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Neurobiological Substrates of Depression and Treatment Response
From Brain Structure to Sex Hormones

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2. **Jensen KHR**, Aarestrup MR, Larsen SV, Köhler-Forsberg K, Knudsen GM, Jørgensen MB, Frokjaer VG (2024) Psychoneuroendocrine profiles of unmedicated men with major depressive disorder and associations to treatment effects and sexual side-effects. ***Neuroscience Applied***

3. **Jensen KHR**, Larsen SV, Köhler-Forsberg K, Knudsen GM, Jørgensen MB, Frokjaer VG (2024) EEG abnormalities are not associated with poor antidepressant treatment outcome – a NeuroPharm study. ***European Neuropsychopharmacology***

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- **Jensen KHR**, McCulloch DE, Olsen AS, Bruzzone SP, Larsen SV, Fisher PM, Frokjaer VG (2022) Effects of an oral contraceptive on dynamic brain states and network modularity in a serial single-subject study. ***Frontiers in Neuroscience***
- Mikkelsen AT, **Jensen KHR**, Jørgensen MB, Frokjaer VG, Dam VH (2024) No association between serotonin 4 receptor brain binding and personality trait Neuroticism – a positron emission tomography study in depressed patients and healthy individuals. ***Neuroscience Applied***
- Tyron JM, Ip CT, Jørgensen MB, **Jensen KHR** (2024) Loudness Dependent Auditory Evoked Potentials and Suicidality in Depression – a meta-analysis with replication in unmedicated patients. ***Journal of Affective Disorders***

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SUMMARY

Major depressive disorder (MDD) is a complex and heterogeneous mental disorder causing suffering worldwide. While effective treatments exist, including pharmacotherapy and psychotherapy, more than half of patients do not respond adequately to their first medication. The search for reliable biomarkers to guide treatment selection has largely focused on individual domains, e.g. symptoms, social demographics, neuroimaging, genetics, or blood markers. Yet no single marker has proven clinically useful. This reflects both the disorder's heterogeneity and our limited understanding of the underlying biology.

This thesis investigates neurobiological mechanisms and markers of treatment response in MDD through three studies using data from the NeuroPharm-1 study and presents the ongoing BrainDrugs-Depression cohort study.

Study I challenges traditional views about antidepressant mechanisms by showing that successful SSRI treatment was associated with decreased rather than increased hippocampal volume. This suggests therapeutic effects may involve circuit refinement rather than growth. We also found sex-specific relationships between hippocampal volume and serotonin 4 receptor binding, with correlations present only in women.

Study II failed to replicate previous findings that EEG abnormalities predict poor response to escitalopram. However, patients with EEG abnormalities showed greater emotional disturbance and poorer verbal memory, potentially marking a distinct clinical phenotype rather than treatment resistance.

Study III revealed that in men, low pretreatment sex hormone levels predicted sexual side effects from SSRIs but not treatment response. The finding that higher testosterone levels were associated with vegetative symptoms like weight loss likely reflects metabolic consequences of depression rather than causation.

These studies highlight how depression and its treatment involve complex and sex-specific mechanisms that cannot be reduced to simple biomarker-outcome relations. The work provides a foundation for a more nuanced approach to precision psychiatry that accounts for sex differences and multiple interacting biological systems.

With this in mind, I present the BrainDrugs-Depression cohort study, which aims to enable deep phenotyping of first-episode depression patients in a naturalistic clinical setting in the Capital Region of Denmark. I showcase the study's current status and how it may enable studies of predictors and mechanisms of treatment response to identify and understand *who benefits from what*.

DANSK RESUMÉ

Depression er en meget almindelig psykisk lidelse med store konsekvenser, særligt for det enkelte menneske. Der er stor variation i, hvor gavnlig samtaleterapi og medicin er - nogle oplever fremragende effekt, mens andre kun mærker en lille eller ingen bedring. Mere viden kan hjælpe os med at tilpasse behandlingen til den enkelte. Specifikt ved at identificere faktorer i hjernen, målbare stoffer i blodet, psykosociale forhold og andre markører, som kan bruges til at forudsige, hvilken effekt forskellige behandlinger vil have hos den enkelte patient. Denne forskning kan bidrage til, at behandlingsvalg ved depression kan målrettes og skræddersys, frem for udelukkende at basere sig på behandlerens skøn eller et standardiseret behandlingspakketilbud.

Gennem tre studier baseret på NeuroPharm-1 studiet og det igangværende BrainDrugs-Depression-kohortestudie undersøger denne afhandling neurobiologiske mekanismer og markører for behandlingseffekter:

Studie I undersøger hvordan antidepressiva påvirker volumen af hippocampus. Vi fandt at vellykket SSRI-behandling var forbundet med en reduktion i hippocampus-volumen, hvilket peger på, at de terapeutiske effekter kan involvere optimering af neuronale kredsløb snarere end vækst. Der blev også afdækket kønsmæssige forskelle i forholdet mellem hippocampus-volumen og serotonin-receptor-niveauer.

Studie II kunne ikke bekræfte tidligere fund om, at EEG-abnormiteter forudsiger dårlig behandlingsrespons på escitalopram. I stedet viste patienter med abnormiteter større humørforstyrrelser og dårligere verbal hukommelse, hvilket indikerer en specifik klinisk fænotype snarere end behandlingsresistens.

Studie III viste, at lave niveauer af kønshormoner forud for behandling forudsagde seksuelle bivirkninger af antidepressiva, men ikke selve behandlingsresponset. Derudover korrelerede højere testosteronniveauer med vegetative symptomer som vægttab, hvilket sandsynligvis afspejler depressionens metaboliske konsekvenser snarere end en årsagssammenhæng.

Samlet set understreger disse studier, at depression og dens behandling involverer komplekse, kønsspecifikke mekanismer, som ikke kan reduceres til simple biomarkør-udfaldsforhold. Dette danner grundlag for mere nuancerede tilgange til præcisionspsykiatri, der tager højde for kønsforskelle og flere interagerende biologiske systemer.

Med dette som afsæt præsenteres planen for det igangværende kohortestudie, BrainDrugs-Depression, som sigter mod dybdegående fænotypning af patienter med førstegangsdepression i en naturalistisk klinisk kontekst i RegionH. Formålet er at undersøge prædiktorer og mekanismer for behandlingseffekt med henblik på at opnå en bedre forståelse af *hvem har gavn af hvad*.

ABBREVIATIONS

ADHD	Attention-Deficit/Hyperactivity Disorder
AUC	Area Under the ROC Curve
BDD	BrainDrugs-Depression cohort study
BMI	Body Mass Index
BP_{ND}	Non-Displaceable Binding Potential
BSI	Brief Symptom Inventory
CAMH	Child and Adolescent Mental Health Services
CATS	Child Abuse and Trauma Scale
CI	95% confidence interval
COBRA	Cognitive Complaints in Bipolar Disorder Rating Assessment
CONSORT	Consolidated Standards of Reporting Trials
CPH	Copenhagen
DDD	Defined Daily Dose
DSM	Diagnostic and Statistical Manual of Mental Disorders
EEG	Electroencephalography
EMOTICOM	Emotional Test Battery
ENIGMA	Enhancing Neuro Imaging Genetics through Meta-Analysis
GP	General Practitioner
HAMD	Hamilton Depression Rating Scale
HRRT	High-Resolution Research Tomograph
ICD	International Classification of Diseases
IDS	Inventory of Depressive Symptomatology
IQR	Interquartile Range
iSPOT-D	International Study to Predict Optimized Treatment for Depression
LDAEP	Loudness Dependence of Auditory Evoked Potentials
LOD	Limit of Detection
LPFS	Level of Personality Functioning Scale
MDD	Major Depressive Disorder
MPRAGE	Magnetization-Prepared Rapid Gradient-Echo
MRI	Magnetic Resonance Imaging
NAT	Norepinephrine Transporter
NEQ	Negative Effects Questionnaire
NESDA	Netherlands Study of Depression and Anxiety
NP1	NeuroPharm-1 study
OR	Odds ratio
PAQ	Perth Alexithymia Questionnaire
PET	Positron Emission Tomography
PMDD	Premenstrual Dysphoric Disorder
POMS	Profile of Mood States
PRISE	Patient-Reported Inventory of Side-Effects
PSQI	Pittsburgh Sleep Quality Index
PSST	Premenstrual Symptoms Screening Tool
PVE	Partial Volume Effects
QIDS	Quick Inventory of Depressive Symptomatology
ROC	Receiver Operating Characteristic
SAPAS	Standardized Assessment of Personality - Abbreviated Scale
SCL	Symptom Checklist
SDS	Sheehan Disability Scale
SERT	Serotonin Transporter
SHAPS	Snaith-Hamilton Pleasure Scale
SLIDE	Significant Latent Factor Interaction Discovery and Exploration
SNRI	Serotonin-Norepinephrine Reuptake Inhibitor
SSRI	Selective Serotonin Reuptake Inhibitor
STAR*D	Sequenced Treatment Alternatives to Relieve Depression
TCA	Tricyclic Antidepressant
UKU	Udvalg for Kliniske Undersøgelser
VAMT	Verbal Affective Memory Task
WHO	World Health Organization

PREFACE

The burden of depression extends far beyond mood - it reshapes relationships, derails vocations, and fundamentally alters how people experience the world and themselves (Poem). Depression is associated with an increased risk of suicide and affects not only the individual but also families and communities. The heterogeneity in presentation, underlying biology, and treatment options challenge our ability to treat patients effectively. While poets like Teasdale captured the experience of melancholia in their work, some argue that romanticising depression has prevented us from recognising it as the severe condition it is [1].

Poem 1: I Shall Not Care by Sara Teasdale.

Teasdale died from an overdose of sleeping pills in her bathtub at age 48 in January 1933 in New York City. She struggled with chronic pneumonia and depression and died shortly after the end of her marriage in 1929 and the suicide of her close friend and lover in 1931. The poem is from 1915 [2].

When I am dead and over me bright April
Shakes out her rain-drenched hair,
Though you should lean above me broken-hearted,
I shall not care.

I shall have peace, as leafy trees are peaceful
When rain bends down the bough,
And I shall be more silent and cold-hearted
Than you are now.

In this thesis, I investigate depressive psychopathology, antidepressant treatment mechanisms and outcomes while working to build a large cohort study to enable research into more precise psychiatric care[1]. Rather than pursuing *personalised medicine* - a somewhat misleading term given that clinicians already strive to provide personable, individualised care within existing treatment structures, I aim better to understand depression heterogeneity and variations in treatment response.

Standardised treatment packages ensure minimum standards of care and may be necessary for effective healthcare delivery. However, they may constrain how treatment can be tailored to each patient's needs. This tension reflects broader challenges in organising healthcare. Also, we do not adequately know each patient's needs when starting treatment. *Precision psychiatry* aims to identify the needed treatments and the structures for delivery.

During recruitment, a participant asked if the research was political. "Of course not." But after reflection, yes. Better understanding depression, reducing suffering, improving quality of care, and ensuring more equitable access to effective treatments are inherently political goals. To achieve meaningful change, we need data that reveals where our current approaches fall short and how they might be improved.

Through careful scientific investigation, I hope to contribute knowledge that helps identify which existing treatments may work best for whom, with the least burden of side effects. The goal is not to personalise medicine - the healthcare system is already deeply personal for those seeking and providing care - but to make treatment selection more precise, ultimately working toward better mental healthcare for all.

[1] Footnotes traditionally provide technical clarifications. I also use them to provide context and reflections on the scientific process, acknowledging that research exists within a broader human and societal context rather than in an empirical vacuum.

BACKGROUND

Major depressive disorder (MDD) is characterised by at least one discrete depressive episode lasting at least two weeks and involving changes in mood, interests, pleasure, and cognition, and neurovegetative symptoms. While there are core symptoms, none of the symptoms are pathognomonic and they feature in other psychiatric and medical illnesses. MDD represents more than just persistent sadness and anhedonia - it is a heterogeneous condition that manifests differently across individuals. There are 227 possible symptom combinations[2] to meet the criteria for MDD in DSM-IV and -5, yet in clinical practice, a quarter to half do not occur [3,4].

The illness course and treatment response also vary between people and may also vary between episodes in the same person, possibly due to age and life circumstance dynamics [5]. Studies tracking symptom trajectories show that different symptoms follow distinct courses, particularly in response to treatment [6]. Sleep problems, fatigue, and cognitive difficulties often show a residual or pre-existing pattern [7–10].

1. Depression Subtypes and Multifactorial Aetiology

Different forms of depression have been recognised early on and are clinically described by symptom profiles (e.g. melancholic, anxious, and psychotic), patterns of relapse (e.g. recurrent, seasonal, or rapid cycling) and degrees of severity [11]. Multiple attempts have been made to subtype depression based on these specifiers, other factors (e.g., trauma, age of onset, treatment resistance) and biological markers. Large meta-reviews have identified common MDD subtypes but no consistent differences in clinical presentation or outcomes between them [12,13].

The incidence of depression is increasing globally [14], in Denmark, particularly among young adults [15]. The heritability of MDD is estimated to be only 30-40%, which is lower than bipolar disorder and schizophrenia [16–18]. Increased mental health awareness and changes in diagnostic practices may contribute to this increase, but it may also be due to changes in risk factor exposure [15,19]. MDD appears to be caused by a combined effect of genetics, environmental, biological and psychological factors [20]. Particularly childhood maltreatment and negative life events influence the onset [21,22] and course [23,24] of depression. Early understandings and successes came from drugs affecting monoamine neurotransmission [25].

2. From monoamines to SSRIs

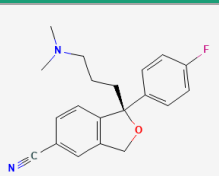
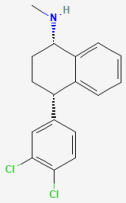
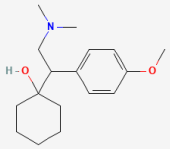
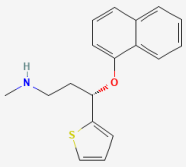
The monoamine neurotransmitters have long been implicated in depressive pathophysiology based on the early observation that monoamine-depleting drugs could induce depressive symptoms [26]. The success of tricyclic antidepressants

[2] This increases to 1816 possible combinations if you consider the opposing direction of symptoms such as sleep disturbance, i.e. insomnia versus hypersomnia, agitation versus retardation and appetite loss versus gain.

(TCA) and monoamine oxidase inhibitors, which enhance monoamine signalling, supported this theory. Selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) in the late 1980s marked a significant advance in the pharmacological treatment of MDD. They selectively target the reuptake of serotonin and/or norepinephrine, offering improved tolerability (Table 1) and quickly replacing TCAs and are the current first-line treatment for MDD However, they exhibit only modest efficacy, with response rates around 50% [27].

The monoamine hypothesis has been criticised as oversimplified, as changes in monoamine systems occur rapidly after drug administration [32], while the therapeutic effects of antidepressants typically take several weeks to emerge. While modulation of monoamines may initiate the therapeutic cascade, the clinical effects likely involve downstream changes in neuroplasticity and inflammation [33–35]. Furthermore, treatment response shows considerable variation, potentially influenced by factors such as age and sex, and may depend on pretreatment levels of central monoamines, such as serotonin [36–39].

Table 1: Common antidepressants and their characteristics.
Escitalopram and Duloxetine were used in the NeuroPharm-1 study, and Escitalopram, Sertralin, and Venlafaxine in iSPOT-D. [1]From PubChem. [2]Compared to placebo [29]. [3]Based on [30,31].

	Escitalopram	Sertralin	Venlafaxine	Duloxetine
Chemistry[1]				
Dosage range	10-20 mg	50-200 mg	75-375 mg	30-120 mg
SERT specificity	●●●●	●●●○	●●○○	●●●○
NAT specificity	○○○○	●○○○	●●○○	●●○○
Efficacy and acceptability, OR [CI][2]				
Response rate	1.68 [1.50; 1.87]	1.67 [1.49; 1.87]	1.78 [1.61; 1.96]	1.85 [1.66; 2.07]
Dropout rate	0.90 [0.80; 1.02]	0.96 [0.85; 1.08]	1.04 [0.93; 1.15]	1.09 [0.96; 1.23]
Side effects[3]				
Nausea	●●○	●●○	●●●	●●○
Sexual	●●●	●●●	●●●	●●○
Insomnia	●●○	●●○	●●●	●●●
Somnolence	●●●	●●○	●●○	●●●

3. Sex Differences in Depression: From Risk to Treatment

Women experience MDD at twice the rate of men [40], and have an earlier onset of depression, higher anxiety, lower alcohol use, and a higher prevalence of atypical depression compared to men, with no significant differences in medication use or counselling [41,42]. Women with depression also show higher levels of inflammatory, neurotrophic, and serotonergic markers [43], and there is considerable sexual

dimorphism in transcriptional patterns in MDD [44,45]. The sex differences in depression likely reflect complex interactions between biological and sociocultural factors such as gender roles and differences in risk factor exposure [46]. Evidence also points to women responding differently to SSRIs, with potentially higher response rates but also more adverse effects [47].

The adult female brain undergoes considerable changes during reproductive transitions, e.g. pregnancy and menopause [48–50]. Furthermore, widespread use of hormonal contraceptives may influence the brain's serotonin system and, consequently, both depression risk and treatment outcomes [51–54]. Sex hormones contribute to mood regulation and anxiety through receptors in brain regions [55,56] particularly in the hippocampus [57]. While the role of sex hormones in depression has been well-studied in women, less so in men despite evidence linking low testosterone to MDD in men [58].

This suggests that while the presentation of depression may appear similar across sexes, the underlying biological mechanisms into and out of depression may differ.

4. Structural Brain Changes in MDD and During SSRI Treatment

Despite symptom overlap with other disorders, the hippocampus appears uniquely affected in MDD. A large meta-analysis of the brain structure of nearly 16,000 individuals across psychiatric disorders suggests that hippocampus volume reduction may be relatively specific to MDD compared to schizophrenia, anxiety, and other conditions [59]. Early meta-analysis indicated reduced hippocampal volume might be present at illness onset [60], although not confirmed by larger studies [61,62]. Nonetheless, the reduced hippocampus volume in MDD has been a replicable finding in humans [61,62] and animal models [63]. Why it has been the focus of several biomarker studies [64], including in NeuroPharm-1 (NP1), where it did not predict treatment outcome [65].

The hippocampus is richly serotonergic innervated [66]. Preclinical studies indicate that SSRIs enhance hippocampal neuroplasticity and neurogenesis [67–70], as well as increase volume [68,70,71]. However, findings from human studies have been mixed [72]. This inconsistency may reflect the greater heterogeneity of human depression than animal depression models and that while serotonin plays a central role in depression [73], it may not be central to every type of depression [74,75].

5. Serotonin, Synaptic Plasticity and the 5-HT4 Receptor

Serotonin acts as a neurotrophic factor during development and modulates neuroplasticity in adulthood through multiple receptor subtypes [76]. Research shows reduced synaptic density in depression, which may reflect impaired plasticity [77–79].

The postsynaptic serotonin-4 receptor (5-HT4R) is implicated in familial risk for depression [80], and reduced hippocampal 5-HT4R levels are observed in MDD and

correlated with memory deficits [81,82]. The receptor regulates hippocampal plasticity [83,84], and 5-HT4R agonism promotes hippocampal neurogenesis and has anxiolytic and antidepressant-like effects in rodents [85]. Agonists have also been shown to improve memory in humans and rodents [86,87], making the receptor a promising drug target [85,88,89]. This and early work that suggested 5-HT4R levels as an inverse marker of serotonergic tonus [90] made 5-HT4R the central focus of NP1, which investigated mechanisms and predictors of SSRI treatment response.

The BrainDrugs-Depression (BDD) cohort omits 5-HT4R but maintains a focus on serotonin, e.g. by EEG markers associated with serotonin activity.[3] BDD focuses more broadly on neuroplasticity by using measures of synaptic density, neurite density, and intracortical myelin alongside functional measures from fMRI and EEG [91].

6. Biomarker Research Challenges

Treatment biomarkers can enable clinicians to move beyond the current trial-and-error approach, shortening the time to remission and minimising exposure to potential adverse effects. Yet, no clinically validated biomarkers are currently in routine use in psychiatry [92], and not all biomarker types may be easily clinically implemented.[4]

PET provides detailed molecular information, but is impractical for widespread use in psychiatry due to costs, availability and invasiveness. MRI is more available but has multiple contraindications, including claustrophobia. In contrast, EEG and cognitive testing offer more scalable brain-based biomarkers and could be implemented even in small or rural practices. Genetic and digital biomarkers raise privacy concerns and can feel intrusive to patients,[5] particularly in young patients [93].

Nonetheless, a key limitation in clinical use is also that many biomarkers lack external validation (or remain unpublished) and are based on small samples, leading to inconsistent results [12,94,95]. A concrete example of this is ‘abnormal EEG activity’ from the International Study to Predict Optimized Treatment for Depression (iSPOT-D) [96] finding its way into an EEG-guided treatment algorithm without validation [97].

In summary, the search for biomarkers is compounded by the heterogeneity of MDD and an incomplete understanding of how biomarkers reflect pathophysiological states [92]. Instead of pursuing perfect prediction for single treatments like SSRIs, a more practical approach might be stratification and matching patients to their individually best-fitting treatment [98]. Lastly, biomarker research has primarily focused on

[3] LDAEP: loudness dependence of auditory evoked potentials [36]

[4] There are also ethical considerations around using impersonal methods like neuroimaging, algorithms, and artificial intelligence in psychiatry, which may feel alienating to patients seeking care. Biomarkers may sound more *objective*, but biomarker and algorithmic approaches can also perpetuate existing societal biases and health disparities if not carefully developed and validated across diverse populations.

[5] The limit of genetics testing in BDD was chosen not to include full genome sequencing, as this would force participation to include the transfer of their data to the Danish National Genome Center, which at the time was politically debated [211]. While Danes are heavily tracked in the civil and health registries, the use of their genetic data remains a concern, particularly in psychiatric research [212].

predicting treatment response and remission, understandably, with less attention given to treatment dropout and side effects, which are also part of finding the best fit.

7. Current State and Future Needs

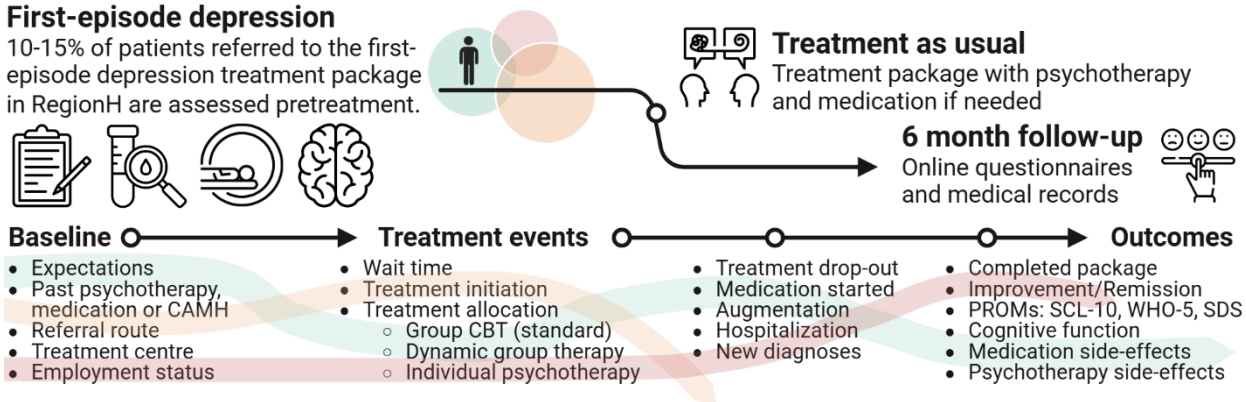
Considering the aforementioned, the large-scale BDD study uses a deep phenotyping approach with patients with first-episode MDD [91]. This approach moves away from controlled studies by acknowledging that treatment effects likely involve multiple interacting systems not predictable by single markers. Linking the cohort with electronic medical records and healthcare and civil registries enables both treatment monitoring and long-term outcome tracking and additional context that biomarker studies often lack (Figure 1).

While the BDD cohort is underway, we continue to investigate mechanisms and potential biomarkers of treatment response using the available data from the open-label clinical SSRI trial NP1 [39]. The three interconnected studies based on NP1 examine how selected markers from structure to sex hormones relate to clinical symptoms and treatment outcomes.

Study I investigated hippocampal volume changes during SSRI treatment in relation to treatment response and 5HT4R levels. Study II attempted to replicate previous findings linking EEG abnormalities to poor SSRI response. Study III investigated how pretreatment sex hormone levels in men were associated with psychopathology and SSRI treatment outcomes.

The BDD design is briefly displayed below and described in the methods (section 9.2, page 21) and the protocol paper (Appendix D). Since this work is ongoing, Study IV presents the current status of the cohort, including how well the cohort demographics match the treatment population and the response rate during one-year follow-up. This provides context for interpreting the dataset that will emerge from this cohort.

Figure 1: Overall structure of the BrainDrugs-Depression cohort study.
Overview of the treatment pathway from referral through baseline assessment, treatment delivery, and 6-month follow-up. The figure illustrates key treatment events and outcomes captured through the treatment package, including group and individual psychotherapy options, medication use, and package completion. Primary outcomes include clinical response and remission (based on IDS) and secondary outcomes based on other Patient-Reported Outcome Measures (PROMs), along with cognitive function and treatment side effects. The study has a total 18-month observation period with an additional 12- and 18-month follow-ups. CAMH: Child and Adolescent Mental Health Services.



Aims & Hypotheses

This thesis aimed to investigate neurobiological markers of treatment response in MDD, focusing on neuroimaging, cognition and hormones and establishing a deep phenotyping cohort. Through three interconnected studies using NP1 data, how selected markers relate to clinical symptoms and treatment outcomes is examined. The BDD cohort study and its current progress and potential are presented.

Study I

Prior studies have shown mixed results about whether SSRI antidepressants increase hippocampal volume in humans with depression despite more consistent findings in rodents and non-human primates. Additionally, while the serotonin 5-HT4 receptor is implicated in hippocampal development and depression, it is unclear whether individual differences in 5-HT4 receptor levels moderate hippocampal plasticity and verbal memory changes during SSRI treatment.

The aim of Study I was, therefore, to examine hippocampal plasticity during SSRI treatment and its relationship to clinical improvement and cognitive function. We tested the following hypotheses: 1) that hippocampal volume would increase during SSRI treatment, particularly in treatment responders; 2) that pretreatment hippocampal 5-HT4 receptor binding would be associated with volume changes during treatment; 3) that changes in hippocampal volume would correlate with improvements in verbal memory.

As the value of hippocampus volume for predicting treatment response has already been investigated in NP1 [65], this is not investigated here.

Study II

Research suggested that EEG abnormalities like isolated epileptiform discharges and slowing could predict non-response to escitalopram [96]. However, this finding lacks independent replication in a well-characterized clinical cohort to determine if EEG abnormalities are reliable biomarkers for antidepressant selection.

The aim of Study II was, therefore, to replicate and extend previous findings. We tested the following hypotheses: 1) that EEG abnormalities would be associated with poor response to escitalopram treatment; 2) that EEG abnormalities would be associated with distinct symptom profiles, including poorer cognitive function.

Study III

The role of sex hormones in MDD has been well-studied in women but less so in young men [99]. Specifically, it remains unclear whether sex hormones in men with depression are associated with depressive symptom profiles and SSRI treatment effects and side-effects response [100] .

The aim of Study III was, therefore, to investigate the role of sex hormones in male patients with MDD and their relationship to treatment outcomes. We tested the following hypotheses: 1) that testosterone and estradiol levels would be associated with depression severity and symptom profiles before medication; 2) that the sex hormone levels would be associated with SSRI treatment response; and 3) treatment-related sexual side effects.

Study IV

The BrainDrugs-Depression cohort aims to establish the large-scale deep phenotyping clinical cohort of patients with first-episode MDD to enable future precision psychiatry [91]. The overall aim is to a) identify single or composite biomarkers that can reliably predict treatment outcomes; b) establish a comprehensive dataset combining clinical, cognitive, psychometric, and biological data from 800 patients; c) examine disease trajectories using multimodal neuroimaging in a subcohort of 600 patients; and d) investigate presynaptic density using PET imaging in 60 antidepressant-naïve patients.

Since this work is ongoing, we present descriptive statistics and, using the currently available data, evaluate 1) the status of recruitment, 2) how well the cohort demographics match the treatment population, 3) assess potential recruitment biases within subgroups, and lastly 4) the response rate during one-year follow-up. The challenges and opportunities of the cohort are discussed.

METHODS

8. Ethics, approvals, and registrations

Studies 1-3 are based on patient data from the NeuroPharm-1 study (clinicaltrail.gov: NCT02869035). NP1 was approved by the Committees on Health Research Ethics for the Capital Region of Copenhagen (H-15017713). A description of NP1 elements for part of Study I-III is given here. For a detailed description, see the protocol [39].

The ongoing BrainDrugs-Depression study has been collecting patient data since July 2021. BDD was approved by the Committees on Health Research Ethics for the Capital Region of Denmark (H-20083013). A description of the study design is given here. For a detailed description of the protocol, see Appendix D.

In NP1 and BDD, investigators explain study aims, methods, potential hazards, and benefits verbally and in writing, and participants provide written consent.

Data collection was conducted in accordance with the Declaration of Helsinki (2013). To align with the recent 8th revision of the Declaration of Helsinki (2024), we will review current BDD study practices to ensure we maintain high ethical standards, particularly in protecting our vulnerable patient participants.[6]

9. Study designs

9.1 Studies I-III based on NeuroPharm-1

NP1 was a longitudinal, open-label, multimodal neuroimaging clinical trial investigating potential biomarkers for SSRI treatment response in MDD.

One hundred antidepressant-free patients with moderate to severe MDD ($HAMD_{17}$ ≥18) were recruited from outpatient clinics and primary care physicians in Denmark's capital region. Patients were diagnosed according to ICD-10 criteria, verified by a psychiatrist, and screened using the Mini International Neuropsychiatric Interview version 6 [101]. Participants were required to be antidepressant-free for >2 months before and with a depression duration of <2 years and no other primary psychiatric disorder. Exclusion criteria included psychosis, suicidality, substance use disorders, prior SSRI non-response, severe physical illness, medical and neuroimaging contraindications, e.g. pregnancy/breastfeeding, and concurrent medications that could interfere with the study protocol. The study ran from 08-2016 to 04-2019.

9.1.1 Drug treatment

Following baseline assessments, patients were treated with 10-20 mg daily escitalopram adjusted based on response and side effects. Escitalopram was selected

[6] In NP1, subjects are mostly referred to as *patients* as they received treatment and support from Dr Köhler-Forsberg and the research team. In BDD, although subjects are patients receiving clinical care, the term *participant* is used here as they contribute data without being in a treatment relationship with the research team.

for its high SERT selectivity (Table 1, page 11). Per standard clinical practice, patients with inadequate response (<25% reduction in $HAMD_6$) or intolerable side effects at week four could switch to duloxetine. For drug differences, see Table 1.

Treatment effects and side effects were assessed at weeks 4, 8 and 12. Treatment adherence was confirmed through pill counting and plasma drug concentrations at week 8. After eight weeks of treatment, a subset of about half of the patients completed follow-up MRI, PET, and EEG.

9.1.2 Structural MRI

We acquired T1w images using the common magnetization-prepared rapid gradient-echo (MPRAGE) sequence (TR: 1900 ms, TE: 2.58 ms, TI: 900 ms, 9° flip angle, 224 slices and 0.9 mm isotropic resolution) using a 64-channel head coil on the Siemens 3T Prisma scanner with a 64-channel head coil. For hippocampal segmentation, these T1w images are processed using FreeSurfer 7.2, a pipeline that combines intensity normalisation, registration to standard space, and probabilistic classification based on a manually labelled training dataset [102]. FreeSurfer's hippocampal segmentation algorithm specifically accounts for the complicated hippocampus's architecture, using both geometric and intensity information to delineate its boundaries [103]. While hippocampal subfield segmentation offers more granular anatomical investigation, we used total hippocampal volume due to reliability concerns with automated subfield segmentation without a high-resolution in-plane T2 thick-slab. The subfield segmentation algorithm, while validated for total hippocampal volume, shows lower reliability for individual subfields at standard resolution [104,105].

9.1.3 PET 5-HT4R

Positron Emission Tomography (PET) enables in vivo quantification of molecular targets by using radioactively labelled molecules that bind selectively to targets of interest. We used [^{11}C]SB207145, a selective antagonist radioligand for the 5-HT4R.

Dynamic PET data (256 × 256 × 207 voxels; 1.22 mm isotropic) were acquired over 120 minutes and reconstructed into time frames of increasing duration. Done on High-Resolution Research Tomograph (HRRT, CTI/Siemens) scanner developed by CTI (later Siemens Medical Solutions) as a dedicated brain PET scanner for research.

The reconstruction process included corrections for random coincidences, scattered photons, and tissue attenuation. To correct for head motion during the long scan duration, post-reconstruction motion correction was performed using AIR 5.2.5 [106]. The PET images were co-registered to individual T1w images to delineate regions of interest, e.g. the hippocampus using PVElab [107]. Time-activity curves representing decay-corrected radioactivity concentration were extracted from hemisphere-averaged GM volumes. These curves were analysed using the simplified reference tissue model with the cerebellum without the vermis as the reference (as it has negligible 5-HT4R levels) [108]. The non-displaceable binding potential (BP_{ND}) represents the ratio of specifically bound radioligand to non-displaceable radioligand in the tissue at equilibrium and serves as the measure reflecting 5-HT4R availability.

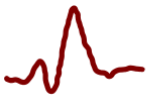
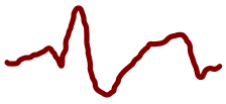
Partial volume effects (PVE) occur when the scanner's limited spatial resolution causes signal spillover between adjacent regions, leading to underestimation in small structures and regions bordering tissues with different radiotracer concentrations. PVE has been observed in subcortical structures in early [^{11}C]SB207145 studies on an older PET scanner in an older population[7] [109]. We did not apply PVE correction because 1) our higher resolution HRRT scanner is less affected by PVE, 2) our young cohort (mean age 27 years) has minimal age-related atrophy, and 3) our longitudinal analyses focus on within-subject changes over a short timeframe where partial volume effects would remain relatively constant.

9.1.4 EEG abnormalities

Electroencephalography (EEG) measures the electrical activity of neurons, primarily cortical pyramidal cells [110]. Abnormal EEG patterns, including the slowing of background rhythms and epileptiform discharges, may indicate underlying neurophysiological disturbances.

In study II, we used resting-state EEG data recorded using a 256-channel HydroCel Sensor Net system at 1 MHz with 0.1-100 Hz analogue filtering and impedance maintained <50 kΩ during recording. The vertex electrode as the reference and the recording consisted of four 3-minute periods alternating between eyes-open and eyes-closed conditions counterbalanced across subjects. Only eyes-closed EEG data were included in the analysis. A board-certified clinical neurophysiologist, blinded to clinical data, visually inspected the EEGs according to international guidelines [111]. The evaluation included an assessment of posterior rhythmic activity, alpha peak frequency, background activity, and the presence of interictal activity. Abnormal activity encompassing EEG slowing or potential epileptiform activity was noted together with Wicket spikes (Table 2), though their significance remains uncertain.

Table 2: EEG abnormalities.
Descriptions adapted from [111].

Sharp wave: A pointed waveform that stands out from the background activity, lasting 70-200 ms with a typically steeper upward slope than a downward slope. It must be distinguished from normal activity patterns like sleep waves and clearly visible against the background EEG.	
Sharp-and-slow-wave complex: A pattern where a sharp wave is immediately followed by a slower wave. Both clearly stand out from the background activity and can occur as single events or in repetitions.	
Theta activity/Continuous slow activity: Ongoing slow waves that differ from normal activity patterns. It can be regular or irregular in rhythm and varies in size. Persists without interruption and does not respond to external stimuli. Appears more frequently than normal for age.	
Wicket spikes: Arc-shaped waves that appear over one side of the temporal region during drowsiness. Usually harmless and more common in older adults, though sometimes seen in epilepsy patients. Typically occur in sequences and have a distinctive curved appearance.	

[7] An 18-ring Advance scanner GE Healthcare with an approximate in-plane resolution of 6 mm (reconstructed into 128 × 128 × 35 voxels; 2.0 × 2.0 × 4.25 mm) with subjects aged 20-86 years (mean 44 years).

9.1.5 Cognition

NP1 had an extensive assessment of both "cold" (emotion-independent) and "hot" (emotion-dependent) cognitive domains at baseline and week 12 [113].

To investigate hippocampal-dependent cognitive function in Study I, we used the Verbal Affective Memory Task-26 (VAMT-26). This task assesses learning and recall of valenced words (10 positive, 10 negative, and 6 neutral), with participants recalling words across five immediate trials, followed by short-term and 30-minute delayed recall tests. We used total word recall (averaged across all trials) to assess explicit non-affective verbal memory function [114].

To investigate cognitive function in relation to abnormal EEG activity in Study II, we expanded our analysis to include both total word recall and affective memory bias (calculated as positive minus negative word recall). Additionally, working memory capacity was assessed using the Letter-Number Sequence (LNS) task, where participants reorganise mixed sequences of letters and numbers into ascending/alphabetical order, with increasing difficulty [115].

9.1.6 Sex hormone levels

Plasma testosterone and estradiol were quantified at Rigshospitalet's clinical laboratory. Estradiol had a lower quantitation limit of 0.09 nM (LOD). For statistical analysis, estradiol values <0.09 nM were values 0.05-0.09 nM were imputed based on the mean and SD of quantified values. The lower limit was based on a comparable male study using an earlier assay at Rigshospitalet with a lower LOD [57].

9.1.7 Treatment response and effect

Treatment response represents a clinically meaningful improvement in depressive symptoms following treatment. While complete and sustained remission is the goal, response is often used as an intermediate outcome measure in clinical trials and practice and is defined as a ≥50% reduction in depression scale from baseline.

NP1 specifically used the 6-item subscale of the Hamilton Depression Rating Scale ($HAMD_6$) to assess the response [39], as this subscale captures core depressive symptoms and shows greater sensitivity to treatment effects compared to the full 17-item version, i.e. $HAMD_6$ [116,117]. However, most studies, such as the iSPOT-D, use the full $HAMD_{17}$ [96]. To replicate the Arns et al. in study II, we use both.

In addition, mood disturbance measured by Profile of Mood States (with six states: tension, depression, anger, vigour, fatigue and confusion) was used as part of study II.

9.1.8 Treatment side effects

Sexual side effects of SSRIs are common and can significantly impact treatment adherence. These effects are mediated through serotonergic pathways and potentially through interactions with sex hormones [118]. SSRI-related side effects were assessed using the UKU Side Effect Rating Scale, a standardised clinician-rated evaluation [119,120]. In study III, we specifically assessed erectile and ejaculatory dysfunction as well as decreased libido during weeks 8 to 12, when depression symptoms are decreased and overall sexual function increases [121].

9.2 Study IV based on Brain Drugs-Depression

The BrainDrugs-Depression study aims to establish a cohort of 800 patients with first-episode MDD referred for standardised treatment packages in the Capital Region of Denmark during 2021-2028 [91]All patients undergo comprehensive baseline phenotyping, including clinical, cognitive, psychometric, and biological assessments. A subgroup (subcohort I, n=600) additionally undergoes MRI and EEG. From subcohort I, antidepressant-naïve patients are invited to participate in a PET subcohort II (n=60) with [^{11}C]UCB-J PET imaging to assess synaptic density.

The study includes adults aged 18-65 with first-episode depression meeting ICD-10 criteria. Exclusion criteria included psychosis, severe head trauma, known brain abnormalities, and poor Danish proficiency. Additional restrictions apply for neuroimaging: MRI contraindications (e.g., pregnancy, metal implants, and claustrophobia) and PET contraindications (i.e. psychotropic drug use, recent radiation exposure, and pregnancy/breastfeeding).

9.2.1 Patient recruitment and treatment

The participants are recruited from a central visitation unit or by clinicians in the treatment centres (Figure 2). The participants are not randomised to the neuroimaging subgroup, but the patients decide and is also limited by scheduling conflicts and the exclusion criteria. There is a considerable difference in time commitment between the basic assessment without neuroimaging (Figure 4, page 23). Following baseline assessments, patients receive standard care through the treatment package in the six clinics (Figure 4), typically consisting of group-based cognitive behavioural therapy with the option of antidepressant medication (Figure 1, page 14).

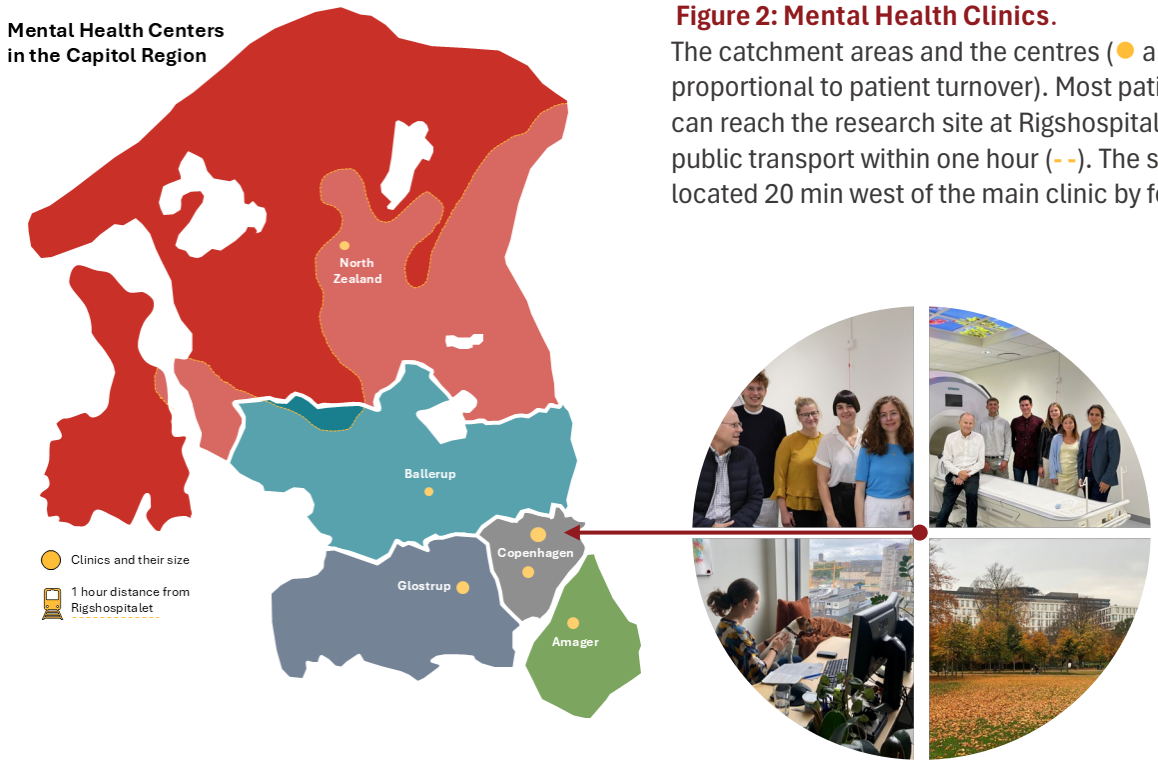


Figure 2: Mental Health Clinics.
The catchment areas and the centres (● area proportional to patient turnover). Most patients can reach the research site at Rigshospitalet by public transport within one hour (- -). The site is located 20 min west of the main clinic by foot.

9.2.2 Baseline assessment

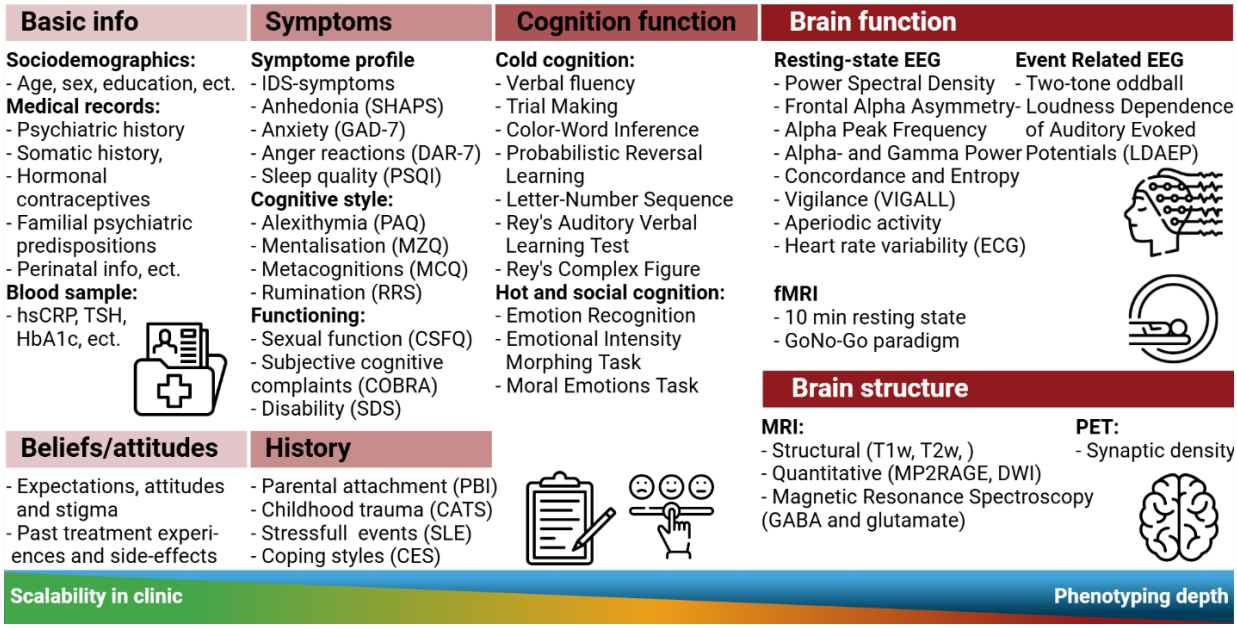
The BrainDrugs-Depression study employs comprehensive baseline phenotyping before treatment initiation (Figure 3).

All participants (n=800) undergo short clinical interviews, psychometric assessments, and cognitive testing, including standard 'cold' tasks and 'hot' tasks from the EMOTICOM test battery [122,123] assessing emotion recognition and social cognition (Figure 3). Full blood, serum, plasma and urine samples are collected and biobanked for later analyses, enabling the investigation of hormone levels, inflammatory markers, neurotrophic factors, and genetic variations.[8] The Standardised Assessment of Personality - Abbreviated Scale (SAPAS) was added to the inclusion interview in late October 2022.[9]

A subcohort (n=600) additionally undergoes neuroimaging (Figure 4), including 90 min structural and functional MRI using a 3-Tesla Siemens Magnetom Prisma scanner[10], and EEG with the same setup as in NP1 with the addition of a one-lead ECG. A smaller subcohort of unmedicated patients (n=60) also undergo 90 min PET imaging with [^{11}C]-UCB-J, which requires arterial cannulation[11] for proper modelling of tracer kinetics.

Figure 3: Baseline phenotyping metrics in the BrainDrugs-Depression cohort

The baseline phenotyping metrics, including medical records and lab values, while symptom assessments cover depressive symptoms, anxiety, sleep, and cognitive styles. Cognitive function is evaluated through both non-emotional tasks and social-emotional processing. Brain function and structure are characterised through EEG, MRI, and PET measurements in a subset of patients (EEG and MRI n=600; PET n=60). The gradients at the bottom indicate clinical scalability from **easily implementable** to **challenging** and phenotyping depth from **readily available but superficial** to **deeper but resource-intensive**.



[8] BDD does not include cerebrospinal fluid, microbiome samples and ecological momentary assessment; limits.

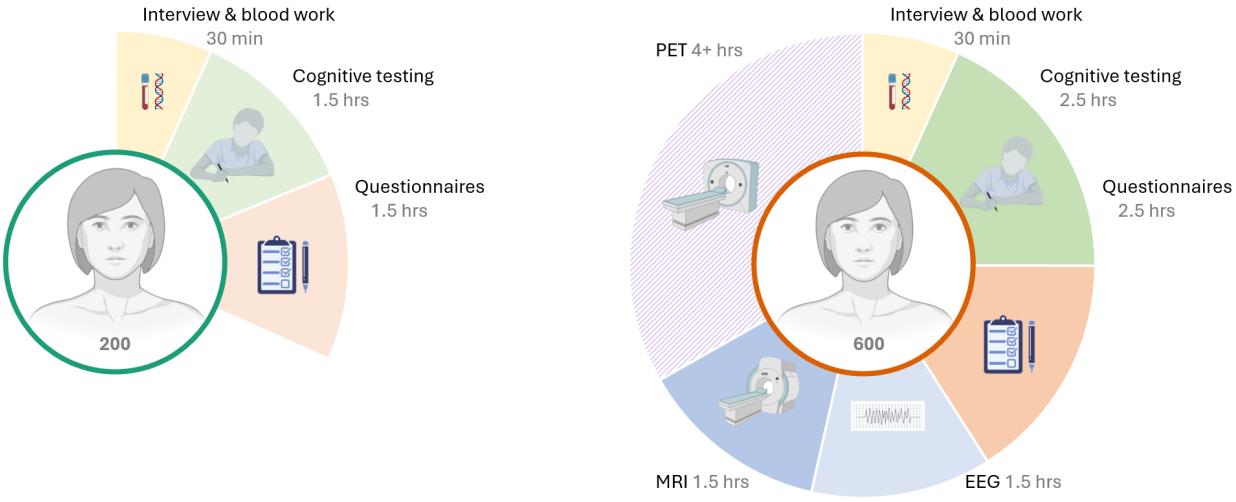
[9] On the recommendation of Emilie Hestbæk, who reached out to me on LinkedIn after a BDD talk. Tak.

[10] A newer scanner than NP1. BDD uses a 32-channel head coil, which provides a marginally lower signal-to-noise ratio than the 64-channel coil used in NP1, its less restrictive design offers better participant comfort and compliance.

[11] Not needed in NP1 due to the availability of a proper reference region.

Questionnaires assess demographics, medical history, depressive symptoms, and various psychometric domains through a secure web-based platform (Table 3). Depression severity is evaluated using the Inventory of Depressive Symptomatology (IDS), while additional scales, e.g., measuring anxiety (GAD-7), stress (PSS), and well-being (WHO-5). The study leverages Danish health registers to obtain comprehensive electronic medical records and sociodemographic data.

Figure 4: Approximate time commitments for patient subgroups.
The basic assessment takes 2-3 hours on-site, with questionnaires mostly filled out at home. The neuroimaging subgroup has a longer cognitive assessment and the addition of EEG and MRI. Depending on scheduling, this can be done on one long day but is often split over two. The 10% of patients from the neuroimaging group who also undergo PET imaging (not depicted) commit an additional half day.



The selection of questionnaires and other metrics intentionally overlaps to some degree with the concurrent BrainDrugs-Epilepsy study [124] to enable investigations, e.g., of how depression may differ in epilepsy and to be compatible with other projects at the research unit [124]. This did mean compromises, e.g., the use of lesser-used questionnaires, e.g., the Child Abuse and Trauma Scale (CATS) [125], used in NP1.

9.2.3 Follow-up assessments

Clinical outcomes in BDD are assessed at 6, 12, and 18 months (Table 4) using the Quick Inventory of Depressive Symptomatology (QIDS, from the IDS), with remission defined as QIDS ≤ 5 and treatment response as a ≥50% reduction [91].

Secondary outcomes include changes in anxiety and depressive symptoms (SCL-10), well-being (WHO-5), and disability (mSDS) which are the established treatment effect parameters used by the Mental Health Services of the Capital Region of Denmark [19] .

Tertiary endpoints are three measurements of psychosocial remission defined as a WHO-5 score of ≥50, an SCL-10 score of <26 and an mSDS score of <10.

Treatment side effects are evaluated using PRISE for medication [126] , and the NEQ [127] for psychotherapy, and full medical record access and healthcare registries enable knowing what and when treatment events happen.

Table 3: Baseline questionnaires.

The questionnaires that all participants get (●) and additional (○) for the neuroimaging subgroup.

[1]The mPSST, NEQ and LPFS-BF were added later and are not mentioned in [91].

Symptom Profile and Severity	
Inventory of Depressive Symptomatology – self-report (IDS-SR)	●
Dimension of Anger Reactions (DAR-7)	●
Generalised Anxiety Disorder 7-item (GAD-7)	●
Cohen's Perceived Stress Scale (PSS)	●
Brief Symptom Inventory (BSI)	●
Symptom checklist (SCL-10)	●
Snaith-Hamilton Pleasure Scale (SHAPS)	●
Modified premenstrual symptoms screening tool (mPSST) [1]	●
Pittsburgh Sleep Quality Index (PSQI)	○
Cognitive style	
Mentalisation Questionnaire (MZQ)	●
Ruminative Response Scale (RRS)	●
Perth Alexithymia Questionnaire (PAQ)	●
Mindful Attention Awareness Scale (MAAS)	○
Short form of Metacognitions Questionnaire (MCQ-30)	○
Coping Self-Efficacy Scale (CSES)	○
Upbringing, life history and past treatment experiences	
Online Stimulant and Family History Assessment Module (OS-FHAM)	●
Attitudes Towards Depression and its Treatment (ATDT)	●
Child abuse and trauma scale (CATS)	●
Negative Effects Questionnaire (NEQ) [1]	●
Parental Bonding Instrument (PBI)	○
Stressful Life Events (SLE)	○
Functioning and quality of life	
Cognitive Complaints in Bipolar Disorder Rating Assessment (COBRA)	●
Modified Sheehan Disability Score (mSDS)	●
WHO 5 wellbeing index (WHO-5)	●
Changes in Sexual Functioning Questionnaire (CSFQ)	●
Questions from the Copenhagen Aging and Midlife Biobank (CAMB)	●
Revised Sociosexual Orientation Inventory (SOI-R)	●
Level of Personality Functioning Scale – Brief Form (LPFS-BF) 2.0[1]	●

Table 4: Follow-ups questionnaires.

The questionnaires for all participants (●) and those dependent on the given treatment modality (○).

[1]The LPFS-BF and PAQ-S were added after the protocol paper was published [91].

Questionaries	Follow-up:	6	12	18
Symptoms				
Inventory of Depressive Symptomatology – self-report (IDS-SR)		●	●	●
Brief Symptom Inventory 18-item (BSI-18)		●	●	●
Symptom checklist (SCL-10)		●	●	●
Perth Alexithymia Questionnaire-Short Form (PAQ-S) [1]		●	●	●
Functioning and quality of life				
Modified Sheehan Disability Score (mSDS)		●	●	●
WHO 5 wellbeing index (WHO-5)		●	●	●
Level of Personality Functioning Scale – Brief Form (LPFS-BF) 2.0[1]		●	●	●
Treatment side effects				
Patient-Reported Inventory of Side-Effects (PRISE)		○		
Negative Effects Questionnaire (NEQ-20)		○		

10. Statistics

Statistical tests and graphs were done in R version 4.2.2 or 4.3.2 [128] with several packages: lme4 (1.1-35.1) for mixed modelling and BuyseTest for Gehan's test [129,130] or JASP [131]. We present mean (SD) or median (IQR) and two-sided 95% confidence intervals [CI]. *P*-values <0.05 were considered statistically significant.

10.1 Study I

Bilaterial hippocampal volume was used in all analyses due to the high correlation between hemispheres. For verbal memory models due to lateralisation [132–134].

10.1.1 Change in hippocampus volume and treatment response

A linear mixed-effects model was used to estimate the average change in hippocampus volume between baseline and week 8. The model included hippocampus volume as the outcome variable, with time, hemisphere, age, and sex as fixed effects. Subject and hemisphere nested within subject were included as random effects.

The relation between volume changes and treatment response was analysed using a linear mixed model as above, with the addition of a fixed effect for the interaction between treatment response and time (there was no main effect of treatment response as we assumed pre-treatment volume to be independent of treatment response).

10.1.2 Hippocampus volume and 5-HT4R binding

Linear mixed-effects models evaluated relationships between hippocampal 5-HT4R binding and volume changes during treatment. The models included hippocampus volume as the outcome, with time, 5-HT4R binding, PET tracer mass, age, and sex as fixed effects, and subject as a random effect. A sex interaction term was included to examine potential gender differences in these relationships.

10.1.3 Left hippocampus volume and verbal memory

Linear regression analysed the relationship between left pretreatment hippocampus volume and verbal memory (VAMT-26), adjusted for intracranial volume, age and sex.

A linear mixed-effects model examined the volume change in the left hippocampus at week 8 and the change in verbal memory at week 12, with volume, time, age, and sex as fixed effects and subject as a random effect.

10.2 Study II

10.2.1 EEG abnormality and treatment response

Demographics and depression severity were compared between patients with and without EEG abnormalities using Welch's t-tests and Fisher's exact tests.

Treatment response (≥50% reduction on the $HAMD_{17}$ and $HAMD_{6}$) between groups was assessed in week eight using Fisher's exact test. The published results [135] are based on the four categories of abnormal EEG activity (Table 2). Wicket spikes were not part of Arns et al.'s study [96]. Therefore, we supplement the findings here with analyses of treatment response, ignoring Wicket spikes.

10.2.2 EEG abnormality and mood and cognition

ANCOVAs adjusted for age and sex were employed to examine differences in clinical profiles. Post hoc, to identify the drivers of the observed mood disturbance, logistic regression was performed with EEG abnormality as the dependent variable and POMS subscale items as predictors, controlling for age and sex. Effect sizes were reported as Cohen's *d* and omega-squared (ω^2).

10.3 Study III

10.3.1 Sex hormones and SSRI treatment response

Pretreatment sex hormone levels between treatment responders (defined as ≥50% HAMD6 reduction) and non-responders were compared using Welch t-tests, followed by linear regression models with age and BMI as covariates. Linear regression models with age and BMI were also used to investigate associations between sex hormones and %-change in $HAMD_6$ at weeks 8 and 12 from baseline.

10.3.2 Sex hormones and SSRI sexual side effects

Plasma sex hormone levels were compared between individuals experiencing and not experiencing sexual side effects using Welch t-tests. For estradiol values below the detection limit (0.09 nM, LOD), Gehan's test was used to account for left-censoring.

A logistic regression analysis examined the relation between the sex hormones and sexual side effects during 8-12 weeks of treatment, with age and BMI. The discriminative ability of hormone levels to predict sexual side effects was assessed using the area under the ROC curve (AUC).

10.3.3 Sex hormones and depressive symptoms

Linear regression models with age and BMI were used to examine whether sex hormone levels were associated with depression severity ($HAMD_{17}$) before treatment initiation. Post-hoc, Spearman's rank correlations between sex hormone levels and the $HAMD_{17}$ items conditioned on age and BMI were used to identify which symptoms drove the observed associations.

10.4 Study IV

We use data from the participants in the BDD cohort from recruitment starting in August 2021 until and including October 2024. Data from March 2019 to April 2021 in the Capitol region was provided by the Mental Health Centre Copenhagen (CPH). Data on patients referred for and starting in the first-episode depression treatment package (codes: AFV01E1 and AGB04A) and using antidepressants (code: N06A) stratified by sex and age groups across catchment areas (Figure 2).

10.4.1 Status of recruitment and demographics

A potential sex difference in the proportion of possible comorbid personality disorders in the BDD cohort (Table 11) was investigated using Fisher's exact test. The age and sex distributions and antidepressant use in the BDD cohort to date were compared with the regional CPH treatment data (Figure 13) using chi-square goodness-of-fit. We compared the demographic and clinical characteristics of study participants who

received the basic assessment and those who underwent neuroimaging (Table 13) using Fisher's exact, Mann-Whitney U and Welch's t tests.

10.4.2 Current follow-up response rates

We compared not responding to a 6-month follow-up between participants in the basic assessment and neuroimaging groups using a Welch's t-test. A sensitivity analysis with only participants included from 2022 and on as neuroimaging was first initiated and offered to participants then. A logistic regression was used to further identify groups not responding based on baseline depression severity and sex in stratified age groups with and without neuroimaging. Given only two subjects to date have died, this reason for censoring is ignored.

RESULTS

In the following, summaries of the main results from Study I-III and an overview of the data acquired from Study IV are presented. Full details are provided in each of the manuscripts (Appendix A-D). Studies I-III are all based on NP1 data and a brief overview of the NP1 study is therefore presented below.

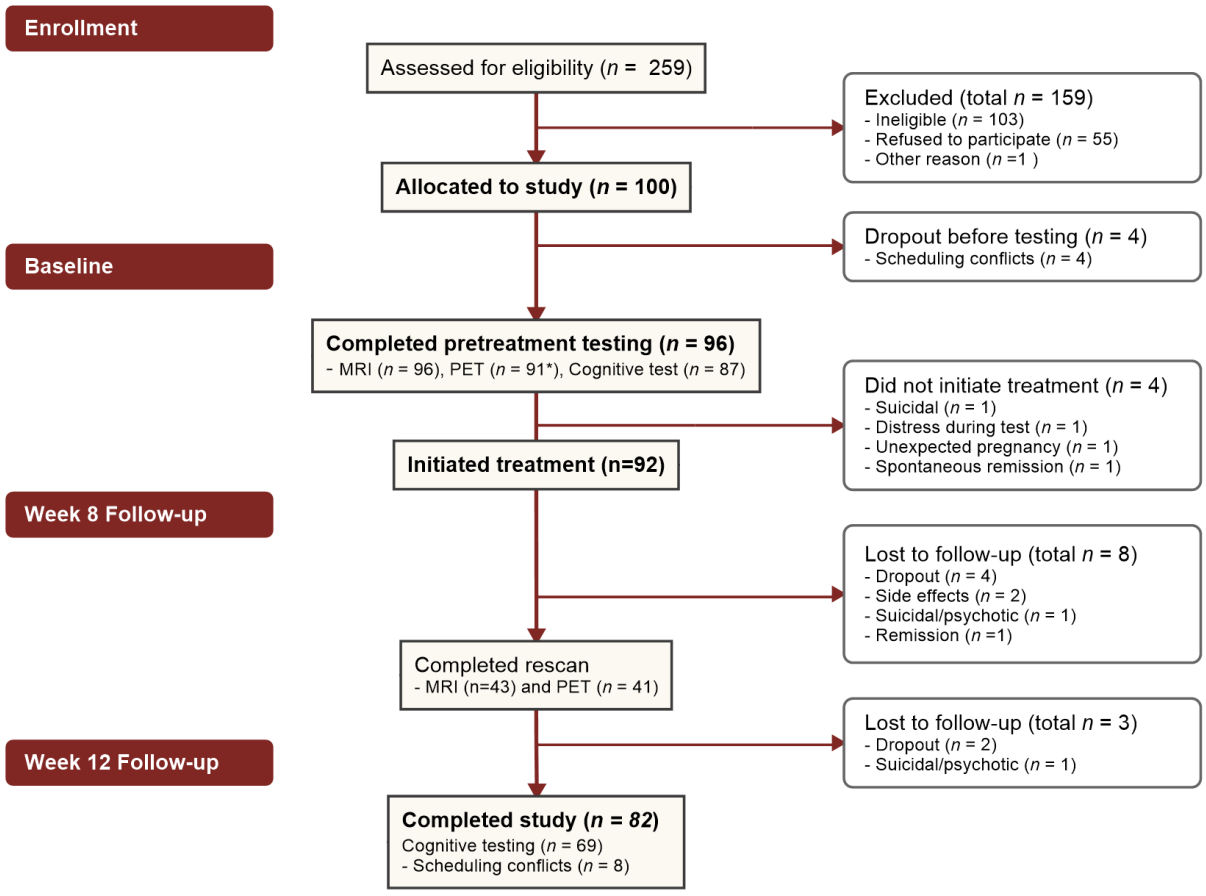
Table 5: Brief overview of NeuroPharm-1.

Data is mean (SD) unless n (%). [1]Information on the episode was unavailable for one patient, and [2]education information was unavailable for 20.

	All	Female	Male
n	96	69	27
First episode[1]	42 (44%)	27 (40%)	15 (56%)
Age (years)	27.2 (8.2)	27.3 (12.2)	26.9 (7.8)
Years of education[2]	11.6 (1.1)	11.6 (1.1)	11.6 (0.9)
BMI	24.7 (5.6)	24.7 (6.2)	24.5 (3.9)
$HAMD_{17}$	22.9 (3.5)	23.3 (3.4)	22.2 (3.2)
$HAMD_{6}$	12.4 (1.7)	12.5 (1.7)	12.0 (1.5)

Figure 5: CONSORT diagram of NeuroPharm-1.

*One pretreatment PET scan was excluded due to data quality. From [136].



from KHRJ et al. J. Psychiatr. Res. 181, 197-205 2023. (doi.org/10.1016/j.jpsychires.2024.11.043)

11. Study I

This study included 96 patients with MRI from NP1 aged 18 to 57 (Table 5, page 28). Only 92 initiated treatment. Of these, 43 were rescanned at week 8. Four patients were not treatment adherent, two of whom were rescanned.

11.1 Change in hippocampus volume and treatment response

We observed no overall increase in hippocampus volume after eight weeks of SSRI treatment. Instead, we estimated a mean 27 [-4; 58] mm^3 reduction (p=0.086).

Stratified by treatment response (Figure 2), we estimated a significant mean 45 [-81; -8]mm^3 reduction in treatment responders (p=0.019), while non-responders showed a non-significant change of 8 [-46; 61] mm^3 (p=0.78). The estimated group difference was 52 [13; 117] mm^3, which was not statistically significant (p=0.12).

Changes in hippocampal volume did not correlate with improvements in depression severity as measured by %-change in $HAMD_6$ (β=0.79 [0.30; 1.87] mm^3/%, p=0.16).

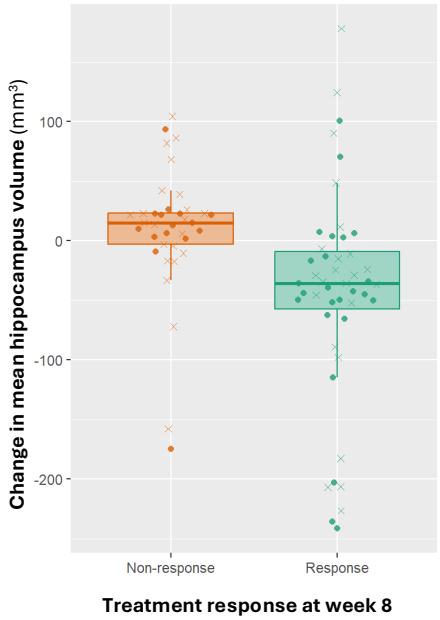


Figure 6: Hippocampus volume changes and treatment response. We estimated a -45 [-81; -8] mm^3 mean change in **responders** (p=0.019) and 8 [-46; 61] mm^3 in **non-responders** (p=0.78). However, there was not a difference group difference (p=0.12). Dots are patients who have both baseline and rescan. Crosses are patients only with baseline scans, where the change was computed based on the mixed model estimate of the mean rescan value conditional on the observed baseline. Modified from [136].

from KHRJ et al. J. Psychiatr. Res. 181, 197-205 2023. (doi.org/10.1016/j.jpsychires.2024.11.043)

11.2 Hippocampus volume and 5-HT4R binding

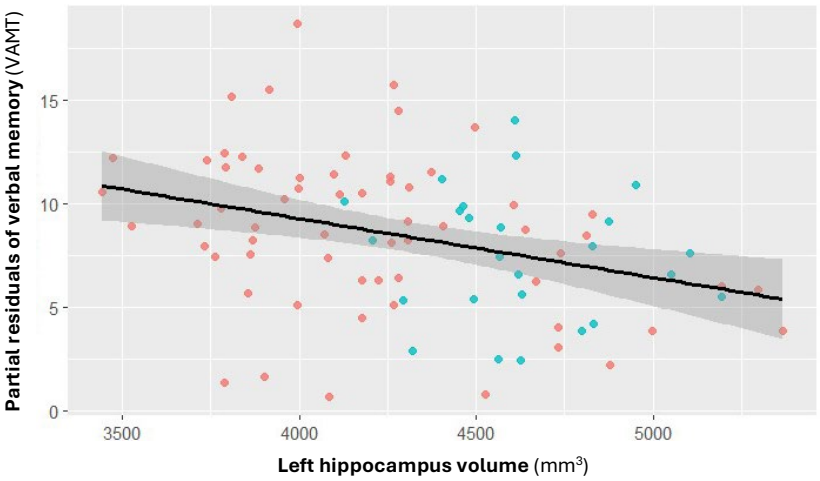
We found notable sex differences in the relationship between changes in hippocampal 5-HT4R and volume changes during treatment (Figure 7). In female patients, increased 5-HT4R binding was significantly associated with decreased hippocampal volume both before treatment (β=-276 [-459; -94], p=0.006) and at week eight (β=-319 [-508, -131], p=0.002). Male patients showed opposite trends with positive associations, though these were not statistically significant (pretreatment: β=110 [-92; 310], p=0.30; week eight: β=89 [-116; 293], p=0.41). Despite these apparent sex-specific patterns, the sex interaction effect was not statistically significant (β=-264 [-632; 104], p=0.15).

11.3 Left hippocampus volume and verbal memory

We found a significant negative correlation between left hippocampus volume and verbal memory performance before treatment (β=-2.84 [-0.34; -5.34] score/cm^3, p=0.026). This association remained significant after adjusting for depression severity (β=-2.76 [-0.27; -5.25] score/cm^3, p=0.030). However, changes in left hippocampus volume from baseline to week 8 did not correlate with changes in verbal memory performance from baseline to week 12 (β=-0.10 [-1.80; 1.58], p=0.90).

Figure 7: Changes in hippocampal 5-HT4R binding and volume during treatment.
Change in mean hippocampus volume and 5-HT4R binding was obtained from the linear mixed model. For **females**, the relation was negative (β=-268 [-529; -6], p=0.045) but near zero for **males** (β=-4 [-271; 263], p=0.98). Dots are patients who have both baseline and rescan. Crosses are patients only with baseline scans, where the change was computed based on the model estimate of the mean rescan value conditional on the observed baseline. From [136].

Figure 8: Pretreatment left hippocampus volume and verbal memory performance.
Partial residuals of the effect of hippocampus volume on verbal memory minus effects of age, sex (**female**/**male**) and intracranial volume (β= -2.8 [-0.3; -5.3]/1000mm^3, n = 87, p = 0.026). [136].



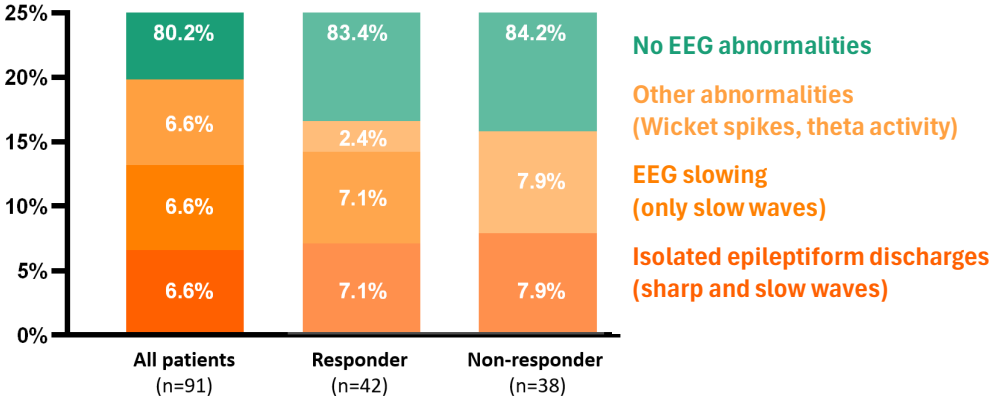
12. Study II

This study included 91 patients with EEG from NP1 aged 18 to 57 (Table 5, page 28) who initiated treatment.

12.1 EEG abnormality and treatment response

Abnormal EEG findings were present in 20% of the patients (n=18, Figure 9): 13% had isolated epileptiform discharges and EEG slowing, and 7% had other findings with unclear significance (i.e. Wicket spikes and theta activity). The abnormal activity was predominantly located bilaterally in frontotemporal regions. The demographics and depression and anxiety severity did not differ between patients with and without EEG abnormalities (*p-values*>0.11, see Table 2 in Appendix B).

Figure 9: Frequency of EEG abnormalities.
Clinical response was defined using relative $HAMD_{17}$ scores from to pretreatment week 8, with Responders having a ≥50% symptom reduction. The frequency of EEG abnormalities was not significantly different between responders and non-responders (OR=0.94 [0.23; 3.65], *p*=1.00). Data from [135].



At week 8, the frequency of EEG abnormality was not significantly different between treatment responders and non-responders (OR=0.94 [0.23; 3.65], *p*=1.00). This analysis included Wicket spikes and theta activity, which Arns et al. did not include in their analysis [96]. Ignoring Wicket spikes and theta activity, did not change the conclusion (OR=0.52 [0.08; 2.67], *p*=0.52).

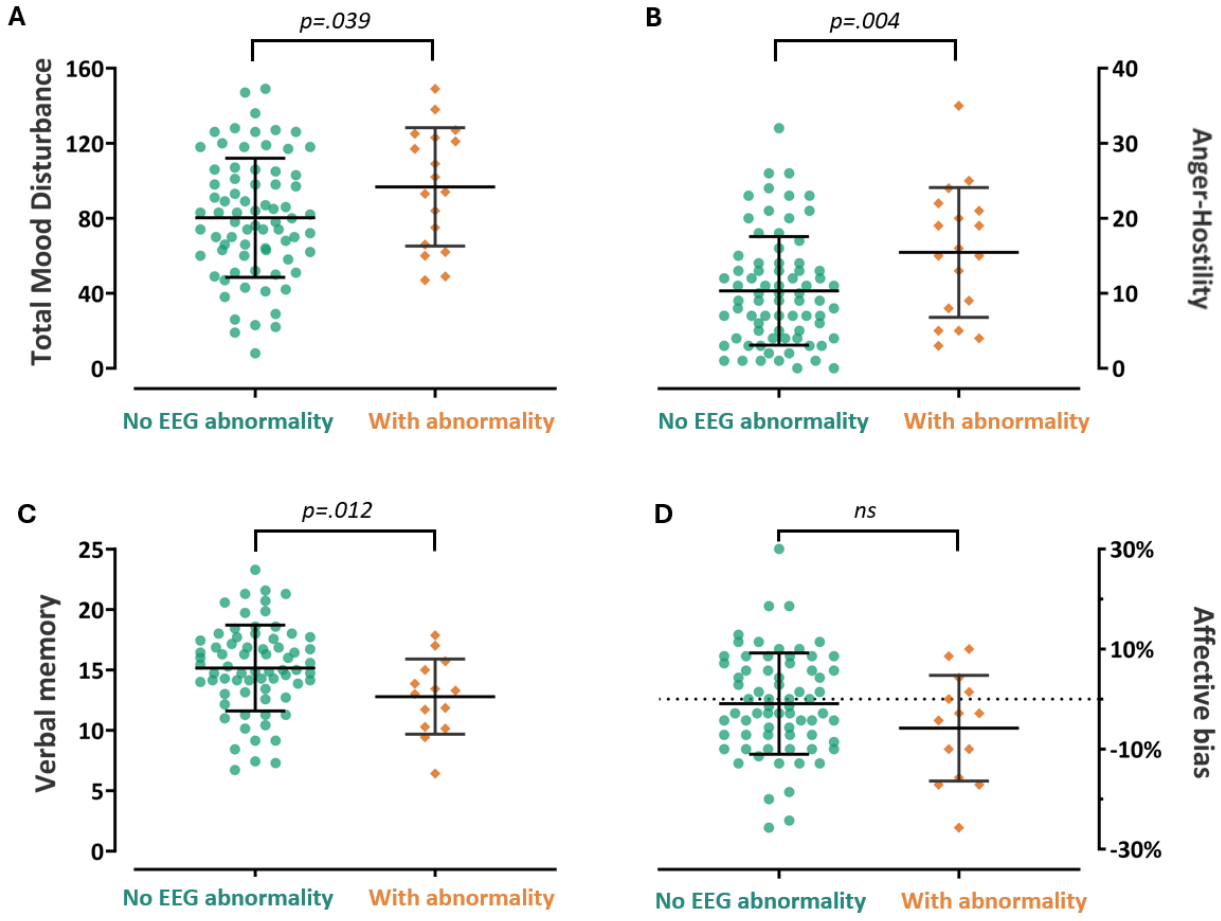
12.2 EEG Abnormality and Mood and Cognition

Though patients with and without EEG abnormalities showed similar levels of depression and anxiety symptoms, those with EEG abnormalities exhibited significantly higher total mood disturbance (ω^2=0.04, *p*=0.039). On average, their mood disturbance scores were 17.6 [0.9; 34.2] points higher (Cohen's *d*=0.55, Figure 10A), which was driven by elevated anger-hostility (Figure 10A-B). Specifically, patients with abnormalities scored 5.7 [1.9; 9.6] points higher on the anger-hostility subscale (*d*=0.78) compared to those without abnormalities (ω^2=0.07, *p*=0.004).

Working memory performance did not differ between groups (ω^2=0.00, p=0.38). However, verbal memory was significantly impaired in those with EEG abnormalities (Figure 10C), who recalled an average of 2.6 [0.6; 4.7] fewer words (d=0.76) compared to those without abnormalities (ω^2=0.07, p=0.012).

Given the patients with EEG abnormalities showed heightened negative emotions and poorer verbal memory, we post hoc examined affective memory bias (Figure 10C). Patients with EEG abnormalities showed a non-significant better recall of negative words compared to positive words (mean bias: -5.8%) versus those without abnormalities (mean bias: -0.9%, (ω^2=0.01, p=0.17). There was a positive linear relation between poorer verbal memory and total mood disturbance after controlling for age, sex, and EEG abnormality (p=0.031), yet no interaction with abnormality.

Figure 10: Pretreatment differences in mood and cognitive disturbances.
Descriptive plots of mean and SD with *p*-values are from ANCOVAs with age and sex from [100].



from KHRJ et al. Eur. Neuropsychopharmacol. 79 59-65 2024 (doi.org/10.1016/j.euroneuro.2023.11.004)

13. Study III

This study included 26 men from NP1 aged 18 to 48 (Table 5, page 28).

13.1 Sex Hormones and SSRI Treatment Response

Among the 24 men assessed at week eight, 15 responded to SSRI treatment (defined as ≥50% $HAMD_6$ reduction). Neither pretreatment plasma testosterone nor estradiol levels differed significantly between responders and non-responders at this timepoint (p>0.28), including after adjustment for age and BMI (p>0.44).

At week 12, five patients had still not responded to treatment. They had a 6 [1.6; 10.4] nM lower mean pretreatment testosterone than responders (Hedges' g=0.98, p=0.0097). However, this difference was only 5.2 [-2.5; 12.9] nM and non-significant after adjusting for age and BMI (p=0.17). Pretreatment testosterone was not associated with a %-change in $HAMD_6$ at week 12 (β=-1.63 [3.45; 0.19], p=0.076).

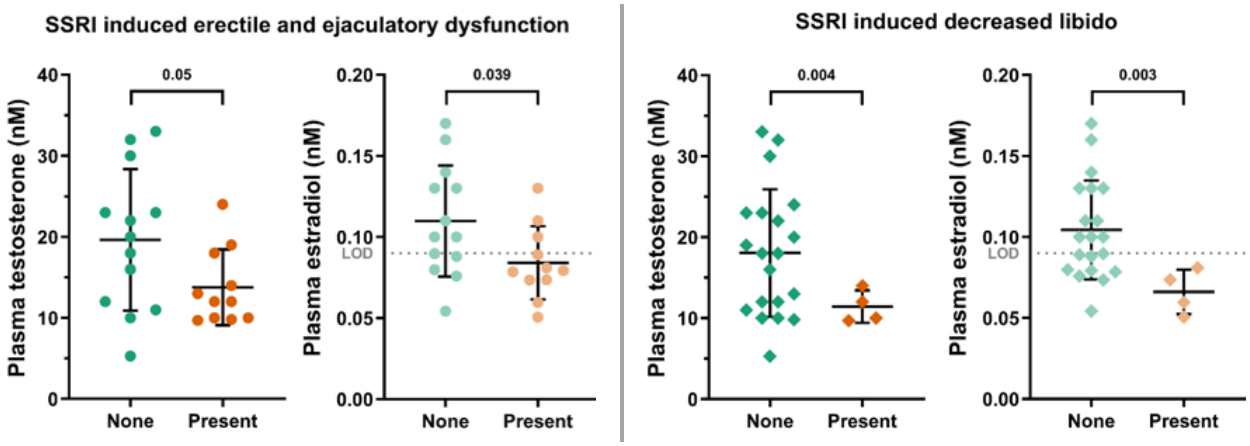
13.2 Sex Hormones and SSRI Sexual Side Effects

Among the 11 men who experienced erectile and ejaculatory dysfunction attributed to escitalopram (Figure 11), mean testosterone (13.8 nM) and estradiol (0.084 nM) were lower compared to those without these side effects (19.6 and 0.110 nM). This difference was borderline significant for testosterone (g = 0.81, p = 0.050) and significant for estradiol (g = 0.86, p = 0.039).

The four men who experienced decreased libido also had erectile/ejaculatory issues. Their hormone levels were markedly lower (testosterone: 11.4 nM; estradiol: 0.066 nM) than men without decreased libido (18.1 and 0.104 nM), with significant differences for both testosterone (g = 1.6, p = 0.004) and estradiol (g = 1.6, p = 0.003).

To better account for the left-censored estradiol (Figure 11) than imputation, we used Gehan's test. In this analysis, the lower estradiol in men with erectile/ejaculatory dysfunction was not significant (p=0.056) but remained highly significant in decreased libido (p=0.0001).

Figure 11 Pretreatment sex hormone levels and sexual SSRI side effects in men.
Values below the limit of detection (LOD) were imputed, and displayed p-values from Welch's t-tests [100].



Higher pretreatment estradiol was associated with a lower likelihood of sexual side effects (β=-34.3 [-68.6; -0.01], p=0.0499), with moderate predictive ability (AUC=0.75 [0.55, 0.95]). Testosterone showed a similar yet non-significant relationship (β=-0.12 [-68.6; -0.01], p=0.096) with an AUC of 0.71 [0.50; 0.93].

13.3 Testosterone and Vegetative Symptoms

Before treatment, higher plasma testosterone was surprisingly *positively* correlated with greater overall depression severity on the $HAMD_{17}$ (β=0.22 [0.04; 0.39], p=0.016). A post-hoc investigation examining testosterone's relation with only core symptoms (i.e. the $HAMD_6$) showed no association (β=-0.001 [-0.08; 0.08], p=0.97).

Rather, the relationship between testosterone and depression severity appeared to be driven primarily by vegetative symptoms. Specifically, we found strong positive correlations between testosterone and gastrointestinal symptoms - namely, *reduced appetite* and *weight loss* (Table 6), The composite score of these gastrointestinal symptoms showed a stronger correlation with testosterone (*rho*=0.74 [0.43; 0.81], p<0.0001), and the combined score of three insomnia items also demonstrated a moderate positive correlation with testosterone (*rho*=0.45 [-0.07; 0.75], p=0.027).

Estradiol showed no relation with $HAMD_{17}$ or individual symptoms (p≥0.40).

Table 6 Plasma testosterone and depression individual symptoms

Spearman's rank correlations of $HAMD_{17}$ items (conditioned on age and BMI). *$HAMD_6$- items. [1]Includes loss of appetite, heavy feeling in the abdomen and constipation. Modified from [100].

$HAMD_{17}$ item		*rho*	[CI]	*p*-value
12.	Gastrointestinal somatic symptoms[1]	0.71	[0.47; 0.87]	0.0001
17.	Weight loss	0.54	[0.07; 0.81]	0.006
4.	Insomnia: early in the night	0.35	[-0.17; 0.72]	0.097
5.	Insomnia: middle of the night	0.30	[-0.12; 0.60]	0.16
6.	Insomnia: early hours of the morning	0.30	[-0.24; 0.62]	0.16
7.	Work and activities*	0.28	[-0.17; 0.65]	0.18
14.	Sexual dysfunction	0.25	[-0.17; 0.60]	0.25
16.	Lack of insight	0.20	[-0.22; 0.61]	0.34
13.	General somatic symptoms*	0.13	[-0.30; 0.56]	0.54
11.	Somatic anxiety	0.04	[-0.36; 0.46]	0.84
2.	Feelings of guilt*	0.02	[-0.41; 0.45]	0.95
8.	Psychomotor retardation*	-0.09	[-0.50; 0.34]	0.67
15.	Hypochondriasis	-0.15	[-0.54; 0.35]	0.50
9.	Agitation	-0.16	[-0.55; 0.32]	0.45
3.	Suicide	-0.21	[-0.62; 0.21]	0.32
10.	Psychic anxiety*	-0.27	[-0.67; 0.16]	0.21
1.	Depressed mood*	-0.37	[-0.71; 0.10]	0.072

14. Study IV

14.1 Status of Recruitment and Demographics

According to the Capitol Region (CPH), about 1000 patients annually enter the treatment package for first-episode depression. From August 2021 until October 2024, clinicians provided the research team with contact information for 540 patients (17% of all patients). Of them, 338 patients were included in the study, meaning 10% of patients were included in the cohort. However, 158 declined, and 44 were excluded.

We tracked eight overlapping reasons for declining participation (Table 7). Reaching potential participants is difficult, as one-third (36%) did not answer their phones. Patients often mention anxiety about taking calls from unknown numbers and opening electronic mail. The majority cited time constraints with work and studies and *manglende overskud*[12]. We also observed other patterns of declining participation (Table 7). The reasons for exclusion were mostly being sent to private clinics[13] due to a lack of capacity at the public clinics or being too far in treatment.

Primary baseline clinical measures are presented in Table 8. Unfortunately, 4.6% of participants did not answer the primary outcome measure questionnaires.[14]

Table 7: Reasons for declining and exclusion

Participants can have more than one reason.

Reasons for declining	Reasons for exclusion
36% Did not answer phone calls	30% Sent to a private clinic
30% *Manglende overskud*	25% Too far in treatment
22% Did not wish to participate	11% Sent back to GP (not for treatment)
20% Cancelling their baseline visit	9% Language barrier
8% Time constraints	7% Change of primary diagnosis
	5% Severe medical conditions (e.g. recent aneurysm)

Table 8: Baseline symptom characteristics.

Inventory of Depressive Symptomatology (IDS) with ≤13 being normal, World Health Organization Five Well-Being Index (WHO-5) with <50 indicating poor well-being, modified Sheehan Disability Score (mSDS) with ≥12 indicating significant overall functional impairment.

Symptom	N (missing)	Mean (SD)	Median (min; max)
IDS	323 (15)	39.7 (9.4)	40 (12; 63)
WHO-5	318 (20)	22.5 (13.3)	20 (0; 76)
mSDS	320 (18)	19.8 (5.2)	20 (1; 30)

[12] The literal translation is *a lack of surplus or excess*, capturing that feeling of being depleted or not having any capacity or reserves left to draw on. This is a common reason for declining participation in Nordic cultures.

[13] This is 50-100 patients in the region yearly. Their health care records and not available and they do not adhere to the treatment package and are therefore not part of the study.

[14] Anecdotally, one young man fainted upon seeing the blood sampling needle, fled, and was never heard from again.

Table 9: Patient demographics at study inclusion.
Variables are presented as % and Median (IQR). 1) Sedatives during the day, e.g. benzodiazepines, quetiapine or hydroxyzine. 2) Sleep aids, i.e. melatonin and sedatives. 3) 74% were using an SSRI, 14% an SNRI and 12% other (e.g. Mirtazapine). *Data only available from 39 women and 19 men.

	All	Female	Male
n	338	243	95
Age	25.8 (11.5)	24.9 (9.3)	28.7 (18.3)
Current episode information			
Leave/absence from work/studies	53.6%	51.9 %	57.9%
Duration (months)	7 (10)	6.0 (7.8)	11 (17)
Tried nothing (treatment naïve)	25.9%	27.6%	21.1%
Tried antidepressants	37.3%	33.8%	45.8%
Tried psychotherapy	54.6%	53.1%	57.9%
Tried sedatives[1]	4.2%	4.1%	4.2%
Tried sleep aids[2]	11.9%	10.3%	15.8%
Other treatments (e.g. bodywork)	21.2%	23.7%	14.9%
Current antidepressant use[3]	39.1%	32.5%	55.8%
- Duration in weeks*	9 (16)	10 (22)	8 (12)
Past episodes information			
Have had past episodes	52.6%	54.3%	48.4%
Number of past episodes	2 (2)	2 (2)	2 (2)
Mean duration of past episodes	6 (9)	6 (6)	4.5 (9)
Cannabis use during the past month			
None	88.6%	92.2%	80.0%
Within a month	4.6%	3.0%	7.6%
Within a week	5.1%	4.2%	7.1%
Yesterday	1.7%	0.6%	4.3%
Experienced discrimination			
No	64.2%	58.8%	76.9%
Due to sex	12.3%	17.5%	0%
Due to colour/ethnicity	8.5%	9.8%	5.2%
Due to mental illness or disability	7.4%	9.3%	2.6%
Due sexual orientation	4.2%	4.9%	2.6%
Due to gender identity	1.5%	1.6%	1.3%

Two-thirds of patients scored ≥3 (Table 11), indicating a co-morbid personality disorder rate above the 47% scoring ≥3 in a Danish hospital sample of remitted and low-severity MDD patients [137]. If a one-point higher cut-off is used for greater accuracy, only 42% of BDD patients potentially have a co-morbid personality disorder. As expected, the most common direction of disturbance is in cluster C with ≥60% reporting worried or perfectionist. While there does not appear to be a sex difference in the frequency of potential co-morbid personality disorders (score ≥3, $\chi^2(1)=0.96$, $p=33$), there is a possible different direction of disturbance (Table 11).

Table 10: Education and childhood characteristics.
Data from 289 participants (49 missing). *Including half-siblings.

Characteristics		
Childhood trauma	Mean (SD)	Median (min; max)
Punishment	1.4 (0.8)	1.3 (0; 3.7)
Emotional abuse	1.1 (0.8)	0.9 (0; 3.9)
Sexual abuse	0.2 (0.4)	0.0 (0; 2.7)
Negative home atmosphere	1.2 (0.8)	1.1 (0; 3.3)
Education score	Mean (SD)	Median (min; max)
Patient	3.4 (1.6)	4 (1; 5)
Mother	3.4 (1.6)	4 (1; 5)
Father	3.4 (1.6)	3 (1; 5)
1st-degree relative with	Without	With*
Mania	95.5%	4.5%
Suicide attempt	95.2%	4.8%
Drug problems	88.6%	11.4%
Anxiety (nerve problems)	87.9%	12.1%
Alcohol problem	79.6%	20.4%
Depression	58.1%	41.9%

Table 11: Assessment of Personality.
Distribution of the Standardised Assessment of Personality - Abbreviated Scale (SAPAS) interview scores during inclusion (n = 212, 68% female). The median (IQR) total score was 3 (2) for all groups.

Personality clusters	Question	All	Female	Male
Cluster A	Loner	30%	21%	48%
Odd/Eccentric	Distrust of others	34%	37%	30%
Cluster B	Loses temper	22%	23%	21%
Dramatic/Emotional/Erratic	Impulsive	35%	31%	43%
	Difficulty with friends	25%	25%	27%
Cluster C	Worried	82%	83%	78%
Anxious/Fearful	Perfectionist	63%	66%	55%
	Dependent on others	34%	40%	25%
Cut-offs in clinical populations	Total score ≥3	67%	65%	72%
	Total score ≥4	42%	41%	45%

A review of the medical records of the latest 154 participants reveals that 84% were referred to the treatment package from their GP, 10% from another outpatient clinic, and the rest from either a private practising psychiatrist or the psychiatric emergency department. Of the records, self-harm was mentioned in 24%, suicide attempts in 6%, and 10% had a past association with Child and Adolescent Mental Health Services. From these records, it was possible to assess beginning treatment resistance on the Maudsley Staging (Figure 12). 21% had a past treatment failure to 1-2 medications, and the majority scored mild on the treatment resistance score, driven mainly by illness severity and duration.

We also assess past psychotherapy types and negative experiences using the NEQ-20 [127]. Of the subjects included since December 2023, when this was added, 67% have had psychotherapy, most often through public, employee or private health insurance (Table 12). The majority had 8 sessions (2-52) of individual therapy (92.5 %), with cognitive behavioural therapy (40%), by GP referral (30%) 2 months ago (0 months – 24 years). The most common negative effects were u*npleasant memories resurfacing* (72%) and *not always understanding the treatment* (55%).

Given past treatment attempts, we also assess treatment resistance using the Maudsley Staging [138]. The majority are classified as mild treatment-resistant resistance primarily driven by illness severity and duration (Figure 12).

Table 12: Past psychotherapy

Type of psychotherapy	Access to psychotherapy
40% Cognitive behavioural therapy	30% GP referral (public subsidised)
19% Psychodynamic	25% Work or private insurance
9% Metacognitive	19% Parental support
4% Mindfulness-based	13% School or municipal
2% Psychoanalysis	13% Self-payment
26% *Unknown*	

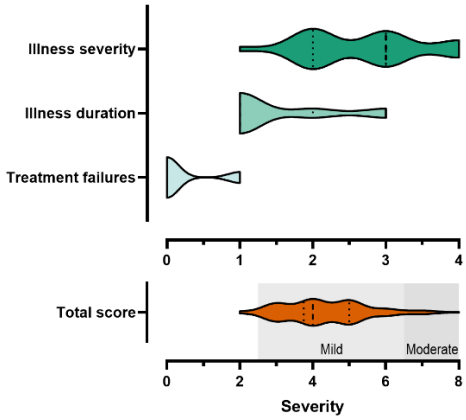
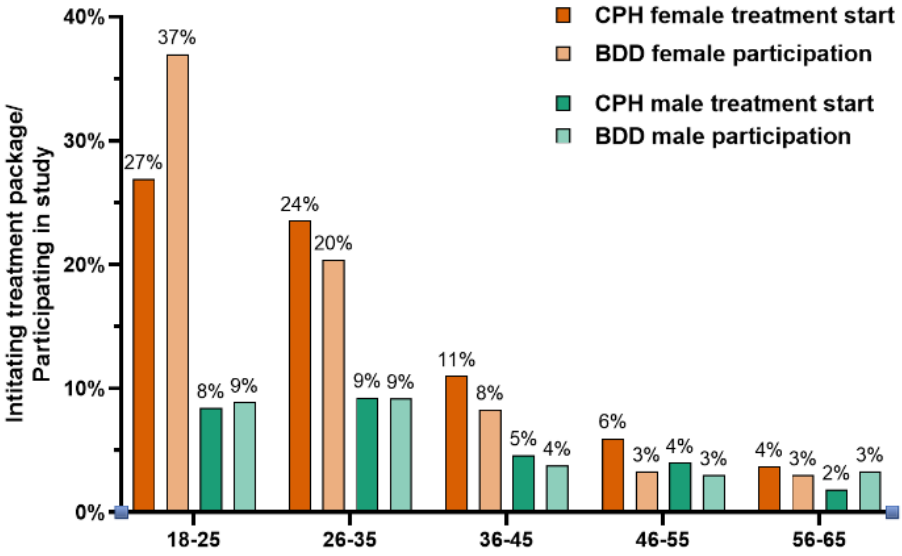


Figure 12 Treatment resistance at study inclusion. Violin plots of Maudsley Staging from the latest 142 participants [138]. A score of 1 on treatment failures indicates failure to 1–2 medications. Duration is scored as 1 for Acute (≤12 months), 2 for Sub-acute (13–24 months), and 3 for Chronic (>24 months). Symptom severity from Subsyndromal, Mild, Moderate to Severe based on IDS scored as 1-4. None of the participants had augmentation (i.e. score 0). The total Treatment Resistance staging score (grey) is mild (3–6), Moderate (7–10) and Severe (11-15).

14.1.1 Potential age and sex bias in the cohort

An annual inclusion rate of 100-120 patients represents 10-12% of the treatment population (CPH). The gender distribution of included participants (72% female) matches the treatment population (72% female). The male age distribution for men in BDD similarly matches the population, but young women are overrepresented (37% compared to 27%, Figure 13). Antidepressant use was significantly higher in BDD (39%, Table 9) than in the treatment sample (30%, $\chi^2(1)=12.47$, $p<0.001$).

Figure 13 Age and sex distribution.
Patients entering the Copenhagen Region treatment package (CPH) during 2019-2021 and the BrainDrugs-Depression (BDD) participants included during 2021-2024.



14.1.2 Potential recruitment biases in the neuroimaging subgroup

Patients contribute different amounts of time depending on which cohort they participate in (Figure 4, page 23), i.e. 2-3 hours on-site without neuroimaging and more than 6 hours on-site with neuroimaging[15]. A few participants declined neuroimaging due to claustrophobia and anxiety. However, the majority cited a lack of time and *excess*. Some, particularly young men from technical science backgrounds, showed a keen interest in neuroimaging. All participants could receive a picture of their brain scan afterwards. While some declined due to anxiety, the prospect of obtaining a brain scan could be appealing to those with somatisation.

To assess potential recruitment bias in the two groups, we assessed key demographic and clinical variables (Table 13). The neuroimaging and basic assessment groups showed similar characteristics, with no significant differences in demographics, illness duration (8 vs 6 months, p=0.08), depression severity (IDS: 39.5 vs 40.3, p=0.49), or somatisation (BSI: 1.4 vs 1.4, p=0.59). The proportion of females (71% vs 75%), prior antidepressant use (38.2% vs 34.8%), and work/study absence (51.8% vs 58.2%) were also similar between groups.

However, the neuroimaging cohort was first initiated and offered to patients in January 2022. The remaining 48 patients who did not take part in neuroimaging had a median current episode duration of 8 (IQR=12) months, like the neuroimaging cohort (Table 13), and work/study absence was 61.2%. However, the estimated mean differences did not change considerably, and there remained no sign of increased anxiety or somatisation in participants not undergoing neuroimaging.

[15] Due to scheduling conflicts or cancellations, of the 247 currently in the neuroimaging group, only 235 had EEG (17 without MRI), and 230 had MRI (12 without EEG). Thus, only 218 have both EEG and MRI.

14.1.3 The PET imaging subgroup

From the neuroimaging group, 24 were planned to undergo PET brain imaging, which succeeded in 19 participants. This subgroup has additional inclusion criteria, i.e., being antidepressant naïve, willing to undergo radio imaging and arterial cannulation, and an additional half-day. Participation was also limited by imaging resources.

This group was predominantly female (72%), with a median age of 25.8 (IQR: 5.6). Over half (53%) had taken a leave of absence from work or studies. The median illness duration was 6 months (IQR: 7), and participants showed moderate to severe depression symptoms with a mean IDS of 37.5 (SD: 9.5). They were thus similar to the rest of the neuroimaging cohort (Table 13).

Table 13: Cohort subgroup differences.

Inventory of Depressive Symptomatology (IDS), World Health Organization Five Well-Being Index (WHO-5), modified Sheehan Disability Score (mSDS), Cognitive Complaints in Bipolar Disorder Rating Assessment (COBRA), General Anxiety Disorder-7 (GAD-7) Brief Symptom Inventory (BSI).

	Basic group	**Neuroimaging**	**Difference [*CI*]**	***p***
%	*n=91*	*n=247*	*Odds Ratio*	*Fisher's*
Female	75%	71%	1.2 [0.7; 2.2]	0.59
Tried antidepressants	34.8%	38.2%	1.2 [0.7; 2.0]	0.61
Leave/Absence	58.2%	51.8%	0.7 [0.5; 1.3]	0.33
median (IQR)			*Hodges-Lehmann*	*Mann-Whitney*
Age (years)	25.5 (11.2)	25.9 (11.5)	0.4 [-2.0; 1.1]	0.56
Duration (months)	6 (8)	8 (13)	1.0 [3.0; 0.0]	0.08
Mean (SD)			Mean	*Welch*
IDS	40.3 (10.0)	39.5 (9.2)	0.8 [-1.6; 3.3]	0.49
WHO-5	21.3 (14.2)	23.0 (7.0)	-1.7 [-5.2; 1.8]	0.34
mSDS	20.4 (5.1)	19.6 (5.2)	0.8 [-0.4; 2.2]	0.18
COBRA	24.2 (8.4)	24.4 (7.0)	-0.2 [-2.2; 1.8]	0.82
GAD-7	10.7 (4.8)	10.8 (4.4)	-0.1 [-1.3; 1.0]	0.80
BSI somatisation	1.4 (0.8)	1.4 (0.7)	0.06 [-0.15; 0.26]	0.59

14.2 Current Follow-up Response Rates

Patients are sent 6- and 12-month follow-up questionnaires. Examining the response rate to follow-up during the first year of participation, we observe that despite "assertive follow-up", only 43% respond to the 6-month follow-up. Others respond later or first respond at the subsequent one-year follow-up. While patients appear to improve overall in their depression severity, clinical remission (IDS ≤13) is rare during one year (Figure 14). However, with this temporal resolution, we cannot clearly track potential remission and relapses during this period.

The timely response proportion to the 6-month follow-up questionaries (i.e. 5-7 months from baseline) was 61.6% for men and 55.5% for women included before October 2023 (Table 14). Patients who did not participate in neuroimaging had significantly higher non-response rates (73.7%) compared to those with neuroimaging

(48.0%; mean difference = 25.7 [12.8; 38.6] %, p<0.001). More granularly, participants aged 18-30 and 30-45 without neuroimaging had significantly higher odds of non-response (OR=3.28 [1.08-10.19] and 3.35 [0.84-15.50]), suggesting that these groups may need extra assertive or *supportive* follow-up to avoid missing data.

Figure 14: Baseline and first follow-up depression scores.
Baseline IDS scores from participants included before October 2023 (i.e. have been in the study for at least 13 months) and when their *first* follow-up response arrives. Despite "assertive follow-up", only 43% respond to 6-month follow-up. IDS depression severity cut-offs to the right with remission ≤13.

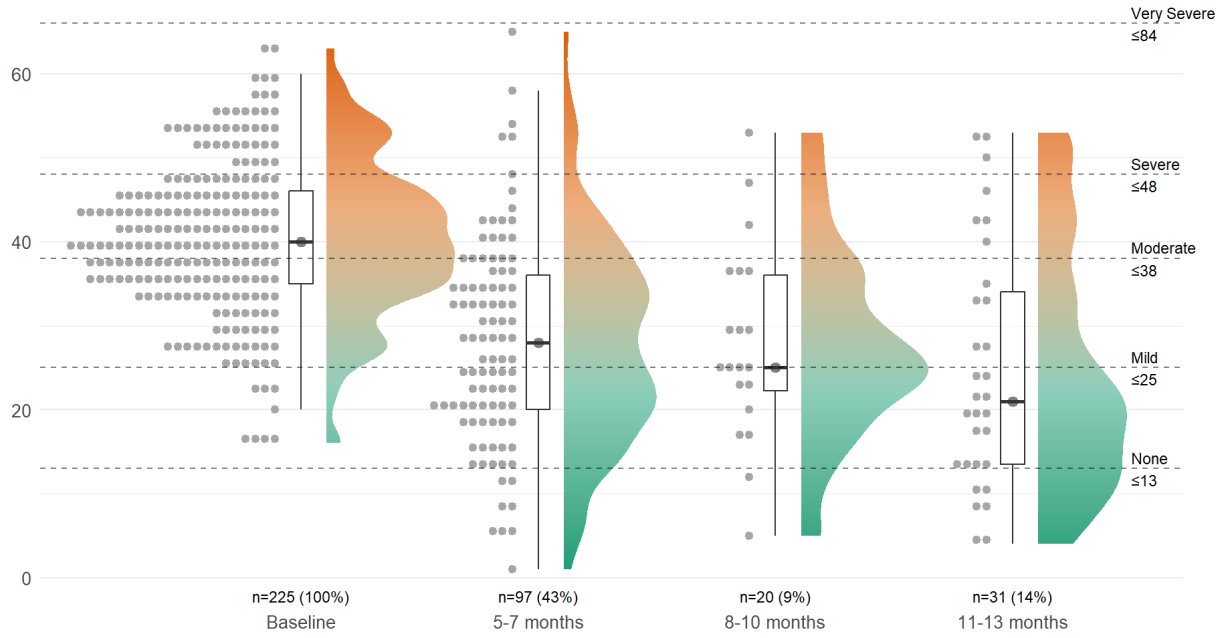


Table 14: The proportion of not responding to 6-month follow-up questionnaires.
Percent of questionnaire non-response at 6-month follow-up by subgroups of participants included before October 2023 (as in Figure 12 above). [1]Only two in this group.

Sex		Age	Without -	With neuroimaging
Female	56%	18-30	77%	45%
Female		31-45	73%	39%
Female		46-65	75%	40%
Male	62%	18-30	67%	52%
Male		31-45	83%	73%
Male		46-65	0%[1]	60%
			74%	48%

DISCUSSION

15. Study I

In the 96 patients with MDD treated with escitalopram for 8 weeks, hippocampal volume decreased in treatment responders but not non-responders. In women, decreased hippocampal volume correlated with increased 5-HT4R binding, but not in men. Surprisingly, smaller left hippocampal volume was associated with better verbal memory performance before treatment.

15.1 Change in Hippocampus Volume and Treatment Response

Contrary to preclinical evidence showing antidepressant-induced hippocampal growth, we found that if hippocampal volume did change, it appeared to decrease only in treatment responders. This challenges traditional neuroplasticity models suggesting that antidepressants increase hippocampal volume. Rather than representing atrophy, the observed volume reduction in responders may reflect synaptic refinement and circuit optimisation [139]. Recent studies have shown that SSRIs can increase synaptic density without increasing gross volume [140].

Our findings align with recent evidence suggesting that neuroplastic changes during antidepressant treatment may involve qualitative circuit-level modifications rather than quantitative volumetric changes. The 'new serotonin theory' supports this view, arguing that SSRIs trigger adaptations in neural circuitry rather than simply correcting a serotonin deficiency [26]. The reduced hippocampal volume we observed specifically in treatment responders warrants further investigation into the underlying mechanisms and suggests a potentially overlooked 'refinement through reduction' process in successful antidepressant treatment.

Our finding may be specific to the young (mean age 27), where hippocampus size is negatively correlated with verbal memory [141]. Similarly, reduced hippocampus volume in this cohort compared to age-matched healthy controls was not found either [142]. MDD may impact brain structure dynamically in different life stages [143].

15.2 Hippocampal Volume and 5-HT4R

The relationship between hippocampal volume and 5-HT4R binding showed potential sex differences. While pretreatment 5-HT4R levels were not associated with volume changes across both sexes, increased receptor binding correlated with decreased volume during treatment in females only. This aligns with known sex differences in 5-HT4R [109,144,145] and its relation to endogenous and exogenous hormone levels [52,57,145,146]. The sex-specific pattern suggests potentially distinct mechanisms of SSRI action on hippocampal plasticity between males and females. The tight coupling between 5-HT4R and volume changes in females suggests this receptor may be particularly relevant for women's treatment outcomes. However, due to our limited male sample size, we lacked power to definitively demonstrate sex interaction – a

common problem in depression research [99]. Nonetheless, these findings suggest that antidepressant effects may operate through sex-specific pathways.

15.3 Left Hippocampus Volume and Verbal Memory

The negative correlation between left hippocampal volume and verbal memory performance before treatment appears counterintuitive but aligns with previous findings in young adults [141]This relationship differs markedly from that of older adults, where a larger volume likely indicates better tissue preservation.

Despite larger left hippocampal volume being associated with poorer verbal memory at baseline, memory function improved during treatment without corresponding volume increases. This dissociation between structural and functional changes suggests memory enhancement can occur independently of macroscopic volumetric changes [147]. The improvements may instead reflect changes in hippocampal subregions more closely linked to verbal memory [148–150], that our whole-hippocampus approach could not detect. The observed improvement in verbal memory alongside volume reduction in treatment responders further supports that antidepressant effects may involve circuit refinement rather than growth. This aligns with theories stressing synaptic efficiency over size increases [76,139,151].

15.4 Methodological Constraints

Our study lacks a placebo control, making it difficult to distinguish treatment effects from natural course. The relatively young female sample and exclusion of SSRI non-responders limit generalizability and our ability to fully characterise sex differences.

15.5 Summary and Perspectives

Contrary to the idea that SSRIs increase the reduced hippocampus volumes observed in depression, we observed a mean decrease in the patients who responded to the SSRI. Thus, antidepressant efficacy may depend more on the quality of neural circuits rather than size. The sex-specific 5-HT4R relationship adds to the growing recognition of sex differences in both depression pathophysiology and treatment response. These insights on the interplay between serotonergic signalling and clinical improvement may help the development of more effective and targeted depression treatments.

16. Study II

EEG is a cost-effective and clinically scalable tool [152]. NP1 and BDD use a 256-channel setup, which enables complex analysis, but for most cases, a simpler setup like the International 10-20 system, e.g. used in iSPOT-D or slightly higher resolution variants like the 10-10 and 10-5 systems are adequate. However, the clinical implementation of EEG biomarkers is limited by the underreporting of negative findings and a lack of out-of-sample validation [95].

16.1 EEG Abnormality and Treatment Response

The iSPOT-D data suggests that abnormal EEG activity could predict poor treatment response to escitalopram and a good response to Sertralin in MDD [96]. Arns and Vinne et al. argued that sertraline uniquely normalises abnormal EEG activity supported by limited EEG follow-up (n=57; 19 on Sertralin) in iSPOT-D [153]. Early case reports raised concerns about antidepressants having *pro-convulsive* effects [154]. However, SSRIs/SNRIs generally have anticonvulsant properties through reduced glutamate release [155,156].

Our study failed to support the iSPOT-D findings that abnormal EEG activity was associated with poor escitalopram treatment response [96]. Here, we supplemented our published analysis that included Wicket spikes [135], with an analysis ignoring them to most closely replicate Arns et al. In both analyses, we did not find an association with escitalopram treatment response.

16.2 EEG Abnormality and Mood and Cognition

While depression severity was comparable across groups, individuals with EEG abnormalities experienced greater emotional disturbance and lower verbal memory performance than those without abnormalities. The latter aligns with the abnormalities primarily being detected in frontotemporal areas. Yet, current literature presents mixed evidence regarding the impact of epileptiform activity on cognition. While acute disturbances are well-documented [157], the long-term cognitive effects remain debated [158], and the effect on emotional regulation is understudied.

The observed memory deficits may explain the increased emotional disturbance, given the established connections between cognitive function and mood regulation [159]. The simple explanation of this connection may be that poorer verbal memory leads to poorer everyday function, more misunderstandings, and, in turn, more feelings of frustration and anger. This is supported by the positive relation between poor verbal memory and mood disturbance with no interaction with EEG abnormality.

16.3 Methodological Constraints

While our study conducted longer EEG recordings (six compared to two minutes), which, combined with higher channel density, may have enabled more reliable detection of EEG abnormalities than iSPOT-D [96], the rarity of EEG abnormalities limits statistical power.

16.4 Summary and Perspectives

While EEG abnormalities may be linked to poorer cognitive and emotional functioning in depression, their utility as a biomarker guiding antidepressant selection requires further rigorous evaluation before implementation in clinical practice.

Although we did not establish a link between abnormal EEG and treatment response, the association with verbal memory warrants further investigation, particularly since cognitive impairment predicts poorer psychotherapy outcomes [160]. This could be more thoroughly explored in the larger BDD cohort [91], which offers an opportunity to examine connections between EEG markers, cognition, and response to both pharmacological and psychological interventions. Furthermore, the link between epileptiform activity and mood and cognitive disturbances and the bidirectional relationship between MDD and epilepsy can be further investigated with the concurrent BrainDrugs-Epilepsy cohort study [123], which uses overlapping assessments to assess and also shares a few participants.

While we wait for BDD, the search for relevant EEG biomarkers based on NP1 continues. The inconsistent findings regarding the utility of EEG abnormalities as a predictor of treatment response echo our recent work on the Loudness Dependent Auditory Evoked Potentials (LDAEP) and its relationship to suicidality in depression. Our recent meta-analysis and replication study using NP1 data found no associations between LDAEP and suicidality [161], underscoring the need for replication and validation before clinical use and to limit research waste [162].

Further, we are currently investigating the potential interaction between hormonal contraceptive use and the predictive value of common EEG biomarkers of treatment response, which has not been investigated despite the common use of hormonal contraceptives and their link to depression [163], and some EEG biomarkers, such as Alpha Peak Frequency, are sex-specific [164].

17. Study III

In this study, using the 26 men from the NeuroPharm study, we examined associations between pretreatment plasma sex hormone levels and treatment response, sexual treatment side effects, and depressive symptomatology.

17.1 Sex Hormones and SSRI Treatment Response

Pretreatment estradiol and testosterone levels were not associated with SSRI treatment response at week eight. However, a trend emerged at week 12 where non-responders had lower testosterone levels, suggesting a potential hormonal involvement in treatment resistance. This aligns with evidence that testosterone has antidepressant-like effects [165], though this relationship is possibly age-dependent. The lack of clear associations between sex hormones and SSRI response in our (small) sample of (mostly young) men is in contrast with women. In women, there is a remarkable SSRI efficacy in Premenstrual dysphoric disorder (PMDD) [166,167], where hormonal fluctuations drive the depressive symptomatology [168]. Furthermore, the suggested lower SSRI efficacy in postmenopausal women [169], and their benefit of low-dose estradiol augmentation [170,171]. In summary, our findings suggest potential sex-dependent hormonal influences on SSRI treatment response, but further research is needed to clarify this.

17.2 Sex Hormones and SSRI Sexual Side Effects

Low sex hormone levels were associated with increased sexual side effects. This supports previous observations of lower sex hormones in depressed men experiencing SSRI sexual side effects compared to those without such effects [172]. Notably, estradiol emerged as a strong predictor for erectile and ejaculatory dysfunction. This fits with SSRIs increasing central serotonin levels leading to inhibition of desire, ejaculation, and orgasm, primarily by $5HT_{2A}$ receptor agonism [173], which cortical receptor levels are associated with plasma estradiol levels in men [55]. Nonetheless, it may also reflect estradiol's role in erectile function through abundant estrogen α and β receptors in both the brain and genital tract, particularly the epididymal cauda [174,175]. Which highlights estradiol’s role in regulating contractility during ejaculation [176–178].

17.3 Testosterone and Vegetative Symptoms

Contrary to previous literature associating low testosterone with depression in men [179], we found testosterone levels *positively* correlated with depression severity. This was driven by vegetative symptoms - weight loss, gastrointestinal symptoms, and insomnia. Rather than indicating a causal relationship, this likely reflects the metabolic consequences of depression, as weight loss can increase testosterone levels [180]. This interpretation is supported by findings that androgen levels correlate with weight loss symptoms in depression [181]. The relationship between sex hormones

and depression appears age-dependent, with associations between low testosterone and depression primarily emerging in older populations [182].

Our findings contribute to understanding depression subtypes. Patients with atypical features show different hormonal patterns compared to those with melancholic features [183]. While hormonal differences between subtypes may reflect downstream consequences of the underlying pathophysiology rather than causal mechanisms, addressing these hormonal disruptions - through either direct intervention or successful treatment of core symptoms - could potentially improve both depressive symptoms and treatment side effects [165,184,185].

17.4 Methodological Constraints

Our study is based on a relatively small sample of young men, limiting generalizability to older populations where age-related hormonal changes may influence outcomes. Without a placebo, we cannot definitively attribute the effects and side effects to treatment versus natural symptom fluctuation or nocobo. By excluding individuals with previous SSRI non-response, our findings may not generalise to treatment-resistant populations.

17.5 Summary

The association between low pretreatment sex hormone levels and SSRI-induced sexual side effects suggests potential benefits of hormonal assessment before treatment initiation, even in young men. This could be particularly relevant given that sexual side effects significantly impact treatment adherence [186], and men tend to report these effects more frequently than women [47].

While it is premature to guide treatment based on hormone levels alone, our findings suggest two pragmatic approaches: First, sex hormone levels might help identify patients at higher risk for SSRI-related sexual dysfunction, enabling proactive management strategies. Second, hormone augmentation or lifestyle interventions targeting weight management and exercise could be valuable therapeutic adjuncts, as testosterone improves SSRI-induced sexual dysfunction and mood in men [184], and weight loss to improve sexual function [187].

These observations warrant validation in larger cohorts but point toward the potential of hormone levels in optimising treatment outcomes and minimising side effects.

18. Study IV

The BrainDrugs-Depression cohort is well underway and includes approximately 10% of the treatment population it aims to study. Nearing the halfway point, it is worth investigating the status of recruitment and follow-up to identify potential biases and areas of improvement to maximise representation and minimise missing data.

18.1 Status of Recruitment and Demographics

We observed a slight overrepresentation of young women (18-25 years) in the cohort during 2021-2024 compared to the treatment population data from 2019-2021. The incidence of depression is increasing particularly among young adults in Demark [15]. However, the COVID-19 pandemic coincided with the initial cohort recruitment, representing a period of increased psychological distress in the Danish population [19], particularly among young women [188]. Therefore, the overrepresentation may reflect a shift in patient demographics rather than recruitment bias. However, this could also stem from geographical factors, as participants living closer to the research site may be more likely to participate.

Rather than speculate on this, we plan to conduct a formal non-participant analysis comparing non-participants and declined participants with the cohort on demographics and electronic healthcare measures (e.g. $HAMD_{17}$) using anonymised aggregate data from the clinics and follow the whole treatment population in registries. This will enable us to quantify potential selection bias and adjust our findings. To address these potential biases statistically, we are considering using inverse probability weighting to adjust for demographic differences between our cohort and the treatment population [189].

A possible demographic change ahead is among young adults (<25), the current largest age group in BDD (Figure 13). Guidelines from 2015 mandated that psychiatrists rather than GPs should prescribe SSRIs for this age group [190]. This means they could enter treatment earlier and more frequently than older groups. However, recognising the need to improve treatment accessibility for adolescents, the restriction has recently been reversed [191]. Therefore, we may see a change in demographics, with GPs initiating SSRIs before the young patients arrive at the clinics and the cohort.[16]

The higher active antidepressant-use in the cohort (39%) compared to the 2019-2021 treatment population (30%) may represent the increased psychotropic drug use during the COVID-19 pandemic [192] or that we measured some participants after starting their treatment package, and the clinicians (and not the GPs before entering the package) may have initiated the treatment. Healthcare records and temporally matched data can resolve this.

[16] We have monthly communication with the largest treatment centres and regular engagement with the central visitation unit to mitigate recruitment biases and monitor changes in treatment provider practices.

The transition ahead to ICD-11 in Denmark presents opportunities and challenges for BDD. The criteria for MDD have undergone minimal changes. While core diagnostic criteria remain largely unchanged [193], ICD-11 better recognises mixed anxiety-depression presentations and aligns more closely with DSM-5 through the inclusion of bipolar II disorder and PMDD. This could enhance international research compatibility. Premenstrual exacerbation, which has only received limited attention in MDD [194], is screened for in the cohort.[17] However, the implementation of PMDD may take time to be reliably captured in registries.[18]

Irritability, although poorly defined in the DSM [195], can substitute depressed mood as a core symptom of depression, yet exclusively in youth. ICD-11 acknowledges the research showing irritability as central to male depression presentations, particularly in young men [196], and allows both irritability and emotional emptiness to qualify as core affective symptoms for diagnosing depression, regardless of age [197]. Currently, only 28% of patients in the treatment package are men (Figure 13, page 39). In group therapy, men aged 45+ are outnumbered 5:1 by women aged 18-35. Expanded criteria may improve depression recognition in men, but whether standard treatments are equally effective for this minority is an open question.

Lastly, the introduction of Transcranial Magnetic Stimulation in clinical practice is an interesting new modality used exclusively for MDD in Denmark [198].

18.2 How Should Treatment Response and Remission be Defined?

While treatment response (≥50% symptom reduction) and remission (score below threshold) are standard metrics, their arbitrary nature poorly captures individual patient trajectories. A 48% improvement becomes a "non-response" despite both potentially representing meaningful clinical change [199]. Our measurement tools further complicate this picture [200]. The $HAMD_{17}$ emphasises somatic symptoms unidirectionally, while the Beck Depression Inventory probes cognitive features like hopelessness and worthlessness [201]. Each scale tells a different story of recovery.[19] Others emphasise broader markers, including quality of life and occupational and social functioning [202]. Cognitive function, while often the last domain to improve [7,8] and one of the most important outcomes for patients [7,8], may better indicate genuine recovery than mood symptoms alone, as patients can report improved mood while still struggling with concentration and decision-making that impair daily functioning. This complexity has led to calls for composite outcome measures that capture recovery's multidimensional nature [203,204].

BDD addresses these limitations through multiple complementary measures. We selected the comprehensive IDS to capture symptom heterogeneity while maintaining

[17] Using a modified version of the premenstrual symptoms screening tool (PSST) [213].
[18] It may take several years before we can trust the PMDD diagnosis code in the registries, as there has been push-back to the diagnosis [168]. Why Danish women with PMDD have been awaiting the inclusion into the ICD as a recognition of their distress [214], and are still waiting for the Danish implementation of ICD-11.
[19] For further reading, see the recent struggles by Weiss et al. [215].

correlation with the $HAMD_{17}$ used in Danish clinics [205]. Unlike the $HAMD_{17}$, the IDS assesses bidirectional symptoms like insomnia versus hypersomnia. Beyond symptom measures, we assess psychosocial functioning (Table 4, page 24), and recent funding enables adding online follow-up cognitive testing as well. [20] Registry data provides long-term tracking of employment, education and other real-world outcomes. While analytically complex, this multimodal approach may better characterise the heterogeneous paths to recovery.

18.3 Current Follow-up Response Rates

Lastly, the current one-year response rate of 66% in the cohort is comparable to other large studies like STAR*D's 67% [27] and iSPOT-D's 70% [206], but lower than shorter and more intensive studies like NeuroPharm-1's 85%. While statistical methods can handle irregular response timing, access to clinical data from medical records provides additional outcome measures (e.g. $HAMD_{17}$, WHO-5, mSDS, and SCL-10) for both participants and non-participants. Thus, we have a reasonable basis for modelling missing data. Our planned online cognitive testing may also incentivise participants. Lastly, we are considering shorting or omitting questionnaires and more formalised patient feedback and involvement in how participant contact and assessments are organised [207].

18.4 Summary and Perspectives

The BDD cohort is off to a promising start despite the challenges of launching during the COVID-19 pandemic and initially limited staff.[21] There is room for improvement to ensure representative sampling and minimal attrition. The deep phenotyping approach provides a solid foundation for investigating predictors and mechanisms of benefit and for whom.

Looking ahead, the focus remains on optimising data collection while being mindful of participant burden, ultimately working toward a better understanding of treatment outcomes in depression.

[20] Cognition and Brain Biomarkers and Determinants of Treatment Trajectory and Outcomes in First-Episode Depression, €600.000 from the Independent Research Fund Denmark.

[21] As the cohort size has increased, so have the number of students associated with the project. One has relapsed and will return as a PhD student. Others I look forward to meeting again in the clinic.

THE ROAD AHEAD

The three NP-1 studies represent focused mechanistic investigations in a selected group of patients seeking medication and willing to undergo extensive neuroimaging [39]. While informative about SSRI mechanisms, this controlled approach captures only a narrow subset of depression. Despite this, these studies emphasise that depression and its treatment involve complex and possibly sex-specific mechanisms that cannot be reduced to simple biomarker-symptom or biomarker-outcome relations. They highlight the importance of considering multiple interacting systems when investigating treatment mechanisms and attempting to predict outcomes.

19. Treatment Heterogeneity in Natural Settings

BDD moves beyond controlled mechanistic studies and clinical trials to capture depression's real-world heterogeneity. By observing approximately 10% of first-episode patients in the Copenhagen region, BDD enables the investigation of naturalistic treatment paths and their relationship to social, cognitive, and neurobiological factors. Unlike previous depression cohorts (i.e. STAR*D and iSPOT-D) that focused primarily on medication [27,206], BDD examines the interplay between psychotherapy, the backbone of current treatment packages, and pharmacological interventions. This allows for investigating questions like the effect of comorbidities and optimal treatment sequencing and identifying patient profiles likely to benefit from psychotherapy alone versus combined treatment.

19.1 Advanced Analytics for Complex Data

BDD was designed with complexity in mind, embracing heterogeneity through a multimodal deep phenotyping approach that observes treatment outcomes in their natural clinical context. While resource-intensive, the deep phenotyping approach provides a foundation for developing clinically implementable predictive markers. The current consideration is to complement standard statistical approaches with machine learning methods more suited for high-dimensional multi-omic data like HYDRA and SLIDE [208,209]. We aim to predict outcomes and understand underlying causal mechanisms. SLIDE's ability to identify interpretable latent factors may be particularly useful for understanding how different markers interact to influence treatment effects. This addresses a fundamental limitation of "black box" machine learning approaches while maintaining predictive power.

19.2 Novel Research Applications

The BDD cohort design enables several novel investigations:

- We can examine how treatment preferences and paths evolve, providing insight into real-world effectiveness rather than controlled trial efficacy.

- The comprehensive phenotyping allows the identification of minimal marker sets needed for accurate predictions, potentially enabling scalable and cost-effective clinical implementation in different settings.
- Registry linkage enables tracking both short-term clinical changes and long-term trajectories across future decades, a unique advantage of conducting research within the Danish healthcare system.

The cohort can serve as a platform for targeted mechanistic studies. The extensive phenotyping data can aid in selecting patients for upcoming trials and facilitate comparisons between novel interventions and standard care. Studies on experimental treatments for MDD can recruit BDD participants, utilising both their thorough baseline characterisation and the presence of matched controls receiving standard care. This approach maximises the scientific value of participants' contributions - an ethical consideration given the burden of extensive phenotyping during their suffering. Why data sharing with other researchers and consortiums is also a goal.

19.3 Long-term Potential and Scientific Value

The BDD cohort represents an important step towards precision psychiatry by bridging mechanistic insights from controlled studies like NP1 [39] and epidemiological studies with moderate phenotyping like NESDA [210] with real-world treatment patterns and long-term outcomes. The ability to follow patients through the Danish registries while enabling a uniquely valuable resource for understanding immediate treatment effects and lifelong trajectories.

APPENDIX

[A] Study I

Jensen KHR, Dam VH, Köhler-Forsberg K, Ozenne B, Stenbæk DS, Ganz M, Fisher PM, Frokjaer VG, Knudsen GM, Jørgensen MB (2024) Changes in Hippocampal Volume, 5-HT4 receptor binding, and Verbal Memory Over the Course of Antidepressant Treatment in Major Depressive Disorder. *Journal of Psychiatric Research*

[B] Study II

Jensen KHR, Aarestrup MR, Larsen SV, Köhler-Forsberg K, Knudsen GM, Jørgensen MB, Frokjaer VG (2024) Psychoneuroendocrine profiles of unmedicated men with major depressive disorder and associations to treatment effects and sexual side-effects. *Neuroscience Applied*

[C] Study III

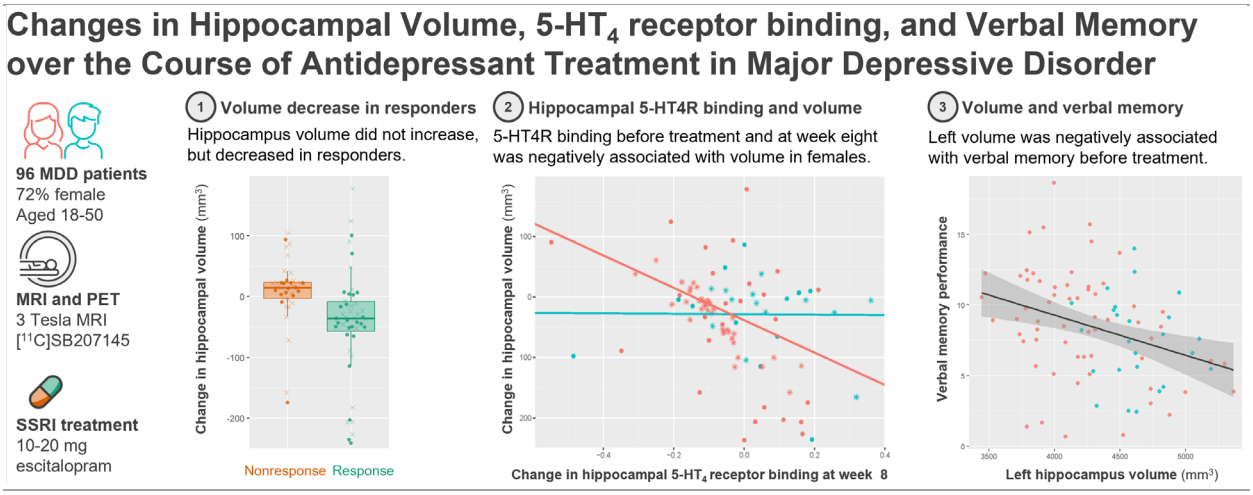
Jensen KHR, Reveles MR, Larsen SV, Köhler-Forsberg K, Knudsen GM, Jørgensen MB, Frokjaer VG (2024) EEG abnormalities are not associated with poor antidepressant treatment outcome - A NeuroPharm study. *European Neuropsychopharmacology*

[D] Study IV

Jensen KHR, Dam VH, Ganz M, Fisher PM, Ip CT, Sankar A, Marstrand-Joergensen MR, Ozenne B, Osler M, Penninx BWJH, Pinborg LH, Frokjaer VG, Knudsen GM, Jørgensen MB (2023) Deep phenotyping towards precision psychiatry of first-episode depression — the Brain Drugs-Depression cohort. *BMC Psychiatry*

Study I

Graphical abstract



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Changes in hippocampal volume, 5-HT$_4$ receptor binding, and verbal memory over the course of antidepressant treatment in major depressive disorder

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ABSTRACT

Serotonin reuptake inhibitors have been reported to increase hippocampal volume and improve memory function in patients with Major depressive disorder (MDD). The postsynaptic 5-HT4 receptor (5-HT4R) is involved in hippocampal development, familial risk for depression and depressive pathology. In an open-label trial with 91 patients (72% female, mean 27.2 years) with MDD, we investigated the relation between changes in hippocampal volume, 5-HT4R, and verbal memory during 12 weeks treatment with 10–20 mg escitalopram. Depression severity, verbal memory, MRI-determined hippocampus volume and PET-determined 5-HT4R were measured pretreatment. Forty-three patients were rescanned at week 8. $HAMD_{17}$ was reassessed at week 8 and together with verbal memory at week 12. We used mixed-effects models and linear regressions. We estimated a 27 mm^3 ($p = 0.086$) reduction in mean hippocampus volume over the course of eight weeks. In patients clinically responding to treatment, we estimated a 45 mm^3 reduction ($p = 0.019$), 8 mm^3 increase in non-responders ($p = 0.78$), and a 52 mm^3 group difference ($p = 0.12$). Hippocampal 5-HT4 receptor binding before treatment and at week eight was negatively associated with hippocampal volume in females, regardless of treatment response (p-values$\leq$0.006). However, no clear evidence for an association in males or sex interaction could be established (p-values$\geq$0.16). Although the hippocampus volume did not increase with treatment, we found a decrease in clinically responsive patients. Our findings suggest an association between 5-HT4R signalling and changes in hippocampal volume in females with MDD during antidepressant treatment, highlighting the need for further investigation into the role of serotonergic mechanisms in hippocampal plasticity.

1. Introduction

The hippocampus plays a critical role in emotion processing and memory functioning and is richly innervated by serotonergic fibres (E Dale et al., 2016). In Major Depressive Disorder (MDD), studies have shown hippocampus volume reduction in both human (TC Ho et al., 2022; L Schmaal et al., 2016) and animal studies (SL Willard et al., 2009), as well as impaired memory (Dam et al., 2020; McIntyre et al., 2013), that may persist even during periods of symptom remission (McIntyre et al., 2013; Semkovska et al., 2019). Preclinical studies in rodents and non-human primates indicate that antidepressants targeting the serotonin system, such as selective serotonin reuptake inhibitors (SSRIs), can enhance hippocampal neuroplasticity and neurogenesis (M Benekareddy et al., 2008; Chen et al., 2010; Sairanen et al., 2007; Santarelli et al., 2003), as well as increase hippocampus volume (Chen et al., 2010; Sairanen et al., 2007; Willard et al., 2015). Recent research

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has demonstrated that SSRIs can increase hippocampal synaptic density in healthy adults (Johansen et al., 2023). However, human neuroimaging studies of SSRI effects on hippocampal volume in depression have yielded mixed results (Arnone et al., 2013; Enneking et al., 2020; Godlewska et al., 2014; Schermuly et al., 2011; Vakili et al., 2000; Vythilingam et al., 2004), possibly due to heterogeneity in serotonergic dysfunction among MDD patients (Ip et al., 2023; Köhler-Forsberg et al., 2021).

The fact that not all patients respond to SSRI treatment (Rush et al., 2006) may be due to the possibility that not all patients have a 'serotonergic depression' (Ip et al., 2023; Köhler-Forsberg et al., 2021). This could help explain the inconsistent findings in human studies on the effects of SSRIs on hippocampal volume changes and improvements in depressive symptoms.

The postsynaptic serotonin-4 receptor (5-HT4R) is a key regulator of hippocampal plasticity and memory (L Agrawal et al., 2019; Hagena and Manahan-Vaughan, 2017). In rodents, 5-HT4R agonists promote hippocampal neurogenesis (Mendez-David et al., 2014), enhance memory (Lamirault and Simon, 2001), and exert rapid anxiolytic and antidepressant-like effects (Mendez-David et al., 2014; Nogovitsyn et al., 2020; Samuels et al., 2016; Segi-Nishida, 2017a). In humans, 5-HT4R levels in the hippocampus are reduced and correlate with memory deficits in MDD (Dam et al., 2024; Köhler-Forsberg et al., 2023). These receptors are also implicated in familial risk for depression (K Madsen et al., 2015), and 5-HT4 receptor agonism has been shown to improve memory (Murphy et al., 2019).

Interestingly, sub-chronic SSRI treatment downregulates 5-HT4R both in the rodent and human hippocampus (ME Haahr et al., 2014; KA Jennings et al., 2012; CL Licht et al., 2009; R Vidal et al., 2009), likely reflecting desensitisation to elevated serotonin levels. Evidence suggests that SSRI-induced synaptic plasticity may be a key mechanism of SSRI action (Castrén, 2013; Johansen et al., 2023), which could potentially be enhanced by 5-HT4R agonism.

We hypothesise that the ability of SSRIs to induce hippocampal volume changes depends on the capacity to mount an adaptive serotonergic response to the intervention, and we propose that changes may only occur in clinical responders and that variations in pretreatment 5-HT4R levels influence the magnitude of treatment-related changes in hippocampal volume.

To test these hypotheses, we used data from the NeuroPharm-1 depression trial (Köhler-Forsberg et al., 2020, ; Köhler-Forsberg et al., 2023) to examine whether hippocampus volume increases following antidepressant treatment and whether changes are associated with clinical response and hippocampal 5-HT4R levels. Additionally, we explored whether hippocampal volume changes correlated with verbal memory function.

2. Methods

Patient data was available from the NeuroPharm-1 study, a longitudinal, open-label multimodal neuroimaging clinical trial investigating potential biomarkers for antidepressant treatment (clinicaltrail.gov: NCT02869035). The study was approved by the Ethics committee for the Capital Region of Copenhagen (protocol: H-15017713). For a detailed description of the full protocol, see (Köhler-Forsberg et al., 2020).

2.1. Subjects and study design

One hundred non-psychotic, antidepressant-free patients with moderate to severe depressive episodes (Hamilton Depression Rating Scale-17 ($HAMD_{17}$) >17) were initially included in the trial. Patients were eligible for inclusion if they were 18–64 years old; their depressive episode had lasted less than two years; they had been free of antidepressant medication for >2 months; had not previously exhibited non-response to SSRIs; and had not undergone more than one antidepressant treatment attempt in the current depressive episode. Patients were recruited through their primary care center or a central referral site at the Mental Health Services of the Capital Region of Copenhagen. MDD diagnosis was confirmed by a certified psychiatrist and corroborated by a Mini-International Neuropsychiatric Interview (MINI) version 6.

Patients completed a comprehensive assessment program, including structural magnetic resonance imaging (MRI), positron emission tomography (PET) neuroimaging of 5-HT4R brain levels, and cognitive testing of verbal memory function. Baseline MRI, PET and cognitive data were available for 96, 90 and 87 patients. After 8 weeks of antidepressant treatment, a subset was rescanned, with 43 patients completing MRI and 41 patients completing PET scans. Eighty-seven patients underwent cognitive testing after 12 weeks of antidepressant treatment. Fifteen patients were lost to follow-up (see Fig. S1 for a CONSORT diagram).

2.2. Treatment and adherence

After completing the neuroimaging and cognitive investigation program, patients started antidepressant treatment with the SSRI escitalopram, individually adjusted to 10–20 mg daily depending on response and side effects. Following standard clinical practice, patients experiencing intolerable side effects or <25% reduction in $HAMD_6$ from baseline at week 4 were offered to switch to the serotonin-norepinephrine reuptake inhibitor, duloxetine (n = 16), individually adjusted (30–120 mg daily). Serum concentrations of escitalopram and duloxetine were determined at week 8; quantification was performed at the Epilepsy Hospital, Filadelfia, Dianalund, Denmark, using a routine UPLC-MS/MS method developed in-house. Assessment of treatment adherence was based on self-reported adherence and, ultimately, serum concentrations.

2.3. Clinical outcome and treatment response

Depression symptom severity was assessed with the $HAMD_{17}$ interview at baseline and weeks 4, 8, and 12. Regular co-ratings between study investigators were implemented to ensure inter-rater reliability. As the 6-item subscale ($HAMD_6$) of $HAMD_{17}$ has been shown to capture core depressive symptoms and is more sensitive to antidepressant treatment response (SD Østergaard et al., 2016), this was used as the primary clinical outcome at all follow-up time points. Relative change in $HAMD_6$ ($\Delta HAMD_6$ in %) was calculated as the change in $HAMD_6$ from baseline to follow-up at week 8 or 12 divided by baseline $HAMD_6$. Treatment response was defined as ≥50% $HAMD_6$-reduction from pretreatment.

2.4. MRI and PET acquisition

MRI and PET acquisition, pre-processing, and PET quantification were performed as previously described (Köhler-Forsberg et al., 2023). Briefly, all patients were scanned with a Siemens 3-T Prisma MRI scanner. High-resolution structural T1-weighted magnetization-prepared rapid gradient-echo (1900 ms TR, 2.58 ms TE, 900 ms TI, 9° flip, 224 slices, 0.9 x 0.9 × 0.9 mm voxels) and T2-weighted spin echo (3200 ms TR, 408 ms TE, 120° flip, 208 slices, 0.9 x 0.9 × 0.9 mm voxels) MR images were acquired.

PET images (256 × 256 × 207 voxels; 1.22 × 1.22 × 1.22 mm) were acquired during a 120-min dynamic scan using a high-resolution research tomography PET scanner (CTI/Siemens, Knoxville, TN, USA) after intravenous injection of the [^{11}C]SB-207145 radiotracer. PET scans were motion-corrected using the AIR 5.2.5 (RP Woods et al., 1992). 3D T1-weighted MR images were co-registered to PET images to obtain structural information, and PVElab was used to extract radioactivity counts from regions of interest automatically delineated on the individuals' MRI (C Svarer et al., 2005). The mean tissue time activity for hemisphere-averaged grey matter volumes was used for kinetic modelling with the Simplified Reference Tissue Model with the cerebellum

(excluding vermis) as a reference region (L Marner et al., 2009). The calculated non-displaceable binding potential (BP_{ND}) was the outcome measure for the 5-HT4R binding. PET characteristics are shown in Supplementary Table S1.

2.5. MRI volumetry of the hippocampus

Registration and segmentation of the hippocampus were performed with FreeSurfer version 7.1.0 (Fischl, 2020). The MR images from each subject were processed with the default *recon-all* script. The segmentation quality was visually inspected using FreeSurfer, and R. Intracranial volume was quantified using SPM12 (v7219, https://www.fil.ion.ucl.ac.uk/spm) in Matlab (R2019a, MathWorks, Natick, MA, USA) by segmenting the grey matter, white matter, and cerebrospinal fluid and summing their combined volume (Malone et al., 2015).

2.6. Verbal memory

Verbal memory was assessed with the Verbal Affective Memory Task 26 words (VAMT-26) (Dam et al., 2020; LV Hjordt et al., 2020; CG Jensen et al., 2015). Participants were shown a list of 26 words (10 positive, 10 negative and 6 neutral) on a screen and were asked to recite as many words as they could remember from the list across five trials (immediate recall) and again after being shown the interference list (short-term recall) and lastly after 30 min (long-term recall). Total word recall, calculated as the average number of words (positive, negative, and neutral) recalled across immediate, short-term, and long-term recall in the VAMT-26, was used to assess overall verbal memory function (Dam et al., 2020).

2.7. Statistical analysis

Bilateral hippocampal volume was used in all analyses due to the high correlation in volume between hemispheres (Pearson's r = 0.88), except for models comparing the change in volume between the hemispheres and the association with VAMT-26.

2.7.1. Change in hippocampus volume

We estimated the average change in hippocampus volume ($E[\Delta V_{hippo}]$) between week 0 and week 8 using a linear mixed-effects model. The outcome variable was hippocampus volume, while time, baseline $HAMD_{17}$, hemisphere, age, and sex were fixed effects; subject and hemisphere nested within subject were used as random effects. The model will be denoted LMM1.

2.7.2. Change in hippocampus volume and depression severity

We compared average changes in bilateral hippocampus volume between treatment responders and non-responders at week 8 using a linear mixed model corresponding to LMM1 with one additional fixed effect for the interaction between treatment response and time (no main effect of treatment response as we assumed pre-treatment volume to be independent of treatment response).

2.7.3. Pretreatment hippocampus volume and depression severity

We performed a linear regression of the relationship between pretreatment mean hippocampus volume and %-change in $HAMD_6$ from pretreatment at weeks 8 and 12 adjusted for intracranial volume, age and sex.

2.7.4. Hippocampus volume and 5-HT4R binding

We performed a linear mixed-effects model of the relationship between pretreatment 5HT4R binding in the hippocampus and change in hippocampus volume over the course of treatment. In this model, the outcome variable was hippocampus volume. Time, pretreatment total hippocampal 5HT4R binding, and PET tracer injected mass, age, and sex were fixed effects, and subject was a random effect. We also performed a linear mixed-effects model of the relationship between change in mean hippocampal 5HT4R binding and change in hippocampal volume and a sex interaction. In this model, the outcome variable was the mean hippocampus volume of left and right. Time, mean of left and right hippocampal 5HT4R binding and PET tracer mass, age, and sex were fixed effects, and subject was a random effect.

2.7.5. Left hippocampus volume and verbal memory

Verbal memory function is primarily lateralised to the left hippocampus (PM Aslaksen et al., 2018; A Ezzati et al., 2016; JA Witt et al., 2019). We, therefore, performed a linear regression of the relationship between pretreatment left hippocampus volume and pretreatment VAMT-26 adjusted for intracranial volume, age and sex.

Finally, we performed a linear mixed-effects model of the relationship between the change in left hippocampal volume at week 8 and the change in VAMT-26 at week 12. In this model, the outcome variable was VAMT-26 performance. Time, left hippocampus volume, age, and sex were fixed effects, and subject was a random effect.

The linear mixed-effects models were estimated using restricted maximum likelihood (REML) estimation via the lme4 (1.1–35.1) package in R (4.3.2.). Statistical inferences were based on Satterthwaite's degrees of freedom method and likelihood ratio tests. We present the estimates with 95% confidence intervals (CI) and unadjusted *p*-values. The significant level was set to 0.05. Detailed descriptions of sensitivity and exploratory analyses are provided in the Supplementary Materials.

3. Results

Ninety-six patients completed the pretreatment cognitive and structural neuroimaging assessment, of which 92 initiated treatment, and 43 were rescanned at week 8 (Table 1). Pretreatment left and right hippocampal volumes were highly correlated (Pearson correlation 0.88, $p < 0.001$). The two groups were comparable for all characteristics except HAMD, e.g. patients who were rescanned scored an average of 2.2 points higher on the $HAMD_{17}$ pretreatment than those who did not. Four patients were not adherent to treatment at week eight, of which two were rescanned (Supplementary Table S2).

3.1. Change in hippocampus volume

Based on the pretreatment volumes from all patients and follow-up volumes from the adherent rescanned patients, we estimated an average reduction of 27 mm^3 (95% confidence interval [−4 mm^3; 58 mm^3], $p = 0.086$) in hippocampus volume over the course of eight weeks of adherent medication. In a sensitivity analysis, we included

Table 1
Pretreatment characteristics
$HAMD_{17/6}$: Hamilton Depression Rating scale 17 or 6 items. VAMT-26: Total word recall from the Verbal Affective Memory Task 26. [a]Information on episode was missing for one patient, [b]Education information was missing for 20 patients, and [c]VAMT-26 was missing for nine patients. Values are mean (SD) unless *n* (%).

Pretreatment characteristics	All subjects	Not Rescanned	Rescanned
N	96	53	43
Female, n (%)	69 (72%)	41 (77%)	28 (65%)
First episode/recurrent[a]	42 (44%)/53 (56%)	23 (44%)	19 (44%)
Age (years)	27.2 (8.2)	27.6 (9.3)	26.6 (6.80)
Years of education[b]	11.6 (1.1)	11.3 (1.4)	11.8 (0.5)
Total Education score[b]	14.9 (2.1)	14.1 (2.3)	15.6 (1.7)
$HAMD_{17}$	22.9 (3.5)	24.0 (3.3)	21.7 (3.0)
$HAMD_6$	12.4 (1.7)	12.7 (1.5)	12.0 (1.7)
VAMT-26[c]	14.6 (3.6)	14.3 (3.9)	15.0 (3.1)
Initiated treatment, N	92	49	43
% treatment dropout	4%	7.5%	0%

pretreatment depression severity as a covariate, yielding similar mean volume reduction estimates (27 mm^3, $p = 0.084$). As a sensitivity analysis, we included an interaction of time and hemisphere in which there was no evidence for a difference in volume changes between hemispheres ($p = 0.75$, Supplementary Fig. S2).

3.2. Change in hippocampus volume and depression severity

We investigated changes in hippocampus volume in treatment responders and non-responders in a linear mixed-effects model (Fig. 1). We estimated a mean change of −45 mm^3 (CI: −81; −8 mm^3) in hippocampus volume in responders ($p = 0.019$) and a non-significant mean change of 8 mm^3 (CI: −46; 61 mm^3) in non-responders ($p = 0.78$). However, the estimated group difference of 52 mm^3 (CI: 13; 117 mm^3) was not significant ($p = 0.12$). For complete case plots, see Supplementary Fig. S3A and Supplementary Table S3.

Changes in mean hippocampus volume were not associated with a change in depression severity (i.e., %-change in $HAMD_6$) over eight weeks of treatment (Supplementary Fig. S3B), as evaluated with a mixed-model (β = 0.79 mm^3/%-change, CI: −0.30; 1.87, $p = 0.159$).

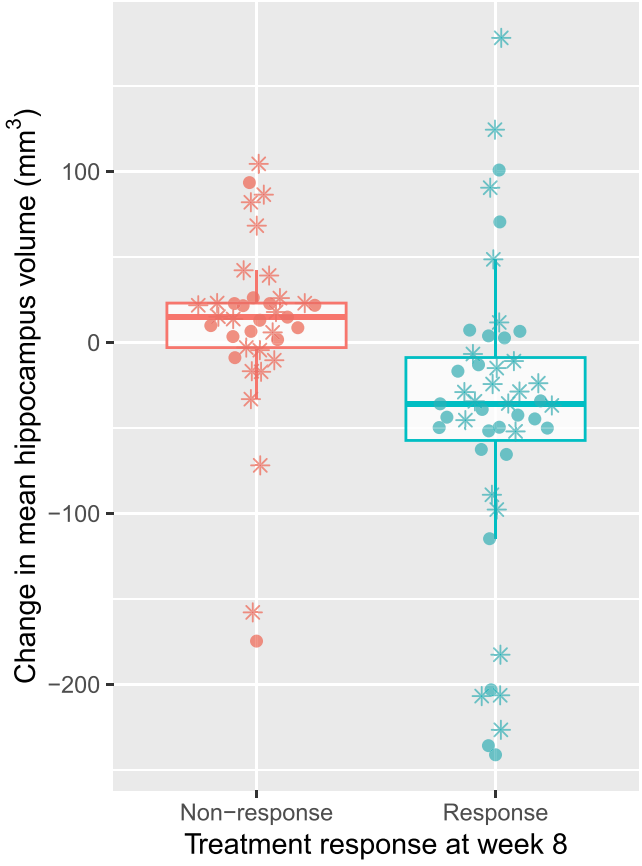

Fig. 1. Change in hippocampus volume and treatment response
We estimated a mean change of −45 mm^3 (CI: −81; −8 mm^3, $p = 0.019$) in treatment responders (i.e., ≥50% $HAMD_6$-reduction) and a mean change of 8 mm^3 (CI: −46; 61 mm^3, $p = 0.78$) in non-responders at week eight. The estimated group difference of 52 mm^3 (CI: 13; 117 mm^3) was not significant ($p = 0.12$). Dots indicate patients having both baseline and re-scan data. Stars indicate patients with only baseline data where the change was computed based on the mixed model estimate of the mean re-scan value conditional on the observed baseline value.

3.3. Pretreatment hippocampus volume and depression severity

In linear regressions, there was no (clear) evidence for pretreatment hippocampus volumes to be associated with pretreatment depression severity ($p = 0.88$) or changes in depression severity at weeks 8 or 12 of treatment ($p > 0.22$).

3.4. Hippocampus volume and 5-HT_4 receptor binding

We conducted a linear mixed-effects model to examine the relationship between change in hippocampal volume and change in hippocampal 5-HT4R binding during treatment (Fig. 2). For females, 5-HT4R binding was negatively associated with hippocampal volume both before treatment and at week eight (β = −276, CI: −459; −94, $p = 0.006$; and β = −319 CI: −508; −131, $p = 0.002$, respectively). For males, the point estimates had the opposite sign, i.e., they were positive (pretreatment: β = 110; week eight: β = 89), but no clear evidence for an association could be established (pretreatment: CI: −92; 310, $p = 0.30$; week eight CI: −116; 293, $p = 0.41$). However, there is insufficient evidence for a sex interaction (β = −264, CI: −632; 104, $p = 0.154$).

In a linear mixed-effects model, we estimated a non-significant association of pretreatment 5-HT4R binding in the hippocampus with changes in hippocampus volume (β = 136, CI: −40; 313, $p = 0.14$).

3.5. Left hippocampus volume and verbal memory

When tested with a linear regression model, we found that before treatment, the left hippocampus volume was negatively associated with VAMT-26 (Fig. 3, β = −2.84 score/1000 mm^3, CI -0.34; −5.34 score/1000 mm^3, $p = 0.026$), this remained statistically significant also when adjusting for depression severity (β = −2.76 score/1000 mm^3, CI -0.27; −5.25 score/1000mm^3 $p = 0.030$). As expected, right hippocampus volume was not associated with VAMT-26 (β = −0.001 score/cm^3, CI: 0.001; −0.004 score/cm^3, $p = 0.23$).

Meanwhile, we found no evidence that changes in left hippocampus volume from baseline to week 8 were associated with changes in VAMT-26 from baseline to week 12 in a linear mixed model (β = −0.10, CI: −1.80; 1.58, $p = 0.90$).

4. Discussion

We could not confirm that hippocampus volume increases in response to eight weeks of SSRI treatment in patients with moderate to severe depressive episodes, as the largest plausible increase was 4 mm^3. In contrast, in the sub-group analysis, we found that hippocampal volume decreased for those who clinically responded to treatment. However, we did not have enough evidence to show a group difference between responders and non-responders.

Pretreatment hippocampal 5-HT4R levels were not associated with a change in hippocampal volume over the course of treatment across sexes. Increased hippocampal 5-HT4R levels were associated with decreased volume during treatment in females but not in males. However, there was not sufficient evidence of a sex interaction. Pretreatment left hippocampus volume was negatively associated with pretreatment verbal memory performance. Yet, change in verbal memory performance was not associated with changes in left hippocampus volume.

4.1. Hippocampal remodeling during antidepressant treatment

An increase in hippocampal volume over the course of antidepressant treatment has been observed in rodents and non-human primates (Chen et al., 2010; Sairanen et al., 2007; Willard et al., 2015). Meanwhile, human studies of hippocampus volume and serotonergic antidepressants have been limited and contradicting (Enneking et al., 2020). Two small studies (15 and 24 patients) using 1.5 T MRI have observed increased hippocampal volume after 8 and 23 weeks of antidepressants

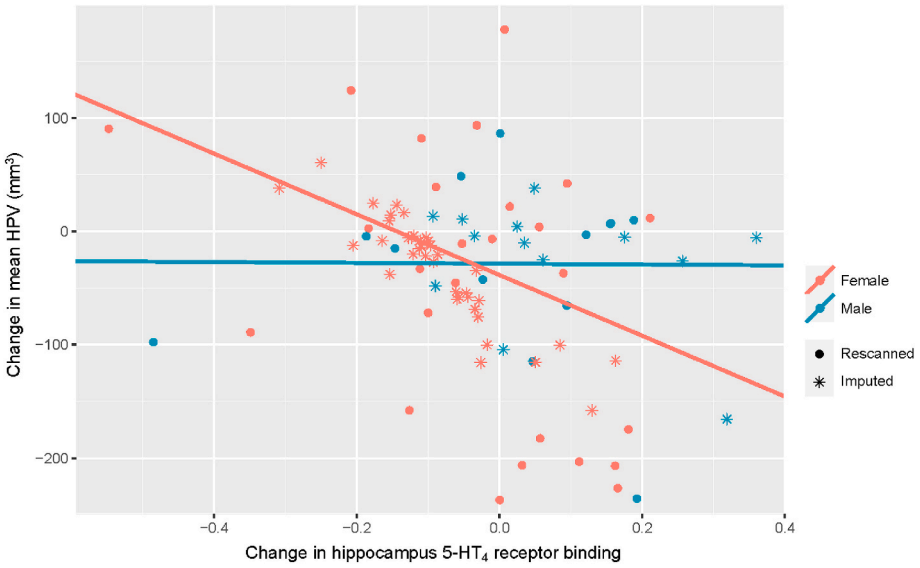

Fig. 2. Changes in hippocampal 5-HT$_4$ receptor binding and volume during treatment
Plot of change in mean hippocampus volume and changes in hippocampus 5-HT4R binding from the linear mixed model. For female patients, the relation was negative (β = −268, CI: −529; −6, p = 0.045), but near zero for men (β = −4, CI: −271; 263, p = 0.978). However, there is insufficient evidence for a sex-interaction (β = −264, CI: −632; 104, p = 0.154). Dots indicate patients having both baseline and re-scan data. Stars indicate patients with only baseline data where the change was computed based on the mixed model estimate of the mean re-scan value conditional on the observed baseline value.

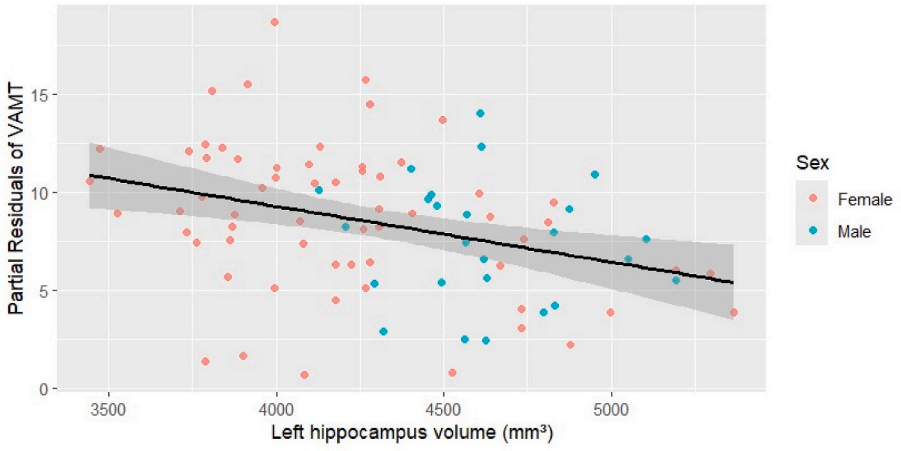

Fig. 3. Pretreatment left hippocampus volume and verbal memory performance
Partial residuals plot of male and female patients, i.e. VAMT observed value minus the age, sex and intracranial volume effects (β = −2.8 score/1000 mm^3, CI -0.3; −5.3 score/1000 mm^3, n = 87, p = 0.026).

(Arnone et al., 2013; Schermuly et al., 2011). In contrast, 11 other studies (13–70 patients) with 1.5–3 T MRI did not find changes in hippocampus volume during six weeks to a year of antidepressant treatment (Enneking et al., 2020). Our results also do not support an increase in hippocampus volume over the course of SSRI treatment; if anything, we observed a decrease in volume in patients clinically responding to SSRI treatment. However, there was no general association between the change in $HAMD_6$ and the volume change.

It is worth noting that the mean age in the aforementioned studies was in the third to fourth life decade. Noteworthy, the study closest in age and design (mean age 29.9, eight weeks of 20 mg escitalopram, and 3 T MRI) to our study showed similar, but non-significant, reductions in hippocampal volume in treatment responders (Godlewska et al., 2014). This pattern is mirrored in the frontal cortex, with early small studies showing increases, which were not replicated in more extensive and higher-resolution studies (Enneking et al., 2020).

While our observation of no increase in hippocampus volume, or even a possible reduction in response to SSRI treatment, challenges the notion that antidepressants promote hippocampal growth, the underlying mechanisms remain unclear. Changes in hippocampal volume could be attributed to various factors, including alterations in neuronal or glial cell numbers or dendritic branching (Duman, 2004). Our study design does not allow us to distinguish between these potential mechanisms.

However, our findings do not necessarily refute the neuroplastic theory of antidepressants (Kraus et al., 2017). SSRIs may exert their therapeutic effects in young adults through more subtle neuroplastic changes that are not reflected in overall hippocampal volume, e.g. changes in synaptic density (Johansen et al., 2023). This process could enhance neural connectivity and optimise hippocampal function without increasing overall volume and may explain the pro-cognitive effects of SSRI treatment (Dam et al., 2022).

Lastly, pretreatment hippocampus volume has been proposed as a prognostic marker of antidepressant treatment, with greater volume

associated with treatment response (Enneking et al., 2020; G MacQueen and Frodl, 2011; N Nogovitsyn et al., 2020). However, in line with several other studies, we did not find evidence to support this (EA Bartlett et al., 2018; V Beliveau et al., 2022; Enneking et al., 2020; Poirot et al., 2024; Vakili et al., 2000). We also did not find an association between hippocampus volume and depression severity in the unmedicated state. This is similar to the study by J Keller et al., 2008) but in contrast to smaller studies (n < 40) with lower MRI resolution (1.5 T) and older patients where greater depression severity was associated with smaller volume (CE Bearden et al., 2009; Vakili et al., 2000). Since our patients were young, some of these differences could be partly explained by a scarring effect where reduced hippocampal volume is a consequence of repeated depressive episodes (P Videbech and Ravnkilde, 2004).

4.2. Potential sex differences in serotonin architecture and neuroplasticity

SSRIs may reactivate developmental plasticity (Kraus et al., 2017; Larsen et al., 2020), although not all patients with depression respond to SSRIs (Rush et al., 2006). One theory is that perhaps not all patients have a "serotonergic depression" (Ip et al., 2023; Köhler-Forsberg et al., 2023). We found no evidence that pretreatment hippocampal 5-HT4R levels were related to hippocampal volume changes during treatment. However, we observed sex-specific associations after treatment. In females, a decrease in hippocampal volume was significantly linked to an increase in 5-HT4R levels, while in males, the association was near zero. Despite these apparent differences, there was insufficient evidence for a statistically significant sex interaction, possibly due to the relatively small sample size, particularly for males. Thus, we cannot conclusively state that the relationship between 5-HT4R binding and hippocampal volume changes differs between sexes. Nonetheless, our findings add to growing evidence of sexual dimorphism in serotonergic signaling and its role in affective disorders. Previous studies have reported sex differences in cortical and subcortical 5-HT4R binding related to hormonal factors (2022; E Perfalk et al., 2017) and behavioural traits like aggression (S da Cunha-Bang et al., 2016). The sex-specific relationship between 5-HT4R binding and hippocampal volume changes may reflect differential mechanisms of SSRI action on hippocampal plasticity in males and females. This may relate to broader sex differences in depression neurobiology, e.g. sex differences in functional connectivity of frontal and limbic regions in MDD (Mohammadi et al., 2023). Additionally, sex differences in stress responses and hypothalamic-pituitary-adrenal (HPA) axis function may interact with serotonergic signaling to produce differential effects (Bangasser and Valentino, 2014). However, it is also essential to consider convergent sex differences, where similar phenotypes occur via sex-specific mechanisms (Bangasser and Cuarenta, 2021). While our study did not directly investigate this, the observed changes in hippocampal volume could be driven by different underlying mechanisms in males and females despite similar outcomes. This possibility underscores the need for more detailed mechanistic and clinical studies considering sex as a biological variable, with adequate male representation for robust conclusions (Rosa and Bücker, 2024).

4.3. Verbal memory

Verbal memory deficits are common in depression, and hippocampal dysfunction may contribute to these deficits (JD Bremner et al., 2000). We explored the relationship between hippocampus volume and verbal memory before and after SSRI treatment. This depressed sample previously demonstrated verbal memory deficits compared to matched healthy controls before medication, along with improvements during SSRI treatment. (2022).

We found that pretreatment left hippocampus volume was negatively associated with verbal memory performance, i.e., a smaller hippocampus was associated with better performance in the untreated depressed state. This may seem counterintuitive; is bigger, not better (JK Foster et al., 1999; ST Pohlack et al., 2014), as exemplified by the skilled London taxi drivers with enlarged hippocampi (Maguire et al., 2000). However, a large meta-analysis found little evidence supporting a relationship between hippocampal volume and memory ability *unless* stratified by age (Petten, 2004). In elderly individuals, volume was positively associated with performance, likely reflecting variation in atrophy and cognitive decline, while a stronger association emerged from studies of children and young adults, which uniformly reported negative correlations (Petten, 2004). The latter is consistent with our finding in an age group with a mean age of 27; however, in depression, the association between hippocampal volume and verbal learning may be decoupled (O Ajilore et al., 2015).

SSRIs have been shown to improve verbal memory in MDD (CE Prado et al., 2018; JE Schulkens et al., 2022), as observed in our sample (, ; Dam et al., 2020; Dam et al., 2022). Thisaligned with studies showing improved hippocampal function during antidepressant treatment without detectable volume changes (Vythilingam et al., 2004). Recent work by our group in the same patient sample observed that changes in verbal memory between baseline and week 12 were positively associated with changes in brain 5-HT4R binding (using a latent variable of neocortical, hippocampal and neostriatal 5-HT4R), indicating that downregulation of 5-HT4R binding leads to larger improvements in verbal memory (Dam et al., 2024).

4.4. Strengths and limitations

A major strength of the study is its inclusion of a large sample of unmedicated patients with moderate to severe depressive episodes. However, this work is not without limitations. Firstly, in the absence of a placebo group, spontaneous remission, measurement error, and regression to the mean could make interpreting these changes as solely treatment effects somewhat challenging. Secondly, as we investigated the effects of escitalopram (and duloxetine as a second-line treatment), our findings may not be generalisable to treatments with other antidepressants. Thirdly, the patients were relatively young and the results may not generalize to older patients. Lastly, excluding individuals with previous non-response to SSRIs may limit the generalizability of the findings to those with MDD who did not respond to SSRIs in the past.

5. Conclusion and future perspectives

Our findings contribute to the ongoing debate about the neurobiological mechanisms of antidepressants. Contrary to the traditional suggestion that SSRIs increase hippocampal volume, we observed no mean increase. Instead, we observed a mean decrease in the patients who responded to SSRI treatment. These results align with recent evidence suggesting that antidepressant efficacy may depend more on the quality of neural circuits rather than gross volumetric changes. Our observation that decreased volume can accompany clinical improvement supports emerging theories emphasizing synaptic refinement and circuit efficiency over simple growth models. Indeed, this is supported by our finding of a negative correlation between hippocampal volume and verbal memory performance, indicating that smaller volumes may be associated with enhanced cognitive function in certain domains.

The sex-specific relationship we found between 5-HT4R binding and hippocampal volume adds to the growing recognition of sex differences in both depression pathophysiology and response to different treatments. This is particularly relevant given the higher prevalence of depression in women and known sex differences in serotonergic signaling.

These results indicate the need to investigate the cellular and molecular mechanisms underlying volume changes in treatment responders and a more detailed examination of sex differences in serotonergic signaling and their therapeutic implications. As the field moves toward precision psychiatry, these insights into the complex relationships between serotonergic signaling, brain structure, and clinical improvement

may help guide the development of more targeted and effective antidepressant treatments.

CRediT authorship contribution statement

Kristian.H.Reveles Jensen: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Conceptualization. **Vibeke H. Dam:** Writing – review & editing, Investigation. **Kristin Köhler-Forsberg:** Writing – review & editing, Investigation. **Brice Ozenne:** Writing – review & editing, Visualization, Formal analysis. **Dea S. Stenbæk:** Writing – review & editing. **Melanie Ganz:** Writing – review & editing, Formal analysis, Data curation. **Patrick MacDonald Fisher:** Writing – review & editing, Data curation. **Vibe Gedsoe Frokjaer:** Writing – review & editing, Investigation, Funding acquisition, Conceptualization. **Gitte M. Knudsen:** Writing – review & editing, Funding acquisition, Conceptualization. **Martin Balslev Jørgensen:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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Declaration of competing interest

GMK has been a speaker for Abbvie, Angelini, H. Lundbeck, Compass and Cybin and is a consultant for Onsero, Gilgamesh, Pure Technologies, Pangea and Sanos. VGF has served as a consultant for SAGE therapeutics and given talks sponsored by H. Lundbeck Pharma, Gedeon-Richter and Janssen-Cilag. MBJ has given talks sponsored by Boehringer Ingelheim and H. Lundbeck. VHD has given talks sponsored by H. Lundbeck Pharma. All other authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2024.11.043.

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Changes in Hippocampal Volume, 5-HT$_4$ receptor binding, and Verbal Memory Over the Course of Antidepressant Treatment in Major Depressive Disorder

Supplementary Material

Supplementary statistical analyses:

2.7.1 Change in hippocampus volume

We did sensitivity analyses for $E[\Delta V_{hippo}]$ where it was estimated by adding pretreatment depression severity as an additional fixed effect in LMM1 and including an interaction of time and hemisphere. Due to an average two-point difference in pretreatment depression severity ($HAMD_{17}$) between patients rescanned and not rescanned, we also did sensitivity analyses using pretreatment depression severity as a covariate.

2.7.2 Change in hippocampus volume and depression severity

As a sensitivity analysis, we performed a mixed-effects model of the relationship between change in depression severity and change in hippocampal volume at week 8. In this model, the outcome variable was hippocampus volume; with time, the interaction between time and %--change in $HAMD_6$ from pretreatment, age, and sex were fixed effects and subject and hemisphere nested within the subject, where the subject was used as random effects.

2.7.3 Change in hippocampus volume

We also performed a linear regression of the relation between pretreatment mean hippocampus volume and pretreatment depression severity ($HAMD_{17}$) adjusted for intracranial volume, age and sex.

2.7.5 Left hippocampus volume and verbal memory

As a sensitivity analysis, we performed a linear regression of the relation between pretreatment left hippocampus volume and pretreatment VAMT-26 adjusted for intracranial volume, age, sex *and* $HAMD_{17}$. As a negative control, we did the same analysis with the *right* hippocampus.

Table S1 Pretreatment MRI and PET characteristics

*One pretreatment PET scan was excluded due to poor data quality.

Pretreatment	**mean (SD)**
MRI, *n*	**96**
Left hippocampus volume (mm^3)	4327 (434)
Right hippocampus volume (mm^3)	4494 (485)
Mean hippocampus volume (mm^3)	4410 (445)
Intracranial volume (cm^3)	1450 (150)
PET *n*	**90***
Total hippocampus BP_{ND}	1.060 (0.182)
Injected SB dose (MBq)	577 (56)
Injected SB mass (µg/kg)	0.013 (0.015)
Cerebellum AUC (kBq/ml)	10251 (2572)

Table S2 Pretreatment and longitudinal characteristics of the patients with MDD

$HAMD_{17/6}$: Hamilton Depression Rating scale 17 or 6 items. VAMT-26: Total word recall from the Verbal Affective Memory Task 26. [a]Information on episode was unavailable for one patient, [b]Education information missing for 20 patients, and [c]VAMT-26 missing for nine patients. Values are mean and (SD) unless *n* and (%).

Pretreatment characteristics	**All subjects**	**Not Rescanned**	**Rescanned**
N	96	53	43
Female, n (%)	69 (72%)	41 (77%)	28 (65%)
First episode / recurrent[a]	42 (44%) / 53 (56%)	23 (44%)	19 (44%)
Age (years)	27.2 (8.2)	27.6 (9.3)	26.6 (6.80)
Years of education[b]	11.6 (1.1)	11.3 (1.4)	11.8 (0.5)
Total Education score[b]	14.9 (2.1)	14.1 (2.3)	15.6 (1.7)
$HAMD_{17}$	22.9 (3.5)	24.0 (3.3)	21.7 (3.0)
$HAMD_6$	12.4 (1.7)	12.7 (1.5)	12.0 (1.7)
VAMT-26[c]	14.6 (3.6)	14.3 (3.9)	15.0 (3.1)
Initiated treatment			
N (% dropout)	92 (4%)	49 (7.5%)	43 (0%)
Week 8 characteristics			
N (% dropout)	84 (8%)	41 (16%)	43 (0%)
Treatment non-adherent, N (%)	4 (4.8%)	2 (4.9%)	2 (4.7%)
Escitalopram dose (mg)	16.8 (4.0)	16.7 (4.1)	16.9 (3.8)
Switched to duloxetine by week 6, N (%)	10 (11%)	6 (13%)	4 (9%)
Duloxetine dose (mg)	63 (10)	66 (13)	60 (0)
$HAMD_6$ %-change from pretreatment	-51 (31) %	-45 (30) %	-57 (30) %
Week 12 characteristics			
N (% dropout)	82 (10%)	39 (20%)	43 (0%)
Escitalopram dose (mg)	18 (3.8)	18 (3.8)	17 (3.7)
Switched to duloxetine by week 11, N (%)	17 (18%)	9 (18%)	8 (19%)
Duloxetine dose (mg)	81 (22)	83 (21)	79 (22)
$HAMD_6$ %-change from pretreatment	-59 (31) %	-53 (35) %	-66 (28) %

Table S3 Hippocampal volumes of rescanned patients

43 patients with MDD were rescanned after eight weeks of treatment; based on blood samples collected at week 8, two patients were deemed non-adherent to medication. Change in volume estimated by paired Welch and one-sample student *t*-tests for absolute volume and %-change, respectively. Treatment response is defined as ≥50% $HAMD_6$-reduction from pretreatment.

Group	**N**	**Pretreatment** mean (SD)	**Week 8** mean (SD)	**Change** mean (CI)	**Cohen's *d***	***p*-value**
All patients	43	4570 (411) mm^3	4539 (399) mm^3	-30 (-64; 4) mm^3 -0.6 (-1.3: 0.1) %	0.27 0.25	0.082 0.108
- Adherent only	41	4589 (409) mm^3	4557 (398) mm^3	-32 (-67; 3) mm^3 -0.6 (-1.4; 0.1) %	0.29 0.27	0.072 0.096
Treatment responders	28	4619 (378) mm^3	4569 (365) mm^3	-49 (-95; -4) mm^3 -1.0 (-2.0; -0.03) %	0.42 0.40	0.034 0.045
Non-responders	15	4478 (466) mm^3	4485 (467) mm^3	7 (-40; 53) mm^3 0.2 (-0.9; 1.2) %	0.08 0.09	0.763 0.734
- Adherent only	13	4526 (478) mm^3	4531 (477) mm^3	5 (-49; 59) mm^3 0.1 (-1.4; 1.1) %	0.06 0.07	0.840 0.803

Figure S1. CONSORT flow diagram of study participants

*One pretreatment PET scan was excluded due to data quality, i.e., the scan was interrupted halfway and could not properly be reconstructed.

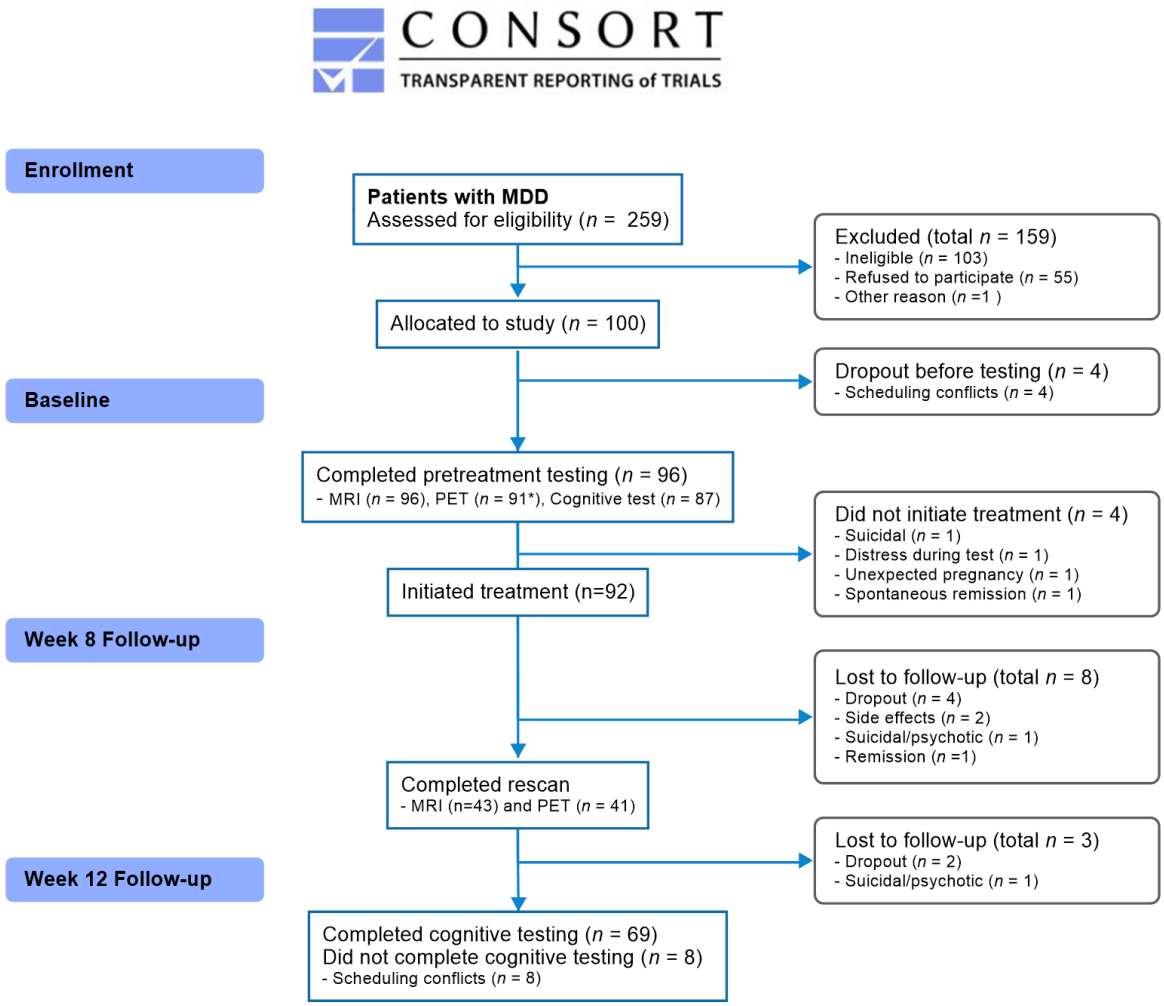


Figure S2: Hippocampus volume before and after eight weeks of adherent SSRI/SNRI treatment.

Raincloud plots of left and right hippocampus volume changes during adherent treatment (n=41). Estimated changes on the left -0.60% (CI: -1.5%; 0.39%) and right -0.65% (CI: -1.7%; 0.39%).

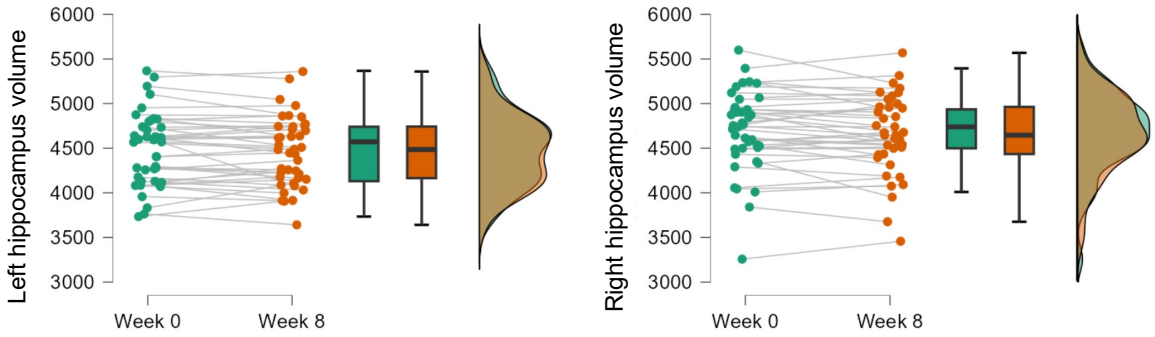
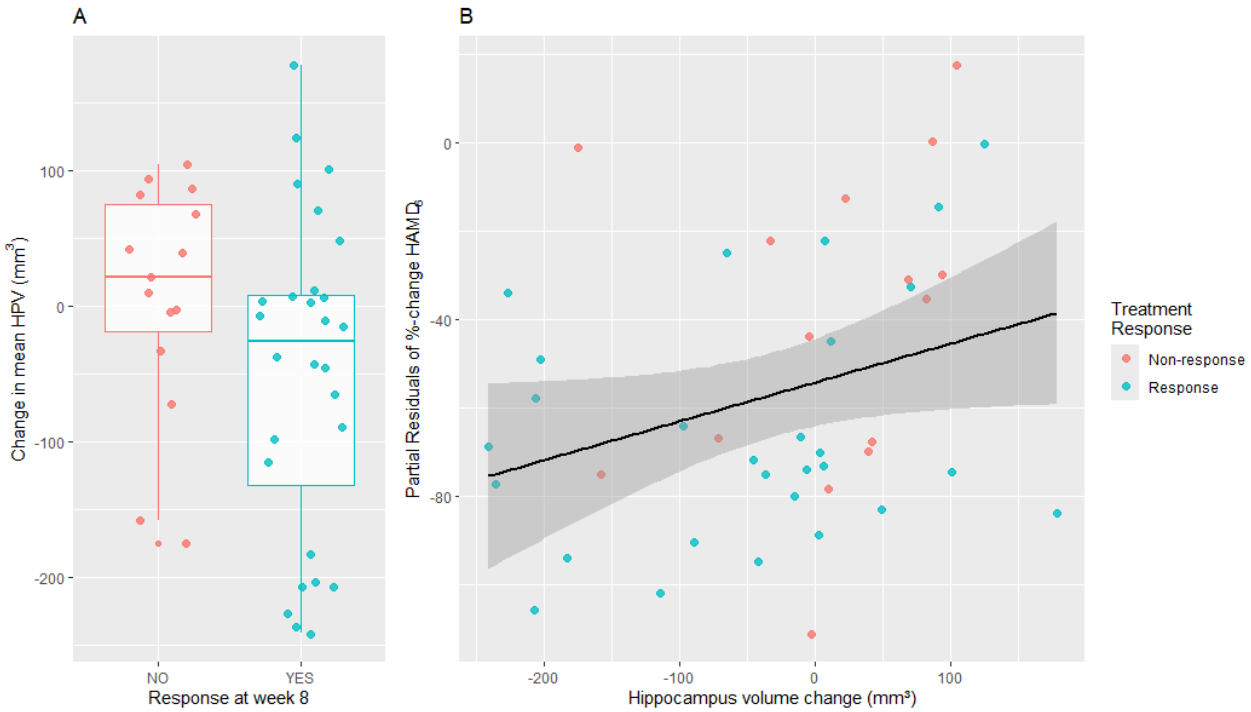


Figure S3 Change in hippocampus volume and change in depression severity in complete cases

A) Patients with **treatment response** and **non-response** (i.e., <50% $HAMD_6$-reduction) at week eight had a mean increase of 6.8 mm3 (SD: 86 mm3) in hippocampal volume compared to treatment responders who had a mean decrease of 49.4 mm3 (SD: 117.2 mm3); an estimated difference of 56.2 mm3 (CI: -120; 7.3 mm3) in a Welch t-test (Cohen's *d*=0.55, *p*=0.081). Further, see Supplementary Table 2 above.

B) Partial residuals of change in depression severity (i.e. %-$HAMD_6$) in **treatment responders** and **non-responders** adjusted for age, sex and intracranial volume in linear regression (β= 0.07 %$HAMD_6$/1000mm^3, CI: -0.02; 0.15, *p*=0.130).



Study II

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EEG abnormalities are not associated with poor antidepressant treatment outcome - A NeuroPharm study

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ABSTRACT

EEG brain abnormalities, such as slowing and isolated epileptiform discharges (IEDs), has previously been associated with non-response to antidepressant treatment with escitalopram and venlafaxine, suggesting a potential need for treatment with anticonvulsant property in some patients. The current study aims to replicate the reported association of EEG abnormality and treatment outcomes in an open-label trial of escitalopram for major depressive disorder (MDD) and explore its relationship to mood and cognition. Pretreatment, 6 min eyes-closed resting-state 256-channel EEG was recorded in 91 patients with MDD (age 18–57) who were treated with 10–20 mg escitalopram for 12 weeks; patients could switch to duloxetine after four weeks. A certified clinical neurophysiologist rated the EEGs.

IED and EEG slowing was seen in 13.2%, and in 6.6% there were findings with unclear significance (i.e., Wicket spikes and theta activity). We saw no group-difference in remission or response rates after 8 and 12 weeks of treatment or switching to duloxetine. Patients with EEG abnormalities had higher pretreatment mood disturbances driven by greater anger (p=.039) and poorer verbal memory (p=.012). However, EEG abnormality was not associated with improved mood or verbal memory after treatment. Our findings should be interpreted in light of the rarity of EEG abnormalities and the sample size. While we cannot confirm that EEG abnormalities are associated with non-response to treatment, including escitalopram, abnormal EEG activity is associated with poor mood and verbal memory. The clinical utility of EEG abnormality in antidepressant treatment selection needs careful evaluation before deciding if useful for clinical implementation.

1. Introduction

Depression is a complex and prevalent mental illness that remains difficult to treat efficiently despite several available antidepressant options. Unfortunately, first-line antidepressants such as selective serotonin reuptake inhibitors (SSRIs) fail to facilitate remission in most patients with major depressive disorder (MDD) (Rush et al., 2006). There is no clear evidence-based strategy for medication when patients inadequately respond to several antidepressants. This has led to a search for predictive markers of treatment response to guide treatment selection and provide precision psychiatric care.

Electroencephalography (EEG) is a cost-effective and scalable clinical tool that has shown promise for predicting treatment outcomes (Olbrich and Arns, 2013), but is limited by underreporting of negative results and a lack of out-of-sample validation and replication of previous findings (Widge et al., 2019). For example, research based on data from the international Study to Predict Optimized Treatment in Depression (iSPOT-D) suggests that in patients with depression, abnormality EEG activity such as isolated epileptiform discharges (IEDs) and EEG slowing is a biomarker for non-response to escitalopram and venlafaxine but not

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sertraline (Arns et al., 2015). The authors hypothesised that the association between EEG abnormalities and better treatment response to sertraline could be attributed to a mild anticonvulsant property (Arns et al., 2015; Vinne et al., 2021).

The conventional view is that the slowing of EEG rhythms and the presence of IEDs indicate different pathological processes (Markand, 2003). EEG slowing refers to a decrease in the frequency of the electrical activity. It can be seen in a variety of conditions, including brain injury, neurological disorders, and certain medications. But can also occur naturally during sleep or states of relaxation or meditation. IEDs are abnormal electrical discharges that indicate a hyperexcitable state of the brain. Although interictal epileptiform discharges are typically found in patients with epilepsy, isolated epileptiform discharges are also found in other brain disorders such as autism spectrum disorder and attention-deficit/hyperactivity disorder (Swatzyna et al., 2016). However, their association with affective disorders such as MDD and anxiety is not well understood. Previous studies suggest an incidence of 3–5% in MDD (Shelley et al., 2008), similar to the 1–6% prevalence of paroxysmal EEG in normal child and adult populations (Borusiak et al., 2010; Okubo et al., 1994; Richter et al., 1971; Shelley et al., 2008). A couple of studies suggest that the prevalence of EEG abnormalities in patients with MDD is approximately 10–17% (Arns et al., 2015; Vinne et al., 2021), similar when compared to healthy controls (Arns et al., 2015).

Abnormal EEG activity has been associated with poorer cognitive function (Meghdadi et al., 2021), and patients with panic disorder and epileptiform activity have been found to respond clinically to anticonvulsant medication (McNamara and Fogel, 1990). Additionally, the antiepileptic drug lamotrigine is used as a mood stabilizer and antidepressant in bipolar affective disorder. Rodent studies also suggest that SSRIs, such as fluoxetine in low and moderate doses, have an anticonvulsant effect (Aygun, 2019). Sertraline is also suggested to normalize IEDs due to a possible dopaminergic action (Vinne et al., 2019). In contrast, the antipsychotic clozapine is clinically known to frequently induce EEG slowing and epileptiform discharges (Jackson and Senevirratne, 2019). Thus, the role of IEDs in depression, its associations with anxiety and cognitive dysfunction, and its treatment effects are not well understood.

We here seek to replicate the putative associations of abnormal EEG activity and treatment outcome, to mood, anxiety, and cognition, in an independent cohort from an open-label 12-week study of escitalopram treatment with close clinical follow-up in MDD (Köhler-Forsberg et al., 2020). Our primary research question is whether the presence of EEG abnormalities is associated with poorer response to serotonergic treatment and, secondarily, whether this association EEG abnormalities are associated with differences in mood, anxiety, or cognitive function.

2. Materials and methods

We report findings from the NeuroPharm study, a longitudinal, open-label multimodal neuroimaging clinical trial investigating potential biomarkers in the antidepressant treatment of MDD. The NeuroPharm-1 study was approved by the Ethics committee for the Capital Region of Copenhagen (protocol: H-15017713), was conducted in accordance with the Declaration of Helsinki, and was pre-registered at www.clinicaltrial.gov (reg. nr. NCT02869035). For a detailed protocol description, see (Köhler-Forsberg et al., 2020).

2.1. Subjects and treatment

Non-psychotic antidepressant-free patients, 18–65 years of age, suffering from a moderate to severe depressive episode lasting less than two years were assessed for inclusion in the study. Patients were eligible if they had been free of antidepressant medication for >2 months, had not previously exhibited non-response to SSRIs, and had not undergone more than one antidepressant treatment attempt in the current depressive episode. Patients were recruited through their primary care centre or a central referral site at the Mental Health Services of the Capital Region of Copenhagen. MDD diagnosis was confirmed by certified psychiatrists and corroborated by a Mini-International Neuropsychiatric Interview (MINI) version 6 (Sheehan et al., 1998). The detail inclusion and exclusion criteria are listed in Köhler-Forsberg et al., 2020. Briefly, patients were screened for and excluded neurological and substance or alcohol use disorders, the use of any CNS drug that could not be washed out prior to participation (e.g., metoclopramide, ondansetron, serotonergic migraine medicine, clonidine, antiepileptic medication); a history of brain injury (i.e., loss of consciousness and amnesia or symptoms of concussion disorder), or medical conditions interfering with measurements (e.g., epilepsy).

Ninety-one patients with MDD completed the pretreatment investigation program, and 86 patients started antidepressant treatment with escitalopram, individually adjusted to 10–20 mg daily depending on response and side effects. In addition, per standard practice, patients experiencing intolerable side effects or <25% reduction in $HAMD_6$ from pretreatment at week four were offered to switch to the serotonin-norepinephrine reuptake inhibitor (SNRI), duloxetine (n= 17), individually adjusted (30–120 mg daily). Treatment adherence was assessed by plasma levels of medication at week 8. See supplementary figure S1 for the CONSORT diagram.

2.2. Depression severity and treatment response

Depression symptom severity was assessed with the Hamilton Depression Rating Scale ($HAMD_{17}$) interview before and at weeks 4, 8, and 12 of treatment. Treatment responder was defined as > 50% improvement in $\Delta HAMD_{17}$ (from pretreatment to week 8) or else patients were defined as non-responders. In the NeuroPharm trial, we also use the 6-item subscale ($HAMD_6$) to assess the treatment effect because $HAMD_6$ captures core depressive symptoms and is more sensitive to antidepressant treatment response (Østergaard et al., 2016; Timmerby et al., 2017).

2.3. Mood disturbance and anxiety

Mood disturbance was assessed by the Profile of Mood States (POMS) as the Total Mood Disturbance (TMD) based on the six mood states Tension-Anxiety, Anger-Hostility, Vigor, Fatigue-Inertia, Depression-Dejection, Confusion-Bewilderment. Anxiety was assessed pretreatment by the 10-item Generalized Anxiety Disorder (GAD-10) scale (Bech et al., 2005).

2.4. Working memory and verbal memory

Trained neuropsychologists conducted testing in standardised test rooms before drug intervention.

The Letter Number Sequence (LNS) task assessed working memory capacity (Wechsler, 1997). One outlier from the LNS task was excluded as the patient had misunderstood the test instructions.

The Verbal Affective Memory Task 26 (VAMT-26) was used to assess the learning and recall of affective words (Dam et al., 2020; Hjordt et al., 2020). Here, total word recall, calculated as the average number of words (positive, negative, and neutral) recalled across immediate, short-term, and long-term recall in the VAMT-26, was used to assess explicit non-affective verbal memory function.

Affective memory bias was calculated by subtracting negative word scores from positive word scores.

2.5. EEG recording

Patients were seated in a comfortable armchair in a quiet room. Resting EEG was recorded with both eyes closed and open. Participants were instructed to remain quiet and relaxed, avoid eye blinks and movements, and relax chin muscles during recording. Resting EEG was

recorded during four 3 min periods with a counterbalanced order of OCOC (O for eyes open, C for eyes closed) or COCO between subjects. EEG data were recorded using a 256-channel HydroCel Sensor Net system (EGI, Inc., Eugene, OR) at 1000 Hz with 0.1–100 Hz analog filtering, the vertex electrode served as the reference channel. Impedances were kept <50 kΩ during the recording.

2.6. EEG classification

Only eyes-closed EEG data were included in the analysis. A board-certified neurophysiologist (O.U.C.) visually inspected the EEGs, blinded to patients' clinical data. The abnormal EEG activity was rated according to international guidelines (Kane et al., 2017) to identify the prevalent posterior rhythmic activity, which included alpha peak frequency (APF) and background. Moreover, interictal activity was evaluated and categorized as either focal or generalized, which entailed EEG slowing or potential epileptiform activity. The diffuse slowing manifested when the background frequency persistently remained below the alpha range (α, 8–13 Hz). Conversely, focal slowing occurred if rhythms slower than alpha, such as theta (θ, 4–7.9 Hz) or delta (δ, 0.5–3.9 Hz), were continually detected in a particular region.

Epileptiform or paroxysmal activities refer to any EEG pattern emerging and disappearing paroxysmally from ongoing background activity (Grant et al., 2014). In contrast, non-paroxysmal slow wave activities of both focal and generalized types existed frequently with some waxing and waning - note that records pertained mostly to fully awake subjects. Finally, controversial waveforms such as Wicket spikes (single waveforms that occur in brief trains or clusters) were also scored. These waveforms are paroxysmal but are of uncertain significance.

2.7. Statistics

We compared features between the unmedicated MDD patients with and without EEG abnormality using Welch's *t*-test and Fisher's exact test, and ANCOVAs to adjust for age and sex. To identify the primary mood disturbance in the Total Mood Disturbance from POMS, we used logistic regression with EEG abnormality as the dependent variable and POMS subscales, with age and sex as covariates.

We used Fisher's exact test to assess the frequency of EEG abnormality in clinical groups i.e., treatment responders and non-responders.

To assess clinical, mood, and cognitive changes during treatment, we used repeated measures ANCOVA with age and sex. In addition, to align with the prior study (Arns et al., 2015), we performed partial correlations conditioned on age between APF and the percentage improvement in the $HAMD_{17}$ at week 8. The change in prevalence in EEG abnormality during treatment was assessed with McNemar chi-square.

We present the estimates with 95% confidence intervals (CI) and *p*-values unadjusted for multiple comparisons. Greenhouse-Geisser correction was used when the sphericity assumption for ANOVAs was not met. Effect sizes are given in Cohen's *d* and omega-squared (ω^2) The significant level was set to 0.05. Statistics were done in JASP version 0.17.1 (JASP-team, 2023).

3. Results

3.1. Sociodemographic and clinical characteristics

Abnormal EEG findings were present in 20% of the patients (n=18): 13% had IED and EEG slowing, and 7% had other findings with unclear significance (Wicket spikes and theta activity, Table 1, supplementary Figure S2). The abnormal activity was predominately located in the frontotemporal regions and mostly bilateral. The demographics and other clinical profiles of patients with and without EEG abnormalities were not significantly different (*p-values* >0.11, Table 2), except patients with EEG abnormalities had a borderline significant higher self-reported total mood disturbance ($t(89)$=1.92, Cohen's d=0.51, p=.058). Patients with EEG abnormalities did not have greater familial disposition for affective disorders (p=.81, Table 2). One patient in our study was undergoing treatment with disulfiram to prevent a relapse of alcoholism, and another patient had a history of migraines but did not use sumatriptan during the study period. Importantly, neither of the patients exhibited EEG abnormalities. The rest of the participants did not have any history of neurological disorders or substance use disorders.

Table 1
Frequency of EEG abnormalities.

EEG abnormality	Pretreatment	Week 8 (per-protocol analysis)	
	(n=91)	Responder[a] (n=42)	Non-responder (n=38)
Isolated epileptiform	6.6%	7.1%	7.9%
- Sharp and slow waves	6	3	3
EEG slowing	6.6%	7.1%	0%
- Only slow waves	6	3	0
Abnormality with unclear significance	6.6%	2.4%	7.9%
- Wicket spikes and theta activity	6	1	3

91 EEG were assessed.
[a] Clinical response was defined using relative $HAMD_{17}$ scores from week 8 to pretreatment, with Responders having *a* >50% improvement. One patient was not assessed at week 8, but was assessed at week 12 as a non-responder and is included as a non-responder at week 8. The frequency of EEG abnormality was not significantly different between treatment responders and non-responders at week 8 (Fisher's exact test, Odds ratio=0.94 CI: 0.23; 3.65, p=1.00).

Table 2
Demographic and clinical profile.

	No abnormality (n=73)		With abnormality (n=18)		
	Mean (SD)	Range	Mean (SD)	Range	*p*-value
Demographic					
Sex (male/female)	19/54	(26/74%)	6/12	(33/67%)	.57[d]
Age (years)	27.8 (8.6)	18.6–57.3	25.8 (7.3)	19.0–44.4	.37
Education score[a]	15.1 (2.2)	8–17	14.2 (2.0)	11–17	.11
Clinical					
Single/Recurrent episode	32/41	(44/56%)	7/10[b]	(41/59%)	1.00[d]
1st-degree disposition score[a]	1.3 (1.5)	0–6	1.2 (1.2)	0–4	.81
$HAMD_{17}$	22.9 (3.4)	18–31	22.7 (3.4)	18–30	.85
$HAMD_6$	12.3 (1.7)	7–17	12.4 (1.5)	9–15	.84
TMD	80.7 (31.8)	8–149	96.7 (31.5)	47–119	.06
GAD-10[c]	23.6 (9.9)	7–47	26.9 (8.1)	17–43	.22

MDD: Major Depressive Disorder. [a]Education level and disposition based on first-degree relatives were only available for 61 without and 13 with EEG abnormality. [b]Information on episode was not available for one participant with EEG abnormality. [c]GAD-10, a self-report of anxiety during the previous two weeks, was only available for 71 without and 15 with EEG abnormality. TMD: Total Mood Disturbance measured by Profile of Moods. $HAMD_{17}$: 17-item Hamilton Depression Rating scale. $HAMD_6$: 6-item subscale. *P*-values calculated by student's *t*-tests and [d]Fishers exact test.

3.2. EEG abnormality and treatment effects on depression and mood

The remission and response rates were not significantly different between groups with and without EEG abnormality at 8 or 12 weeks of antidepressant treatment, nor was the rate of switching to duloxetine

(Fig. S2, Supplementary Table 1). A repeated ANOVA with age and sex as covariates were performed to compare the effect of EEG abnormality on depression severity at 4, 8, and 12 weeks of treatment, where EEG abnormality did not interact with changes in severity assessed by $HAMD_{17}$ ($F(3216)=0.09$, $\omega^2=0.00$, $p=.95$) or $HAMD_6$ ($F(3216)=0.36$, $\omega^2=0.00$, $p=.79$). Removing the four patients (one with abnormal EEG activity) deemed non-adherent to treatment from analyses did not change the findings.

Similar to a prior study (Arns et al., 2015), we also observed a non-significant negative partial correlation between APF and improvement on $HAMD_{17}$ ($r(80)=-0.10$, $p=.38$).

While patients with EEG abnormality reported more Anger-Hostility before treatment, there was no interaction with changes in Anger-Hostility during treatment and EEG abnormality ($F(2, 112)=1.87$, $\omega2=0.01$, $p=.17$).

3.3. EEG abnormality and mood and cognition

Patients with and without EEG abnormality did not differ in either anxiety- or depression severity assessed by $HAMD_{17}$ and the $HAMD_6$ subscale (Table 2).

However, patients with an EEG abnormality had significantly higher total mood disturbance score ($F(1, 87)=4.38$, $\omega^2=0.04$, $p=.039$, Fig. 1), on average 17.6 higher score on the POMS (CI: 0.9–34.2, Cohen's $d=0.55$). The difference was mainly driven by Anger-Hostility ($p=.017$). Patients with an EEG abnormality scored 5.7 (CI: 1.9–9.6, Cohen's $d=0.78$) higher on the Anger-Hostility subscale than those without ($F(1, 87)=8.75$, $\omega^2=0.07$, $p=.004$, Fig. 1).

Patients with EEG abnormality did not have poorer working memory assessed by LNS as evaluated. in an ANCOVA test adjusted for sex and age ($F(1,74)=0.80$, $\omega^2=0.00$, $p=.38$). However, they had poorer verbal memory and remembered on average 2.6 (CI: 0.6–4.7, Cohen's $d=0.76$)

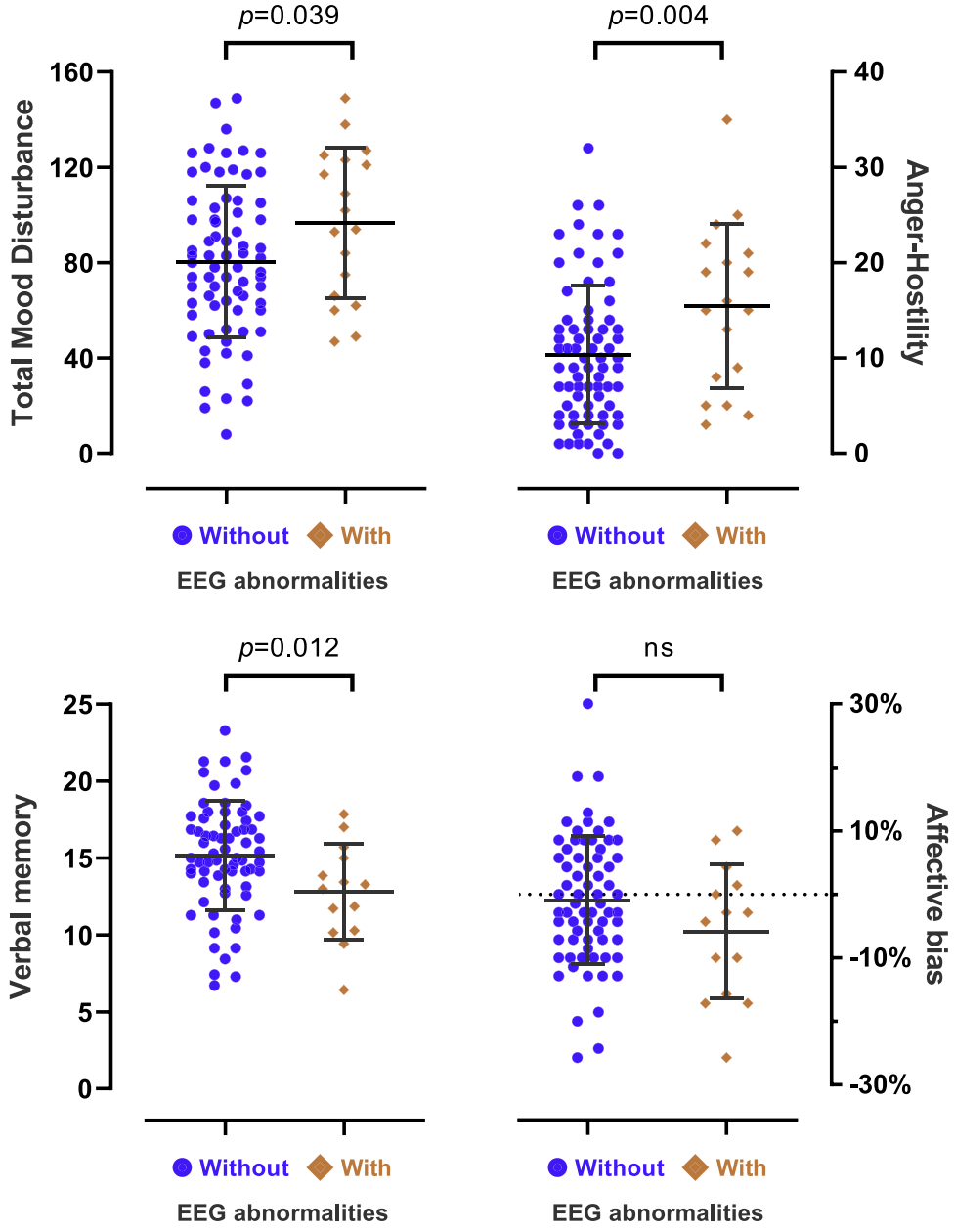

Fig. 1. Pretreatment mood and cognitive differences
Descriptive plots from ANCOVA of Total Mood Disturbance and the Anger-Hostility factor from the Profile of Mood States and verbal memory and affective bias from VAMT-26 with pretreatment and EEG abnormalities (adjusted for age and sex). P-values are from ANCOVAs with age and sex. Error bars are standard deviations.

fewer words ($F(1, 79)=6.575$, $p=.012$, Fig. 1).

Since the patients with EEG abnormalities had poorer verbal memory and also reported more negative emotions in the form of anger and hostility, we, post-hoc, also examined differences in affective memory bias. The average affective memory bias in patients with EEG abnormalities was −5.8% indicating a trend that they recalled negative words (e.g., revenge and hate) better than positive words (e.g., joy and peace) compared to patients without EEG abnormalities whose mean affective bias was −0.9% (Cohen's $d=0.48$, $p=.11$). However, when adjusted for age and sex, this group difference was not significant ($F(1, 79)=1.9$, $\omega^2=0.01$,$p=.17$, Fig. 1).

There was a positive linear relationship between poorer verbal memory and total mood disturbance adjusted for age, sex and EEG abnormality ($p=.031$, supplementary Figure S3) with no interaction with EEG abnormality.

3.4. EEG abnormality and treatment effects on verbal memory

While patients with EEG abnormality had poorer verbal memory before treatment, they had similar improvements in verbal memory over the 12 weeks of treatment ($F(1, 61)=0.31$, $\omega^2=0.00$, $p=.58$).

3.5. EEG abnormality after treatment

At week 8, follow-up EEG measurements were obtained from 38 patients. After 8 weeks of treatment, there were no new instances of EEG abnormalities, and one patient no longer exhibited pre-treatment EEG abnormalities. There was no statistically significant change in the prevalence of EEG abnormalities between pretreatment and follow-up ($p=1.00$).

4. Discussion

Previous research has highlighted abnormal EEG activity as a potential prognostic biomarker of non-response to specific SSRIs and SNRIs, such as escitalopram and venlafaxine, in MDD (Arns et al., 2015). We do not find that unmedicated patients with MDD with and without EEG abnormalities differ in depression severity. We also do not replicate that those with abnormal EEG activity have a poorer response to treatment including escitalopram at 8- and 12-weeks follow-up. Thus, our data do not support using EEG abnormalities as a predictive biomarker to guide antidepressant treatment selection.

Based on the iSPOT-D data, Vinne et al. reported that some patients who responded to another SSRI, sertraline, and had an abnormal EEG before treatment did not have an abnormal EEG at follow-up (based on only 2 min EEG recording) and suggested that this phenomenon could be attributed to a sertraline-specific mechanism involving the normalization of EEG patterns through the modulation of dopamine transporters. Initially, there were concerns about antidepressants having pro-convulsive effects, based mainly on small case studies or individual patient reports (Kondziella and Asztely, 2009; Ware and Stewart, 1989). Although sertraline may indeed possess this capability, it is important to consider broader evidence from animal and clinical studies that suggest a general anticonvulsant effect of serotonergic and noradrenergic antidepressants (Hong and Bainbridge, 2014; Jobe and Browning, 2005; Kondziella and Asztely, 2009). This effect is thought to be mediated by the reduction of the potassium-evoked release of glutamate (Jobe and Browning, 2005). Inconsistent with the iSPOT-D study findings (Arns et al., 2015), our research did not establish a association between EEG abnormalities and non-response to treatment options, including escitalopram (Arns et al., 2015). Taken together, it appears there may not be substantial evidence to suggest that sertraline performs more effectively in addressing EEG abnormalities among patients with depression when compared to other SSRIs or SNRIs.

The patients with EEG abnormality did have more severe mood disturbances and poorer verbal memory pretreatment. The abnormal EEG activity was predominantly bilaterally in frontotemporal regions, consistent with verbal memory issues; however, there were no detectable effects on their working memory.

There is evidence supporting that epileptiform discharges disrupt short-term cognitive processes in humans (Fernández et al., 2015). However, collectively, there is currently limited and conflicting evidence that IEDs cause chronic cognitive deficits in humans (Fernández et al., 2015; Meekes and Jennekens-Schinkel, 2018).

Although the patients with EEG abnormality had similar levels of depressive symptomatology, including anxiety prior to treatment, they did report worse mood and more anger and hostility. There are limited studies on EEG abnormalities and anger or aggression. Epileptiform discharges in healthy children aged 6–12 are not associated with emotional and behavioural problems (Okubo et al., 1994). In autism, IED is associated with more severe illness, behavioural problems, and social impairment (Veerappan et al., 2018).

Cognitive impairments can greatly affect the patient's ability to function in everyday life and are often associated with high levels of subjective distress. Memory impairments have been associated with mood disturbances and anger (Lindert et al., 2021). This could explain why the patients with EEG abnormalities also reported greater mood disturbance, particularly feelings of anger.

EEG abnormality did not interact with improvement in mood or verbal memory during treatment, which suggests that the resting-state brain activity reflects an underlying brain pathophysiology or that it may take longer for the symptomatology and aberrant brain activity to normalise following treatment.

Although EEG abnormalities did not affect the results of medical treatment in this study, it is important to consider that having more severe verbal memory problems could have an impact on the effectiveness of psychotherapy. Previous research has shown that patients with post-traumatic stress disorder who have difficulties with verbal memory tend to have poorer outcomes with cognitive therapy (Wild and Gur, 2008).

Our findings should be interpreted in the light of several considerations.

The EEG data were rated by a blinded, single expert rater (O.U.C), as has been done elsewhere (Arns et al., 2015; Vinne et al., 2019). This approach may introduce bias; however, the inter-rater reliability of EEG abnormalities is generally high (Grant et al., 2014; Jing et al., 2020).

Our study found a 13.2% prevalence of IED and EEG slowing, slightly higher than the 9.4% reported in the study by Arns et al. (2015). This difference could be due to our three times longer EEG recording duration, which may have facilitated a more comprehensive evaluation and fewer false negatives. To further investigate the incidence of EEG abnormality in patients with MDD, future studies should ideally include longer recordings, e.g., a clinical EEG of ≥20 min, to limit false negatives. Provocation methods such as hyperventilation, intermittent photic stimulation, or sleep deprivation may also be used further to induce IEDs (Mendez and Brenner, 2006).

Additionally, it is worth noting that our study does not assess the association between EEG abnormalities and treatment response to sertraline and venlafaxine, as was done in the iSPOT-D dataset (Arns et al., 2015).

The rarity of abnormal EEG activity and the limited sample size constrains the statistical power and the precision in estimating effect sizes. Future research could clarify the relationship between EEG abnormalities and antidepressant treatment response using large datasets such as the CAN-BIND-1 study (Zhdanov et al., 2020) and a current large-scale cohort study (Jensen et al., 2023).

5. Conclusion

In conclusion, we could not replicate previous findings of an association between pretreatment EEG abnormalities and non-response to escitalopram. This suggests that abnormal EEG may not be a reliable,

generalisable, and operable biomarker for predicting SSRI treatment outcomes and, therefore, not appropriate to use in routine clinical practice without further validation. Our study also reveals that abnormal EEG activity in the depressed state, pretreatment, is associated with mood disturbance and poor verbal memory. This emphasizes the potential of further research to advance the understanding of the relationship between EEG abnormalities, cognitive and affective deficits, and treatment outcomes to optimise treatment strategies and improve outcomes for patients with brain disorders.

Declaration of competing interest

CTI is a shareholder and has served as a consultant at DeepPsy AG. MBJ has given talks sponsored by H. Lundbeck and Boehringer Ingelheim. GMK has served as a consultant for SAGE Therapeutics and Sanos. VGF has served as a consultant for SAGE Therapeutics and given lectures at seminars sponsored by Lundbeck A/S, Jannsen-Cilag A/S and Gedeon-Richter A/S. The other authors declare no competing interests.

Data availability

The data analysed in this study is subject to the following licenses/restrictions: A Cimbi database application is required to access the dataset through the following procedures: https://cimbi.dk/index.php/documents/category/3-cimbi-database.

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Author contributions

KRJ and CTI contributed to the study's conception and design and wrote the manuscript's initial draft. OUC performed the clinical EEG evaluations, and KRJ and CTI performed the statistical analyses. VGF, MBJ and GMK conceptualised the Neuropharm study and the data was collected by CTI, VND, KKF. All authors contributed to the interpretation of the analyses and the manuscript revision and have approved the submitted version.

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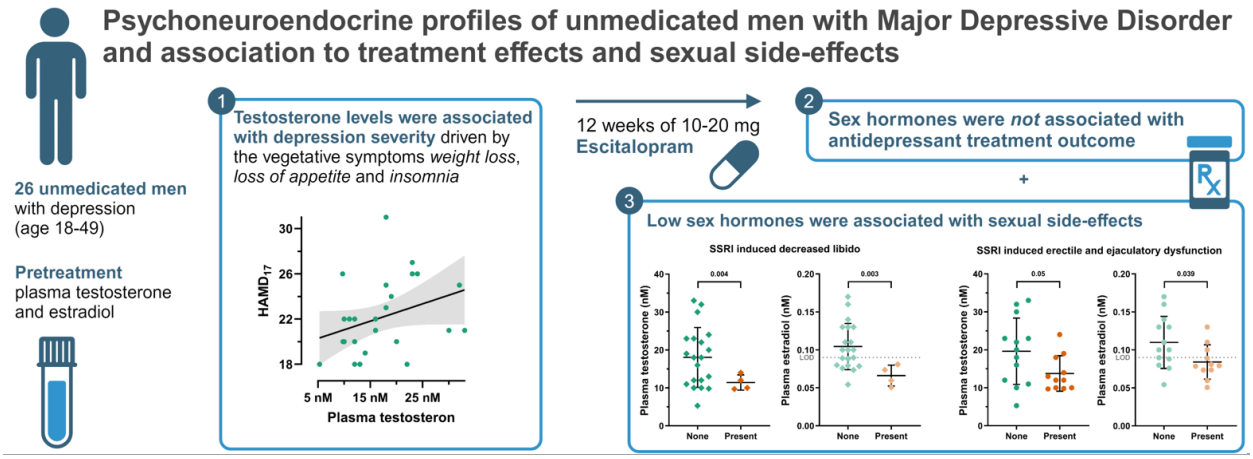
Study III

Graphical abstract



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Research Articles

Psychoneuroendocrine profiles of unmedicated men with major depressive disorder and associations to treatment effects and sexual side-effects

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ABSTRACT

Sex hormones are potentially involved in major depressive disorder (MDD) as there are sex differences in risk for depression, symptomatology and treatment efficacy. Such hormonal contributions to MDD have mainly been studied in women and scarcely in men. We, therefore, investigate whether testosterone and estradiol levels in unmedicated men with MDD are associated with depressive symptom profiles, SSRI treatment response, and sexual side-effects. Pretreatment plasma testosterone and estradiol were available from 26 unmedicated men (age 18–49). The patients were treated with 10–20 mg escitalopram for 12 weeks. Depression severity was measured by Hamilton Depression Rating Scale 17 (HAMD17), and treatment response was the change in HAMD6 from baseline. Sexual side-effects were assessed with a standardized clinician-rated scale. Pretreatment testosterone was positively associated with depression severity ($p = 0.016$), the association was primarily driven by vegetative symptoms: weight loss, gastrointestinal symptoms, and insomnia ($p \leq 0.027$). Pretreatment sex hormone levels were not associated with antidepressant treatment outcome. However, pretreatment sex hormones were lower in patients who experienced sexual side-effects after 8–12 weeks of escitalopram treatment; low estradiol was associated with erectile and ejaculatory dysfunction ($p = 0.039$) and low testosterone and low estradiol were both associated with decreased libido ($p \leq 0.004$). In conclusion, we find low testosterone in men with MDD coupled with depression severity, particularly vegetative symptoms. However, pretreatment sex hormone levels were not associated with treatment efficacy but with sexual side-effects. Taken together, our findings highlight sex hormone profiles as potential prognostic biomarkers of SSRI-induced sexual dysfunction. *Data from:* Clinical study (NCT02869035).

1. Introduction

The incidence of depression differs by sex, i.e., men are diagnosed with Major Depressive Disorder (MDD) half as often as women (McHenry et al., 2014). The pathogenesis of MDD is likely affected by sex hormones, which has been well studied in women (Albert and Newhouse, 2019; Frokjaer, 2020; Morssinkhof et al., 2020; Barth et al., 2023); yet less studied in men (Thériault and Perreault, 2019; Brietzke et al., 2019). Men and postmenopausal women respond poorer to Selective Serotonin Reuptake Inhibitors (SSRI) than women in their reproductive age (LeGates et al., 2018). Further understanding sex differences and the potential hormonal contribution to MDD psychopathology in both sexes may improve future treatment (Silva et al., 2023).

Low free testosterone has been associated with depression in men aged 71–89 (Almeida et al., 2008), and testosterone replacement therapy has been shown to reduce depressive symptoms in men with MDD (Zarrouf et al., 2009). Men with the atypical depressive subtype have been found to have lower testosterone than men with melancholic depression (Rodgers et al., 2015). In a group of middle-aged males with MDD treated with an SSRI, plasma estradiol was lower than in healthy men, and estradiol was negatively correlated with depression severity

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(Arinami et al., 2021).

Sex hormones may play a key role in mood regulation as testosterone and estrogen receptors are abundant in brain regions associated with mood and anxiety, such as the hippocampus and amygdala (McHenry et al., 2014; Behl, 2002). Studies have found lower levels of androgen receptors and higher levels of estrogen receptors in the post-mortem brains of men and women who suffered from MDD (Wang et al., 2008). One way sex hormones may play a role in depression could be through modulation of the serotonergic neurotransmission (Cahill, 2006; Cosgrove et al., 2007). We, therefore, investigate whether plasma testosterone and estradiol are associated with depressive symptoms and SSRI treatment response.

SSRIs are commonly associated with sexual side effects, including decreased libido and erectile and orgasmic dysfunction (Schweitzer et al., 2009); and more so in men than women (LeGates et al., 2018; Frackiewicz et al., 2000). Higher testosterone and estradiol levels are associated with better erectile function and libido in healthy men (Finkelstein et al., 2013). Lower plasma testosterone has been observed in depressed men with SSRI-induced sexual dysfunction compared to those without dysfunction (Safarinejad, 2008). In depressed men and women taking SSRIs and experiencing sexual side-effects, exogenous testosterone treatment improves sexual function (Amiaz et al., 2011; Fooladi et al., 2014). Low pretreatment hormone levels may precipitate sexual side-effects, and therefore, we also examine associations with sexual SSRI side-effects in this dataset.

2. Materials and methods

2.1. Study population and treatment

We included data from 26 Caucasian patients of male sex with moderate to severe depression from the NeuroPharm-1 study, an open-label clinical trial uncovering potential biomarkers for antidepressant treatment response (Köhler-Forsberg et al., 2020). The study was pre-registered on clinicaltrials.gov (NCT02869035) and approved by the Danish Data Protection Agency (04711/RH 2016-163), the Committees on Health Research Ethics in the Capital Region of Denmark (H15017713), and the Danish Medicines Agency (NeuroPharmNP1, EudraCT-number 2016-001626-34).

Participants did not suffer from any severe somatic illness confirmed by basic somatic screening, including routine blood testing. One patient had Type 1 diabetes mellitus treated with an insulin pump. This was regarded as a stable and well-treated chronic illness, and the participant was not excluded. Drugs of abuse and anabolic steroid abuse were screened during the interview. No screened participants were excluded based on somatic illness or abuse (including anabolic steroids).

Educational level was scored on a 5-point scale, with the highest commenced degree being the highest (one point for no vocational degree and five points for more than four years of higher university education).

2.2. Plasma sex hormones

Plasma estradiol and testosterone were measured using Elecsys® Estradiol III kits on Cobas 8000 e602 module and Elecsys® Cobas 8000, e801 module (Roche Diagnostic) in a routine hospital laboratory at Rigshospitalet, Copenhagen University Hospital. The lower limit of quantitation for plasma estradiol level was 0.09 nM.

2.3. Treatment

The treatment was 10–20 mg of escitalopram daily. If no treatment response was seen after four weeks, escitalopram was substituted for 60–90 mg duloxetine. Serum levels confirmed drug adherence after eight weeks.

2.4. Depression severity, treatment effect and side-effects

Depression symptom severity was assessed with the Hamilton Depression Rating Scale 17 items ($HAMD_{17}$) interview before treatment. We used the 6-item subscale ($HAMD_6$) to assess the treatment effect because $HAMD_6$ primarily captures core depressive symptoms and is particularly well-suited to monitor antidepressant treatment response over time (Østergaard et al., 2016). The primary clinical outcome was categorical treatment status at eight weeks, classified as either 'responder' or 'non-responder' defined as a $\geq$50% or $<$50% reduction in $HAMD_6$. The secondary clinical outcome was the relative change in $HAMD_6$ score ($\Delta HAMD_6$ in % from $HAMD_6$ pretreatment) at weeks 8 and 12.

SSRI side-effects were assessed at weeks 1, 2, 4, 8 and 12 with a standardized semi-structured interview for clinician-rated evaluation of SSRI side effects, i.e., Udvalg for Kliniske Undersogelser Side Effect Rating Scale (UKU) (Bech et al., 2018), in which the clinician-rated the reported side-effects and categorised them as unlikely, possible and likely SSRI-induced. The rating was performed by the clinician, who also assessed depression severity and individuals' symptoms using the Hamilton Depression Rating Scale. The presence of SSRI-induced nausea was evaluated during the initial two weeks. Sexual side-effects were evaluated during weeks 8–12.

2.5. Statistics

Estradiol values below the quantitation limit of 0.09 nM were imputed in the 0.05–0.09 nM range from a normal distribution based on the mean and SD of quantified estradiol measures. The lower estradiol of 0.05 was chosen based on the lowest measured value in a male sample of similar age using a similar assay with a lower detection limit (Perfalk et al., 2017).

We performed multiple linear regression models adjusted for age and BMI to investigate whether plasma sex hormones were associated with depression severity before treatment initiation. Post hoc, we tested Spearman's rank correlations between testosterone and the individual items in the $HAMD_{17}$ (adjusted for age and BMI).

We compared plasma sex hormones between treatment responders and non-responders using Welch t-tests. We performed multiple linear regression models of plasma sex hormones between treatment responders and non-responders adjusted for age and BMI. We performed multiple linear regression models adjusted for age and BMI to investigate whether plasma sex hormones were associated with %-change in $HAMD_6$ at weeks 8 and 12 from baseline.

We compared plasma sex hormones between men experiencing treatment side-effects and those not experiencing side-effects using Welch t-tests. We performed multiple linear regression models of plasma sex hormones between treatment responders and non-responders adjusted for age and BMI. A logistic regression analysis was conducted to examine the relationship between sex hormones and sexual side effects during 8–12 weeks of treatment, with age and BMI as covariates.

In group comparisons of estradiol using Welch *t*-test on imputed values below the limit of quantitation, we also used Gehan's test to account for left-censored estradiol values.

Statistical tests and graphs were done in *R* 4.2.2 and JASP (Version 0.18.1) (JASP -team, 2023). We used the *R* package BuyseTest for Gehan's test (Ozenne and Peron, 2022; Buyse, 2010). We present two-sided 95% confidence intervals (CI) and *p*-values unadjusted for multiple comparisons with p-values < 0.05 considered statistically significant. Graphical abstract made with Biorender.com.

3. Results

3.1. Testosterone levels were associated with depression severity, and in particular with vegetative symptoms

26 men aged 18–48 with moderate to severe MDD part of the study; 14 were first-episode depression (Table 1). Depression severity measured by $HAMD_{17}$ was positively associated with plasma testosterone levels (β = 0.22, CI: 0.04; 0.39, $p = 0.016$, Fig. 1). Post-hoc, we examined if testosterone mapped onto the core symptoms of depression as indexed by $HAMD_6$, which was not the case (β = −0.001, CI -0.08; 0.08, $p = 0.97$, Fig. 1). However, we identified that the vegetative symptoms, loss of weight, appetite, and sleep, were driving the positive association (Table 2). The sum of the items related to *gastrointestinal somatic symptoms,* i.e., *loss of appetite* (*rho* = 0.71, CI: 0.47; 0.87, $p = 0.000099$) and *weight loss* (*rho* = 0.54, CI: 0.07; 0.81, $p = 0.006$), was positively correlated to testosterone (*rho* = 0.74, CI: 0.43; 0.81, $p=0.000037$) and similarly was the sum of the three *insomnia* items (*rho* = 0.45, CI: 0.07; 0.75, $p = 0.027$, Table 2).

Estradiol was not associated with depression severity measured by $HAMD_{17}$ ($p=0.40$), and estimated correlations between estradiol and $HAMD_{17}$-items are available in Supplementary Table 1.

3.2. Sex hormones were not associated with antidepressant outcomes of treatment

We investigated the putative association between pretreatment sex hormone levels and treatment response. 15 of the 24 men responded to SSRI treatment at week eight (≥50% $HAMD_6$ reduction). We found no significant difference in testosterone and estradiol between treatment responders and non-responders at week eight (mean escitalopram dose 17 mg (SD: 4.2 mg), Supplementary Table 2, Supplementary Table 3, $p > 0.28$), and results were similar when adjusted for age and BMI ($p > 0.44$). Pretreatment testosterone and estradiol were not associated with the change in depressive symptoms from baseline at week 8, measured as %-change in $HAMD_6$ from baseline (Estradiol: β = −159, CI: 645; 328, $p = 0.50$; Testosterone: β = −0.67, CI: 2.84; 1.51, $p = 0.53$). The results did not change when a non-adherent patient and a patient who switched from escitalopram to duloxetine at week 4 were removed.

However, at week 12 we observed that the 5 men who had not responded to treatment had 6 nM (CI: 1.6; 10.4) lower pretreatment testosterone than the treatment responders (Supplementary Table 3, Hedges' $g = 0.98$, $p=0.0097$). When adjusted for age and BMI, this difference was estimated to 5.2 nM (CI: 2.5; 12.9), $p = 0.17$). Pretreatment testosterone and estradiol were not associated with the change in depressive symptoms from baseline at week 12, measured as %-change in $HAMD_6$ from baseline (Estradiol: β = −97, CI: 538; 343, $p = 0.50$; Testosterone: β = −1.63, CI: 3.45; 0.19, $p = 0.076$). The results were similar when data from the non-adherent patients were removed.

Table 1
Demographic and clinical profile.

Men with MDD ($n = 26$)	Mean (SD)	Range
Demographic		
Age (years)	27.1 (7.8)	18–48
Educational level[a]	3.6 (2.2)	1–5
Clinical		
Body mass index (kg/m²)	24.5 (3.9)	17.1–35.8
Hamilton Depression Rating scale 17 items	22.1 (3.3)	18–31
Hamilton Depression Rating 6 items	12.0 (1.5)	8–15
Recurrent depression, n (%)	12 (46%)	
Hormone levels		
Plasma testosterone (nM)	17.0 (7.3)	5.3–33.0
Plasma estradiol > 0.09 nM (nM)	0.123 (0.026)	0.10–0.17
Plasma estradiol ≤ 0.09 nM, n (%)	15 (57%)	

MDD: Major Depressive Disorder.
[a] Education level was only available for 18 men.

3.3. Sex hormones were associated with sexual side-effects across treatment

We investigated the putative association between pretreatment sex hormone levels and sexual side-effects of escitalopram reported at weeks 8 and 12 (Fig. 2). One patient was switched to duloxetine at week four due to lack of effect and presence of side-effects, i.e., high degree of erectile, ejaculatory dysfunction, and decreased libido, which was not present during the SNRI and was included in the analysis as escitalopram-induced SSRI.

The 11 men who had erectile and ejaculatory dysfunction as a plausible escitalopram side-effect had lower mean testosterone (13.8 ± 4.7 nM) and estradiol (0.084 ± 0.023 nM) than the men without the side-effects (19.6 ± 8.7 nM and 0.110 ± 0.034 nM), which was borderline significant for testosterone (Hedges' $g = 0.81$, t (18.9) = 2.1, $p = 0.050$) and significant for estradiol using Welch's tests (Hedges' $g = 0.86$, t (20.9) = 2.2, $p = 0.039$). We used Gehan's test to account for the left-censored estradiol values below the quantitation limit ($p = 0.056$).

The four men with decreased libido during treatment also had erectile and ejaculatory dysfunction. They had lower testosterone (11.4 ± 7.9 nM) and estradiol (0.066 ± 0.014 nM) compared to all the men with decreased libido as a side-effect (18.1 ± 7.9 nM and 0.104 ± 0.030 nM), which was significant for testosterone (Hedges' $g = 1.6$, t (20.1) = 3.275, $p = 0.004$) and estradiol (Hedges' $g = 1.6$, t (10.3) = 4.0, $p = 0.003$). This difference remained significant for estradiol in Gehan's test ($p = 0.00011$).

Greater pretreatment estradiol was associated with less likelihood of sexual side effects (β = −34.3, CI: 68.6; −0.01, $p = 0.0499$) with an area under the ROC curve (AUC) of 0.748 (CI: 0.545, 0.952) suggesting moderate discriminative ability of estradiol within the sample (Fig. 3). Pretreatment testosterone showed a similar non-significant relationship (β = −0.12, CI: 68.6; −0.01, $p = 0.096$) with an AUC of 0.713 (CI: 0.499; 0.928, Supplementary Fig. 1).

Given the association between pretreatment plasma testosterone and gastrointestinal symptoms and insomnia, we also examined pretreatment hormone levels and SSRI-induced nausea and insomnia commonly associated with treatment initiation. Testosterone and estradiol levels were not different in patients experiencing or not experiencing nausea or insomnia during the initial two weeks of escitalopram treatment (Supplementary Table 4, Hedgs' *g*-values <0.41, p-values ≥0.30).

4. Discussion

This study of young adult men with MDD demonstrates that testosterone is associated with depression severity; a phenomenon driven by the magnitude of vegetative symptoms. Although sex hormones in the unmedicated state do not appear to affect antidepressant treatment outcome, low pretreatment testosterone and estradiol levels were associated with the emergence of sexual side-effects.

4.1. Testosterone and vegetative symptoms in depression

Our initial finding that testosterone levels were positively associated with depression severity ($HAMD_{17}$) contrasts with the literature associating low testosterone with depressive symptoms (McHenry et al., 2014). Indeed, this association was absent for the core depressive symptoms captured by the $HAMD_6$-subscale. Rather, we found that the vegetative symptoms, *weight loss*, *gastrointestinal symptoms* (primarily *loss of appetite*), and *insomnia*, to a smaller degree, drove the positive association between testosterone and $HAMD_{17}$. This is consistent with the metabolic consequence of weight loss, which can lead to elevated testosterone (Corona et al., 2013). In line with our findings for melancholic-like symptoms, a recent study found lower testosterone in men with atypical MDD versus melancholic MDD and healthy controls (Rodgers et al., 2015). Atypical MDD is characterised by reversed vegetative symptoms, i.e., oversleeping (hypersomnia), overeating

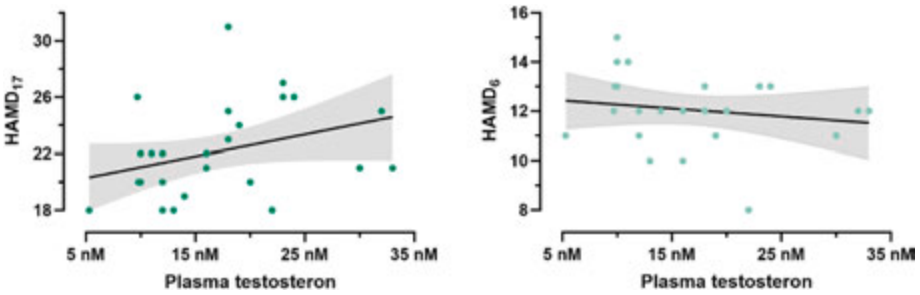

Fig. 1. Plasma testosterone and depression severity in unmedicated men with MDD
Plots of unadjusted associations with 95% CI between plasma testosterone and depression severity (i.e., $HAMD_{17}$) and core depressive symptoms (i.e., $HAMD_6$) in unmedicated men with MDD. $HAMD_{17}$ was positively associated with plasma testosterone in a linear regression adjusted for age and BMI ($\beta = 0.22$, CI: 0.04; 0.39, $p = 0.016$), while $HAMD_6$ was not ($\beta = -0.001$, CI -0.08; 0.08, $p = 0.97$).

Table 2
Plasma testosterone and $HAMD_{17}$-items in men with MDD.

#	$HAMD_{17}$ items	*rho*	95% CI	*p*-value
12.	*Gastrointestinal somatic symptoms*[a]	0.71	0.47; 0.87	0.000099
17.	*Loss of weight*	0.54	0.07; 0.81	0.006
4.	*Insomnia: early in the night*	0.35	−0.17; 0.72	0.097
5.	*Insomnia: middle of the night*	0.30	−0.12, 0.60	0.16
6.	*Insomnia: early hours of the morning*	0.30	−0.24; 0.62	0.16
7.	*Work and activities*[b]	0.28	−0.17; 0.65	0.18
14.	*Sexual dysfunction*	0.25	−0.17; 0.60	0.25
16.	*Insight*	0.20	−0.22; 0.61	0.34
13.	*General somatic symptoms*[b]	0.13	−0.30; 0.56	0.54
11.	*Anxiety (somatic)*	0.04	−0.36; 0.46	0.84
2.	*Feelings of guilt*[b]	0.02	−0.41; 0.45	0.95
8.	*Retardation*[b]	−0.09	−0.50; 0.34	0.67
15.	*Hypochondriasis*	−0.15	−0.54; 0.35	0.50
9.	*Agitation*	−0.16	−0.55; 0.32	0.45
3.	*Suicide*	−0.21	−0.62; 0.21	0.32
10.	*Anxiety (psychic)*[b]	−0.27	−0.67; 0.16	0.21
1.	*Depressed mood*[b]	−0.37	−0.71; 0.10	0.072
Vegetative Symptoms				
Sum of items 12 and 17: weight and appetite loss		0.74	0.43; 0.81	0.000037
Sum of items 4, 5, and 6: insomnia items		0.45	−0.07; 0.75	0.027

Partial Spearman's rank correlations (conditioned on age and BMI) with 95% confidence intervals (CI).
[a] Gastrointestinal somatic symptoms include loss of appetite, heavy feeling in the abdomen and constipation.
[b] $HAMD_6$-subscale items.

(hyperphagia), and weight gain, thus indirectly aligning with the positive relationship between testosterone and vegetative symptoms we observe.

Although testosterone levels decline over age, appetite and weight disturbance were not associated with age of onset in the STAR*D trial (Baez and Heller, 2020). In the same study, sleep disturbance was the

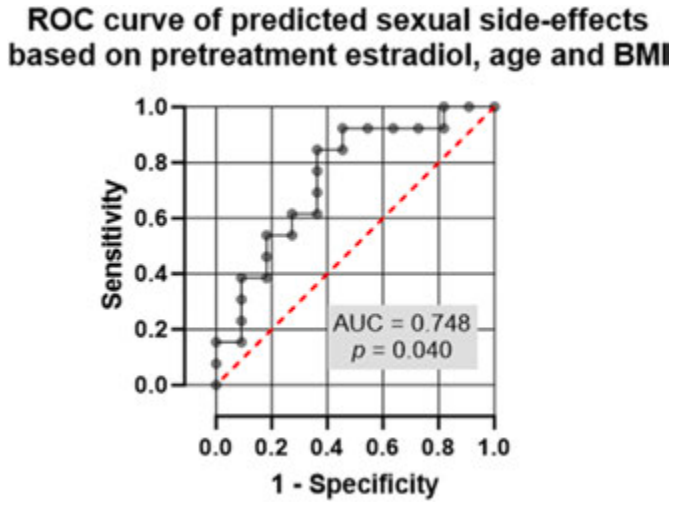

Fig. 3. Pretreatment estradiol and sexual side-effects
Predicted probability of sexual side-effect during 8–12 weeks based on logistic regression of treatment based on pretreatment plasma estradiol, age and BMI.

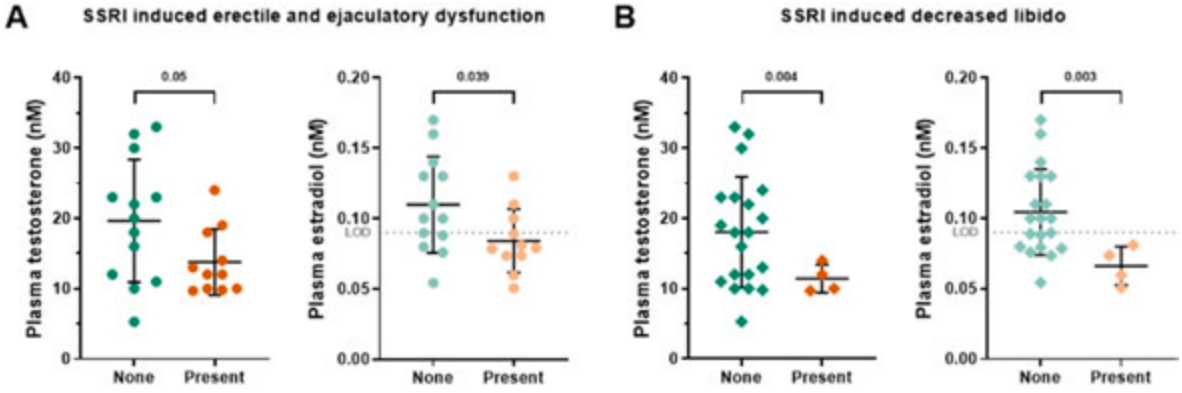

Fig. 2. Pretreatment sex hormone levels and sexual side-effects of escitalopram
Pretreatment plasma testosterone and estradiol in the men with MDD reporting and not reporting A) erectile and ejaculatory dysfunction and B) decreased libido at weeks 8 and 12 evaluated by a clinician as being due to escitalopram treatment. Values below the limit of quantitation (LOQ) were imputed, and *p*-values computed by Welch's t-tests are displayed in the plots. When accounting for the left-censored estradiol values below the LOQ using Gehan's test, the lower estradiol in men with erectile and ejaculatory dysfunction was only borderline significant (A, $p = 0.056$), but remained highly significant in decreased libido (B, $p = 0.00011$).

only symptom that increased in severity with age of onset (Baez and Heller, 2020). Nonetheless, this phenomenon occurs in both individuals without depression and those diagnosed with it (Ancoli-Israel and Ayalon, 2006; Park et al., 2013), indicating that this observation may not be directly linked to depression itself, but instead might be a secondary effect of age-related sleep issues (Baez and Heller, 2020).

In a recent study of 823 middle-aged men (18–65 years of age), androgen levels were not associated with MDD, but androstenedione and dehydroepiandrosterone-sulphate were positively associated with some individual depressive symptoms, including pessimism, suicidality and weight loss (Wit et al., 2021).

That these precursor hormones were associated with specific symptoms of depression (Wit et al., 2021) suggests that the positive association between testosterone and $HAMD_{17}$ and levels reflects biochemical consequences of somatic symptoms (e.g., depression-induced weight loss), i.e., that high testosterone is associated with depression is due to processes of depression rather than causing the symptoms.

Nevertheless, existing literature indicates that subgroups of men with MDD have lower testosterone levels, i.e., men with treatment-resistant depression, melancholic depression, comorbid HIV infection, dysthymia, and men with MDD and above the age of 60 years compared to healthy men (Johnson et al., 2013). In hypogonadal men with depression, testosterone replacement appears to improve depressive symptoms (Zarrouf et al., 2009; Wang et al., 2004; Elliott et al., 2017) again supporting a link between testosterone levels and depression. Also, pharmacologically induced hypogonadism in healthy men can induce low mood (Schmidt et al., 2004; Bloch et al., 2006). On the other hand, high exogenous testosterone due to anabolic steroid abuse can also cause affective disorders; however, depressive symptoms are most prominent during withdrawal (Talih et al., 2007; Pope and Katz, 1994).

We observed a positive association between testosterone and depression severity in a group of young men (mean age 27). Age is essential since testosterone declines in old age and is up to 40% lower at 70 than at 40 years of age (Seidman, 2007), and the prevalence of MDD also rises with age (Johnson et al., 2013). In men aged 50–89 years, lower bioavailable testosterone is associated with more depressive symptoms (Barrett-Connor et al., 1999). These findings might not apply to our study in young men, and as such, the discrepancy in the direction of association between testosterone and depression compared to older men is not surprising. Similarly, the effects of testosterone may be age-specific, as low testosterone levels seem to be associated with suicidal behaviour in older men, while high testosterone might be associated with suicidal behaviour in young men (Walther et al., 2017).

4.2. Sex hormones and SSRI antidepressant effects and side-effects

Antidepressants such as SSRIs affect the serotonergic system, but their interactions with the hormonal system remain elusive. Estrogen (in high doses) has been shown to exert antidepressant effects. Similarly, in low doses, as an augmentation in menopausal women with depression, it also appears to exert an antidepressant treatment response (Keating et al., 2011). In male rats, the antidepressant-like effects of testosterone indeed seem to be partly mediated by its conversion to estradiol (Carrier et al., 2015).

We do not detect an association between pretreatment plasma testosterone or estradiol with SSRI treatment response in our small sample at week 8. However, a trend for lower pretreatment testosterone was seen in depressed men who still did not respond at week 12, highlighting the question of whether low testosterone indexes treatment resistance. Interestingly, most antidepressants influence levels of sex hormones, but studies are inconsistent about how sex hormones are affected (Pavlidi et al., 2021). Thus, it is unknown whether antidepressants influence both the peripheral and the brain levels of testosterone and estrogen and whether they impact the treatment response of antidepressants. Unfortunately, we did not have access to sex hormone measurements during treatment and, therefore, cannot elucidate any such antidepressant-induced changes in sex hormone levels.

Typically, sexual dysfunction and reduced libido that are often part of the depressive symptomatology ease as overall depressive symptoms ameliorate during antidepressant treatment (Weber et al., 2023). Nonetheless, sexual side-effects such as erectile and orgasmic dysfunction in men are frequent and disturbing adverse event of SSRIs that influences a patient's desire to continue long-term antidepressant treatment (Clayton et al., 2002), and indeed, men tend to report greater sexual side-effects than women (LeGates et al., 2018; Frackiewicz et al., 2000).

We found that pretreatment sex hormone levels did not correlate with sexual dysfunction, but nevertheless, low pretreatment levels were associated with SSRI sexual side-effects in men. This is in line with the observation of lower sex hormones (testosterone, follicle-stimulating hormone, and luteinising hormone) in depressed men with SSRI sexual side-effects compared to those without sexual side-effects (Safarinejad, 2008), and in depressed men and women on SSRIs who experience sexual dysfunction, exogenous testosterone treatment improves sexual function (Amiaz et al., 2011; Fooladi et al., 2014).

However, this is possibly mediated by testosterone's aromatisation into estradiol since men with decreased estradiol who reported low libido improved by estrogen administration (Finkelstein et al., 2013). Estrogen can sustain libido and affect the abundance of serotonin receptors and transporters in the brain, modulating mood, mental state, cognition, and emotion. Estradiol levels have been demonstrated to correlate with the incidence and severity of erectile dysfunction (Schulster et al., 2016; Chen et al., 2020). This supports our finding that low pretreatment estradiol is a more informative marker than testosterone of expected SSRI-induced erectile and ejaculatory dysfunction. This is further supported by estrogen α and β receptors and aromatase being widely expressed in the male brain and genital tract (Vignozzi et al., 2008; Cooke et al., 2017). Estrogen receptors are abundant in the epididymal cauda, suggesting a role in regulating epididymal function and contractility during ejaculation (Corona et al., 2012). Animal experiments find that aromatase inhibitors reduce ejaculatory and sexual activity in males, which could be improved by estradiol administration (Cooke et al., 2017). Similarly, estrogen α receptor and aromatase knockout mice have decreased sexual activity and lack ejaculation altogether (Bakker et al., 2004; Ogawa et al., 1997).

SSRI-induced decreased libido was associated with pretreatment sex hormone levels. Estrogen receptors are expressed in the serotonergic neurons and target neurons, by which estrogen can support serotonergic neurotransmission by modulating gene expressions, including serotonin receptors (Bethea et al., 2002). Our group has shown a positive correlation between endogenous plasma estradiol levels, but not testosterone levels, nd cortical 5-HT2A receptor binding in men (Frokjaer et al., 2010). When serotonin binds to 5-HT2A receptors in the cortex, limbic system, hypothalamus, and midbrain, sexual desire is inhibited with subsequent induction of refractoriness and sexual satiety (Pfaus, 2009). Low pretreatment sex hormones could thus be associated with low 5-HT2A receptor availability, resulting in greater 5-HT2A receptor saturation during SSRI elevated synaptic serotonin and thus inhibited sexual desire and decreased libido.

Whether SSRI-induced sexual dysfunction is mediated solely by a serotonergic effect on the brain or a possible change in plasma sex hormone levels is unclear. Nonetheless, we observed that the patients with sexual dysfunction had lower pretreatment sex hormone levels.

Wit et al. question the clinical relevance of single measurements of circulating androgens as testosterone in men with MDD as they were not associated with MDD and do not aid in diagnosis (Wit et al., 2021). We propose that low sex hormone levels may be a clinically relevant risk factor for SSRI-induced sexual dysfunction in men and a possible tool for stratified or personalised treatment. Weight loss intervention studies have pointed towards improvement in sexual dysfunction with reduced weight (Larsen et al., 2007). Our data also support that personalised treatment should consider the potential benefits of weight loss on sexual

health outcomes of treatment when relevant, e.g., for patients with atypical depression.

4.3. Methodological considerations

The presented findings should be interpreted in light of at least three limitations. First, we have data from a smaller group of MDD participants, and as the analyses are not adjusted for multiple comparisons, they should be considered explorative and not be firmly interpreted until replicated. Second, this study used total testosterone and estradiol levels, which do not directly represent the biologically active form. Only 1–3% of total testosterone and estradiol are unbound from sex hormone-binding globulin and albumin. Yet, total testosterone and estradiol levels correlate with their free hormone levels (Johnson et al., 2013; Mounib et al., 1988; Södergard et al., 1982). Therefore, the total levels serve as a valid proxy for the biologically active levels. Third, the men in this study are young (mean age 27); thus, our results most likely do not generalise to older men or men in andropause.

4.4. Summary

However, we find a positive association between testosterone and overall depression severity, driven by vegetative symptoms, i.e., weight loss, gastrointestinal symptoms, and insomnia.

Pretreatment sex hormone levels were not associated with antidepressant treatment outcomes but were associated with sexual side-effects of SSRI treatment. Our results highlight the possibility that plasma estrogen and testosterone can serve as potential prognostic biomarkers of SSRI-induced sexual dysfunction in men, which should be evaluated in larger patient groups.

Data availability

The data analysed in this study is subject to the following licenses/restrictions: A Cimbi database application is required to access the dataset through the following procedures: https://cimbi.dk/index.php/documents/category/3-cimbi-database.

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Author contributions

KRJ, MRA, and VGF contributed to the conception and design of the study. KRJ and MRA wrote the initial draft of the manuscript. KRJ and MRA performed the statistical analyses. All authors contributed to the interpretation of the analyses and the manuscript revision and have approved the submitted version.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nsa.2024.104050.

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Supporting information for:

Psychoneuroendocrine profiles of unmedicated men with Major Depressive Disorder and associations to treatment effects and sexual side-effects

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Supplementary Table 1: Correlations of plasma estradiol and $HAMD_{17}$-items in men with MDD

Partial Spearman's rank correlations (conditioned on age and BMI) with 95% confidence intervals (CI) based on 1000 bootstrap replicates. **HAMD$_6$-subscale items.*

#	$HAMD_{17}$ item	*rho*	CI 95%	*p*-value
12.	*Gastrointestinal somatic symptoms*	0.39	-0.03; 0.81	0.059
17.	*Loss of weight*	0.29	-0.14; 072	0.16
14.	*Sexual dysfunction*	0.24	-0.21; 0.62	0.27
13.	*General somatic symptoms**	0.20	-0.24; 0.62	0.34
2.	*Feelings of guilt**	0.18	-0.26; 0.61	0.41
16.	*Insight*	0.18	-0.23; 0.61	0.41
6.	*Insomnia: early hours of the morning*	0.16	-0.29; 0,58	0.44
11.	*Anxiety (somatic)*	0.12	-0.33; 0.58	0.57
4.	*Insomnia: early in the night*	0.12	-0.29; 0.58	0.58
7.	*Work and activities**	0.12	-0.33; 0.51	0.58
5.	*Insomnia: middle of the night*	0.09	-0.35; 0,50	0.67
8.	*Retardation**	0.09	-0.37; 0.48	0.67
15.	*Hypochondriasis*	-0.13	-0.55; 0.42	0.56
3.	*Suicide*	-0.21	-0.59; 0,20	0.32
1.	*Depressed mood**	-0.34	-0.65; 0.07	0.11
9.	*Agitation*	-0.40	0.73; 0.48	0.051
10.	*Anxiety (psychic)**	-0.43	-0.75; 0.00	0.037

Supplementary Table 2: Medication doses during the treatment protocol

Weeks	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Escitalopram (*n*)	26	26	25	24	23	23	23	23	21	21	21	20
Mean dose (mg)	9.8	10.4	14.8	15.0	16.7	17.2	16.5	17.0	17.6	17.6	17.6	17.5
Median dose (mg)	10	10	15	15	20	20	20	20	20	20	20	20
Duloxetine (*n*)					1	1	1	1	3	3	3	4
Mean dose (mg)					60	60	60	60	70	70	80	75
Median dose (mg)					60	60	60	60	60	60	90	75

Supplementary Table 3: Pretreatment hormone levels in treatment responders and non-responders

Comparison of pretreatment hormone levels in men classified as either 'responder' or 'non-responder' at eight weeks of treatment defined as a ≥50% or <50% reduction in $HAMD_6$. Lowest escitalopram dose was 10 mg and median escitalopram dose was 20 mg at week 8 and 12. The Duloxetine doses at week 12 were two on 60 mg and two on 90 mg Welch t-tests. [1]When treatment response group differences for Estradiol are compared using Gehan's t-test on non-imputed data to account for left-censoring the groups remain non-significant (*p*-values >0.61). [2]One man with a pretreatment plasma testosterone of 18.0 nM showed non-response to treatment at week 8 and dropped out of the study at week 12. If he is assumed to continue to not response to treatment (Week 12 calculation A) or respond to treatment (Week 12 calculation B), there is still a significant 5 or 6 nM lower plasma testosterone in non-responders.

Treatment response	**Non-response** mean (SD)	**Response** mean (SD)	**Mean difference** mean (CI 95%)	**Hedges' *g***	***p*-value**
Week 8	n=9	n=15			
Testosterone (nM)	15.2 (6.8)	18.6 (7.8)	3.4 (-9.7; 2.9)	0.45	0.28
Estradiol (nM)	0.093 (0.037)	0.101 (0.029)	0.014 (-0.039; 0.22)	0.25	0.56[1]
Week 12	n=5	n=19			
Testosterone (nM)	12.2 (2.3)	18.2 (8.1)	6.0 (1.6; 10.4)	0.98	0.0097
Estradiol (nM)	0.101 (0.042)	0.097 (0.030)	0.004 (-0.047;0.055)	0.10	0.85[1]
Week 12 - A[2]	n=6	n=19			
Testosterone (nM)	13.2 (3.1)	18.2 (8.1)	5.0 (0.4; 9.7)	0.69	0.0359
Week 12 - B[2]	n=5	n=20			
Testosterone (nM)	12.2 (2.3)	18.2 (7.9)	6.0 (1.8; 10.2)	0.83	0.0074

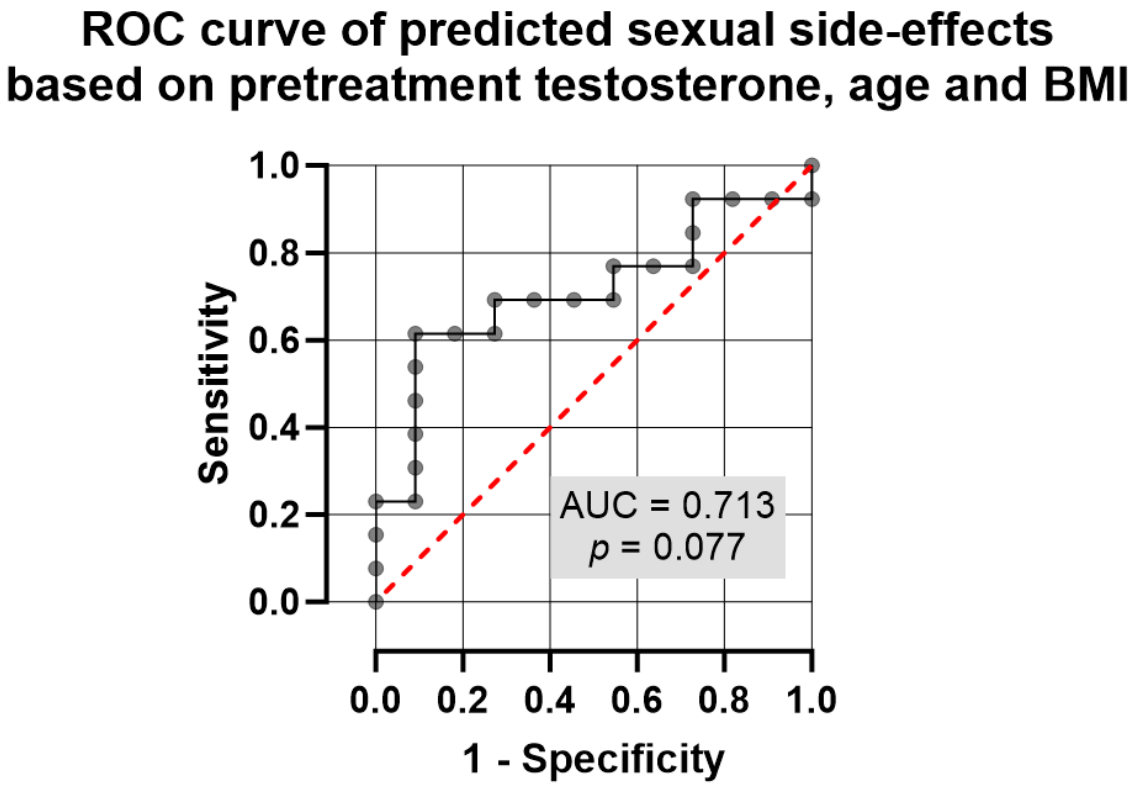
Supplementary Table 4: Pretreatment hormone levels and SSRI-induced nausea and insomnia at treatment week 2

Comparison of pretreatment hormone levels in men experiencing nausea and insomnia during the initial two weeks of escitalopram treatment. Welch t-tests.

Side-effect during initial two weeks	**Absent** mean (SD)	**Present** mean (SD)	**Mean difference** mean (CI 95%)	**Hedges' *g***	***p*-value**
Nausea	n=14	n=10			
P-testosterone (nM)	17.3 (8.1)	16.5 (7.4)	0.8 (-5.8; 7.4)	0.10	0.80
P-estradiol (nM)	0.098 (0.031)	0.099 (0.034)	0.001 (-0.029; 0.027)	0.03	0.94
Insomnia	n=16	n=8			
P-testosterone (nM)	15.8 (7.5)	19.4 (7.7)	-3.6 (-10.7; 3.5)	0.47	0.30
P-estradiol (nM)	0.097 (0.037)	0.100 (0.201)	-0.003 (-0.027; 0.021)	0.10	0.80

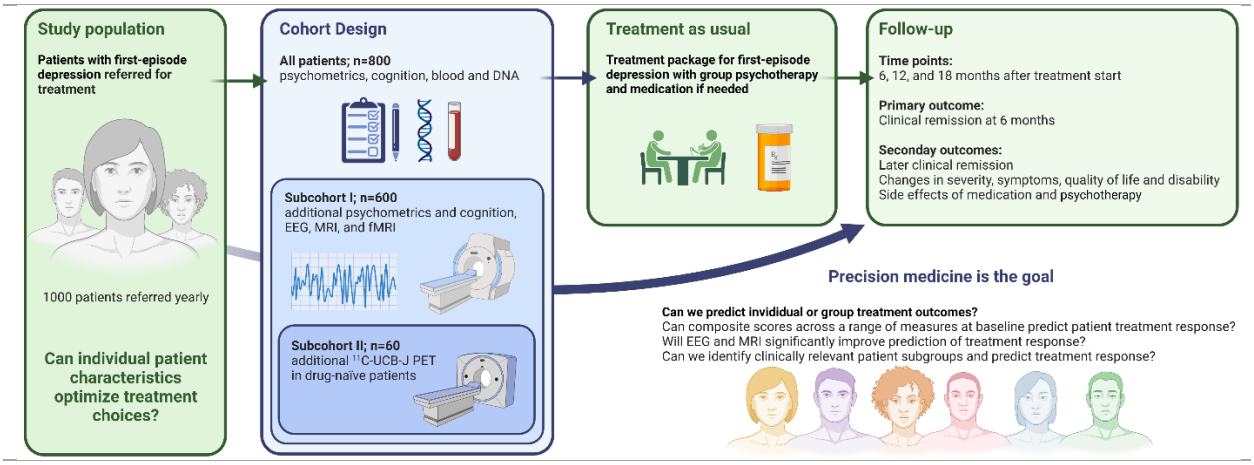
Supplementary Figure 1: Pretreatment testosterone and sexual side-effects

Predicted probability of sexual side-effect during 8-12 weeks based on logistic regression of treatment based on pretreatment plasma testosterone, age and BMI.



Study IV

Graphical abstract



BMC Psychiatry

STUDY PROTOCOL

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Deep phenotyping towards precision psychiatry of first-episode depression — the Brain Drugs-Depression cohort

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Abstract

Background Major Depressive Disorder (MDD) is a heterogenous brain disorder, with potentially multiple psychosocial and biological disease mechanisms. This is also a plausible explanation for why patients do not respond equally well to treatment with first- or second-line antidepressants, i.e., one-third to one-half of patients do not remit in response to first- or second-line treatment.

To map MDD heterogeneity and markers of treatment response to enable a precision medicine approach, we will acquire several possible predictive markers across several domains, e.g., psychosocial, biochemical, and neuroimaging.

Methods All patients are examined before receiving a standardised treatment package for adults aged 18–65 with first-episode depression in six public outpatient clinics in the Capital Region of Denmark. From this population, we will recruit a cohort of 800 patients for whom we will acquire clinical, cognitive, psychometric, and biological data. A subgroup (subcohort I, $n=600$) will additionally provide neuroimaging data, i.e., Magnetic Resonance Imaging, and Electroencephalogram, and a subgroup of patients from subcohort I unmedicated at inclusion (subcohort II, $n=60$) will also undergo a brain Positron Emission Tomography with the [^{11}C]-UCB-J tracer binding to the presynaptic glycoprotein-SV2A. Subcohort allocation is based on eligibility and willingness to participate. The treatment package typically lasts six months.

Depression severity is assessed with the Quick Inventory of Depressive Symptomatology (QIDS) at baseline, and 6, 12 and 18 months after treatment initiation.

The primary outcome is remission (QIDS ≤ 5) and clinical improvement ($\geq 50\%$ reduction in QIDS) after 6 months. Secondary endpoints include remission at 12 and 18 months and %-change in QIDS, 10-item Symptom Checklist, 5-item WHO Well-Being Index, and modified Disability Scale from baseline through follow-up. We also assess psychotherapy and medication side-effects.

We will use machine learning to determine a combination of characteristics that best predict treatment outcomes and statistical models to investigate the association between individual measures and clinical outcomes.

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We will assess associations between patient characteristics, treatment choices, and clinical outcomes using path analysis, enabling us to estimate the effect of treatment choices and timing on the clinical outcome.

Discussion The BrainDrugs-Depression study is a real-world deep-phenotyping clinical cohort study of first-episode MDD patients.

Trial Registration Registered at clinicaltrials.gov November 15th, 2022 (NCT05616559).

Keywords Major depressive disorder, Biomarker, Synaptic density, PET, MRI, SSRI, EEG, Cognition, Psychotherapy, Precision medicine

Background

Depression is the leading cause of disability worldwide, with an estimated 322 million people currently suffering from a depressive episode [1]. We know that pharmacotherapy and psychotherapy, individually or combined, can efficaciously treat Major Depressive Disorder (MDD) [2, 3].

Nevertheless, more than half of patients do not respond to the initial medication prescribed [4] and require sequential trials of different treatments that may not alleviate symptoms, resulting in treatment-resistant MDD [5, 6]. After the first depressive episode, approximately half of patients will experience a relapse or re-emergence of depressive symptoms [7]. Current treatment guidelines emphasise depression severity as the primary or exclusive element on which to base treatment choice [8]. Despite numerous studies on definitions, frequency, and determinants of relapse and treatment-resistant MDD [5, 9], we continue to lack clinically relevant markers to guide treatment choice in first-episode MDD.

MDD is highly heterogeneous, with complex disease mechanisms and diverse biological and psychological causes [10], complicating drug discovery and optimal patient care. Deep phenotyping with a subsequent analysis to stratify patients' response to treatment is an essential tool to resolve this heterogeneity and move away from a 'one-size-fits-all' approach [11]. The prospect of this strategy is that patients instead can be stratified based on objective and replicable psychosocial, biochemical and/or neurobiological characteristics. Interventions would ideally be tailored to these individual profiles and thereby maximise clinical response [12]. Furthermore, precision medicine enables the optimisation of resource allocation within patient populations, e.g., by efficient allocation of relapse prevention and interventions to high-risk patient groups.

Previous studies have focused primarily on individual course predictors from just one or two domains, e.g., genetic [13], structural and functional neuroimaging [14–18], electroencephalographic biomarkers [19, 20], blood markers [21], cognitive disturbances [22, 23], and demographic or clinical data [24, 25]. So far, no single marker has proven reliable enough to be implemented in clinical practice [19, 26]. A multimodal approach to depression is essential as the disorder's aetiology is likely multi-causal. That is, prediction models that combine multiple candidate biomarkers might have more predictive utility [24], but must still be generalizable and cross-validated across different cohorts [27]. Other issues include small sample sizes, using data from randomised control trials, lack of clinically and biologically relevant predictive markers, short follow-up time, and poorly validated and biased prediction models [9, 26, 28].

So far, most studies have focused on predictors of response to pharmacological antidepressant treatment without examining the effect of other commonly used treatment modalities such as psychotherapy. Factors such as safety, tolerability, and the effect of the patient's preferences (e.g., dislike of medication or psychotherapy) on overall treatment outcome are also underexplored [26]. Together with substantial methodological variance across studies, these limitations have hampered the ability to draw ecologically relevant and generalizable conclusions from meta-analyses [26, 29, 30].

With this cohort-based study, we will generate a large longitudinal observational multi-modality clinical dataset that allows for a thorough analysis of which phenotypic components enable the best participant stratification for optimal treatment success. We will leverage the Danish healthcare system, which provides standardised treatment packages for all patients. Currently, group cognitive-behavioural therapy (CBT) constitutes the backbone of the treatment package with possibilities for initiation or changes in pharmacotherapy. The treatment package is nationally uniform and designed by Mental Health Services in the Capital Region. The treatment package was introduced in 2013 and revised in 2017 based on clinical and patient experience. However, while the treatment components in the package are research and evidence-based, the treatment effect in real life has not been examined.

The standardised treatment package system enables us to characterise newly diagnosed patients with depression and monitor their clinical response to different treatment modalities, combinations, and paths within the treatment package and deviations from it. Further, the

individual Danish person identification number (CPR), combined with several national health and civil registers, will allow us to obtain additional information and follow the patients' clinical progress longitudinally [31].

Objectives

The primary objective of this study is to identify single or composite biomarkers that can reliably identify clinical profiles of MDD and predict their treatment outcomes.

To achieve this aim, we will establish a large, single-site cohort of adult patients diagnosed with first-episode MDD and referred for a treatment package for first-episode depression in secondary care, phenotyping patients before treatment initiation. All patients in the cohort ($n=800$) will contribute with basic clinical, cognitive, psychometric, and biological data, i.e., genetics and blood biochemistry. A subcohort (subcohort I, $n=600$) will provide Magnetic Resonance Imaging (MRI) and Electroencephalogram (EEG), and a second subcohort (subcohort II, $n=60$) of subcohort I with patients unmedicated at inclusion will also undergo a Positron Emission Tomography (PET) brain scan with the presynaptic PET tracer [^{11}C]-UCB-J. Clinical depression symptom severity is assessed with the Quick Inventory of Depressive Symptomatology (QIDS) at baseline (T_0), and 6 (T_1), 12 (T_2) and 18 (T_3) months after treatment initiation (Fig. 1). The treatment package typically lasts six months.

To examine disease trajectories, we will combine collected data with information from Danish national health and social registers, allowing us to further characterise and follow the patients before, during, and after treatment. Together, this might enable us to identify clinically relevant biomarkers and suggest treatment response algorithms, aiding treatment choices and improving patient care (Figure 1).

Furthermore, synaptic loss and deficits in functional connectivity are hypothesized to contribute to depressive symptoms, i.e., cognitive dysfunction, anhedonia, and anxiety and treatment effect. Therefore, we will examine the relationship between presynaptic density and cognitive dysfunction, depressive symptoms, and treatment effect in antidepressant naïve patients (subcohort II) and compare their cerebral presynaptic density to healthy controls (HC).

Hypotheses

The entire cohort

Primary hypotheses:

1.1 Clinical, cognitive, psychometric, genetic, and blood biomarker measures at inclusion can predict clinical remission (defined as QIDS ≤ 5) at the first follow-up.

1.2 Clinical, cognitive, psychometric, genetic, and blood biomarker measures at inclusion can predict clinical improvement (a $\geq 50\%$ reduction in QIDS from pretreatment) at the first follow-up.

Secondary hypotheses:

1.3 Composite scores across a range of clinical, cognitive, psychometric, genetic, and blood biomarker measures at inclusion can cluster patients into MDD subgroups associated with treatment trajectories and outcomes.

1.4 Clinical, cognitive, psychometric, genetic, and blood biomarker measures at inclusion are associated with clinical outcome defined as a change in QIDS.

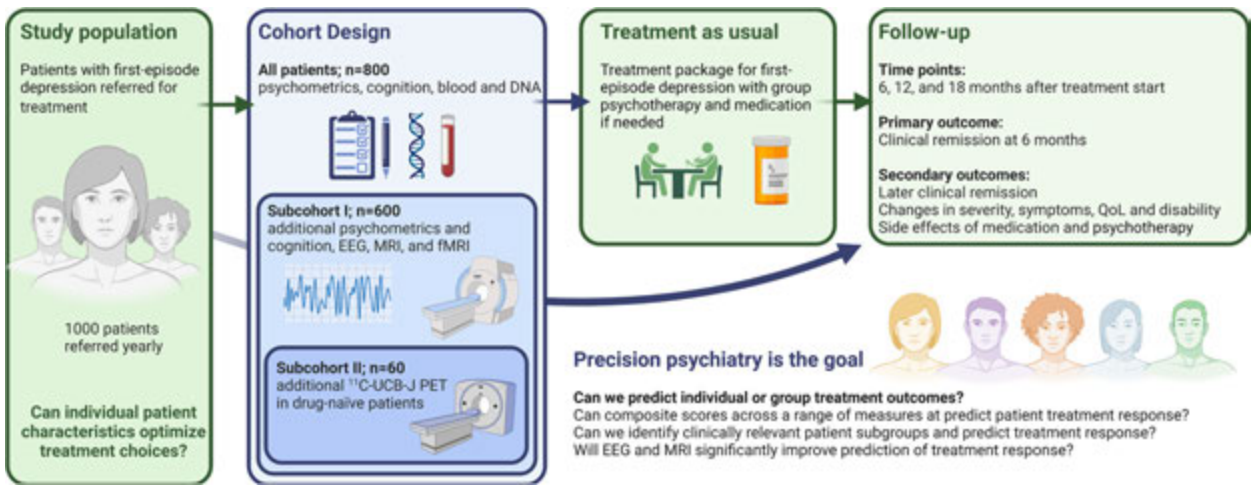

Fig. 1 Study design of the BrainDrugs-Depression prospective cohort. *QoL* Quality of life. Figure created by K.H.R.Jensen with BioRender.com

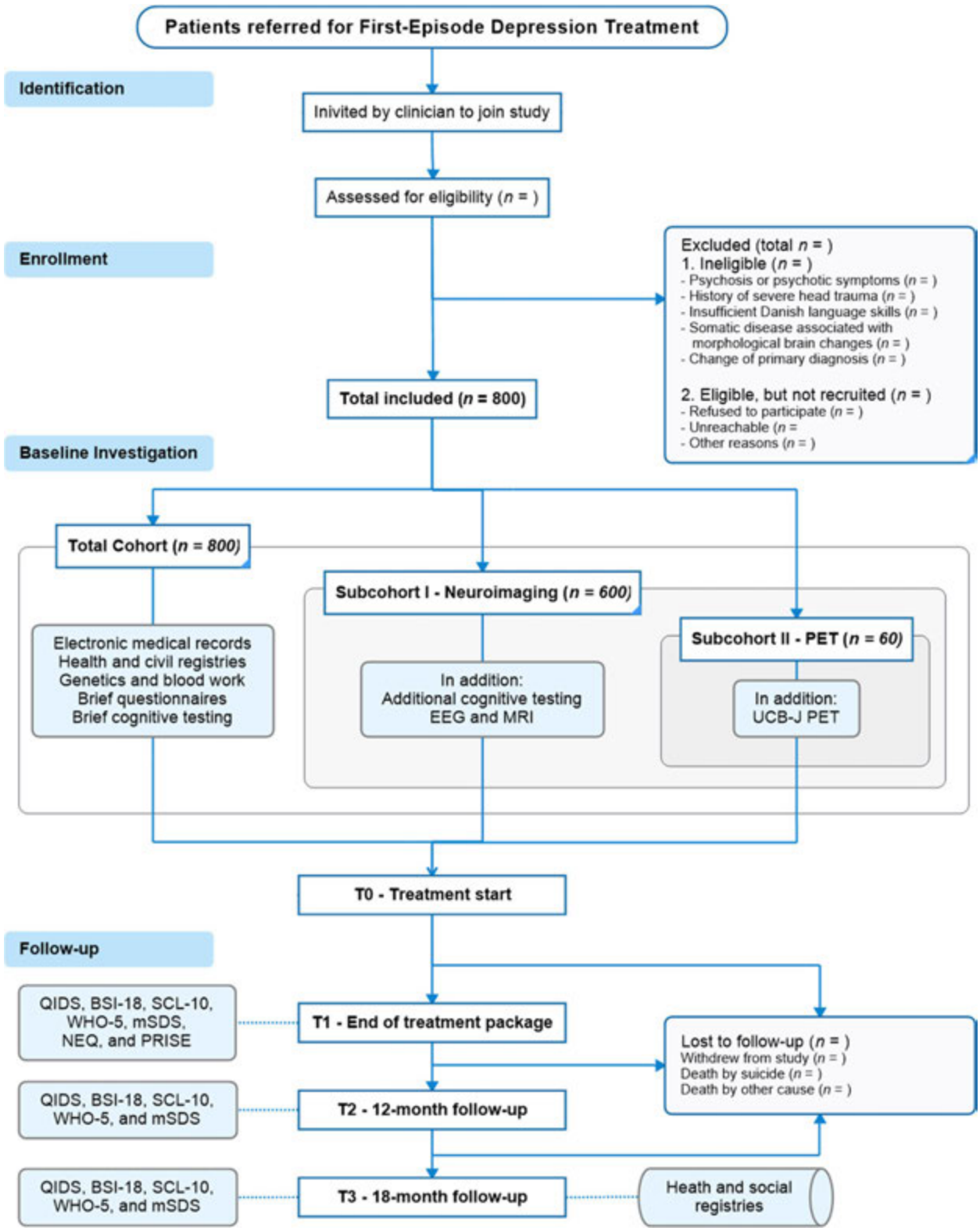

Fig. 2 Flow diagram (STROBE) of the BrainDrugs-D cohort study. CVD: (Center for Visitation og Diagnostik) the central referral center within the mental health services in the capital region of Denmark, QIDS-SR: Quick Inventory of Depressive Symptomatology – Self Report, BSI-18: 10 item Brief Symptom Inventory, SCL-10: 10 item Symptom Checklist, mSDS: Modified Sheehan Disability Score, WHO-5: World Health Organisation—Five Well-Being Index (WHO-5), NEQ: Negative Effects Questionnaire, PRISE: Patient-Related Inventory of Side Effects

1.5 Path analysis of baseline patient characteristics and treatment tracks can uncover causal paths for clinical improvements, i.e., estimate the effect of treatment on clinical outcomes.

Subcohort I

Primary hypotheses:

2.1 MRI, fMRI, and EEG patterns at inclusion may be associated with depressive phenotypes.
2.2 Adding EEG, MRI, and fMRI measures at inclusion to the classifier model (defined in hypotheses 1.1 and 1.2) may significantly improve the prediction of clinical remission and improvement.

Secondary hypotheses:

2.3 Adding EEG, MRI, and fMRI measures at inclusion to the composite score (defined in hypothesis 1.3) may significantly improve the clustering of patients into MDD subgroups.

Subcohort II

Primary hypotheses:

3.1 Cerebral [^{11}C]-UCB-J binding is lower in patients with MDD than in healthy controls.
3.2 Domain-specific cognitive function correlates positively with [^{11}C]-UCB-J binding in associated cortical and subcortical areas.

Secondary hypotheses:

3.3 Depression severity, anxiety, and anhedonia correlate with [^{11}C]-UCB-J binding in associated cortical and subcortical areas.
3.4 Addition of [^{11}C]-UCB-J binding, EEG, and MRI measures at inclusion to the composite score (defined in hypotheses 2.1) can significantly improve the prediction of clinical improvement and remission beyond clinical, cognitive, psychometric, fluid biomarker, EEG, and MRI measures in antidepressant naïve patients.

Methods and design

Setting

The Capital Region of Denmark has a population of 1.6 million people. Patients in the Capital Region of Denmark are referred by their general practitioner (GP) or other treatment providers to a central diagnostic and referral centre within the mental health services that yearly assesses 20.000 referrals. About 4000 patients are further evaluated in person and diagnosed by the centre.

Five mental health centres in the region provide treatment packages for first-episode depression and will include participants in the study. The Mental Health Centre Amager and the Copenhagen centre consisting of two clinics located in the City of Copenhagen and treat approximately half of all patients, whereas Ballerup and Glostrup treat approximately a third of patients in the surrounding suburb (Sup. Figure 2). The Psychiatric Centre Northern Zealand treats approximately 16% of patients and is located north of Copenhagen, in a region of intermediate urbanisation with individual municipalities classified as rural.

Study population

We aim to establish a cohort of 800 patients referred to the Danish treatment packages for unipolar first-episode, non-psychotic depression during 2021–2025. We recruit patients from all six clinics in the region. Each clinic receives approximately 100–250 treatment referrals yearly, and approximately 1100 patients are referred yearly. Approximately 80% of referrals are sent directly to the clinics. Patients are recruited during evaluation at the central diagnostic and referral centre or the first consultation in the clinics. Approximately 88% of referrals result in treatment package initiation.

During 2019–2020, 37% of patients were on an antidepressant (usually the selective serotonin reuptake inhibitor (SSRI) Sertraline from their GP) when starting the treatment package, and 54% of patients ended the treatment package on an antidepressant medication. 13% of patients were transferred to a treatment package for a different primary diagnosis group, e.g., generalised, social anxiety, post-traumatic stress disorder, emotionally unstable personality, avoidant personality disorder, eating disorder or obsessive–compulsive disorder. 20% dropped out of treatment. 5% of patients were hospitalised during their treatment package; hospitalization does not preclude the continuation of the treatment package.

The treatment package is a program with manualised psychotherapy in groups of eight patients as the core treatment module together with psychoeducation for the patient and relative (Sup. Table 1). In brief, a treatment package consists of 15–18 h: 2–3 h of initial workup followed by 6 h of individual therapy or 12 sessions of 2 h group therapy (8 patients per group); 1–2 h of engagement and psychoeducation of relatives; 1–5 h of medication clinic; and 2 h of relapse prevention. The program is designed around group-based CBT, but clinics also offer alternatives to CBT, e.g., psychodynamic and schema therapy, and groups for specific demographics, e.g., men

or adolescents, and individual therapy. Medication is available as needed.

The research and assessment at baseline for recruited participants is conducted at the Neurobiology Research Unit (NRU) at the Copenhagen University Hospital Rigshospitalet and followed by clinicians from the Mental Health Centre Copenhagen who are not involved in the patient's treatment.

Inclusion and exclusion criteria for patients

Patients between 18 and 65 years of age referred to a treatment package for single-episode depression will be recruited (Table 1) with minimal exclusion criteria to recruit representative adult outpatients who would typically receive treatment in routine practice (Table 1), of which the majority are women (71%) and aged 18–35 (68%) (S. Figure 1). Patients over 65 (approximately 0.7% of the target population) are excluded because of potential age-related cognitive decline, concomitant medical conditions, or medications that could interact with assessments or treatment (S. Figure 1). Allocation into the subcohorts is based on eligibility, e.g., MRI compatibility, scheduling, and patient willingness to participate.

The primary depressive episode, consistent with the International Statistical Classification of Diseases and Related Health Problems version 10 (ICD-10) criteria for MDD without psychotic features (F32.1, F32.2, F32.8 and F32.9), is confirmed by a specialist in psychiatry at the central diagnostic and referral centre.

Inclusion and exclusion criteria for healthy controls

Data from HCs for comparisons to patients with MDD are available on-site from recent and concurrent projects, stored in the Cimbi database described in [32] including the BrainDrugs-Epilepsy study [33]. Apart from psychiatry-related issues (e.g., no current or history of mental illness or unstable somatic condition), the HC meet the same inclusion and exclusion criteria as required for patients.

Data collection

The patients will undergo a multi-modal investigative program at inclusion and will be followed up after treatment and 12 and 18 months after treatment initiation with questionnaires assessing clinical status (Figs. 1 and 2). Apart from follow-up measures and information extracted from patient files and Danish health and social registries, all data collection will occur before the patient starts treatment.

Baseline

Questionnaires Questionnaires will be completed through a secure, web-based survey system hosted by the research centre so that participants can complete questionnaires electronically, either at home or during their visit to the research centre. Online questionnaires are sent via a national secure mail platform used by citizens in regular correspondence with public institutions and the health care system.

Measures include several salient domains in the clinical characterisation of the patient, among others, assessments of demographics (e.g., ethnicity, education, and marital status); medical and psychiatric history; depressive symptoms and impact of depression behaviour and day-to-day life; treatment preferences and expectations, life experiences; and a broad range of state and trait psychometrics. Some questionnaires will only be given to patients in subcohorts I-II (Table 2).

Table 1 Inclusion and exclusion criteria for patients

Patient inclusion criteria:
• Fulfilment of ICD-10 diagnostic criteria for a primary depressive episode (i.e., not secondary to known organic or other psychiatric disorder) • Referral to a treatment package for single-episode depression • Age between 18 and 65 years
Exclusion criteria:
• Psychosis or psychotic symptoms • History of severe head trauma involving hospitalization or unconsciousness for more than 5 min • Known, substantial structural brain abnormalities • Insufficient Danish language skills to complete questionnaires and cognitive testing
Additional exclusion criteria for subcohort I:
• Severe somatic disease • Contraindications for MRI (e.g., metal implants, claustrophobia, or back problems)
Additional exclusion criteria for subcohort I:
• Use of psychotropic drugs • Exposure to radioactivity > 10 mSv within the last year • Pregnancy or breastfeeding

Table 2 Questionaries Additional questionnaires for the subcohort I-II only are in bold

Symptom profile and Severity	Cognitive style	Upbringing and life history	Functioning and quality of life
Inventory of Depressive Symptomatology – self-report (IDS-SR) [34]	Mentalisation Questionnaire (MZQ) [35]	Online Stimulant and Family History Assessment Module (OS-FHAM) [11]	Cognitive Complaints in Bipolar Disorder Rating Assessment (COBRA) [36]
Dimension of Anger Reactions (DAR-5) [37]	Ruminative Response Scale (RRS) [38]	Child abuse and trauma scale (CATS) [39]	Modified Sheehan Disability Score (mSDS)
Generalised Anxiety Disorder 7-item (GAD-7) [40]	Perth Alexithymia Questionnaire (PAQ) [41]	**Parental Bonding Instrument (PBI)** [42]	WHO 5 wellbeing index (WHO-5)
Cohen's Perceived Stress Scale (PSS) [43]	**Mindful Attention Awareness Scale (MAAS)** [44]	**Stressful Life Events (SLE)** [45]	Changes in Sexual Functioning Questionnaire (CSFQ) [46]
Brief Symptom Inventory (BSI) [47]	**Short form of Metacognitions Questionnaire (MCQ-30)** [48]		Questions from the Copenhagen Aging and Midlife Biobank (CAMB) [49]
Symptom checklist (SCL-10) [50]	**Coping Self-Efficacy Scale (CSES)** [51]		Revised Sociosexual Orientation Inventory (SOI-R) [52]
Snaith-Hamilton Pleasure Scale (SHAPS) [53]			
Pittsburgh Sleep Quality Index (PSQI) [54]			

Medical records and registry data Detailed medical information about previous illness, medication usage, hereditary dispositions, drug, tobacco, and alcohol intake will be acquired for all participants through interviews, self-report questionnaires, electronic medical records (EMR), and registry data.

Data extracted from the EMR will include treatment codes from the MDD treatment package, dates for treatment package start and completion, psychiatric comorbidities; and standard clinical blood work (e.g., HBA1c, TSH, CRP, and cholesterol). In addition, hormonal contraceptive and psychotropic medication prescription and usage (from 1995 onward) will be extracted from the Danish National Prescription Registry [55, 56]. This information includes prescribed medication and dosage and when the patient redeems a prescription. We will retrieve information on lifetime comorbidity from The Danish National Patient Registry (DNPR) [57]. From the Medical Birth Registry, we will obtain data on maternal and maternal perinatal health [58]. We will also collect information on alcohol and drug abuse treatment from the National Registry of Alcohol Treatment and Registry of Drug Abusers Undergoing Treatment. From the social registers in Statistics Denmark, we add data on marital status, occupational history, ethnicity, and educational level [59].

Cognitive testing All patients are assessed with a ~ 1-h neuropsychological test battery, including 'cold' (emotion-independent) cognitive tasks indexing reaction time; psychomotor speed; verbal learning and memory; working memory; and executive functions, as well as 'hot' (emotion-dependent) cognitive tasks from the Danish version of the EMOTICOM test-battery indexing emotion recognition; emotion detection; and moral emotions in social situations [60].

Patients in subcohorts I-II will complete an additional ~ 1 h of testing with tasks assessing mental flexibility, verbal fluency, and visuospatial learning and memory (see Additional questionnaires for the subcohort I-II only are in bold.

Table 3 for a complete overview of all cognitive tasks). In addition, patients' subjective experiences of cognitive disturbances will be assessed by the Cognitive Complaints in Bipolar Disorder Rating Assessment (COBRA) questionnaire [36].

Patients in subcohorts I-II will complete an additional ~ 1 h of testing with tasks assessing mental flexibility, verbal fluency, and visuospatial learning and memory (see Additional questionnaires for the subcohort I-II only are in bold.

Blood biochemistry, genetics, and gene expression Venous blood samples will be collected for serum, plasma, DNA, and RNA extraction. Identifying biomarkers relevant to the course of depression is an area of research that is evolving rapidly. Thus, based on an ongoing critical literature review, the search for and analysis of specific biomarkers may change during the study period. Currently, the blood biomarkers include inflammation parameters (e.g., high sensitivity CRP) [61, 62] and neurotrophic factors (e.g., BDNF and S100B) [63, 64].

Table 3 Cognitive testing before treatment

Cognitive Test	Cognitive Domaine
Whole Cohort	
Simple Reaction Time task (SRT)	Reaction time
Trail Making Test A & B	Psychomotor speed/executive function
Symbol Digit Modality Task (SDMT)	Psychomotor speed/working memory
Letter-Number Sequence (LNS)	Working memory
D-KEFS Color-Word Interference Test (Stroop)	Executive function
Rey Auditory Verbal Learning Test (RAVLT)	Learning/memory
EMOTICOM Emotional Recognition Task (ERT)	Emotion recognition accuracy
EMOTICOM Emotional Intensity Morphing Task (IMT)	Emotion perceptual detection threshold
EMOITCOM Moral Emotions Task (MET)	Social cognition: guilt and shame
Additional testing in the subcohorts	
D-KEFS Verbal Fluency	Executive function
Rey Complex Figure Test (RCFT)	Visuo-spatial learning/memory
Probabilistic Reversal Learning task	Learning within a feedback context
Screen for Cognitive Impairments in Psychiatry—Depression (SCIP-D)	Memory, working memory, vocabulary, psychomotor speed

DNA from blood samples will be used for microarray-based genotyping of MDD candidate genes, genes of relevance for MDD (e.g., rs41271330, 5-HTTLPR, COMT, and BDNFval66met), drug metabolism (e.g., CYP2D6, CYP2C19, UGT1A1, ABCB1, ABCC1) and to compute polygenic risk scores in all participants after genome-wide genotyping in the future. DNA will also be used for epigenetic analysis, and circular extrachromosomal DNA, a form of decomposed free DNA [65], will be extracted and characterised. RNA will be extracted for gene transcription profiles using microarray or TAG-based methods (mRNA and microRNA).

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Gene analyses will be based on a priori models of genetic variations known to modulate pharmacotherapy and psychotherapy responses. The results will be used to calculate a polygenic risk score for diagnosis and treatment response and meta-analyses with established polygenic risk scores for MDD and those currently developed for anxiety and anxiety disorders, including treatment response [66].

Electroencephalogram In subcohort I, we will record resting state EEG and event-related potentials (ERPs) with simultaneous two-lead electrocardiography (ECG) to measure autonomic nervous system activation. EEG will be recorded using a 256-channel HydroCel Sensor Net system (MagstimEGI, USA) at 1000 Hz, where the vertex electrode serves as the reference. Impedances across all electrodes will be kept below 50 kΩ. ECG will be acquired at 1000 Hz using a Physio 16 device (MagstimEGI, USA). EEG/ERP recording: resting EEG (6 min eyes closed and eyes open), two-tone auditory oddball and the LDAEP tasks.

MRI Participants in subcohort I will undergo MRI using a Siemens 3-Tesla Magnetom Prisma scanner. High-resolution structural T1-, T2-, and diffusion-weighted MR images will be acquired as well as ultra-fast functional magnetic resonance encephalography (MREG) asses cardiovascular brain pulsations [67]. Resting-state and task-based blood oxygen level-dependent (BOLD) fMRI scans will be acquired to measure related brain function. To assess distributed and intrinsic brain functional connectivity patterns, we will acquire a resting-state fMRI scan (10 min), during which participants are asked to close their eyes, let their minds wander and not fall asleep. Participants will complete established tasks to assess processes involved in cognition and mood, e.g., the Cyberball task, a ball-tossing game during which the participant interacts with fictitious characters to simulate

experiences of social inclusion, exclusion, rejection and ostracism [68]. Trained research personnel will instruct participants on how to perform all tasks.

PET imaging Participants in subcohort II will undergo PET neuroimaging with [^{11}C]-UCB-J, which binds to the presynaptic vesicle glycoprotein 2A (SV2A). SV2A is ubiquitously and homogeneously located in synapses across the brain and allows for the determination of SV2A binding and presynaptic density in the brain [69, 70]. However, due to the ubiquitous distribution of SV2A, there is no proper reference region in the brain, and we, therefore, measure the arterial input function. PET scanning is conducted using a High-Resolution Research Tomography (HRRT) PET scanner (CTI/Siemens, Knoxville, TN, USA). First a 6 min transmission scan, then an intravenous bolus of < 400 MBq of [^{11}C]-UCB-J administered over 20 s followed by a 90-min dynamic acquisition ($256 \times 256 \times 207$ voxels; $1.22 \times 1.22 \times 1.22$ mm).

Treatment and life events

As the study is observational, we will not interfere with or delay treatment. EMR and registry data during treatment and until the 18 months from inclusion will be extracted to inform the individual treatment path, i.e., what treatment the individual patient received, e.g., amount of individual psychotherapy sessions and when, timing, type and dose of medication and switches, and participation in group therapy. Using registry data, we can also follow life events, e.g., change of residence, divorce, and employment.

Follow-up

Patient-reported outcome measures assessing depression symptom severity and clinical status are sent via the national secure mail platform at three time points: 6, 12 and 18 months after treatment start (Table 4). Complete registry follow-up is done at 18 months as well.

Primary and secondary outcome measures The primary clinical outcomes are categorical: *improvement* defined as ≥ 50% reduction in QIDS and *remission* after the treatment package defined as a QIDS ≤ 5. The secondary outcome is *change* in depression severity as measured by QIDS.

Tertiary outcome measures The ten-item depression and anxiety symptom checklist (SCL-10) [50, 71], well-being measured by WHO-5 [50, 72], and disability measured by a modified Sheehan Disability Scale (mSDS) are the established treatment effect parameters by the Mental Health Services of the Capital Region of Denmark.

Tertiary endpoints are three measurements of psychosocial remission defined as a WHO-5 score of > 49, an SCL-10 score of < 26 and an mSDS score of < 10. Additional tertiary clinical endpoints are changes in wellness (WHO-5), disability (mSDS), and symptomatology on the Brief Symptom Inventory 18 (BSI-18) and the SCL-10.

Two questionnaires assessing the negative effects of psychological and antidepressant treatment will be sent at the first follow-up, at the end of the treatment package. We use the Patient-Reported Inventory of Side-Effects (PRISE), originally developed and validated in Danish and also used in the STAR*D trial [71, 73]. The questionnaire is omitted if the patient has not been or is not on antidepressant medication. We use the short 20-item form of the Negative Effects Questionnaire (NEQ) to assess adverse and unwanted events in psychological treatment, i.e., new symptoms, dependency, stigma, hopelessness, and the experienced quality of treatment [74, 75]. Both baseline characteristics and treatment experiences, e.g., negative effects on the NEQ, will be used to investigate reasons for CBT and treatment package drop-out.

Registry follow-up After the last 18-month follow-up, the study dataset will be sent to Statistics Denmark with a list of all invited participants to allow a non-participant analysis and long-term follow-up. The study data will be linked with data from the Danish Civil Registration System [76] e.g., the DNPR [57], the Danish National Prescription Registry [55, 56], and other registries indexing, e.g., hospital admittance, diagnosis- and treatment codes, prescription medications, employment status, living

Table 4 Follow-up Measurements [a]Omitted if the patient did not receive antidepressant medication

Measures	6 months	12 months	18 months
Quick Inventory of Depressive Symptomatology (QIDS-SR)	X	X	X
BSI-18, SCL-10, WHO-5 and mSDS	X	X	X
Patient-Reported Inventory of Side-Effects (PRISE)[51][a]	X	X	X
Negative Effects Questionnaire (NEQ)	X		

situation (e.g., partner information), and income. We will also examine diagnostic stability [77], e.g., change of primary diagnosis from first episode depression to an anxiety or personality disorder or later recurrent depressive episode or conversion to bipolar affective disorder.

Statistical analyses

We plan to investigate the outlined hypotheses using both data- and hypothesis-driven approaches. We will publish more specific analysis plans on the web (e.g., PROSPERO) before starting detailed analyses.

Data-driven analyses

We will use machine learning algorithms to determine a combination of baseline characteristics that best predict treatment outcomes. In contrast to hypothesis-driven analyses, data-driven machine learning frameworks enable us to identify novel associations between patient characteristics and treatment response and interactive effects of complementary patient characteristic information in prediction. Recent interest in machine learning approaches to prediction is to develop *classifiers* that combine a range of patient-level data to provide a patient-level prediction of treatment response. The most common classifiers are based on patient characteristics or biomarkers assessed before treatment initiation [78, 79]. This information may be used in clinical settings to select a specific treatment if it is predicted to have a higher chance of success or to suggest that a patient may generally be treatment-resistant, which could justify earlier use of second-line therapies [26].

In the present study, we will first use machine learning to train classifiers based on broad non-imaging data collected from all patients in the cohort. Neuroimaging data from EEG, MRI, and PET in subcohorts I and II will subsequently be included, enabling us to make meaningful statements about the marginal improvement in model performance with or without specific neuroimaging measures. This is critical for optimising patient care with costs associated with data acquisition. Following the recommendations for best practice [80, 81], we will employ randomized k-fold nested cross-validation, i.e., splitting the collected data into training and testing datasets that reported model performance measures are not upwardly biased due to data leakage.

We will use latent class analysis to identify different MDD subgroups that share characteristics measured at baseline (hypotheses 1.2 and 2.3) [25, 82, 83]. To investigate if adding neuroimaging data improves clustering (hypothesis 2.3), we will compare the heterogeneity of change on clinical outcome, i.e., mean and variance.

Hypothesis-driven analyses

To answer the hypothesis-driven research questions, i.e., hypotheses 3.1 – 3.4, we will use appropriate parametric models, including multiple linear regression or linear latent variable models to investigate group differences in [^{11}C]-UCB-J binding between patients and HCs and multiple linear regression to determine associations between cognitive scores and [^{11}C]-UCB-J binding in patients. We will adjust for age and sex, as our current data on HCs with [^{11}C]-UCB-J are of equal sex distribution with a mean age of approximately 30 years old, which is not expected for the patient sample expecting to be predominately women and below 25 years old (Supplementary Fig. 1). Furthermore, dependent on additional funding, we will include more HCs and attempt to match their age distribution with the patients better.

We will also use statistical models such as logistic regression to investigate associations between individual measures and dichotomous clinical outcomes (e.g., the association between early childhood trauma and treatment response) when testing secondary hypotheses. Lastly, we will assess the associations between baseline patient characteristics, treatment events, and clinical outcomes using path analysis, enabling us to estimate the effect of treatments over time on the clinical outcome.

Power calculation

Data-driven analyses

To answer primary hypotheses 1.1 – 3.1, we will use a machine learning approach as outlined above. Statistical power calculations are not well adapted to data-driven machine learning model frameworks because any such calculation depends on broad assumptions about model structure and feature space. However, the number of patients in the cohort ($N=800$) represents one of the largest cohorts to date, looking to determine prediction classifiers in MDD [84].

To limit the strain on patients as well as costs, we collect neuroimaging data in a subset of patients ($N=600$) and not in the entire cohort. By contrast, questionnaire and EMR data collection is relatively cheap, fast, and non-invasive. Therefore, this data's potential predictive value need not be very high to be clinically relevant. Acquisition of neuroimaging data, i.e., MR and PET, is costly and time-consuming and must exhibit higher predictive value to be relevant as a clinical tool. Thus, fewer patients are needed in Cohorts II-III to determine the relevance of neuroimaging biomarkers in MDD, as the power of these biomarkers would have to be large enough to be detectable even in smaller patient samples.

Hypothesis-driven analyses

To answer hypothesis 3.1, we will use [^{11}C]-UCB-J PET data from healthy controls ($N=40$) currently available from the Cimbi database with [^{11}C]-UCB-J PET data collected from patients in the PET subcohort II (expected $n=60$). These sample sizes will provide us with a statistical power of 0.99 to detect group differences with a Cohen's d effect size of 0.95 as reported in previous study [85]. With the samples size and a statistical power of 0.80 we can defect a group difference with a Cohen's d 0.58 or higher using a significance threshold of $p \leq 0.05$ in a two-sample t-test. The previous study by Holmes et al. (2019) reported group differences in [^{11}C]-UCB-J binding between healthy controls and a small cohort with mixed psychiatric diagnoses, including MDD. Based on their findings in frontal cortex binding (which were similar to other brain regions), our study is statistically powered to detect group differences in binding of ~ 6.8%; notably, Holmes et al. found a group difference of 12.5% in this region, so our study should be adequately powered.

To answer hypothesis 3.2, we will have a statistical power of 0.8 to detect a significant association between [^{11}C]-UCB-J binding and cognitive scores in the PET subcohort II equivalent to a correlation coefficient of $r \geq 0.35$ at a statistical significance threshold of $p \leq 0.05$.

Ethics and data availability

The study is conducted according to the principles of the seventh revision of the Declaration of Helsinki (2013) and was reviewed and approved by the Committees on Health Research Ethics in the Capital Region of Denmark (reference number: H-20083013). Before undertaking any study-related procedures, each participant receives verbal and written explanations of study aims, methods, potential hazards, and benefits from investigators and provides written informed consent. All participating patients are asked to consent to disclose relevant information from their EMR to extract health-related information relevant to the study.

Data management and monitoring during the study adhere to the rules protecting personal data. Paper-based material (e.g., cognitive test results) will be stored in a secured archive. Identifiable electronic data files will be stored in password-secured files behind a firewall per regulations.

Biological material will be coded with a unique identification number. Access to de-identification keys is restricted to authorised personnel only and stored in a temporary biobank located in secured areas in the laboratory facility. The biomaterial will later be analysed in batches to reduce noise, and potential extra material after the end of the study will be transferred to the CIMBI biobank [32]. All biological material will ultimately be anonymised after 15 years after the end of the study.

The study results will be presented following relevant reporting guidelines, i.e., STROBE and TRIPOD [86, 87]. After publication of results from our primary hypothesis, the data can upon request be made available to other scientists or consortia, through the Cimbi database (or similar platform).

Discussion

MDD is a brain disorder with etiological heterogeneity in the interplay between biological, social, psychological, and behavioural factors and pathophysiology with several neurobiological mechanisms. In the BrainDrugs-Depression study, we employ a broad, multi-modal biopsychosocial characterisation of each patient. This, combined with a large sample size, follow-up over 18 months, and further complete follow-up in the social- and health registries, offers a unique opportunity to uncover potential single or combined predictors of treatment outcome, identify MDD subtypes, advance the understanding of MDD aetiology, and map neurobiological predictors of treatment response; ultimately paving the way for a precision medicine approach for optimised MDD treatment.

Further, the ability to track and follow patients in the Danish health care system and registers provides a unique opportunity to obtain large amounts of data collected independent of the patient's mood and enables us to perform sensitivity analyses and account for selective participation and attrition. The study also makes it possible to track the long-term consequences of various degrees of treatment response and point to areas where the current treatment could be improved to obtain better prognostic outcomes.

Cognitive dysfunction is a core dimension in MDD, both in first- and multiple-episode patients [88] and mediates a significant degree of psychosocial impairment and reduction in workplace productivity [89]. The presence of cognitive dysfunction may also significantly impact medication [90] and psychotherapy [91] and may even persist even past remission of the depressive episode [92, 93]. Recently, researchers have begun to explore the potential of cognitive markers to inform clinical decision-making in the treatment of depression [94] and as a specific treatment target [95]. We include an extensive neurocognitive battery at inclusion and hope to extend the follow-up assessments to include a brief internet-based self-administered cognitive assessment [96] to follow long-term cognitive function.

This study is embedded in a more extensive network of closely related studies in the BrainDrugs Research Alliance (braindrugs.nru.dk), which will significantly increase its scientific scope and value. Specifically, a

concurrent prospective cohort study, the BrainDrugs-Epilepsy study at our research unit, studies newly diagnosed patients with epilepsy using a similar multi-modal precision medicine approach [33]. Depression and other psychiatric disorders (e.g., anxiety) are frequent in patients with epilepsy, and a bidirectional relationship has been proposed [97–100]. Shared measures enable further exploration of the relationship between depression and epilepsy.

During the last decades, new developments in the pharmacological treatment of depression have been modest. Emphasis has been paid to the effect of other treatment modalities, such as psychotherapy, lifestyle modification and a combination of treatments. However, we do not know which specific groups of patients benefit from different treatment modalities. In the present project, we develop tools for prediction and uncover causal paths for treatment response in a real-world setting. Thus, the project goes beyond a traditional evaluation of existing health services with the potential to develop a more targeted treatment to be implemented and tested in the clinical setting. Furthermore, to maximise the utility of the participants' contribution, the project in also intended for cross-validating models from other research groups, data sharing and multi-centre collaborations. The datasets generated by this study will be available in the Cimbi database, which researchers can request access to [32].

Abbreviations

5-HTTLPR	Serotonin-transporter-linked promoter region
ABCB1	ATP Binding Cassette Subfamily B Member 1
ABCC1	ATP Binding Cassette Subfamily C Member 1
BDNF	Brain Derived Neurotrophic Factor
BOLD	Based blood oxygen level-dependent
BSI	Brief Symptom Inventory
BSI-18	18-Item Brief Symptom Inventory
CAMB	Copenhagen Aging and Midlife Biobank
CATS	Child abuse and trauma scale
CBT	Cognitive behavioral therapy
CIMBI	Center for Integrated Molecular Brain Imaging
COBRA	Cognitive Complaints in Bipolar Disorder Rating Assessment
COMT	Catechol-O-methyltransferase
CPR	Centrale Personregister
CSES	Coping Self-Efficacy Scale
CSFQ	Changes in Sexual Functioning Questionnaire
CVD	Center for Visitation og Diagnostik
CYP2C19	Cytochrome P450 Family 2 Subfamily C Member 19
CYP2D6	Cytochrome P450 Family 2 Subfamily D Member 6
DAR-5	Dimension of Anger Reactions
D-KEFS	Delis-Kaplan Executive Function System
DNA	Deoxyribonucleic acid
DNPR	Danish National Patient Registry
EEG	Electroencephalogr
EMR	Electronic medical records
ERP	Event-related potentials
ERT	Emotional Recognition Task
GAD	Generalised Anxiety Disorder 7-item
GP	General practitioner
HBA1c	Hemoglobin A1C
HC	Healthy control
HRRT	High resolution research tomograph
ICD	International Classification of Diseases
IDS	Inventory of depressive symptomatology
IMT	Emotional Intensity Morphing Task
LDAEP	Loudness dependence of auditory evoked potentials
LNS	Letter-Number Sequence
MAAS	Mindful Attention Awareness Scale
MCQ-30	Short form of Metacognitions Questionnaire
MDD	Major Depressive Disorder
MET	Moral Emotions Task
MREG	Ultra-fast functional magnetic resonance encephalography
MRI	Magnetic Resonance Imaging
mSDS	Modified Sheehan Disability Scale
MZQ	Mentalization Questionnaire
NEQ	Negative Effects Questionnaire
NRU	Neurobiology Research Unit
OS-FHAM	Online Stimulant and Family History Assessment Module
PAQ	Perth Alexithymia Questionnaire
PBI	Parental Bonding Instrument
PET	Positron emission tomography
PRISE	Patient Reported Inventory of Side-Effects
PSQI	Pittsburgh Sleep Quality Index
PSS	Cohen's Perceived Stress Scale
QIDS	Quick Inventory of Depressive Symptomatology
RAVLT	Rey Auditory Verbal Learning Test
RCFT	Rey Complex Figure Test
RRS	Ruminative Reponse Scale
S100B	S100 calcium-binding protein B
SCIP-D	Screen for Cognitive Impairments in Psychiatry—Depression
SCL-10	10-Item depression and anxiety Symptom Checklist
SDMT	Symbol Digit Modality Task
SHAPS	Snaith-Hamilton Pleasure Scale
SLE	Stressful life events
SOI-R	Revised Sociosexual Orientation Inventory
SRT	Simple Reaction Tim
SSRI	Selective serotonin reuptake inhibitor
STROBE	Strengthening the reporting of observational studies in epidemiology
SV2A	Synaptic vesicle glycoprotein 2A
TRIPOD	Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis
TSH	Thyroid-stimulating hormone
UGT1A1	UDP glucuronosyltransferase 1 family, polypeptide A1
WHO	World Health Organization
WHO-5	WHO 5 wellbeing index

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12888-023-04618-x.

Additional file 1: Supplementary Figure 1. The age and sex distribution of patients enteringthe treatment package during 2019-2021 before inclusion start.

Additional file 2: Supplementary Figure 2. The annual distribution of treatment initiation at the Mental Healthcare Centres in the Capital Region of Denmark. *The Mental Health Centre Copenhagen comprises two clinics, i.e., in Frederiksberg and Nørrebro. B) The Mental Health Centres admission area and geographical locations (image made by K. R. Jensen).

Additional file 3.

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Authors' contributions
GMK, MBJ, MO and VGF conceptualised the study. KHRJ wrote the manuscript together with MBJ. VNHD, MG, PMF, CI, AS, MRM, BO, MO, BWJHP, LHP, VGF, and

GMK contributed to different aspects of the study design and critically revised the manuscript. All authors have approved the submitted version.

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Availability of data and materials
The datasets generated by this study will be available in the Cimbi database. All researchers can request access to data from the Cimbi database (www.cimbi.dk/db).

Declarations

Ethics approval and consent to participate
The study is conducted according to the principles of the seventh revision of the Declaration of Helsinki (2013) and was reviewed and approved by the Committees on Health Research Ethics in the Capital Region of Denmark (reference number: H-20083013). Before undertaking any study-related procedures, each participant receives verbal and written explanations of study aims, methods, potential hazards, and benefits from investigators and provides written informed consent. The study will be carried out in accordance with relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
GMK has received honoraria as speaker for SAGE Therapeutics/Biogen and H. Lundbeck and as advisor for Sanos, and VF as a consultant for SAGE Therapeutics. MJ and VF have given talks sponsored by Lundbeck Pharma and MJ for Boehringer Ingelheim. The remaining authors declare that the research is conducted without any commercial or financial relationships that could be construed as a potential conflict of interest. MRMJ received sponsorship from Jazz Pharmaceuticals for attendance at the 14th European Epilepsy Congress.

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Publisher's Note

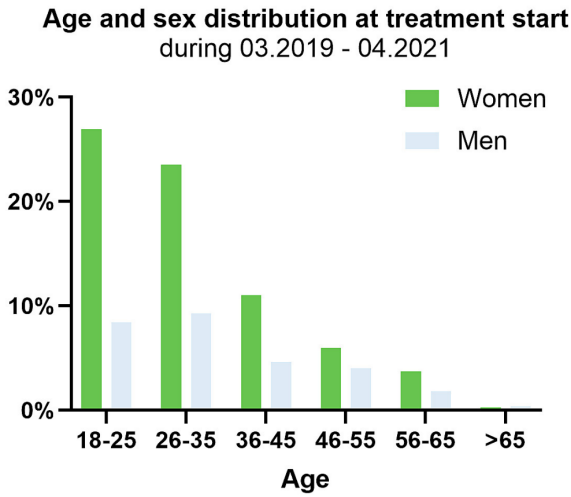
ADDITIONAL MATERIAL

For:

Jensen KHR, Dam VH, Ganz M, Fisher PM, Ip CT, Sankar A, Marstrand-Joergensen MR, Ozenne B, Osler M, Penninx BWJH, Pinborg LH, Frokjaer VG, Knudsen GM, Jørgensen MB (2023) Deep phenotyping towards precision psychiatry of first-episode depression – the Brain Drugs-Depression cohort. ***BMC Psychiatry***

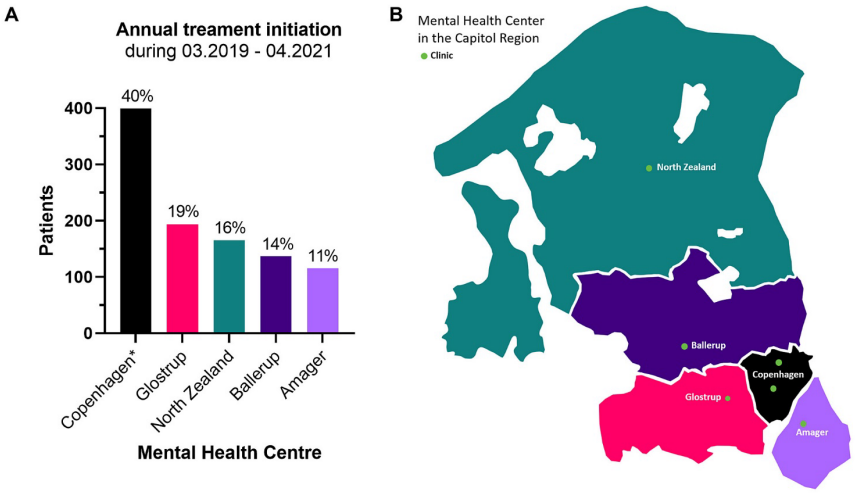
Supplementary Figure 1.

The age and sex distribution of patients entering the treatment package during 2019-2021 before inclusion starts.



Supplementary Figure 2.

The annual distribution of treatment initiation at the Mental Healthcare Centres in the Capital Region of Denmark. *The Mental Health Centre Copenhagen comprises two clinics, i.e., in Frederiksberg and Nørrebro. B) The Mental Health Centres admission area and geographical locations (image made by K. R. Jensen).



Supplementary Table 1

Treatment		Cumulative Time	Content
Examination and Monitoring	Initial examination	2 hours	The patient is seen and assessed by a specialist in psychiatry Investigation of psychopathology with diagnostic interview
	Psychometric monitoring	1 hour	Hamilton Depression Scale rating
	Continuous examination		Examination of cognitive function in the remitted phase and in case of suspected cognitive difficulties Investigation of social support needs Somatic and neurological examination
Non-pharmacological Treatment (in groups and individually as needed)	Individual	6 hours *as required*	Psychotherapy - Cognitive behavioural therapy or other similar short-term therapy - Psychoeducation
	In group (e.g., 8 patients)	6 hours *as required* *(12 sessions of two hours)*	Psychotherapy - Cognitive behavioural therapy or other similar short-term therapy Behavioural training Psychoeducation
	Relatives	2 hours	Relative involvement Psychoeducation
Pharmacological Treatment		5 hours	Treatment with antidepressants Additional treatment Restrictions on the use of benzodiazepines Monitoring of side effects and metabolic disorders Systematic adverse reaction detection
Network		2 hours	Network meetings with coordination of efforts - Contact with partners, etc. Follow-up on treatment and relapse prophylaxis

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