suggesting a similar degree of association between whole brain-PAD and disorder between the two findings.

While speculative, there are some possible explanations for the accentuated brain aging observed in individuals with SAD in the present chapter. One possible explanation is neuroinflammation, which may be underlined by continuous antigenic load and stress (Finlay et al., 2022; McEwen, 2008). As SAD is often characterized by persistent and excessive fear or anxiety in social situations (APA, 2013), it is possible that chronic stress may lead to dysregulation of the HPA-axis and the release of cortisol. Elevated levels of salivary cortisol have been documented in SAD (Åhs et al., 2006), but plasma levels have been found to be lower in individuals with SAD suggesting a hypo-responsiveness of the HPA-axis (Petrowski et al., 2021). Lower plasma cortisol levels have also been negatively correlated with inhibitory serotonergic receptor (5-HT_{1A}) binding in the hippocampus and amygdala, which may lead to the decreased release of GABA in the limbic areas and therefore increase anxiety and stress (Lanzenberger et al., 2010). Of note, findings on cortisol stress response after exposure to social challenges in SAD have been mixed (Petrowski et al., 2021), as is the literature on proinflammatory cytokines in SAD (Caldiroli et al., 2023). These inconsistent findings may be due to methodological differences across the studies and the non-standardization of stressors used to assess cortisol stress reactivity.

In terms of the immune system, some studies suggest that the immune-kynurenine pathway is dysregulated in SAD suggesting immunosuppression, however, these findings have not yet been replicated or correlated with alterations in brain structures involved in the neurobiological model of SAD (Butler et al., 2022). In the context of brain aging in anxiety disorders, Han et al. (2021) did not find a correlation between biological stress variables (i.e., inflammatory markers and HPA-axis) and brain-PAD in individuals with anxiety disorders compared to HCs. There are likely a myriad of other biological mechanisms and confounders that are associated with brain aging in anxiety and related disorders. The underlying mechanism may involve genetic factors, lifestyle factors, and medication use, all of which warrant further investigation.

Higher regional brain aging in SAD.

Individuals with SAD had significantly higher regional brain-PAD in the parietal (d=0.38), temporal (d=0.37), occipital (d=0.33), and frontal regions (d=0.33). The effect sizes observed in the present chapter are comparable to those observed in Kaufmann et al. (2019) specifically for the temporal lobe in MDD (d=0.26), though higher effect sizes in the frontal lobe are observed in schizophrenia (d=0.71). However, the findings in the present chapter should be interpreted with caution as it is challenging to achieve accurate brain age predictions for small regions with a relatively small number of features. Moreover, the model fits were particularly high for the significant regions in individuals with SAD in main analysis and subgroup analysis SAD group; main analysis results: parietal (MAE=13.927, R=0.504, R^2=0.254), temporal (MAE=11.153, R=0.503, R^2=0.253), occipital (MAE=14.852, R=0.187, R^2=0.035), and the frontal regions (MAE=9.788, R=0.687, R2=0.472)(see table x). The high MAEs for the regional models suggest that the regional brain age model was a poor fit for the data used in the present chapter.

Subgroup analysis: association of whole and regional brain-PAD and clinical characteristics

SAD without a comorbid anxiety disorder

Individuals with SAD without comorbid anxiety disorder had significantly higher whole brain-PAD compared to HCs. The whole brain-PAD for individuals with SAD without comorbid anxiety disorder (+2.39 years, d=0.31, p_{FDR}=0.024) remained significant and comparable, in terms of the predicted difference and effect size, to the main analysis (+2.5 years, d=0.33, p_{FDR}=0.010), despite the smaller sample size (n=86). Significantly higher brain-PAD in the absence of comorbid anxiety disorder suggests that brain aging may not be driven by psychiatric comorbidity. However, it would be expected that psychiatric comorbidity would increase the predicted brain age due to shared neurobiological pathways among comorbid disorders (Koyuncu et al., 2019). Indeed, it is shown that psychiatric comorbidity enhances the chronicity of symptoms in SAD (Lydiard, 2001). However, given that the association between brain-PAD and comorbidity in individuals with anxiety disorders (+2.23 years, d=0.21, p_{FDR}=0.08) has been shown to be marginally significant (Han et al., 2021), and the fact that the present chapter was unable to model brain age in the individuals with SAD with comorbid anxiety disorder due to small sample sizes (n=21), the relationship between whole brain-PAD and comorbid anxiety disorder in SAD remains unclear.

SAD without comorbid MDD

Similar to our findings in individuals with SAD without comorbid anxiety disorder, individuals with SAD without comorbid MDD, had significantly higher whole brain-PAD compared to HCs. The brain-PADs and effect sizes in individuals with SAD without comorbid MDD were comparable to the main analysis (+2.34 years, d=0.30, p_{FDR}=0.031; +2.5 years, d=0.33, p_{FDR}=0.010 respectively), suggesting that accentuated brain aging remained even after excluding MDD comorbidity. In MDD, findings on brain aging are mixed as both accentuated brain aging (Koutsouleris et al., 2014), and null findings have been reported (Besteher et al., 2019; Kuo et al., 2020). While it would be expected that comorbidity with MDD would increase brain aging in SAD due to cumulative stress responses, studies find that MDD comorbidity modulates stress reactivity by decreasing the cortisol responses found in SAD (Yoon & Joormann, 2012). This is speculated to be because depression is associated with reduced overall emotional reactivity, including blunted physiological responding (Bylsma et al., 2008), whilst anxiety is associated with increased emotional reactivity (Lang & McTeague, 2010). However, due to small sample sizes (n=24), the present chapter was unable to investigate brain age in individuals with SAD with comorbid MDD. Further analysis in large-scale studies rich with clinical characteristics are required to elucidate the relationship between brain age and MDD (stratified by medication status, i.e., individuals with MDD with and without medication use).

SAD psychotropic without medication use

Individuals with SAD without psychotropic medication use had significantly higher whole brain-PAD compared to HCs. Compared to the main analysis (n=107), the subgroup analysis had less sample size (n=89), however, the brain-PADs and effect size in the subgroup analysis was slightly higher (+3.15 years, d=0.41, p_{FDR}=0.003) than the main analysis (+2.5 years, d=0.33, p_{FDR}=0.010). These findings suggest that while we observed an association between accentuated brain aging in individuals with SAD compared to HCs, this effect is slightly more pronounced (with a high level of statistical significance; p_{FDR}=0.003) in individuals with SAD without psychotropic medication use. Additionally, the effect size in this subgroup increased (d=0.41) suggesting a stronger association between brain-PAD and individuals with SAD without psychotropic medication use than in the main analysis. While our findings seem contrary to studies that show an association between anxiety disorders and brain-PAD (only significant when controlling for antidepressants) (Han et al., 2021), and significantly higher in antidepressant users compared to medication-free individuals with MDD (Luo et al., 2022), the present chapter did not have enough sample to investigate brain-PAD in individuals with SAD with psychotropic medication (n=19). We speculate that individuals with SAD with psychotropic medication use had less of an association with whole brain-PAD resulting in lower brain-PADs and effect sizes in the mixed sample of the main analysis, however, to date very little evidence to infer how brain-PAD responds to pharmacotherapy. Further analysis is required to elaborate on this speculation.

SAD symptom severity

There was no association between whole or regional brain-PADs and SAD symptom severity (n=84). There are very few studies linking brain-PAD to anxiety symptom severity, Han et al. (2021) found that higher brain-PAD was associated with anxiety symptoms, however, the sample included other anxiety disorders including panic disorder (Han et al., 2021). In a meta-analysis, Blake et al. (2023) found that higher brain-PAD was associated with symptom severity mainly in psychotic disorders (Blake et al., 2023). With respect to SAD symptom severity across brain features, some reports found a positive association between gray matter volume and SAD symptom severity (Bas-Hoogendam et al., 2017), while others found a negative association between SAD symptom severity and cortical thickness in the right rostral anterior cingulate cortex (Frick et al., 2013) and postcentral cortex (Syal et al., 2012). In a mega-analysis, Groenewold et al. (2023) did not find an association between subcortical volumes and SAD symptom severity in individuals with SAD, after multiple comparison corrections (Groenewold et al., 2023). Additionally, due to a lack of sufficient LSAS data from the various research sites that were utilized in the present chapter, the sample size was too small to perform further inspection, specifically, investigating whether brain-PAD differs by symptom dimension and sub-types of SAD. Given that there is some evidence of an association between brain volume, cortical thickness, and SAD symptom severity (although findings are mixed), future analysis using larger samples with multiple categories of SAD symptomatology is required to determine whether there is a link between brain-PAD and SAD symptom severity and sub-types.

Partial correlation analysis

A partial correlation analysis was performed to investigate the association between hippocampal and amygdala subfield volumes and whole brain-PADs within the groups (SAD and HCs). In the SAD group, there was a trend association between brain-PAD and the lateral nucleus (R=-0.277, p=0.010) and the CA3 (R=0.269, p=0.012). In the HCs, there was a trend association between the CAT (R=-0.229, p=0.013) and the CA3 (R=0.228, p=0.014). These associations were not significant after correction for multiple comparisons (Benjamini Hochberg FDR). Findings on the hippocampal CA3 subfield volume concerning aging have been mixed, some studies find it to be influenced by chronological age (Daugherty et al., 2017) whilst others do not (Malykhin et al., 2017). Heterogeneity in the volume of the CA3 across studies is associated with chronological age, according to a systematic review of hippocampal subfield volumes in psychiatric disorders (Sun et al., 2023). While the lateral nucleus (Aghamohammadi-Sereshki et al., 2019), CAT (Kurth et al., 2019), and CA3(Daugherty et al., 2017) have been associated with aging, of note none of these findings reach significance in the present analysis.

6.5. Limitations

One notable strength of the present chapter is the use of a large multisite sample (SAD: n=107; HC: n=137) which allowed for the subgroup analysis to investigate the association between brain-PAD and clinical characteristics. However, for some of the subgroup analyses (individuals with SAD with comorbid anxiety disorder (n=21), comorbid MDD (n=24), and psychotropic medication use (n=19)) the samples were too small and therefore we could not compare these results to those reported for individuals with SAD without comorbid anxiety disorder (n=86), MDD (n=83), and psychotropic medication use (n=89). While the brain age model used in this study was trained on a large independent sample (n=35474) using a variety of brain features (thickness, volume surface area) (Kaufmann et al., 2019), a noteworthy limitation was the high MAE (reflecting the performance of the prediction model) observed for the brain-PADs for both the SAD and HCs groups, which were larger than those observed in other brain age studies (Clausen et al., 2021; Han et al., 2021; Liu et al., 2022). However, studies suggest that models with tighter fits (MAE=3 years) tend to prioritize age-related characteristics minimizing disease-related characteristics (Bashyam et al., 2020). Because the presented chapter used inherited data from a previously published study (Bas-Hoogendam et al., 2017), there was limited information on several variables including sociodemographic data (socioeconomic status), lifestyle variables (smoking, alcohol and physical activity) and clinical data (inflammatory markers, ANS, and HPA axis) that have been association with biological aging (Cribbs et al., 2012; Jansen et al., 2021; Norden & Godbout, 2013).

6.6. Conclusion

The findings in the present chapter suggest a link between diagnosis and accentuated whole brain aging in a large sample of individuals with SAD (n=107) and HCs (n=137). Additionally, I provide evidence of regional accentuated brain aging in the cortical brain structures that would be associated with dysregulation in SAD, mainly the temporal (d=0.37), parietal (d=0.38), occipital (d=0.33) and frontal regions (d=0.33) (with comparable effect sizes). However, since the MAEs for these models were high in both the SAD and HCs groups, the interpretation of these findings has been limited. Of note, considering that the positive brain-PADs found in individuals with SAD in the main analysis remained significant (even when considering individuals without comorbid MDD, anxiety disorder, or psychotropic medication use) suggests that the observed group differences could not be solely explained by comorbid conditions. Future investigation should evaluate moderating factors of brain age including the influence of overlapping comorbidity and how brain aging may be associated with clinical and neurocognitive data in SAD.

6.7. Tables and figures

Table 6.1a: Correlation matrix for HC regional brain-PAD estimates, after regressing out site and chronological age

	Cingulate	Frontal	Insula	Occipital	Parietal	Subcortex	Temporal
Cingulate	1	0.561	0.481	0.521	0.568	0.188	0.333
Frontal	0.561	1	0.407	0.477	0.585	0.261	0.555
Insula	0.481	0.407	1	0.415	0.539	0.094	0.269
Occipital	0.521	0.477	0.415	1	0.466	0.247	0.419
Parietal	0.568	0.585	0.539	0.466	1	0.218	0.486
Sub-cortical	0.188	0.261	0.094	0.247	0.218	1	0.263
Temporal	0.333	0.555	0.269	0.419	0.486	0.263	1

Table 6.1b: Model fit (MAE, R, R^2) for whole and regional brain age model in individuals with SAD (n=107) and HCs (n=137)

	SAD (n=107)			**HCs (n=137)**			
	MAE	**R**	**R^2**		**MAE**	**R**	**R^2**
Whole brain age	7.0401	0.7642	0.5839		5.8524	0.7933	0.6293
Cingulate	14.3020	0.5020	0.2520		13.1220	0.6420	0.4120
Insula	13.0710	0.4150	0.1720		11.1260	0.6340	0.4020
Temporal	11.1530	0.5030	0.2530		7.8180	0.6540	0.4280
Subcortex	8.5720	0.6240	0.3890		7.5060	0.6130	0.3760
Occipital	14.8520	0.1870	0.0350		10.6770	0.5950	0.3540
Frontal	9.7880	0.6870	0.4720		8.0690	0.7520	0.5660
Parietal	13.9270	0.5040	0.2540		9.7320	0.7110	0.5060

Table 6.2: Demographic and clinical characteristics of individuals with SAD (n=107) and HCs (n=137)

	SAD (n=107)		**HCs (n=137)**		**Statistical analysis**		
Characteristics	**mean**	**SD**	**mean**	**SD**	**t**		**p**
Age (years)	32.21	10.43	34.75	10.51	-2.47		0.001
Education level (years)	14.65	2.94	15.07	3.04	3.51		< 0.001
LSAS score mean[a]	78.1	19.5	--	--	--		--
Age of onset[b]	14.8	7.1	--	--	--		--
Euler	-200.92	153.81	-284.25	190.47	1.63		0.10
	N	**%**	**N**	**%**			
Female	65	61.0	179	56	X2= 1.2		0.27
Medication use at time of scan	19	17.75	--	--	--		--

[a] Liebowitz Social Anxiety Scale (LSAS). [b] measured using LSAS.

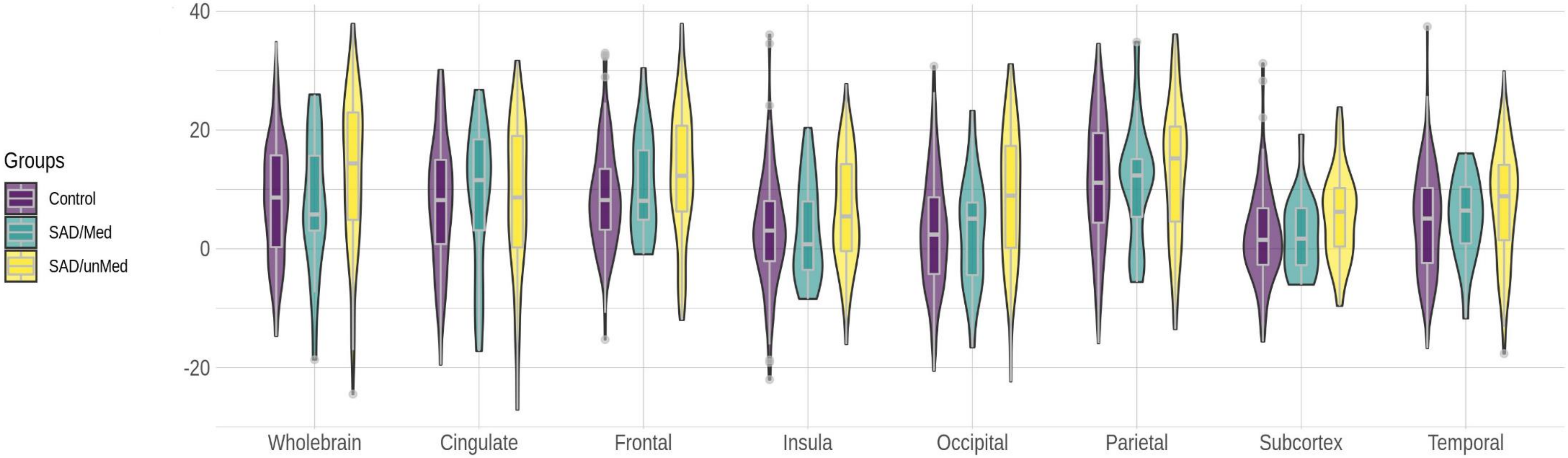

Figure 6.1: violin plot showing whole and regional brain-PADs in SAD (n=107) with (n=15) and without (n=86) psychotropic medication use (n=137), compared to HCs (n=137).

Table 6.3a: Partial correlation between hippocampal and amygdala subfield volumes and brain-PADs in individuals with SAD (n=107)

SAD group partial correlation.	**R**	**P value**	**P value corrected (p_{FDR})**	**Statistics**
Amygdala subfields				
Lateral	-0.276	0.010	0.124	-2.652
Basal	0.037	0.730	0.929	0.346
Accessory Basal	-0.033	0.765	0.929	-0.300
AAA	0.058	0.595	0.929	0.533
Central	0.012	0.913	0.958	0.110
Medial	0.084	0.442	0.929	0.773
Cortical	-0.005	0.965	0.965	-0.044
CAT	0.035	0.745	0.929	0.326
Paralaminar	0.027	0.802	0.929	0.252
Hippocampal subfields				
Parasubiculum	-0.067	0.538	0.929	-0.619
Presubiculum	0.063	0.562	0.929	0.582
Subiculum	0.069	0.524	0.929	0.639
CA 1	0.081	0.456	0.929	0.749
CA 3	0.269	0.012	0.124	2.572
CA 4	-0.149	0.167	0.929	-1.394
DG	0.106	0.328	0.929	0.984
ML	-0.143	0.185	0.929	-1.337
HATA	-0.123	0.255	0.929	-1.147
Fimbria	0.022	0.840	0.929	0.202
Hippocampal fissure	-0.030	0.783	0.929	-0.276
Hippocampal tail	0.080	0.462	0.929	0.739

Table 6.3b: Partial correlation between hippocampal and amygdala subfield volumes and brain-PADs in individuals with SAD (n=137)

SAD HCs group partial correlation.	**R**	**P value**	**P value corrected (p_{FDR})**	**Statistics**
Amygdala subfields				
Lateral	-0.035	0.710	0.925	-0.373
Basal	0.023	0.804	0.925	0.249
Accessory Basal	-0.010	0.912	0.946	-0.111
AAA	0.019	0.837	0.925	0.206
Central	-0.081	0.388	0.801	-0.866
Medial	-0.043	0.649	0.908	-0.457
Cortical	0.184	0.047	0.328	2.010
CAT	-0.229	0.013	0.141	-2.525
Paralaminar	0.006	0.946	0.946	0.068
Hippocampal subfields				
Parasubiculum	-0.029	0.760	0.925	-0.306
Presubiculum	0.097	0.300	0.801	1.042
Subiculum	0.129	0.165	0.782	1.396
CA 1	0.068	0.466	0.801	0.732
CA 3	0.228	0.013	0.141	2.510
CA 4	-0.123	0.186	0.782	-1.330
DG	0.092	0.322	0.801	0.995
ML	-0.113	0.225	0.789	-1.219
HATA	-0.055	0.558	0.837	-0.588
Fimbria	0.072	0.443	0.801	0.770
Hippocampal fissure	-0.087	0.349	0.801	-0.940
Hippocampal tail	0.064	0.496	0.801	0.683

6.8. References

Åhs, Fredrik, Tomas Furmark, Åsa Michelgård, Bengt Långström, Lieuwe Appel, Oliver T. Wolf, Clemens Kirschbaum, and Mats Fredrikson. 2006. “Hypothalamic Blood Flow Correlates Positively with Stress-Induced Cortisol Levels in Subjects with Social Anxiety Disorder.” *Psychosomatic Medicine* 68(6):859–62. doi: 10.1097/01.psy.0000242120.91030.d8.

APA. 2013. *Diagnostic and Statistical Manual of Mental Disorders: DSM-5^{TM}, 5th Ed.* Arlington, VA, US: American Psychiatric Publishing, Inc.

Baecker, Lea, Rafael Garcia-Dias, Sandra Vieira, Cristina Scarpazza, and Andrea Mechelli. 2021. “Machine Learning for Brain Age Prediction: Introduction to Methods and Clinical Applications.” *EBioMedicine* 72:103600. doi: 10.1016/j.ebiom.2021.103600.

Bas-Hoogendam, Janna Marie, Henk van Steenbergen, J. Nienke Pannekoek, Jean Paul Fouche, Christine Lochner, Coenraad J. Hattingh, Henk R. Cremers, Tomas Furmark, Kristoffer N. T. Månsson, Andreas Frick, Jonas Engman, Carl Johan Boraxbekk, Per Carlbring, Gerhard Andersson, Mats Fredrikson, Thomas Straube, Jutta Peterburs, Heide Klumpp, K. Luan Phan, Karin Roelofs, Dick J. Veltman, Marie José van Tol, Dan J. Stein, and Nic J. A. van der Wee. 2017. “Voxel-Based Morphometry Multi-Center Mega-Analysis of Brain Structure in Social Anxiety Disorder.” *NeuroImage: Clinical* 16(May):678–88. doi: 10.1016/j.nicl.2017.08.001.

Bashyam, Vishnu M., Guray Erus, Jimit Doshi, Mohamad Habes, Ilya Nasralah, Monica Truelove-Hill, Dhivya Srinivasan, Liz Mamourian, Raymond Pomponio, Yong Fan, Lenore J. Launer, Colin L. Masters, Paul Maruff, Chuanjun Zhuo, Henry Völzke, Sterling C. Johnson, Jurgen Fripp, Nikolaos Koutsouleris, Theodore D. Satterthwaite, Daniel Wolf, Raquel E. Gur, Ruben C. Gur, John Morris, Marilyn S. Albert, Hans J. Grabe, Susan Resnick, R. Nick Bryan, David A. Wolk, Haochang Shou, and Christos Davatzikos. 2020. “MRI Signatures of Brain Age and Disease over the Lifespan Based on a Deep Brain Network and 14 468 Individuals Worldwide.” *Brain* 143(7):2312–24. doi: 10.1093/brain/awaa160.

Besteher, Bianca, Christian Gaser, and Igor Nenadić. 2019. “Machine-Learning Based Brain Age Estimation in Major Depression Showing No Evidence of Accelerated Aging.” *Psychiatry Research - Neuroimaging* 290(June):1–4. doi: 10.1016/j.pscychresns.2019.06.001.

Blake, Kimberly V., Ziphozihle Ntwatwa, Tobias Kaufmann, Dan J. Stein, Jonathan C. Ipser, and Nynke A. Groenewold. 2023. “Advanced Brain Ageing in Adult Psychopathology: A Systematic Review and Meta-Analysis of Structural MRI Studies.” *Journal of Psychiatric Research* 157:180–91. doi: 10.1016/j.jpsychires.2022.11.011.

Butler, Mary I., Caitriona Long-smith, Gerard M. Moloney, Sabrina Morkl, Siobhain M. O. Mahony, John F. Cryan, Gerard Clarke, and Timothy G. Dinan. 2022. “Brain Behavior and Immunity The Immune-Kynurenine Pathway in Social Anxiety Disorder.” *Brain Behavior and Immunity* 99(November 2021):317–26. doi: 10.1016/j.bbi.2021.10.020.

Bylsma, Lauren M., Bethany H. Morris, and Jonathan Rottenberg. 2008. “A Meta-Analysis of Emotional Reactivity in Major Depressive Disorder.” *Clinical Psychology Review* 28(4):676–91. doi: 10.1016/j.cpr.2007.10.001.

Caldiroli, Alice, Enrico Capuzzi, Letizia M. Affaticati, Teresa Surace, Carla L. Di Forti, Antonios Dakanalis, Massimo Clerici, and Massimiliano Buoli. 2023. “Candidate Biological Markers for Social Anxiety Disorder : A Systematic Review.”

Chen, Tianqi, and Tong He. 2014. “Xgboost: Extreme Gradient Boosting.” *R Lecture* (2016):1–84.

Clausen, Ashley N., Kelene A. Fercho, Molly Monsour, Seth Disner, Lauren Salminen, Courtney C. Haswell, Emily Clarke Rubright, Amanda A. Watts, M. Nicole Buckley, Adi Maron-Katz, Anika Sierk, Antje Manthey, Benjamin Suarez-Jimenez, Bunmi O. Olatunji, Christopher L. Averill, David Hofmann, Dick J. Veltman, Elizabeth A. Olson, Gen Li, Gina L. Forster, Henrik Walter, Jacklynn Fitzgerald, Jean Théberge, Jeffrey S. Simons, Jessica A. Bomyea, Jessie L. Frijling, John H. Krystal, Justin T. Baker, K. Luan Phan, Kerry Ressler, Laura K. M. Han, Laura Nawijn, Lauren A. M. Lebois, Lianne Schmaal, Maria Densmore, Martha E. Shenton, Mirjam van Zuiden, Murray Stein, Negar Fani, Raluca M. Simons, Richard W. J. Neufeld, Ruth Lanius, Sanne van Rooij, Saskia B. J. Koch, Serena Bonomo, Tanja Jovanovic, Terri deRoon-Cassini, Timothy D. Ely, Vincent A. Magnotta, Xiaofu He, Chadi G. Abdallah, Amit Etkin, Christian Schmahl, Christine Larson, Isabelle M. Rosso, Jennifer Urbano Blackford, Jennifer S. Stevens, Judith K. Daniels, Julia Herzog, Milissa L. Kaufman, Miranda Olff, Richard J. Davidson, Scott R. Sponheim, Sven C. Mueller, Thomas Straube, Xi Zhu, Yuval Neria, Lee A. Baugh, James H. Cole, Paul M. Thompson, and Rajendra A. Morey. 2022. “Assessment of Brain Age in Posttraumatic Stress Disorder: Findings from the ENIGMA PTSD and Brain Age Working Groups.” *Brain and Behavior* 12(1):1–14. doi: 10.1002/brb3.2413.

Cole, James H., and Katja Franke. 2017. “Predicting Age Using Neuroimaging: Innovative Brain Ageing Biomarkers.” *Trends in Neurosciences* 40(12):681–90. doi: 10.1016/j.tins.2017.10.001.

Cole, James H., Riccardo E. Marioni, Sarah E. Harris, and Ian J. Deary. 2019. “Brain Age and Other Bodily ‘Ages’: Implications for Neuropsychiatry.” *Molecular Psychiatry* 24(2):266–81. doi: 10.1038/s41380-018-0098-1.

Cribbs, David H., Nicole C. Berchtold, Victoria Perreau, Paul D. Coleman, Joseph Rogers, Andrea J. Tenner, and Carl W. Cotman. 2012. “Extensive Innate Immune Gene Activation Accompanies Brain Aging, Increasing Vulnerability to Cognitive Decline and Neurodegeneration: A Microarray Study.” *Journal of Neuroinflammation* 9:1–18. doi: 10.1186/1742-2094-9-179.

Danese, Andrea, and Bruce S. McEwen. 2012. “Adverse Childhood Experiences, Allostasis, Allostatic Load, and Age-Related Disease.” *Physiology and Behavior* 106(1):29–39. doi: 10.1016/j.physbeh.2011.08.019.

Emdin, Connor A., Ayodele Odutayo, Christopher X. Wong, Jenny Tran, Allan J. Hsiao, and Benjamin H. M. Hunn. 2016. “Meta-Analysis of Anxiety as a Risk Factor for Cardiovascular Disease.” *American Journal of Cardiology* 118(4):511–19. doi: 10.1016/j.amjcard.2016.05.041.

Finlay, Sabine, Cody Roth, Tiansha Zimsen, Tahnee Lee Bridson, Zoltan Sarnyai, and Brett McDermott. 2022. “Adverse Childhood Experiences and Allostatic Load: A Systematic Review.” *Neuroscience and Biobehavioral Reviews* 136(June 2021):104605. doi: 10.1016/j.neubiorev.2022.104605.

Fischl, Bruce, David H. Salat, Evelina Busa, Marilyn Albert, Megan Dieterich, Christian Haselgrove, Andre Van Der Kouwe, Ron Killiany, David Kennedy, Shuna Klaveness, Albert Montillo, Nikos Makris, Bruce Rosen, and Anders M. Dale. 2002. “Whole Brain Segmentation: Automated Labeling of Neuroanatomical Structures in the Human Brain.” *Neuron* 33(3):341–55. doi: 10.1016/S0896-6273(02)00569-X.

Franke, Katja, and Christian Gaser. 2019. “Ten Years of Brainage as a Neuroimaging Biomarker of Brain

Aging: What Insights Have We Gained?” *Frontiers in Neurology* 10(JUL). doi: 10.3389/fneur.2019.00789.

Frick, Andreas, Katarina Howner, Håkan Fischer, Simon Fristed Eskildsen, Marianne Kristiansson, and Tomas Furmark. 2013. “Cortical Thickness Alterations in Social Anxiety Disorder.” *Neuroscience Letters* 536(1):52–55. doi: 10.1016/j.neulet.2012.12.060.

Gogtay, Nitin, Jay N. Giedd, Leslie Lusk, Kiralee M. Hayashi, Deanna Greenstein, A. Catherine Vaituzis, Tom F. Nugent, David H. Herman, Liv S. Clasen, Arthur W. Toga, Judith L. Rapoport, and Paul M. Thompson. 2004. “Dynamic Mapping of Human Cortical Development during Childhood through Early Adulthood.” *Proceedings of the National Academy of Sciences of the United States of America* 101(21):8174–79. doi: 10.1073/pnas.0402680101.

Groenewold, Nynke A., Janna Marie Bas-Hoogendam, Alyssa R. Amod, Max A. Laansma, Laura S. Van Velzen, Moji Aghajani, Kevin Hilbert, Hyuntaek Oh, Ramiro Salas, Andrea P. Jackowski, Pedro M. Pan, Giovanni A. Salum, James R. Blair, Karina S. Blair, Joy Hirsch, Spiro P. Pantazatos, Franklin R. Schneier, Ardesheer Talati, Karin Roelofs, Inge Volman, Laura Blanco-Hinojo, Narcís Cardoner, Jesus Pujol, Katja Beesdo-Baum, Christopher R. K. Ching, Sophia I. Thomopoulos, Andreas Jansen, Tilo Kircher, Axel Krug, Igor Nenadić, Frederike Stein, Udo Dannlowski, Dominik Grotegerd, Hannah Lemke, Susanne Meinert, Alexandra Winter, Michael Erb, Benjamin Kreifelts, Qiyong Gong, Su Lui, Fei Zhu, Benson Mwangi, Jair C. Soares, Mon Ju Wu, Ali Bayram, Mesut Canli, Raşit Tükel, P. Michiel Westenberg, Alexandre Heeren, Henk R. Cremers, David Hofmann, Thomas Straube, Alexander G. G. Doruyter, Christine Lochner, Jutta Peterburs, Marie José Van Tol, Raquel E. Gur, Antonia N. Kaczkurkin, Bart Larsen, Theodore D. Satterthwaite, Courtney A. Filippi, Andrea L. Gold, Anita Harrewijn, André Zugman, Robin Bülow, Hans J. Grabe, Henry Völzke, Katharina Wittfeld, Joscha Böhnlein, Katharina Dohm, Harald Kugel, Elisabeth Schrammen, Peter Zwanzger, Elisabeth J. Leehr, Lisa Sindermann, Tali M. Ball, Gregory A. Fonzo, Martin P. Paulus, Alan Simmons, Murray B. Stein, Heide Klumpp, K. Luan Phan, Tomas Furmark, Kristoffer N. T. Månsson, Amirhossein Manzouri, Suzanne N. Avery, Jennifer Urbano Blackford, Jacqueline A. Clauss, Brandee Feola, Jennifer C. Harper, Chad M. Sylvester, Ulrike Lueken, Dick J. Veltman, Anderson M. Winkler, Neda Jahanshad, Daniel S. Pine, Paul M. Thompson, Dan J. Stein, and Nic J. A. Van der Wee. 2023. “Volume of Subcortical Brain Regions in Social Anxiety Disorder: Mega-Analytic Results from 37 Samples in the ENIGMA-Anxiety Working Group.” *Molecular Psychiatry* (December 2022). doi: 10.1038/s41380-022-01933-9.

Han, Laura K. M., Hugo G. Schnack, Rachel M. Brouwer, Dick J. Veltman, Nic J. A. van der Wee, Marie José van Tol, Moji Aghajani, and Brenda W. J. H. Penninx. 2021. “Contributing Factors to Advanced Brain Aging in Depression and Anxiety Disorders.” *Translational Psychiatry* 11(1). doi: 10.1038/s41398-021-01524-2.

Han, Laura K. M., Josine E. Verhoeven, Audrey R. Tyrka, Brenda W. J. H. Penninx, Owen M. Wolkowitz, Kristoffer N. T. Månsson, Daniel Lindqvist, Marco P. Boks, Dóra Révész, Synthia H. Mellon, and Martin Picard. 2019a. “Accelerating Research on Biological Aging and Mental Health: Current Challenges and Future Directions.” *Psychoneuroendocrinology* 106(September 2018):293–311. doi: 10.1016/j.psyneuen.2019.04.004.

Han, Laura K. M., Josine E. Verhoeven, Audrey R. Tyrka, Brenda W. J. H. Penninx, Owen M. Wolkowitz, Kristoffer N. T. Månsson, Daniel Lindqvist, Marco P. Boks, Dóra Révész, Synthia H. Mellon, and Martin Picard. 2019b. “Accelerating Research on Biological Aging and Mental Health: Current Challenges and Future Directions.” *Psychoneuroendocrinology* 106(January):293–311. doi:

10.1016/j.psyneuen.2019.04.004.

Hilbert, Kevin, Ulrike Lueken, and Katja Beesdo-Baum. 2014. “Neural Structures, Functioning and Connectivity in Generalized Anxiety Disorder and Interaction with Neuroendocrine Systems: A Systematic Review.” *Journal of Affective Disorders* 158:114–26. doi: 10.1016/j.jad.2014.01.022.

Ipser, Jonathan C., John Joska, Tatum Sevenoaks, Hetta Gouse, Carla Freeman, Tobias Kaufmann, Ole A. Andreassen, Steve Shoptaw, and Dan J. Stein. 2022. “Limited Evidence for a Moderating Effect of HIV Status on Brain Age in Heavy Episodic Drinkers.” *Journal of NeuroVirology* 28(3):383–91. doi: 10.1007/s13365-022-01072-5.

Jansen, Rick, Laura K. M. Han, Josine E. Verhoeven, Karolina A. Aberg, Edwin C. G. J. van den Oord, Yuri Milaneschi, and Brenda W. J. H. Penninx. 2021. “An Integrative Study of Five Biological Clocks in Somatic and Mental Health.” *ELife* 10:1–20. doi: 10.7554/eLife.59479.

Johnson, Thomas E. 2006. “Recent Results: Biomarkers of Aging.” *Experimental Gerontology* 41(12):1243–46. doi: 10.1016/j.exger.2006.09.006.

Kaufmann, Tobias, Dennis van der Meer, Nhat Trung Doan, Emanuel Schwarz, Martina J. Lund, Ingrid Agartz, Dag Alnæs, Deanna M. Barch, Ramona Baur-Streubel, Alessandro Bertolino, Francesco Bettella, Mona K. Beyer, Erlend Bøen, Stefan Borgwardt, Christine L. Brandt, Jan Buitelaar, Elisabeth G. Celius, Simon Cervenka, Annette Conzelmann, Aldo Córdova-Palomera, Anders M. Dale, Dominique J. F. de Quervain, Pasquale Di Carlo, Srdjan Djurovic, Erlend S. Dørum, Sarah Eisenacher, Torbjørn Elvsåshagen, Thomas Espeseth, Helena Fatouros-Bergman, Lena Flyckt, Barbara Franke, Oleksandr Frei, Beathe Haatveit, Asta K. Håberg, Hanne F. Harbo, Catharina A. Hartman, Dirk Heslenfeld, Pieter J. Hoekstra, Einar A. Høgestøl, Terry L. Jernigan, Rune Jonassen, Erik G. Jönsson, Lars Farde, Lena Flyckt, Göran Engberg, Sophie Erhardt, Helena Fatouros-Bergman, Simon Cervenka, Lilly Schwieler, Fredrik Piehl, Ingrid Agartz, Karin Collste, Pauliina Victorsson, Anna Malmqvist, Mikael Hedberg, Funda Orhan, Peter Kirsch, Iwona Kłoszewska, Knut K. Kolskår, Nils Inge Landrø, Stephanie Le Hellard, Klaus Peter Lesch, Simon Lovestone, Arvid Lundervold, Astri J. Lundervold, Luigi A. Maglanoc, Ulrik F. Malt, Patrizia Mecocci, Ingrid Melle, Andreas Meyer-Lindenberg, Torgeir Moberget, Linn B. Norbom, Jan Egil Nordvik, Lars Nyberg, Jaap Oosterlaan, Marco Papalino, Andreas Papassotiropoulos, Paul Pauli, Giulio Pergola, Karin Persson, Geneviève Richard, Jaroslav Rokicki, Anne Marthe Sanders, Geir Selbæk, Alexey A. Shadrin, Olav B. Smeland, Hilkka Soininen, Piotr Sowa, Vidar M. Steen, Magda Tsolaki, Kristine M. Ulrichsen, Bruno Vellas, Lei Wang, Eric Westman, Georg C. Ziegler, Mathias Zink, Ole A. Andreassen, and Lars T. Westlye. 2019. “Common Brain Disorders Are Associated with Heritable Patterns of Apparent Aging of the Brain.” *Nature Neuroscience* 22(10):1617–23. doi: 10.1038/s41593-019-0471-7.

Koutsouleris, Nikolaos, Christos Davatzikos, Stefan Borgwardt, Christian Gaser, Ronald Bottlender, Thomas Frodl, Peter Falkai, Anita Riecher-Rössler, Hans Jürgen Möller, Maximilian Reiser, Christos Pantelis, and Eva Meisenzahl. 2014. “Accelerated Brain Aging in Schizophrenia and beyond: A Neuroanatomical Marker of Psychiatric Disorders.” *Schizophrenia Bulletin* 40(5):1140–53. doi: 10.1093/schbul/sbt142.

Koyuncu, Ahmet, Ezgi İnce, Erhan Ertekin, and Raşit Tükel. 2019. “Comorbidity in Social Anxiety Disorder: Diagnostic and Therapeutic Challenges.” *Drugs in Context* 8:1–13. doi: 10.7573/dic.212573.

Kuo, Chen Yuan, Pei Lin Lee, Sheng Che Hung, Li Kuo Liu, Wei Ju Lee, Chih Ping Chung, Albert C. Yang, Shih Jen Tsai, Pei Ning Wang, Liang Kung Chen, Kun Hsien Chou, and Ching Po Lin. 2020. "Large-Scale Structural Covariance Networks Predict Age in Middle-to-Late Adulthood: A Novel Brain Aging Biomarker." *Cerebral Cortex* 30(11):5844–62. doi: 10.1093/cercor/bhaa161.

Lang, P. .., and Lisa M. McTeague. 2010. "The Anxiety Disorder Spectrum." *Anxiety Stress Coping* 22(1):5–25. doi: 10.1080/10615800802478247.The.

Lanzenberger, Rupert, Wolfgang Wadsak, Christoph Spindelegger, Markus Mitterhauser, Elena Akimova, Leonhard Key Mien, Martin Fink, Ulrike Moser, Markus Savli, Georg S. Kranz, Andreas Hahn, Kurt Kletter, and Siegfried Kasper. 2010. "Cortisol Plasma Levels in Social Anxiety Disorder Patients Correlate with Serotonin-1A Receptor Binding in Limbic Brain Regions." *International Journal of Neuropsychopharmacology* 13(9):1129–43. doi: 10.1017/S1461145710000581.

Liu, Liang, Junhong Liu, Li Yang, Baohong Wen, Xiaopan Zhang, Junying Cheng, Shaoqiang Han, Yong Zhang, and Jingliang Cheng. 2022. "Accelerated Brain Aging in Patients With Obsessive-Compulsive Disorder." *Frontiers in Psychiatry* 13(May):1–9. doi: 10.3389/fpsyt.2022.852479.

López-Otín, Carlos, Maria A. Blasco, Linda Partridge, Manuel Serrano, and Guido Kroemer. 2013. "The Hallmarks of Aging." *Cell* 153(6):1194. doi: 10.1016/j.cell.2013.05.039.

Luo, Yunsong, Wenyu Chen, Jiang Qiu, and Tao Jia. 2022. "Accelerated Functional Brain Aging in Major Depressive Disorder: Evidence from a Large Scale FMRI Analysis of Chinese Participants." *Translational Psychiatry* 12(1):1–9. doi: 10.1038/s41398-022-02162-y.

Lydiard, R. Bruce. 2001. "Social Anxiety Disorder: Comorbidity and Its Implications." *Journal of Clinical Psychiatry* 62(SUPPL. 1):17–23.

McEwen, Bruce S. 2000. "Allostasis and Allostatic Load: Implications for Neuropsychopharmacology." *Neuropsychopharmacology* 22(2):108–24. doi: 10.1016/S0893-133X(99)00129-3.

McEwen, Bruce S. 2008. "Central Effects of Stress Hormones in Health and Disease: Understanding the Protective and Damaging Effects of Stress and Stress Mediators." *European Journal of Pharmacology* 583(2–3):174–85. doi: 10.1016/j.ejphar.2007.11.071.

Meier, Sandra M., Manuel Mattheisen, Ole Mors, Preben B. Mortensen, Thomas M. Laursen, and Brenda W. Penninx. 2016. "Increased Mortality among People with Anxiety Disorders: Total Population Study." *British Journal of Psychiatry* 209(3):216–21. doi: 10.1192/bjp.bp.115.171975.

Monereo-Sánchez, Jennifer, Joost J. A. de Jong, Gerhard S. Drenthen, Magdalena Beran, Walter H. Backes, Coen D. A. Stehouwer, Miranda T. Schram, David E. J. Linden, and Jacobus F. A. Jansen. 2021. "Quality Control Strategies for Brain MRI Segmentation and Parcellation: Practical Approaches and Recommendations - Insights from the Maastricht Study." *NeuroImage* 237(January). doi: 10.1016/j.neuroimage.2021.118174.

Niu, Xin, Alexei Taylor, Russell T. Shinohara, John Kounios, and Fengqing Zhang. 2022. "Multidimensional Brain-Age Prediction Reveals Altered Brain Developmental Trajectory in Psychiatric Disorders." *Cerebral Cortex (New York, N.Y. : 1991)* 32(22):5036–49. doi: 10.1093/cercor/bhab530.

Norden, D. M., and J. P. Godbout. 2013. "Review: Microglia of the Aged Brain: Primed to Be Activated and Resistant to Regulation." *Neuropathology and Applied Neurobiology* 39(1):19–34. doi: 10.1111/j.1365-2990.2012.01306.x.

Pannekoek, J. N., van der Werff, S. J., Stein, D. J., & van der Wee, N. J. (2013). Advances in the neuroimaging of panic disorder. *Human Psychopharmacology: Clinical and Experimental*, *28*(6), 608-611.

Petrowski, Katja, Ileana Schmalbach, Anne Strunk, Jürgen Hoyer, Clemens Kirschbaum, and Peter Joraschky. 2021. “Cortisol Reactivity in Social Anxiety Disorder: A Highly Standardized and Controlled Study.” *Psychoneuroendocrinology* 123(October 2020):104913. doi: 10.1016/j.psyneuen.2020.104913.

Syal, Supriya, Coenraad J. Hattingh, Jean Paul Fouché, Bruce Spottiswoode, Paul D. Carey, Christine Lochner, and Dan J. Stein. 2012. “Grey Matter Abnormalities in Social Anxiety Disorder: A Pilot Study.” *Metabolic Brain Disease* 27(3):299–309. doi: 10.1007/s11011-012-9299-5.

Thompson, Paul M., Neda Jahanshad, Christopher R. K. Ching, Lauren E. Salminen, Sophia I. Thomopoulos, Joanna Bright, Bernhard T. Baune, Sara Bertolín, Janita Bralten, Willem B. Bruin, Robin Bülow, Jian Chen, Yann Chye, Udo Dannlowski, Carolien G. F. de Kovel, Gary Donohoe, Lisa T. Eyler, Stephen V. Faraone, Pauline Favre, Courtney A. Filippi, Thomas Frodl, Daniel Garijo, Yolanda Gil, Hans J. Grabe, Katrina L. Grasby, Tomas Hajek, Laura K. M. Han, Sean N. Hatton, Kevin Hilbert, Tiffany C. Ho, Laurena Holleran, Georg Homuth, Norbert Hosten, Josselin Houenou, Iliyan Ivanov, Tianye Jia, Sinead Kelly, Marieke Klein, Jun Soo Kwon, Max A. Laansma, Jeanne Leerssen, Ulrike Lueken, Abraham Nunes, Joseph O’ Neill, Nils Opel, Fabrizio Piras, Federica Piras, Merel C. Postema, Elena Pozzi, Natalia Shatokhina, Carles Soriano-Mas, Gianfranco Spalletta, Daqiang Sun, Alexander Teumer, Amanda K. Tilot, Leonardo Tozzi, Celia van der Merwe, Eus J. W. Van Someren, Guido A. van Wingen, Henry Völzke, Esther Walton, Lei Wang, Anderson M. Winkler, Katharina Wittfeld, Margaret J. Wright, Je Yeon Yun, Guohao Zhang, Yanli Zhang-James, Bhim M. Adhikari, Ingrid Agartz, Moji Aghajani, André Aleman, Robert R. Althoff, Andre Altmann, Ole A. Andreassen, David A. Baron, Brenda L. Bartnik-Olson, Janna Marie Bas-Hoogendam, Arielle R. Baskin-Sommers, Carrie E. Bearden, Laura A. Berner, Premika S. W. Boedhoe, Rachel M. Brouwer, Jan K. Buitelaar, Karen Caeyenberghs, Charlotte A. M. Cecil, Ronald A. Cohen, James H. Cole, Patricia J. Conrod, Stephane A. De Brito, Sonja M. C. de Zwarte, Emily L. Dennis, Sylvane Desrivieres, Danai Dima, Stefan Ehrlich, Carrie Esopenko, Graeme Fairchild, Simon E. Fisher, Jean Paul Fouche, Clyde Francks, Sophia Frangou, Barbara Franke, Hugh P. Garavan, David C. Glahn, Nynke A. Groenewold, Tiril P. Gurholt, Boris A. Gutman, Tim Hahn, Ian H. Harding, Dennis Hernaus, Derrek P. Hibar, Frank G. Hillary, Martine Hoogman, Hilleke E. Hulshoff Pol, Maria Jalbrzikowski, George A. Karkashadze, Eduard T. Klapwijk, Rebecca C. Knickmeyer, Peter Kochunov, Inga K. Koerte, Xiang Zhen Kong, Sook Lei Liew, Alexander P. Lin, Mark W. Logue, Eileen Luders, Fabio Macciardi, Scott Mackey, Andrew R. Mayer, Carrie R. McDonald, Agnes B. McMahon, Sarah E. Medland, Gemma Modinos, Rajendra A. Morey, Sven C. Mueller, Pratik Mukherjee, Leyla Namazova-Baranova, Talia M. Nir, Alexander Olsen, Peristera Paschou, Daniel S. Pine, Fabrizio Pizzagalli, Miguel E. Rentería, Jonathan D. Rohrer, Philipp G. Sämann, Lianne Schmaal, Gunter Schumann, Mark S. Shiroishi, Sanjay M. Sisodiya, Dirk J. A. Smit, Ida E. Sønderby, Dan J. Stein, Jason L. Stein, Masoud Tahmasian, David F. Tate, Jessica A. Turner, Odile A. van den Heuvel, Nic J. A. van der Wee, Ysbrand D. van der Werf, Theo G. M. van Erp, Neeltje E. M. van Haren, Daan van Rooij, Laura S. van Velzen, Ilya M. Veer, Dick J. Veltman, Julio E. Villalon-Reina, Henrik Walter, Christopher D. Whelan, Elisabeth A. Wilde, Mojtaba Zarei, and Vladimir Zelman. 2020. “ENIGMA and Global Neuroscience: A Decade of Large-Scale Studies of the Brain in Health and Disease across More than 40 Countries.” *Translational Psychiatry* 10(1):1–28. doi: 10.1038/s41398-020-0705-1.

Wang, Xiuli, Bochao Cheng, Qiang Luo, Lihua Qiu, and Song Wang. 2018. “Gray Matter Structural

Alterations in Social Anxiety Disorder: A Voxel-Based Meta-Analysis." *Frontiers in Psychiatry* 9(SEP). doi: 10.3389/fpsyt.2018.00449.

Wolkowitz, Owen W., Elissa S. Epel, Victor I. Reus, and Synthia H. Mellon. 2010. "Depression Gets Old Fast: Do Stress and Depression Accelerate Cell Aging?" *Depression and Anxiety* 27(4):327–38. doi: 10.1002/da.20686.

Yoon, K. Lira, and Jutta Joormann. 2012. "Stress Reactivity in Social Anxiety Disorder with and without Comorbid Depression." *Journal of Abnormal Psychology* 121(1):250–55. doi: 10.1037/a0025079.

Zou, James, Mikael Huss, Abubakar Abid, Pejman Mohammadi, Ali Torkamani, and Amalio Telenti. 2019. "A Primer on Deep Learning in Genomics." *Nature Genetics* 51(1):12–18. doi: 10.1038/s41588-018-0295-5.

CHAPTER 7: DISCUSSION

In chapter 7, I present a concise summary of the key findings observed throughout the various chapters of the dissertation. Furthermore, I address the limitations of my work and propose areas of future research and recommendations.

Overview of the research approach

The overarching aim of this dissertation was to investigate hippocampal and amygdala subfield volumes and brain aging in anxiety and related disorders. To achieve this aim, this dissertation conducted five separate investigations. First (chapter 2), was a systematic review of studies measuring biological aging in anxiety disorders, as indexed by molecular, cellular, and brain aging indicators. The second (chapter 3) and third (chapter 4) investigations were original empirical studies investigating hippocampal and amygdala subfield volumes in OCD and SAD, respectively. For both of these studies, separate subgroup analysis was performed to examine the association between clinical characteristics (psychotropic medication use and psychiatric comorbidity) and subfield volumes. In an additional analysis, a diagnosis-by-age interaction term was included in the general linear model, to assess whether there was an association between chronological age and subfield volumes. Chapters 5 and 6, investigated whether individuals with OCD (chapter 5) and those with SAD (chapter 6) had accentuated brain aging. This was achieved by using a previously trained MLA to predict brain-PADs (predicted brain age- chronological age) in individuals and HCs. A separate subgroup analysis was performed to examine the association between clinical characteristics and brain-PADs. In addition, a partial correlation analysis was conducted to investigate the within-group association between hippocampal and amygdala volume and brain-PAD in OCD and SAD.

Summary of key findings

The results from the systematic review revealed that there were very few studies investigating biological aging in anxiety disorders. After an extensive literature search and study selection based on the eligibility criteria, 14 studies were identified for the systematic review. The majority of studies that were identified focused on TL in PTSD which is warranted given the well-documented association between trauma exposure and biological aging (Wolf et al., 2018), and the high number of papers conducted on TL to date (Jylhävä et al., 2017). While there were few other indicators of biological aging identified by the systematic review, the general impression was that 1) findings for an association between TL in PTSD (Boks et al., 2015; Wolf et al., 2018), and for DNA methylation in anxiety disorders were mixed, 2) anxiety and related disorders were associated with higher predicted brain age dependent on medication use, sex, and chronological age (Clausen et al., 2022; Han et al., 2021), and

3) there is very little correlation between the different indicators, supporting the idea that they are measuring various aspects of aging throughout the body (Cole et al., 2018; Han et al., 2021; Jansen et al., 2021).

Chapter 3 revealed that there were no significant differences in hippocampal and amygdala subfield volumes in individuals with OCD (n=381) compared to HCs (n=338). However, medicated individuals with OCD (n=161) had significantly smaller hippocampal DG and ML, and larger lateral and basal amygdala subfields than HCs. Chapter 4 revealed that individuals with SAD (n=107) showed smaller amygdala subfield volumes in the basal, accessory basal, and CAT, and larger hippocampal volume in the CA3, CA4, DG, and ML, compared to HCs (140). In chapters 3 and 4, chronological age did not moderate the association between subfield volume and OCD or SAD, respectively. Chapter 5 revealed that individuals with OCD (n=375) had significantly higher whole brain-PAD compared to HCs (n=335) and that the lateral and CAT subfield of the amygdala and the hippocampal fimbria and fissure were correlated to brain-PAD within the OCD group. Chapter 6 found that individuals with SAD (n=107) had significantly higher whole brain-PAD compared to HCs (n=137). There was no evidence of an association between subfield volume and brain age in the diagnosis group.

General findings

The findings presented in this dissertation provide valuable insights into the structural differences in the hippocampal and amygdala subfield volumes in individuals with OCD and SAD, and their association with chronological age and brain-PADs. In chapter 3, It was hypothesized that individuals with OCD would have differences in both hippocampal and amygdala subfield volumes and that these effects may be particularly apparent in subfields previously implicated in OCD, including the BLA and central nucleus of the amygdala, as well as hippocampal subiculum and presubiculum (Zhang et al., 2019, Zhang et al., 2020).

One of the notable findings that emerged in OCD was that there were no differences in subfield volumes in individuals with OCD, compared to HCs. These findings could be partially explained by the fact that OCD likely involves other brain regions i.e., the CSTC as the predominant neurobiological model (van den Heuvel et al., 2016), and perhaps the hippocampus and amygdala are less central in the pathophysiology of the disorder. The involvement of the hippocampus and amygdala in OCD may be related to certain subtypes of OCD (Boedhoe & van den Heuvel, 2018). Pujol et al. (2004) found that individuals with prominent aggressive obsessions and checking compulsions had a relative decrease in gray matter volume in the right amygdala compared to other individuals with OCD within the sample (Pujol et al., 2004). In another study, van den Heuvel et al. (2009) found that harm and checking were associated with a reduction in gray and white matter volumes in the bilateral temporal regions (van den Heuvel et al., 2009). Rees et al. (2018) found smaller hippocampal volumes in individuals with OCD with high severity of ordering and checking symptoms suggesting that there is a relationship between hippocampal volume and symptom profiles in OCD (Reess et al., 2018). However, due to limited data, chapter 3 did not have enough sample to investigate the association between OCD subtypes and subfield volumes. Therefore, future large-sample analysis might elucidate whether hippocampal volumes differ by symptom categories and whether this association is moderated by clinical characteristics like medication status.

In chapter 4, It was hypothesized that individuals with SAD would have differences in the volume of the BLA amygdala given its involvement in other anxiety disorders (Asami et al., 2018) and its role in threat evaluation (Besnard & Sahay, 2016; Etkin et al., 2004; Hortensius et al., 2016). The positive findings in chapter 4 were to be expected as some studies propose that the amygdala may be the neurobiological candidate endophenotype of social anxiety (Bas-Hoogendam et al., 2020). While the neurobiological model of SAD is not yet fully delineated, both the hippocampus and amygdala are an integral part of the neurocircuitry of fear (Brühl et al. 2014; Shin & Liberzon 2010).

Fear is a prominent feature in anxiety disorders and a large body of rodent research suggests that the key structures involved in the neurocircuitry of fear are the amygdala and its subfields (Felix-Ortiz et al., 2016; Janak & Tye, 2015; Tye et al., 2011b), the hippocampus (Felix-Ortiz & Tye, 2014), the insula and other brain regions (Shin & Liberzon, 2010). While these structures have their respective roles in various aspects of fear processing, including threat perception, fear learning/conditioning, execution of fear responses, and fear extinction, the precise role of the structures in fear processing is still debated (Shin & Liberzon, 2010). A recent review of candidate biological markers for SAD showed that most functional studies in SAD report greater activation in the amygdala and/or amygdala-hippocampal fronto-limbic regions in response to different emotional paradigms, whilst structural studies point to various brain regions that are associated with the fear network (Caldiroli et al., 2023). On the other hand, it is important to note that while both functional and structural studies point to the involvement of the hippocampus and amygdala in SAD, most studies have reported mixed findings (Caldiroli et al., 2023) and further research is needed to corroborate the findings presented in chapter 4.

The findings of increased hippocampal subfield volumes in SAD compared to HCs were intriguing. While these observations add new insight into the association between hippocampal volume and adult SAD, they warrant a more nuanced interpretation. Despite some evidence of larger hippocampal volume in SAD (Koç et al., 2017; Machado-de-Sousa et al., 2014), there is also contrary evidence (Baksh et al., 2021; Irle et al., 2010; Liao et al., 2011). Adolescent neuroimaging data points to smaller hippocampal volumes in young individuals with anxiety disorders, although in post-hoc analysis these differences were linked to generalized anxiety disorder rather than SAD (Gold et al., 2017).

One possible explanation for the larger hippocampal volumes observed in Chapter 4 could be the exhaustive use of the hippocampus in managing chronic social anxiety given its extensive role in emotional regulation. This could reflect a compensatory mechanism, where the hippocampus becomes more robust due to its frequent activation in socially stressful scenarios. Rodent models demonstrate a positive relationship between anxiety and hippocampal volumes, where anxiety-related behaviour had a more pronounced effect on hippocampal volume than depression-like behaviour (Kalisch et al., 2006). However, some studies found that rodents exposed to chronic stress had smaller hippocampal volumes than controls (Lee et al., 2009). To explore this further, future analysis is required to investigate the relationship between hippocampal volume and symptom severity including subclinical levels of anxiety, to establish whether larger hippocampal volumes are associated with higher levels of anxiety symptoms.

Of note, one interesting finding that emerged in chapter 3 was that subfield volumes differed according to medication status but not psychiatric comorbidities in OCD. These findings are partially in line with work from ENIGMA-OCD where between-group differences for hippocampal volume, thickness, and shape were only found in individuals with and/or without medication use (Fouche et al., 2017, 2022). Recently, a mega-analysis found that an MRI-based MLA was selective for medication status rather than diagnosis status in individuals with OCD, suggesting that medication status was a stronger predictor of subcortical brain structures in OCD than diagnosis status (individuals with OCD n=2304, HCs n= 2068) (Bruin et al., 2020).

Medication status was also an important predictor in the brain age analysis presented in chapter 5. While there was a between-group difference in brain-PAD in individuals with OCD compared to HCs (+1.6 years, p_{FDR}=0.006, d=0.20), the brain-PAD was observably higher in individuals with medication use (+2.98 years, d=0.38, p_{FDR}<0.001). As discussed in chapter 5, medication status has been shown to influence brain-PADs in anxiety and related disorders (Han et al., 2021). Notably, there are a variety of mechanisms that could explain the medication findings in OCD including, neuroinflammation (Ivanov et al., 2022). However, while neuroinflammation has been demonstrated in OCD (Attwells et al., 2017), it is unclear how psychotropic medication could exacerbate brain aging by increasing neuroinflammation as cell culture studies show that SSRIs inhibit the release of pro-inflammatory factors from activated microglia and thereby prevent neurotoxicity (Zhang et al., 2012).

Subfield volumes associated with chronological age

The studies included in this dissertation support the conclusion that chronological age is a less informative predictor of subfield volumes in OCD and SAD than brain-PAD. The evidence for this finding emerged from chapter 3 and 4, where chronological age did not significantly moderate the association between subfield volumes and OCD and SAD, respectively, as modelled by age-by-diagnosis interactions. This observation is not consistent with evidence suggesting that amygdala subfields are differentially associated with chronological age (Kurth et al., 2019). Several possible reasons may account for these findings. In OCD, chronological age has been associated with greater volume loss in the limbic regions which may be related to chronic stress and exaggerated emotional responsiveness (De Wit et al., 2014).

Chronic stress as a potential mechanism for volume loss in the hippocampal subfield is plausible considering that animal models show a decrease in glucocorticoid receptor expression in the hippocampal CA1, CA3, and DG, and volume loss in all the hippocampal subfields in response to physiological stress (Li et al., 2019).

Early life stress, such as childhood maltreatment, has been found to significantly impact hippocampal structure, particularly leading to smaller volumes in several key subfields. Specifically, adults (age 18-25, n=193) with histories of childhood maltreatment showed smaller volumes in the hippocampal subfields CA3, the DG, and the subiculum (Teicher et al., 2012). These findings are corroborated by several neuroimaging studies demonstrating an association between early life stress and smaller hippocampal volume (Andersen et al., 2008; Frodl et al., 2010; Weniger et al., 2009). In older adults, chronic stress associated with ongoing life circumstances has been associated with smaller CA2/3 and CA4/DG, suggesting a link between psychological stress and specific hippocampal subfield volumes in otherwise healthy older individuals (mean age 79.42 years (SD=5.04), n=116) (Zimmerman et al., 2016).

At the cellular level, early life stress is suggested to suppress neurogenesis in the dentate gyrus and induce dendritic remodelling in the CA3 subfield (McEwen, 2000, 2002). Rodent models demonstrate a reduction in the overall synaptic density within the hippocampus in response to early life stress, persisting long after the stressor has been removed (Andersen & Teicher, 2004). Taken together, these observations highlight the sensitivity of the hippocampus to stress during critical developmental stages.

Subfield volumes association with brain-PAD

In OCD, the partial correlation analysis showed that brain-PAD was differentially associated with subfield volumes. Perhaps the most interesting of the findings is the distinct and opposing pattern in which the amygdala and hippocampal subfields related to brain-PAD in OCD. Of note, larger amygdala subfield volumes were correlated with smaller brain-PAD estimates, suggesting that larger volumes were associated with less brain aging in individuals with OCD, and the opposite was observed for the hippocampus. The finding that only the volumes of LA and CAT subfield of the amygdala were correlated to brain age in OCD is in agreement with evidence of subfield-specific age-related effects, with strong association for subfields that belong to the superficial area (superficial area is suggested to contain the CAT, anterior amygdaloid area and the cortical nucleus of the amygdala (Schmitz-Koep et al., 2021)) (Kurth et al., 2019). Concerning the hippocampal subfield volumes in OCD, the findings that larger hippocampal subfield volumes correlated with larger brain-PAD estimates suggest that larger hippocampal subfield volumes were associated with greater brain aging. Studies suggest smaller whole hippocampal volumes with increasing chronological age in healthy individuals (Nobis et al., 2019) and that with the exception of the entorhinal cortex, all of the hippocampal subfields including the CA, fascia dentata subiculum, HATA are smaller with increasing age (Kurth et al., 2017). Interestingly, large hippocampal fissure volumes have been associated with OCD but not in the context of aging (Jiang et al., 2022). A large fissure is thought to be indicative of irregular hippocampal formation during human development (Humphrey, 1967), as the fissure gradually becomes smaller as the embryo develops, eventually forming a small opening among the DG, presubiculum, and parasubiculum in the healthy adult brain (Kier et al., 1997) In the context of aging, large fissures are linked to brain atrophy and are strongly correlated with chronological age, as well as being proposed as a useful biomarker for the diagnosis of AD (Li et al., 2006).

Future recommendations

The chapters presented in this dissertation had some novel findings in terms of (1) subfield volume differences in SAD, (2) the influence of medication status on subfield volumes in OCD, and (3) differences in brain aging observed in both SAD and OCD. Larger cross-sectional and longitudinal analyses with harmonized protocols (MRI field strength, scan sequence, FreeSurfer segmentation version, and brain age model) are required to replicate, confirm, and extend our findings. The brain age prediction model used in this dissertation was constructed using feature extraction based on shallow learning (Kaufmann et al., 2019), however, deep learning techniques such as convolutional neural networks (Jonsson et al., 2019) are suggested to enhance model performance which improves prediction estimates (Cole et al. 2017). For example, Amoroso et al. (2019) found that the deep learning technique surpassed the performance of the standard MLA for brain age estimation including ridge and lasso regression, random forest, and support vector machines (Amoroso et al., 2019). Another way to improve brain age model performance is by combining multiple imaging modalities (i.e., T2 FLAIR, susceptibility-weighted imaging, diffusion MRI, or functional neuroimaging), instead of using select features from one modality (Dinsdale et al., 2021). The predictive ability should be validated by correlating the predicted brain age to the chronological age (for example see Clausen et al. 2021). In doing so, studies will be able to select the best prediction model that accurately fits the data (as indicated by the MAE), as an ideal model should not overfit the data by exactly predicting the chronological age (Bashyam et al., 2020). Additionally, studies will be able to gauge the precision and reliability of the brain age model used and ensure a cautious interpretation of the findings. For the prediction of regional brain age, it is important to note that this aspect of brain aging research is still in its infancy. While it would be useful to identify regional specificity in brain aging patterns across different disorders (Kaufmann et al., 2019), regional brain age models still require systematic evaluation in large sample sizes using a wide age range. In the context of aging, since it is a biologically complex process that occurs at differential rates throughout the body (Han et al., 2019; López-Otín et al., 2013), a more comprehensive approach includes combining multiple indicators of biological aging to improve the prediction of aging (Cole et al., 2018). Further evidence is required to ascertain whether the findings of subfield volume and brain age differences in OCD and SAD observed in this dissertation relate to differences in cognitive or affective function. Moreover, future studies could investigate how other factors, like poverty, low economic status, and food insufficiency, are associated with subfield volume and brain age.

Strengths and limitations

A noteworthy strength of this dissertation is that it benefitted from the secondary use of large-scale data that was collected by the OBIC and EUSARNAD consortiums (previously published in Bas-Hoogendam et al. 2017; De Wit et al. 2014). The sharing of this data provided access to research data that might not have been obtainable by an independent doctoral researcher. The OCD and SAD data that was provided was multisite, included both sexes and had a wide age range (18-65 years old). These study characteristics increased the statistical power of the analyses and enhanced the generalizability of the dissertation's findings. However, there were some limitations to consider. Given the cross-sectional nature of our study, it was not possible to ascertain whether the association between subfield volume and disorder, the association between brain-PAD and disorder, or the association between subfield volume and brain-PAD was causal. Additionally, it is unknown whether the observed differences in subfield volume or brain age were pre-existing to SAD or OCD, or occurred during the development of the disorders, or both. Lastly given that brain aging is a continuous and non-linear process (Dinsdale et al., 2021), it is unclear whether the observed differences in brain age or association between subfield volume and brain age remain stable or if they change over time.

Chapter 5 and 6 reports the correlations between age and PAD for the control groups. Notably, a significant negative association was observed for OCD controls ($R = -0.322$, $p < 0.001$), but not for SAD controls (R = -0.074, p = 0.390). The mean brain-PAD for the controls for the OCD and SAD chapters were also significantly different from zero (t = 18.510, p < 0.001 and t = 3.146, p = 0.002, respectively), possibly signifying sub-optimal model fit to these novel data.

The moderate correlation between chronological age and predicted brain age, along with the high MAE, suggests potential issues with the model's fit and its generalization capability across different populations. As reported in the discussion chapter of chapter 5 and 6, the MAEs observed in the present thesis were higher than those in the literature (Clausen et al., 2022; Han et al., 2021). One plausible reason for this suboptimal fit could be the inherent variability within the sample, including differences in demographic factors such as age, gender, and health status, which may not have been adequately captured by the model. Future research should focus on refining the model by incorporating additional relevant features and using more sophisticated modelling techniques. In chapter 3 and 4, to reduce the statistical burden of multiple comparisons, the hippocampal subfield divisions (head, body, and tail along the longitudinal axis) were combined for each subfield so that the number of tests conducted in the mixed linear model for the bilateral hippocampus and amygdala subfields was 21, instead of 28 tests. Some studies suggest that the head body and tail divisions may be differentially affected in psychiatric disorders, specifically those belonging to the anterior hippocampal CA subfields (McHugo et al., 2018). Moreover, the horizontal length of the hippocampus is thought to have some functional specialization due to projections to different brain regions (Daugherty et al., 2017; Duvernoy, 2013). By combining these subdivisions, we were unable to determine whether subfield volume differences occurred along the longitudinal axis of the hippocampus.

Another limitation is due to the test re-test reliability of the hippocampal parasubiculum and fissure segmentation. Based on the FreeSurfer 6.0 segmentation atlas (Iglesias et al., 2015; Saygin et al., 2017), both the hippocampal parasubiculum and fissure have been shown to have relatively low-test re-test reliability, with mean volume differences greater than 5%, and overlap less than 70%, and ICC scores ranging from 0.78 to 0.89 (Brown et al., 2020). This complicates the interpretation of the finding in chapter 5 that the hippocampal fissure was correlated with whole brain-PAD estimates in individuals with OCD. Some studies suggest that the hippocampus is surrounded by several fissures (the peri-hippocampal fissures). For example, the transverse fissure of Bichat is the lateral extension of the ambient cistern separating the thalamus superiorly from the parahippocampal gyrus inferiorly, the choroidal fissure is the superolateral extension of the transverse fissure, and the hippocampal fissure is the inferolateral extension of the transverse fissure extending between the DG and the subiculum- all of which may be useful in indicating mesial temporal atrophy and parietal atrophy in some of the Dementia syndromes (Dekeyzer et al., 2017; Duvernoy, 2013).

Given that the gold standard for hippocampal and amygdala segmentation is manual tracing, this approach would not be feasible for the large sample employed in this dissertation. The reliability and reproducibility of the amygdala subfields have only been tested by combining the smaller subfields like the medial and central nucleus into the centromedial complex (Quattrini et al., 2020), which would defeat the purpose of investigating subfield-specific differences in OCD and SAD for this dissertation. However, consistent with most reports in the literature, both the hippocampal fissure and parasubiculum were included in the analysis of this dissertation (Sun et al., 2023).

Another limitation of the thesis is the potential confounding effects of medication use and symptom severity on hippocampal subfield volumes (reported in chapter 3). This confounder is critical as it complicates the interpretation of whether the differences in hippocampal volume are attributable to the effects of the medication itself or to the underlying severity of the disorder. Although our analysis did not find a significant association between symptom severity and subfield volume, the possibility that medication effects are intertwined with severity differences cannot be entirely ruled out. Future research should aim to disentangle these potential effects by including longitudinal data and controlling for symptom severity more rigorously to better understand the independent impact of medication on brain volume.

Although large-scale multisite datasets enhance the statistical power needed to detect small effect sizes, differences in data acquisition across multiple sites is a known source of heterogeneity (Bayer et al., 2022). In the present thesis, scanner site was included as random effects term in the linear regression model to adjust for differences between sites. However, the present thesis could have benefitted from utilizing advanced harmonization techniques such as ComBat, which may have provided a more accurate adjustment for site-specific variability and reduced residual biases (Bayer et al., 2022). The use of sophisticated harmonization techniques could have enhanced the optimal performance of the brain age prediction model used in the present thesis.

The brain serves as a central hub for processing and responding to the diverse environmental factors that shape our experiences including physical factors (e.g., nutrition), psychosocial factors (e.g., family stability, childhood adversity), and socio-economic status (Farah, 2017). Epidemiological research consistently demonstrates that childhood adversity like abuse, neglect, and exposure to traumatic events (including retrospectively reported events) increases the risk of developing anxiety disorders in adult life (Oliveira et al., 2021). For example, in a national comorbidity survey of individuals who experienced multiple forms of childhood adversity, Green et al. (2010) found that childhood adversity predicted 32.4% of first onset anxiety disorders (Green et al., 2010). Furthermore, the prevalence of childhood adversity and the associated risk of psychiatric disorders differs between low-to-middle and high-income areas, suggesting that socioeconomic status may play a role in the exposure to and impact of childhood adversity (Cano & Takeuchi, 2020). Several studies are reporting the influence of environmental stressors, including childhood trauma, adversity, and low socioeconomic status, on brain structure and function (Nelson et al., 2020).

In PTSD, Ahmed-Leitao et al., 2019 reported smaller parasubiculum volume in PTSD group with a history of early childhood trauma. Additionally, smaller HATA volumes were observed in the PTSD group with early childhood trauma when compared to both HCs and the SAD group with early childhood trauma (Ahmed-Leitao et al., 2019). However, it remains unclear how these factors influence brain aging in the context of OCD and SAD. The data used in the present dissertation did not include information on childhood adversity, early life stress, or socio-economic factors and therefore these variables could not be accounted for. Lastly, while some studies suggest that there is very little correlation between the biological indicators of aging including brain age, TL, and epigenetic clocks (Cole et al., 2018; Jansen et al., 2021), this dissertation did not have the data to perform a comprehensive analysis of biological aging at various cellular and molecular levels. Therefore, it remains unknown whether the brain-PADs observed in OCD and SAD would be associated with other aspects of biological aging.

Conclusion

The findings presented in this dissertation collectively contribute to our understanding of the association between hippocampal and amygdala subfield volumes and brain aging in OCD and SAD. Of note, the findings highlight the nuanced interplay between medication status, subfield volumes, and brain-PAD whilst demonstrating unique patterns of subfield volume differences in OCD and SAD. Specifically, in SAD, subfield volume differences were observed in areas associated with social processing and threat evaluation. While there were no significant differences in subfield volumes in OCD overall, the subfield volume differences were dependent on medication status specifically in regions associated with adult-born neurogenesis. The findings of an association between medication status, subfield volumes, and brain-PAD in OCD, prompt further research on the specific mechanism through which psychotropic medication affects subcortical brain volumes in OCD and how this informs treatment optimization. Moreover, while the findings of accentuated brain aging in OCD and SAD expand our understanding of brain aging, it also highlights that very little is known about the neurobiological complexities of anxiety disorders and processes of biological aging. Further research is required to uncover the precise mechanisms underlying the association between subcortical brain volume attenuation in anxiety disorders and brain aging.

References

Aghamohammadi-Sereshki, Arash, Nicholas J. Coupland, Peter H. Silverstone, Yushan Huang, Kathleen M. Hegadoren, Rawle Carter, Peter Seres, and Nikolai V. Malykhin. 2021. “Effects of Childhood Adversity on the Volumes of the Amygdala Subnuclei and Hippocampal Subfields in Individuals with Major Depressive Disorder.” *Journal of Psychiatry and Neuroscience* 46(1):E186–95. doi: 10.1503/jpn.200034.

Ahmed-Leitao, F., Rosenstein, D., Marx, M., Young, S., Korte, K., & Seedat, S. (2019). Posttraumatic stress disorder, social anxiety disorder and childhood trauma: Differences in hippocampal subfield volume. Psychiatry Research - Neuroimaging, 284(December 2018), 45–52. https://doi.org/10.1016/j.pscychresns.2018.12.015

Andersen, S. L., & Teicher, M. H. (2004). Delayed effects of early stress on hippocampal development. Neuropsychopharmacology, 29(11), 1988–1993. https://doi.org/10.1038/sj.npp.1300528

Andersen, S. L., Tomada, A., Vincow, E. S., Valente, E., Polcari, A., & Teicher, M. H. (2008). Preliminary Evidence for Sensitive Periods in the Effect of Childhood Sexual Abuse on Regional Brain Development. Journal of Neuropsychiatry, 20(3), 292–301. https://doi.org/10.1176/appi.neuropsych.20.3.292

Asami, Takeshi, Ryota Nakamura, Masao Takaishi, Haruhisa Yoshida, Asuka Yoshimi, Thomas J. Whitford, and Yoshio Hirayasu. 2018. “Smaller Volumes in the Lateral and Basal Nuclei of the Amygdala in Individuals with Panic Disorder.” *PLoS ONE* 13(11):1–8. doi: 10.1371/journal.pone.0207163.

Attwells, Sophia, Elaine Setiawan, Alan A. Wilson, Pablo M. Rusjan, Romina Mizrahi, Laura Miler, Cynthia Xu, Margaret Anne Richter, Alan Kahn, Stephen J. Kish, Sylvain Houle, Lakshmi Ravindran, and Jeffrey H. Meyer. 2017. “Inflammation in the Neurocircuitry of Obsessive-Compulsive Disorder.” *JAMA Psychiatry* 74(8):833–40. doi: 10.1001/jamapsychiatry.2017.1567.

Baksh, R. A., Ritchie, C. W., Terrera, G. M., Norton, J., Raymont, V., & Ritchie, K. (2021). The association between anxiety disorders and hippocampal volume in older adults. Psychology and Aging, 36(2), 288–297. https://doi.org/10.1037/pag0000597

Bas-Hoogendam, Janna Marie, Henk van Steenbergen, Nic J. A. van der Wee, and P. Michiel Westenberg. 2020. “Amygdala Hyperreactivity to Faces Conditioned with a Social-Evaluative Meaning– a Multiplex, Multigenerational FMRI Study on Social Anxiety Endophenotypes.” *NeuroImage: Clinical* 26(October 2019):102247. doi: 10.1016/j.nicl.2020.102247.

Bayer, J. M. M., Thompson, P. M., Ching, C. R. K., Liu, M., Chen, A., Panzenhagen, A. C., Jahanshad, N., Marquand, A., Schmaal, L., & Sämann, P. G. (2022). Site effects how-to and when: An overview of retrospective techniques to accommodate site effects in multi-site neuroimaging analyses. *Frontiers in Neurology*, *13*. https://doi.org/10.3389/fneur.2022.923988

Boedhoe, Premika S. W., and Odile A. van den Heuvel. 2018. "Morphological Brain Alterations in Individuals with Obsessive–Compulsive Disorder." Pp. 429–50 in *Palmetto Rose*. Vol. 136.

Brühl, Annette Beatrix, Aba Delsignore, Katja Komossa, and Steffi Weidt. 2014. "Neuroimaging in Social Anxiety Disorder-A Meta-Analytic Review Resulting in a New Neurofunctional Model." *Neuroscience and Biobehavioral Reviews* 47:260–80. doi: 10.1016/j.neubiorev.2014.08.003.

Bruin, Willem B., Luke Taylor, Rajat M. Thomas, Jonathan P. Shock, Paul Zhutovsky, Yoshinari Abe, Pino Alonso, Stephanie H. Ameis, Alan Anticevic, Paul D. Arnold, Francesca Assogna, Francesco Benedetti, Jan C. Beucke, Premika S. W. Boedhoe, Irene Bollettini, Anushree Bose, Silvia Brem, Brian P. Brennan, Jan K. Buitelaar, Rosa Calvo, Yuqi Cheng, Kang Ik K. Cho, Sara Dallaspezia, Damiaan Denys, Benjamin A. Ely, Jamie D. Feusner, Kate D. Fitzgerald, Jean Paul Fouche, Egill A. Fridgeirsson, Patricia Gruner, Deniz A. Gürsel, Tobias U. Hauser, Yoshiyuki Hirano, Marcelo Q. Hoexter, Hao Hu, Chaim Huyser, Iliyan Ivanov, Anthony James, Fern Jaspers-Fayer, Norbert Kathmann, Christian Kaufmann, Kathrin Koch, Masaru Kuno, Gerd Kvale, Jun Soo Kwon, Yanni Liu, Christine Lochner, Luisa Lázaro, Paulo Marques, Rachel Marsh, Ignacio Martínez-Zalacaín, David Mataix-Cols, José M. Menchón, Luciano Minuzzi, Pedro S. Moreira, Astrid Morer, Pedro Morgado, Akiko Nakagawa, Takashi Nakamae, Tomohiro Nakao, Janardhanan C. Narayanaswamy, Erika L. Nurmi, Joseph O'Neill, Jose C. Pariente, Chris Perriello, John Piacentini, Fabrizio Piras, Federica Piras, Y. C. Janardha. Reddy, Oana G. Rus-Oswald, Yuki Sakai, João R. Sato, Lianne Schmaal, Eiji Shimizu, H. Blair Simpson, Noam Soreni, Carles Soriano-Mas, Gianfranco Spalletta, Emily R. Stern, Michael C. Stevens, S. Evelyn Stewart, Philip R. Szeszko, David F. Tolin, Ganesan Venkatasubramanian, Zhen Wang, Je Yeon Yun, Daan van Rooij, Nerisa Banaj, Nuria Bargalló, Marcelo C. Batistuzzo, Daniel Brandeis, Geraldo F. Busatto, Anna Calvo, Valentina Ciullo, Renate Drechsler, Madalena Esteves, Andrea Falini, Yu Fang, Martijn Figee, Martine Fontaine, Margot Gueguen, Sayo Hamatani, Gregory L. Hanna, Bjarne Hansen, Keisuke Ikari, Luisa Lázaro, Ricardo Magalhães, Yasutaka Masuda, Koji Matsumoto, Euripedes C. Miguel, Astrid Morer, Christopher Pittenger, Sara Poletti, Yuki Sakai, Nuno Sousa, Jumpei Takahashi, Anders L. Thorsen, Aki Tsuchiyagaito, Daniela Vecchio, Dick J. Veltman, Susanne Walitza, Anri Watanabe, Xiufeng Xu, Jian Xu, Kei Yamada, Tokiko Yoshida, Mojtaba Zarei, Qing Zhao, Cong Zhou, Froukje E. de Vries, Stella J. de Wit, Daan van Rooij, Guido A. van Wingen, Odile A. van den Heuvel, Ysbrand D. van der Werf, Paul M. Thompson, Odile A. van den Heuvel, Dan J. Stein, and Guido A. van Wingen. 2020.

"Structural Neuroimaging Biomarkers for Obsessive-Compulsive Disorder in the ENIGMA-OCD Consortium: Medication Matters." *Translational Psychiatry* 10(1). doi: 10.1038/s41398-020-01013-y.

Caldiroli, Alice, Enrico Capuzzi, Letizia M. Affaticati, Teresa Surace, Carla L. Di Forti, Antonios Dakanalis, Massimo Clerici, and Massimiliano Buoli. 2023. "Candidate Biological Markers for Social Anxiety Disorder : A Systematic Review."

Cano, M., & Takeuchi, D. T. (2020). Recent/Childhood Adversities and Mental Disorders Among US Immigrants. Frontiers in Psychiatry, 11(November), 1–19. https://doi.org/10.3389/fpsyt.2020.573410

De Wit, Stella J., Pino Alonso, Lizanne Schweren, David Mataix-Cols, Christine Lochner, José M. Menchón, Dan J. Stein, Jean Paul Fouche, Carles Soriano-Mas, Joao R. Sato, Marcelo Q. Hoexter, Damiaan Denys, Takashi Nakamae, Seiji Nishida, Jun Soo Kwon, Joon Hwan Jang, Geraldo F. Busatto, Narcís Cardoner, Danielle C. Cath, Kenji Fukui, Wi Hoon Jung, Sung Nyun Kim, Euripides C. Miguel, Jin Narumoto, Mary L. Phillips, Jesus Pujol, Peter L. Remijnse, Yuki Sakai, Na Young Shin, Kei Yamada, Dick J. Veltman, and Odile A. Van Den Heuvel. 2014. "Multicenter Voxel-Based Morphometry Mega-Analysis of Structural Brain Scans in Obsessive-Compulsive Disorder." *American Journal of Psychiatry* 171(3):340–49. doi: 10.1176/appi.ajp.2013.13040574.

Farah, M. J. (2017). The Neuroscience of Socioeconomic Status: Correlates, Causes, and Consequences. Neuron, 96(1), 56–71. https://doi.org/10.1016/j.neuron.2017.08.034

Farah, Martha J. 2017. "The Neuroscience of Socioeconomic Status: Correlates, Causes, and Consequences." *Neuron* 96(1):56–71. doi: 10.1016/j.neuron.2017.08.034.

Felix-Ortiz, A. C., A. Burgos-Robles, N. D. Bhagat, C. A. Leppla, and K. M. Tye. 2016. "Bidirectional Modulation of Anxiety-Related and Social Behaviors by Amygdala Projections to the Medial Prefrontal Cortex." *Neuroscience* 321:197–209. doi: 10.1016/j.neuroscience.2015.07.041.

Felix-Ortiz, Ada C., and Kay M. Tye. 2014. "Amygdala Inputs to the Ventral Hippocampus Bidirectionally Modulate Social Behavior." *Journal of Neuroscience* 34(2):586–95. doi: 10.1523/JNEUROSCI.4257-13.2014.

Fouche, Jean Paul, Nynke A. Groenewold, Tatum Sevenoaks, Sarah Heany, Christine Lochner, Pino Alonso, Marcelo C. Batistuzzo, Narcis Cardoner, Christopher R. K. Ching, Stella J. de Wit, Boris Gutman, Marcelo Q. Hoexter, Neda Jahanshad, Minah Kim, Jun Soo Kwon, David Mataix-Cols, Jose M. Menchon, Euripedes C. Miguel, Takashi Nakamae, Mary L. Phillips, Jesus Pujol, Yuki Sakai, Je Yeon Yun, Carles Soriano-Mas, Paul M. Thompson, Kei Yamada, Dick J. Veltman, Odile A. van den Heuvel, and Dan J. Stein. 2022. “Shape Analysis of Subcortical Structures in Obsessive-Compulsive Disorder and the Relationship with Comorbid Anxiety, Depression, and Medication Use: A Meta-Analysis by the OCD Brain Imaging Consortium.” *Brain and Behavior* 12(10):1–12. doi: 10.1002/brb3.2755.

Fouche, Jean Paul, Stefan Du Plessis, Coenie Hattingh, Annerine Roos, Christine Lochner, Carles Soriano-Mas, Joao R. Sato, Takashi Nakamae, Seiji Nishida, Jun Soo Kwon, Wi Hoon Jung, David Mataix-Cols, Marcelo Q. Hoexter, Pino Alonso, Stella J. De Wit, Dick J. Veltman, Dan J. Stein, and Odile A. Van Den Heuvel. 2017. “Cortical Thickness in Obsessive-Compulsive Disorder: Multisite Mega-Analysis of 780 Brain Scans from Six Centres.” *British Journal of Psychiatry* 210(1):67–74. doi: 10.1192/bjp.bp.115.164020.

Frodl, T., Reinhold, E., Koutsouleris, N., Reiser, M., & Meisenzahl, E. M. (2010). Interaction of childhood stress with hippocampus and prefrontal cortex volume reduction in major depression. Journal of Psychiatric Research, 44(13), 799–807. https://doi.org/10.1016/j.jpsychires.2010.01.006

Gold, A. L., Steuber, E. R., White, L. K., Pacheco, J., Sachs, J. F., Pagliaccio, D., Berman, E., Leibenluft, E., & Pine, D. S. (2017). Cortical thickness and subcortical gray matter volume in pediatric anxiety disorders. Neuropsychopharmacology, 42(12), 2423–2433. https://doi.org/10.1038/npp.2017.83

Green, J. G., McLaughlin, K. A., Berglund, P. A., Gruber, M. J., Sampson, N. A., Zaslavsky, A. M., & Kessler, R. C. (2010). Childhood adversities and adult psychiatric disorders in the national comorbidity survey replication I: Associations with first onset of DSM-IV disorders. Archives of General Psychiatry, 67(2), 113–123. https://doi.org/10.1001/archgenpsychiatry.2009.186

Groenewold, Nynke A., Janna Marie Bas-Hoogendam, Alyssa R. Amod, Max A. Laansma, Laura S. Van Velzen, Moji Aghajani, Kevin Hilbert, Hyuntaek Oh, Ramiro Salas, Andrea P. Jackowski, Pedro M. Pan, Giovanni A. Salum, James R. Blair, Karina S. Blair, Joy Hirsch, Spiro P. Pantazatos, Franklin R. Schneier, Ardesheer Talati, Karin Roelofs, Inge Volman, Laura Blanco-Hinojo, Narcís Cardoner, Jesus Pujol, Katja Beesdo-Baum, Christopher R. K. Ching, Sophia I. Thomopoulos, Andreas Jansen, Tilo Kircher, Axel Krug, Igor Nenadić, Frederike Stein, Udo Dannlowski, Dominik Grotegerd, Hannah Lemke, Susanne Meinert, Alexandra Winter, Michael Erb, Benjamin Kreifelts, Qiyong Gong, Su Lui, Fei Zhu, Benson Mwangi, Jair C. Soares, Mon Ju Wu, Ali Bayram, Mesut Canli, Raşit Tükel, P. Michiel Westenberg, Alexandre Heeren, Henk R. Cremers, David Hofmann, Thomas Straube, Alexander G. G. Doruyter, Christine Lochner, Jutta Peterburs, Marie José Van Tol, Raquel E. Gur, Antonia N. Kaczkurkin, Bart Larsen, Theodore D. Satterthwaite, Courtney A. Filippi, Andrea L. Gold, Anita Harrewijn, André Zugman, Robin Bülow, Hans J. Grabe, Henry Völzke, Katharina

Wittfeld, Joscha Böhnlein, Katharina Dohm, Harald Kugel, Elisabeth Schrammen, Peter Zwanzger, Elisabeth J. Leehr, Lisa Sindermann, Tali M. Ball, Gregory A. Fonzo, Martin P. Paulus, Alan Simmons, Murray B. Stein, Heide Klumpp, K. Luan Phan, Tomas Furmark, Kristoffer N. T. Månsson, Amirhossein Manzouri, Suzanne N. Avery, Jennifer Urbano Blackford, Jacqueline A. Clauss, Brandee Feola, Jennifer C. Harper, Chad M. Sylvester, Ulrike Lueken, Dick J. Veltman, Anderson M. Winkler, Neda Jahanshad, Daniel S. Pine, Paul M. Thompson, Dan J. Stein, and Nic J. A. Van der Wee. 2023. "Volume of Subcortical Brain Regions in Social Anxiety Disorder: Mega-Analytic Results from 37 Samples in the ENIGMA-Anxiety Working Group." *Molecular Psychiatry* (December 2022). doi: 10.1038/s41380-022-01933-9.

Han, Laura K. M., Richard Dinga, Tim Hahn, Christopher R. K. Ching, Lisa T. Eyler, Lyubomir Aftanas, Moji Aghajani, André Aleman, Bernhard T. Baune, Klaus Berger, Ivan Brak, Geraldo Busatto Filho, Angela Carballedo, Colm G. Connolly, Baptiste Couvy-Duchesne, Kathryn R. Cullen, Udo Dannlowski, Christopher G. Davey, Danai Dima, Fabio L. S. Duran, Verena Enneking, Elena Filimonova, Stefan Frenzel, Thomas Frodl, Cynthia H. Y. Fu, Beata R. Godlewska, Ian H. Gotlib, Hans J. Grabe, Nynke A. Groenewold, Dominik Grotegerd, Oliver Gruber, Geoffrey B. Hall, Ben J. Harrison, Sean N. Hatton, Marco Hermesdorf, Ian B. Hickie, Tiffany C. Ho, Norbert Hosten, Andreas Jansen, Claas Kähler, Tilo Kircher, Bonnie Klimes-Dougan, Bernd Krämer, Axel Krug, Jim Lagopoulos, Ramona Leenings, Frank P. MacMaster, Glenda MacQueen, Andrew McIntosh, Quinn McLellan, Katie L. McMahon, Sarah E. Medland, Bryon A. Mueller, Benson Mwangi, Evgeny Osipov, Maria J. Portella, Elena Pozzi, Liesbeth Reneman, Jonathan Repple, Pedro G. P. Rosa, Matthew D. Sacchet, Philipp G. Sämann, Knut Schnell, Anouk Schrantee, Egle Simulionyte, Jair C. Soares, Jens Sommer, Dan J. Stein, Olaf Steinsträter, Lachlan T. Strike, Sophia I. Thomopoulos, Marie José van Tol, Ilya M. Veer, Robert R. J. M. Vermeiren, Henrik Walter, Nic J. A. van der Wee, Steven J. A. van der Werff, Heather Whalley, Nils R. Winter, Katharina Wittfeld, Margaret J. Wright, Mon Ju Wu, Henry Völzke, Tony T. Yang, Vasileios Zannias, Greig I. de Zubicaray, Giovana B. Zunta-Soares, Christoph Abé, Martin Alda, Ole A. Andreassen, Erlend Bøen, Caterina M. Bonnin, Erick J. Canales-Rodriguez, Dara Cannon, Xavier Caseras, Tiffany M. Chaim-Avancini, Torbjørn Elvsåshagen, Pauline Favre, Sonya F. Foley, Janice M. Fullerton, Jose M. Goikolea, Bartholomeus C. M. Haarman, Tomas Hajek, Chantal Henry, Josselin Houenou, Fleur M. Howells, Martin Ingvar, Rayus Kuplicki, Beny Lafer, Mikael Landén, Rodrigo Machado-Vieira, Ulrik F. Malt, Colm McDonald, Philip B. Mitchell, Leila Nabulsi, Maria Concepcion Garcia Otaduy, Bronwyn J. Overs, Mircea Polosan, Edith Pomarol-Clotet, Joaquim Radua, Maria M. Rive, Gloria Roberts, Henricus G. Ruhe, Raymond Salvador, Salvador Sarró, Theodore D. Satterthwaite, Jonathan Savitz, Aart H. Schene, Peter R. Schofield, Mauricio H. Serpa, Kang Sim, Marcio Gerhardt Soeiro-de-Souza, Ashley N. Sutherland, Henk S. Temmingh, Garrett M. Timmons, Anne Uhlmann, Eduard Vieta, Daniel H. Wolf, Marcus V. Zanetti, Neda Jahanshad, Paul M. Thompson, Dick J. Veltman, Brenda W. J. H. Penninx, Andre F. Marquand, James H. Cole, and Lianne Schmaal. 2021. "Brain Aging in Major Depressive Disorder: Results from the ENIGMA Major Depressive Disorder Working Group." *Molecular Psychiatry* 26(9):5124–39. doi: 10.1038/s41380-020-0754-0.

Irle, E., Ruhleder, M., Lange, C., Seidler-Brandler, U., Salzer, S., Dechent, P., Weniger, G., Leibing, E., & Leichsenring, F. (2010). Reduced amygdalar and hippocampal size in adults with generalized social phobia. Journal of Psychiatry and Neuroscience, 35(2), 126–131. https://doi.org/10.1503/jpn.090041

Ivanov, Iliyan, Premika S. W. Boedhoe, Yoshinari Abe, Pino Alonso, Stephanie H. Ameis, Paul D. Arnold, Srinivas Balachander, Justin T. Baker, Nerisa Banaj, Nuria Bargalló, Marcelo C. Batistuzzo, Francesco Benedetti, Jan C. Beucke, Irene Bollettini, Silvia Brem, Brian P. Brennan, Jan Buitelaar, Rosa Calvo, Yuqi Cheng, Kang Ik K. Cho, Sara Dallaspezia, Damiaan Denys, Juliana B. Diniz, Benjamin A. Ely, Jamie D. Feusner, Sónia Ferreira, Kate D. Fitzgerald, Patricia Gruner, Gregory L. Hanna, Yoshiyuki Hirano, Marcelo Q. Hoexter, Chaim Huyser, Keisuke Ikari, Anthony James, Fern Jaspers-Fayer, Hongyan Jiang, Norbert Kathmann, Christian Kaufmann, Minah Kim, Kathrin Koch, Jun Soo Kwon, Luisa Lázaro, Yanni Liu, Christine Lochner, Rachel Marsh, Ignacio Martínez-Zalacaín, David Mataix-Cols, José M. Menchón, Luciano Minuzzi, Astrid Morer, Pedro Morgado, Akiko Nakagawa, Takashi Nakamae, Tomohiro Nakao, Janardhanan C. Narayanaswamy, Erika L. Nurmi, Sanghoon Oh, Chris Perriello, John C. Piacentini, Maria Picó-Pérez, Fabrizio Piras, Federica Piras, Y. C. Janardhan Reddy, Daniela Rodriguez Manrique, Oana Yuki Sakai, Eiji Shimizu, H. Blair Simpson, Noam Soreni, Carles Soriano-Mas, Gianfranco Spalletta, Emily R. Stern, Michael C. Stevens, S. Evelyn Stewart, Philip R. Szeszko, David F. Tolin, Daan van Rooij, Dick J. Veltman, Ysbrand D. van der Werf, Guido A. van Wingen, Ganesan Venkatasubramanian, Susanne Walitza, Zhen Wang, Anri Watanabe, Lidewij H. Wolters, Xiufeng Xu, Je-Yeon Yun, Mojtaba Zarei, Fengrui Zhang, Qing Zhao, Paul M. Thompson, Dan J. Stein, Odile A. van den Heuvel, Joseph O'Neill, Sara Poletti, Egill Axfjord Fridgeirsson, Toshikazu Ikuta, Stella J. de Wit, Chris Vriend, Selina Kasprzak, Masaru Kuno, Jumpei Takahashi, Euripedes C. Miguel, Roseli G. Shavitt, Morgan Hough, Jose C. Pariente, Ana E. Ortiz, Sara Bertolín, Eva Real, Cinto Segalàs, Pedro Silva Moreira, Nuno Sousa, Jin Narumoto, Kei Yamada, Jinsong Tang, and Jean-Paul Fouche. 2022. "Associations of Medication with Subcortical Morphology across the Lifespan in OCD: Results from the International ENIGMA Consortium." *Journal of Affective Disorders* 318(August):204–16. doi: 10.1016/j.jad.2022.08.084.

Ivanov, Iliyan. 2019. "Effects of Psychopharmacological Treatment for OCD on Brain Morphometry

Across the Lifespan." *Biological Psychiatry* 85(10):S32. doi: 10.1016/j.biopsych.2019.03.091.

Janak, Patricia H., and Kay M. Tye. 2015. "From Circuits to Behaviour in the Amygdala." *Nature* 517(7534):284–92. doi: 10.1038/nature14188.

Kalisch, R., Schubert, M., Jacob, W., Keßler, M. S., Hemauer, R., Wigger, A., Landgraf, R., & Auer, D. P. (2006). Anxiety and hippocampus volume in the rat. Neuropsychopharmacology, 31(5), 925–932. https://doi.org/10.1038/sj.npp.1300910

Kier, E. Leon, Jung H. Kim, Robert K. Fulbright, and Richard A. Bronen. 1997. "Embryology of the Human Fetal Hippocampus: Mr Imaging, Anatomy, and Histology." *American Journal of Neuroradiology* 18(3):525–32.

Koç, M., Kuloğlu, Ö., Yıldırım, H., & Atmaca, M. (2017). The investigation of hippocampus and amygdala volume changes with MRI in patients with social anxiety disorder. Anatolian Journal of Psychiatry, 19(2), 1. https://doi.org/10.5455/apd.268817

Kurth, Florian, Nicolas Cherbuin, and Eileen Luders. 2019. "Age but No Sex Effects on Subareas of the Amygdala." *Human Brain Mapping* 40(6):1697–1704. doi: 10.1002/hbm.24481.

La Joie, Renaud, Marine Fouquet, Florence Mézenge, Brigitte Landeau, Nicolas Villain, Katell Mevel, Alice Pélerin, Francis Eustache, Béatrice Desgranges, and Gaël Chételat. 2010. "Differential Effect of Age on Hippocampal Subfields Assessed Using a New High-Resolution 3T MR Sequence." *NeuroImage* 53(2):506–14. doi: 10.1016/j.neuroimage.2010.06.024.

Lee, T., Jarome, T., Li, S. J., Kim, J. J., & Helmstetter, F. J. (2009). Chronic stress selectively reduces hippocampal volume in rats: A longitudinal magnetic resonance imaging study. NeuroReport, 20(17), 1554–1558. https://doi.org/10.1097/WNR.0b013e328332bb09

Li, Y., J. Li, S. Segal, J. Wegiel, S. De Santi, J. Zhan, and Mony J. De Leon. 2006. "Hippocampal Cerebrospinal Fluid Spaces on MR Imaging: Relationship to Aging and Alzheimer Disease." *American Journal of Neuroradiology* 27(4):912–18.

Li, Yuefeng, Jiasheng Qin, Jinchuan Yan, Ningning Zhang, Yuhao Xu, Yan Zhu, Liangju Sheng, Xiaolan Zhu, and Shenghong Ju. 2019. "Differences of Physical vs. Psychological Stress: Evidences from Glucocorticoid Receptor Expression, Hippocampal Subfields Injury, and Behavioral Abnormalities." *Brain Imaging and Behavior* 13(6):1780–88. doi: 10.1007/s11682-018-9956-3.

Liao, W., Xu, Q., Mantini, D., Ding, J., MacHado-De-Sousa, J. P., Hallak, J. E. C., Trzesniak, C., Qiu, C., Zeng, L., Zhang, W., Crippa, J. A. S., Gong, Q., & Chen, H. (2011). Altered gray matter morphometry and resting-state functional and structural connectivity in social anxiety disorder. Brain Research, 1388, 167–177. https://doi.org/10.1016/j.brainres.2011.03.018

Machado-de-Sousa, J. P., De Lima Osório, F., Jackowski, A. P., Bressan, R. A., Chagas, M. H. N., Torro-Alves, N., DePaula, A. L. D., Crippa, J. A. S., & Hallak, J. E. C. (2014). Increased amygdalar and hippocampal volumes in young adults with social anxiety. PLoS ONE, 9(2), 1–5. https://doi.org/10.1371/journal.pone.0088523

McEwen, B. S. (2000). The neurobiology of stress: From serendipity to clinical relevance. Brain Research, 886(1–2), 172–189. https://doi.org/10.1016/S0006-8993(00)02950-4

McEwen, B. S. (2002). Sex, stress and the hippocampus: Allostasis, allostatic load and the aging process. Neurobiology of Aging, 23(5), 921–939. https://doi.org/10.1016/S0197-4580(02)00027-1

Nelson, C. A., Scott, R. D., Bhutta, Z. A., Harris, N. B., Danese, A., & Samara, M. (2020). Adversity in childhood is linked to mental and physical health throughout life. The BMJ, 371, 1–9. https://doi.org/10.1136/bmj.m3048

Oliveira, J., Paixão, V., Cardoso, G., Xavier, M., Caldas de Almeida, J. M., & Oliveira-Maia, A. J. (2021). Childhood adversities and the comorbidity between mood and general medical disorders in adults: Results from the WHO World Mental Health Survey Portugal. Brain, Behavior, and Immunity - Health, 17(January). https://doi.org/10.1016/j.bbih.2021.100329

Pantazatos, Spiro P., Ardesheer Talati, Franklin R. Schneier, and Joy Hirsch. 2014. “Reduced Anterior Temporal and Hippocampal Functional Connectivity during Face Processing Discriminates Individuals with Social Anxiety Disorder from Healthy Controls and Panic Disorder, and Increases Following Treatment.” *Neuropsychopharmacology* 39(2):425–34. doi: 10.1038/npp.2013.211.

Prater, Katherine E., Avinash Hosanagar, Heide Klumpp, Mike Angstadt, and K. Luan Phan. 2013. “Aberrant Amygdala-Frontal Cortex Connectivity during Perception of Fearful Faces and at Rest in Generalized Social Anxiety Disorder.” *Depression and Anxiety* 30(3):234–41. doi: 10.1002/da.22014.

Pujol, Jesús, Carles Soriano-Mas, Pino Alonso, Narcís Cardoner, José M. Menchón, Joan Deus, and Julio Vallejo. 2004. “Mapping Structural Brain Alterations in Obsessive-Compulsive Disorder.” *Archives of General Psychiatry* 61(7):720. doi: 10.1001/archpsyc.61.7.720.

Raz, Naftali, Ana M. Daugherty, Andrew R. Bender, Cheryl L. Dahle, and Susan Land. 2015. “Volume of the Hippocampal Subfields in Healthy Adults: Differential Associations with Age and a pro-Inflammatory Genetic Variant.” *Brain Structure and Function* 220(5):2663–74. doi: 10.1007/s00429-014-0817-6.

Reess, Tim Jonas, Oana Georgiana Rus, Deniz A. Gürsel, Benita Schmitz-Koep, Gerd Wagner, Götz Berberich, and Kathrin Koch. 2018. "Association between Hippocampus Volume and Symptom Profiles in Obsessive–Compulsive Disorder." *NeuroImage: Clinical* 17(November 2017):474–80. doi: 10.1016/j.nicl.2017.11.006.

Shin, Lisa M., and Israel Liberzon. 2010. "The Neurocircuitry of Fear, Stress, and Anxiety Disorders." *Neuropsychopharmacology* 35(1):169–91. doi: 10.1038/npp.2009.83.

Teicher, M. H., Anderson, C. M., & Polcari, A. (2012). Childhood maltreatment is associated with reduced volume in the hippocampal subfields CA3, dentate gyrus,and subiculum. Proceedings of the National Academy of Sciences of the United States of America, 109(9). https://doi.org/10.1073/pnas.1115396109

Tye, Kay M., Rohit Prakash, Sung Yon Kim, Lief E. Fenno, Logan Grosenick, Hosniya Zarabi, Kimberly R. Thompson, Viviana Gradinaru, Charu Ramakrishnan, and Karl Deisseroth. 2011. "Amygdala Circuitry Mediating Reversible and Bidirectional Control of Anxiety." *Nature* 471(7338):358–62. doi: 10.1038/nature09820.

van den Heuvel, Odile A., Guido van Wingen, Carles Soriano-Mas, Pino Alonso, Samuel R. Chamberlain, Takashi Nakamae, Damiaan Denys, Anna E. Goudriaan, and Dick J. Veltman. 2016. "Brain Circuitry of Compulsivity." *European Neuropsychopharmacology* 26(5):810–27. doi: 10.1016/j.euroneuro.2015.12.005.

Van Den Heuvel, Odile A., Peter L. Remijnse, David Mataix-Cols, Hugo Vrenken, Henk J. Groenewegen, Harry B. M. Uylings, Anton J. L. M. Van Balkom, and Dick J. Veltman. 2009. "The Major Symptom Dimensions of Obsessive-Compulsive Disorder Are Mediated by Partially Distinct Neural Systems." *Brain* 132(4):853–68. doi: 10.1093/brain/awn267.

Weniger, G., Lange, C., Sachsse, U., Irle, E., & Irle, E. (2009). Asjdgbjkadsbgl. De J Psychiatry Neurosci, 34(5), 383–391.

Zhang, Feng, Hui Zhou, Belinda C. Wilson, Jing Shan Shi, Jau Shyong Hong, and Hui Ming Gao. 2012. "Fluoxetine Protects Neurons against Microglial Activation-Mediated Neurotoxicity." *Parkinsonism and Related Disorders* 18(SUPPL. 1):S213–17. doi: 10.1016/s1353-8020(11)70066-9.

Zimmerman, Molly E., Ali Ezzati, Mindy J. Katz, Michael L. Lipton, Adam M. Brickman, Martin J. Sliwinski, and Richard B. Lipton. 2016. "Perceived Stress Is Differentially Related to Hippocampal Subfield Volumes among Older Adults." *PLoS ONE* 11(5):1–10. doi: 10.1371/journal.pone.0154530.