CHANGES IN PERIPHERAL INFLAMMATION AND NEUROTROPHIC FACTORS AND BRAIN NETWORK CONNECTIVITY IN DEPRESSIVE PATIENTS UNDER TREATMENT WITH ELECTROCONVULSIVE THERAPY (ECT)

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Abstract

Depression is characterized by a range of symptoms including persistent low mood, loss of interest or pleasure, and fatigue. Furthermore, depressed patients experience this state as chronic and difficult to escape. Notably, treatment resistant depression accounts for 30% of cases in major depressive disorder, representing a huge humanistic and economic burden. Electroconvulsive therapy (ECT), an electrical stimulation of the brain which triggers a generalized seizure, provides a chance of relief for such cases. Although beneficial, the precise mechanisms of ECT remain to be determined. Based on previous knowledge, ECT induces an acute inflammatory immune response that might reinforce the expression of neurotrophins. Here we aim to unveil the potentially beneficial role of inflammation observed during ECT. We also hypothesize that ECT induces changes in brain network communication, as a result of changes in metabolism. Hence, studying brain functional connectivity is of great importance to understand the pathophysiological mechanisms of ECT.

Within a repeated measures design study of six weeks, nine patients diagnosed with depressive disorder received three ECT sessions per week for a total of 16 sessions on average. To investigate the pathophysiological mechanisms of ECT treatment, we implemented a multi-level strategy which involved tracking the clinical progress using the Hamilton Depression rating scale (HAMD-21) and Beck's depression inventory, collecting peripheral blood samples, and performing functional Magnetic Resonance Imaging (fMRI).

ECT led to successful clinical response affecting both molecular pathways and brain network communication. ECT increased BDNF serum expression, which negatively correlated with HAMD-21 scores. Moreover, ECT induced an increase in TNF-α levels and altered the expression of IL-4. At the brain level, ECT induced an overall increase in regional connectivity, modulated the amplitudes of low frequency fluctuations (fALFF) in a region-specific fashion, and improved brain network integration in terms of node degree, betweenness centrality, and communication efficiency.

The present work revealed that ECT appears to impact multiple pathways, which act in synergy to generate the desired clinical improvement: the reactivation of BDNF-mediated cortical plasticity induced by ECT may lead to a new balance in brain functional network properties to better adapt to environmental challenges.

Zusammenfassung

Depressionen sind durch eine Reihe von Symptomen gekennzeichnet, darunter anhaltende schlechte Laune, Verlust von Interesse oder Freude und Müdigkeit. Außerdem erleben depressive Patienten diesen Zustand als chronisch und schwer zu überwinden. Behandlungsresistente Depressionen machen 30 % der Fälle von schweren depressiven Störungen aus und stellen eine enorme soziale und wirtschaftliche Belastung dar. Die Elektrokrampftherapie (EKT), eine elektrische Stimulation des Gehirns, die einen generalisierten Krampfanfall auslöst, bietet in solchen Fällen eine Chance auf Linderung. Die genauen Mechanismen der EKT sind jedoch noch nicht geklärt. Nach bisherigen Erkenntnissen löst EKT eine akute entzündliche Immunreaktion aus, die die Expression von Neurotrophinen verstärken könnte. Hier wollen wir die potenziell positive Rolle der während der EKT beobachteten Entzündung aufklären. Wir stellen außerdem die Hypothese auf, dass die EKT als Folge der Veränderungen im Stoffwechsel Veränderungen in der Netzwerkkommunikation des Gehirns hervorruft. Daher ist die Untersuchung funktioneller Konnektivität des Gehirns von großer Bedeutung für das Verständnis der pathophysiologischen Mechanismen der EKT.

Im Rahmen einer sechswöchigen Studie mit wiederholten Messungen erhielten neun Patienten, bei denen eine depressive Störung diagnostiziert wurde, drei EKT-Sitzungen pro Woche mit insgesamt durchschnittlich 16 Sitzungen. Zur Untersuchung der pathophysiologischen Mechanismen der EKT-Behandlung setzten wir eine mehrstufige Strategie ein, die die Verfolgung des klinischen Fortschritts anhand der Hamilton Depression Rating Scale (HAMD-21) und des Beck'schen Depressionsinventars, die Entnahme peripherer Blutproben und die Durchführung funktioneller Magnetresonanztomographie (fMRT) umfasste.

Die EKT führte zu einer erfolgreichen klinischen Reaktion, die sich sowohl auf molekulare Signalwege als auch auf die Kommunikation im Gehirnnetzwerk auswirkte. EKT erhöhte die BDNF-Serumexpression, die negativ mit den HAMD-21-Scores korrelierte. Außerdem führte die EKT zu einem Anstieg der TNF-α-Spiegel und veränderte die Expression von IL-4. Auf der Ebene des Gehirns führte die EKT zu einem allgemeinen Anstieg der regionalen Konnektivität, modulierte die Amplituden der niederfrequenten Fluktuationen (fALFF) regionalspezifisch und verbesserte die Integration des Gehirnnetzwerks in Bezug auf Knotengrad, Betweenness-Zentralität und Kommunikationseffizienz.

Die vorliegende Arbeit hat gezeigt, dass die EKT offenbar mehrere Signalwege beeinflusst, die synergetisch wirken, um die gewünschte klinische Verbesserung zu bewirken: Die Reaktivierung der BDNF-vermittelten kortikalen Plastizität, die durch die EKT ausgelöst wird, könnte zu einem neuen Gleichgewicht der funktionellen Netzwerkeigenschaften des Gehirns führen, um sich besser an die Herausforderungen der Umwelt anzupassen.

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Abbreviations

5HT	Serotonin
ACC	Anterior Cingulate Cortex
ACTH	Adrenocorticotrophin
Ala	Alanine
ALFF	Amplitude of Low Frequency Fluctuations
AM	Acquisition Matrix
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ANT	Adenine Nucleotide Transporter
AROMA	Automatic Removal of Motion Artifacts
BC	Betweenness Centrality
BDI	Beck's Depression Inventory
BDNF	Brain-derived Neurotrophic Factor
BIDS	Brain Imaging Data Structure
BIL	Bilateral
BMI	Body Mass Index
BOLD	Blood Oxygenation Level-Dependent
CBT	Cognitive Behavioral Therapy
CCL2	C–C motif Chemokine Ligand 2
CCN	Cognitive Control Network
CNS	Central Nervous System
CRH	Corticotropin-Releasing Hormone
CRHR1	Corticotropin-Releasing Hormone Receptor 1
CRP	C-reactive protein
CV	Coefficient of Variance
DA	Dopamine
DAN	Dorsal Attention Network
DFG	Deutsche Forschungsgesellschaft
DLPFC	Dorsolateral Prefrontal Cortex
DMN	Default Mode Network
ECG	Electrocardiogram

ECS	Electroconvulsive Seizures
ECT	Electroconvulsive Therapy
EDTA	Ethylenediaminetetraacetic Acid
EEG	Electroencephalogram
ELISA	Enzyme-Linked Immunosorbent Assay
EMG	Electromyogram
FA	Flip Angle
fALFF	Fractional Amplitude of Low Frequency Fluctuations
FC	Functional Connectivity
FCD	Functional Connectivity Dynamics
FLAIR	Fluid-Attenuated Inversion Recovery
FWHM	Full Width at Half Maximum
GCP	Good Clinical Practice
GG	Greenhouse-Geisser
GRK	Graduiertenkolleg (engl.: graduate college)
HAMD-21	Hamilton Depression Rating Scale
HPA	Hypothalamic–Pituitary–Adrenal
HRP	Horseradish Peroxidase
ICA	Independent Component Analysis
ICD-10	International Classification of Diseases
ICH	International Conference on Harmonization
IDO	Indoleamine 2,3-dioxygenase
IFG	Inferior Frontal Gyrus
IFN- γ	Interferon gamma
IL	Interleukin
IP	Intraparietal
KYN	Kynurenine
KYNA	Kynurenic Acid
LPS	Lipopolysaccharide
MAOI	Monoamine Oxidase Inhibitors
MNI	Montreal Neurological Institute
MP-RAGE	Magnetization Prepared Rapid Gradient Echo
MR	Magnetic Resonance

MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NaSSA	Noradrenergic and Specific Serotonergic Antidepressants
NDRI	Norepinephrine and Dopamine Reuptake Inhibitors
NE	Norepinephrine
NGF	Nerve Growth Factor
NSAID	Non-Steroidal Anti-Inflammatory Drugs
OD	Optical Density
OFC	Orbitofrontal cortex
PBMC	Peripheral Blood Mononuclear Cells
PBS-T	Phosphate-Buffered Saline with Tween
PCC	Posterior Cingulate Cortex
PET	Positron Emission Tomography
QUIN	Quinolinic Acid
rCBF	Regional Cerebral Blood Flow
rCMRGlc	Regional Metabolic Rate for Glucose
RCT	Randomized Controlled Trial
ReHo	Regional Homogeneity
rmCorr	Repeated measures Correlation
ROI	Region of Interest
ROS	Reactive Oxygen Species
rs-fMRI	Resting state functional Magnetic Resonance Imaging
RT	Repetition Time
rU	Relative Units
RUL	Unilateral
SEM	Standard Error of the Mean
SERT	Serotonin Transporter
SN	Salience Network
SNRI	Serotonin and Norepinephrine Reuptake Inhibitors
SSRI	Selective Serotonin Reuptake Inhibitors
StAR	Steroidogenic Acute Regulatory protein
STG	Superior Temporal Gyrus
STS	Superior Temporal Sulcus

TE	Echo Time
TGF- β	Transforming Growth Factor-β
Thr	Threonine
TMS	Transcranial Magnetic Stimulation
TNF	Tumor Necrosis Factor
TRD	Treatment Resistant Depression
TSPO	Translocator Protein
VDAC-1	Voltage-Dependent Anion Channel
WHO	World Health Organization

CHAPTER 1 Introduction

Take a moment to reflect on a challenging time in your life when you felt completely overwhelmed, trapped, and devoid of hope. Perhaps it seemed like every door was closed, leaving you devoid of motivation to pursue even the most basic life goals. The truth is, we have, or we will all experience such moments. They are an inevitable part of our journey on this earth. I genuinely hope that you were able to rise above those moments, finding the inner strength to fight for what truly matters to you. However, it's important to recognize that we are all unique, and our responses to life's hardships differ greatly. Some of us may need more time to heal, others may require support, and sadly, there are those who lose their will to live, longing for an escape from the overwhelming pain. Depression is a profound and complex condition that touches countless lives. By raising awareness, fostering understanding, and offering support, we can create a more compassionate and inclusive society where those struggling with depression can find the help and empathy, they need to reclaim their lives.

Nadia Falhani

1.1 Psychiatric disorders & depression

Psychiatric disorders encompass a wide range of mental health conditions that affect an individual's thoughts, emotions, behaviors, and overall well-being. These disorders can manifest in various forms, such as depression, anxiety, schizophrenia, bipolar disorder, and more, impacting individuals across different age groups and cultures.

Depression is a common and serious mental illness that negatively affects how one feels, what one thinks, and how one acts. It is classified under the group of mood disorders in the International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-10) of the World Health Organization (WHO, 1993).

Depression is different from usual mood fluctuations and short-lived emotional responses to challenges in everyday life. Especially when long-lasting and with severe intensity, depression may become a serious health condition.

In the typical mild, moderate, or severe episodes, the affected patient suffers from depressed mood and a reduction in drive and activity. The capacity for pleasure, interest and concentration is reduced. Pronounced fatigue may occur after the slightest exertion. Sleep is usually disturbed, and appetite reduced. Self-esteem and self-confidence are almost always impaired. Even in the mild form, feelings of guilt or thoughts about one's own worthlessness occur. The depressed mood changes little from day to day and is unresponsive to life circumstances. Depending on the number and severity of symptoms, a depressive episode is classified as mild, moderate or severe. Furthermore, symptoms must last at least *two weeks* and must represent a change in the previous level of functioning for a diagnosis of depression. At its worst, depression can lead to suicide. Close to 700 000 people die due to suicide every year, which is the fourth leading cause of death for people between 15 and 29 years of age (WHO, 2023).

1.1.1 Types of depressive disorders

Depression is classified within the 5th chapter of the ICD-10 under 'Mental, Behavioral and Neurodevelopmental disorders'. However, depression encompasses a range of distinct types of depressive episodes, such as persistent depressive disorder, perinatal depression, seasonal depression, and depression with symptoms of psychosis, including delusions and hallucinations. In our study, we specifically focused on three distinct categories within the broad spectrum of depression:

- **Bipolar mood disorder (ICD-10, F31):** this disorder consists of episodes characterized by unusual, elevated mood, increased drive, and activity (hypomania or mania) alternated with episodes with lowered mood, hopelessness and decreased drive and activity (depression).
- **Depressive episodes (ICD-10, F32):** this class includes the most known type of depression as described above, differentiating into subtypes depending on the severity or on the presence of psychotic symptoms. The official descriptor of F32 refers to single episodes and it includes agitated depression, depressive reaction, major depression, psychogenic depression, reactive depression, and vital depression.

- **Recurrent depressive disorder (ICD-10, F33):** this is a disorder characterized by repeated episodes of depression (F32.-). There are no independent episodes of high spirits and increased drive (mania) in the anamnesis. However, the risk that a patient with recurrent depressive disorder will develop a manic episode is never completely eliminated, no matter how many depressive episodes have occurred.

Depression has been increasing in the recent years, with approximately 280 million people affected worldwide and a prevalence between 4.4–20% in the general population. The 12-month prevalence of major depressive disorder varies considerably across countries but is approximately 6% overall (Kessler & Bromet, 2013). The lifetime risk of depression is three times higher (15–18%) (Malhi & Mann, 2018), meaning major depressive disorder is common, with almost one in five people experiencing one episode at some point in their lifetime. Depression can affect people of all ages, ethnicities, and genders. However, statistics indicate that depression is approximately 50% more common among women compared to men (WHO, 2023). Since men may be less likely to recognize, talk about, and seek help for their feelings or emotional problems, they are more likely to have undiagnosed or undertreated symptoms of depression. Additionally, the COVID-19 pandemic has further exacerbated the issue, with many people experiencing increased levels of stress, anxiety, and depression due to the pandemic's social and economic impacts (Pourdehghan et al., 2022).

1.1.2 Etiology

Depressive symptoms such as feelings of sadness, pessimism, and lethargy are universal human experiences, considered natural responses to life’s challenges, disappointments, or important losses. However, for some individuals the severity and persistence of those symptoms are not typical. Hence the question arises about why some individuals undergo prolonged and intense depressive experiences while others do not.

While there is no single cause of depression, most experts believe there is a combination of biological, social, and psychological factors that contribute to depression risk. Among the biological factors, the interaction between various mechanisms - including genetic vulnerabilities, brain structure and function, neurotransmitters and neuroendocrine processes, and immune system processes - may play a role in the onset of depression or emerge as a consequence of the disorder itself.

1.1.2.1 Monoamine hypothesis of depression

The involvement of three major monoamine systems - serotonin (5-hydroxytryptamine, 5HT), norepinephrine (NE) and dopamine (DA) – has been widely supported (Charney, 1998; Dunlop & Nemeroff, 2007; Ressler & Nemeroff, 2001). According to the monoamine hypothesis of depression a deficiency or imbalance in the monoamine neurotransmitters is associated with depression. This emerged with the observation that reserpine, a drug used to control blood pressure, and which depleted monoamines, led to an increased rate of depression and suicide among patients. Interestingly, effective first line antidepressant medication, such as serotonin or norepinephrine reuptake inhibitors, act on these monoaminergic systems.

A link between decreased serotonin and depression was first suggested in the 1960s (Coppen, 1967). Subsequent work showed also that plasma levels of the essential amino acid tryptophan, the key precursor of serotonin, were reduced in depressed patients (Coppen et al., 1973). Further evidence was given by postmortem and positron emission tomography (PET) imaging studies reporting reduced activity of serotonergic neurons, reduction in the number of serotonin transporter (SERT) binding sites, and increased $5HT_2$ receptor density probably as a compensatory mechanism (Drevets et al., 1999; Mann et al., 1996). Nevertheless, it is important to note that this theory has been recently questioned, claiming that consistent evidence of the association between serotonin activity and depression is lacking (Moncrieff et al., 2022).

The role of NE in the pathophysiology of depression may be due to the importance of this neurotransmitter in processes such as memory, attention, and stress response. Therefore, abnormalities in neural circuits mediated by NE may contribute to the development of depressive symptoms (Ressler & Nemeroff, 2001). Furthermore, similarly to medication that increases 5HT availability, NE reuptake inhibitors proved to be effective antidepressants (Saveanu & Nemeroff, 2012).

While DA-mediated neuronal pathways have long been associated with the pathophysiology of schizophrenia, some evidence suggested a significant contribution of DA brain circuits in the context of depression (Dunlop & Nemeroff, 2007). The involvement of DA in the pathogenesis of depression emerged particularly from the association with the inability to experience pleasure, anhedonia, which is characteristic of depression. Indeed, pleasure, whether associated with eating, social, or sexual behavior, is primarily mediated by activation of DA neurons.

Nevertheless, it is important to note that, considering the variety of depressive symptoms, none of the above-mentioned neurotransmitter systems seems to be solely responsible of such a complex disorder.

1.1.2.2 Neurotrophic hypothesis: brain derived neurotrophic factor

One of the most important discoveries in the last century has been the identification, in the adult brain, of pluripotent stem cells from which new neurons can be generated, a process known as *neurogenesis*. The growth and adaptability at a neuronal level has been more broadly named *neuroplasticity*, and it is possible that neuroplasticity at a cellular level is altered by inflammation and hypothalamic–pituitary–adrenal (HPA) axis dysfunction, both caused by environmental stress (Egeland et al., 2015). The process of neurogenesis is controlled by regulatory proteins, such as brain-derived neurotrophic factor (BDNF), which was interestingly found diminished in patients with major depressive disorder (Molendijk et al., 2013). The neurotrophic hypothesis proposes that reduced neurotrophic support can lead to neuronal atrophy, decreased hippocampal neurogenesis, and loss of synaptic connections, contributing to the development of depression. BDNF also influences the differentiation of serotonergic and dopaminergic neurons and plays a crucial role in dynamic synaptic organization, longterm potentiation (Knusel et al., 1991; L. Shen et al., 1997), and learning and memory (Yamada et al., 2002). Animal studies have shown a correlation between stress, reduced BDNF expression in the brain, and depressive-like behavior (Filuś & Rybakowski, 2005). However, longitudinal studies in humans suggest that the decrease in serum BDNF may be a consequence of depression rather than a cause (Bus & Molendijk, 2016). Nonetheless, strong evidence supports that BDNF is required for a response to antidepressant treatments, and BDNF neurotrophic effects have been proposed as target for antidepressant medication (Castrén & Rantamäki, 2010; Duman & Li, 2012). An interesting perspective on the role of BDNF in the antidepressant effects of drugs has been suggested by Castrén: stress and adverse life experiences may induce plasticity and lead to an unfavorable reorganization of cortical networks; the reactivation of BDNF-mediated cortical plasticity induced by antidepressant treatment could allow the rewiring of the miswired networks to better adapt to environmental challenges.

Furthermore, BDNF genetic variants seem to influence susceptibility and resilience towards depression. A BDNF single nucleotide polymorphism, Val66Met, has been associated with decreased processing and release of BDNF, reduced episodic memory and executive function, and decreased hippocampal volume (Casey et al., 2009). While there is no direct link

to depression, the BDNF Met allele increases susceptibility to developing depression in individuals who have experienced early life stress or trauma (Gatt et al., 2009; Kim et al., 2007).

1.1.2.3 The stress diathesis hypothesis of depression

The neuroendocrine system and particularly the HPA axis have been the focus of depression research for many decades (Goodyer et al., 2000; Harris et al., 2000; Keller et al., 2017; Knorr et al., 2010; Stetler & Miller, 2011). The stress-diathesis hypothesis of depression states that excessive secretion of cortisol and other hormones of the HPA axis plays a significant role in the pathogenesis of depression. Indeed, patients suffering from Cushing syndrome (hypercortisolism) often manifest depressive or anxiety-related symptoms. The physiological stress response is an essential and adaptive mechanism. Nonetheless, extended and excessive exposure to stress can trigger maladaptation and result in illness in vulnerable individuals (De Kloet et al., 2005). Of particular interest here is that patients with severe, particularly recurrent depression often experience disturbances of the HPA axis with consequent increases in corticotropin-releasing hormone (CRH), adrenocorticotrophin (ACTH) and glucocorticoid levels (Holsboer, 2000; Nagaraja et al., 2007), especially in cortisol (Jia et al., 2019). These elevated levels of glucocorticoids can in turn affect neurogenesis and neuroplasticity, contributing to the reinforcement of depression illness. Animal studies show in fact that increases in glucocorticoid levels associated with stress can decrease cell proliferation and neurogenesis in the dentate gyrus of the hippocampus (Hellsten et al., 2002; Sheline et al., 1996). Notably, HPA axis function was found to be heterogeneous among patients with depression, leading to different subtypes: patients with melancholia tend to show hypercortisolemia, while patients with atypical depression exhibited a decreased HPA axis activity (Juruena et al., 2018). Furthermore, differences were found also in unipolar compared to bipolar depressed patients (Becking et al., 2015; Rybakowski & Twardowska, 1999). This could clarify the conflicting results and the reason why HPA axis dysfunction has not been incorporated into clinical practice as a risk biomarker.

1.1.2.4 The immune-inflammatory hypothesis

Depression is also characterized by chronic inflammation and sustained activation of the immune system (Köhler et al., 2017). Increased expression of proinflammatory cytokines such as interleukin 6 (IL-6), interleukin 1 (IL-1), tumor necrosis factor–alpha (TNF-alpha),

interferon-gamma (IFN-γ), as well as C-reactive protein (CRP, an acute phase protein) have been consistently found in depressed patients. Pro-inflammatory cytokines, in turn, modulate neuroendocrine function (CRH and HPA axis activation), neuroplasticity processes, and neurotransmitters metabolism.

Major depressive disorder and treatment-resistant depression (TRD) appear to be, in part, related to dysfunction of the immune and inflammatory response (Haack et al., 2001; Maes et al., 2011; Miller & Raison, 2016). In the context of depression, external stress can be a precipitating factor, which can increase levels of circulating cytokines, both peripherally and centrally (Miller, 2009; Miller et al., 2009). In the central nervous system (CNS) these cytokines result from activation of the microglia. The diversity and plasticity of microglial cells lead to the identification of several *functional polarization states*, which are ultimately dependent on the macrophage extracellular environment (fig. 1; Nakagawa & Chiba, 2014). The presence of activating factors, such as lipopolysaccharide (LPS) or IFN-γ, stimulates the polarization of resting microglia to **M1** phenotype, which can produce pro-inflammatory cytokines/mediators including IL-1β, IL-6, TNF-α, C–C motif chemokine ligand 2 (CCL2), reactive oxygen species (ROS), and nitric oxide (NO). On the other hand, IL-4 and IL-13 can induce alternative activation and polarization of **M2** microglia which express anti-inflammatory cytokines, such as IL-10. In addition, M2 microglia can promote tissue remodeling by producing neuroprotective factors, TGFβ, IGF-1, and BDNF. Based on this evidence, there is a possibility that M1/M2 polarization of microglia plays an important role in controlling the balance between promotion and resolution of neuroinflammation in the CNS, and subsequently in the onset and maintenance of depression (fig. 1).

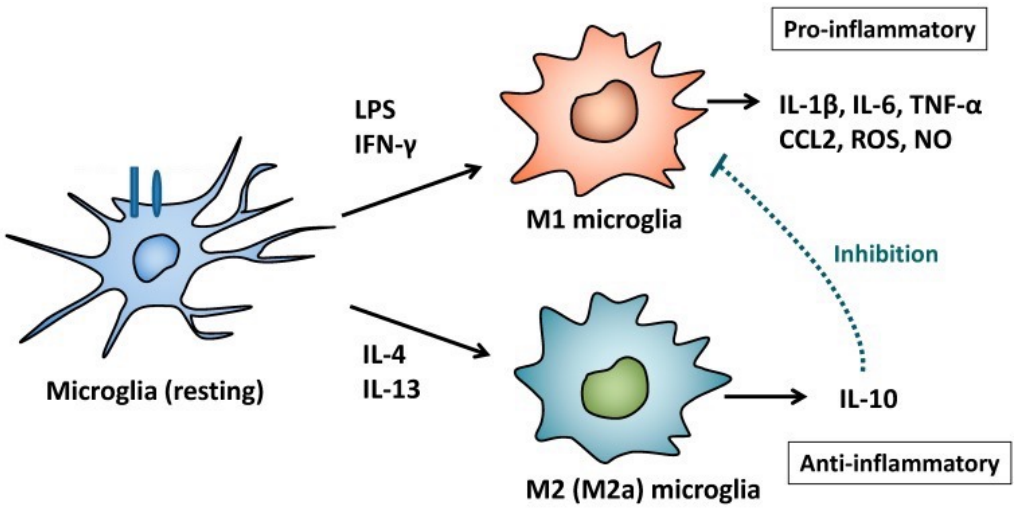

Figure 1: M1/M2 polarization of microglia and their immunoregulatory functions. Depending on the presence of pro-inflammatory activating factors or anti-inflammatory promoters, M1 or M2 phenotype of microglia is induced respectively. Adapted from (Nakagawa & Chiba, 2015).

1.1.2.4.1 TSPO and the neuroactive steroids cascade

A widely recognized marker of neuroinflammation is the translocator protein (TSPO). TSPO is an 18 kDa protein located on outer mitochondrial membranes in microglia and increased expression of TSPO occurs when microglia cells are activated during neuroinflammation (Rupprecht et al., 2010; Setiawan et al., 2015). TSPO is considered a multifunctional protein, being implicated in various mitochondrial functions, such as cholesterol transport and steroid hormone synthesis (Papadopoulos et al., 2015; Wolf et al., 2015), mitochondrial bioenergetics and metabolism (Liu et al., 2017), production of ROS (Gatliff et al., 2014), as well as apoptosis and cell proliferation (Veenman et al., 2007; Papadopoulos et al., 2018; Papadopoulos & Lecanu, 2009). Together with a voltage-dependent anion channel (VDAC), the adenine nucleotide transporter (ANT) and the steroidogenic acute regulatory protein (StAR), TSPO forms a complex that serves as bridge from the outer to the inner of mitochondria (Mcenery et al., 1992). Notably, the transport of cholesterol from the outer to the inner mitochondrial membrane represents the first step in the synthesis of pregnenolone from cholesterol by the cholesterol monooxygenase (P450scc) in the mitochondrion (fig. 2) (Nothdurfter et al., 2012; Papadopoulos et al., 2015).

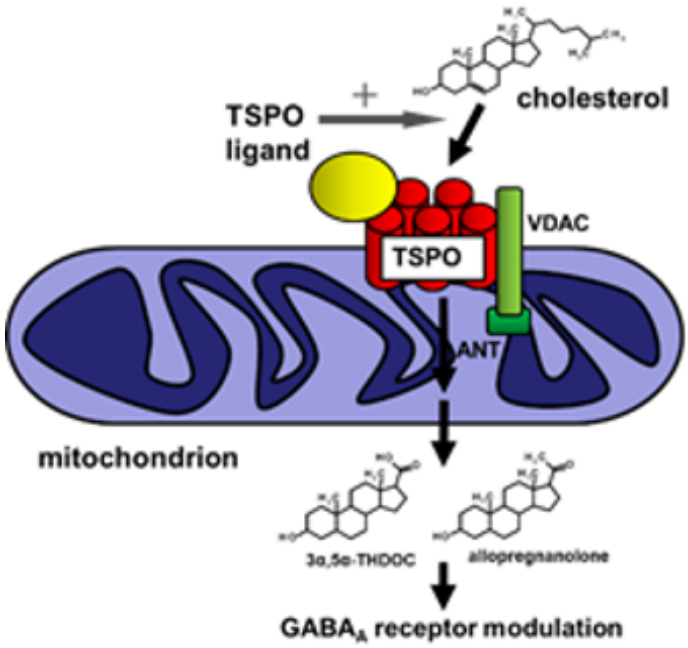

Figure 2: Structure of the Translocator Protein 18 kDa (TSPO). Overview of the TSPO complex located at the outer membrane of mitochondria including the voltage-dependent anion channel (VDAC) and the adenine nucleotide transporter (ANT). Adapted from (Nothdurfter et al., 2012).

In the brain, pregnenolone is metabolized in astrocytes and microglia by further enzymatic modifications in the cytoplasm of the cell to so-called *neuroactive steroids* (fig. 3) (Papadopoulos et al., 2006). These neurosteroids can in turn modulate the neuronal transmission (e.g. of GABAA receptors) in the CNS. Particularly efficient are the so-called 3-alpha-reduced steroids (e.g. allopregnanolone), which play a key role in the regulation of emotions and thus in the development of depression (Rupprecht & Holsboer, 2001; Schüle et al., 2011). Since TSPO is involved in several mechanisms related to mitochondrial function and inflammatory alterations, its role in the pathophysiology of psychiatric disorders has been broadly investigated. TSPO was found to be up-regulated in several inflammatory and neurodegenerative diseases, while its expression was decreased in peripheral blood from psychiatric patients (Barichello et al., 2017). Moreover, PET studies revealed that TSPO expression was increased in various brain regions of depressed patients (Enache et al., 2019; Setiawan et al., 2015).

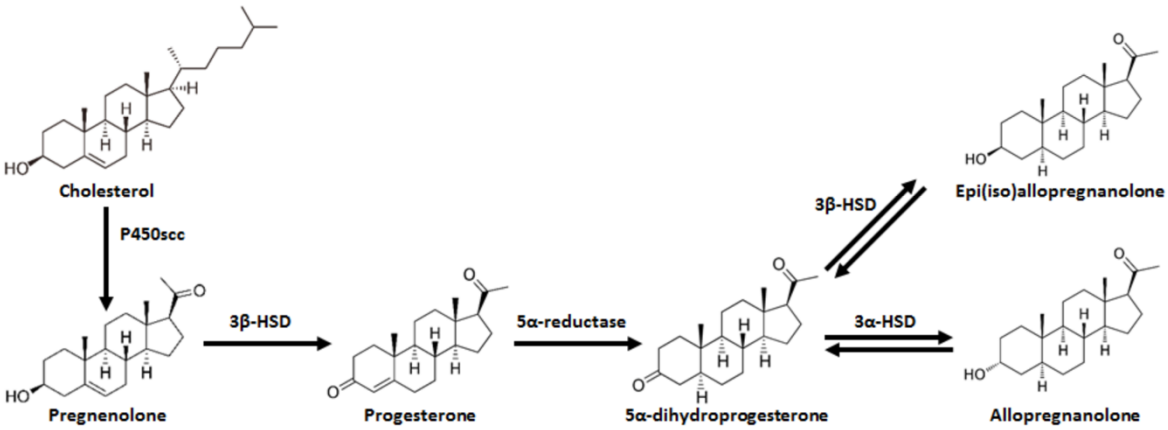

Figure 3: Biosynthesis of neuroactive steroids. The cholesterol, inside the mitochondrion is converted in pregnenolone by the cytochrome P450scc. The 3β-hydroxysteroid dehydrogenase (3β-HSD) and 5α-reductase enzymes convert pregnenolone in progesterone and 5αdihydroprogesterone, respectively. The 3α-hydroxysteroid dehydrogenase (3α-HSD) and 3β-hydroxysteroid dehydrogenase (3β-HSD) enzymes regulate the chemical equilibrium of the conversion of 5α-dihydroprogesterone in allopregnanolone (3α,5α-tetrahydroprogesterone) and epiallopregnanolone (or isoallopregnanolone; 3β,5α-tetrahydroprogesterone), respectively. Adapted from (Tomaselli & Vallée, 2019).

Nevertheless, the role of TSPO in immune reactions and neuroinflammation is still controversial. Rather than merely being a marker of neuroinflammation, TSPO has been shown to be able to modulate the two different states of microglial activation: the M1 pro-inflammatory and the M2 anti-inflammatory state (Kim & Yu, 2015). In particular, the binding of TSPO to its ligand, or up regulation of its expression level, enhances M2 anti-inflammatory microglial activation. M2 polarization, as mentioned above (see fig. 1), results in up regulation of anti-inflammatory genes to resolve neuroinflammation and promote recovery from tissue damage. In contrast, M1 pro-inflammatory microglial activation seems to be reduced by the up regulation of TSPO or its ligand.

1.1.2.4.1.1 TSPO rs6971 polymorphism

A key aspect worth to be considered when investigating the role of TSPO in the context of depression and its therapy is the single nucleotide polymorphism rs6971. This polymorphism leads to a substitution from alanine to threonine at position 147 in the transmembrane of TSPO (Owen et al., 2012). Consequently, this polymorphism alters mitochondrial TSPO protein structure, reducing cholesterol transport into the mitochondria and therefore decreasing production of pregnenolone as compared to that occurring in the presence of the wild type genotype (Costa et al., 2009). More recent evidence suggests that this common TSPO

polymorphism is associated with dysregulated steroid production in humans (Owen et al., 2017). Moreover, it has been suggested that rs6971 TSPO polymorphism is associated with bipolar disorder, eventually by reducing both cholesterol binding or transport and subsequent cortisol production, increasing vulnerability to environmental stress, and consequently, facilitating development of stress related psychiatric disorders, such as bipolar disorder (Colasanti et al., 2013). Prossin and colleagues also found that the presence/absence of the TSPO functional polymorphism (rs6971) predicted differences in cortisol's diurnal rhythm in healthy control and bipolar disorder volunteers with and without comorbid alcohol dependence (Prossin et al., 2018). Importantly, this polymorphism further determines the affinity with which ligands bind to TSPO resulting in three different patterns (low- / medium- / and high-affinity binders) that might even influence therapeutic effects (Berroterán-Infante et al., 2019; Owen et al., 2011). Given its effect on steroidogenesis, rs6971 TSPO polymorphism becomes an interesting factor in the framework of stress-exacerbated disorders, such as depression. However, limited evidence exists regarding the implications of this polymorphism in depression and its treatment and further light should be shed on this crucial aspect (Setiawan et al., 2015).

1.1.2.5 Brain structure and function in depression

Alterations in brain structure and function have been reportedly implicated in the pathogenesis of depression. Critical alterations of brain regions (e.g., limbic or frontal cortical regions) and associated communication pathways have been identified in depressed patients (Pandya et al., 2012; Scheepens et al., 2020; Zhang et al., 2018). Imaging studies show that the brains of depressed people and healthy control subjects differ in terms of both metabolism (Luykx et al., 2012) and functional connectivity, and there is evidence that functional connectivity as a biomarker may be able to identify different biotypes of depression (Drysdale et al., 2017). Furthermore, recent approaches in clinical neuroscience (Deco & Kringelbach, 2014; Holtzheimer & Mayberg, 2011; Tognoli & Kelso, 2014) suggest that depression is manifested in reduced connectivity dynamics.

To investigate the changes in brain function in the context of depression, resting-state functional MRI (rs-fMRI) has been widely performed. In particular, the analysis of the amplitude of low frequency fluctuations (ALFF) and regional homogeneity (ReHo) are two fundamental rs-fMRI parameters describing local properties of resting-state brain function (Li et al., 2022). ALFF can be used to test the magnitude of spontaneous blood-oxygen-level-

dependent (BOLD) signals, since it directly correlates to the intensity of spontaneous neural activity in the resting state in terms of energy metabolism (Nugent et al., 2015). Moreover, considering only a portion of the low frequency spectrum reduces the effects of physiological noise (Zou et al., 2008). Therefore, *fractional* ALFF has been introduced to measure functional abnormalities in depressed patients. Various studies reported significant alterations in depressed patients in some brain regions, such as orbital gyrus, precentral gyrus, the limbic system and the cerebellum (W. Guo et al., 2013; Lai & Wu, 2015; Wang et al., 2012). Regional homogeneity (ReHo) measures if a given voxel is temporally synchronized to its neighbors, providing a notion about local coherence throughout the brain (Zang et al., 2004). Previous studies have shown that ReHo values are decreased in the precuneus, angular gyrus, left orbitofrontal cortex, and anterior cingulate cortex in depressed patients (Hao et al., 2019; Lai, 2018; Späti et al., 2015). One study has found that the ReHo value in the left precentral gyrus was positively correlated with HAMD scores (Geng et al., 2019).

Using graph theory-based methods, researchers have explored topological properties of brain functional networks. Specific topological features of brain networks, such as the combination of high local and global efficiency and high clustering, are thought to support information processing and to be characteristic of a healthy brain (Bullmore & Sporns, 2009). Therefore, depression could represent alterations in the topological features of functional brain networks, such as aberrant global and local efficiency (Bullmore & Sporns, 2012). For instance, a large-scale multisite study reported disrupted topological network properties, particularly decreased global and local efficiency in depressed patients compared to healthy controls (Yang et al., 2021). However, these results were driven mostly by patients with recurrent depression. Conversely, other studies reported that depressed patients show increased global efficiency (Guo et al., 2014; Zhang et al., 2011) and local efficiency (Ye et al., 2015). Given those inconsistencies, more reproducible and reliable findings are needed in the field.

1.1.2.6 The gene x environment interaction model

The current view of the etiology of depression is best summarized as a prototypical Gene × Environment (G × E) interaction model (Saveanu & Nemeroff, 2012). Approximately one-third of the risk for the development of depression is inherited, and two-thirds is environmental (Sullivan et al., 2000). Genetic susceptibility to depression is closely linked to the effects of early traumatic events during crucial developmental periods. Even though early life stress increases the risk of depression, there are important differences in the way individuals respond

to the same stressful event, and these differences may be explained in part by genetic factors. A variety of genetic polymorphisms seem to exert control over the degree of sensitivity to adverse events in early life, including protective polymorphisms in the serotonin transporter, CRH receptor ($CRHR_1$), and BDNF genes. The environment as well can directly impact the expression of genetic information through epigenetic mechanisms, such as DNA methylation and demethylation, histone modifications and noncoding RNA-mediated regulation of gene expression (Saveanu & Nemeroff, 2012). Clearly, the combination of genetic and environmental influences is a key factor in determining whether an individual will be vulnerable or resilient to developing depression.

1.1.3 Treatment

When treating a depressive episode (see Table 1), the initial objective is clinical response to treatment, which is scientifically defined as a significant reduction in symptoms and is normally quantified as a 50% reduction in the total score on a standardized rating scale, such as the HAMD; secondary objective is complete remission of depressive symptoms, formally defined by a cut-off score of less than 9 on the HAMD scale (Hamilton, 1960; Malhi & Mann, 2018). Broadly speaking, these objectives can be achieved by use of psychotherapy, pharmacotherapy, or both. For many cases of major depressive disorder, psychological treatment alone can suffice and evidence-based psychotherapy, such as cognitive behavioral therapy or interpersonal psychotherapy, has been shown to have a comparable efficacy to that of antidepressant medication (Beck et al., 1961; Feijo De Mello et al., 2005). To achieve a faster therapeutic response, medication is likely to be needed, and a combination of pharmacotherapy and psychological treatment appears to be preferable. In cases of severe major depressive disorder, medication should be considered as first-line treatment. Most prescribed antidepressants act on the enhancement of monoaminergic neurotransmission by producing initial effects within the synapse, which then impact intracellular signaling and second messenger pathways (Willner et al., 2013). These pathways culminate in changes in gene expression, neurogenesis, and synaptic plasticity, and ultimately, these adaptive changes lead to therapeutic benefit (Sharp, 2013). Over the last quarter of a century, selective serotonin reuptake inhibitors (SSRIs) have become the first-line antidepressant medication class, although producing a measurable benefit could take weeks. Furthermore, SSRIs can also produce significant side-effects that patients do not tolerate, including sleep deprivation, sexual dysfunction, weight gain, nausea, and headaches (Moret et al., 2008).

Table 1: Management of depression.

Goal

The main objective of treatment is the complete remission of depression with full functional recovery and the development of resilience

General Measures

- Taper and cease any drugs that can potentially lower mood
- Institute sleep hygiene and address substance misuse if relevant
- Implement appropriate lifestyle changes (e.g., smoking cessation, adopt regular exercise, and achieve a healthy diet)

Interventions

Psychological Therapy

- Cognitive behavioral therapy
- Interpersonal therapy
- Acceptance and commitment therapy
- Mindfulness-based cognitive therapy

Pharmacotherapy

First line

- SSRIs, NaSSAs, NDRIs, or SNRIs
- Melatonin agonist, serotonin modulator

Second line

- Tricyclic antidepressants
- MAOIs
  Augmentation
- Mood stabilizers

TRD therapies

- Ketamine
- Electroconvulsive therapy
  - Unilateral
  - Bilateral
- TMS

Strategies

- Start with psychotherapy
- Combine pharmacotherapy and psychotherapy
- Change/Increase dose of antidepressant medication (up to three times)
- Electroconvulsive therapy

1.1.3.1 Treatment resistance

Despite advances in the understanding of the psychopharmacology and biomarkers of major depression and the introduction of several novel classes of antidepressants, only 60%–70% of patients with depression respond to antidepressant therapy (Al-Harbi, 2012). Of those who do not respond, 10%–30% exhibit treatment-resistant symptoms coupled with difficulties in social and occupational function, decline of physical health, suicidal thoughts, and increased health care utilization. We speak about treatment-resistant depression when the response to two adequate (optimal dosage and duration) trials of at least two different classes of antidepressants is poor or unsatisfactory (Al-Harbi, 2012). When a suboptimal response is observed, a common approach is to combine psychological therapy with pharmacotherapy (Jobst et al., 2016). In

cases where the desired effect of antidepressant medication is not achieved, one strategy is to increase the dosage of the antidepressant (Adli et al., 2005). However, it should be noted that merely increasing the dosage does not necessarily lead to increased efficacy, and clinical studies have not found significant benefits when dose escalation was attempted after initial non-response to standard-dose pharmacotherapy (Dold et al., 2017). Nevertheless, increasing the dosage may help to overcome certain pharmacokinetic limitations. Another strategy to address non-response is augmentation, which involves adding a supplementary medication, such as mood stabilizers or neuroleptics, to enhance the antidepressant effects of the current prescription. If this approach also proved to be ineffective, switching to a new antidepressant with a different mechanism of action is considered (Ruhé et al., 2006). Unfortunately, for some cases none of the previous attempts reveals to be helpful. For such cases, electroconvulsive therapy (ECT) represents the most consistently effective intervention in patients with treatment-resistant depression, with a response rate ranging from 50% to 70% (Shelton et al., 2012).

1.1.4 Electroconvulsive therapy – ECT

In the upcoming section, I will present a collection of firsthand testimonials (parts in italics) shared by patients who have directly undergone electroconvulsive therapy. I believe that by focusing on these individual cases, one can genuinely comprehend the advantages of this treatment approach.

"I am a man who - almost 30 years ago - had his life saved by two long courses of electroshock therapy[...]

I would schedule all my major surgical cases for 12, 1 o'clock in the afternoon, because I couldn't get out of bed before about 11 o'clock […] I couldn't even put the covers off myself.

I got increasingly depressed until I thought: 'oh my God, I can't work anymore'.

By the time I got out of that unit, I was not functional at all. I could hardly see five feet in front of myself. I shuffled when I walked, I was bowled over. I rarely bathed. I sometimes didn't shave. It was dreadful. And it was clear - not to me, because nothing was clear to me at that time anymore - that

I would need long term hospitalization in that awful place called a 'mental hospital'

They tried everything they had, they tried the usual psychotherapy, they tried every medication available in those days… and finally they decided they better have a meeting of the senior staff. They put all their heads together and they decided that there was nothing that could be done for this surgeon who had essentially separated himself from the world, who by that time had become so overwhelmed, not just with depression and feelings of worthlessness and inadequacy, but with obsessional thinking, obsessional thinking about coincidences, just awful, awful stuff […] every moment was a scream so they decided there was no therapy, there was no treatment.

Well, he said 'can't we try a course of electroshock therapy?' […] Six didn't work, seven didn't work, eight didn't work. At nine, I noticed a change and at 10, I noticed a real change. By 16, by 17, there were demonstrable differences in the way I felt. By 18 and 19, I was sleeping through the night. And by 20, I really had the sense, that I could overcome this. I was now strong enough that I thought, by an act of will, I could blow the obsessional thinking away, I could blow the depression away […]

My children came back, the career resuscitated, even better than it had been before.

Anything can happen to you, things change, accidents happen, something from childhood comes back to haunt you, you can be thrown off the track, I hope it happens to none of you, but it will probably happen to a small percentage of you. To those to whom it doesn't happen there will be adversities […] if I can find my way back from this, believe me, anybody can find their way back from any adversity that exists in their lives. There is recovery and there is resurrection."

Sherwin B. Nuland, American surgeon, and writer (1939-2014).

ECT is a somatic treatment consisting of an electrical stimulation of the brain, which triggers a generalized seizure (Grözinger et al., 2013). Nevertheless, a single session is not sufficient to have an antidepressant effect. Therefore, a series of six to twelve individual treatments is needed with usually three treatments a week. Although beneficial, the precise mechanism by which ECT works remains to be determined (Hoy & Fitzgerald, 2010).

Approximately 80% of patients presenting for ECT treatment have a diagnosis of major depression. ECT also continues to be used, although less frequently, to treat schizophrenia, the condition in which it was originally applied, as well as catatonia and acute mania (Jaffe, 2002).

1.1.4.1 ECT throughout history

Figure 4: Dr.Ugo Cerletti, Rome, 1939.

Although the specific mechanism of action of ECT has not been isolated, the notion that convulsions may promote wellness has existed for centuries (Payne & Prudic, 2009). In the 16th century, Paracelsus, a Swiss alchemist, administered camphor orally to induce convulsions and "cure lunacy". Cases of chemically induced convulsions using camphor in oil were reported in the 18th and 19th centuries. In 1934, Ladislaw Meduna, a Hungarian psychiatrist, explored the potential inverse link between seizures and schizophrenia. Based on neuropathological research and a review of studies conducted in the past century, Meduna suggested a potential correlation between the reduced presence of glial cells in individuals with schizophrenia and the excessive proliferation of these cells in those with epilepsy. Hoping to treat patients with schizophrenia by inducing epilepsy, he administered an injection of camphor in oil to a patient with catatonic schizophrenia, triggering a 60 second grand mal seizure. Following a brief series of such treatments, the patient made a full recovery; by the end of that year, five more patients had undergone the same treatment. Camphor was replaced by metrazol, and the therapy became popular throughout Europe. The idea of applying electricity to individuals with mental health issues emerged due to the highly uncomfortable sensations experienced by patients treated with metrazol. This led scientists to explore alternative ways of inducing convulsions. Researchers from Switzerland developed a method of inducing seizures in dogs through the use of direct electric current. This was later refined by the Italian scientists Cerletti and Bini, who successfully established the requisite parameters for administering electricity directly to the human scalp. In 1938, they treated an unidentified 39-year-old man who was found in a

delusional state in a train station. Following several sessions, his delusions began to fade, and he experienced a full recovery after 11 treatments, without any negative effects. Thus, "electroconvulsive" therapy was born. Although widely used, the effectiveness of ECT as an antidepressant was primarily based on anecdotal reports and individual case studies. Guidelines for empirical research on depression treatment would not emerge until a later stage, when pharmacological trials were initiated.

Figure 5: Dr. Lucio Bini, Rome, 1938.

1.1.4.2 Efficacy of ECT

Between the 1960s and the 1980s, with the introduction of antidepressant medication, the efficacy of ECT for major depressive disorder was tested with many randomized trials comparing these new medications to ECT, which was the standard treatment at that time. Patients were generally randomly assigned to ECT or medication, particularly imipramine, and monoamine oxidase inhibitors (MAOIs) (Gangadhar et al., 1982; Hutchinson & Smedberg, 1963; Medical Research Council, 1965). A meta-analysis in 1985 (Janicak et al., 1985) reported that the average response rate to ECT was 20% higher than response to tricyclic antidepressants and 45% higher than response to MAOIs. Later, the general efficacy of ECT was investigated by comparing *real* ECT to *sham* ECT (i.e., anesthesia alone) (Brandon et al., 1984; Gregory et al., 1985; Johnstone et al., 1980; West, 1981). Those studies as well revealed significant differences in response rates, favoring real ECT over sham ECT, as defined by between-group differences in HAMD-21 scores.

Today, ECT's efficacy is determined by both electrodes' placement and electrical stimulus properties. It is important to note that achieving efficacy is not the sole objective of a successful therapy; minimizing adverse effects, such as memory loss, is equally essential.

First, as regards the electrical stimulus, ECT was initially delivered using a sinusoidal waveform (Payne & Prudic, 2009). However, this pattern of electrical stimulation was less effective as it delivered a substantial amount of electricity below the threshold needed to depolarize neuronal tissue. This lack of efficacy not only failed to improve clinical outcomes but also led to notable cognitive side effects. In contrast, the brief pulse stimulus delivered full current amplitude "instantaneously". Nevertheless, this technique did not lead to any substantial difference in clinical response, but rather to a more favorable side-effect profile (Moriarty & Siemens, 1947). Based on this knowledge, modern ECT devices employ a brief pulse waveform stimulation (Prudic et al., 2004).

Secondly, the intensity of the electrical stimulation can influence ECT's efficacy and side effect profile. Clinicians had previously assumed that the induction of a generalized seizure of adequate duration - that was both "necessary and sufficient" – was essential for antidepressant effects (Sackeim et al., 1993; Weiner et al., 1986). Sackeim et al. developed and employed a method of *seizure threshold approximation*, involving a series of progressively increasing stimulations (Sackeim et al., 1987). This incremental method was utilized to determine the minimum electrical dose required for brief-pulse ECT to induce a grand mal seizure of sufficient duration, in a particular individual. The subsequent research had the goal of preventing patients from receiving a fixed electrical dosage that could either be insufficient to achieve the desired clinical effects or exceed optimal stimulus dosing and thus intensify unfavorable cognitive effects.

Electrode placement also plays a crucial role in terms of both efficacy and safety. Initially, ECT was administered bilaterally, but in 1949, Goldman introduced the practice of unilateral electrode placement to prevent the seizure induced by ECT from affecting speech areas (Goldman, 1949). This modification led to a decrease in post-ECT confusion experienced by patients.

1.1.4.3 Side effects: between perception and reality

> *In 2003, Ms. A, a teacher with a master's degree in education, was referred for electroconvulsive therapy (ECT) by her psychiatrist because she was experiencing a severe melancholic depression with profound agitation and had failed to respond to multiple medications. Ms. A. was screened for ECT, which was considered an appropriate option for her treatment. During the interview, she cried continuously and exhibited palpable fear. When asked to express the source of her fear, she replied, "The only ECT I've ever seen was in 'Cuckoo's Nest.'" Following our explanation of the treatment in its present form and urging from her psychiatrist, therapist, and family, Ms. A. did agree to the treatment and signed consent.*
>
> From (Payne & Prudic, 2009)

The response of Ms. A., a woman with postgraduate education is emblematic of how influential, and potentially destructive, distorted views of ECT can be. The film mentioned by

Ms. A., "*One Flew Over the Cuckoo's Nest"*, based on Ken Kesey's novel from 1962, showed ECT used for the wrong condition and delivered in an unmodified, outmoded fashion without anesthetic or muscle relaxant (Payne & Prudic, 2009). Although the treatment process and indications for use had significantly changed by the time the film was released, the public has only recently become aware of these developments.

The pervasive fear of ECT, derived from past inadequate treatment practices and distorted media depictions, has served as a major obstacle discouraging individuals from considering this treatment option. The fear of serious medical and psychiatric consequences, coupled with the stigma attached to ECT, may seem counterintuitive given its demonstrated efficacy and safety (Wilkinson & Daoud, 1998). The idea that ECT leads to brain damage or "fries the brain" has been propagated since the introduction of this therapy. However, there are no data to support this idea and research found no evidence that ECT produces any damage to the brain on a structural or cellular level (Devanand et al., 2006).

Severe medical consequences associated with ECT are relatively uncommon. The most problematic adverse effects are of cognitive nature (Table 2). Common cognitive side effects fall into four basic categories (Payne & Prudic, 2009). The first type of cognitive effect is the transient **postictal disorientation** that patients experience immediately after ECT treatment, which is a function of the seizure itself and of the anesthesia that was administered. However, patients subjectively experience this period quite differently. Some patients quickly regain consciousness and can return to their normal routines. Others may sleep for several hours, after which they are able to eat and continue with their day. A second form of cognitive impact is **anterograde amnesia**, where the capacity to retain information acquired during and soon after a series of ECT sessions is compromised. Anterograde amnesia can contribute significantly to a patient's inability to retain important information; hence the use of a daily log or diary is highly recommended. A third type of effect on the cognitive level is short-term **retrograde amnesia**, which results in memory lapses for events that occurred within a few weeks or months before the ECT series. Typically, this kind of amnesia improves within the initial months following the acute ECT phase, although some patients may not fully recover. There is a fourth type of cognitive impairment which is fortunately rare. This entails more extensive retrograde memory, with individuals facing severe and persistent memory deficits reaching back several months or even years (Sackeim, 2000).

Some patients may encounter **physical side effects** following ECT, such as headaches, nausea, and muscle pain, especially in the early stages of treatment. These effects may be the

result of the seizure, the anesthesia, or some combination of the two and are not medically serious (Payne & Prudic, 2009).

Table 2: Main aversive effects experienced by ECT patients.

Cognitive effects	Physical effects
• Postictal disorientation • Anterograde amnesia • Retrograde amnesia	• Headache • Nausea • Muscle pain

It is important to keep in mind that patients receiving ECT are being treated for a potentially life-threatening psychiatric condition. ECT, like most serious medical treatments, has a range of possible side effects, which can be unpleasant and subjectively disturbing, but not dangerous. Therefore, it is ethically, medically, and psychologically necessary to provide education and support to both patients and families.

To conclude this paragraph, public perceptions and revelations about ECT abound, and range from highly positive to intensely negative. Several people have elaborated on their personal stories, describing severe depressive illnesses and positive, possibly life-saving experiences with ECT. However, many individuals have also discussed the highly stigmatizing responses they received from family, friends, and colleagues upon going public. Martha Manning's experience is a case in point (Manning, 1994):

> *Telling people I've had ECT is a real conversation killer. People seem more forthright these days about discussing depression. Hell, the cashier in the grocery store told me yesterday that she's on Prozac. But ECT is in a different class. For months I have glossed over ECT's contribution to the end of my depression with most people. But lately I've been thinking, "Damn it. I didn't rob a bank. I didn't kill anybody. I have nothing to be ashamed of." I've started telling people about ECT. My admission is typically met with uncomfortable silences and abrupt shifts in topics. An acquaintance at a party is outraged. "How could you let them do that to you?" I bristle and answer, "I didn't let them do it to me. I asked them to do it" (p. 164).*

Manning also observed that the idea of administering an electrical stimulus to the human brain evokes significantly distinct sentiments compared to the application of electricity to rescue a life during a cardiac event:

> *No one bats an eye when electricity is delivered to a stalled heart. There is no outcry; in fact, it's considered a miracle. A person passes from life to death to life again through the application of electric current to the heart. But try talking about the same thing with the brain, and it's no miracle. Suddenly, words like torture and mind control populate the descriptions (p. 165).*

1.1.4.4 How does ECT work? - Potential mechanisms of action

ECT has proven to be remarkably beneficial in clinical practice, effectively improving the condition of depressed patients, even in seemingly hopeless situations. However, the specific mechanisms through which ECT operates remain to be elucidated.

Several theories have been proposed, yet no consensus has been reached on the mode of action of ECT. Undoubtedly, unraveling the precise mechanisms of ECT would significantly enhance therapeutic efficacy and improve the overall profile of side effects. Additionally, investigating the effects of ECT on the human body, may shed light or provide valuable insights on the intricate pathophysiology of depression.

In this section, we will briefly explore some of the most supported theories regarding the mechanisms of action of ECT.

1.1.4.4.1 The immune-inflammatory response

Studies have associated ECT with a neuroinflammatory immune response (Guloksuz et al., 2014; van Buel et al., 2015; Yrondi et al., 2018). A recent meta-analysis revealed that ECT induces an acute and transient *pro-inflammatory* response followed by *anti-inflammatory* effects in the long-term (Gay et al., 2021). In fact, ECT has been associated with an immediate increase in IL-1 and IL-6 (Järventausta et al., 2017; Lehtimäki et al., 2008; Rush et al., 2016; Zincir et al., 2016) and longer-term reduction in TNF-α and IL-6 (Freire et al., 2017; Järventausta et al., 2017), though reports on TNF-α are conflicting (Freire et al., 2017). While some researchers attribute only negative or adverse effects to immune system activation,

interestingly, others suggest a positive effect associated with it (van Buel et al., 2015). The latter highlight that the acute increases in cytokines following ECT could stimulate release of neurotrophins, which in turn could lead to hippocampal neurogenesis and subsequent clinical response.

1.1.4.4.2 Neuroproliferation hypothesis: brain derived neurotrophic factor

Antidepressant therapies, be it pharmacotherapy or psychological interventions, have the potential to restore reduced levels of BDNF in people experiencing depression (Molendijk et al., 2013). In animal studies, limiting neurogenesis prevents antidepressant action and has been shown to result in depression-like symptoms, especially in stressful situations. Therefore, neurogenesis has been suggested to facilitate resilience against stress, which could be the basis of antidepressant clinical effects (Kraus et al., 2017). ECT also increases levels of BDNF in the peripheral blood of depressed patients (Bouckaert, Dols, et al., 2016; Rocha et al., 2016). Moreover, similar findings were reported in animal studies using electroconvulsive seizures (ECS), an animal model for ECT (Jonckheere et al., 2018). ECS could increase BDNF and nerve growth factor (NGF) levels in several brain regions, including prefrontal cortex, hippocampus and amygdala (Angelucci et al., 2002; Conti et al., 2006) and block the stress-induced downregulation of BDNF in brains of rats (Altar et al., 2004). Importantly, some studies did not report any evidence of increases of BDNF following ECT (Lin et al., 2013; Ryan et al., 2018).

Some researchers suggest that the stimulation of BDNF release relates to the activation of the immune system by ECT. In particular, it seems that there is a positive association between secretion of cytokines, including IL-6 and TNF-α, and BDNF release (Patas et al., 2014; Schulte-Herbrüggen et al., 2005).

These findings of ECT-induced neurogenesis do not unequivocally explain how ECT exerts its therapeutic effects. However, they are compelling in that they may begin to clarify ECT's effects on brain regions putatively involved in the clinical presentation of depression.

1.1.4.4.3 ECT and HPA axis

An ECT series strongly and repetitively activates the HPA axis. Measures of hormone activity in patients consistently show a significant increase after ECT in ACTH, cortisol, and arginine vasopressin in the blood and saliva (Apéria et al., 1985; Bernardo et al., 1993; Florkowski et al., 1996; Zis et al., 1996). The increases are abrupt and seem to normalize to

baseline levels within 1 h post-treatment in human studies (Eşel et al., 2003; Zis et al., 1996). More invasive rodent studies indicate a longer lasting increase in ACTH, with sustained elevated levels at least 24 h post-treatment (Brady et al., 1994).

The intriguing connection between depression, glucocorticoid levels, and memory impairment holds significance in the context of discussing both the *therapeutic* and *cognitive effects* of ECT. It is known that depression is associated with memory impairment (Burt et al., 1995), and disruptions of the HPA axis with resulting elevated glucocorticoid levels (Gold et al., 2002), and that elevated cortisol is associated with impaired cognitive functioning (Gold et al., 2002; Nagaraja et al., 2007; Newcomer et al., 1999). Therefore, the fact that ECT produces an acute rise in serum cortisol following a single ECT session, may lead to an acute overstimulation of steroid receptors, overlaying the chronic activation induced by depression or stress, especially in the hippocampus, potentially contributing to short-term cognitive impairments (Nagaraja et al., 2007). However, ECT often elicits a decrease in overall serum cortisol over a course of treatment and may reverse the noxious effects of stress-related cortisol elevations (Burgese & Bassitt, 2015). This effect is noted particularly in treatment responders and has been investigated by Schwartz and Chen (Schwartz & Chen, 1985). These authors assumed that the abnormalities in cortisol regulation seen in many depressed patients would result in larger ECT-induced cortisol releases early in a course of treatment, when the patient was still ill.

In summary, the acute and rapid elevation in stress-related hormones following ECT might be a consequence of seizure activity, without significance on clinical or cognitive effects. However, the normalizing of the HPA axis as an outcome of successful ECT provides impetus for continuing inquiry into the relationship between ECT stimulus and the pathophysiology of depressive illness and associated cognitive effects.

1.1.4.4.4 Brain imaging insights

Researchers have used structural magnetic resonance imaging (sMRI) to explore the changes in brain structure associated with ECT and have consistently reported volume increases in limbic regions such as the hippocampus, the amygdala or the anterior cingulate cortex (Bouckaert, De Winter, et al., 2016; Nordanskog et al., 2014; Sartorius et al., 2016; Tendolkar et al., 2013), which are brain regions that have consistently been reported as critical in accounting for depressive symptomatology and disease remission (Lemke et al., 2022; Pandya et al., 2012). Such ECT-induced structural changes have been explained as a result of

neurogenesis induction in neurogenic regions (dentate gyrus), or general structural neuroplastic changes such as synaptogenesis, gliogenesis or angiogenesis (Bouckaert et al., 2014). Alternatively, other reports have suggested that ECT-induced structural changes might partially depend on neuroinflammatory mechanisms (Andrade & Bolwig, 2014; Bouckaert, De Winter, et al., 2016). For instance, an increase of the permeability of the blood–brain barrier could lead to a local swelling of adjacent brain tissue (*vasogenic edema*) (Andrade & Bolwig, 2014; Bolwig et al., 1977).

Because ECT is an effective antidepressant treatment, researchers hypothesized that there should be some significant functional alterations in specific brain regions in depressed patients before and after ECT (Porta-Casteràs et al., 2021). Using functional MRI, Kong et al. found decreased ReHo in the bilateral superior frontal gyrus after ECT in elder depressed patients (Kong et al., 2017); while another study found that ReHo values in the left angular gyrus (LAG) significantly increased in depressed patients after ECT (Mo et al., 2020). ReHo and fALFF are considered two reliable algorithms of whole brain rs-fMRI signals (Kong et al., 2017; Zou et al., 2008), which provide a valuable contribution to the research field in the context of depression and its treatment.

1.2 Aim of the thesis

Based on the current literature, electroconvulsive therapy is one of the most powerful and rapidly acting treatments in severe depression. Although ECT has been successfully applied in clinical practice for decades, the underlying mechanisms of action remain unclear. A large number of studies have attempted to propose a coherent explanatory framework for ECT's mode of action. Unfortunately, as of now, no definite mechanism has been confirmed. It is important to highlight that only a handful of studies in the extensive literature examine the complete course of ECT from initiation to conclusion, while also capturing several intermediate timepoints. This would allow to unveil the intermediate alterations that ultimately lead to the clinical improvement at the end of the therapy. Additionally, a limited number of studies have tackled the research question from multiple perspectives, thus hindering their ability to uncover potential associations between molecular and network-scale effects. In the present study, by sampling at multiple timepoints and using functional magnetic resonance imaging (fMRI) and peripheral blood samples analysis, we were aiming at examining the fluctuating effects of ECT treatment of severe depression.

Our hypotheses regarding the mechanisms of ECT are summarized below:

- **Immune-inflammatory acute response followed by long-term normalization**: as mentioned above, there is evidence that TSPO negatively regulates microglial activation and suppresses neuroinflammation (Kim & Yu, 2015). On the other hand, there is evidence that ECT induces an acute inflammatory response. Based on this knowledge, we hypothesize that ECT induces an acute release of inflammatory cytokines, which in turn triggers the increase of TSPO expression as adaptive response, which resolves the inflammatory response in the long term.
- **Neurotrophic effects enhance neuronal plasticity**: ECT favors neurogenesis, synaptogenesis, and neuronal survival in key brain regions, reinforcing the ability of our brain to adapt to new potentially positive information circuits, which in turn affect region to region communication at a larger scale. This hypothesis was driven by the neurotrophic hypothesis of depression, which states that depression is secondary to an altered expression of BDNF in the brain (Molendijk et al., 2013).
- **Molecular level changes indirectly result in brain communication changes**: brain communication is thought to be a prerequisite of complex cognition. Depression expresses itself as a disorder of cognition (Clark & Beck, 2010; Hammen, 2018). Here,

we argue that brain areas which change their metabolism change their activity and communication, and therefore cognitive processes. In this framework, spontaneous activity, functional connectivity (FC) and functional connectivity dynamics (FCD) are biomarkers for complex cognition as they denote flexible communication between brain areas (Donnelly-Kehoe et al., 2019). In fact, it has been suggested that depression as a psychological disorder can be better conceptualized as the inability to switch from a negative mood state to a non-negative one, rather than the depressive state itself (Holtzheimer & Mayberg, 2011), i.e. that depression may be a disorder of brain dynamics.

CHAPTER 2 Materials and Methods

2.1 Study design

The study was realized within the scope of the graduate school GRK 2174 Neurobiology of emotion dysfunctions (spokesperson: Prof. Dr. rer. nat. Inga Neumann) funded by the German research foundation (DFG; subproject P8). The clinical trial was conducted monocentric at the Clinic for Psychiatry and Psychotherapy at the University of Regensburg (medical facilities of the District Hospital – medbo – KU-) under the supervision of: Prof. Dr. med. Caroline Nothdurfter as principal investigator; Prof. Dr. rer. nat. Jens Schwarzbach as project co-investigator; Prof. Dr. rer. nat. Inga Neumann as project mentor.

The clinical study had a repeated measures design including one observational group that received electroconvulsive therapy (ECT) as treatment (see Figure 6). We performed measurements weekly throughout the course of the therapy from baseline until the 6th week. Every week (on different days) included the following appointments:

- Assessment of the clinical course:
    - Hamilton Depression Rating Scale – HAMD-21 (Hamilton, 1960)
    - Beck Depression Inventory – BDI (Beck et al., 1961))
- Collection of blood samples:
    - 2 x 9 ml Serum tubes
    - 2 x 9 ml Ethylenediaminetetraacetic Acid (EDTA) tubes
    - 1 x 9 ml citrate tube
- Magnetic Resonance Imaging (MRI) scan:
    - Functional
    - Structural
    - Spectroscopy

The study plan was approved by the ethics committee of the University of Regensburg (vote # 20-1691-101). It was conducted according to the guidelines of the International Conference on Harmonization (ICH, 2006) and Good Clinical Practice (GCP) as well as the Declaration of Helsinki (World Medical Association, 2013). The clinical trial was registered at the Clinical Trials Register (study ID number: DRKS00026738) and the regional authorities

(government of Upper Franconia). We conducted the clinical trial from October 2020 to April 2023.

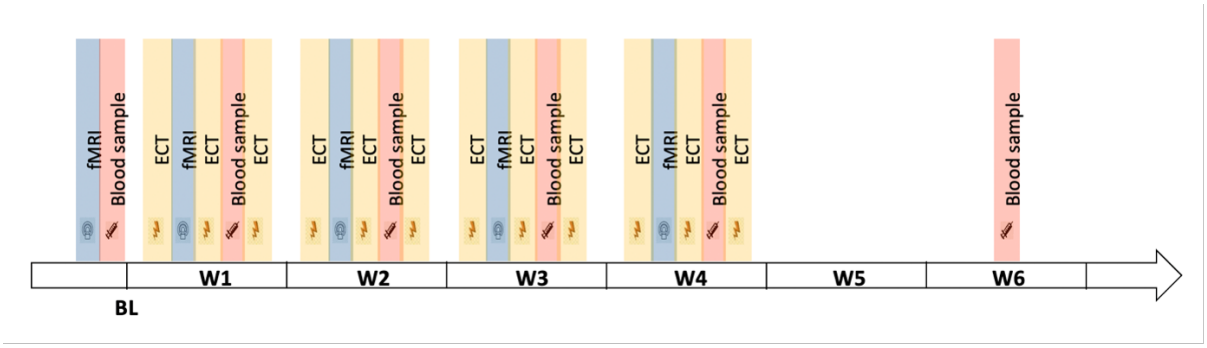

Figure 6: Study design. Patients received three electroconvulsive therapy (ECT) sessions per week. Functional magnetic resonance imaging (fMRI) and blood samples collection were performed every week. Follow up measurements of blood parameters occurred in week six.

2.2 Patients

2.2.1 Recruitment

We planned to include $N = 10$ patients aged between 18 and 60, diagnosed with a depressive episode and selected to receive an ECT treatment series.

In total, we asked thirteen patients who were admitted to the Clinic for Psychiatry and Psychotherapy of the University of Regensburg at the District Hospital to participate in our study. Prof. Dr. med. Nothdurfter showed and explained in detail the structure of the study, including the number of appointments, medical and scientific investigators involved in the study, and the content of the consent form. We cared to keep any additional task for the patients at minimum: the clinical ratings and the collection of blood samples were already included in the routine clinical practice; the additional part consisted of five MRI sessions. Eleven patients out of thirteen agreed to participate in the study. Two of the recruited patients were unable to complete the study due to high BMI (therefore increased risk of complications related to anesthesia during ECT (Domi & Laho, 2012)) or due to adverse side effects (e.g., localized and persistent headaches). Ultimately, nine patients successfully completed the study (fig. 7).

Source data of the participants were collected in case report forms in an anonymized form. We assigned a pseudonymous code with the form "*GRK-EKT XX*" (*XX*, 01 to 10) to each patient to ensure confidentiality and protect their identity throughout the study. Participants received a compensation of 200 euros at the end of the study for their contribution to our research.

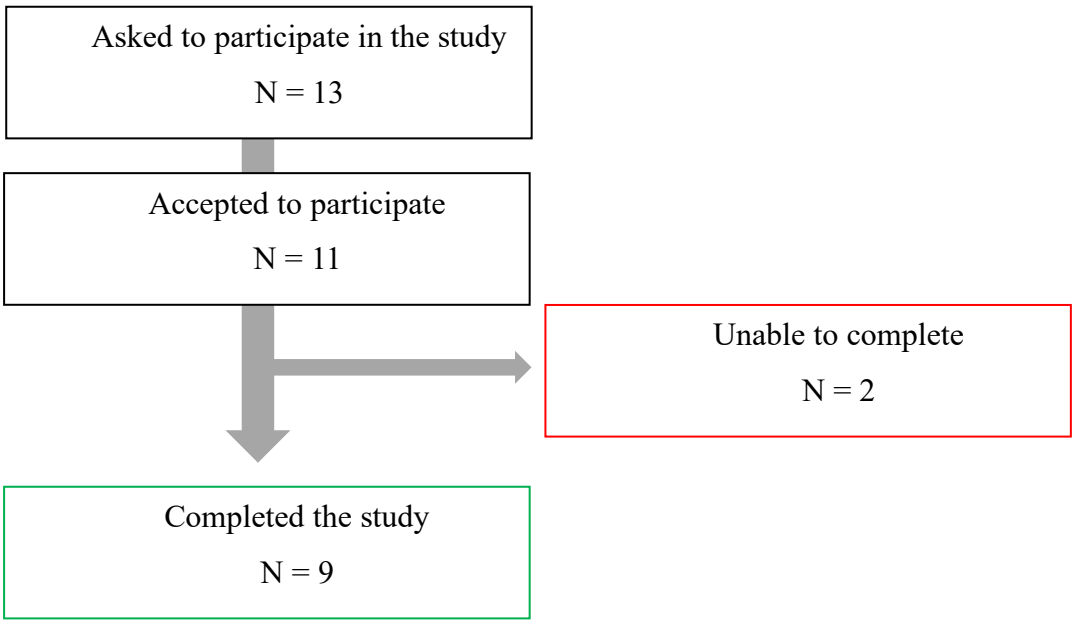

Figure 7: Flowchart of participants recruitment: from 13 patients asked, 9 were able to complete the study and provide compete MRI and blood data.

2.2.2 Inclusion criteria

We planned to include both male and female patients in our study. Inpatients with a depressive episode for whom ECT represented the best treatment option were suitable candidates for the study. A psychiatrist diagnosed the depressive episode based on the International Classification of Diseases, tenth revision (ICD-10) if the patient had bipolar affective disorder (F31), depressive episode (F32), or recurrent depressive disorder (F33). Additionally, the diagnosis was confirmed by a score higher than 17 in the HAMD-21 score.

Participants had to be between 18 and 60 years of age and to be able to understand the nature and the meaning of the study, including their rights (e.g., the right to terminate their participation at any time if they wanted) and the eventual risks associated with the trial. It should be noted that the risk for the patient associated with the present study was assessed as extremely low, since it was a purely observational study, which had no influence on the treatment of the patient. Most importantly, the signature of the consent form, after oral and written information, was necessary to be able to begin the study. Patients had at least two days between receiving the relevant information and providing their written consent.

2.2.3 Exclusion criteria

The most important exclusion criterion was an incapability of a patient to understand the nature and scope of the study, therefore an incapability of giving informed consent. Patients were also not included if diagnosed with a mental disorder, such as dementia, substance dependence (drug, alcohol, or others), depressive episode secondary to somatic or neurological disease, or additional severe psychiatric comorbidities. Moreover, participants should not suffer from any severe neurological or internal concomitant disease that may affect the interpretability of the results (e.g., fresh myocardial infarction, Cushing's disease, underlying rheumatic diseases). Given the scope of the investigation (steroidal and inflammation-related parameters), candidates who regularly underwent systemic administration of steroids or non-steroidal anti-inflammatory drugs (NSAID), could not be included in the study. A major part of the study was represented by the MRI sessions. Therefore, incompatibility with MR measurements was a crucial exclusion criterion (see Appendix B - MR questionnaire). Finally, the condition of pregnancy or breastfeeding precluded participation in the study.

2.2.4 Control group – MITDEP

Thanks to the collaboration with a colleague from our research group, we were able to compare our data to data from a group of depressed patients who received similar antidepressant medication but did not undergo ECT treatment. Data collection has been performed within the MITDEP study conducted by Dr. Lisa-Marie Brunner and Prof. Dr. Caroline Nothdurfter at the District Hospital – medbo – KU- from April 2021 until March 2022.

Since the two studies were not planned in parallel, we were not able to precisely pair each patient with an individual healthy control. Therefore, we included $n = 11$ patients trying to maximize matching between the two groups by age and gender. Within the MITDEP study, three timepoints have been considered for data collection: baseline (BL), week two (W2), and week six (W6). Therefore, the comparison between ECT and MITDEP data was possible only for those timepoints.

2.3 ECT

Electroconvulsive therapy was administered thanks to the teamwork of psychiatrists, including Prof. Dr. med. Nothdurfter leading and coordinating the procedure, anesthesiologists, and nursing staff. Before starting the procedure, a psychiatrist evaluated the patients' condition, their medical eligibility for the therapy and concluded that ECT was the most suitable treatment. The psychiatrist then proceeded with proposing the therapy to the patient and, after the informed consent form was signed, the therapy began.

2.3.1 Procedure

ECT started around 9:15 a.m., patients were prepared for the procedure, including not eating nor drinking before the session. The procedure was performed under general anesthesia (propofol and ketamine) and muscle relaxant (succinylcholine followed by a non-depolarizing muscle relaxant) to avoid the peripheral convulsion effects but being able to induce the effects in the brain. Electrodes were then applied choosing right unilateral (RUL) or bilateral (BIL) method (fig. 8; table 3). Following the recommended RUL administration protocol, one electrode was positioned at the vertex of the patient's head, while another electrode was placed on the right side of the head. Initially, all patients received treatment using the RUL method. However, if this approach did not yield a satisfactory response, we switched to the BIL administration method. In the BIL method, one electrode was placed on the right side of the forehead, and the other electrode was placed on the left side. Then a small electrical current was delivered to the brain for ~ 20 seconds. This stimulation triggered a therapeutic general seizure of variable duration in each patient. During ECT, several parameters including anesthesia, electroencephalogram (EEG), electrocardiogram (ECG), electromyogram (EMG), blood pressure, blood oxygenation were carefully monitored to assess the success of the procedure. After each session patients were monitored for any adverse effect, including confusion, memory loss, and muscle ache.

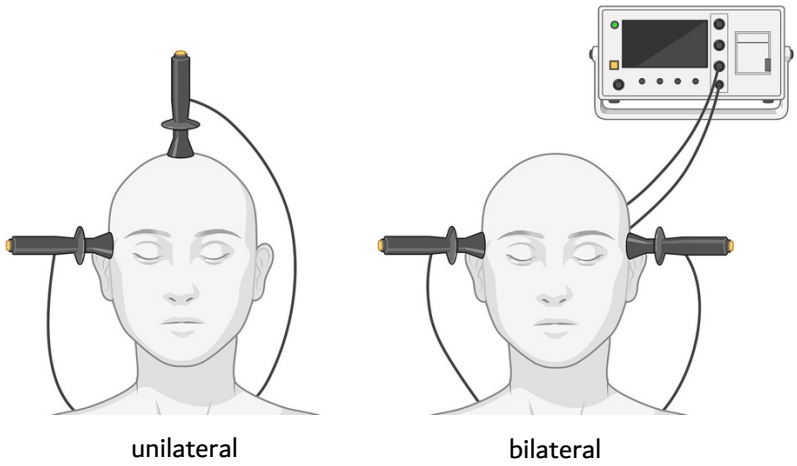

Figure 8: Electrode placement for right unilateral (RUL) and bilateral (BIL) administration methods in electroconvulsive therapy (ECT).

Depending on the severity of the condition of the patients and how they responded to the treatment, a different number of sessions was required for each patient (see Table 3). Our sample required an average of 15 sessions in total (M =15, SD = 5), usually administered 3 times per week (Table 3).

Table 3: ECT administration details and additional medication for each patient.

Patient	Days of ECT	Nr. Sessions	Electrode placing	Additional medication
GRK-EKT03	Mo, Wed, Thu	14	45-95% RUL	Antidepressant (SSRI), Benzodiazepine, Atypical antipsychotic
GRK-EKT04	Mo, Wed, Fr	15	50-80% RUL	Antidepressant (SSRI), Benzodiazepine, Atypical antipsychotic
GRK-EKT05	Mo, Wed, Fr	23	35-70% RUL/BIL	Antidepressant (NaSSA), Benzodiazepine, Atypical antipsychotic
GRK-EKT06	Mo, Wed, Fr	17	50-80% RUL	Antidepressant (Agomelatine), Methylphenidate
GRK-EKT07	Mo, Wed, Fr	21	50-100% RUL/BIL	Antidepressant (NaSSA, SSRI), Atypical antipsychotic
GRK-EKT08	Mo, Wed, Fr	11	50-70% RUL	Antidepressant (SNRI), Atypical antipsychotic
GRK-EKT09	Mo, Wed, Fr	15	50-100% RUL	Antidepressant (SSRI), anxiolytic, dopamine agonist
GRK-EKT10	Mo, Wed, Fr	8	60-80% RUL	Antidepressant (SNRI), Atypical antipsychotic
GRK-EKT11	Mo, Wed, Fr	11	40-50% RUL	Antidepressant (Bupropion), Atypical antipsychotic

2.4 Clinical course rating scales

We monitored the severity of depression using both an external and a self-assessment method to ensure the greatest possible certainty of diagnostic assessment. For the external assessment, a psychiatrist conducted the Hamilton Depression Rating Scale (HAMD-21), whereas patients filled the Beck Depression Inventory (BDI) questionnaire as self-evaluation (Appendix A) once a week. We performed the evaluation at baseline (BL), at week one (W1), week two (W2), week three (W3), week four (W4) and repeated at week six (W6) as follow up measurement.

2.4.1 Hamilton Depression Rating Scale

The HAMD-21 is a clinical assessment scale for evaluating the severity of depression, introduced by Max Hamilton in 1960. It consists of 21 structured questions that evaluate various aspects of depression, including mood, feelings of guilt, suicidal thoughts, sleep disturbances, and cognitive impairments. Each item on the scale is scored on a standardized scale, with higher scores indicating more severe depressive symptoms. At the end of the test, we summed the scores of each question, obtaining a total score which indicates the severity of depression (Table 4). The advantage of using HAMD-21 questionnaire is that it provides a standardized and objective evaluation of depression course.

Table 4: HAMD-21 scores and associated outcomes.

Score	Outcome
0-9	No depression / remission
10-20	Mild depression
21-30	Medium depression
> 30	Severe depression

2.4.2 Beck Depression Inventory

Beck's Depression Inventory (BDI) was developed by psychiatrist Aaron T. Beck in 1961. It comprises 21 multiple-choice items that measure various emotional, cognitive, and physical symptoms associated with depression. In this case as well, each item was assigned a score and

the sum of all scores showed the overall level of depressive symptomatology, with higher scores reflecting more severe symptoms (Table 5). We gave the BDI to the patients on the same day of the HAMD-21 rating, but patients were allowed to fill in the questionnaire at the most convenient time for them during the week. We then collected the BDI and compared the total score with the HAMD-21 total score.

Table 5: BDI scores and associated outcomes.

Score	**Outcome**
0-12	No depression / remission
13-19	Mild depression
20-28	Medium depression
29-63	Severe depression

2.5 Blood samples

2.5.1 Collection procedure

We collected blood samples, as the other measures, at the following timepoints: BL, W1, W2, W3, W4 and W6. Given that the patients were hospitalized, blood samples were obtained once a week, coinciding with the day of the regular clinical blood analysis (Monday or Friday). This approach aimed to prevent the need for multiple blood draws during the week, reducing discomfort and inconvenience for the patient. The blood collection procedure was conducted in the morning, around 7 a.m., ensuring that the patient was in a sober state. At each appointment, either the unit nurse or the study nurse conducted the blood withdrawal under sterile conditions. We collected 5 tubes (Sarstedt, Nümbrecht, Germany) of 9 ml of full blood each:

- 2 x 9 ml Serum tubes → for inflammatory biomarkers
- 2 x 9 ml Ethylenediaminetetraacetic Acid (EDTA) tubes→ for peripheral blood mononuclear cells (PBMCs) isolation, TSPO gene polymorphism
- 1 x 9 ml citrate tube → for TSPO and VDAC-1 protein expression

Each tube was labelled with the pseudonymous patient's code to ensure the correct identification of the samples during the whole process until reaching the laboratory of the Chair of Psychiatry and Psychotherapy located at the University Hospital Regensburg (UKR, D4 department; responsible person: Prof. Dr. med. Caroline Nothdurfter). Serum tubes had to be immediately placed on ice to preserve the integrity of the biological molecules in the samples. After collection, we transported the samples to the lab at UKR and preprocessed them within two hours.

2.5.2 Preprocessing

2.5.2.1 2 x 9 ml serum

To separate serum from cellular blood components *serum tubes* were first centrifuged at 2877 g (Megafuge 2.0 R, Heraeus, Thermo Scientific, Schwerte, Germany) for 10 minutes at 4 °C. Then the isolated serum was pipetted into four aliquots of 0.1 ml and six epicups of 0.2 ml and frozen at -80°C until further analyzing.

2.5.2.2 1 x 9 ml citrate

After centrifuging the full blood samples (9 ml citrate blood tubes) at 2100 g for three minutes at RT, we placed 2400 µl of the platelet rich plasma and 400 µl acid-citrate-dextrose solution (Sigma-Aldrich, Taufkirchen, Germany) into 15 ml centrifuge tubes and homogenized the components. Following further centrifugation at 2100 g for three minutes at RT, the supernatant was decanted and the pellets containing platelets were frozen at -80 °C until determination of TSPO and VDAC-1 expression using Western blots.

Figure 9: Thrombocyte isolation from plasma. Supernatant: plasma; white ring: platelets; red sediment: erythrocytes.

2.5.3 Analysis

We collected peripheral blood samples to analyze a range of inflammatory markers, including cytokines, TSPO, and the associated channel VDAC-1. Additionally, we assessed the levels of the neurotrophic factor BDNF to examine its role during ECT, cortisol as an indicator of stress levels, and pregnenolone as crucial molecule for neurosteroids biosynthesis.

2.5.3.1 TSPO and VDAC-1

To quantify protein concentrations of lysates we used the Bradford method (M. M. Bradford, 1976) with the Bio-Rad Protein Assay Dye Reagent Concentrate (Bio-Rad Laboratories, Munich, Germany). First, we separated 50 µg of protein lysates using sodium dodecyl sulfate gel electrophoresis with 3.5 % stacking and 15% running in 4 µl buffer and water. After incubating the lysates at 95° C for five minutes, we applied 3 µl of molecular weight markers and the first gel run started (150 V, 90 min., RT). We then transferred the lysates onto a nitrocellulose membrane (0.2 A /100 V, 2.5 h, RT), and performed immune detection on the membranes that were first colored with Ponceau S (AppliChem GmbH,

Darmstadt, Germany). Separation of the membrane was conducted from left to right at 25 kDa and 35 kDa. We then further incubated the membranes with 5 % non-fat dry milk in 10 ml TBS- T (Tris-buffered saline with Tween20) for one hour at RT. To detect TSPO we implemented a rabbit-monoclonal-anti-TSPO antibody (5 µl / 5 ml; 1:1000) (Davids Biotechnologie GmbH, Regensburg, Germany), while for ß-tubulin we used a rabbit-anti-ß-tubulin antibody (300µl / 2,7 ml; 1:10) (Abcam, Berlin, Germany) overnight at 4°C. For VDAC-1, a mouse-anti-VDAC-1 antibody (5 µl / 5 ml; 1:1000) was used (Abcam, Berlin, Germany). On the following day, we washed the blots with PBS-T three times for ten minutes, then we performed incubation with secondary horseradish peroxidase-conjugated antibodies (1 µl / 5 ml; 1:2000) for two hours at RT and washed again 3 x 10 minutes with PBS-T.

To detect proteins bands, we used chemiluminescence with a digital imaging system (Image Quant LAS 4000, GE Healthcare Europe, Freiburg, Germany). Densitometric analyses were performed with ImageJ Software (Wayne Rasband, National Institute of Health, USA). Finally, we normalized TSPO and VDAC-1 values to ß-tubulin values of the same sample and to a control sample.

2.5.3.1.1 TSPO gene polymorphism rs6971

To determine the TSPO gene polymorphism rs6971 we extracted genomic DNA from the whole blood samples with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), and we assessed quality with optical absorbance and gel electrophoresis. We then performed polymerase chain reaction to amplify exon 4 of the TSPO gene containing the polymorphism rs6971 (alanine or threonine at position 147). Next, the samples were sequenced according to the Sanger method (Sanger, Nicklen, & Coulson, 1977) with the following primers: hTSPO-exon4-F (5'-AGT TGG GCA GTG GGA CAG-3'), hTSPO-exon 4-R (5'-GCA GAT CCT GCA GAG ACG A-3') (Metabion, Planegg, Germany). The obtained data were analyzed using SnapGene software (GSL Biotech, Chicago, IL, USA).

2.5.3.2 BDNF

BDNF serum levels were determined using an enzyme-linked immunosorbent assay (ELISA) kit (Thermo Fisher Scientific, Carlsbad, USA; catalog number: EH42RB) according to the manufacturers' protocol. First, 20X diluted serum samples were added to a microplate well coated with capture antibodies specific to BDNF protein. After incubation at 4°C overnight, unbound components were washed away 4 times with wash buffer (available in the

kit) to remove any nonspecific binding. Next, biotinylated detection antibodies, which are labeled with horseradish peroxidase (HRP), were added to the well. During incubation at room temperature for 1 h and 45 min, these antibodies bound to the BDNF protein that has been captured by the immobilized antibodies. Following another wash step (4X) to remove unbound detection antibodies, TMB substrate specific to the enzyme was added. The substrate undergoes a reaction with the enzyme, resulting in a soluble blue reaction product, which is proportional to the amount of protein present in the sample. The reaction time plays a crucial role in the formation of the product: if the substrate is allowed to react for too long, it can lead to an excessive increase in protein levels, surpassing the maximum threshold of detection. To prevent this, we carefully monitored the concentration of the highest standard solution at a wavelength of 620 nm. We initiated measurements after 10 minutes of the reaction and continued monitoring every 5 minutes thereafter. The aim was to determine the optimal point at which the reaction should be stopped. We established that when the optical density (OD) value reached approximately 0.95, it was the appropriate time to halt the reaction. This value served as a reliable indicator that the desired protein concentration had been achieved, ensuring that it remained within the detectable range. After stopping the reaction, the signal intensity was measured by spectrophotometry at 450 nm. A standard curve generated using known concentrations of BDNF was used to determine the protein concentration in the unknown samples. The assay sensitivity was 80 pg/ml. Intra-assay variation was below 10 %, inter- assay variation <12 %.

2.5.3.3 Pregnenolone

We utilized ELISA to measure pregnenolone levels. For this purpose, we employed a kit from IBL, Tecan (Hamburg, Germany, catalog number: DB52031). Our assay protocol involved using 50 µl of undiluted serum. The principle of this assay followed the common competitive binding scenario observed in ELISAs. To determine the concentrations of pregnenolone, we extrapolated the values from a 4 Parameter Logistic (lin/log) standard curve, which allowed for accurate quantification.

2.5.3.4 Cortisol

We analyzed serum levels of cortisol using ELISA (Demeditec Diagnostic, Kiel, Germany; catalog number: DEH3388). Following the manufacturers' protocol, we needed 10 µl of undiluted serum samples. We calculated the concentration of the samples using a 4

Parameter Logistic (log/log) curve fit. For each sample, we conducted two measurements and calculated the mean value to be used in subsequent statistical analyses. In order to exclude measurements with significant variability, we applied a criterion of coefficient of variation (CV) greater than 20 %. For any samples that met this criterion, we repeated the analysis to obtain more reliable and consistent results. The assay sensitivity was 3.79 ng/ml. Intra-assay variation ranged between 6.3 % and 8 %, inter- assay variation between 4.2 % and 6.4 %.

2.5.3.5 Cytokines

To evaluate various pro and anti-inflammatory cytokines in the serum of patients, we chose to use a highly regarded ELISA kit (MSD Mesoscale - multi-spot Proinflammatory Panel 1, USA). This kit offered the advantage of simultaneous detection and quantification of nine distinct cytokines: IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, and TNF-α. By employing this kit, we were able to efficiently analyze and measure the concentrations of these cytokines in a single assay. MSD provides a plate pre-coated with capture antibodies on independent and well-defined spots, as shown in the layout below (fig. 10). We then added the sample and a solution containing detection antibodies conjugated with electro-chemiluminescent labels. Analytes in the sample bound to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completed the sandwich. Next, we added an MSD buffer that created the appropriate chemical environment for electrochemiluminescence (ECL), and we loaded the plate into an MSD reader where a voltage applied to the plate electrodes caused the captured labels to emit light. The instrument measured the intensity of emitted light (which is proportional to the amount of analyte present in the sample) and provided a quantitative measure of each analyte in the sample. Intra-run CVs were typically below 7%, and inter-run CVs below 15%.

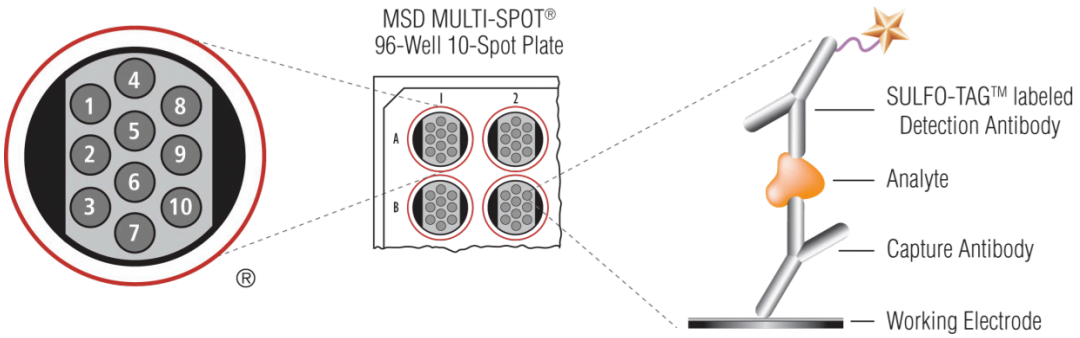

Figure 10: MSD Multi-Spot ELISA system.

2.5.4 Statistical analysis

We conducted statistical analyses on a dataset of $n = 9$ patients (GRK-EKT 03 - 11), for whom we had complete data available. Analyses concerning the formulated hypotheses were conducted using the statistics software SPSS (version 26, IBM Statistics) with a significance level at $\alpha = .05$ for all analyses. Given our weekly sample collection, we conducted a repeated measures analysis of variance (rm-ANOVA) to assess each blood parameter. The analysis included the factor of time, with categories including baseline (BL), week 1 (W1), week 2 (W2), week 3 (W3), week 4 (W4), and week 6 (W6). First, we used Levene's tests to test for homogeneity of variances. In case of violation of sphericity indicated by a significant p-value of the Mauchly-test, we report Greenhouse-Geisser (GG) corrected values. Effect sizes are reported as partial eta (η_p^2). In case of significant main effects produced by the ANOVA, we computed post-hoc t-tests to determine differences between the single timepoints. To account for multiple comparisons and the concomitant accumulation of the alpha error, we conducted Bonferroni correction.

In relation to TSPO and VDAC-1 expression, we calculated the relative measure by comparing TSPO or VDAC-1 protein levels to those observed in a healthy control subject. As a result, we present the findings in relative units (rU). This methodology enables us to account for the disparities in TSPO and VDAC-1 levels between patients and healthy individuals, while also highlighting any within-patient changes throughout the course of therapy.

The remaining parameters were all reported in ng/mL, representing the standard unit of measurement used for these specific parameters.

To investigate whether the observed changes were linked to the patient's response to therapy, we performed an analysis comparing responders and non-responders. The criterion used to define responsiveness was based on the HAMD-21 scores. Following the recommended guidelines, we classified patients as responders if their HAMD-21 score at week six demonstrated a reduction of at least 50% compared to the baseline score (Malhi et al., 2015). By employing this criterion, we aimed to identify and differentiate individuals who showed a positive response to the treatment from those who did not.

2.5.4.1 Correlation between blood parameters and clinical outcome

To further investigate whether changes in the blood parameters were associated with clinical outcome, we conducted a correlation analysis. Considering our repeated measures design, we performed a repeated measures correlation (rmcorr) using Python (Bakdash & Marusich, 2017). Differently from simple regression or correlation, rmcorr accounts for non-independence among observations using analysis of covariance (ANCOVA) to statistically adjust for inter-individual variability. Rmcorr estimates the common regression slope, i.e., the association shared among individuals. We report results as r = correlation value, $\alpha = .05$ for significance.

2.6 Magnetic Resonance Imaging (MRI)

To investigate how ECT affects brain network connectivity and dynamics, we acquired MRI data at the following timepoints: BL, W1, W2, W3, and W4. It is important to note, however, that we did not collect a follow-up measurement at week six as we did for the blood samples. We made this decision after careful consideration of the ethical implications of requiring patients to undergo an additional scan session.

Each scanning session lasted around 1 h and included a structural brain scan, a 20-minutes resting-state scan and, depending on the patient's condition, an optional magnetic resonance spectroscopy (MRS) scan to assess GABAergic activity in the praecuneus. We were able to acquire MRS scans from 8 patients. Additionally, we included a diffusion scan at baseline for later fiber tracking (table 6).

Table 6: MRI scanning plan throughout the study.

BL		W1		W2		W3		W4	
AAHead_Scout	00:14	AAHead_Scout	00:14	AAHead_Scout	00:14	AAHead_Scout	00:14	AAHead_Scout	00:14
GRKP8_mb04_1000TR_rs-Fmri	22:10	GRKP8_mb04_1000TR_rs-Fmri	22:10	GRKP8_mb04_1000TR_rs-Fmri	22:10	GRKP8_mb04_1000TR_rs-Fmri	22:10	GRKP8_mb04_1000TR_rs-Fmri	22:10
gre_field_mapping	02:20	gre_field_mapping	02:20	gre_field_mapping	02:20	gre_field_mapping	02:20	gre_field_mapping	02:20
sag T2 Flair space3d	05:42	T1 mp-rage3d we sag 1mm	04:27	sag T2 Flair space3d	05:42	sag T2 Flair space3d	05:42	sag T2 Flair space3d	05:42
DWI_MSMT_2.5mm_full_AP	15:37	sag T2 Flair space3d	05:42						
DTI b0 only 2.5mm_96_PA	01:10								
OPTIONAL: SPECTROSCOPY		OPTIONAL: SPECTROSCOPY		OPTIONAL: SPECTROSCOPY		OPTIONAL: SPECTROSCOPY		OPTIONAL: SPECTROSCOPY	
t1_fl2d_sag_p2	00:28	t1_fl2d_sag_p2	00:28	t1_fl2d_sag_p2	00:28	t1_fl2d_sag_p2	00:28	t1_fl2d_sag_p2	00:28
t1_fl2d_cor_p2	00:32	t1_fl2d_cor_p2	00:32	t1_fl2d_cor_p2	00:32	t1_fl2d_cor_p2	00:32	t1_fl2d_cor_p2	00:32
t1_fl2d_tra_p2	00:24	t1_fl2d_tra_p2	00:24	t1_fl2d_tra_p2	00:24	t1_fl2d_tra_p2	00:24	t1_fl2d_tra_p2	00:24
PRESS_short	03:27	PRESS_short	03:27	PRESS_short	03:27	PRESS_short	03:27	PRESS_short	03:27
GABA_short_single_averages	08:36	GABA_short_single_averages	08:36	GABA_short_single_averages	08:36	GABA_short_single_averages	08:36	GABA_short_single_averages	08:36
GABA_short	08:36	GABA_short	08:36	GABA_short	08:36	GABA_short	08:36	GABA_short	08:36
TIME with spect	01:09:16		00:56:56		00:52:29		00:52:29		00:52:29
TIME without spect	00:47:13		00:34:53		00:30:26		00:30:26		00:30:26

2.6.1 Procedure

Imaging took place at the Brain Imaging Center of the University of Regensburg with a 3 Tesla scanner (Siemens Magnetom Prisma, Erlangen, Germany) and a 64-channel head-coil. We scheduled the timing of the scans to ensure that patients did not undergo electroconvulsive therapy (ECT) and MRI scans on the same day. As ECT sessions were conducted three times per week (typically on Mondays, Wednesdays, and Fridays), the remaining two days (Tuesdays and Thursdays) were selected as eligible days for the MRI scans. The scans were consistently scheduled in the afternoon, specifically between 1 and 5 pm. After arrival at the scanner facility,

one of the two MRI operators repeated the MR specific screening for possible exclusion criteria (Appendix B). We ensured that all points had been understood by the subjects and we repeated the screening before every session to account for any changes that may have occurred during the week. Inside the scanner room, we first attached the built-in photoplethysmography sensor to the index finger as well as the respiration belt to monitor pulse and respiration, respectively, throughout scanning. Participants were equipped with in-ear earplugs (3M E-A-R Classic, 3M, Neuss, Germany) to reduce exposure to acoustic noise. They further received an alarm bell, which they should ring in case of emergency. After positioning the patient at the scanner table as comfortably as possible, cushions were put between the head and the coil to avoid movement. After information about the duration of the different scanning sequences, the operator left the room and further communication in between the scans took place via a speaker system. Patients were asked to close their eyes during the measurements, to think of nothing in particular, and to try not to fall asleep.

2.6.2 Resting state scan

Resting state functional data were obtained using echo planar imaging (EPI), a rapid MRI data acquisition technique initially introduced by Mansfield (1977).

During 22 minutes of scanning, we acquired a whole-brain EPI sequence of 1320 volumes with axial slices of 3 mm thickness (voxel size = 3.0 × 3.0 × 3.0 mm^3, field of view (FOV) = 192 × 192 mm^2, acquisition matrix (AM) = 64 × 64, echo time (TE) = 30 ms, TR = 1000 ms, flip angle (FA) = 60°). We used a multi-band acceleration factor of 4 in a descending order (Seidel et al., 2020).

To reduce distortion that might occur due to B0 magnetic field inhomogeneities we acquired field map images after each functional scan. For this scan we used a double-echo spoiled gradient echo sequence, with TR = 715 ms, TE = 5.81 / 8.27 ms, FA = 40°, with an isotropic voxel size of 3 mm, generating two magnitude images and one phase difference image. The field map was estimated from the phase difference image.

2.6.3 Structural scan

To obtain more detailed anatomical information, additional structural images were acquired for the purpose of later registering the functional data. For this, a Magnetization Prepared Rapid Gradient Echo (MP-RAGE) scan was utilized. MP-RAGE is a 3D imaging

sequence designed to rapidly capture high-resolution structural scans. This is achieved through the implementation of double phase coding in the y- and z-directions. Initially, a 180° high-frequency pulse is applied to align the magnetization antiparallel to the magnetic field. This results in specific T1-relaxation depending on the particular tissue, causing the transversal magnetization to flip back into the z-direction and release energy. Following an inversion time, the transversal magnetization and corresponding measurable signal are elicited (Stöcker & Shah, 2013). By employing the MP-RAGE sequence, a more precise and detailed representation of the underlying anatomy can be obtained to enhance the accuracy of subsequent functional data registration.

Two different types of structural scans were obtained during the study. The first type was T1-weighted scans, which were acquired specifically at week 1. The second type of scans was T2-weighted fluid-attenuated inversion recovery (FLAIR), which were collected at all timepoints. FLAIR images offer the advantage of identifying brain areas that exhibit inflammation, providing valuable insights into regions of interest associated with inflammatory processes.

The T1-weighted structural images consisted of 160 axial slices with a thickness of 1 mm using an MP-RAGE sequence (voxel size = 1.0 × 1.0 × 1.0 mm^3, FOV = 250 × 250 mm^2, AM = 256 × 256, TR = 1910 ms, TE = 3.67 ms, FA = 9°).

The T2-weighted FLAIR structural images consisted of 160 axial slices with a thickness of 1 mm using an MP-RAGE sequence (voxel size = 1.0 × 1.0 × 1.0 mm^3, FOV = 250 × 250 mm^2, AM = 256 × 256, TR = 5000 ms, TE = 386 ms, flip angle = 120°).

2.6.4 Data analysis

We analyzed data from a total of n = 9 patients (GRK-EKT 03-11) at multiple timepoints: BL, W1, W2, W3 and W4. To perform the analysis, we developed a dedicated pipeline that was specifically designed to handle the unique features of the data. The pipeline was implemented using MATLAB, Python and R programming languages.

2.6.4.1 Preprocessing

First, we converted each dataset into a validated Brain Imaging Data Structure (BIDS) (Gorgolewski et al., 2016), which is a simple and easy-to-adopt way of organizing neuroimaging data. During this step, DICOM-files were converted in NIfTI format. After that, we performed basic pre-processing steps using fMRIprep pipeline (version 20.2.1 – see Appendix C) (Esteban et al., 2017). The processing steps include brain tissue segmentation and transformation of the T1-weighted image to the Montreal Neurological Institute (MNI) standard space (MNI152NLin2009cAsym). For fMRI data preprocessing we applied bias field correction, motion correction, slice timing corrections and spatial normalization. The preprocessed fMRI volumes were projected onto the cortical surface generating high-resolution gray ordinate time courses. Finally, the fMRI images were spatially smoothed with a Gaussian kernel of full width at half maximum (FWHM) of 3 mm (Glasser et al., 2013).

We finally analyzed the preprocessed data to study functional network properties, regional homogeneity (ReHo), and modulations in fractional amplitude of low frequency fluctuations (fALFF) of the BOLD signal (fig.11).

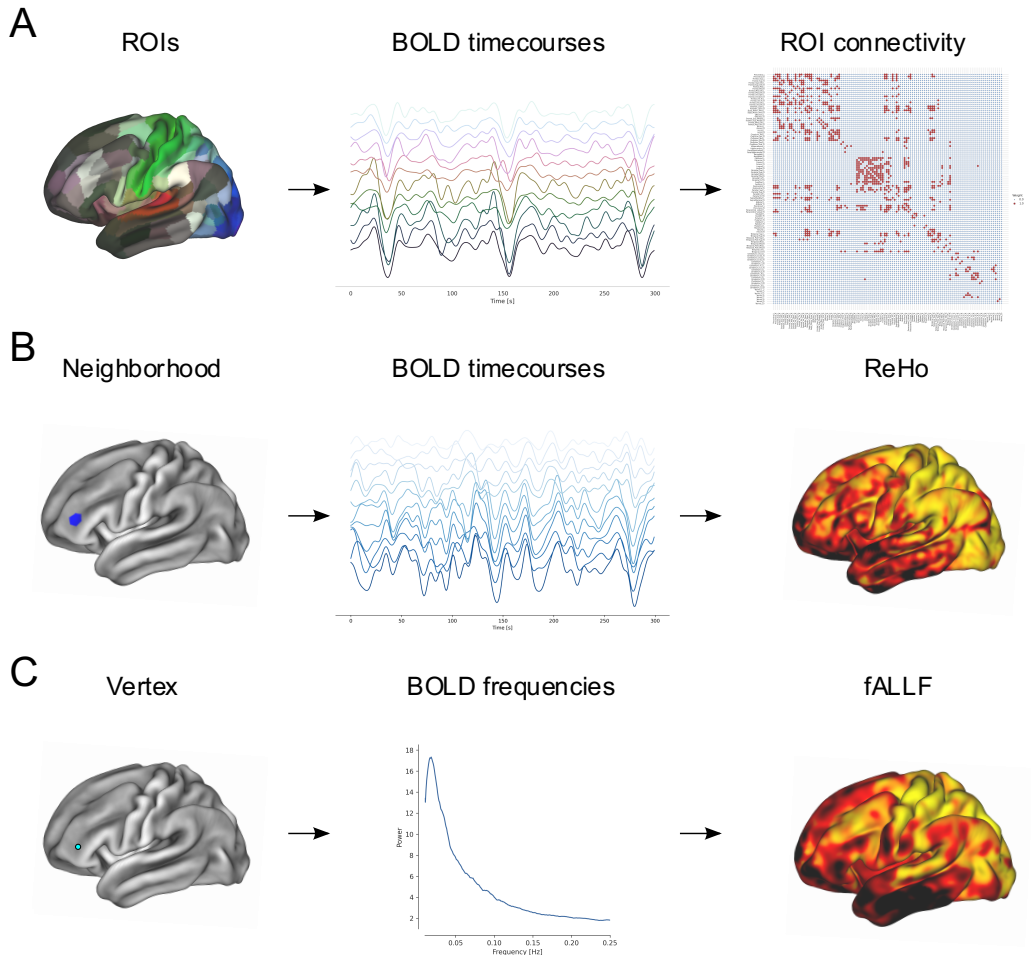

Figure 11: Analysis strategies used in our study to characterize activity in rs-fMRI. Panel A illustrates how resting-state FC between different ROIs is computed. An atlas can be used to subdivide the cortex into segregated ROIs and by correlating the average BOLD time courses within each ROI, a functional connectivity matrix can be computed. Using different graph theoretical measures allows us then to analyze changes of the region-wise or whole-brain connectivity. Panel B shows how ReHo maps that were derived from rs-fMRI data. For each vertex on the surface the neighboring vertices within a pre-defined radius were selected to define a local neighborhood (marked in blue in this example). By computing the similarity between all time courses within this neighborhood, we obtained a ReHo value for each vertex on the surface. Panel C illustrates the steps to compute a fALFF map from rs-fMRI data. For each vertex, the power spectrum of its BOLD time course was computed, and fALFF resulted from the ratio of the low frequency power to the power of the entire frequency range. Adapted from (Wein et al., 2023 - under review).

2.6.4.2 Functional network properties

To investigate changes in functional network properties during the therapy, we used graph theory analysis. According to graph theory, the complex function of the brain can be mathematically described as a graph which is defined as a set of nodes (brain regions) linked by edges (statistical dependencies that describe functional associations) (fig. 12).

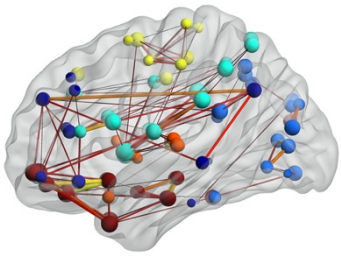

Figure 12: Graph theory used to build a brain graph. Brain regions represent the "nodes" of the graph and functional connections are the graph "edges". (Printed from www.nitrc.org).

First, we applied the multi-modal parcellation proposed by Glasser et al. (Glasser et al., 2016), subdividing each hemisphere into 180 segregated brain areas. We then computed the average activity time course within each region and filtered the resting-state BOLD signal within the 0.01Hz − 0.1Hz low frequency range. To compute FC strength, we used the Pearson correlation coefficient between time courses of pairs of regions to obtain a 360 × 360 FC matrix for each resting state fMRI session (fig. 11A). By selecting a predefined correlation threshold σ above which two regions were considered as functionally connected, we obtained a binary connectivity matrix, also called the "adjacency matrix" because it records which pairs of nodes are "adjacent" (i.e., directly connected by an edge) (Sporns, 2010). In our study, we used a threshold of $\sigma = \pm 0.6$ to construct the adjacency matrices. This correlation coefficient threshold was selected because a correlation coefficient smaller than ±0.4 increases false-positive rates and a coefficient greater than ±0.7 results in matrices with lower sensitivity because of reduced dynamic range (Tomasi & Volkow, 2010; Wang et al., 2018).

Once a brain graph has been constructed, we analyzed it by means of graph metrics which report key features of network organization and functionality. In our study, we measured node degree, nodal network efficiency, and betweenness centrality.

Node degree

Given a graph $G(N, K)$ with N nodes and K edges, node degree d_i of a brain region i in the network can then be defined as the total number of edges connected to node i (k_i) (Bullmore & Sporns, 2009):

$$d_i = \sum_{i \in N} k_i \qquad \textbf{Equation (1)}$$

This is the most fundamental network measure and most other measures are ultimately linked to node degree.

Nodal Network efficiency

Efficiency is defined as the reciprocal of the shortest path length l_{ij} between any two nodes i and j of the network, with the shortest path being the minimal number of edges passed to get from one node to another:

$$E = \frac{1}{(N-1)} \sum_{i,j \in N, i \neq j} \frac{1}{l_{ij}} \qquad \textbf{Equation (2)}$$

In a network with high efficiency, short communication paths can be identified between most or all pairs of nodes (Sporns, 2010).

Betweenness centrality

Betweenness centrality $\sigma(i)$ captures how much a given node i is important in short, efficient communication paths. This metric is measured with the number of shortest paths (between any couple of nodes in the graphs) that passes through the target node i divided by the total number of shortest paths existing between any couple of nodes of the graph:

$$B_i = \sum_{i,v,w \in N, i \neq v \neq w} \frac{\sigma_{v,w}(i)}{\sigma_{v,w}} \qquad \textbf{Equation (3)}$$

The target node will have a high betweenness centrality if it appears in many shortest paths. A node with high betweenness centrality is thus crucial to efficient communication (Freeman, 1977).

2.6.4.3 Regional Homogeneity (ReHo)

While classical FC analysis offers us a possibility to study long-distance relationships between segregated brain areas, regional homogeneity (ReHo) allows to quantify local connectivity across the cortex at a scale of millimeters (Jiang & Zuo, 2016). In fMRI, ReHo is typically defined as the temporal coherence or synchrony of the BOLD signal of neighboring voxels or vertices. In our study we implemented a surface-based approach, by selecting for each vertex all their k-hop neighboring vertices, and quantified ReHo by computing the average Pearson correlation between these neighboring BOLD time courses (fig. 11B). This metric allowed us to study the changes in coherence of spontaneous resting-state activity during ECT.

In a neighborhood containing in total N vertices, we computed a ReHo measure as the average Pearson correlation coefficient r_{ij} between all pairs time courses *i* and *j*:

$$ReHo = \frac{\sum_{i \neq j} r_{ij}}{N(N-1)} \qquad \textbf{Equation (4)}$$

2.6.4.4 Low frequency fluctuations

Classical FC approaches do not directly provide information of the amplitude of brain activity of each brain region within a network. Alternatively, the analysis of the amplitude of low-frequency fluctuation (ALFF) of the resting-state fMRI signal offers the possibility to study the intensity of regional spontaneous brain activity (Zou et al., 2008). To compute this metric, we used a *fractional* ALFF (fALFF) approach, i.e., the ratio of power spectrum of low-frequency (0.01–0.05 Hz) to that of the entire frequency range (fig. 11C).

For each vertex *i* fALFF can be computed as the ratio of the power of the BOLD signal Si(t), after being filtered with a bandpass filter *h(t)*, to the power of the unfiltered signal Si(t):

$$fALFF = \sqrt{\frac{\sum_t \big(h(t) * S_i(t)\big)^2}{\sum_t S_i(t)^2}} \qquad \textbf{Equation (5)}$$

Here $*$ denotes the convolution operation and t the temporal index. In our study we focused on the very low frequency range $0.01\text{Hz} - 0.05\text{Hz}$ to increase sensitivity and specificity.

2.6.4.5 Functional connectivity dynamics

Based on our hypothesis regarding depression as a disorder of brain dynamics, we investigated functional connectivity dynamics. After preprocessing the data as described above with an additional step of denoising the data using ICA-AROMA (Automatic Removal of Motion Artifacts) (Pruim et al., 2015), we extracted time-course activity from each region of interest (ROI) implementing an atlas based parcellation of the brain or ICA-based components. At this point, using two different methods: the sliding window approach followed by cluster analysis (fig. 13), and the computation of the Kuramoto parameter and metastability (fig. 14).

In the first approach, instead of using the entire time course to compute pairwise correlations, we divided the time course into *n* time-windows (fig. 13B). For each time-window, we computed pairwise Pearson's correlation, and we created a (ROI x ROI) FC matrix. Thus, we obtained a series of *n* FC matrices, which described time varying FC for each patient. Next, we used k-means clustering or temporal ICA on the windowed-FC matrices from all patients to identify distinct and recurring dynamic FC patterns, called *(meta)states.* Each window was then expressed as a weighted sum of states (fig. 13C), and dynamic properties could be measured for each patient. In our study, we assessed four dynamic's measures including 1) metastates number, 2) metastates switching times, 3) occupied metastates range, and 4) overall traveled distance. For this approach we implemented the GIFT MATLAB toolbox (Calhoun & Adali, 2016).

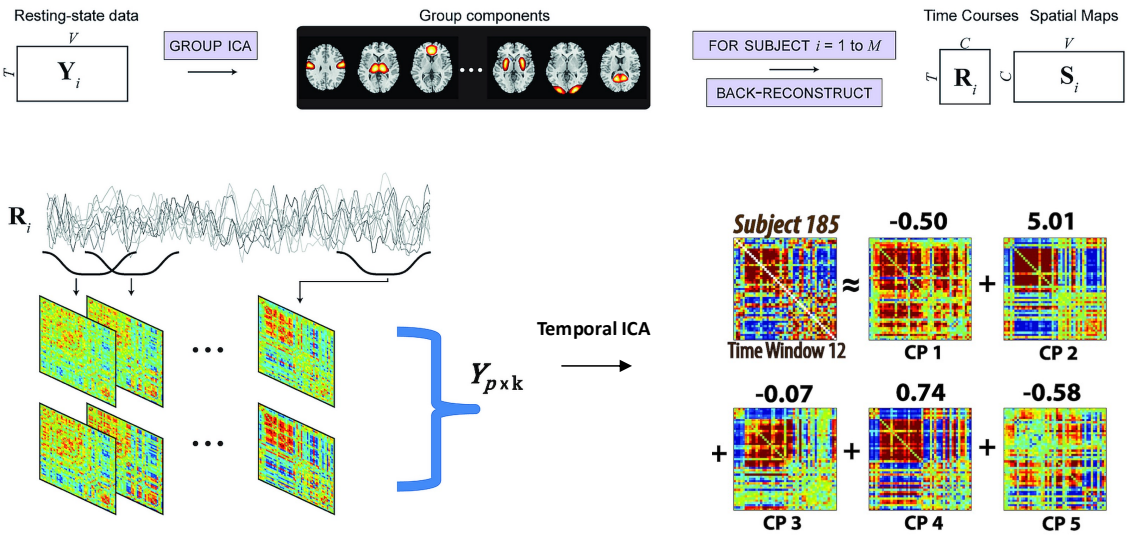

Figure 13: Metastates analysis pipeline for functional connectivity dynamics. A) Initial decomposition of fMRI data into network spatial maps and corresponding timecourses using group spatial ICA (GICA); B) Computation of window-indexed correlation matrices on sliding windows through the network timecourses, which are decomposed into temporally independent connectivity patterns using temporal ICA; C) Example of an observed window-FC expressed as weighted sum of the five displayed connectivity patterns. Adapted from (Miller et al., 2016).

The second method for estimating FCD was computing the Kuramoto order parameter and metastability measure between ROIs timecourses (Deco & Kringelbach, 2016). The Kuramoto parameter is a measure of the level of synchrony between two oscillating signals, while metastability is mathematically the standard deviation of the Kuramoto parameter (fig. 14B). Metastability is a measure of the variability of the states of phase configurations as a function of time. In other words, it describes the level of flexibility of the system: the higher metastability, the more dynamic the system.

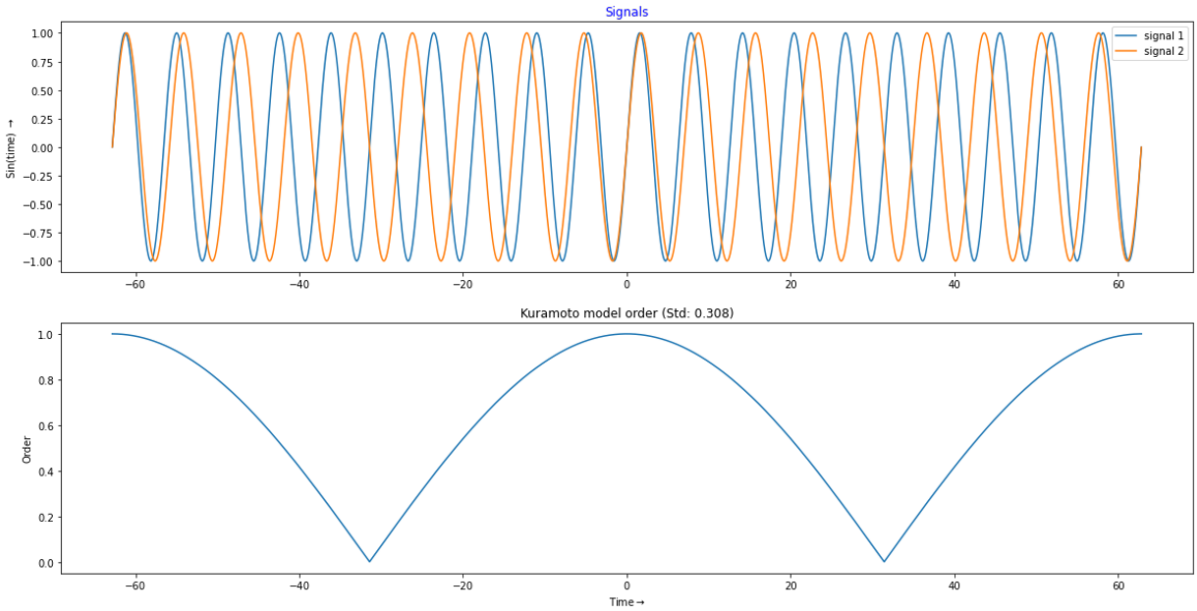

Figure 14: Kuramoto order parameter and metastability. A) Two simulated signals (oscillators). B) Kuramoto order parameter computed for each timepoint: the two signals can be either in phase k=1 (regions are connected) or not in phase (k=0). Metastability is the standard deviation of this curve.

Results of functional connectivity dynamics are not reported in this work; however, the analysis pipeline was developed within the scope of this project and will be continued by the upcoming doctoral candidate.

CHAPTER 3 Results

In this chapter, I will present the comprehensive results of our study, which explores various aspects of interest at multiple levels. For clarity, I have divided this chapter into three sections, each focusing on a distinct facet: clinical course ratings, blood parameters, and brain network properties. Before uncovering the details of each section, I will provide a brief overview of the demographic information of the participants included in the study. Finally, I will report the outcomes of the correlation analyses, which interlink the findings from the three levels of investigation described above.

3.1 Demographics

We included a total of 9 patients (1 female, 8 males) with ages ranging from 35 to 61 years ($M = 47$, $SD = 9$). Table 7 includes all the demographic variables of the patients included. All patients were employed ($n = 7$ fulltime; $n = 2$ parttime) and lived alone at the time of inclusion. Except for one patient, all had experienced at least one previous depressive episode ($M = 4$, $SD = 4$). Four of the patients had relatives who also had experienced depression. For three of them depression was accompanied by psychotic symptoms. Additionally, there was one case with a high risk of suicidality, having experienced 3 previous suicidal attempts. All patients had undergone both psychotherapy and pharmacological treatment, but without success. Only two patients had previously received electroconvulsive therapy (ECT), making it a novel treatment attempt for most of the participants.

Table 7: Demographic variables of the patients included in the study.

Patient	Sex	Age	Nr previous episodes	Psychotic symptoms	Familiar history	Previous ECT
GRK-EKT03	M	47	10	-	+	-
GRK-EKT04	M	44	10	+	-	2020
GRK-EKT05	M	35	0	-	-	-
GRK-EKT06	M	54	10+	-	-	2019
GRK-EKT07	M	53	2	-	-	-
GRK-EKT08	M	49	5	+	+	-
GRK-EKT09	M	49	3	-	-	-
GRK-EKT10	W	61	3	+	+	-
GRK-EKT11	M	35	1	-	+	-

3.2 ECT clinical outcome: Hamilton scale and Beck Depression Inventory ratings

Our initial question revolved around the effectiveness of ECT: did it yield positive results, and if so, for how many patients? To address this question, we relied on two evaluation methods: an objective, external assessment conducted by a professional – HAMD-21 – and a self-evaluation performed by the patients themselves – the BDI (see Materials & Methods, 2.4). Responsiveness was defined as reduction of at least 50% of the HAMD-21 score at week six compared to the baseline score. Remission, instead, was considered by a cut-off score of less than 9 on the Hamilton scale.

We observed a significant main effect of ECT on HAMD-21 scores ($F(5) = 16.96$, $p < .001$, $\eta_p^2 = .680$; fig. 15, table 8). Out of the nine patients included in our study, seven demonstrated a successful response to ECT, resulting in a response rate of 77.8% (calculated as the number of responders divided by the total number of patients). Even though the HAMD-21 score decreased, patients 05 and 06 could not be categorized as responders (fig. 15; see Appendix D for subject-specific graph).

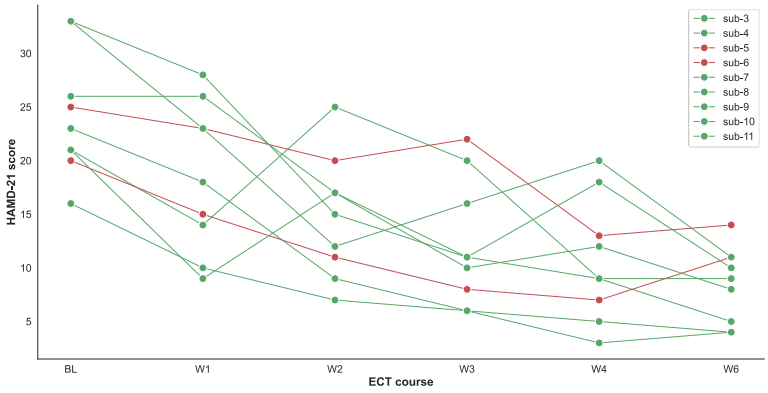

Figure 15: Hamilton score (HAMD-21) during the course of ECT, depicted in green for responders and in red for non-responders. Responsiveness is defined as reduction of at least 50% of the HAMD-21 score at week six compared to the baseline score.

We repeated the analysis for the BDI scores, and we found a significant main effect of ECT ($F(1.78) = 9.93$, $p = .002$, $\eta_p^2 = .554$; fig. 16, table 8). In the case of BDI, an additional three patients - 07, 09, and 11 - did not surpass the response threshold.

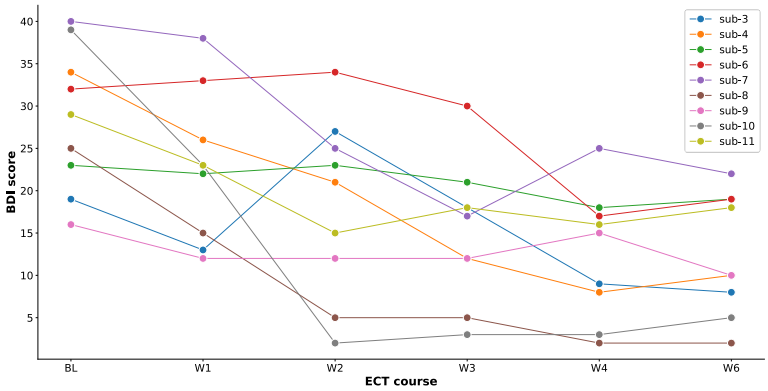

Figure 16: Beck Depression Inventory (BDI) scores of all patients (n=9) over six weeks of ECT treatment.

Regarding remission, a total of five out of nine patients (55,6%) achieved complete remission, according to both HAMD-21 and BDI scores (table 8).

Table 8: HAMD-21 and BDI scores for all patients during ECT.

Patient	BL	W1	W2	W3	W4	W6
HAMD-21						
GRK-EKT03	21	14	25	20	9	5
GRK-EKT04	26	26	17	11	9	9
GRK-EKT05	20	15	11	8	7	11
GRK-EKT06	25	23	20	22	13	14
GRK-EKT07	33	28	15	11	18	10
GRK-EKT08	16	10	7	6	5	4
GRK-EKT09	21	9	17	10	12	8
GRK-EKT10	23	18	9	6	3	4
GRK-EKT11	33	21	12	16	20	11
BDI						
GRK-EKT03	19	13	27	18	9	8
GRK-EKT04	34	26	21	12	8	10
GRK-EKT05	23	22	23	21	18	19
GRK-EKT06	32	33	34	30	17	19
GRK-EKT07	40	38	25	17	25	22
GRK-EKT08	25	15	5	5	2	2
GRK-EKT09	16	12	12	12	15	10
GRK-EKT10	39	23	2	3	3	5
GRK-EKT11	29	21	15	18	16	18

3.3 Effects of ECT on peripheral blood parameters

3.3.1 TSPO and VDAC-1 protein expression

TSPO protein expression in the peripheral blood of the patients did not significantly change across therapy nor at the follow up timepoint at week six ($F(5) = 1.45$, $p = .228$, $\eta_p^2 = .153$; fig. 17).

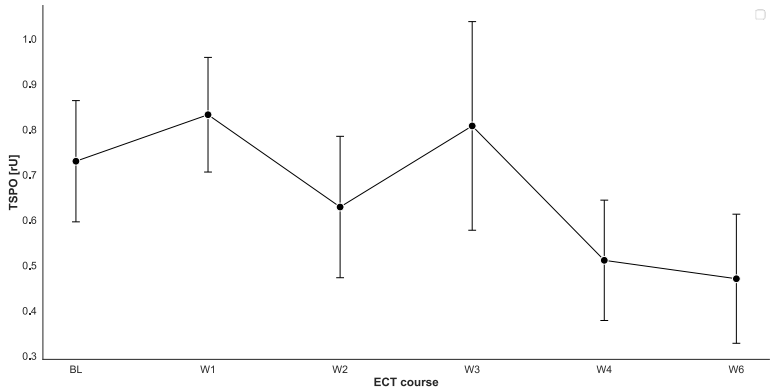

Figure 17: TSPO protein expression in platelets of depressed patients under ECT (n=9) measured by Western Blot. ECT does not affect TSPO expression in the peripheral blood. Data represent mean ± SEM.

The channel protein VDAC-1, which is associated to TSPO function, did not change its expression during and after ECT ($F(2.03) = .24$, $p = .942$, $\eta_p^2 = .029$; fig. 18).

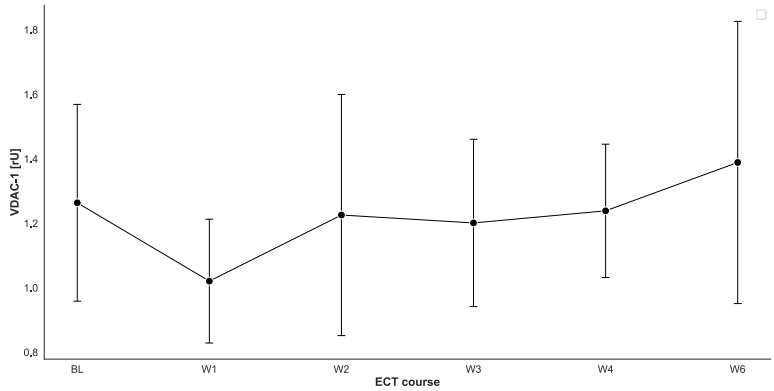

Figure 18: VDAC-1 protein expression in platelets of depressed patients under ECT (n=9) measured by Western Blot. ECT does not affect VDAC-1 expression in the peripheral blood. Data represent mean ± SEM.

3.3.1.1 TSPO polymorphism rs6971

The analysis of the TSPO polymorphism rs6971 revealed that within our sample, there were *n = 5* patients (GRK-EKT 03, 06, 09, 10, 11) who exhibited homozygosity with the GCG / GCG genotype, resulting in Ala / Ala configuration in position 147 of the TSPO protein. Additionally, *n = 4* patients (GRK-EKT 04, 05, 07, 08) displayed heterozygosity with the GCG / ACG genotype, leading to an Ala / Thr configuration at position 147.

3.3.1.2 Correlation of TSPO expression with Hamilton score during ECT

Figure 19 shows that TSPO expression levels in the peripheral blood positively correlate with HAMD-21 scores in our sample (r(44) = +.39, 95%-CI[.11, .61], p = .007), suggesting that lower values of TSPO levels may be associated with better clinical state.

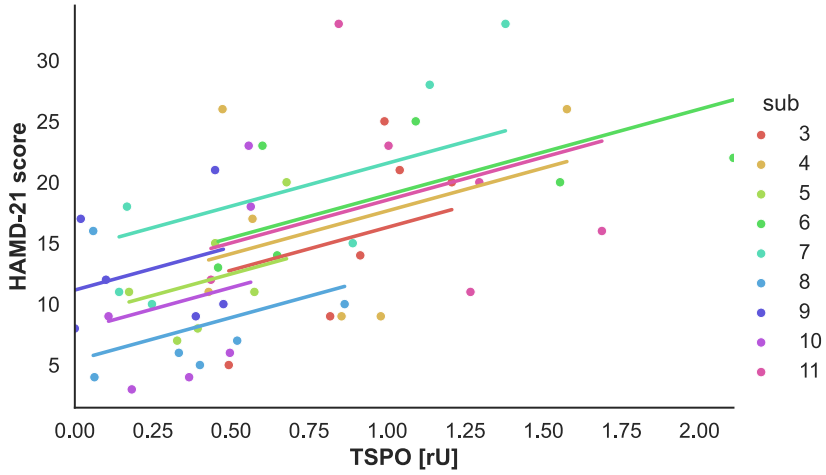

Figure 19: Repeated measures correlation (rmcorr) between TSPO levels and HAMD-21 scores for each patient. TSPO positively correlates with HAMD-21 scores. Colored lines represent regression lines for each patient during the six weeks.

3.3.2 BDNF expression

During the course of ECT, BDNF protein expression assessed in the serum of the patients significantly increased over time (F(5) =3.188, p = .016, η_p^2 = .285; fig. 20). Follow-up tests showed that BDNF increased progressively from baseline (BL) to week three (W3) (mean

difference = -20.48, 95%-CI[-33.71, -7.24], p = .007); BDNF maintained higher levels at week four (W4) compared to baseline (mean difference = -16.67, 95%-CI[-32.61, -.73], p = .042) and at week six (W6) compared to baseline (mean difference = -23.02, 95%-CI[-34.9, -11.15], p = .002).

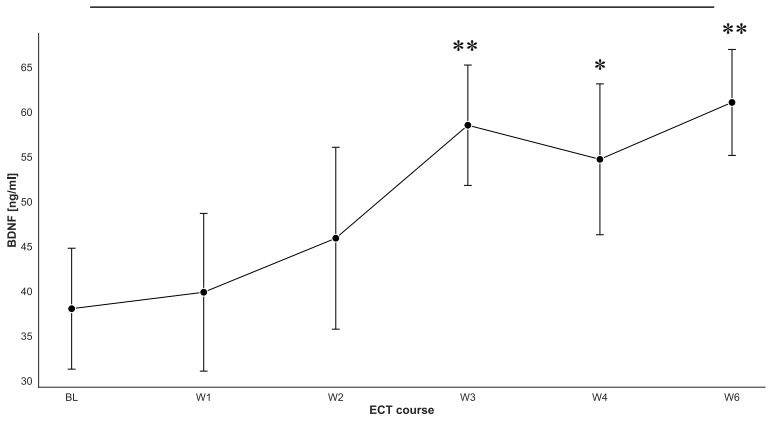

*Figure 20: BDNF protein expression in serum of depressed patients under ECT (n=9) measured by ELISA. ECT progressively increases BDNF expression in the peripheral blood. Data represent mean ± SEM. Asterisks mark significant difference to baseline (BL), *p < .05, **p < .01.*

After obtaining these results, we sought to investigate whether the observed effect on BDNF serum levels was specifically attributed to ECT or if it could be a general outcome of antidepressant therapy. To examine this, we conducted a similar analysis on a separate sample of depressed patients (n=11) who were inpatients at the same hospital and received similar antidepressant medication but did not undergo ECT treatment (MITDEP; see Materials & Methods, 2.2.4). Consequently, the only significant difference between these two groups was the administration of ECT. The findings revealed that BDNF levels remained unchanged during usual antidepressant therapy (fig. 21; F(2) =.013, p = .987, η_p^2 = .001). This result would hint that the observed increase in BDNF levels in our ECT sample was specifically attributed to the ECT treatment.

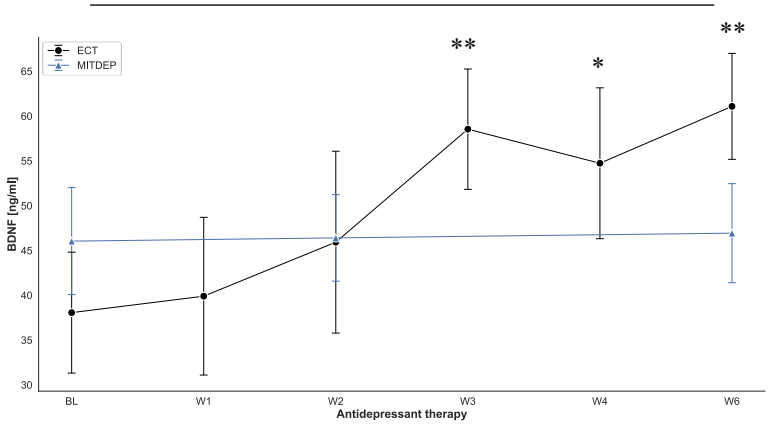

*Figure 21: BDNF serum levels measured by ELISA in two groups of depressed patients undergoing antidepressant therapy. The 'ECT' group (black line) received both antidepressant medication and ECT treatment. The 'MITDEP' control group (blue line) received only antidepressant medication. Data represent mean ± SEM. Asterisks mark significant difference to baseline (BL), *p < .05, **p < .01.*

However, the fact that we did not find changes in BDNF levels in the MITDEP sample may be due to a low response rate in this group. Therefore, we checked for the response rate and the correlation between the BDNF levels and HAMD score in the MITDEP sample as well as in the ECT sample (see 3.3.2.1). Antidepressant treatment as usual was effective in nine patients out of eleven in the MITDEP group with a response rate of 81.81%.

3.3.2.1 Correlation of BDNF levels with Hamilton score during ECT and treatment as usual

To further investigate the relationship between the increase in BDNF levels and the effects of ECT, we conducted a repeated measures correlation between BDNF levels and Hamilton score for each patient. The analysis revealed a significant negative correlation between BDNF and HAMD-21 score ($r(44) = -.35$, 95%-CI[-.58, -.06], $p = .018$), indicating that - as expected - higher BDNF levels were associated with lower HAMD-21 scores and a more favorable clinical condition (fig. 22).

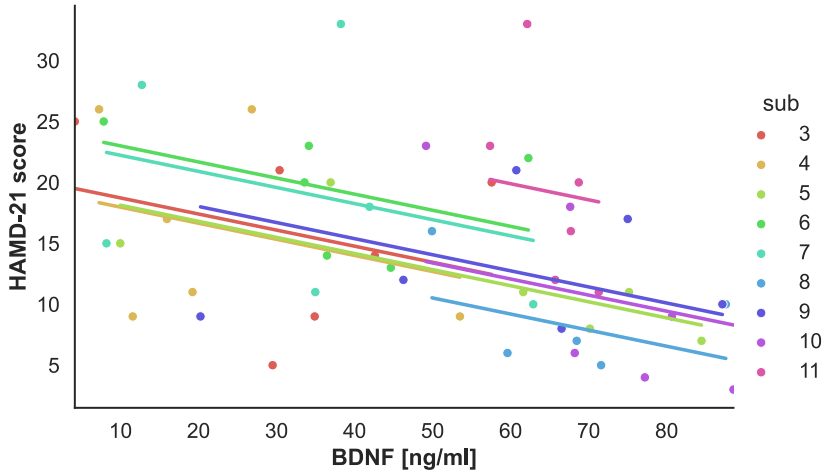

Figure 22: Repeated measures correlation (rmcorr) between BDNF levels and HAMD-21 scores for each patient. BDNF negatively correlates with HAMD-21 scores. Colored lines represent regression lines for each patient during the six weeks.

We did not find a significant correlation between the variables BDNF and HAMD-21 scores in the MITDEP sample (see Appendix E; r(21) = -.16, 95%-CI[-.53, +.27], p = .48).

3.3.3 Pregnenolone in serum

As the primary and crucial molecule in the biosynthesis of neuroactive steroids, we examined whether ECT had an impact on the levels of pregnenolone in the peripheral blood. Despite observing fluctuations in pregnenolone levels during the treatment, we did not find any significant change (F(2) =2.02, p = .166, η_p^2 = .201; fig. 23).

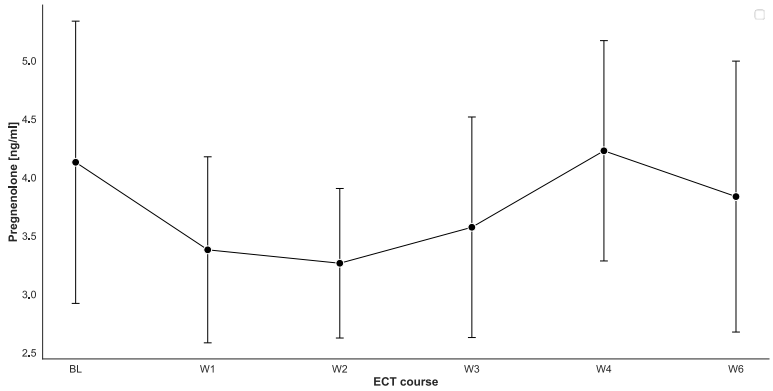

Figure 23: Pregnenolone levels in serum of depressed patients undergoing ECT measured by ELISA. Pregnenolone expression does not change significantly in the peripheral blood after ECT. Data represent mean ± SEM.

3.3.4 ECT effects on the HPA axis: Cortisol

While it appears that ECT may lead to a slight increase in cortisol levels in the serum of depressed patients over time, the change was not statistically significant ($F(5)=1.48$, $p = .217$, $\eta_p^2 = .156$; fig. 24).

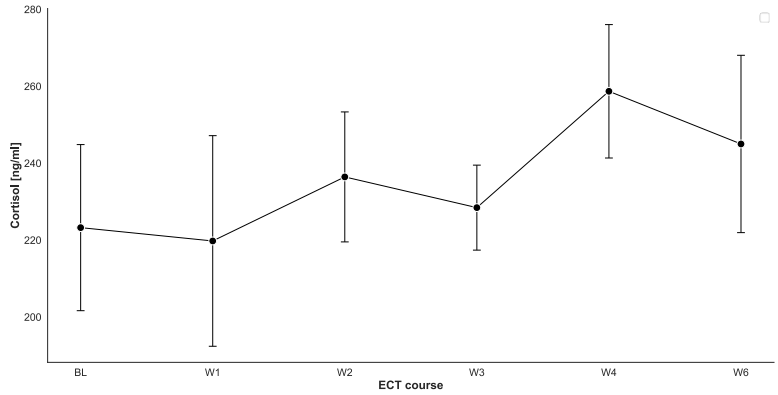

Figure 24: Cortisol levels in serum of depressed patients during ECT (n=9) measured by ELISA. ECT does not significantly change cortisol levels. Data represent mean ± SEM.

3.3.5 Pro and anti-inflammatory cytokines

Among the pro- and anti-inflammatory cytokines that we assessed in our sample (IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, and TNF-α), only TNF-α ($F(5)=4.01$, $p = .005$, $\eta_p^2 = .334$; fig. 25) and IL-4 ($F(5)=3.75$, $p = .011$, $\eta_p^2 = .428$; fig. 26) displayed a significant change (see Appendix F for statistics). TNF-alpha levels increased from week one (W1-W4: mean difference = -.440, 95%-CI[-.780, -.100], p = .017) and continued to rise through week two (W2-W4: mean difference = -.389, 95%-CI[-.672, -.106], p = .013) and three (W3) until week four (W3-W4: mean difference = -.253, 95%-CI[-.495, -.012], p = .042). However, a subsequent drop in TNF-α levels was observed from W4 to W6 in the patients' serum (mean difference = +.246, 95%-CI[.042, .449], p = .024). Collectively, these findings indicate that ECT may lead to short-term inflammation but eventually normalize inflammation levels after treatment and in the long term.

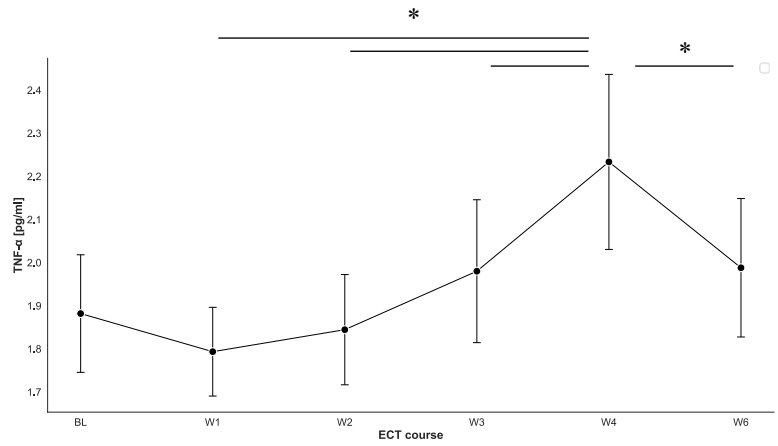

*Figure 25: TNF-alfa levels in serum during ECT (n=9) measured by ELISA. Data represent mean ± SEM. Asterisks mark significant difference to baseline (BL), *p < .05.*

We also observed fluctuating peaks in the expression of the anti-inflammatory cytokine IL-4 (fig. 26). Significant alterations occurred after one week of ECT (BL-W1: mean difference = +.006, 95%-CI[.000, .012], p = .041); at week two IL-4 increased again (W1-W2: mean difference = -.005, 95%-CI[-.010, .000], p = .046); a decrease peak occurred at week three (W2-W3: mean difference = +.005, 95%-CI[.001, .009], p = .030); finally concentration increased again at week four (W3-W4: mean difference = -.006, 95%-CI[-.011, -.002], p = .019).

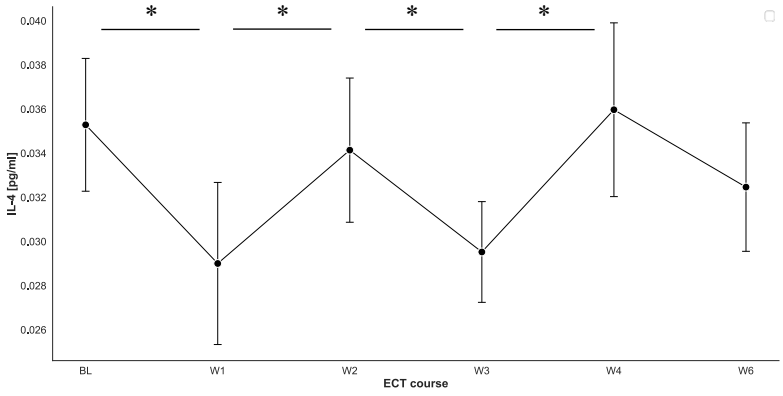

*Figure 26: IL-4 levels in serum during ECT (n=9) measured by ELISA. Data represent mean ± SEM. Asterisks mark significant difference, *p < .05*

We report here also the results regarding the key inflammatory cytokine IL-6 (fig. 27). IL-6 did not show significant change during ECT ($F(1.97)= 1.357$, $p = .286$, $\eta_p^2 = .145$). However, a trend of increase could be noticed from the graph.

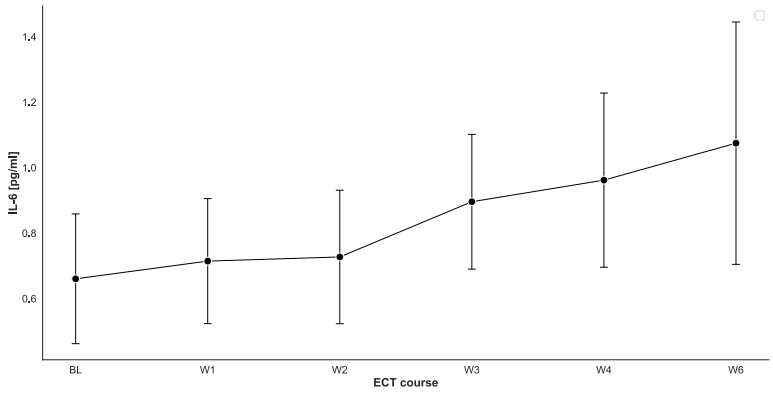

Figure 27: IL-6 levels in serum during ECT (n=9) measured by ELISA. Data represent mean ± SEM. No significant change was detected.

3.3.5.1 Correlation of cytokine expression with Hamilton score during ECT

We performed a repeated measure correlation (rmcorr) analysis to assess the relationship between cytokines levels and the clinical outcome. We observed that among all the measured cytokines, TNF-α negatively correlates with HAMD-21 scores ($r(44) = -.31$, 95%-CI[-.55, -.02], $p = .038$). This result suggests that higher levels of TNF-α in our sample were associated with lower depression score, therefore better mood state (fig. 28).

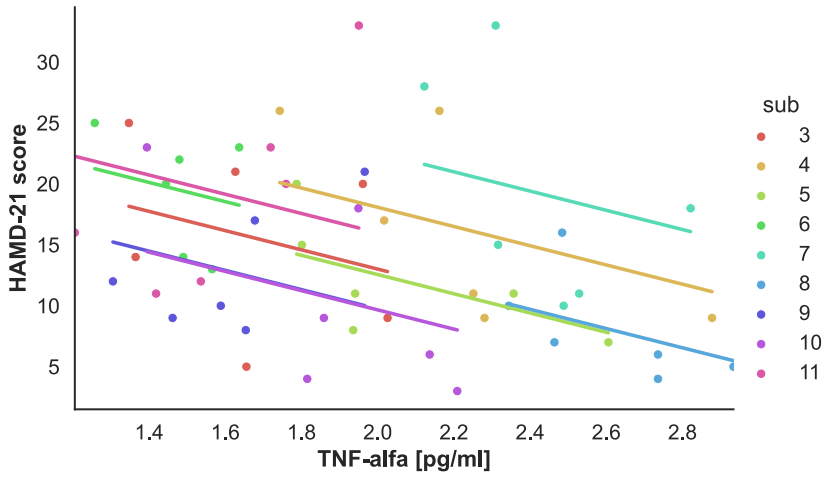

Figure 28: Repeated measures correlation (rmcorr) between TNF-α levels and HAMD-21 scores for each patient. TNF-α negatively correlates with HAMD-21 scores. Colored lines represent regression lines for each patient during the six weeks.

3.4 Effects of ECT on brain network connectivity and topology

We studied the effects of ECT on brain functional connectivity and topology by means of graph theory analysis, regional homogeneity (ReHo) and analysis of the amplitudes of low frequency fluctuations (fALFF) of the BOLD signal spectrum.

3.4.1 ECT effects on functional network topology/graph theory metrics

In our first analysis we investigated brain network topology by means of graph theory measures. For this purpose, we considered brain regions of interest (ROIs) from the multi-modal parcellation of the Glasser atlas (Glasser et al., 2016) as nodes of the graph. We restricted graph-based topological scores to measures of functional integration (i.e., ROIs efficiency estimates reflecting the efficiency of the interaction between distributed brain areas) and centrality (i.e., degree and betweenness-centrality both reflecting the importance of nodes for functional integration). We selected an intermediate adjacency matrix threshold of $\sigma = 0.6$ and we applied a paired t-test between timepoints after starting the therapy (W1, W2, W3, W4) compared to baseline (BL). I report here results for $p < 0.01$, uncorrected for multiple comparisons. Statistics of graph metrics are reported in Appendix G and altered regions in Appendix H.

Degree

Figure 29 shows that the first effect of ECT on degree appears at the second week of treatment: degree increases in the left inferior frontal sulcus (L_IFSp) (row A). At week four (W4) of treatment degree increases in area 31a in the left posterior cingulate cortex (row B).

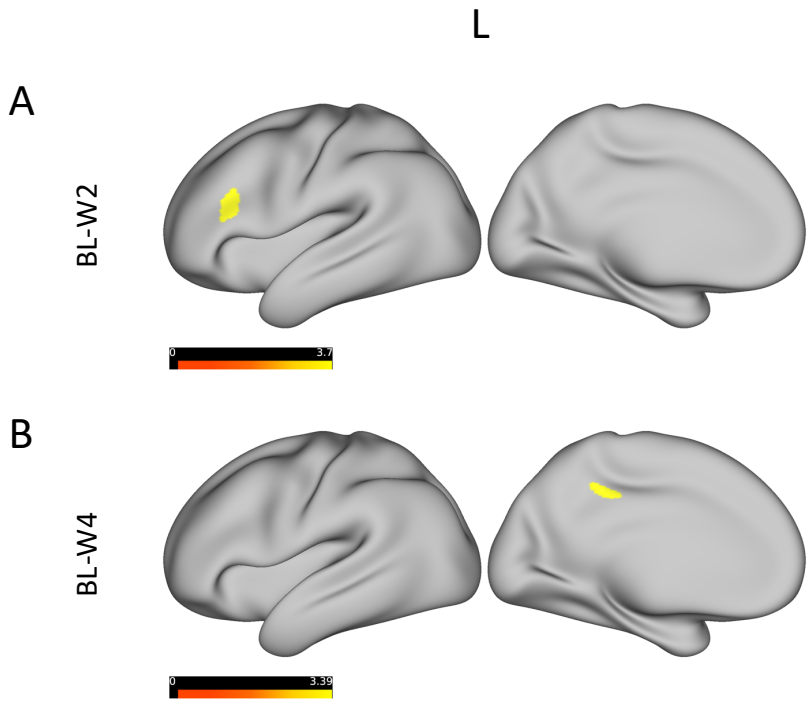

Figure 29: Degree changes in left hemisphere of the brain of depressed patients under ECT. The first row (A) shows an increase in degree in the left inferior frontal sulcus (L_IFSp) from baseline to the second week of treatment (W2). The second row (B) illustrates that degree increases in area 31a in the left posterior cingulate cortex at the fourth week compared to baseline (W4). The color scale depicts t-values (e.g., yellow: W[x]>BL). L=left hemisphere.

Betweenness centrality

Betweenness centrality (BC) decreases in the right ventral intraparietal complex (R_VIP) after one week (W1) of treatment (fig. 30, row A). At the second week BC decreases in the medial belt complex (R_MBelt), while it increases in the inferior frontal cortex (R_p47r) in early auditory cortex (fig. 30, row B).

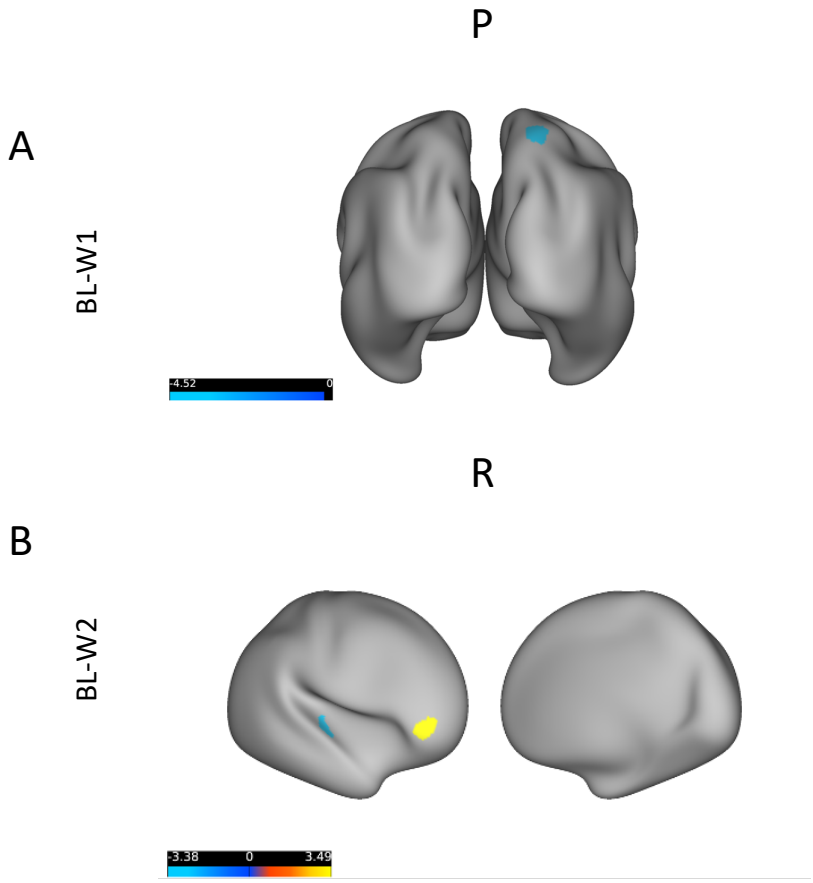

Figure 30: Betweenness centrality (BC) changes during ECT course. The first row (A) shows that BC decreases in the right ventral intraparietal complex after one week (W1) of treatment. The second row (B) illustrates that BC decreases in the medial belt complex in the early auditory cortex, while it increases in the inferior frontal cortex at the second week (W2) of ECT compared to baseline. Warm and cold colors depict t-values (e.g., blue: W[x]<BL, yellow: W[x]>BL). P=posterior view; R=right hemisphere.

Region of interest (ROI) efficiency

ECT increased efficiency (E) in different brain regions over time. Figure 31 (row A) shows an increase of E after one week of treatment in several regions of the left hemisphere including: primary visual cortex (L_V1), third visual area (L_V3), area 23d (L_23d) in the posterior cingulate cortex, area 8c (L_8C) and area 9-46v in dorsolateral prefrontal cortex, parabelt complex (L_PBelt) and lateral belt complex (L_LBelt) in early auditory cortex. After three weeks of treatment, the increase compared to baseline involves only area STS in the auditory association cortex (fig. 31, row B). At week four ECT efficiency of area 13 in the right orbital polar frontal cortex increases with respect to baseline (fig. 31, row C).

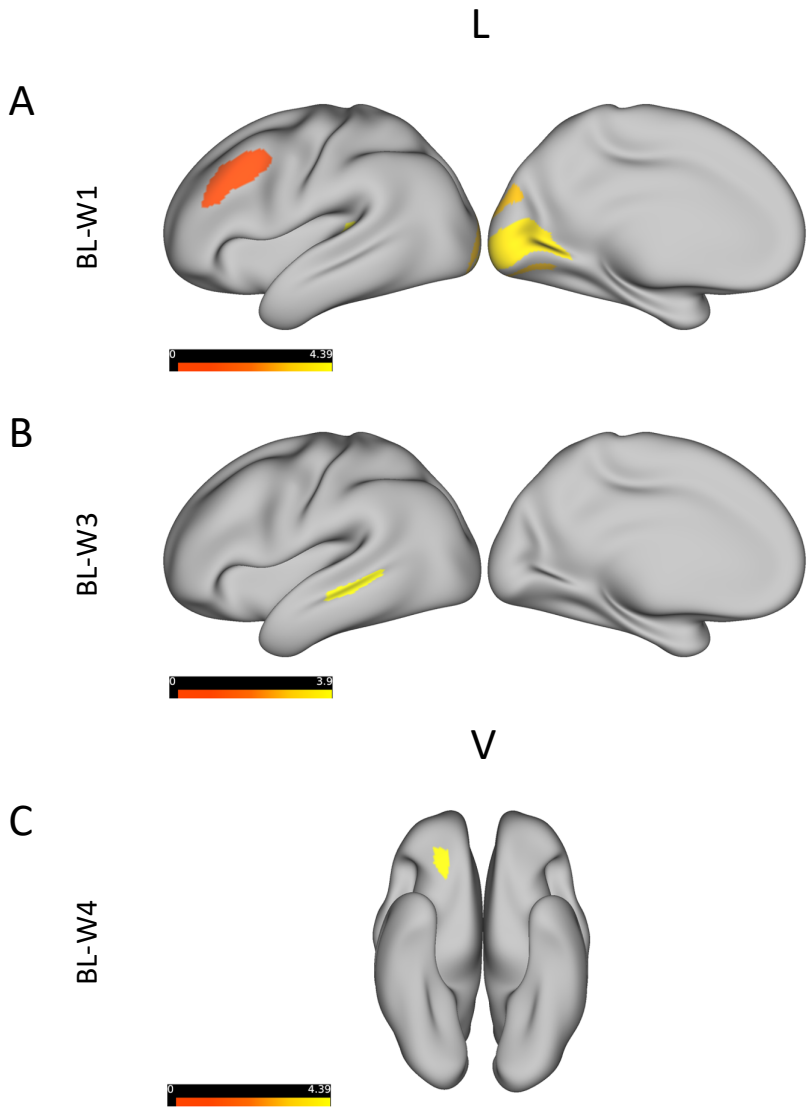

Figure 31: Efficiency changes in depressed patients under ECT. The first row (A) shows an increase in efficiency in regions of the left visual cortex, dorsolateral prefrontal cortex, and early auditory cortex after one week of treatment (W1). The second row (B) depicts that efficiency increases in area STS in the auditory association cortex at the third week (W3) of treatment compared to baseline. The third row (C) reveals an increase in efficiency in area 13 in the right orbital polar frontal cortex at week four (W4) compared to baseline. The color scale depicts t-values (e.g., warm colors: W1>BL). L=left hemisphere; V=ventral view.

3.4.2 Effects of ECT on Regional Homogeneity (ReHo)

To investigate local connectivity changes we computed ReHo using a medium-sized neighborhood radius of 4 vertices (≈ 5.2mm). We compared each fMRI session during treatment with the baseline session performing a paired t-test. I report here results for $p < 0.01$, corrected for multiple comparisons using a cluster-based permutation test (Friston et al., 1994; Han et al., 2013).

Figure 32 reports the changes in ReHo throughout therapy from baseline to week four (*rows*) in the left and right hemispheres (*columns*). We observed that after one week of treatment ReHo increases in some regions of the left hemisphere including the inferior parietal cortex, the temporo-parietal-occipital junction, and a small area in the right dorsolateral prefrontal cortex (DLPFC); but also decreases in the right parahippocampal area. From the second week of ECT until the fourth week, we noted only increases of ReHo distributed across the brain, including regions mainly from three brain areas: frontal/prefrontal cortex, intraparietal cortex, and temporal cortex. All the regions are reported in Appendix I.

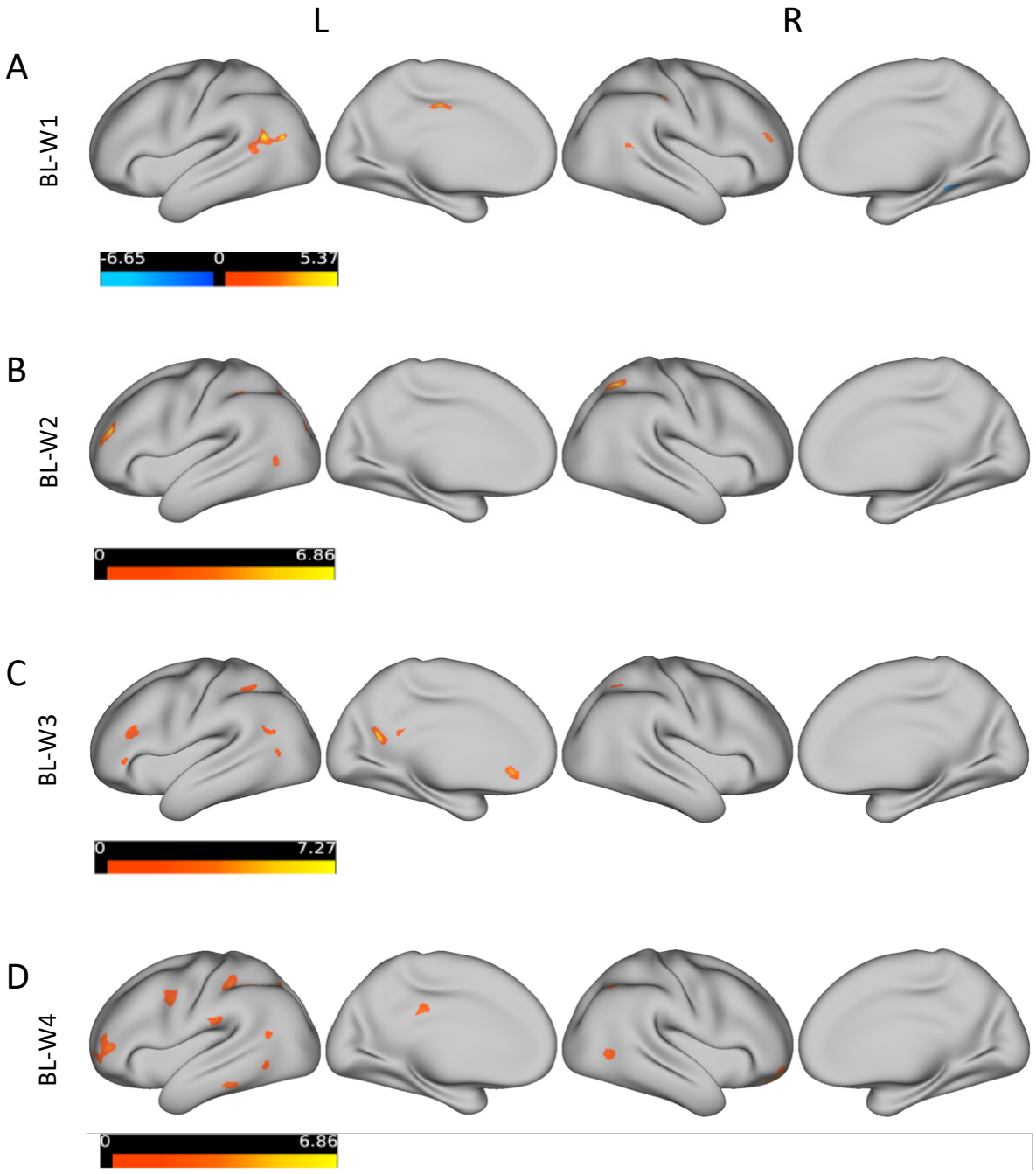

Figure 32: Changes in brain local connectivity, defined as Regional Homogeneity (ReHo) during the course of electroconvulsive therapy (ECT). The first row (A) shows that ReHo increases in some regions including the inferior parietal cortex and the temporo-parietal-occipital junction but also decreases in the right parahippocampal area from baseline to the first week (W1) of ECT. The second row (B) depicts that ReHo only increases in regions of the frontal cortex, parietal and temporal cortex at week two (W2) of ECT compared to baseline. The third row (C) reports a more diffuse increase of ReHo in regions of the left hemisphere after three weeks (W3) of ECT compared to baseline. The fourth row (D) shows that additional regions of the left hemisphere increase their ReHo after four weeks (W4) of ECT. Appendix I includes a complete list of affected regions. Warm and cold colors depict t-values (e.g., blue: W1<BL, yellow: W1>BL). L=left hemisphere; R=right hemisphere.

3.4.3 Effects of ECT on amplitudes of low frequency signal spectrum (fALFF)

To assess the amplitude of BOLD signal fluctuations, we measured fractional amplitude of low frequency fluctuations (fALFF). We compared each fMRI session during treatment with the baseline session performing a paired t-test. I report here results for $p < 0.01$, corrected for multiple comparisons using cluster-based permutation test.

Figure 33 illustrates the changes in fALFF throughout therapy from baseline to week four (*rows*) in the left and right hemispheres (*columns*). We found that at week one (W1) compared to baseline fALFF increased in regions of the left temporal lobe, left frontal lobe, but also decreased in regions of the right hemisphere in the parietal and occipital cortex. At the second week of ECT (W2), we observed only decreases of fALFF in regions of the paracentral lobular and middle cingulate cortex in both left and right hemispheres, and some regions in the frontal lobe. At the third week (W3) fALFF values increased again compared to baseline in the left and right frontal cortex, left inferior parietal cortex, and temporal cortex, while they decreased in one region of the right paracentral lobular and middle cingulate cortex. Finally, at week four (W4) we observed again a decrease of fALFF in regions in the paracentral lobular and middle cingulate cortex, in the anterior cingulate and medial prefrontal cortex, DLPFC, and superior parietal cortex. All the regions are reported in Appendix I.

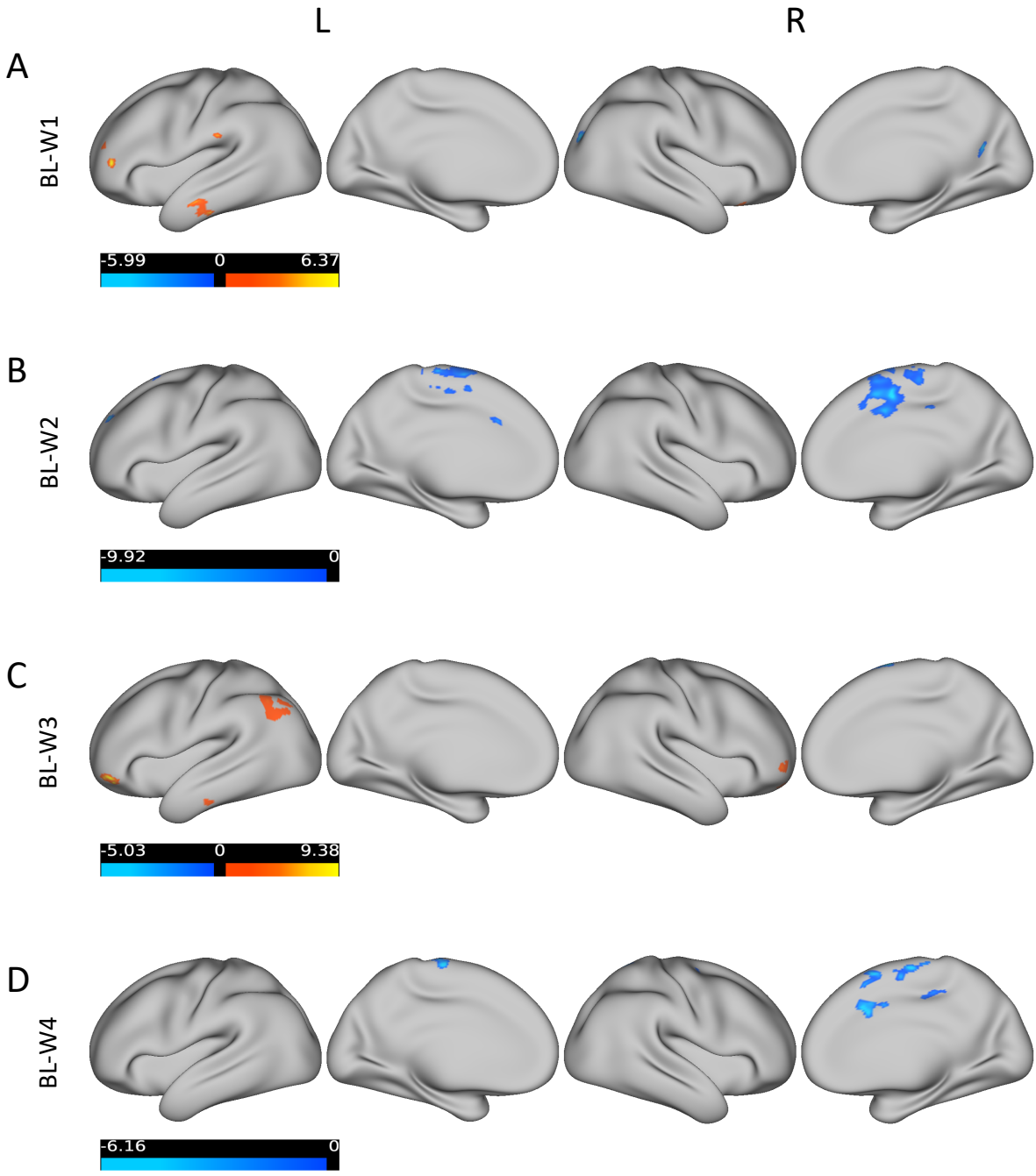

Figure 33: Changes in low frequency amplitudes, as derived from fALFF, from baseline to each week of treatment. The first row (A) shows that after one week of ECT (W1), fALFF increase in regions of the left temporal lobe, left frontal lobe, but also decreased in regions of the right parietal and occipital cortex. The second row (B) illustrates that fALFF globally decrease, particularly in the paracentral lobular and middle cingulate cortex at week two (W2) compared to baseline. The third row (C) shows that at the third week (W3) fALFF increase again compared to baseline in the left and right frontal cortex, left inferior parietal cortex, and temporal cortex. The last row (D) reports a decrease in regions in the paracentral lobular and middle cingulate cortex, in the anterior cingulate and medial prefrontal cortex. Appendix I includes a complete list of affected regions. Warm and cold colors depict t-values (e.g., blue: W1<BL, yellow: W1>BL). L=left hemisphere; R=right hemisphere.

CHAPTER 4 Discussion

The current chapter will include a summary of the findings of our study, followed by a detailed discussion of each result for each research level – clinical outcome, blood molecular parameters, brain network connectivity – aiming at unraveling the effects of ECT in severely depressed patients.

4.1 Summary of findings

In the present study we followed the course of six weeks of electroconvulsive therapy in depressed patients who were resistant to standard pharmacological therapy. We investigated the effects of ECT on multiple research levels to gain broader understanding on this matter. We found that ECT leads to successful clinical response in 77.8% of the cases included in the trial, affecting both molecular pathways and brain network communication.

On the level of blood molecular measurements, we learned that ECT increased BDNF serum levels, and that BDNF expression negatively correlated with HAMD-21 scores. Moreover, ECT induced an increase in TNF-α levels around week four of treatment and altered the expression of IL-4 in the patients' serum. The other measured cytokines did not show any significant alteration during ECT course. Furthermore, expression of TSPO, VDAC-1, and pregnenolone was not significantly affected by ECT, and there were no substantial changes detected in cortisol levels.

On the level of brain network connectivity, we identified an overall increase in local connectivity (ReHo) with ECT. We observed differential region-specific increase and decrease in the amplitudes of low frequency fluctuations (fALFF). Furthermore, we detected that ECT could improve brain network integration by means of number of connections (node degree), betweenness centrality, and efficiency in between-region communication.

4.2 Clinical outcome

In our sample, seven out of nine patients successfully responded to ECT treatment, according to HAMD-21 scores, resulting in a response rate of 77.8%. Conversely, according to BDI scores, only four out of nine patients responded to ECT. The BDI was mainly conducted to point out any major discrepancy between the observer-rated and the self-reported outcome. In our study, we found discrepancies between the two evaluation methods for three patients, who were considered responders based on the HAMD-21 but not the BDI score. Nevertheless, it is essential to highlight that self-assessment using the BDI may yield distorted results, given that the disorder itself can impact one's ability to evaluate symptoms accurately, potentially leading to an underestimation or exacerbation of symptom evaluation (Wang & Gorenstein, 2021).

Regarding remission, a total of five out of nine patients (55,6%) achieved complete remission, according to HAMD-21 scores.

It is important to acknowledge that our sample size was relatively small, comprising only nine patients in total. Moreover, most of the participants were men, leading to a gender imbalance and potentially impacting the generalizability of the findings to the broader population. Literature reports indicate that the average response rate for ECT in patients with treatment-resistant depression (TRD) is 65.9% (Nygren et al., 2023). Additionally, a meta-analysis encompassing 1175 patients across 11 studies revealed a response in 58% of patients who experienced resistance to at least one medication, compared to 70% in patients without medication resistance before undergoing ECT (Haq et al., 2015).

4.3 ECT effects on peripheral blood molecular markers

4.3.1 BDNF

The dominant result of our study was in terms of BDNF levels changes in the serum of patients under electroconvulsive therapy. ECT increased serum levels of BDNF after three weeks of treatment - i.e., after around nine sessions – and continued to increase at week four until week six (see Results, 3.3.2). This result is in line with a massive portion of the literature, including reviews and meta-analyses, which supports that ECT acts through neurotrophic effects at a brain level or restores neuroplastic homeostasis, respectively (Bocchio-Chiavetto et al., 2006; Brunoni et al., 2014; Bumb et al., 2015; Pelosof et al., 2023; Rocha et al., 2016; Wang et al., 2023). However, another important portion of studies does not support this hypothesis, as they claim that BDNF cannot be considered a reliable biomarker for antidepressant effects of ECT (Allen et al., 2015; Fernandes et al., 2009; Huang et al., 2020; Lin et al., 2013; Ryan et al., 2018). Clearly, the literature offers a contradictory scene of what is the effect of ECT on BDNF function in the brain. Nevertheless, by looking closely at some studies, one may attribute the lack of consistency in the results to the way of measuring peripheral BDNF. For instance, Vanicek and colleagues assessed BDNF levels before, during and after ECT both in *serum* and *plasma* of peripheral blood (Vanicek et al., 2019). They observed that ECT increased BDNF levels in serum but not in plasma. In the same way, Ryan and colleagues as well as Lin and colleagues, who did not find any change, measured BDNF levels in plasma (Lin et al., 2013; Ryan et al., 2018). BDNF is known to be stored in human platelets and to circulate in plasma, but BDNF concentration in plasma is affected by the handling of the blood sample, and it is released upon platelet degranulation (Huang et al., 2020). Therefore, circulating BDNF is usually measured in the serum. There is also evidence for a correlation between serum and plasma BDNFs, nevertheless BDNF levels in serum seem to be quite stable over one year and reliably measured (Naegelin et al., 2018; Polyakova et al., 2015). To eliminate additional source of doubt during future studies, one suggestion could be to test the reproducibility of the results in both serum and plasma for each patient.

Another crucial aspect in measuring peripheral BDNF is the timing. This aspect could, to some extent, account for the conflicting results observed in various studies. Increases in serum levels of BDNF were found 5 weeks after starting ECT (Okamoto et al., 2008) and during the treatment period (Bilgen et al., 2014; Marano et al., 2007); in other studies, the timing of the sampling varied from 1 day after ECT treatment to 1 month after the completion of ECT

treatment (Bocchio-Chiavetto et al., 2006; Bumb et al., 2015; Piccinni et al., 2009; Vanicek et al., 2019). On the contrary, in studies which failed to find a link between ECT and peripheral BDNF levels, the sampling was performed right after the second session up to the 8th session (Huang et al., 2020; Ryan et al., 2018).These findings indicate that changes in serum levels of BDNF may occur in the long term rather than at the beginning or during the therapy. Animal studies also suggested that the time course of BDNF changes in the brain and periphery differ following ECT, and that a delay may be required for brain changes to be detectable in the blood (Sartorius et al., 2009). This is in accordance with the results of our study since we also found that BDNF levels increase only after around nine sessions (W3) of ECT.

To summarize, our findings support the *neurotrophic hypothesis* stating that depression is secondary to an altered expression of BDNF in the brain: ECT would induce neurotrophic effects in the brain and these neurotrophic effects would promote antidepressant effects (Henn et al., 2004). In support of this idea, studies showed that ECT can increase brain volume in the limbic structures, including the hippocampus and amygdala (Takamiya et al., 2018).

Important to note is that our patients continued usual antidepressant pharmacotherapy during ECT course. This might be considered a confounder for the real effects of ECT on the measured parameters. To rule out this occurrence, we measured BDNF level in another sample of depressed patients who received only pharmacotherapy (MITDEP – see Materials & Methods, 2.2.4). We observed that BDNF serum levels increased only in the ECT sample and not in the MITDEP group. On one hand this result supports the fact that the increased levels of serum BDNF can be attributed directly to ECT. On the other hand, this result would be in contrast with the large portion of studies showing an increase in BDNF blood levels after pharmacotherapy (Çakici et al., 2021; Molendijk et al., 2013; Zhou et al., 2017). Mounting evidence shows that antidepressant drugs can block or even reverse hippocampal atrophy: the neurotrophic effects of antidepressants would upregulate BDNF which, in turn, would increase neurogenesis within the hippocampus (Schmidt & Duman, 2007). One potential explanation for the absence of an increase in BDNF levels in the MITDEP group may be a low response rate in this group. In fact, if the therapy was not successful it would be difficult to detect a rise in BDNF levels. However, quite interestingly, we observed a relatively high response rate in the MITDEP group as well, suggesting that the observed BDNF increase in the ECT group may be attributed to the mechanism of action specific to ECT, rather than solely the antidepressant effect of the treatment. Nevertheless, we cannot ignore the extensive literature associating neurotrophic effects with antidepressant therapy. To gain a more comprehensive understanding of the distinct mechanisms of antidepressants and ECT on brain neurotrophins,

future studies should incorporate a more structured comparison between ECT and treatment as usual groups.

In our study, we also found a significant negative correlation between serum levels of BDNF and HAMD-21 scores, meaning that higher levels of BDNF are associated with lower scores in the Hamilton scale, hence to a better clinical outcome. This result suggests that serum BDNF may be considered as a biomarker for treatment outcome. However, despite previous studies being consistent with our results (Chauhan et al., 2023; Kurita et al., 2012; Polyakova et al., 2015), other studies did not find any significant correlation (Brunoni et al., 2014; Bumb et al., 2015; Huang et al., 2020). One possible explanation of our result is that we performed a *repeated measures correlation*, which is better suited for the type of our study design. This method is ideal for assessing a common association across individuals, specifically a homogenous intra-individual linear association between two paired measures (Bakdash & Marusich, 2017). Additionally, this method finds its strength in its potential for high statistical power, which is precious for small sample sized studies. Nonetheless, data are still quite conflicting in this regard and defining BDNF as a biomarker for successful treatment requires more coherent findings. Indeed, we did not observe a significant correlation between serum levels of BDNF and HAMD-21 scores in the MITDEP group. However, despite the absence of statistical evidence, it is important to highlight that the trend towards correlation is consistent across patients in the MITDEP group. We speculate that with a more robust study design, this correlation could strengthen, potentially unveiling BDNF as a general biomarker for therapy outcome.

4.3.2 Cytokines

The effects of ECT on the immune-inflammatory system represent another central aspect in our study. We found that TNF-α levels in serum started raising after one week of ECT until reaching a peak at week four (see Results, 3.3.5). However, from week four to week six, we observed a drop in TNF-α levels. IL-4 also showed an altered expression during ECT course, alternating between low and high peaks. Other crucial cytokines, including IL-6, IL-1β, IL-10, did not show significant changes during therapy.

A large number of studies and recent literature reviews support the hypothesis that ECT induces an acute and transient increase in the expression of pro-inflammatory cytokines, followed by long-term decrease in the immune-inflammatory response (Gay et al., 2021; Järventausta et al., 2017; Kruse et al., 2018; Lehtimäki et al., 2008; Rush et al., 2016; Ryan &

McLoughlin, 2022; Yrondi et al., 2018). However, studies examining the effect of ECT on inflammatory mediators show contrasting results. For instance, Hestad et al. reported that TNF-α concentrations increase 1 h after a single ECT session (Hestad et al., 2003), though another recent study showed a decrease of TNF-α by 2 and 4 h after each ECT session (Sorri et al., 2018). Results regarding *long-term* changes in TNF-α show an overall reduction following a treatment course (24 h and one week post-ECT course), with concentrations at one week post-ECT comparable to concentrations of healthy controls (Hestad et al., 2003). However, others have noted no significant change in TNF-α post-ECT (Rush et al., 2016; Ryan & McLoughlin, 2022; Zincir et al., 2016). Notably, in our study we could not really consider the increase at week four as an acute increase, since *"acute"* normally refers to 1 or 2 sessions of ECT. Nevertheless, we also observed a prominent decrease in TNF-α levels at week six, around the end of the therapy. Hence, our result would support the hypothesis that ECT decreases the pro-inflammatory response in depression in the long-term. One possible explanatory mechanism is that cytokines can activate the enzyme indoleamine 2,3-dioxygenase (IDO), which catabolizes tryptophan, a precursor of serotonin, into kynurenine (KYN) and leads to reduced serotonin synthesis (Campbell et al., 2014). KYN is metabolized to kynurenic acid (KYNA) and 3-hydroxykynurenine, which is further metabolized to quinolinic acid (QUIN), a neurotoxic compound for the central nervous system (Bay-Richter et al., 2015; Campbell et al., 2014). This process potentially contributes to depressive symptoms and increases glutamate-mediated excitotoxicity (Kruse et al., 2019). Notably, it has been documented that pro-inflammatory cytokines, particularly interferon-γ and TNF-α, induce the expression of IDO. In fact, TNF-α alone can induce IDO expression 24 hours after the start of the culture in primary human macrophages (Wolf et al., 2004). Additionally, the blockade of TNF-α inhibits expression of IDO and protects cortical neurons from stress-induced depression in mice (Liu et al., 2015). Interestingly, reports suggest that ECT is associated with an increase of KYNA, known for its neuroprotective properties, and a decrease in the QUIN/KYNA ratio (Yrondi et al., 2018). Thus, it is plausible to hypothesize that the long-term reduction of TNF-α levels induced by ECT may contribute to the decreased expression of IDO and potentially explains the increased neuroprotective effects induced by the KYN pathway post-ECT (Gay et al., 2021; Guloksuz et al., 2014).

Another hypothesis suggests that the *immune* and *neurotrophic* systems may mutually act with a bidirectional interaction (van Buel et al., 2015). However, there are conflicting data about this topic. On one hand, it has been shown that IL-6 and TNF-α directly induce BDNF secretion in human monocytes (Schulte-Herbrüggen et al., 2005). Lehtimäki et al. (Lehtimäki

et al., 2008) reported a correlation between the release of IL-6 induced by ECT and the dosage of the stimulus, suggesting that neuronal depolarization might trigger cytokine release. On the other hand, Belge et al. showed that the diminution of both IL-6 and TNF-α levels was associated with an increase of the hippocampus volume (Belge et al., 2020). They propose that the reduction of IL-6 could be a crucial factor mediating the central neuroplastic effect. In excessive concentrations, peripheral cytokines interact with the central nervous system, through the blood-brain barrier, and stimulate astrocytes and microglia cells which produce more inflammatory mediators and cytokines (Yang et al., 2019). Microglial activation and cerebral inflammatory cytokines may in turn induce oxidative stress and a decrease of neurotrophic factors (Maeng & Hong, 2019; Yang et al., 2019). Taken together, these findings suggest that a relationship between a part of inflammatory processes and neuroplasticity may explain the therapeutic effect of ECT. However, our findings did not show any significant change in IL-6. Based on the previous literature, we expected that the increase of BDNF would have been accompanied by a decrease in IL-6 levels. This aligns with established knowledge wherein IL-6 serves as a recognized marker in depression. Notably, it is often elevated in depressed individuals compared to healthy controls (Haapakoski et al., 2015; Köhler et al., 2017), decreasing after antidepressant therapy (Bauer et al., 2021; Köhler et al., 2018). Surprisingly, our study showed a tendency toward increased IL-6 levels during the weeks of ECT treatment, contrasting with usual pharmacotherapy. This may imply that despite similar clinical outcomes, the mechanism of action in ECT significantly differs from typical pharmacotherapy. It is plausible that ECT initially causes an immune system perturbation, leading to subsequent beneficial effects.

TNF-α levels also negatively correlated with HAMD-21 scores in our sample of patients. This finding implies that increased TNF-α levels correspond to a more favorable clinical condition, which might seem counterintuitive since this is a pro-inflammatory cytokine. However, instead of interpreting a heightened inflammatory state as beneficial, we could propose that an elevated inflammatory response serves as a mechanism for the efficacy of ECT treatment. Indeed, the assumption that successful treatment of depression is linked to a reduction in inflammation is not always accurate. For instance, some studies reported that antidepressants such as serotonin and norepinephrine reuptake inhibitors (SNRIs) induce an increase in IL-6 and TNF-α expression (Hannestad et al., 2011; Piletz et al., 2009; Warner-Schmidt et al., 2011). ECT has been associated with microglial activation in rats (Wennström et al., 2006). In some cases, enhanced activation of the immune system is a predictor for greater clinical improvement. For example, Jha et al. found that higher levels of IL-17 at baseline

predict a better clinical outcome in patients treated with a combination of bupropion and SSRI (Jha et al., 2017). Furthermore, Uher and colleagues reported that baseline CRP levels differentially predicted treatment outcome with either an SSRI or an SNRI (Uher et al., 2014). Nevertheless, rather than focusing on one single factor like genetics, cytokines, or microbiome composition to predict antidepressant therapy response, future studies should integrate multiple parameters of each individual patient. Analyzing multivariate patterns could offer a more accurate understanding of how these factors collectively influence treatment response. Ideally, we aim to input specific patient data into a predictive algorithm to anticipate the most suitable treatment option, its duration, and eventual outcome.

Our results also revealed no significant change in IL-10, but literature reports on IL-10 are also conflicting with both, reports of increases (Zincir et al., 2016) as well as of no changes (Carlier et al., 2021). The heterogeneity of these results reflects the several issues that continue to characterize research on inflammatory markers in patients undergoing ECT. These issues include, for example, small sample sizes, diverse study population, lack of standardized measurement methodologies (e.g., plasma versus serum), variation in sampling time points, and the influence of anesthesia and muscle relaxants (Ryan & McLoughlin, 2022). An additional challenge is that cytokines are not always detectable in the majority of the samples. In our case, IL-1β and IL-13 were not detectable. The same complication was encountered by Ryan and colleagues and Järventausta et al. (Järventausta et al., 2017; Ryan & McLoughlin, 2022). One potential explanation may lie in the assessment method: Ryan and colleagues also employed the V-PLEX pro-inflammatory panel 1 kit, identical to the one utilized in our study.

With regard to IL-4, the meta-anlysis by Gay et al. revealed no significant change in the expression of this anti-inflammatory cytokine in the peripheral blood of patients treated with ECT. Our finding of fluctuations in the blood levels of IL-4 may demonstrate an attempt by the innate immune system to restore homeostasis within the central nervous system by increasing the expression of an anti-inflammatory cytokine.

4.3.3 TSPO, VDAC1, Pregnenolone

Our findings indicated no significant changes in TSPO levels within peripheral blood platelets during ECT. To the best of our knowledge, limited evidence exists regarding the role of TSPO and VDAC in the context of ECT and our study represents the most recent exploration of this associations. In fact, only one article reports TSPO levels changes in depressed patients undergoing ECT (Weizman et al., 1996). Weizman and colleagues showed that TSPO levels

in platelets significantly decreased after six sessions of ECT. More evidence is available about the effects of antidepressant pharmacological therapy on peripheral blood TSPO levels. Sarubin et al. reported that TSPO expression in platelets of depressed patients decreased after six weeks of antidepressant therapy (Sarubin et al., 2016). Li et al. showed that cognitive behavioral therapy (CBT) decreased TSPO total distribution volume in the brain (V_T) and this reduction correlated with the amelioration of depressive symptoms (Li et al., 2018). It is noteworthy that, while our findings did not achieve a significant level, a trend of decrease of peripheral TSPO might be observed from the results graph (see Results section, 3.3.1). With a larger sample size, and therefore a higher power analysis, this decrease may potentially become more pronounced. Previous studies showed reduced platelet TSPO levels in depression and bipolar disorder (Abelli et al., 2010; Barichello et al., 2017a; Chelli et al., 2008). While other data reported no differences in TSPO platelet levels between depressed patients and healthy controls (Weizman et al., 1995). Considering that TSPO may have a neuroprotective role, capable of influencing microglia polarization towards an M2 anti-inflammatory state (Kim & Yu, 2015), our hypothesis was that successful ECT would result in an initial short-term increase in TSPO levels, followed by a subsequent long-term normalization. The potential mechanism is that TSPO upregulation during neuroinflammation may be an adaptive response to facilitate recovery. However, it is important to note that peripheral blood expression of TSPO, does not necessarily reflect central expression. Indeed, Setiwawan et al. did not find a significant correlation between brain and serum expression of TSPO (Setiawan et al., 2015). Moreover, studies which measured TSPO V_T in the brain showed an increase rather than a decrease of central TSPO in depression (Enache et al., 2019; Li et al., 2018; Setiawan et al., 2015). Another confounder is given by psychopharmacological medication which can impact the expression of TSPO. For instance, research has demonstrated that the neuroleptic clozapine resulted in notable increases in TSPO binding in both the brain and peripheral steroidogenic tissues (Danovich et al., 2008). Overall, our findings remain insufficient to fully elucidate the role of TSPO in the mechanisms of ECT. Clearly, future investigations on ECT should incorporate this crucial marker for a more comprehensive understanding. Nevertheless, the correlation analysis revealed an interesting finding. TSPO expression levels seem to be positively correlated with HAMD-21 scores, meaning that lower values of TSPO expression are associated with better clinical outcome. This aligns with the concept that TSPO serves as a marker of neuroinflammation and plays a neuroprotective role, indicating that an improved clinical condition would require a diminished protective response mediated by TSPO.

Closely related to TSPO function, the anion channel VDAC contributes to the efficiency of mitochondrial quality control, regulating mitochondrial structure and function (Gatliff et al., 2014). We did not detect any significant alterations in the expression of VDAC-1 in platelets during and after ECT. Studies have proposed that TSPO and VDAC-1 interact with each other during the generation of reactive oxygen species (ROS) and this interaction is considered to play a role in the induction of mitochondrial-mediated apoptosis (Gatliff et al., 2014; Shoshan-Barmatz et al., 2019). The absence of changes in our study may indicate that ECT did not trigger ROS-induced pathways. This hints that ECT might exert a positive influence on the molecular mechanisms of the brain by preventing damage caused by ROS. However, this interpretation remains speculative, emphasizing the necessity for more focused investigations to reveal the potential role of VDAC in the mechanisms of ECT. Adding to the complexity, it should be noted that TSPO and VDAC-1 are implicated in various other functions, such as cell proliferation, Ca^{2+} signaling, oxidative stress, and inflammation (Gatliff et al., 2014).

Additionally, the importance of TSPO and VDAC-1 function lies in cholesterol transport through the mitochondrial membrane inside the mitochondrion, where it acts as precursor of pregnenolone. Pregnenolone is the first molecule initiating the neurosteroids synthesis cascade in the brain. Hence, it appears that neurosteroids levels hold more significance for recuperation from depression post successful treatment, as compared to TSPO expression itself (Schüle et al., 2014; Uzunova et al., 1998). Considering this perspective, some researchers endorse the idea that TSPO expression does not necessarily correlate with neurosteroids production. In line with this, Costa et al. discovered that individuals experiencing depression and adult separation anxiety, who demonstrated normal TSPO levels, showed decreased pregnenolone production, while patients with regular pregnenolone production displayed lower TSPO levels (Costa et al., 2012). In our study, we did not find significant changes in pregnenolone peripheral levels (see Results, 3.3.3). However, we could observe a trend of immediate decrease after the first week of ECT, followed by a long-term increase at week four (see Results, 3.3.3). Considering the neuroprotective role of neurosteroids, we could argue that the beneficial effects of ECT may be mediated by the increase of pregnenolone and subsequently of neurosteroids. However, it is important to keep in mind that our measurement of peripheral pregnenolone levels may not precisely reflect its expression at the brain level.

4.3.4 Cortisol

We did not observe significant changes of cortisol levels in the serum of our patients. However, a trend to increase over time could be noticed (see Results, 3.3.4). Based on the evidence that ECT triggers the hyperstimulation of the HPA axis (Apéria et al., 1985; Eşel et al., 2003; Florkowski et al., 1996; Schwartz & Chen, 1985), our hypothesis was that ECT would induce an acute increase of cortisol, yet followed by an overall decrease. In support of the acute increase, Grønli et al. previously found an increase in cortisol levels 1 h after ECT (Grønli et al., 2009). Zis et al. even reported a dose-dependent increase of cortisol 30 minutes after ECT (Zis et al., 1996).

We could not compare baseline levels of cortisol to healthy controls, nevertheless we could observe higher baseline values compared to the normal cortisol range in a healthy population. This is in line with the reports about increased cortisol release in depressed patients compared to healthy controls (Jia et al., 2019). Therefore, we hypothesized that ECT would overall decrease cortisol levels, normalizing them to the healthy population.

The fact that we did not find significant changes is in accordance with other studies (Markianos et al., 2002). Other researchers reported also lower levels of cortisol after ECT (Özsoy et al., 2008). Clearly, it is hard to find a consensus result in the literature and the mechanisms of ECT through cortisol are not explicit. Interestingly, high levels of cortisol after ECT have been associated with the cognitive impairment consequent to ECT. Neylan et al. propose that high cortisol levels may impair brain function because of mechanisms such as changes in the brain glucose metabolism, which potentiate the toxic effect of excitatory amino acids, such as glutamate. This could, in turn, block the action of neurotrophic factors that are crucial for the recovery from brain damage (Neylan et al., 2001). Conversely, in our study, we still observed that BDNF serum levels increased significantly, despite the rise in cortisol levels. Therefore, it is plausible to hypothesize that blocking cortisol release might lead to even higher levels of BDNF, potentially preventing negative cognitive effects. From a clinical perspective, cortisol release blockers could be considered as a supplementary therapy to ECT. Ideally, this combination could offer the beneficial effects of ECT, without having the cognitive side effects. In fact, the HPA system has already been considered as target for antidepressant therapy. For instance, some orally available small molecules entered clinical development as CRH_1 receptor antagonists (Holsboer & Ising, 2008). These compounds were able to improve both the depressed mood and the cognitive symptoms in depressed patients. O'Brien et al. also suggest that high cortisol levels during depression may be associated with cognitive changes.

Chronic hypercortisolism in depression could potentially lead to damage in the hippocampus, explaining why these deficiencies persist even after the affective symptoms have disappeared (O'Brien et al., 2004). Overall, the importance of the changes in serum cortisol in relation to the clinical effects of ECT remains uncertain and it is also possible that a potential increase represents a more general response to the stress of treatment (Florkowski et al., 1996).

4.4 ECT effects on brain network connectivity

4.4.1 Regional homogeneity - Reho

Local functional connectivity assessed by ReHo changed throughout four weeks of ECT treatment. Interestingly, alterations were not limited to a specific brain area or hemisphere. Increased ReHo values denote increased local synchronization of neural activity in a given region (Zang et al., 2004), which in turn may indicate enhanced processing in that region. Alternatively, increased ReHo in a region may imply that another remote brain area has decreased its suppressive influence on that region. Both above mentioned mechanisms are plausible. However, given our small sample size and liberal statistical threshold, we are not able to draw strong conclusions and can only interpret our findings with caution.

After one week of ECT, ReHo increases in regions of the inferior parietal (PGi = parietal G inferior) and temporo-parietal-occipital junction in the left hemisphere and a small area in the right dorsolateral prefrontal cortex (DLPFC); while it decreases in the right parahippocampal area. At week two of treatment, we observe only increases of ReHo: in the left DLPFC, regions of the left parieto-temporal cortex (MIP = medial intra parietal area, IP0 = area intra parietal zero, AIP = anterior intraparietal, FST = fundal superior temporal area) and regions of the right intraparietal cortex (LIPv, LIPd = lateral intraparietal area ventral and dorsal). At week three ReHo increases in the left hemisphere parieto-temporal cortex (POS1 = parietal occipital sulcus area 1, AIP, PGi, MST = medial superior temporal area); areas of the frontal lobe (area s32 = anterior cingulate and medial prefrontal cortex, IFSp = inferior frontal cortex); and LIPd in the right hemisphere. At week four ReHo increases in the left frontal area (p47r = area 47 anterior inferior frontal, a9-46v = anterior dorsolateral prefrontal, a10p = area anterior 10 orbital and polar frontal cortex) and in regions of the left parieto-temporal lobe (AIP, PEF = premotor eye field, PFOP = opercular, TPOJ2 = temporo-parietal-occipital junction two, MIP, TE1m = lateral temporal); in the right hemisphere pareto-temporal cortex (MST = medial superior temporal area, FST, IP1 = intraparietal 1, area 11l = orbital and polar frontal cortex). Overall, we detected three main brain areas affected by ECT: frontal/prefrontal cortex, intraparietal cortex, and temporal cortex.

The DLPFC, an important part of the prefrontal cortex, mainly lies in the medial frontal gyrus (MFG), it is part of the cognitive control network (CCN), and it is involved in emotional regulation. In particular, it plays a crucial role in the top-down regulation of emotional processing (Disner et al., 2011). Moreover, previous studies have suggested that the structural

and functional imbalance of the DLPFC is important in the pathogenesis of depression (Salvadore et al., 2011; Shen et al., 2015; Vasic et al., 2008; Yin et al., 2015). The regional cerebral blood flow (rCBF) and the regional metabolic rate for glucose (rCMRGlc) are associated with the functional activity of neural cells (Jueptner & Weiller, 1995). It has been shown that in most brain areas, there is a significant positive correlation between rCBF/rCMRGlc and ReHo/ALFF values. Therefore, high regional ReHo/ALFF values suggest high rCBF/rCMRGlc, and vice-versa (Aiello et al., 2015; Li et al., 2012; Nugent et al., 2015). In positron emission tomography (PET) studies, Bench et al. observed reduced overall rCBF in the left anterior cingulate cortex (ACC) and the left dorsolateral prefrontal cortex (DLPFC) in individuals with depression (Bench et al., 1992). A study by Guze et al. supported these findings measuring rCMRGlc (Guze et al., 1991). Other researchers also confirmed altered local DLPFC function in depressed patients measured by rCBF, rCMRGlc, ReHo and ALFF. Interestingly, Brody et al. found that in depressed patients, improvement of cognitive disturbance symptoms was associated with increasing DLPFC metabolism (Brody et al., 2001). Based on the previous literature, the increase in ReHo in DLPFC after ECT found in our study, may be related to the improvement mediated by ECT in emotion regulation and cognitive symptoms. In other words, the increase of ReHo in the DLPFC supports the idea that ECT may be efficient by increasing cognitive control over emotion processing.

We observed a decrease in ReHo in the parahippocampal area after one week of ECT. The parahippocampal area, located in the inferior medial temporal lobe, is one of the key regions of the memory circuit. The parahippocampal region plays a major role in detailed memory retrieval of both spatial and temporal context (Eichenbaum & Lipton, 2008). Since it is associated with contextual associations or episodic memory, the parahippocampal area is an hyperactive hub during affective processing tasks (Miller et al., 2015). Previous studies also reported overactivity in the parahippocampus in depressed patients as compared to healthy controls (Miller et al., 2015; Sankar et al., 2015; Wang et al., 2017; Young et al., 2012). Thus, our findings of decreased ReHo values may provide evidence that the parahippocampus function is associated with the efficacy of ECT in depressed patients. A potential mechanism is that ECT normalizes the overactivity characteristic of this region in depressed patients. Consequently, patients would experience weakened spatial and temporal memory retrieval. This might explain both clinical improvement and memory side effects common during ECT treatment. Indeed, we could consider as clinically beneficial that a patient loses the negative memory associations with specific past events. This should be further investigated by integrating memory tasks in fMRI investigations in a follow up study on ECT mechanisms.

Throughout the therapy several regions belonging to the intraparietal sulcus have increased their ReHo. This area of the brain is part of the dorsal attention network (DAN), together with the frontal eye fields, and extra striate visual areas (Corbetta & Shulman, 2002; Fox et al., 2006). The intraparietal sulcus has been shown to be involved in higher-order cognitive processes, such as visuospatial selective attention, top-down control of attentional mechanisms, and goal-directed behavior (Kastner & Ungerleider, 2003; Silver & Kastner, 2009; Szczepanski & Kastner, 2013). The DAN shows increased synchronization during goal-directed processes (Corbetta et al., 1998; Kim, 2010), and depression is characterized by poor performance in cognitive control tasks and bias in attentional processes (Mennen et al., 2019; Veiel, 1997; Zakzanis et al., 1998). Thus, our results support the hypothesis that, by increasing ReHo in the intraparietal region, ECT modulates the attentional and cognitive processes towards goal-directed behaviors and improves the negative attentional bias characteristic of depression.

ReHo increased also in the temporal lobe, particularly in regions of the superior and lateral temporal cortex. The temporal lobe has been shown to participate in the recall of personal experiences, familiar faces and scenes, emotional memory and in prediction of individual behavioral based on personal beliefs and emotions (Gallagher & Frith, 2003; Saxe & Kanwisher, 2003). Abnormal brain activity in the temporal lobe may lead to emotional dysregulation and increased suicide risk in depression. Particularly, the superior temporal gyrus (STG) has been associated with understanding the meaning of stories involving people and the perception of intentional behavior (Brunet et al., 2000). Other researchers suggested that left STG plays a key role in language processing and auditory memory (DeWitt & Rauschecker, 2012; Leff et al., 2009). Interestingly, impaired language processing has been found in depression at the acute phase and recurrent stage of disease (Fossati et al., 2003; Schmid et al., 2011). Right STG, which is an important part of the affective network, is one of the most frequently reported brain regions linked to the neurobiology of depression (Fitzgerald et al., 2008). Moreover, right STG takes part in emotion and cognitive regulation, and social cognition (Allison et al., 2000; Dutta et al., 2014; Gallagher & Frith, 2003). Similarly to our case, Wang et al. found that both left and right STG were closely related to ECT response and had significantly increased local functional connectivity density (FCD) in healthy controls and in depressed patients after ECT (Wang et al., 2018). Other studies also support these data reporting decreased local FCD in left and right STG in depressed patients compared to healthy controls (Guo et al., 2016; Zou et al., 2016). Altogether, these findings suggested that the abnormal activity of left STG may account for the dysfunction of language processing and

auditory memory, and for the aberrant perception of social behaviors in depression and may be reversed by ECT.

4.4.2 Fractional amplitudes of low frequency fluctuations - fALFF

In the present study, we found that spontaneous neural activity, measured by means of voxelwise fALFF, changed during four weeks of ECT. Intriguingly, we observed both increase and decrease of fALFF from week to week throughout therapy, suggesting that ECT may have differential region-dependent effects.

After one week of ECT, fALFF values increase in regions of the left temporal lobe (TE1a, TE2a = area 1 and 2 lateral temporal cortex), and regions of the left frontal lobe (p47r = posterior inferior frontal, 9-46d: dorsolateral prefrontal); while values decrease in regions of the right hemisphere in the parietal and occipital cortex (R_V3B = dorsal stream visual, POS1 = parietal occipital sulcus area 1). At week two of treatment, we observed only decreasing fALFF values. The involved regions were spread essentially over the paracentral lobular and middle cingulate cortex in both left and right hemispheres (SCEF = supplementary and cingulate eye field, 24dv = ventral area 24, 23c = area 23, 24dd = dorsal area 24 d, 6mp = area 6mp, 6ma = area 6m anterior), and some regions in the frontal lobe (24pr = area 24 prime, 9p = area 9 posterior dorsolateral prefrontal cortex). At the third week of treatment, we observed again an increase in fALFF values in the left and right frontal cortex (L_a47r = anterior inferior frontal, R_p47r = posterior inferior frontal), left inferior parietal cortex (PFm = area PFm complex, PGs = area PGs, PGi = area Pgi), and temporal cortex (TE2a = area 2 lateral temporal); and also a decrease in one region of the right paracentral lobular and middle cingulate cortex (6ma). At week four, we observed again only decreased values of fALFF, involving regions in the paracentral lobular and middle cingulate cortex (R_23c, 6mp), in the anterior cingulate and medial prefrontal cortex (R_p32pr, R_a24pr), dorsolateral prefrontal cortex (R_sfl = superior frontal language area), and superior parietal cortex (R_7AL = lateral area 7A).

Our results of decreased fALFF values in the middle cingulate cortex after ECT are consistent with the findings of Kong et al. (Kong et al., 2017). They also found increased ALFF values in the left MFG, right MFG and orbital part, similarly to the increase we observed in the frontal areas. Nevertheless, previous studies investigating ALFF in depression demonstrate

some inconsistencies, showing functional alteration in other different regions (Kong et al., 2017; Qiu et al., 2019; Wang et al., 2018). The midcingulate cortex, an important part of the DMN, has been closely correlated with depression (Graff-Guerrero et al., 2004; Grimm et al., 2012; Hasler et al., 2008; Kühn et al., 2012; Zhang et al., 2016; Zhang et al., 2012), and DMN hyperconnectivity has been detected in depressed patients with respect to healthy controls (Li et al., 2013; Liston et al., 2014; Posner et al., 2013). These findings suggest that ECT's antidepressant effect might be due to a reduction of spontaneous activity in this region at rest. In this way, ECT would act on depressive symptoms such as rumination or exacerbated self-referential processing, which involve an hyperactivation of the DMN. However, it is important to acknowledge that the midcingulate cortex is only one component of the DMN, which weakens our interpretation to some extent.

Notably, we also observed an increase in spontaneous activity throughout the therapy (W1 vs. BL and W3 vs. BL) in some regions which partially overlap with those exhibiting increased local connectivity (ReHo) – frontal, parietal and temporal cortex (see Discussion, 4.4.1). In particular, spontaneous brain activity increased in the lateral temporal cortex after ECT. Temporal poles are higher level cognitive structures which are involved in evaluating emotional significance by integrating contextual information (Park et al., 2019). A study by Guo et al. found that patients with TRD exhibit lower ALFF values in the middle temporal gyrus (Guo et al., 2012). Additionally, brain activity in the temporal lobe negatively correlates with symptom severity among depressed individuals (Park et al., 2019). In line with these findings, it seems that ECT improves emotion regulation and integration of affective responses by increasing the activity in the temporal regions. We also observed that ALFF values increase in regions of the inferior frontal cortex and DLPFC. These findings would reinforce the hypotheses related to ReHo that were previously discussed: having both enhanced local connectivity (ReHo) and increased spontaneous activity (fALFF) in those regions suggests that ECT strengthens the cognitive control over emotional processing and improves the executive functions in depressed patients. Moreover, we observed increased activity in terms of fALFF in regions of the parietal cortex after ECT. The parietal lobe plays a role in information processing, decision making, reward processing, and emotional processing (Zhang et al., 2018). However, findings about the activity of this region in the context of depression are quite conflicting, reporting both increased (Liang et al., 2013) and decreased (Li et al., 2018) values of ReHo in depressed patients. Additionally, altered parietal cortex activity has been associated with a dysfunction in receiving new information and learning (Liang et al., 2013). Therefore,

we can deduce that ECT impacts the processing of information related to emotional stimuli, yet determining the precise direction of this influence remains challenging.

4.4.3 Brain network topology – degree, betweenness centrality and efficiency

To analyze how the topology of the brain's functional network is linked with the course ECT, we assessed graph measures of functional integration and centrality: node degree, betweenness centrality, and node efficiency.

We found that degree measure increased in the left inferior frontal sulcus (L_IFSp) from baseline to the second week of treatment and increased in the left posterior cingulate cortex (area 31a) at the fourth week compared to baseline. Betweenness centrality (BC) decreased in the right ventral intraparietal complex (R_VIP) after one week of treatment. At the second week BC decreased in the medial belt complex (R_MBelt) in the early auditory cortex, while it increased in the inferior frontal cortex (R_p47r). Furthermore, we reported that nodal efficiency (E) increased after one week of treatment in several regions of the left visual cortex (V1 = primary visual cortex, V3 = third visual area), in the left dorsolateral prefrontal cortex (area 8c, area 9-46v) and in early auditory cortex (PBelt = parabelt complex, LBelt = lateral belt complex). After three weeks of treatment, the increase compared to baseline involved only area STS in the auditory association cortex. At week four of ECT, efficiency increased in the right orbital polar frontal cortex (area 13).

Previous studies found aberrant nodal efficiency and centrality of regional connectivity in the dorsal striatum, inferior frontal and orbitofrontal cortex as well as in the occipital and somatosensory cortex of depressed patients (Meng et al., 2014). Particularly, Meng and colleagues reported decreased nodal degree in the inferior frontal gyrus. The inferior frontal gyrus is a hub of the salience network, and specifically the IFS has been associated with semantic processing and working memory (D'Esposito et al., 1998; Gabrieli et al., 1998). Interestingly, we found that ECT increased degree and betweenness centrality in this region, suggesting that ECT antidepressant effect may consist in normalizing functional connectivity in this region. Interestingly, within the frontal lobe also DLPFC increased its nodal efficiency, in line with the effect that we observed on ReHo. This region has been shown to be so relevant for depression, that pretreatment connectivity of DLPFC has been found to be an important predicting feature in predicting the success of ECT (Leaver et al., 2018). Additionally, Zhang

et al. showed decreased regional connectivity in terms of degree, efficiency and betweenness centrality in the DLPFC and occipital regions in depressed patients compared to controls (Zhang et al., 2011).

Posterior cingulate cortex, which is part of the DMN, has shown increased regional cerebral metabolism in depression (Wang et al., 2023). This region has been associated with examination of episodic memory, including autobiographical memory and imagining the future, and also spatial navigation and scene processing (Auger & Maguire, 2013; Leech & Sharp, 2014). Our findings show that ECT leads to an increase in the number of connections of this region, potentially exacerbating a depression-related state. Nonetheless, it is important to notice that degree only informs us about the number of connections, not the specific connections that are altered. It is possible that less effective connections occur within the DMN, in favor of new connections formed with other networks in the brain. Clearly, further investigation is necessary to provide a plausible explanation of our findings.

We reported a decrease in BC in the right ventral intraparietal cortex after one week of ECT. This region is part of the superior parietal lobe, which has been associated with mental imagery and recall of personal experiences (Johns, 2014). It is part of the DMN and is engaged during activities such as daydreaming and introspection. Thus, a decrease in its centrality after ECT may mitigate rumination and self-focused thoughts, characteristic symptoms of depression.

We also observed increased nodal efficiency in the orbitofrontal cortex (OFC) towards the end of the treatment. Evidence obtained using neuroimaging, neuropathologic, and lesion analysis techniques suggests that the OFC plays a role in depression (Drevets, 2007). However, an important functional distinction of the subregions of the OFC must be made between medial and lateral OFC: the lateral OFC (area 12) is implicated in the effects of aversive and subjectively unpleasant stimuli, and in not receiving expected rewards and has increased functional connectivity in depression. In contrast, the medial OFC (areas 13 and 11) is activated by rewarding and subjectively pleasant stimuli and has reduced functional connectivity in depression. In our study, we specifically found area 13 increasing its efficiency after treatment, suggesting a compensatory effect of ECT (Rolls et al., 2020).

Finally, we also found that regions in the visual and auditory cortex increase their efficiency after ECT. Besides the impairments in various cortical and subcortical regions (e.g., PFC, ACC, amygdala and hippocampus), and in brain functional networks (e.g., DMN, SN), evidence from both clinical and preclinical studies has suggested that depression could cause disturbances in sensory perception systems including olfactory, auditory, visual or gustatory

stimulation (Lu et al., 2020). In fact, dysfunction in early auditory processing and visual cortex malfunction have been observed in major depressive disorder (Kähkönen et al., 2007; Wu et al., 2023). Therefore, more efficient communication of those regions induced by ECT, may be associated with treatment antidepressant efficacy.

4.4.4 Concluding remarks on the effects of ECT on brain network connectivity

In the attempt of integrating all the information discussed above, we may conclude that ECT seems to "attack" the disease from different angles. In fact, by changing the activity or connectivity of several regions in the brain, ECT "attempts to fight" depressive symptoms at maximum. Interestingly, the affected regions are all crucial in the context of depression. First, ECT acts on the cognitive control dysfunction characteristic of depression: by increasing the functionality of regions such as DLPFC, IP area, IFG, medial OFC, and STG, ECT improves cognitive performance, semantic processing, working memory, perception of social interactions and the ability to feel reward again from external stimuli. Secondly, ECT influences regions responsible for the negative loops typical of depression: by weakening the activity of regions which are part of the DMN, ECT may alleviate symptoms of rumination, self-focused thoughts, and abnormal emotional appraisal. Finally, we also discussed the possibility that ECT side effects on memory are due to the reduction of local connectivity in the parahippocampal area.

To conclude, we may hypothesize that ECT helps the brain to find a new balance where the level of activity in each region represents the optimal configuration to improve the depressive condition.

CHAPTER 5 Conclusion & Outlook

5.1 General implications

Electroconvulsive therapy revealed itself once again to be among the most effective treatments for depression. Despite our limited sample size, we were able to show that ECT alleviates depressive symptoms.

Truly, the mechanisms of ECT remain still unclear to the scientific world, however, we believe that every single novel insight in the effects of ECT can act as a brick to build a comprehensive understanding in this matter. Here, we share some of those bricks, aspiring that they will be of help for future research.

Based on the above-mentioned findings, we can argue that ECT appears to impact multiple pathways within our organism, rather than having a single specific mechanism of action, as it is often seen in target-specific drugs. All the effects of ECT may act in synergy to help the brain find a new balance and consequently lead to the desired clinical improvement.

First, ECT showed an influence on the neurotrophic function in the brain mediated by BDNF. This neurotrophic factor would exert its trophic effects, such as formation, stabilization, and potentiation of synapses, resulting in enhanced neuronal plasticity. However, how exactly increased neuronal plasticity influences mood remains unclear. Castreń proposes an interesting perspective that connects neuronal plasticity to neuronal network reorganization in the context of antidepressant therapy (Castreń, 2013). He names it *"network hypothesis"* and he explains it as the ability of antidepressants to "achieve their effects via a gradual process in which enhanced plasticity facilitates the reorganization of cortical networks to better adjust to environmental experiences" (Castreń, 2013). He adds that antidepressants improve mood "by reactivating a juvenile-like state of plasticity". In the context of our study, we may adopt this hypothesis to explain how potentially ECT leads to the improvement of mood in our patients. This would link us to our results regarding brain network function, which suggest a reorganization of connections between regions. To note, we did not search for a direct link between the two levels. However, research on simpler models of the human brain (e.g., animal models, cell cultures) may be applied to investigate this relationship in a deeper way.

Interestingly, we found that ECT had region specific effects within the brain, by reinforcing the activity/connectivity of those regions hit negatively by depression and

weakening those regions which “took control” during depression. Thinking about ECT as a tool to “shake” the brain helps to find a description for the effects on brain network organization. This brings us back to Beck’s cognitive model of depression, where “biased attention, biased processing, biased thoughts and rumination, biased memory, and dysfunctional attitudes and schemas” are linked to the development and maintenance of depression (Disner et al., 2011). In this model, latent *schemas* - internally stored representations of stimuli, ideas, or experiences - are activated by internal or external environmental events and then influence how incoming information is processed (Beck, 1967). Disner et al. propose that depression is facilitated by increased influence from subcortical emotion-processing regions combined with attenuated top-down cognitive control. ECT may therefore act on depressive symptoms by affecting molecular pathways in specific regions of the brain, which in turn will have the effect of breaking the *schemas* on a larger scale neuronal network organization.

Conversely, our initial hypothesis regarding the potential advantages of an immediate immune-inflammatory response remains unverified in this particular investigation. Although we did observe a rise in TNF-α levels, followed by a decline after two weeks, it did not align with our anticipation: we would have expected an earlier enhancement of the immune-inflammatory response as an early mechanism of ECT. Nonetheless, we urge subsequent studies to re-evaluate the inflammatory hypothesis to steer the field towards the correct trajectory.

5.2 Limitations & Methodological considerations

We conducted our study with the primary aim of thoroughly investigating the effects of ECT in a sample of severely depressed patients, who demonstrated resistance to standard pharmacological treatment. Thanks to its repeated measures design, the present study was able to provide valuable insights and broaden our knowledge within the field. However, it is essential to acknowledge some general limitations of our study to ensure a comprehensive understanding of the findings.

Since our study was observational, it was subject to various types of bias, such as selection bias, measurement bias, and confounding bias. Indeed, it may be difficult to control variables in an observational study in the same way that randomized controlled trials (RCTs) could (Thadhani, 2006). Furthermore, generalizability may be limited because the study was conducted in a specific hospital setting. In line with this notion, the composition and size of

our sample further restrict the generalizability of our findings. Specifically, our sample comprised nine patients, with eight males and one female, despite the absence of any specific restriction based on sex. This leads to an underrepresentation of the female patient population within the study. Moreover, we believe that to enhance statistical power and ensure the reliability of results, future studies must consider including a larger group of patients. Due to time constraints, which were further aggravated by the Covid-19 pandemic restrictions, we were unable to include a larger number of patients. Nevertheless, in our case, statistical power was enhanced by using a repeated measures design, which allows for tracking effects over time requiring fewer subjects (Guo et al., 2013).

Another limitation was that all patients were receiving pharmacotherapy as it was clinically required throughout the treatment course. An additional confounding factor was that patients received ECT either with unilateral (RUL) or bilateral (BIL) electrode placement.

Furthermore, the absence of matched comparison subjects did not allow us to control for variance induced by repeated measurements. However, the longitudinal design of the study implied that the participants served as their own control for the statistical analysis.

As regards peripheral blood analysis, a crucial consideration is that the relationship between central and peripheral expression of molecular markers is not always a correlation, meaning that the levels of those parameters in the blood might not be indicative of the changes that occur in the brain. However, we tried to bear this notion in mind for interpreting our results.

Concerning MRI statistical analysis, it is important to note that we reported uncorrected results for the graph theory analysis (alpha threshold = 0.01), whereas for ReHo and fALFF results, we applied a cluster-based correction method (with alpha threshold = 0.01). Therefore, the reader should interpret our findings with caution. Additionally, although ReHo and fALFF have been corrected, we acknowledge that the cluster-based correction method has been criticized (Eklund et al., 2016). Eklund and colleagues explain that since this method is more sensitive to the statistical assumptions, false-positive rate may be higher than 5%, leading to inflated reports of statistical significance. Nonetheless, bearing this in consideration, our results might still offer precious insights in the context of ECT mechanisms, which necessitates further exploration.

We also realize that we could have performed intra-individual analysis, to consider that each patient may have a particular reaction to the therapy and a particular set of features prior to the therapy. Moreover, this would also allow to fully profit from a repeated measures design (Cole et al., 2010).

Ultimately, this study did not account for varying subtypes of depression, the influence of psychotic symptoms, nor the differentiation between unipolar and bipolar depression.

5.3 Outlook

The strength of our study lay fundamentally in its multi-level approach and in the six timepoints of measurement, which is rare in the literature exploring ECT. However, considering our limitations, future research which aims at replicating and further clarifying the discussed results needs to be conducted.

Given our interesting results about BDNF expression, it would be of interest to further investigate the significant role of BDNF single nucleotide polymorphism Val66Met (rs6265) in the response to ECT. Previous research investigated the association between BDNF genotype and depression (Verhagen et al., 2008), however little is known about its role in ECT response.

One strong hypothesis of our work was that depression is characterized by a dysfunction in brain dynamics and that ECT may act by "shaking" the brain system and restoring the flexibility in communication between brain regions. Although, the current study did not find significant changes in terms of brain dynamics, I strongly believe that forthcoming research should delve into this aspect.

Another question that future studies should address regards therapy outcome prediction: imagine to be able to predict who will respond to ECT even before starting the therapy, based on their brain connectivity pattern at baseline, which would represent an individual print. For this purpose, machine learning techniques may be implemented and new perspectives towards personalized medicine may be gained. This has been identified as a relevant issue in the context of depression (Insel, 2014), as diagnoses and choice of intervention based on biotypes may increase response rates (Fernandes et al., 2017). Interestingly, Drysdale et al. identified depression subtypes which are able to predict responsiveness to transcranial magnetic stimulation (TMS) therapy (Drysdale et al., 2017). This approach should certainly be explored for ECT as well, since ECT is generally considered a more common and effective therapy compared to TMS (Keshtkar et al., 2011). To note, however, that bigger sample sizes may be required for this type of analysis.

Future studies may investigate the effect size of secondary effects, such as cognitive symptoms or memory impairment, especially if they were correlated to the clinical outcome.

For instance, a task-MRI session may better clarify the association between task performance and brain processes.

With respect to the study design, future trials should consider including a group of matched healthy controls for a more accurate interpretation of the results. Additionally, adding a follow up measurement at a later timepoint (e.g., one month later) may unravel the long-term effects of ECT in the same patients.

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Appendix

A. QUESTIONNAIRES

Beck Depression Inventory

Dieser Fragebogen enthält 21 Gruppen von Aussagen. Bitten lesen Sie jede Gruppe sorgfältig durch. Suchen Sie dann die eine Aussage in jeder Gruppe heraus, die am besten beschreibt, wie sie sich in dieser Woche einschließlich heute gefühlt haben und kreuzen Sie die dazugehörige Ziffer (0, 1, 2 oder 3) an. Falls mehrere Aussagen einer Gruppe gleichermaßen zutreffen, können Sie auch mehrere Ziffern markieren. Lesen Sie auf jeden Fall alle Aussagen in jeder Gruppe, bevor Sie Ihre Wahl treffen.

0 1 2 3	**Gruppe A** O Ich bin nicht traurig. O Ich bin traurig. O Ich bin die ganze Zeit traurig und komme nicht davon los. O Ich bin so traurig oder unglücklich, dass ich es kaum noch ertrage.	0 1 2 3	**Gruppe F** O Ich habe nicht das Gefühl, gestraft zu sein. O Ich habe das Gefühl, vielleicht gestraft zu werden. O Ich erwarte bestraft zu werden. O Ich habe das Gefühl, bestraft zu sein.
0 1 2 3	**Gruppe B** O Ich sehe nicht besonders mutlos in die Zukunft. O Ich sehe mutlos in die Zukunft. O Ich habe nichts, worauf ich mich freuen kann. O Ich habe das Gefühl, dass die Zukunft hoffnungslos ist und dass die Situation nicht besser werden kann.	0 1 2 3	**Gruppe G** O Ich bin nicht von mir enttäuscht. O Ich bin von mir enttäuscht. O Ich finde mich fürchterlich. O Ich hasse mich.
0 1 2 3	**Gruppe C** O Ich fühle mich nicht als Versager. O Ich habe das Gefühl, öfter versagt zu haben als der Durchschnitt. O Wenn ich auf mein Leben zurückblicke, sehe ich bloß eine Menge Fehlschläge. O Ich habe das Gefühl, als Mensch ein völliger Versager zu sein.	0 1 2 3	**Gruppe H** O Ich habe nicht das Gefühl, schlechter zu sein als alle anderen. O Ich kritisiere mich wegen meiner Fehler und Schwächen. O Ich mache mir die ganze Zeit Vorwürfe wegen meiner Mängel. O Ich gebe mir für alles die Schuld, was schief geht.
0 1 2 3	**Gruppe D** O Ich kann die Dinge genauso genießen wie früher. O Ich kann die Dinge nicht mehr so genießen wie früher. O Ich kann aus nichts mehr eine echte Befriedigung ziehen. O Ich bin mit allem unzufrieden oder gelangweilt.	0 1 2 3	**Gruppe I** O Ich denke nicht daran, mir etwas anzutun. O Ich denke manchmal an Selbstmord, aber ich würde es nicht tun. O Ich möchte mich am liebsten umbringen. O Ich würde mich umbringen, wenn ich die Gelegenheit hätte.
0 1 2 3	**Gruppe E** O Ich habe keine Schuldgefühle. O Ich habe häufig Schuldgefühle. O Ich habe fast immer Schuldgefühle. O Ich habe immer Schuldgefühle.	0 1 2 3	**Gruppe J** O Ich weine nicht öfter als früher. O Ich weine jetzt mehr als früher. O Ich weine jetzt die ganze Zeit. O Früher konnte ich weinen, aber jetzt kann ich es nicht mehr, obwohl ich es möchte.

	Gruppe K		**Gruppe Q**
0	O Ich bin nicht reizbarer als sonst.	0	O Ich ermüde nicht stärker als sonst.
1	O Ich bin jetzt leichter verärgert oder gereizt als früher.	1	O Ich ermüde schneller als früher.
2	O Ich fühle mich dauernd gereizt.	2	O Fast alles ermüdet mich.
3	O Die Dinge die mich früher geärgert haben, berühren mich nicht mehr.	3	O Ich bin zu müde, um etwas zu tun.
	Gruppe L		**Gruppe R**
0	O Ich habe nicht das Interesse an Menschen verloren.	0	O Mein Appetit ist nicht schlechter als sonst.
1	O Ich interessiere mich jetzt weniger für Menschen als früher.	1	O Mein Appetit ist nicht mehr so gut wie früher.
2	O Ich habe mein Interesse an anderen Menschen zum größten Teil verloren.	2	O Mein Appetit hat sehr stark nachgelassen.
3	O Ich habe mein ganzes Interesse an anderen Menschen verloren.	3	O Ich habe überhaupt keinen Appetit mehr.
	Gruppe M		**Gruppe S**
0	O Ich bin so entschlussfreudig wie immer.	0	O Ich habe in letzter Zeit kaum abgenommen.
1	O Ich schiebe Entscheidungen jetzt öfter als früher auf.	1	O Ich habe mehr als 2 Kilo abgenommen.
2	O Es fällt mir jetzt schwerer als früher, Entscheidungen zu treffen.	2	O Ich habe mehr als 5 Kilo abgenommen.
3	O Ich kann überhaupt keine Entscheidungen mehr treffen.	3	O Ich habe mehr als 8 Kilo abgenommen.
			Ich esse absichtlich weniger, um abzunehmen: □ Ja □ Nein
	Gruppe N		**Gruppe T**
0	O Ich habe nicht das Gefühl, schlechter auszusehen als früher.	0	O Ich mache mir keine größeren Sorgen um meine Gesundheit als sonst.
1	O Ich mache mir Sorgen, dass ich alt oder unattraktiv aussehe.	1	O Ich mache mir Sorgen über körperliche Probleme, wie Schmerzen, Magenbeschwerden oder Verstopfung.
2	O Ich habe das Gefühl, dass Veränderungen in meinem Aussehen eintreten, die mich hässlich machen.	2	O Ich mache mir so große Sorgen über gesundheitliche Probleme, dass es mir schwer fällt, an etwas anderes zu denken.
3	O Ich finde mich hässlich.	3	O Ich mache mir so große Sorgen über gesundheitliche Probleme, dass ich an nichts anderes mehr denken kann.
	Gruppe O		**Gruppe U**
0	O Ich kann so gut arbeiten wie früher.	0	O Ich habe in letzter Zeit keine Veränderung meines Interesses an Sex bemerkt.
1	O Ich muss mir einen Ruck geben, bevor ich eine Tätigkeit in Angriff nehme.	1	O Ich interessiere mich weniger für Sex als früher.
2	O Ich muss mich zu jeder Tätigkeit zwingen.	2	O Ich interessiere mich jetzt viel weniger für Sex.
3	O Ich bin unfähig zu arbeiten.	3	O Ich habe das Interesse an Sex völlig verloren.
	Gruppe P		
0	O Ich schlafe so gut wie sonst.		_____ **Subtotal Seite 2**
1	O Ich schlafe nicht mehr so gut wie früher.		_____ **Subtotal Seite 1**
2	O Ich wache 1 bis 2 Std. früher auf als sonst und es fällt mir schwer, wieder einzuschlafen.		_____
3	O Ich wache mehrere Stunden früher auf als sonst und kann nicht mehr einschlafen.		_____ **Summenwert**

Hamilton Depression Rating Scale

Anleitung
Bitte jeweils nur die zutreffende Ziffer ankreuzen! **Bitte alle Feststellungen beantworten!**

1. Depressive Stimmung (Gefühl der Traurigkeit, Hoffnungslosigkeit, Hilflosigkeit, Wertlosigkeit)

Keine	0
Nur auf Befragen geäußert	1
Von Patienten spontan geäußert	2
Aus dem Verhalten zu erkennen (z.B. Gesichtsausdruck, Körperhaltung, Stimme, Neigung zum Weinen)	3
Patient drückt FAST AUSSCHLIESSLICH diese Gefühlszustände in seiner verbalen und nicht verbalen Kommunikation aus	4

2. Schuldgefühle

Keine	0
Selbstvorwürfe, glaubt Mitmenschen enttäuscht zu haben	1
Schuldgefühle oder Grübeln über frühere Fehler und ,,Sünden"	2
Jetzige Krankheit wird als Strafe gewertet, Versündigungswahn	3
Anklagende oder bedrohende akustische oder optische Halluzinationen	4

3. Suizid

Keiner	0
Lebensüberdruß	1
Todeswunsch, denkt an den eigenen Tod	2
Suizidgedanken oder entsprechendes Verhalten	3
Suizidversuche (jeder ernste Versuch = 4)	4

4. Einschlafstörung

Keine	0
Gelegentliche Einschlafstörung (mehr als 1/2 Stunde)	1
Regelmäßige Einschlafstörung	2

5.Durchschlafstörung

Keine	0
Patient klagt über unruhigen oder gestörten Schlaf	1
Nächtliches Aufwachen bzw. Aufstehen (falls nicht nur zur Harn- oder Stuhlentleerung	2

6. Schlafstörungen am Morgen

Keine	0
Vorzeitiges Erwachen, aber nochmaliges Einschlafen	1
Vorzeitiges Erwachen ohne nochmaliges Einschlafen	2

7. Arbeit und sonstige Tätigkeiten

Keine Beeinträchtigung	0
Hält sich für leistungsunfähig, erschöpft oder schlapp bei seinen Tätigkeiten (Arbeit oder Hobbies) oder fühlt sich entsprechend.	1
Verlust des Interesses an seinen Tätigkeiten (Arbeit oder Hobbies), muß sich dazu zwingen. Sagt das selbst oder läßt es durch Lustlosigkeit, Entscheidungslosigkeit und sprunghafte Entschlußänderungen erkennen.	2
Wendet weniger Zeit für seine Tätigkeiten auf oder leistet weniger. Bei stationärer Behandlung Ziffer 3 ankreuzen, wenn der Patient weniger als 3 Stunden an Tätigkeiten teilnimmt. Ausgenommen Hausarbeiten auf der Station.	3
Hat wegen der jetzigen Krankheit mit der Arbeit aufgehört. Bei stationärer Behandlung ist Ziffer 4 anzukreuzen, falls der Patient an keinen Tätigkeiten teilnimmt, mit Ausnahme der Hausarbeit auf der Station, oder wenn der Patient die Hausarbeit nur unter Mithilfe leisten kann.	4

8. Depressive Hemmung (Verlangsamung von Denken und Sprache; Konzentrationsschwäche, reduzierte Motorik)

Sprache und Denken normal	0
Geringe Verlangsamung bei der Exploration	1
Deutliche Verlangsamung bei der Exploration	2
Exploration schwierig	3
Ausgeprägter Stupor	4

9. Erregung

Keine	0
Zappeligkeit	1
Spielen mit den Fingern, Haaren usw.	2
Hin- und herlaufen, nicht still sitzen können	3
Händeringen, Nägelbeißen, Haareraufen, Lippenbeißen usw.	4

10. Angst - psychisch

Keine Schwierigkeit	0
Subjektive Spannung und Reizbarkeit	1
Sorgt sich um Nichtigkeiten	2
Besorgte Grundhaltung, die sich im Gesichtsausdruck und in der Sprechweise äußert	3
Ängste werden spontan vorgebracht	4

11. Angst - somatisch
Körperliche Begleiterscheinungen der Angst wie: Gastrointestinale (Mundtrockenheit, Winde, Verdauungsstörungen, Durchfall, Krämpfe, Aufstoßen) - Kardiovaskuläre (Herzklopfen, Kopfschmerzen) - Respiratorische (Hyperventilation, Seufzen) - Pollakisurie - Schwitzen

Keine	0
Geringe	1
Mäßige	2
Starke	3
Extreme (Patient ist handlungsunfähig)	4

12. Körperliche Symptome - gastrointestinale

Keine	0
Appetitmangel, ißt aber ohne Zuspruch. Schweregefühle im Abdomen	1
Muß zum Essen angehalten werden. Verlangt oder benötigt Abführmittel oder andere Magen- Darmpräparate	2

13. Körperliche Symptome - allgemeine

Keine	0
Schweregefühl in Gliedern, Rücken oder Kopf. Rücken-, Kopf- oder Muskelschmerzen. Verlust der Tatkraft, Erschöpfbarkeit	1
Bei jeder deutlichen Ausprägung eines Symptoms 2 ankreuzen	2

14. Genitalsymptome wie etwa: Libidoverlust, Menstruationsstörungen

Keine	0
Geringe	1
Starke	2

15. Hypochondrie

Keine	0
Verstärkte Selbstbeobachtung	1
Ganz in Anspruch genommen durch Sorgen um die eigene Gesundheit	2
Zahlreiche Klagen, verlangt Hilfe etc.	3
Hypochondrische Wahnvorstellungen	4

16. Gewichtsverlust (entweder a oder b ankreuzen)

a. Aus Anamnese

Kein Gewichtsverlust	0
Gewichtsverlust wahrscheinlich in Zusammenhang mit jetziger Krankheit	1
Sicherer Gewichtsverlust laut Patient	2

b. Nach wöchentlichem Wiegen in der Klinik, wenn Gewichtsverlust

Weniger als 0,5 kg/ Woche	0
Mehr als 0,5 kg/ Woche	1
Mehr als 1 kg/ Woche	2

17. Krankheitseinsicht

Patient erkennt, daß er depressiv und krank ist	0
Räumt Krankheit ein, führt sie aber auf schlechte Ernährung, Klima, Überarbeitung, Virus, Ruhebedürfnis etc. zurück	1
Leugnet Krankheit ab	2

18. Tagesschwankungen

a. Geben Sie an, ob die Symptome schlimmer am Morgen oder am Abend sind. Sofern KEINE Tagesschwankungen auftreten, ist 0 (= keine Tagesschwankungen) anzukreuzen.

Keine Tagesschwankungen	0
Symptome schlimmer am Morgen	1
Symptome schlimmer am Abend	2

b. Wenn es Schwankungen gibt, geben Sie die Stärke der SCHWANKUNGEN an. Falls es KEINE gibt, kreuzen Sie 0 (= keine) an.

Keine	0
Gering	1
Stark	2

19. Depersonalisation, Derealisation wie etwa: Unwirklichkeitsgefühle, nihilistische Ideen

Keine	0
Gering	1
Mäßig	2
Stark	3
Extrem (Patient ist handlungsunfähig)	4

20. Paranoide Symptome

Keine	0
Mißtrauisch	1
Beziehungsideen	2
Beziehungs- und Verfolgungswahn	3

21. Zwangssymptome

Keine	0
Gering	1
Stark	2

Bitte prüfen Sie, ob Sie alle Feststellungen zutreffend beantwortet haben!

Score 1 ☐☐

B. MRI QUESTIONNAIRE

Bitte lesen und beantworten Sie alle folgenden Fragen sorgfältig. Für eine sehr kleine Anzahl von Menschen kann Magnetresonanztomografie das Wohlbefinden beeinträchtigen oder eine Bedrohung der Gesundheit oder sogar des Lebens bedeuten. Diese Fragen sollen sicherstellen, dass diese Risiken nicht auf Sie zutreffen.

1. Sind Sie Träger eines Herzschrittmachers, Defibrillators oder einer künstlichen Herzklappe?	JA/NEIN
2. Haben Sie Aneurismaclips, Shunts, Stents, oder ein Cochleaimplantat in Ihrem Körper?	JA/NEIN
3. Hatten Sie jemals Metallsplitter in Ihren Augen oder irgendeinem anderen Körperteil?	JA/NEIN
4. Tragen Sie irgendwelche Metallimplantate, außer Zahnfüllungen oder Kronen, wie z.B. ein künstliches Hüftgelenk, eine Wirbelsäulenfixierung, ein Penisimplantat, ein Ohrimplantat, ein künstliches Auge, eine Augenlidfeder, ein, durch einen Magneten gehaltenes Gerät oder eine Knochenschiene?	JA/NEIN
5. Tragen Sie eine metallhaltige Zahnschiene, Klammer oder Zahnimplantate?	JA/NEIN
6. Tragen Sie ein Hörgerät?	JA/NEIN
7. Tragen Sie irgendwelche Medikamentenpflaster auf Ihrer Haut oder haben Sie eine implantierte Medikamentenpumpe (z.B. Insulin, Chemotherapie, Schmerzmedikation)?	JA/NEIN
8. Hatten Sie jemals eines der Folgenden: Epilepsie, Diabetes, Problem mit der Regelung der Körpertemperatur?	JA/NEIN
9. Haben Sie Kreislauf- oder Atembeschwerden?	JA/NEIN
10. Leiden Sie unter Platzangst?	JA/NEIN
11. Haben Sie Rücken oder Nackenschmerzen?	JA/NEIN
12. Haben Sie irgendwelche Tätowierungen, permanentes Make-up oder Piercings?	JA/NEIN
13. Haben Sie irgendwelchen Schmuck an sich?	JA/NEIN
14. Nehmen Sie z.Zt. irgendwelche Medikamente ein?	JA/NEIN
15. Tragen Sie eine Perücke?	JA/NEIN
16. Wurden Sie mit Clips sterilisiert?	JA/NEIN

Sie haben das Recht, dieses Screening abzubrechen und an der Studie nicht teilzunehmen, wenn Sie diese Fragen als zu aufdringlich oder als in irgendeiner Weise unangemessen empfinden. Ihre Informationen würden dann sofort vernichtet. Falls Sie teilnehmen, werden Ihre Informationen streng vertraulich behandelt und sicher aufbewahrt.
Hatten Sie jemals eine MRT-Untersuchung, bei der es Probleme gab?
Falls ja, bitte beschreiben Sie:

_____
_____
_____

Unzutreffendes streichen

Instruktionen für die/den Patientin/-en

1. Verwenden Sie unbedingt die Ohrstöpsel, die wir Ihnen zur Verfügung stellen.
   Die MRT Untersuchung erzeugt hohe Geräuschpegel, die ohne Schutz Ihr Gehör schädigen können.
2. Entfernen Sie jeglichen Schmuck (z.B. Halsketten, Ringe, Piercings, etc.)
3. Entfernen Sie alle Haarnadeln, -spangen und Clips.
4. Entfernen Sie alle Zahnprothesen, falsche Zähne und Zahnplatten.
5. Entfernen Sie Ihr Hörgerät
6. Legen Sie Ihre Brille ab
7. Legen Sie Ihr(e/n) Uhr, Pager, Handy, Kreditkarte oder andere Karten mit Magnetstreifen ab.
8. Legen Sie Kleidungsstücke mit Metallknöpfen oder -reißverschlüssen ab.

Ich bestätige hiermit, dass die obigen Informationen nach meinem besten Wissen korrekt sind. Ich habe den gesamten Inhalt dieses Formulars gelesen und verstanden und hatte die Möglichkeit, Fragen zu den Informationen auf diesem Formular zu stellen.

Datum: _____

Unterschrift Patient/in: _____

Unterschrift Operator: _____

Name Operator: _____
(Druckbuchstaben)

C. MRI PREPROCESSING PIPELINE

The following boilerplate text was generated automatically by fMRIprep with the express intention that it should be copied and pasted into manuscripts unchanged by users:

```
Results included in this manuscript come from preprocessing
performed using *fMRIPrep* 20.2.1 (@fmriprep1; @fmriprep2;
RRID:SCR_016216), which is based on *Nipype* 1.5.1 (@nipype1;
@nipype2; RRID:SCR_002502).

Anatomical data preprocessing:

A total of 1 T1-weighted (T1w) images were found within the input
BIDS dataset.The T1-weighted (T1w) image was corrected for
intensity non-uniformity (INU) with `N4BiasFieldCorrection`
[@n4], distributed with ANTs 2.3.3 [@ants, RRID:SCR_004757], and
used as T1w-reference throughout the workflow. The T1w-reference
was then skull-stripped with a *Nipype* implementation of the
`antsBrainExtraction.sh` workflow (from ANTs), using OASIS30ANTs
as target template. Brain tissue segmentation of cerebrospinal
fluid (CSF), white-matter (WM) and gray-matter (GM) was performed
on the brain-extracted T1w using `fast` [FSL 5.0.9,
RRID:SCR_002823, @fsl_fast]. Brain surfaces were reconstructed
using `recon-all` [FreeSurfer 6.0.1, RRID:SCR_001847,
@fs_reconall], and the brain mask estimated previously was
refined with a custom variation of the method to reconcile ANTs-
derived and FreeSurfer-derived segmentations of the cortical
gray-matter of Mindboggle [RRID:SCR_002438, @mindboggle]. Volume-
based spatial normalization to two standard spaces
(MNI152NLin2009cAsym, MNI152NLin6Asym) was performed through
nonlinear registration with `antsRegistration` (ANTs 2.3.3),
using brain-extracted versions of both T1w reference and the T1w
template. The following templates were selected for spatial
normalization: *ICBM 152 Nonlinear Asymmetrical template version
2009c* [@mni152nlin2009casym, RRID:SCR_008796; TemplateFlow ID:
MNI152NLin2009cAsym], *FSL's MNI ICBM 152 non-linear 6th
Generation Asymmetric Average Brain Stereotaxic Registration
Model* [@mni152nlin6asym, RRID:SCR_002823; TemplateFlow ID:
MNI152NLin6Asym],

Functional data preprocessing:

For each of the 5 BOLD runs found per subject (across all tasks
and sessions), the following preprocessing was performed. First,
a reference volume and its skull-stripped version were generated
using a custom methodology of *fMRIPrep*. A B0-nonuniformity map
(or *fieldmap*) was estimated based on a phase-difference map
calculated with a dual-echo GRE (gradient-recall echo) sequence,
processed with a custom workflow of *SDCFlows* inspired by the
[`epidewarp.fsl`
script](http://www.nmr.mgh.harvard.edu/~greve/fbirn/b0/epidewarp.
fsl) and further improvements in HCP Pipelines [@hcppipelines].
```

```
The *fieldmap* was then co-registered to the target EPI (echo-
planar imaging) reference run and converted to a displacements
field map (amenable to registration tools such as ANTs) with
FSL's `fugue` and other *SDCflows* tools. Based on the estimated
susceptibility distortion, a corrected EPI (echo-planar imaging)
reference was calculated for a more accurate co-registration with
the anatomical reference. The BOLD reference was then co-
registered to the T1w reference using `bbregister` (FreeSurfer)
which implements boundary-based registration [@bbr]. Co-
registration was configured with six degrees of freedom. Head-
motion parameters with respect to the BOLD reference
(transformation matrices, and six corresponding rotation and
translation parameters) are estimated before any spatiotemporal
filtering using `mcflirt` [FSL 5.0.9, @mcflirt]. BOLD runs were
slice-time corrected using `3dTshift` from AFNI 20160207 [@afni,
RRID:SCR_005927]. The BOLD time-series were resampled onto the
following surfaces (FreeSurfer reconstruction nomenclature):
*fsaverage*. The BOLD time-series (including slice-timing
correction when applied) were resampled onto their original,
native space by applying a single, composite transform to correct
for head-motion and susceptibility distortions. These resampled
BOLD time-series will be referred to as *preprocessed BOLD in
original space*, or just *preprocessed BOLD*. The BOLD time-
series were resampled into standard space, generating a
*preprocessed BOLD run in MNI152NLin2009cAsym space*. First, a
reference volume and its skull-stripped version were generated
using a custom methodology of *fMRIPrep*. *Grayordinates* files
[@hcppipelines] containing 91k samples were also generated using
the highest-resolution ``fsaverage`` as intermediate standardized
surface space. Several confounding time-series were calculated
based on the *preprocessed BOLD*: framewise displacement (FD),
DVARS and three region-wise global signals. FD was computed using
two formulations following Power (absolute sum of relative
motions, @power_fd_dvars) and Jenkinson (relative root mean
square displacement between affines, @mcflirt). FD and DVARS are
calculated for each functional run, both using their
implementations in *Nipype* [following the definitions by
@power_fd_dvars]. The three global signals are extracted within
the CSF, the WM, and the whole-brain masks. Additionally, a set
of physiological regressors were extracted to allow for
component-based noise correction [*CompCor*, @compcor]. Principal
components are estimated after high-pass filtering the
*preprocessed BOLD* time-series (using a discrete cosine filter
with 128s cut-off) for the two *CompCor* variants: temporal
(tCompCor) and anatomical (aCompCor). tCompCor components are
then calculated from the top 2% variable voxels within the brain
mask. For aCompCor, three probabilistic masks (CSF, WM and
combined CSF+WM) are generated in anatomical space. The
implementation differs from that of Behzadi et al. in that
instead of eroding the masks by 2 pixels on BOLD space, the
aCompCor masks are subtracted a mask of pixels that likely
contain a volume fraction of GM. This mask is obtained by
dilating a GM mask extracted from the FreeSurfer's *aseg*
segmentation, and it ensures components are not extracted from
voxels containing a minimal fraction of GM. Finally, these masks
are resampled into BOLD space and binarized by thresholding at
0.99 (as in the original implementation). Components are also
```

```
calculated separately within the WM and CSF masks. For each
CompCor decomposition, the *k* components with the largest
singular values are retained, such that the retained components'
time series are sufficient to explain 50 percent of variance
across the nuisance mask (CSF, WM, combined, or temporal). The
remaining components are dropped from consideration. The head-
motion estimates calculated in the correction step were also
placed within the corresponding confounds file. The confound time
series derived from head motion estimates and global signals were
expanded with the inclusion of temporal derivatives and quadratic
terms for each [@confounds_satterthwaite_2013]. Frames that
exceeded a threshold of 0.5 mm FD or 1.5 standardised DVARS were
annotated as motion outliers. All resamplings can be performed
with *a single interpolation step* by composing all the pertinent
transformations (i.e. head-motion transform matrices,
susceptibility distortion correction when available, and co-
registrations to anatomical and output spaces). Gridded
(volumetric) resamplings were performed using
`antsApplyTransforms` (ANTs), configured with Lanczos
interpolation to minimize the smoothing effects of other kernels
[@lanczos]. Non-gridded (surface) resamplings were performed
using `mri_vol2surf` (FreeSurfer).

Many internal operations of *fMRIPrep* use *Nilearn* 0.6.2
[@nilearn, RRID:SCR_001362], mostly within the functional
processing workflow. For more details of the pipeline, see [the
section corresponding to workflows in *fMRIPrep*'s
documentation](https://fmriprep.readthedocs.io/en/latest/workflow
s.html "FMRIPrep's documentation").

### Copyright Waiver

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fMRIPrep with the express intention that users should copy and
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the[CC0](https://creativecommons.org/publicdomain/zero/1.0/)
license.
```

D. HAMD-21 SCORE FOR ALL PATIENTS

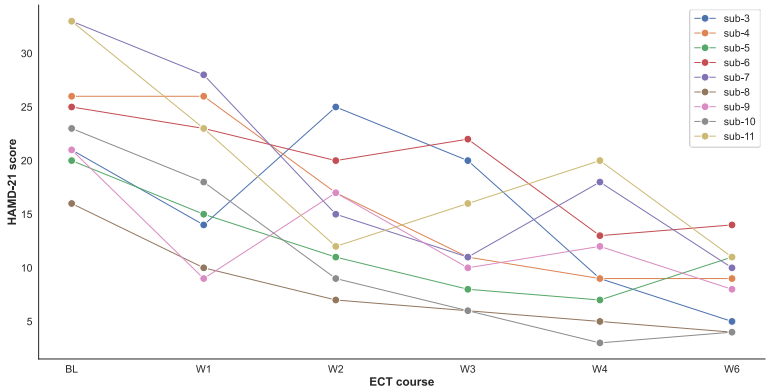

Figure 34: Hamilton score (HAMD-21) of all patients (n=9) across ECT course: from baseline (BL) to week 6 (W6).

E. CORRELATION BDNF LEVELS AND HAMD-21 SCORE IN MITDEP GROUP

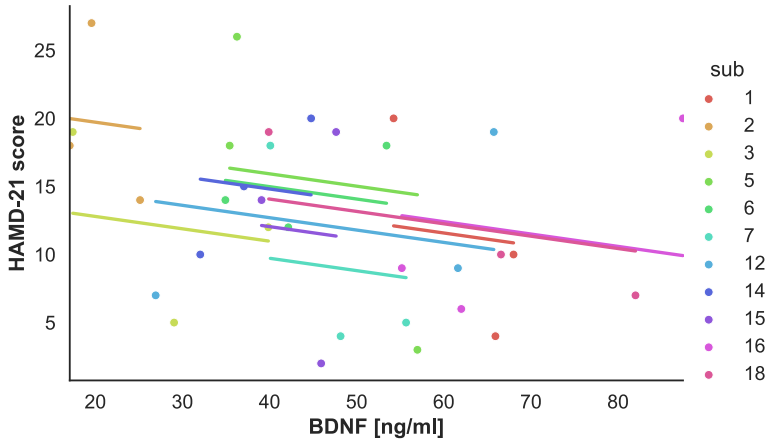

Figure 35: Repeated measures correlation (rmcorr) between BDNF levels and HAMD-21 scores in the treatment as usual group (MITDEP). BDNF does not significantly correlate with HAMD-21 scores. Colored lines represent regression lines for each patient during the six weeks.

F. STATISTICS OF CYTOKINES LEVELS CHANGES DURING ECT

Cytokine	F value	df	p value
IFN-γ	1.317	2.32	0.299
IL-1β	1.001	1.01	0.357
IL-2	1.552	2.24	0.239
IL-4	3.749	5	0.011
IL-6	1.357	1.97	0.286
IL-8	2.025	5	0.096
IL-10	0.737	1.31	0.447
TNF-α	4.013	5	0.005

G. GRAPH MEASURES STATISTICS

Measure	Node	Side	Timepoints	Mean	90% CI		t value	df	p value
					lower	upper			
Degree	Inferior frontal sulcus	L	BL	21.8889	11.2001	32.5777	3.703	8	0.0060
			W2	44.7778	25.6783	63.8772			
	Area 31a	L	BL	33.8889	11.9970	55.7807	3.390	8	0.0095
			W4	75.8889	37.4764	114.3013			
Betweenness centrality	Ventral intraparietal complex	R	BL	0.00494	0.00269	0.00718	-4.523	8	0.0019
			W1	0.00251	0.00068	0.00435			
	Area posterior 47r	R	BL	0.00063	0.00033	0.00093	3.490	8	0.0082
			W2	0.00269	0.00155	0.00383			
	Medial belt complex	R	BL	0.00378	0.00212	0.00543	-3.375	8	0.0097
			W2	0.00134	0.00065	0.00203			
Efficiency	Primary visual cortex	L	BL	0.5020	0.4273	0.5766	4.272	8	0.0027
			W1	0.5783	0.4881	0.6685			
	Third visual area	L	BL	0.4999	0.4539	0.5458	4.128	8	0.0033
			W1	0.5646	0.4991	0.6302			
	Area 23d	L	BL	0.4164	0.3520	0.4807	3.469	8	0.0085
			W1	0.4968	0.4283	0.5653			
	Area 8 c	L	BL	0.4461	0.3989	0.4933	3.546	8	0.0075
			W1	0.5000	0.4524	0.5476			
	Area posterior 9-46v	L	BL	0.4142	0.3767	0.4518	3.581	8	0.0071
			W1	0.4530	0.4115	0.4945			
	Para Belt Complex	L	BL	0.4701	0.3959	0.5444	3.460	8	0.0086
			W1	0.5490	0.4624	0.6357			
	Lateral belt complex	L	BL	0.4569	0.3789	0.5349	4.388	8	0.0023
			W1	0.5219	0.4395	0.6043			
	Area STSv posterior	L	BL	0.3889	0.3420	0.4358	3.902	8	0.0045
			W3	0.4780	0.4118	0.5441			
	Area 13 l	R	BL	0.3148	0.2130	0.4166	4.394	8	0.0023
			W4	0.4391	0.3260	0.5522			

H. GRAPH MEASURES AFFECTED BRAIN REGIONS

		Degree	Betweenness Centrality	Efficiency
BL-W1	↑			L_Primary visual cortex, L_Third visual area, L_Area 23d, L_Area 8 c, L_Area posterior 9-46v, L_ Para Belt Complex
	↓		R_Ventral intraparietal complex	
BL-W2	↑	L_Inferior frontal sulcus	R_Area posterior 47r	
	↓		R_ Medial belt complex	
BL-W3	↑			L_ Area STSv posterior
	↓			
BL-W4	↑	L_Area 31a		R_ Area 13 l
	↓			

I. REGIONAL HOMOGENEITY AND fALFF AFFECTED BRAIN REGIONS

		ReHo	fALFF
BL-W1	↑	L_PGi = parietal G inferior, TPOJ = L_Temporo-parietal-occipital junction, R_DLPFC	L_ TE1a, TE2a = area 1 and 2 lateral temporal cortex, L_p47r = posterior inferior frontal, 9-46d = dorsolateral prefrontal cortex
	↓	Parahippocampal area	R_V3B = dorsal stream visual, R_POS1 = parietal occipital sulcus area 1
BL-W2	↑	L_DLPFC, MIP = medial intra parietal area, IP0 = area intra parietal zero, AIP = anterior intraparietal, FST = fundal superior temporal area, LIPv, LIPd = lateral intraparietal area ventral and dorsal	
	↓		SCEF = supplementary and cingulate eye field, 24dv = ventral area 24, 23c = area 23, 24dd = dorsal area 24 d, 6mp = area 6mp, 6ma = area 6m anterior 24pr = area 24 prime, 9p = area 9 posterior dorsolateral prefrontal cortex
BL-W3	↑	POS1 = parietal occipital sulcus area 1, AIP = anterior intraparietal, PGi = parietal G inferior, MST = medial superior temporal area, area s32 = anterior cingulate and medial prefrontal cortex, IFSp = inferior frontal cortex, R_LIPd = lateral intraparietal area dorsal	L_a47r = anterior inferior frontal, R_p47r = posterior inferior frontal, L_PFm = area PFm complex, L_PGs = area parietal G superior, L_PGi = area parietal G inferior, L_ TE2a = area 2 lateral temporal
	↓		R_6ma = area 6m anterior
BL-W4	↑	p47r = area 47 anterior inferior frontal, a9-46v = anterior dorsolateral prefrontal, a10p = area anterior 10 orbital and polar frontal cortex, AIP = anterior intraparietal, PEF = premotor eye field, PFOP = opercular, TPOJ2 = Temporo-parietal-occipital junction, MIP = medial intra parietal area, TE1m = lateral temporal, R_ MST = medial superior temporal area, FST = fundal superior temporal area, IP1 = intraparietal 1, area 11l = orbital and polar frontal cortex	
	↓		R_23c = area 23, R_6mp = area 6mp, R_p32pr = area p32_prime, R_a24pr = area 24 prime, R_sfl = superior frontal language area, R_7AL = lateral area 7A