Investigating the Effects of the Lithium-Mimetic Drug, Ebselen, on Emotional Processing and Brain Neurochemistry in Patients with Medication-Resistant Depression



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Abstract

Background: Inadequate responses to standard antidepressant treatment is a challenging condition with complicated management. Augmentation with lithium is thought to be effective, but adverse effects limit its widespread use. Ebselen is a lithium-mimetic drug with similar mechanisms of action to lithium, in particular an ability to inhibit the enzyme, inositol monophosphatase (IMPase). Studies in healthy volunteers found that ebselen has antidepressant potential as assessed using emotional processing and is well tolerated.

Objective: To investigate the effects of seven days' ebselen treatment on emotional processing, brain neurochemistry, and resting-state functional connectivity in a clinical population of patients with medication-resistant depression.

Method: We conducted a double-blind, placebo-controlled, parallel-design study and included depressed patients aged 18–70 who were not responsive to current antidepressant therapy. Eligible participants were randomised to receive either 600 mg ebselen or matching placebo twice daily for seven days. Assessments of emotional processing, clinical measures of depression and anxiety, brain neurochemistry, and resting-state functional connectivity, were made at baseline and after seven days of treatment using the Emotional Testing Battery, validated questionnaires, magnetic resonance spectroscopy, and resting state magnetic resonance imaging, respectively.

Results: Compared to placebo, ebselen treatment had no effect on any aspect of emotional processing, except for beta fear. No significant differences were observed between treatment groups in clinical measures of anxiety and depression. Ebselen significantly reduced myo-inositol and glx (glutamate + glutamine) levels in the anterior cingulate cortex (ACC). No significant differences were observed between groups in changes in resting-state functional connectivity using the pregenual ACC as a seed region. Also, a group Independent Component Analysis found no significant differences between groups.

Conclusions: Ebselen, as an add-on treatment, did not alter emotional processing in clinically depressed patients unresponsive to their current antidepressant treatment. Ebselen reduced myo-inositol and glx levels in the ACC, likely due to inhibition of IMPase, glutaminase and glutamate dehydrogenase, respectively.

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Declaration

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Abbreviation

3T: 3 Tesla

5-HT: serotonin

AAL3: Automated Anatomical Labelling atlas 3

ACC: anterior cingulate cortex

ANCOVA: analysis of covariance

ANOVA: analysis of variance

BET: Brain Extraction Tool

BBR: boundary-based registration

CGT: Cambridge Gambling Task

CI: confidence interval

Cr: creatine

CRLB: Cramér-Rao lower bounds

CSF: cerebrospinal fluid

d': d prime

DAG: diacylglycerol

DALYs: Disability-Adjusted Life Years

DAN: dorsal attention network

DMN: default mode network

DOI: 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane

DRC: dynamic reaction cell

DSM-5: Diagnostic and Statistical Manual-5

ECAT: Emotional Categorisation Task

EMEM: Emotional Recognition Memory

EPI: echo-planar imaging

EREC: Emotional Recall

ETB: Emotional Testing Battery

EVs: exploratory variables

FAST: FMRIB's Automated Segmentation Tool

FC: functional connectivity

FDOT: Facial Dot-Probe Task

FERT: Facial Expression Recognition Task

FIX: FMRIB's ICA-based Xnoiseifier

FLIRT: FMRIB's Linear Image Registration Tool

df: degree of freedom

FoV: Field of View

FPN: frontoparietal network

FWHM: full-width at half-height

GLM: general linear model

GM: grey matter

GAD-7: 7-item generalized anxiety disorder

GDH: glutamate dehydrogenase

Glu: glutamate

Glx: combined glutamate and glutamine

HAM-D: Hamilton Depression Rating Scale

HC: healthy controls

ICA: independent component analysis

ICP-MS: inductively coupled plasma mass spectroscopy

IMPase: inositol monophosphatase

FMRIB: Functional MRI of the brain

LCModel: Linear Combination of Model Spectra

MADRS: Montgomery-Asberg Depression Rating Scale

MAO: monoamine oxidase

MAOI: monoamine oxidase inhibitor

MELODIC: Multivariate Exploratory Linear Optimized Decomposition into Independent Components

MDD: major depressive disorder

mGluR5: metabotropic glutamate subtype-5 receptor

MNI: Montreal Neurological Institute

MPRAGE: Magnetization Prepared - RApid Gradient Echo

MRD: medication-resistant depression

MRI: magnetic resonance imaging

MRS: magnetic resonance spectroscopy

NAA: N-acetyl aspartate

STAR*D: Sequenced Treatment Alternatives to Relieve Depression

Ins: inositol

IP_3: inositol triphosphate

PI: phosphoinositide

PIP_2: phosphatidylinositol bisphosphate

Ppm: parts per million (MRS scale)

QIDS-SR-16: 16-item Quick Inventory of Depressive Symptomatology–Self-Rated

RSNs: resting state networks

RT: reaction time

SCID-5: Structured Clinical Interview for DSM-5

SEM: standard error of mean

SD: standard deviation

semi-LASER: semi-Localization by Adiabatic Selective Refocussing sequence

SNR: signal-to-noise

SSRIs: selective serotonin re-uptake inhibitors

SNRIs: selective serotonin-norepinephrine re-uptake inhibitors

TCA: tricyclic antidepressants

T_E: echo time

NHS: National Health Service

NRES: National Research Ethics Service Committee

PET: positron emission tomography

TNR: true negative ratio

TPR: true positive ratio

T_R: repetition time

TRD: treatment-resistant depression

VAPOR: variable power radiofrequency pulses with optimized relaxation delay

VBM: voxel-based morphometry

WM: white matter

CHAPTER 1

1. Introduction

Major depressive disorder (MDD) is one of the most common psychiatric disorders affecting more than 250 million people globally (Santomauro et al., 2021). Treatment outcomes with conventional antidepressant medication are hard to predict, with striking response variability between individual patients (Cuijpers et al., 2020). Nevertheless, pharmacological intervention remains a primary treatment option, particularly in major depression of moderate and greater severity.

Selective serotonin re-uptake inhibitors (SSRIs) are first line medication in patients with major depression. Although moderately effective, about one third of depressed patients treated with SSRIs experience suboptimal response despite adequate treatment duration and dosing (Pigott et al., 2023). After two unsuccessful courses of antidepressant monotherapy, augmentation with another drug class, such as atypical antipsychotic drugs or lithium, is a possible strategy for depressed patients with an inadequate response to antidepressants (Zhou et al., 2015).

Lithium augmentation is a useful option given its demonstrated effectiveness as 'add-on' treatment in controlled trials in depressed patients as well as its likely ability to reduce severe depressive relapse, suicide, and all-cause mortality in patients with unipolar depression (Cipriani et al., 2013; Nelson et al., 2014; Tiihonen et al., 2017). However, the adverse effects of lithium, its narrow therapeutic index, and the need for regular blood testing prevent its wide usage. Therefore, an innovative and helpful solution would be to discover a pharmacological agent with a similar action and efficacy as lithium but with fewer adverse effects.

1.1 Research background

Lithium has important potential as an augmenting agent in unipolar depression as it possesses additional protection against depressive relapse and suicide (Cipriani et al., 2013; Lambrichts et al., 2021; Tiihonen et al., 2017). Inositol monophosphatase (IMPase) inhibition is one of the molecular targets postulated for lithium's anti-depressant and mood stabilising effects (Berridge et al., 1989b). Inositol in the brain is synthesized via several pathways, including phosphoinositide (PI) recycling. IMPase is an essential enzyme in the PI-recycling pathway, which generates inositol from inositol monophosphate (Chhetri, 2019). Clinical and preclinical studies reported a significant reduction in the brain's myo-inositol levels after lithium treatment, potentially due to IMPase inhibition (Berridge et al., 1989b; Davanzo et al., 2001; Moore et al., 1999).

Similarly, in preclinical work, the antioxidant drug, ebselen, has been discovered to block IMPase and lower brain inositol levels (Singh et al., 2013). Ebselen is therefore a potential lithium-mimetic agent; it is also well tolerated in humans with comparable side effects to placebo (Masaki, Sharpley, Cooper, et al., 2016; Sharpley et al., 2020; Singh et al., 2016). In animal models, ebselen reproduces many of the pharmacological effects of lithium, which have been associated with its antidepressant action (Antoniadou et al., 2015; Antoniadou et al., 2018).

Previous studies in healthy volunteers indicate the significant effects of ebselen in reducing myo-inositol levels in anterior cingulate cortex (ACC) and producing positive biases in emotion processing (Masaki, Sharpley, Cooper, et al., 2016; Masaki, Sharpley, Godlewska, et al., 2016). The latter effect has been proposed as a neuropsychological marker of potential antidepressant action (Harmer et al., 2004; Ramli et al., 2022).

Therefore, a key study in the thesis aimed to replicate these findings in a clinical population of depressed patients unresponsive to antidepressant medications. Patients with an inadequate response to at least one antidepressant treatment given for at least four weeks at therapeutic doses, defined as patients with medication-resistant depression (MRD), were included. We decided not to restrict participant inclusion to patients who met formal criteria for treatment-resistant depression (i.e. that is failure to respond to two adequate courses of antidepressant medication in the current episode) but to include depressed patients who had failed to respond satisfactorily to a single antidepressant (MRD). This is because the evidence for the efficacy of lithium as an augmentation treatment in the latter group is somewhat stronger than in patients with formal TRD (Strawbridge et al., 2019; Zhou et al., 2015).

The additional provision of a matched healthy control group allowed an investigation of emotional processing in depressed medicated patients in comparison to healthy controls. It is not known whether negative emotional processing bias is 'normalised' in depressed patients by antidepressant medication even where patients have not shown a therapeutic response to medication. However, the fact that the extent of the positive shift in emotional responses in the early phases of antidepressant treatment correlates with subsequent clinical improvement (Tranter et al., 2009) suggests that in treatment-non-responders, negative biases in emotional processing should still be apparent.

1.2 Problem statement

Treatment-resistant depression (TRD) is a challenging condition, affecting around one-third of patients with MDD. The treatment is usually complex and requires a combination of pharmacological and psychological interventions. Lithium is a historically important drug that has been proven to be effective as an augmentation agent in depression with an extended benefit

as an anti-suicidal agent. However, lithium use is restricted due to several adverse effects. Identifying specific drugs of similar benefits but without the adverse effects associated with lithium can be expected to improve treatment outcomes.

1.3 Objectives and hypotheses

1.3.1 General objectives

A. To investigate the effects of ebselen on emotional processing, clinical measures of depression and anxiety, brain neurochemistry, and resting-state functional connectivity in patients with MRD, using a placebo-controlled design.

B. To investigate the emotional processing of depressed patients taking antidepressant medications with inadequate responses (MRD) in comparison to matched healthy controls.

1.3.2 Specific objectives and hypotheses

Table 1.1. Specific objectives and hypotheses.

No	**Specific objectives**	**Hypotheses**
Study 1A (Patients with Medication-Resistant Depression)		
Primary		
Effects of ebselen on emotional processing		
1	To investigate the effects of ebselen relative to placebo in facial expression recognition (accuracy, reaction time (RT),	Ebselen increases the accuracy, misclassification, target sensitivity (d prime), and response bias (beta), but reduces the RT of recognizing positive

	misclassification, and measures of target sensitivity (d prime), and response bias (beta).	(happy, surprise) relative to negative (sad, fear, disgust, anger) facial expressions.
Secondary		
Effects of ebselen on emotional processing		
1	To investigate the effects of ebselen relative to placebo on the emotional categorization (accuracy and RT) of self-referent words.	Ebselen increases the accuracy but reduces the RT of categorising positive relative to negative self-referent words.
2	To investigate the effects of ebselen relative to placebo on attentional vigilance.	Ebselen reduces the time of dot detection when the dots are located near the happy relative to fearful faces.
3	To investigate the effects of ebselen relative to placebo in the recall of self-referent words (familiar and novel).	Ebselen increases the recall of familiar and novel positive more than negative self-referent words.
4	To investigate the effects of ebselen relative to placebo in recognition of previously presented self-referent words (accuracy, RT, misclassification, measures of signal detection (d prime, beta)).	Ebselen increases the accuracy, misclassification, and signal detection (d prime, beta), but reduces the RT of detecting positive relative to negative self-referent words presented before.
Effects of ebselen on clinical measures of anxiety and depression		

1	To investigate the effects of ebselen relative to placebo on depressive symptoms as measured using MADRS and QIDS-SR-16.	Ebselen reduces depressive symptoms as measured using MADRS and QIDS-SR-16.
2	To investigate the effects of ebselen relative to placebo on anxiety symptoms as measured using GAD-7.	Ebselen reduces anxiety symptoms as measured using GAD-7.
Correlation between blood selenium levels (a surrogate marker of ebselen) and changes in clinical measures of depression and anxiety		
1	To investigate the correlation between blood selenium and changes in clinical measures of depression as measured using MADRS and QIDS-SR-16 in MRD patients who received ebselen.	Blood selenium levels are inversely correlated (negative correlation) with changes in clinical measures of depression as measured using MADRS and QIDS-SR-16 in MRD patients who received ebselen.
2	To investigate the correlation between blood selenium and changes in a clinical measure of anxiety as measured using GAD-7 in MRD patients who received ebselen.	Blood selenium levels are inversely correlated with changes in the clinical measure of anxiety as measured using GAD-7 in MRD patients who received ebselen.
Effects of ebselen on brain neurochemistry		

1	To investigate the effects of ebselen relative to placebo on myo-inositol levels in the ACC.	Ebselen reduces the levels of myo-inositol in the ACC.
2	To investigate the effects of ebselen relative to placebo on glx (glutamate + glutamine) levels in the ACC.	Ebselen reduces the levels of glx in the ACC.
3	To investigate the effects of ebselen relative to placebo on glutamate levels in the ACC.	Ebselen reduces the levels of glutamate in the ACC.
Correlation between blood selenium levels (a surrogate marker of ebselen) and brain neurochemistry		
1	To investigate the correlation between blood selenium and changes in brain myo-inositol levels in MRD patients who received ebselen.	Blood selenium levels are inversely correlated with changes in brain myo-inositol levels in MRD patients who received ebselen.
2	To investigate the correlation between blood selenium and changes in brain glx levels in MRD patients who received ebselen.	Blood selenium levels are inversely correlated with changes in brain glx levels in MRD patients who received ebselen.
3	To investigate the correlation between blood selenium and changes in brain glutamate levels	Blood selenium levels are inversely correlated with changes in brain glutamate levels in MRD patients who received ebselen.

	in MRD patients who received ebselen.	
Effects of ebselen on changes in functional connectivity at resting state		
1	To investigate the effects of ebselen relative to placebo on changes in resting-state functional connectivity using pregenual ACC as the seed region with other parts of the brain.	Ebselen increases or decreases changes in resting-state functional connectivity between pregenual ACC and other brain regions.
2	To investigate the effects of ebselen relative to placebo on changes in resting-state functional connectivity of the whole brain.	No hypothesis, as this is a data-driven method.
Correlation between changes in brain neurochemistry and changes in resting-state functional connectivity in ACC		
1	To investigate the correlation between changes in myo-inositol levels in ACC and changes in resting- state functional connectivity between pregenual ACC and other brain regions in MRD patients who received ebselen.	Changes in myo-inositol levels in ACC are inversely correlated with changes in resting-state functional connectivity between pregenual ACC and other brain regions in MRD patients who received ebselen.

2	To investigate the correlation between changes in glx levels in ACC and changes in resting-state functional connectivity between pregenual ACC and other brain regions in MRD patients who received ebselen.	Changes in glx levels in ACC are inversely correlated with changes in resting-state functional connectivity between pregenual ACC and other brain regions in MRD patients who received ebselen.
3	To investigate the correlation between changes in glutamate levels in ACC and changes in resting-state functional connectivity between pregenual ACC and other brain regions in MRD patients who received ebselen.	Changes in glutamate levels in ACC are inversely correlated with changes in resting-state functional connectivity between pregenual ACC and other brain regions in MRD patients who received ebselen.
Study 1B (Patients with MRD vs Healthy controls)		
Primary		
1	To investigate the difference in facial expression recognition (accuracy, RT, misclassification, measures of signal detection (d prime, beta)) between patients with MRD and healthy controls (HC).	Patients with MRD have higher accuracy, misclassification, and signal detection (d prime, beta), but faster RT for recognizing negative (fear, sad, disgust, anger) than positive (happy, surprise) facial expressions than HC.
Secondary		

1	To investigate the difference in the emotional categorization (accuracy and RT) of self-referent words between patients with MRD and HC.	Patients with MRD have higher accuracy and faster RT for categorizing more negative than positive self-referent words than HC.
2	To investigate the difference in the attentional vigilance between patients with MRD and HC.	Patients with MRD detect dots faster when the dots are located near the fearful than happy faces compared to HC.
3	To investigate the difference in the recall of self-referent words (familiar and novel) between patients with MRD and HC.	Patients with MRD recall more familiar and novel negative than positive self-referent words than HC.
4	To investigate the difference in recognition of previously presented self-referent words (accuracy, RT, misclassification, measures of signal detection (d prime, beta)) between patients with MRD and HC.	Patients with MRD have higher accuracy, misclassification, and signal detection (d prime, beta), and faster RT for recognizing previously presented negative than positive self-referent words than HC.

ACC: anterior cingulate cortex; GAD-7: 7-item generalized anxiety disorder; glx: glutamate + glutamine; HC: healthy controls; MADRS: Montgomery-Asberg Depression Rating Scale; MRD: medication-resistant depression; RT: reaction time; QIDS-SR-16: 16-item Quick Inventory of Depressive Symptomatology–Self-Rated.

1.4 Literature review

1.4.1 Major depressive disorder

MDD is one of the most common psychiatric disorders. The estimated global prevalence in the pre-pandemic year 2020 was 193 million people with a disease burden of 39 million Disability-Adjusted Life Years (DALYs). The pandemic COVID-19 contributed to an additional 53 million MDD cases and 11.7 million DALYs globally. The most significant increase was observed in North Africa and the Middle East regions, contributing to a 37% increase from the pre-pandemic years (Santomauro et al., 2021). The increasing prevalence and burden of MDD warrant more effective plans to combat the disorder.

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (American Psychiatric Association, 2013), MDD is characterised by a set of clinical symptoms, with primary symptoms of depressed mood and anhedonia and numerous secondary symptoms related to appetite, sleep, activity, energy, concentration, decision-making, feeling worthlessness or excessive guilt, and suicidal thinking and behaviour.

To make a diagnosis of MDD in DSM-5, one or both of the primary symptoms must present for most of the day and almost every day for at least two weeks. Certain other symptoms, concerning appetite, sleep, activity, energy, concentration or decision-making, feeling worthless or experiencing excessive guilt, should be present nearly every day; suicidal thoughts should be recurrent or at least one suicide attempt. In DSM-5 at least five symptoms including one or two of the primary symptoms, should be present for at least two weeks to qualify for the diagnosis of MDD(American Psychiatric Association, 2013). In addition to the depressive symptom criteria, impairment or distress attributable to symptoms must present in areas of functioning, such as social, occupational, or other critical areas. Also, the cause of the depression must neither be attributable to physical illnesses nor substances. No previous

hypomanic or manic episode should present, and the illness is not better explained by a psychotic disorder (American Psychiatric Association, 2013).

MDD is a heterogenous illness with likely numerous pathophysiologies. The monoamine hypothesis is the most popular theory explaining the cause of MDD. The hypothesis proposes reductions in monoamine (serotonin, norepinephrine, dopamine) neurotransmission in MDD. Potential factors which could lead to diminished monoamine neurotransmission include increased monoamine oxidase activity, increased monoamine reuptake into presynaptic terminals, and diminished sensitivity of post-synaptic monoamine receptors. Monoamine oxidases (MAOs) degrade various neurotransmitters via oxidation. The isoform A of this enzyme (MAO-A) has higher selectivity towards serotonin than MAO-B, while both isoforms (MAO-A and MAO-B) can degrade norepinephrine and dopamine. Increases in MAO activity can cause significant reductions in neurotransmitters in the synaptic cleft, impairing neuron-neuron signal transmission (Jesulola et al., 2018; Yeung et al., 2019). It is also possible that decreased sensitivity of post synaptic monoamine receptors could lead to decreased neurotransmission at affected synapses (Köhler-Forsberg et al., 2023). It has been hypothesised that reduced monoamine neurotransmission leads to numerous MDD symptoms including changes in mood, interest, energy, cognition, motivation, attention, sleep, appetite, and activity (Jesulola et al., 2018).

1.4.2 Standard antidepressants

Based on the monoamine hypothesis, increasing monoamine neurotransmitters in the synaptic cleft can increase overall neurotransmission. Many antidepressants, including SSRIs, serotonin-norepinephrine reuptake inhibitors (SNRIs), and tricyclic antidepressants (TCAs), block specific monoamine reuptake transporters at the presynaptic neurons. SSRIs, such as citalopram and fluoxetine, are commonly used as the first-line treatment for MDD. As the name

suggests, SSRIs selectively inhibit serotonin reuptake via allosteric inhibition. SNRIs, such as venlafaxine and duloxetine, effectively block serotonergic transporters and, to a lesser extent, noradrenergic transporters. Venlafaxine has a high affinity towards the serotonin transporter but is a relatively poor inhibitor of the noradrenaline transporter. In contrast, other SNRIs, including duloxetine and levomilnacipran, have more balanced affinities towards both transporters, although still somewhat favouring the serotonin transporter (Sharma et al., 2023).

TCAs can also inhibit both transporters, but the affinity towards both transporters is diverse, depending on the individual TCA. For instance, clomipramine has a strong affinity towards the serotonin transporter but a weaker affinity towards the noradrenaline transporter. In contrast, desipramine has more affinity towards the noradrenaline transporter. Other than that, TCAs also block histamine and muscarinic receptors. The blockade of histamine and muscarinic receptors contributes more to the adverse effect profile of TCAs, such as sedation and weight gain. There are also a range of anticholinergic effects, making TCAs' safety profile less favourable than SSRIs and SNRIs. Most TCAs are also dangerous in overdose. As a result, SSRIs and SNRIs are generally preferred to TCAs for treating MDD (Sharma et al., 2023).

Another class of antidepressants is MAO inhibitors (MAOIs), which attenuate MAO's effects in degrading monoamines. MAOIs are classified based on their selectivity towards specific MAO isoforms and the reversibility of their inhibition. Tranylcypromine is an irreversible and non-specific MAOI, thus inhibiting both MAO isoforms. Clorgyline and moclobemide are specific to MAO-A, but the former exerts irreversible inhibition. Examples of irreversible MAO-B inhibitors are selegiline and rasagiline, while safinamide is a reversible MAO-B inhibitor. MAOIs with affinity towards MAO-A are more commonly used for treating depression. Although MAO-B exclusive inhibitors are generally used for Parkinson's Disease, the MAO-B inhibitor selegiline, given at sufficient doses, can also be used to treat depression

(Chamberlain et al., 2020; Sharma et al., 2023; Yeung et al., 2019). MAOIs seem to be an appropriate pharmacological means to dampen the proposed overactivity of MAO in depressed patients (Meyer et al., 2006). However, their use is reserved for TRD and atypical depression due to safety reasons and prescribing complexity (patient psychiatric/medical history, drug or food interaction, stock availability).

1.4.3 Treatment-resistant depression

Despite their pharmacological profiles and ability to potentiate monoamine neurotransmission, the effectiveness of antidepressants for MDD is modest. According to the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study, only 26% of MDD patients treated with the first-line SSRI, citalopram, achieved remission, based on the Hamilton Depression Rating Scale. Various treatment strategies employed in step 2, including switching to other SSRIs, SNRIs or cognitive therapy, or the addition of the norepinephrine-dopamine reuptake inhibitor bupropion or cognitive therapy, only contributed an additional 21% remission rate, making up about one-third (33.3%) of the total remission rate (Pigott et al., 2023). TRD is a common term used to describe a situation where MDD patients have experienced at least two treatment failures of antidepressant therapies. A systematic review published in 2020 reported no definite consensus regarding the TRD definition. Conventionally, after two unsuccessful antidepressant treatments (50% response or remission) with adequate durations and doses, MDD patients are classified as having TRD (Gaynes et al., 2020; Voineskos et al., 2020).

1.4.4 Augmentation therapies

The management of TRD is complex. Augmentation therapy is one further treatment strategy after two treatment failures with antidepressant monotherapy, which adds another class of drug to current ineffective antidepressant treatment. Examples of augmenting agents are

atypical antipsychotics and lithium. The atypical antipsychotic agent, quetiapine, has been reported to produce a significant improvement in MDD when used as an add-on therapy to ongoing antidepressant treatment (Bauer et al., 2010). However, higher withdrawal rates from quetiapine treatment due to adverse effects of sedation and somnolence might compromise its effectiveness (Bauer et al., 2010). Another effective augmenting agent that belongs to the atypical antipsychotic group is aripiprazole (Jha & Mathew, 2023). However, associated adverse effects of akathisia, weight gain, insomnia, and somnolence, particularly at higher doses, might hinder patients from taking the medication (Seshadri et al., 2021).

Lithium is another augmenting agent regarded as effective in TRD. Compared to placebo, a recent network meta-analysis study reported that lithium augmentation was associated with a higher response rate (Nuñez et al., 2022). Also, Cipriani and colleagues found that lithium significantly reduced suicide rates with the odds ratio (OR) of 0.13 (95% confidence interval (CI): 0.02–0.76) and all-cause mortality with the OR of 0.36 (95% CI: 0.13–0.98) compared to placebo in patients with unipolar depression. Intriguingly, lithium has a superior efficacy to carbamazepine in mitigating deliberate self-harms (OR of 0.14, 96% CI: 0.02–0.83) (Cipriani et al., 2013). Another systematic review comparing lithium and atypical antipsychotic olanzapine as an augmenting agent in TRD reported that lithium is more cost-effective than olanzapine (Edwards et al., 2013).

The effectiveness of lithium in treating mood disorders might be attributable to various molecular targets. The most established mechanism was previously proposed by Berridge and thus was called ‘Berridge’s inositol depletion hypothesis’. The hypothesis suggests that lithium works by inhibiting enzymes involved in PI recycling, thus reducing inositol synthesis (Berridge et al., 1989a). Inositol-1,4 bisphosphate 1-phosphatase (which catalyses the conversion of inositol-1,4 bisphosphate to inositol-1 phosphate) and IMPase are two enzymes

inhibited by lithium at therapeutic plasma concentrations. Reduced inositol levels lead to a decreased availability of substrate required to produce phosphatidyl (4,5) bisphosphate. Phosphatidyl (4,5) bisphosphate (PIP_2) is a membrane-bound resource essential for producing the second messengers diacylglycerol (DAG) and inositol triphosphate (IP3). PI recycling is utilised by many serotonergic receptors. Upon receptor stimulation, the G-protein attached to the receptor activates phospholipase C, stimulating second messenger productions and downstream pathways (Harwood, 2005; Ramli et al., 2022).

Another molecular target of lithium at its effective plasma therapeutic levels is glycogen synthase kinase-3 (GSK-3), a widely expressed enzyme in the brain. This enzyme can promote neuronal apoptosis by activating an intrinsic apoptotic pathway (Gómez-Sintes et al., 2011). Lithium's inhibitory effects on the GSK-3-pro-apoptotic-signalling pathway may partly explain its neuroprotective effects (Szałach Ł et al., 2023). Further, lithium stimulates glutamate clearance and blocks glutamatergic post-synaptic receptor binding, attenuating the neurotoxicity effects of glutamate (Jones et al., 2022; Szałach Ł et al., 2023). Interestingly, six weeks lithium treatment increases volumes of various cortical and subcortical structures in patients with bipolar disorder, supporting its role in promoting neurogenesis (Jones et al., 2022). Lithium also has immunoregulating effects, potentially through several mechanisms, including the activation of alpha7 nicotinic acetylcholine receptor-mediated cholinergic anti-inflammatory pathway and the regulation of inflammatory (pro- and anti-) cytokines and immune cells (Liu et al., 2023; Szałach Ł et al., 2023).

Despite lithium's effectiveness, its use is challenging due to the risk of toxicity. The therapeutic index of lithium is narrow; therapeutic blood levels range from 0.5 to 1.0 mmol/l, and levels of more than 1.2 mmol/l may be toxic, particularly in prolonged use (Haussmann et al., 2015). Elevated lithium levels above the therapeutic range induce toxic effects, causing a

range of signs and symptoms. Mild lithium toxicity may cause diarrhoea, weakness, mild ataxia, and worsen existing tremor. Vomiting and neurological manifestations (confusion, lethargy, gross tremors, slurred speech) indicate more severe lithium toxicity (Gitlin, 2016). Many factors can contribute to lithium toxicity, including medical illnesses and drug-drug interactions. Physiological changes due to fever, gastrointestinal symptoms, and dehydration, can disturb body fluid balance and increase blood lithium levels. Some drug classes (angiotensin-converting enzyme inhibitors, diuretics, nonsteroidal anti-inflammatory drugs, cyclooxygenase-2 inhibitors) reduce lithium clearance, thus increasing blood lithium levels (Gitlin, 2016). To ensure patient safety, regular blood lithium monitoring, typically weekly, is essential in the early phase of treatment until the blood lithium level is stabilised (Bauer et al., 2014). Once an appropriate dose has been established, lithium blood checks can be reduced to once every three to six months unless there is any clinical evidence of toxicity or a change in concomitant drug treatment.

Other than lithium levels, kidney function tests should be regularly checked, as lithium has been reported to cause nephropathy. Chronic kidney disease with moderate severity (stage III) is the most common form of lithium-induced nephropathy (Nielsen et al., 2018). End-stage renal disease, although less common, can occur with long-term use of lithium. The risk factors of lithium-induced nephropathy are various, including older age, longer treatment duration, and history of lithium toxicity. Kidney function in patients receiving lithium should be monitored every three to six months (Gitlin, 2016).

Hypothyroidism and goitre are more common forms of lithium-induced thyroid disorders than hyperthyroidism. The development of these disorders might be attributable to lithium's inhibitory effects on the thyroid gland – inhibitions of thyroid hormone synthesis and release and iodine trapping. The prevalence of thyroid disorders varies, ranging from 8% to

27% for overt hypothyroidism and up to 23% for subclinical hypothyroidism (elevated thyroid-stimulating hormone with normal range T4 levels) (Gitlin, 2016; Lieber et al., 2020). General guidelines recommend monitoring serum thyroid-stimulating hormone at baseline and every 3–6 months, as thyroid dysregulation typically occurs in the initial phase after the initiation of lithium therapy. Subsequently, less frequent monitoring (6–12 monthly) is recommended (Gitlin, 2016). Lithium-induced hypothyroidism is reversible in about 80% of patients who discontinued lithium (Lieber et al., 2020).

The adverse effects and complicated monitoring requirements of lithium may cause compliance issues, hindering effective treatment. One potential strategy to manage this issue is to develop a drug with similar mechanisms and efficacy as lithium but with fewer adverse effects. This requires the assumption that the adverse effects of lithium are not necessarily linked to its therapeutic mechanism. Ebselen has been reported to have a similar mechanism of action to lithium (IMPase inhibition) with fewer adverse effects. The subsequent passage will describe ebselen in detail.

1.4.5 Ebselen

Ebselen (2-phenyl-1, 2-benzisoselenazol-3(2H)-one) is an organoselenium compound containing one selenium atom (Figure 1.1). Ebselen was initially developed as an antioxidant for treating inflammatory conditions, including arthritis and liver diseases. However, potential toxicity concerning selenium accumulation and minimal effects in the animal model of adjuvant arthritis caused two pharmaceutical companies in North America and Europe to cease further drug development for rheumatic disease. In contrast, a Japanese company, Daiichi, changed the direction of ebselen's development from the long-term treatment of liver diseases to a short-term treatment of acute ischemic and haemorrhagic strokes. A multicentre study

evaluating 286 patients with acute aneurysmal subarachnoid haemorrhages reported no significant difference in the primary treatment outcome of the incidence of delayed ischemic neurological deficits. However, the ebselen group showed a significantly better treatment outcome, evidenced by reduced incidence and extent of low-density brain areas (Saito et al., 1998). Another multicentre study involving 300 patients with acute ischemic stroke reported that the treatment outcome was better in the ebselen group at one month but not at three months. The efficacy report submitted for approval from the Japanese regulatory body failed to receive approval due to insufficient efficacy data (Parnham & Sies, 2013; Yamaguchi et al., 1998).

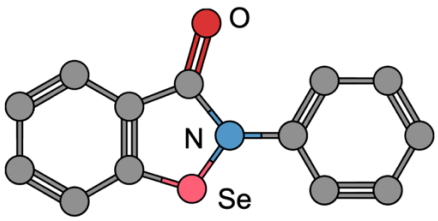


Figure 1.1. Chemical structure of ebselen.

Despite its challenging history in drug development, ebselen remains a drug candidate for repurposing for other indications, including neuropsychiatric illnesses, Covid-19 infection, myocardial infarction, and hearing loss (Antoniadou et al., 2015; Antoniadou et al., 2018; Cheng et al., 2019; Kil et al., 2017; Klann et al., 2020; Lynch & Kil, 2009; Martini et al., 2019; Marzo et al., 2021; Sharpley et al., 2020; Sies & Parnham, 2020; Sun et al., 2021). As noted above, there was early speculation that selenium could be released from ebselen and thereby induce toxicity due to selenium accumulation. However, selenium release from ebselen was found negligible in preclinical and clinical studies (Parnham & Sies, 2013; Ramli et al., 2022).

A multicentre study in many countries, including the US, UK, Korea, Germany, and Japan, is now commencing to assess the efficacy of ebselen in treating Meniere's disease after favourable outcomes of ebselen in improving tinnitus, hearing loss, and word recognition in Phase II studies (Kil et al., 2022). Also, a Phase II clinical trial to assess the safety and efficacy in Covid-19 infected patients with moderate severity is being carried out due to convincing demonstrations of ebselen's ability to inhibit many enzymes involved in Covid-19 virus replication, such as main (M^{pro}) and papain-like (PL^{pro}) proteases, and nsp guanine N7-methyltransferase (Wildner et al., 2023; Zmudzinski et al., 2023).

For drug repurposing in mood disorders, ebselen was initially tested in pre-clinical models. Singh and colleagues expressed human IMPase in bacteria to screen 450 compounds from the National Institutes of Health Clinical Collection to determine potential IMPase inhibitors. The search found ebselen to be an irreversible IMPase inhibitor (IC_{50} 1.5 μM) with a comparable potency to poorly bioavailable IMPase inhibitor, L-690,330 (IC_{50} 0.3 μM), and superior to lithium (IC_{50} 0.8 mM). Using an *ex vivo* model, Singh et al (2013) confirmed the blood-brain barrier penetrability of ebselen, evidenced by reduced IMPase activity in brain homogenate and decreased inositol levels in brain extract. Interestingly, the potency of ebselen to inhibit IMPase is superior to another molecular target (glycogen synthase kinase 3ß) inhibited by lithium at lithium's therapeutic range, confirming ebselen's selectivity to IMPase (Singh et al., 2013).

Ebselen inhibits IMPase by forming a covalent bond with a cysteine residue located away from the active catalytic site (Fenn et al., 2020; Singh et al., 2013). The inhibition reduces inositol pooling for producing PIP_2, thus impairing the PI-signalling pathway (Figure 1.2). The PI-signalling pathway is essential for many serotonergic receptors, and the inhibitory effects of ebselen on PI signalling modify serotonin neurotransmission.

A receptor autoradiography study supported the notion that the effect of ebselen on 5-HT2A receptor-mediated responses occurred at the post-receptor mechanism, as no reduction of 5-HT2A receptor expression was evident (Antoniadou et al., 2018). Behavioural assessments in rodent models confirmed that ebselen (single and repeated doses) attenuated head-twitch response induced by the 5-HT2A receptor agonist 1- (2,5- dimethoxy-4 iodophenyl)-2 aminopropane (DOI), in a way similar to lithium (repeated doses). Ebselen (repeated doses) and lithium (single and repeated doses) also reduced ear-scratch response induced by DOI (Antoniadou et al., 2018; Singh et al., 2013). Interestingly, ebselen also increased 5-hydroxytryptophan and serotonin levels in the presynaptic neurons and potentiated the release of serotonin in the presence of an SSRI (Antoniadou et al., 2018). A possible explanation for this effect is that reduced post-synaptic -5-HT2 receptor sensitivity interrupts a 'long-loop' negative feedback mechanism, stimulating a compensatory increase in pre-synaptic serotonin activity (Boothman et al., 2006).

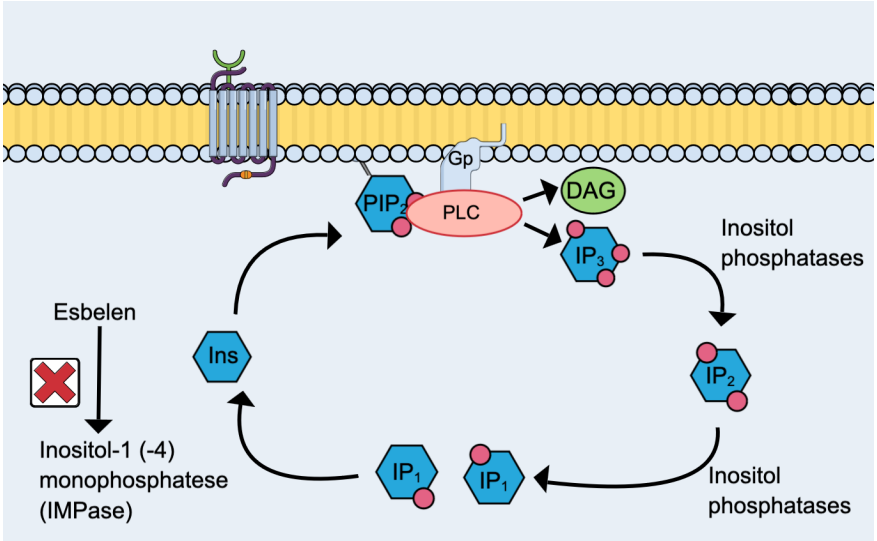


Figure 1.2. Phosphoinositide recycling and the inhibitory effects of ebselen. Adapted from Ramli et al. (2022) and Harwood (2005).

The behavioural changes seen with ebselen coincide with numerous molecular changes in many brain regions, including the prefrontal and cingulate cortices. One molecular change useful to measure neuronal activity is immediate early gene (IEG) expression. IEGs, such as *Arc*, *c-fos,* and *Egr*, encode transcription factors essential for regulating downstream pathways and are often altered in response to specific changes, such as pathological conditions (mental disorders) or pharmacological interventions (antidepressants) (Gallo et al., 2018; Li et al., 2015; Yakout et al., 2021). Ebselen suppresses DOI-induced expression of IEGs in many brain regions, including prefrontal cortex (*Arc*), cingulate cortex (*Arc*, *c-fos*), somatosensory cortices (*c-fos*), caudate nucleus (*Erg2*), and endopiriform cortex (*Erg2*) (Antoniadou et al., 2018). Lithium acts similarly, inhibiting DOI-induced IEGs increase in the cingulate cortex (*Arc*, *c-fos*), somatosensory cortices (*Arc*, *c-fos*), caudate nucleus (*Erg2*), and endopiriform cortex (*Erg2*) (Antoniadou et al., 2015). Ebselen can also diminish 5-HT2C receptor agonist, Ro 60-0175-induced IEGs elevation in the cingulate cortex (*Arc*) and somatosensory cortex (*Arc*, *c-fos*), indicating that ebselen's effects on signal transduction involve 5-HT2C as well as 5-HT2A receptors mechanisms (Antoniadou et al., 2015).

To translate into a clinical application, ebselen was initially tested in healthy volunteers using a surrogate marker of antidepressant potential. Clinical efficacy usually takes a few weeks to appear with most conventional antidepressants, and measuring efficacy is time-consuming and requires extensive funding and commitment from many parties. Thus, a surrogate marker to investigate a drug's antidepressant potential is beneficial before proceeding to Phase II and III clinical trials. Positive biasing of emotional processing is one such marker of antidepressant potential, which appears very early in the course of antidepressant therapy, being apparent after the first dose of antidepressant treatment in both healthy participants and depressed patients (Harmer et al., 2003; Harmer et al., 2009).

A wide range of conventional antidepressants including SSRIs and SNRIs, have been reported to directly affect the processing of emotional information weeks before the onset of obvious clinical therapeutic effects (Harmer et al., 2009; Harmer et al., 2004). The changes appear in various emotional processing domains, promoting a bias shift from negative to positive in the recognition of facial expressions and categorization and memory of self-referent words (Pringle & Harmer, 2015). Also, other studies reported that early positive shifts in emotional processing by antidepressants in depressed patients are predictive of later clinical efficacy (Browning et al., 2019; Tranter et al., 2009). Importantly, two studies investigating the effects of ebselen on emotional processing reported a significant effect of short-term ebselen to increase the accuracy of recognizing happy facial expressions in healthy volunteers (Masaki, Sharpley, Cooper, et al., 2016; Singh et al., 2016).

Interestingly, in MRS studies, ebselen was reported to reduce myo-inositol levels in ACC in healthy volunteers, potentially through a region-specific inhibition of IMPase (Masaki, Sharpley, Godlewska, et al., 2016; Singh et al., 2016). Other incidental findings were significant reductions in glx (a composite MRS measure of glutamate and glutamine), glutamine, and glutamate in the same region. The reductions in these metabolites are postulated to be mediated by ebselen's inhibitory effects on two enzymes, glutamate dehydrogenase and glutaminase, which are both involved in glutamate metabolism. ACC serves many neuropsychological functions, including emotional processing. The neurochemical effects of ebselen in ACC might underpin the mechanistic aspects of ebselen as a potential antidepressant agent.

Another important consideration for developing a lithium-mimetic agent is the safety aspect of lithium treatment. Many studies in healthy volunteers reported comparable side effects between ebselen and placebo (Masaki, Sharpley, Cooper, et al., 2016; Singh et al.,

2016). Ebselen was well tolerated and no dropouts due to adverse effects were reported. Most side effects were mild to moderate in severity for the dose range of 1800–3600 mg over 24 hours and 200–600 mg twice daily for 21 days (Kil et al., 2022; Masaki, Sharpley, Cooper, et al., 2016; Singh et al., 2016). No serious adverse effects were reported in bipolar manic patients receiving either ebselen 600 mg twice daily or matching placebo for three weeks as an add-on therapy. Headache and drowsiness were the most common side effects, but the occurrences were comparable between ebselen and placebo (Sharpley et al., 2020). There were two serious adverse effects in a Phase IIb study involving patients with Meniere's disease; one was classified as ebselen-related (severe liver dysfunction). However, the study drug was continued, and the liver function normalised after treatment ended. Other side effects were mild and moderate (Kil et al., 2022). Similarly, two phase III clinical trials involving patients with acute aneurysmal subarachnoid and ischemic strokes receiving 150 mg twice daily for two weeks reported no significant differences between ebselen and treatment groups in side effects (Saito et al., 1998; Yamaguchi et al., 1998).

As lithium is thought to be effective as an add-on treatment in TRD, it is worth evaluating the potential use of ebselen as an add-on therapy in depressed patients not responsive to standard antidepressant therapy. Given the significant effects of ebselen on emotional processing and brain neurochemistry and good safety profile in healthy volunteers, research in a clinical population is important to confirm ebselen's potential positive effects on surrogate emotional markers on the targeted population before a formal phase 2 clinical trial is conducted.

CHAPTER 2

2. Methodology

2.1 Study 1A

2.1.1 Study design and participants

Study 1A was an experimental medicine, parallel-arm, double-blind, randomised, placebo-controlled trial on patients with MRD to investigate the effects of ebselen on emotional processing, clinical measures of depression and anxiety, brain neurochemistry, and resting-state functional connectivity (Figure 2.1). The study received ethical approval (20/SC/0151) from the National Research Ethics Service Committee (NRES), South-Central Oxford, and was registered at www.clinicaltrials.gov under the US National Institutes of Health (Identifier: NCT05117710).

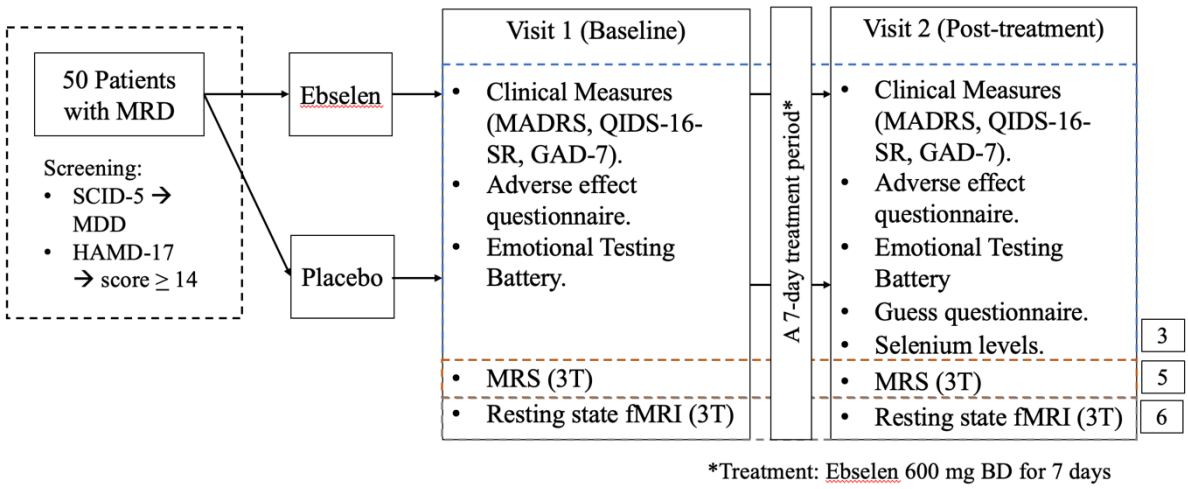


Figure 2.1. Study 1A design.

We screened patients with MDD who had been taking their current antidepressant therapy at therapeutic doses for at least four weeks but were clinically still depressed using the

Structured Clinical Interview for Diagnostic and Statistical Manual (DSM) -5 (SCID-5). We utilised the 17-item Hamilton Depression Rating Scale (HAM-D) to identify patients with depression scores of at least 14. We excluded patients with any history or current bipolar disorder, schizophrenia, or emotionally unstable personality disorder; those who had not responded to standard pharmacological augmentation treatments in the current episode; clinically suicidal; history or ongoing electroconvulsive therapy; history of substance dependence over the past six months; current pregnancy, breastfeeding, or a plan of pregnancy; body mass index outside the range of 18–36; severe claustrophobia and magnetic resonance imaging (MRI) contraindication. Participants who had participated in a similar study involving emotional processing tasks or interventional medication could be included if the previous study had occurred more than three months previously. All participants gave written informed consent before the study.

Eligible participants were randomised in a 1:1 allocation with the sequential assignment of subject numbers as the participants enrolled in the trial. The subject numbers were generated using the computer-generated randomisation algorithm stratified into two variables: sex and number of failed antidepressant treatments (1-2 vs >2 antidepressants).

On the first research visit, participants were interviewed about their depressive symptoms and rated using the Montgomery-Asberg Depression Rating Scale (MADRS). Baseline medication side effects were sought from the participants for comparison with post-treatment side effects. We utilised a study-specific list of adverse effects potentially related to ebselen based on previous studies (nausea, vomiting, dizziness, headache, loss of alertness, drowsiness, and increased appetite) (Masaki, Sharpley, Cooper, et al., 2016; Singh et al., 2016). The severity levels (mild, moderate, severe) were graded at both visits and compared. Also, participants completed self-rated questionnaires (16-item Quick Inventory of Depressive

Symptomatology–Self-Rated (QIDS-SR-16) and 7-item Generalized Anxiety Disorder (GAD-7)) and emotional testing battery (ETB). Magnetic resonance spectroscopy (MRS) was performed to measure brain neurochemistry, followed by functional MRI at resting state to measure functional connectivity. Participants were then given a medication bottle containing either 42 capsules of ebselen (200 mg) or placebo for seven days of treatment. The participants were instructed to take three capsules twice daily, with the last dose taken on the morning of the second study visit. Ebselen and placebo capsules had identical appearances in shape, colour, weight, taste, and smell. Participants and researchers were blinded for the treatment arms.

The same procedures were repeated on the second research visit. Additionally, venous blood samples for measuring the levels of selenium, a surrogate measure for ebselen, were taken. Participants were also asked to guess which treatment (ebselen or placebo) they thought they had received.

The sample size was calculated using the effect size of a previous study, which evaluated the effects of antidepressant treatment given for seven days on emotional processing (Harmer et al., 2004). Using the effect size of 0.4 with a power of 0.9, the required sample size for each treatment arm was 25.

2.1.2 Emotional testing battery (ETB)

Emotional processing was assessed using the ETB, which consists of five different tasks, including Facial Expression Recognition Task (FERT), Emotional Categorisation (ECAT) Task, Facial Dot-Probe Task (FDOT), Emotional Recall (EREC) Task, and Emotional Recognition Memory (EMEM) Task (Harmer et al., 2004; Murphy et al., 2008).

The FERT consisted of a collection of facial expressions of six basic emotions (happy, surprise, anger, disgust, fear, sad) with different intensities, ranging from 0% (neutral) to 100% (full emotion) (Harmer et al., 2004). The participants were required to determine the emotion of each face presented for 500 ms on the computer screen. The accuracy, reaction time, and misclassification for correct choices were recorded. Measures of signal detection, d prime (target sensitivity) and beta (response bias) values were also calculated using the following formula: D prime (d') = 0.5 + ((y - x)(1 + y - x)/4y (1 - x)), $0 < d' < 1$; beta = y(1 - y) - x(1 - x) / y(1 - y) + x(1 - x), $-1 < ß < 1$. X is a false alarm (proportion of incorrect responses), and Y is a hit rate (proportion of correct responses). Higher values of d prime and beta indicate increased accuracy and fewer false alarms, respectively.

The ECAT task consists of a collection of positively- or negatively-valenced words. The participants were required to categorise the words into likeable or dislikeable based on whether they would like or dislike to be referred to by them when they overheard a conversation about that (self-referent). The accuracy and reaction time were recorded.

The third task conducted was the FDOT. In the FDOT, two dots in vertical or horizontal orientation were presented on the screen following the disappearance of two faces (emotional and neutral). The attentional vigilance score was calculated by subtracting the mean reaction time (RT) from the trials where the dots appeared in the place of the emotional face (happy or fear) from when the dots appeared in the opposite position to the emotional face (neutral). After the FDOT, the participants were asked to write as many words as possible from the ECAT task that they could recall within four minutes in this fourth (EREC) task. The number of correctly recalled words (positive and negative) was recorded. Also, incorrectly recalled words were categorized into positive or negative valence words, and the number in each category was

counted. The valences (positive, negative, or neutral) of the incorrectly recalled words were decided based on the consensus of three people.

The last task was the EMEM task in which positively- and negatively-valenced self-referent words from the ECAT and novel distractor words were presented. The participants were required to determine whether they had already seen the word (responded as yes) or not (responded as no). The accuracy, reaction time, and misclassification were calculated. D prime and beta values were also calculated using the formula stated above.

2.1.3 Magnetic resonance spectroscopy

Measurement of myo-inositol, glx, and glutamate levels in the ACC were conducted using 3 Tesla (3T) Siemens MAGNETOM scanner (Siemens, Erlangen, Germany) with a 32-channel receive array head coil at Oxford Centre for Brain Human Activity (OBHA).

A T1-Magnetization Prepared - RApid Gradient Echo (MPRAGE) image (1 mm isotropic, repetition time (T_R) = 1900 ms, echo time (T_E) = 3.96 ms, flip angle = 8°, slice thickness = 1 mm, 192 slices, field-of-view (FoV) read = 256 mm) was acquired first for the voxel placement (Brant-Zawadzki et al., 1992). An 8-ml (2x2x2 cm) voxel was manually placed in the ACC of the T1-MPRAGE image. The screenshots of ACC on three different planes of each participant were taken for the first study visit to ensure placement reproducibility in the second visit. The first- and second-order shims were adjusted using gradient echo shimming to reduce inhomogeneity (Shah, 2009). The spectra were acquired using the semi-Localization by Adiabatic Selective Refocussing (semi-LASER) sequence with variable power radiofrequency pulses with optimized relaxation delay (VAPOR) (T_E=28 ms, T_R=3 s, 128 averages) (Deelchand et al., 2015). Removing residual eddy current effects and reconstructing

the phased array spectra were conducted using the unsuppressed water spectra obtained from the same voxel (Natt et al., 2005).

The paired spectra (both visits) in each participant were visually inspected to detect any significant differences between visits and deviation from the expected spectra. The quantification of myo-inositol, glx, and glutamate concentrations with total creatine and phosphocreatine as reference was conducted using the Linear Combination of Model Spectra (LCModel) in MATLAB 2016 (Provencher, 1993).

The Brain Extraction Tool (BET) of FMRIB Software Library (FSL) was used to extract the brain (Smith, 2002). Segmentation of the brain to obtain percentages of cerebrospinal fluid (CSF), white matter (WM), and grey matter (GM) was conducted using the FMRIB's Automated Segmentation Tool (FAST) with MPRAGE images as the input (Zhang et al., 2011). The levels of myo-inositol, glx, and glutamate were then corrected using the following formula: Corrected metabolite concentration = uncorrected metabolite concentration x(GMx43300+WMx35880+CSFx55556)/(1-CSF)/55556. The Cramér-Rao lower bounds (CRLB) values were inspected for myo-inositol, glx, and glutamate, and a cut-off >30% was used to classify as not detected.

2.1.4 Resting state magnetic resonance imaging

The resting state scan was acquired after the structural and MRS scans. Participants were instructed to open their eyes to look at the cross mark in the centre of the white background and not to fall asleep. The resting state functional MRI (fMRI) was acquired with a voxel size of 2.4 x 2.4 x 2.4 mm, T_R = 735 ms, T_E = 39 ms, flip angle = 52°, slice thickness = 2.4 mm, 192 slices, and FoV read = 210 mm. A total volume of 800 with 64 slices was

obtained. Gradient echo phase and magnitude field maps (a voxel size of 2.4 x 2.4 x 2.4 mm, T_R = 600 ms, T_{E1}/T_{E2}: 4.50/6.96 ms, and flip angle = 46°) were also acquired for correcting any distortions.

The analysis was conducted using FSL (version 6.6.1, FMRIB Analysis Group, University of Oxford, UK) (Jenkinson et al., 2012). Generally, individual subject data were pre-processed, which included brain extractions for T1-MPRAGE and fieldmap magnitude images, and producing fieldmap images in radians/sec (rads). In the next pre-processing step, individual subject independent component analysis (ICA) was conducted using the Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC, version 3.15) function in FSL (Beckmann et al., 2005). The single session ICA using MELODIC included motion correction using FLIRT (FMRIB's Linear Image Registration Tool), unwarping of effective echo-planar imaging (EPI) data using fieldmap magnitude and fieldmap rads images (EPI echo spacing = 0.64 ms, EPI T_E = 39, unwarp direction = -y, percentage signal loss threshold = 10), co-registration of EPI data to the structural scan using boundary-based registration (BBR), and normalization into Montreal Neurological Institute (MNI) standard space using both linear and non-linear registrations (warp resolution = 10 mm, resampling resolution = 2 mm). Variance time course, automatic dimensionality estimation, IC map threshold of 0.5, spatial smoothing with a Gaussian kernel of full-width at half-height (FWHM) of 5 mm, and high-pass temporal filtering of 100 s (0.005 Hz) were also applied in the single ICA. There were odd blobs characterized by circular shapes at random places (including white matter), with the initial data with 800 volumes. The issue was solved by reducing the volume to 490 with the same inputs into MELODIC. Individual output was data-quality checked using the following criteria: movement $\leq$ 2.4 mm in translation along x, y, or z planes and $\leq$ 3° (equivalent to 0.052 radians) rotation in any direction (pitch, roll or yaw) (Sankar et al., 2021).

The training dataset, which consisted of 10 randomly selected participants was manually labelled into signal and noise using FSLeyes in MELODIC view (Griffanti et al., 2017). The training dataset was then trained using FMRIB's ICA-based Xnoiseifier (FIX), and the best threshold was selected (threshold = 20, true positive ratio (TPR) = 97.3, true negative ratio (TNR) = 89.9). Then, FIX was again conducted for all datasets using the training dataset and the selected threshold to clean the noise. The resulting cleaned functional images were registered to standard space.

Subsequently, two types of analysis were conducted; seed-based functional connectivity analysis and independent component analysis. In the first analysis, pregenual ACC was chosen as a region of interest for the seed analysis due to several reasons: 1) Previous studies in healthy volunteers reported a significant reduction in myo-inositol levels in ACC after ebselen treatment relative to placebo (Masaki, Sharpley, Godlewska, et al., 2016; Singh et al., 2016). 2) Significant effects of ebselen on producing a positive bias shift in healthy volunteers, potentially due to ebselen's modulating effects on emotional processing networks including ACC (Masaki, Sharpley, Cooper, et al., 2016; Singh et al., 2016). 3) ACC (consisting of mostly the pregenual ACC) was the region of interest of the current study for the MRS study. 4) A previous study investigating lithium's effect on functional connectivity in bipolar mood disorder patients reported that significantly increased connectivity between the ventromedial prefrontal cortex (of which ACC is a part) and amygdala was associated with clinical improvement (Altinay et al., 2018). To produce a mask for pregenual ACC, Automated Anatomical Labelling atlas 3 (AAL3) was downloaded before the anatomical regions were separated into individual regions. The final mask comprised of pregenual ACC, corresponding to anatomical region number 153 and 154 (right and left, Figure 2.2.) (Rolls et al., 2020).

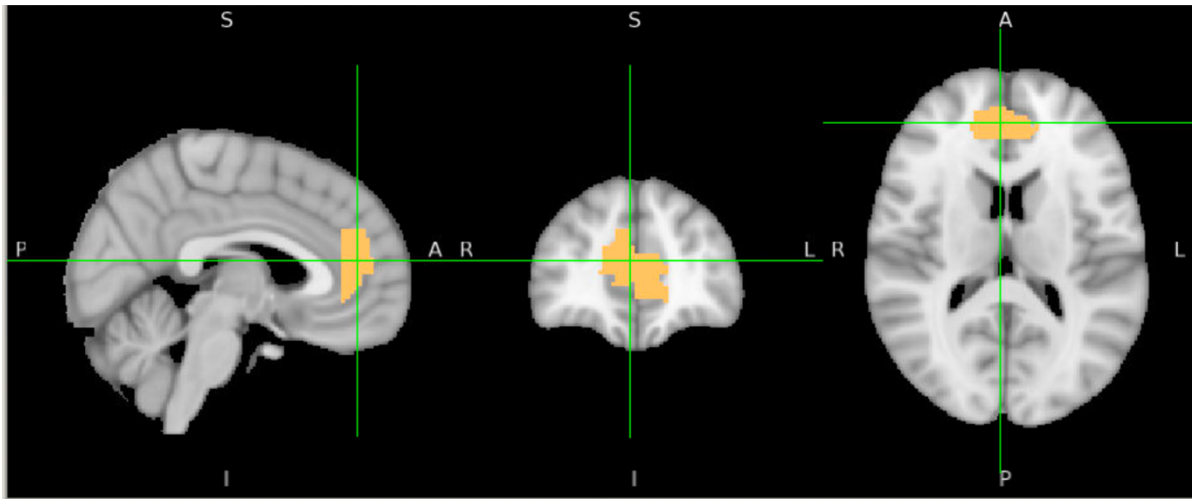


Figure 2.2. The bilateral pregenual ACC mask (in yellow), corresponding to anatomical region number 153 and 154 in the Automated Anatomical Labelling atlas 3 (Cursor coordinate: MNI 43, 86, 43).

Subsequently, the mean time series was extracted from the region of interest, pregenual ACC, as a preparation for the seed-based correlation analysis. Dual regression was then performed as a part of the seed-based correlation analysis. The difference between visits (post-treatment minus baseline) was used to compare between treatment (ebselen vs placebo) in the general linear model (GLM).

Considering a potential confounding factor from structural differences between individuals, further analysis was conducted to measure grey matter volumes. FSL voxel-based morphometry (FSLVBM) was used for quantifying grey matter volumes (Douaud et al., 2007; Good et al., 2001; Smith et al., 2004). Only the first visit T1-weighted-bias-corrected images from 45 participants were utilised to extract grey matter volumes, as we did not expect any significant structural changes during a week of treatment. Generally, the brain extraction was conducted for the first 20 subject before a study-specific template was created. In this step, the

brains were grey matter-segmented and registered to the MNI152 standard space using non-linear registration (Anderson et al., 2007). To produce a study-specific grey matter template, the images were averaged and flipped along the x-axis to produce a right-left symmetric.

Another step was to process all the grey matter images from the dataset (n=45). In this step, all participant images were registered (non-linear) to the study-specific template previously created, and modulated for local effects of expansion or contraction.

The FSL randomised was used to perform permutation-based methods (number of permutations = 5000) with a cluster-based thresholding using the Threshold-Free-Cluster-Enhancement approach and a family-wise error corrected p-value threshold of < 0.05 applied to the suprathreshold clusters (Nichols & Holmes, 2002; Smith et al., 2004; Smith & Nichols, 2009). The grey matter volumes were included as the covariate.

To determine the correlation between changes in neurometabolite concentrations of myo-inositol, glx, and glutamate in the ACC and changes in resting-state functional connectivity between the seed region pregenual ACC and other brain regions, similar methods were conducted with some modifications. The number of participants included based on the MRS data availability was 41. The differences between visits in myo-inositol, glx, and glutamate levels were calculated as MRS input. Similar methods of FSL randomise as described above were used to generate the output.

Other than the seed correlation analysis of pregenual ACC, whole brain resting state fMRI analysis (ICA) was performed to determine changes in resting-state functional connectivity between any parts of the brain. MELODIC was used to perform the group-level ICA to concatenate de-noised functional data temporally and decompose into independent components of 25. Spatial mixture modelling estimates that the average number of individual IC maps is typically about 25 (Martens et al., 2022). Visual inspection of potential networks

was performed after the group-level ICA to identify the network of interest. The correlation between the identified network and published resting state networks (Martens et al., 2022; Smith et al., 2009) was also conducted using the FSL cross-correlation tool with a threshold of 0.3. Subsequently, dual regression was conducted to produce a subject-specific version of the spatial maps and associated time series (Beckmann et al., 2009; Filippini et al., 2009; Martens et al., 2022). In the first step of dual regression, regression was applied to a group-average set of spatial maps for each participant to generate the 4D space-time dataset. A set of subject-specific time series generated were then regressed into the same 4D dataset.

The FSL randomised was used to perform permutation-based methods (number of permutations = 5000) with a cluster-based thresholding using the Threshold-Free-Cluster-Enhancement approach and a family-wise error corrected p-value of < 0.05 applied to the suprathreshold clusters (Nichols & Holmes, 2002; Smith et al., 2004; Smith & Nichols, 2009). The grey matter volumes were included as the covariate. For significant connections, changes in resting-state functional connectivity measures (parameter estimates) were extracted.

2.1.5 Selenium levels

Blood selenium levels were used as a surrogate marker of ebselen as a previous study reported a significant correlation between ebselen and its metabolites and selenium levels (Kil et al., 2017). Venous blood was withdrawn in the second study visit. The blood was then centrifuged on the same day for 10 minutes at 3500 g. The serum was transferred into a microtube and kept in the freezer at -80°C before the measurement took place. After unblinding the treatment, we sent the samples of participants who received ebselen to the Department of Laboratory Medicine, University Hospital Southampton National Health Service (NHS) Foundation Trust for analysis.

A Perkin Elmer NEXION 300D with a dynamic reaction cell (DRC) was used to perform inductively coupled plasma mass spectroscopy (ICP-MS), which analysed the total selenium levels in the blood serum samples. Methane was used as the DRC gas to overcome argon-based interferences. Tuning of the instrument was conducted before the analysis to ensure optimum signal sensitivity and minimise the formation of oxide species and doubly charged ions. The serum was first diluted with 0.5% butanol (1:50 ratio). Rhodium was used as an internal standard. The samples were run against matched calibration solutions prepared with bovine serum (Sigma-Aldrich). Selenium-78 was then measured, and the signal of the analyte isotope was compared against the internal standard. The comparison with the internal standard is essential to compensate for matrix-related effects and signal fluctuation secondary to instrument drift. The analyses of samples, blanks, calibration standards, and quality control samples were conducted in the batch. The calibration plot was used to convert the signal to concentration data.

2.1.6 Clinical measures

Quantitative assessments of depressive and anxiety symptoms were conducted using MADRS (Montgomery & Asberg, 1979b), QIDS-SR-16, and GAD-7 (Table 2.1) (Spitzer et al., 2006a). QIDS-SR-16 (Cronbach $\alpha = 0.86$) is a validated tool to assess the severity of depressive symptoms. The questionnaire contains sixteen items and each item has scores ranging from 0–3 (Rush et al., 2003b). GAD-7 is a validated questionnaire for screening and evaluating the severity of anxiety with excellent internal consistency (Cronbach $\alpha = 0.92$) and test-retest reliability (interclass correlation = 0.83). The questionnaire consists of seven items and each item has scores ranging from 0–3. Higher scores indicate more severe anxiety (Spitzer et al., 2006b). A clinician-rated questionnaire MADRS was utilised to assess the severity of

depression. MADRS (inter-rater reliability ≥ 0.89) has ten items with scores from 0–6 for the first nine items and 0–5 for the last item (Montgomery & Asberg, 1979a).

Table 2.1. Scorings for detection and severity of HAM-D, MADRS, QIDS-SR-16, and GAD-7

Questionnaire	HAM-D-17 (Hamilton, 1960; Zimmerman et al., 2013)	MADRS (Mittmann et al., 1997; Müller et al., 2003)	QIDS-SR-16 (Rush et al., 2003a)	GAD-7 (Spitzer et al., 2006a)
Number of items	17	10	16	7
Rater	Clinician	Clinician	Self	Self
Item score ranges	0-2, 0-4	0-6	0-3	0-3
Detection/diagnostic cut-off				> 10
Severity classification				
None	0-7	0-8	0-5	0-4
Mild	8-16	9-17	6-10	5-9
Moderate	17-23	18- 30	11-15	10-14
Severe	> 24	> 31	16-20*	> 15

* scores of >20 indicates a very severe symptom classification based on GAD-7.

2.2 Study 1B

2.2.1 Study design and participants

To address the potential residual negative emotional processing bias in depressed patients with inadequate responses to current antidepressants, we decided to recruit healthy volunteers to investigate whether emotional processing had been normalized by antidepressant treatment in our patient population, even though the patients had not shown a satisfactory therapeutic response. The study design was cross-sectional, comparing emotional processing between the depressed patients in our sample and age- and sex-matched healthy controls. The study received ethical approval (20/SC/0151) from the NRES Committee, South-Central Oxford.

The inclusion and exclusion criteria for the patient group were described in detail previously. Healthy controls were required to be free from any current or history of major psychiatric disorders, and they were screened using a similar method to our patient group using SCID-5 and 17-item HAM-D. Eligible participants were invited for the study visit and the assessments of the emotional processing and symptoms of depression and anxiety were conducted using ETB and questionnaires (MADRS, QIDS-SR-16, and GAD-7), respectively as described above.

CHAPTER 3

3. Effects of Ebselen on Emotional Processing in Patients with Medication-Resistant Depression

3.1 Introduction

Cognitive theories of depression suggest that negative biases in emotional processing are important in the development and maintenance of MDD (Beck & Bredemeier, 2016). Antidepressants, including SSRIs and SNRIs, have been reported to induce a shift in an emotional processing towards more positively biased emotional information processing in both healthy participants and depressed patients. Interestingly, the effects are immediate, appearing after a single dose of medication or a short course of treatment (Harmer et al., 2009; Harmer et al., 2004; Pringle & Harmer, 2015; Tranter et al., 2009). The positive correlation between early changes in positive bias in emotional processing following the introduction of antidepressant treatment and later clinical improvement makes emotional processing an excellent putative surrogate biomarker to assess the antidepressant potential of pharmacological agents thought to have antidepressant properties.

Previously, two studies in healthy volunteers reported that the ebselen treatment given over 24 hours significantly increased the accuracy in detecting the positive facial expression of happiness (Masaki, Sharpley, Cooper, et al., 2016; Singh et al., 2016). The effect on negative facial expression recognition was inconsistent, with the earlier study reporting a significant increase in disgust recognition. The potential reasons for differing results might be attributable to different study designs (parallel vs cross-over) and doses (1800 mg vs 3600 mg) used. Given the consistent findings in positive facial recognition, these studies supported the antidepressant potential of ebselen in healthy volunteers.

As the intended use of ebselen is to replace lithium as an augmenting agent in depressed patients, a study in a targeted depressed population was necessary to confirm the replicability of the previous findings in healthy participants. This chapter aimed to investigate the effects of seven days' ebselen treatment on emotional processing in depressed patients on current antidepressant treatment but who had not experienced a satisfactory therapeutic response. Such patients are believed to experience a positive response to the addition of lithium to their antidepressant treatment. The outcomes of the study can act as a guide in deciding whether a clinical trial would be warranted to evaluate the clinical effects of ebselen in this depressed population.

3.2 Method

Depressed patients on their current antidepressants received either ebselen 600 mg or matching placebo twice daily for seven days. ETB, clinical measures of depression (MADRS and QIDS-SR-16) and anxiety (GAD-7), and adverse effects were assessed at baseline and 7-day after treatment. The guess of treatment (ebselen or placebo) and venous blood for selenium levels were obtained at the post-treatment visit (refer to the General Methodology Chapter for more detail). Compliance towards medication was assessed using the instruction card in which participants were asked to record the time of medication intake, pill counting, and selenium levels. The acceptable missing dose was less than 10%.

3.2.1 Statistical analysis

Categorical variables are presented in counts and percentages and analysed using a chi-squared test. Continuous variables are presented in the means and standard error of means (SEMs) and analysed using an independent *t*-test for demographic data. The three-way and

two-way analyses of variance (ANOVAs) were used to analyse interaction for FERT (accuracy, RT, misclassification, d prime (d'), beta), ECAT (accuracy and RT), EREC (correct recall, incorrect recall), and EMEM (accuracy, RT, misclassification, d', beta) with emotion or valence and time as within-subject factors and treatment as a between-subject factor. A four-way ANOVA was used to analyse FDOT with valence, time, and condition (masked vs unmasked) as within-subject factors and treatment as a between-subject factor. A significant interaction was followed up with a post-hoc independent *t*-test using change values. Additionally, we performed analysis of covariance (ANCOVA) for all ETB parameters using baseline visit values of ETB parameters as covariates. The differences between visits were used to compare ebselen and placebo in MADRS, QIDS-SR-16, and GAD-7. An additional paired *t*-test was conducted to explore the changes in each group individually. The correlations between selenium levels and changes in clinical measures of MADRS, QIDS-SR-16, and GAD-7 were tested using Pearson's product-moment correlation. The p-value was set at <0.05.

3.3 Result

Fifty-one participants were included in the final analysis. We excluded five participants due to various factors, including bipolar depression (one), device malfunction (one), treatment non-compliance (one), Covid-19 infection (one), and possible allergic reactions towards scanner materials (one). We did not find any significant differences between ebselen and placebo groups in age ($t(49) = 0.156$, $p = 0.88$), sex ($p = 0.552$ (χ^2)), number of failed antidepressant treatments ($p = 0.756$ (χ^2)), and treatment guess ($p = 0.369$ (χ^2)) (Table 3.1).

Table 3.1. Baseline characteristics, treatment guess, and clinical measures of participants included in the study (n=51).

Variable	Ebselen (n=27)	Placebo (n=24)
Age (years)	38.3 ± 2.5	37.7 ± 3.3
Sex	Female: 19 (70.4%), male: 8 (29.6%)	Female: 15 (62.5%), male: 9 (37.5%)
Number of failed antidepressant treatments (1-2 vs >2)	1-2 = 18 (66.7%) >2 = 9 (33.3%)	1-2 = 15 (62.5%) >2 = 9 (37.5%)
Baseline HAM-D	19.6 ± 0.9	19.1 ± 0.8
Treatment guess (percentage)	8/27 (29.6%)	10/24 (41.7%)
Change in clinical measures over seven days of treatment		
MADRS	-3.52 ± 1.21	-3.87 ± 1.68
QIDS-SR-16	-3.74 ± 1.20	-5.04 ± 1.11
GAD-7	-0.96 ± 0.85	-3.12 + 0.74

Continuous variables are presented as means ± standard error of means and categorical variables are presented as counts and percentages.

SSRIs sertraline (39.2%) and citalopram (21.6%) were the most common antidepressants used by the participants, followed by atypical antidepressant mirtazapine (13.7%) (Table 3.2).

Table 3.2. Percentage and count of failed antidepressant treatments used by the participants (n=51).

Antidepressants	Percentage (count)
Selective serotonin reuptake inhibitor	
Sertraline	39.2% (20)
Citalopram	21.6% (11)
Fluoxetine	9.8% (5)
Escitalopram	5.9% (3)
Serotonin and norepinephrine reuptake inhibitor	
Venlafaxine	7.8% (4)
Norepinephrine-dopamine reuptake inhibitor	
Bupropion	2.0% (1)
Atypical	
Mirtazapine	13.7% (7)
Agomelatine	3.9% (2)
Serotonin modulator	
Vortioxetine	2.0% (1)
Tricyclic antidepressant	
Imipramine	2.0% (1)

3.3.1 Effects on emotional testing battery

No significant three-way interaction between treatment, time, and emotion was observed for the accuracy of recognition of facial expressions ($F_{3.9,192.9} = 0.73$, $p = 0.57$, partial

$\eta^2 = 0.015$). A significant two-way interaction between time and emotion for the accuracy of recognition of facial expressions ($F_{3.9,192.9} = 9.82$, $p < 0.001$, partial $\eta^2 = 0.167$) was observed. However, there were no significant treatment interactions with emotion and time in a two-way ANOVA (Table 3.3). On the second testing occasion, both treatment groups showed a reduction in sad accuracy recognition but increased accuracy in recognizing happy and neutral facial expressions (Figure 3.1). However, there were no significant differences between ebselen and placebo groups in the post-hoc independent *t*-tests (Table 3.4).

We found no significant three- way ($F_{2.5,119.8} = 1.15$, $p = 0.33$, partial $\eta^2 = 0.023$) or two-way treatment interactions (treatment x time, treatment x emotion) on the FERT RT. A significant main effect of time ($F_1 = 35.42$, $p < 0.001$, partial $\eta^2 = 0.420$) was observed. We found faster RT for all emotions in both groups on the post-treatment visit, potentially due to practice effects on the repeated task.

No three-way interaction between treatment, time, and emotion for FERT misclassification ($F_{3.0,145.0} = 0.95$, $p = 0.42$, partial $\eta^2 = 0.019$) was observed. There was a significant two-way interaction between time and emotion in the FERT misclassification ($F_{3.0,145.0} = 10.44$, $p < 0.001$, partial $\eta^2 = 0.176$). However, there were no significant treatment interactions with emotion and time in a two-way ANOVA (Table 3.3). Post-hoc independent *t*-tests showed no significant differences between groups (Table 3.4). Generally, both groups had lower sad misclassifications and misclassified more faces as neutral on the second testing occasion, but the results did not reach statistical significance between groups (Figure 3.2).

No three-way interaction between treatment, time, and emotion was found for d' ($F_{3.3,157.3} = 0.61$, $p = 0.63$, partial $\eta^2 = 0.013$). A significant two-way interaction between time and emotion was observed for d' in the FERT ($F_{3.3,157.3} = 6.05$, $p < 0.001$, partial $\eta^2 = 0.114$). However, there were no significant treatment interactions with emotion and time in a two-way

ANOVA (Table 3.3). Post-hoc independent *t*-tests showed no significant differences between groups (Table 3.4; Figure 3.3).

We found no three-way interaction between treatment, time, and emotion for beta ($F_{2.0,98.2} = 0.50$, $p = 0.61$, partial $\eta^2 = 0.01$). A significant two-way interaction between time and emotion was observed for beta in the FERT task ($F_{2.0,98.2} = 8.50$, $p < 0.001$, partial $\eta^2 = 0.15$). However, there were no significant treatment interactions with emotion and time in a two-way ANOVA. Post-hoc independent *t*-tests showed no significant differences between groups (Table 3.4; Figure 3.4).

Interestingly, a significant difference in post-treatment beta values for fear emotion was found ($F1,47 = 4.64$, $p = 0.04$, partial $\eta2=0.09$) in the ANCOVA. A follow-up post-hoc pairwise analysis reported a significantly lower beta value for fear in ebselen (adjusted mean $\pm$ SEM = 0.73 ± 0.02) than in placebo (0.80 ± 0.02) with a mean difference of -0.71 ($\pm$ 0.33 SEM, $p = 0.04$), indicating more false alarms in the ebselen group towards fearful emotions.

Table 3.3. Three-way and two-way ANOVAs of all ETB parameters.

Interaction	Degree of freedom	F	p-value	Partial eta squared
FERT				
Accuracy (percentage)				
Three-way interaction				
Time x Emotion x Treatment Group	3.937	0.734	0.568	0.015

Error (Time x Emotion)	192.93			
Two-way interactions				
Time x Treatment Group	1	0.514	0.477	0.010
Error (Time)	49			
Emotion x Treatment Group	3.867	0.604	0.654	0.012
Error (Emotion)	189.47			
Time x Emotion	3.937	9.815	<0.001*	0.167
Error (Time x Emotion)	192.93			
Reaction time (seconds)				
Three-way interaction				
Time x Emotion x Treatment Group	2.445	1.148	0.328	0.023
Error (Time x Emotion)	119.8			
Two-way interactions				
Time x Treatment Group	1	0.004	0.952	0.000
Error (Time)	49			
Emotion x Treatment Group	3.063	0.285	0.840	0.006
Error (Emotion)	150.1			
Time x Emotion	2.445	4.513	0.008*	0.084
Error (Time x Emotion)	119.8			
Misclassification (percentage)				
Three-way interaction				
Time x Emotion x Treatment Group	2.960	0.953	0.416	0.019
Error (Time x Emotion)	145.04			
Two-way interactions				

Time x Treatment Group	1	0.652	0.423	0.013
Error (Time)	49			
Emotion x Treatment Group	2	0.827	0.441	0.017
Error (Emotion)	98.02			
Time x Emotion	2.960	10.44	<0.001*	0.176
Error (Time x Emotion)	145.04			
d’				
Three-way interaction				
Time x Emotion x Treatment Group	3.347	0.609	0.628	0.013
Error (Time x Emotion)	157.30			
Two-way interactions				
Time x Treatment Group	1	1.216	0.276	0.025
Error (Time)	47			
Emotion x Treatment Group	4.280	1.132	0.343	0.024
Error (Emotion)	201.16			
Time x Emotion	3..347	6.053	<0.001*	0.114
Error (Time x Emotion)	157.30			
beta				
Three-way interaction				
Time x Emotion x Treatment Group	2.047	0.502	0.611	0.010
Error (Time x Emotion)	98.24			
Two-way interactions				
Time x Treatment Group	1	0.118	0.733	0.002
Error (Time)	48			

Emotion x Treatment Group	1.924	1.479	0.234	0.030
Error (Emotion)	92.34			
Time x Emotion	2.047	8.502	<0.001*	0.150
Error (Time x Emotion)	98.24			
ECAT				
Accuracy (percentage)				
Three-way interaction				
Time x Valence x Treatment Group	1	1.474	0.230	0.029
Error (Time x Valence)	49			
Two-way interactions				
Time x Treatment Group	1	0.511	0.478	0.010
Error (Time)	49			
Valence x Treatment Group	1	1.174	0.284	0.023
Error (Valence)	49			
Time x Valence	1	0.007	0.936	0.000
Error (Time x Valence)	49			
Reaction time (seconds)				
Three-way interaction				
Time x Valence x Treatment Group	1	0.372	0.545	0.006
Error (Time x Valence)	49			
Two-way interactions				
Time x Treatment Group	1	0.083	0.775	0.002
Error (Time)	49			

Valence x Treatment Group	1	1.248	0.269	0.025
Error (Valence)	49			
Time x Valence	1	0.280	0.599	0.06
Error (Time x Valence)	49			
FDOT				
Four-way interaction				
Time x Valence x Condition x Treatment Group	1	4.470	0.040*	0.084
Error (Time x Valence x Condition)	49			
Three-way interactions				
Time x Valence x Treatment Group	1	0.248	0.621	0.005
Error (Time x Valence)	49			
Time x Condition x Treatment Group	1	0.726	0.398	0.015
Error (Time x Condition)	49			
Time x Valence x Treatment Group	1	0.248	0.621	0.005
Error (Time x Valence)	49			
Valence x Condition x Treatment Group	1	0.607	0.440	0.012
Error (Valence x Condition)	49			
Two-way interactions				
Time x Treatment Group	1	0.586	0.448	0.012
Error (Time)	49			
Valence x Treatment Group	1	0.016	0.899	0.000
Error (Valence)	49			

Condition x Treatment Group	1	4.011	0.051	0.076
Error (Condition)	49			
Time x Valence	1	0.292	0.592	0.006
Error (Time)	49			
Valence x Condition	1	1.089	0.302	0.022
Error (Valence)	49			
Time x Condition	1	0.034	0.855	0.001
Error (Time)	49			
EREC				
Correctly recalled (count)				
Three-way interaction				
Time x Valence x Treatment Group	1	0.996	0.324	0.021
Error (Time x Valence)	46			
Two-way interactions				
Time x Treatment Group	1	0.194	0.662	0.004
Error (Time)	46			
Valence x Treatment Group	1	0.225	0.638	0.005
Error (Valence)	46			
Time x Valence	1	7.973	0.007*	0.148
Error (Time x Valence)	46			
Incorrectly recalled (count)				
Three-way interaction				
Time x Valence x Treatment Group	1	0.528	0.471	0.011

Error (Time x Valence)	46			
Two-way interactions				
Time x Treatment Group	1	3.543	0.066	0.072
Error (Time)	46			
Valence x Treatment Group	1	0.034	0.855	0.001
Error (Valence)	46			
Time x Valence	1	1.459	0.233	0.031
Error (Time x Valence)	46			
EMEM				
Accuracy (percentage)				
Three-way interaction				
Time x Valence x Treatment Group	1	0.231	0.633	0.005
Error (Time x Valence)	49			
Two-way interactions				
Time x Treatment Group	1	0.776	0.383	0.016
Error (Time)	49			
Valence x Treatment Group	1	0.476	0.494	0.010
Error (Valence)	49			
Time x Valence	1	2.508	0.120	0.049
Error (Time x Valence)	49			
Reaction time (seconds)				
Three-way interaction				
Time x Valence x Treatment Group	1	0.155	0.695	0.003

Error (Time x Valence)	49			
Two-way interactions				
Time x Treatment Group	1	0.773	0.384	0.016
Error (Time)	49			
Valence x Treatment Group	1	0.869	0.356	0.017
Error (Valence)	49			
Time x Valence	1	0.108	0.744	0.002
Error (Time x Valence)	49			
Misclassifications (percentage)				
Three-way interaction				
Time x Valence x Treatment Group	1	0.231	0.633	0.005
Error (Time x Valence)	49			
Two-way interactions				
Time x Treatment Group	1	0.776	0.383	0.016
Error (Time)	49			
Valence x Treatment Group	1	0.476	0.494	0.010
Error (Valence)	49			
Time x Valance	1	2.508	0.120	0.049
Error (Time x Valence)	49			
d’				
Three-way interaction				
Time x Valence x Treatment Group	1	0.296	0.589	0.006
Error (Time x Valence)	49			
Two-way interactions				

Time x Treatment Group	1	0.780	0.381	0.016
Error (Time)	49			
Valence x Treatment Group	1	0.108	0.744	0.002
Error (Valence)	49			
Time x Valance	1	4.468	0.040*	0.084
Error (Time x Valence)	49			
Beta				
Three-way interaction				
Time x Valence x Treatment Group	1	0.005	0.946	0.000
Error (Time x Valence)	49			
Two-way interactions				
Time x Treatment Group	1	0.001	0.971	0.000
Error (Time)	49			
Valence x Treatment Group	1	3.107	0.084	0.060
Error (Valence)	49			
Time x Valance	1	0.101	0.752	0.002
Error (Time x Valence)	49			

No significant three-way and two-way interactions were observed in the ECAT accuracy and RT (Table 3.3). A recall memory of self-referent words from the ECAT task showed no three-way interaction between treatment, time, and valence for the EREC accuracy ($F_{1,46} = 1.00$, $p = 0.32$, partial $\eta^2 = 0.021$) in the EREC task. We observed a significant two-way interaction between time and valence in the accuracy of positive and negative self-referent word recall ($F_{1,46} = 7.97$, $p = 0.007$, partial $\eta^2 = 0.148$) in the EREC task. Trends towards an increase in positive word recall but a decrease in negative word recall on the post-treatment

visit were observed in both ebselen and placebo groups (Figure 3.5) However, post-hoc independent *t*-tests showed no significant differences between groups (Table 3.4).

No significant three-way interaction was observed between treatment, time, and valence for the EMEM d' ($F_{1,49} = 0.30$, $p = 0.59$, partial $\eta^2 = 0.01$). There was a significant two-way interaction between time and valence in EMEM d' ($F_{1,49} = 4.47$, $p = 0.04$, partial $\eta^2 = 0.08$). Trends towards an increase in d' for positive word memory but a decrease in d' for negative word memory on the post-treatment visit were observed in both treatment groups. Follow-up post-hoc independent *t*-tests found no significant differences between groups (Table 3.4). There were no significant three-way and two-way interactions for other parameters in the EMEM tasks, including accuracy, RT, misclassification, and beta (Table 3.3).

A significant four-way interaction between time, valence, condition, and treatment for the FDOT attentional vigilance score ($F_{1,49} = 4.47$, $p = 0.04$, partial $\eta^2 = 0.084$) was observed, but no significant three-way and two-way interactions between all combinations of between- and within-subject factors were observed (Table 3.3).

No significant effects of ebselen for all ETB parameters except beta values for fear for the FERT tasks when ANCOVA was performed using baseline values as the covariates (Table 3.5).

Table 3.4 Post-hoc independent *t*-tests for the FERT accuracy, misclassification, d prime, and beta (n=51), EREC correct and incorrect words recalled accuracy (n=48), and EMEM d prime (n=51).

FERT
Accuracy (percentage)

Valence	t	df	p-value	Mean difference	SEM	95% confidence interval (lower & upper)	
Sad	0.75	49	0.46	2.60	3.46	-4.34	9.55
Fear	1.17	49	0.25	3.74	3.21	-2.70	10.18
Happy	-0.44	49	0.66	-1.08	2.46	-6.02	3.87
Surprise	-1.30	49	0.20	-3.59	2.76	-9.14	1.96
Disgust	0.78	49	0.44	1.93	2.47	-3.04	6.90
Anger	1.31	49	0.20	4.06	3.10	-2.17	10.30
Neutral	-0.13	49	0.90	-0.69	5.29	-11.33	9.94
Misclassification (percentage)							
Sad	0.15	49	0.89	0.16	1.09	-2.03	2.35
Fear	1.63	49	0.11	1.05	0.65	-0.25	2.35
Happy	0.80	49	0.43	0.51	0.64	-0.77	1.78
Surprise	-0.85	49	0.40	-0.56	0.67	-1.90	0.78
Disgust	-0.38	49	0.71	-0.32	0.83	-1.99	1.36
Anger	-0.25	49	0.81	-0.15	0.63	-1.41	1.11
Neutral	-1.29	49	0.20	-1.85	1.43	-4.73	1.03
d’							
Sad	0.54	49	0.59	0.01	0.01	-0.01	0.03
Fear	-0.85	48	0.40	-0.03	0.03	-0.09	0.04
Happy	-0.97	49	0.34	-0.01	0.01	-0.03	0.01
Surprise	-0.80	49	0.43	-0.01	0.01	-0.02	0.01
Disgust	0.21	49	0.83	0.00	0.01	-0.02	0.03

Anger	1.25	48	0.22	0.01	0.01	-0.01	0.03
Neutral	0.33	48	0.74	0.01	0.02	-0.03	0.04
Beta							
Sad	-0.46	49	0.65	-0.03	0.06	-0.14	0.09
Fear	-2.03	48	0.05	-0.09	0.04	-0.18	0.00
Happy	0.48	49	0.63	0.01	0.02	-0.04	0.06
Surprise	0.91	49	0.37	0.03	0.04	-0.04	0.11
Disgust	-0.68	49	0.50	-0.03	0.05	-0.13	0.06
Anger	0.86	49	0.39	0.06	0.07	-0.08	0.19
Neutral	0.51	49	0.61	0.08	0.15	-0.23	0.38
EREC							
Valence	t	df	p-value	Mean difference	SEM	95% confidence interval (lower & upper)	
Positive correct	1.03	40.6	0.31	0.66	0.65	-0.64	1.97
Negative correct	-0.46	46	0.65	-0.28	0.61	-1.51	0.95
Positive incorrect	0.87	46	0.39	0.41	0.48	-0.54	1.37
Negative incorrect	1.63	46	0.11	0.99	0.61	-0.23	2.22
EMEM							
d’							

Valence	t	df	p-value	Mean difference	SEM	95% confidence interval (lower & upper)	
Positive	0.81	49	0.42	0.02	0.03	-0.03	0.07
Negative	0.17	49	0.86	0.003	0.02	-0.03	0.04

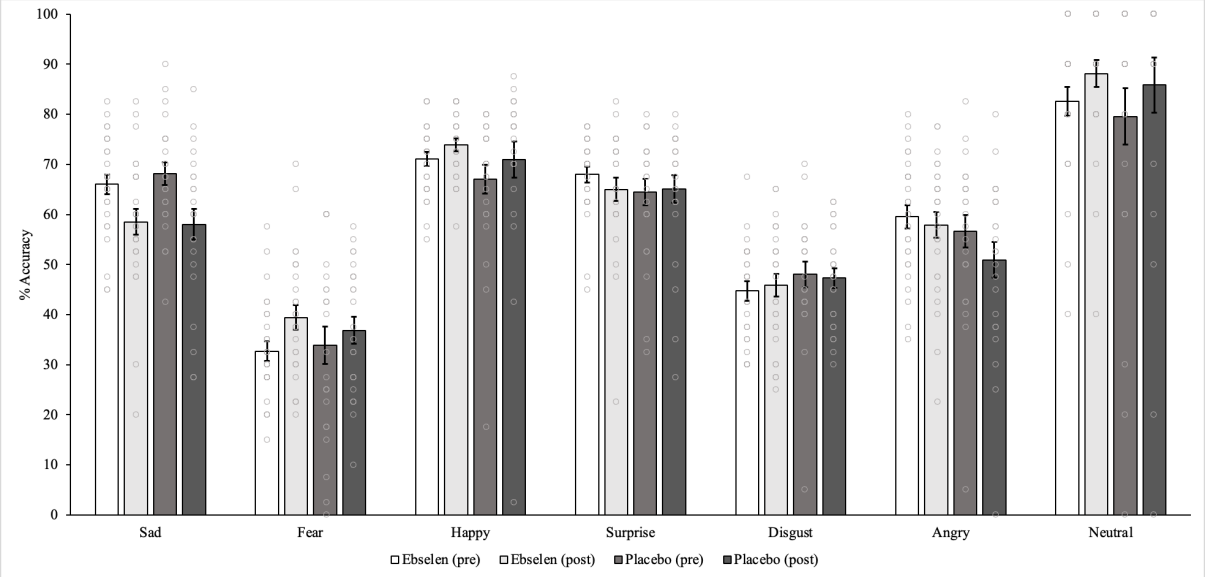


Figure 3.1. Means (± SEMs) per cent accuracy in the FERT. There were no significant effects of treatment (ebselen versus placebo) in the ANOVA. No significant differences between treatment groups in the post-hoc analysis (n=51).

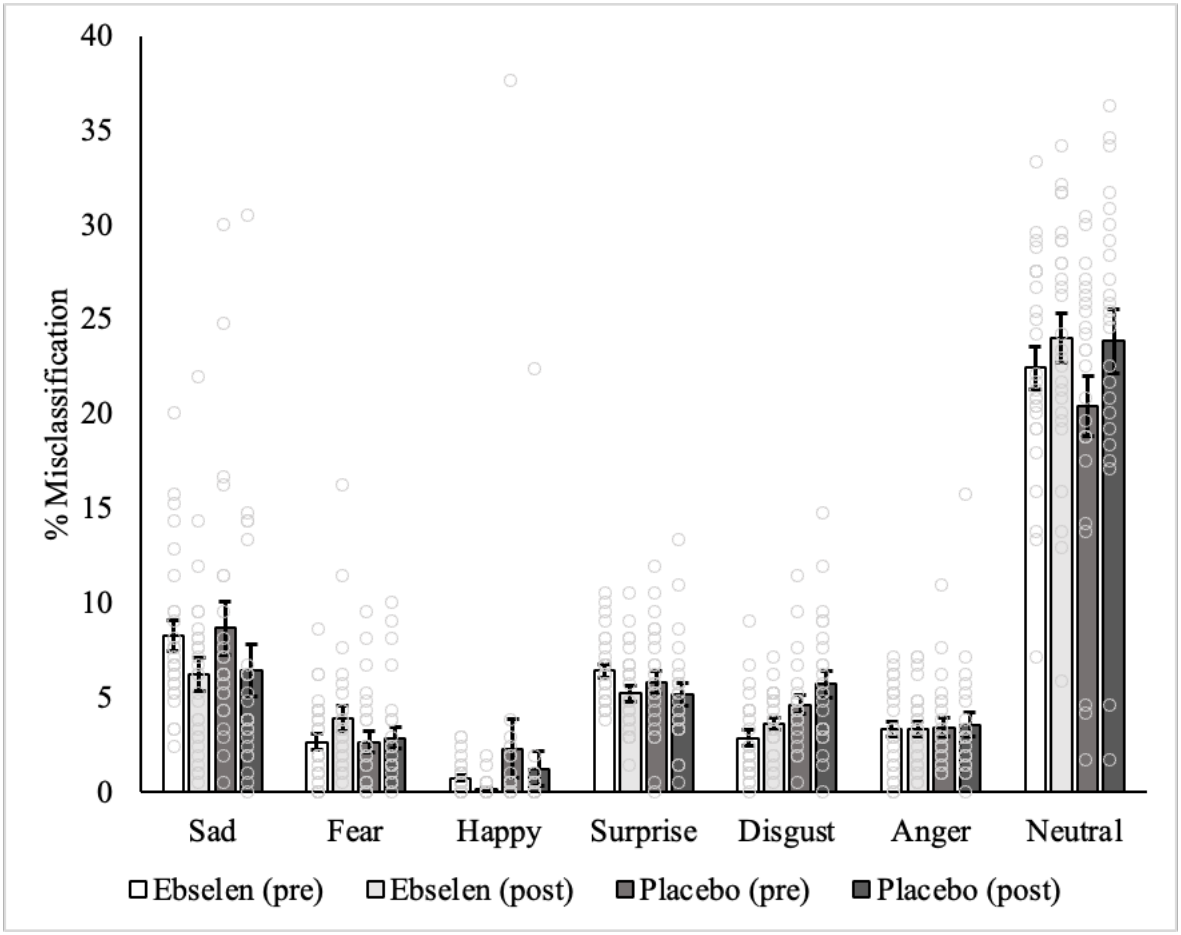


Figure 3.2. Means (± SEMs) per cent misclassification in the FERT. There were no significant effects of treatment (ebselen versus placebo) in the ANOVA. No significant differences between treatment groups in the post-hoc analysis (n=51).

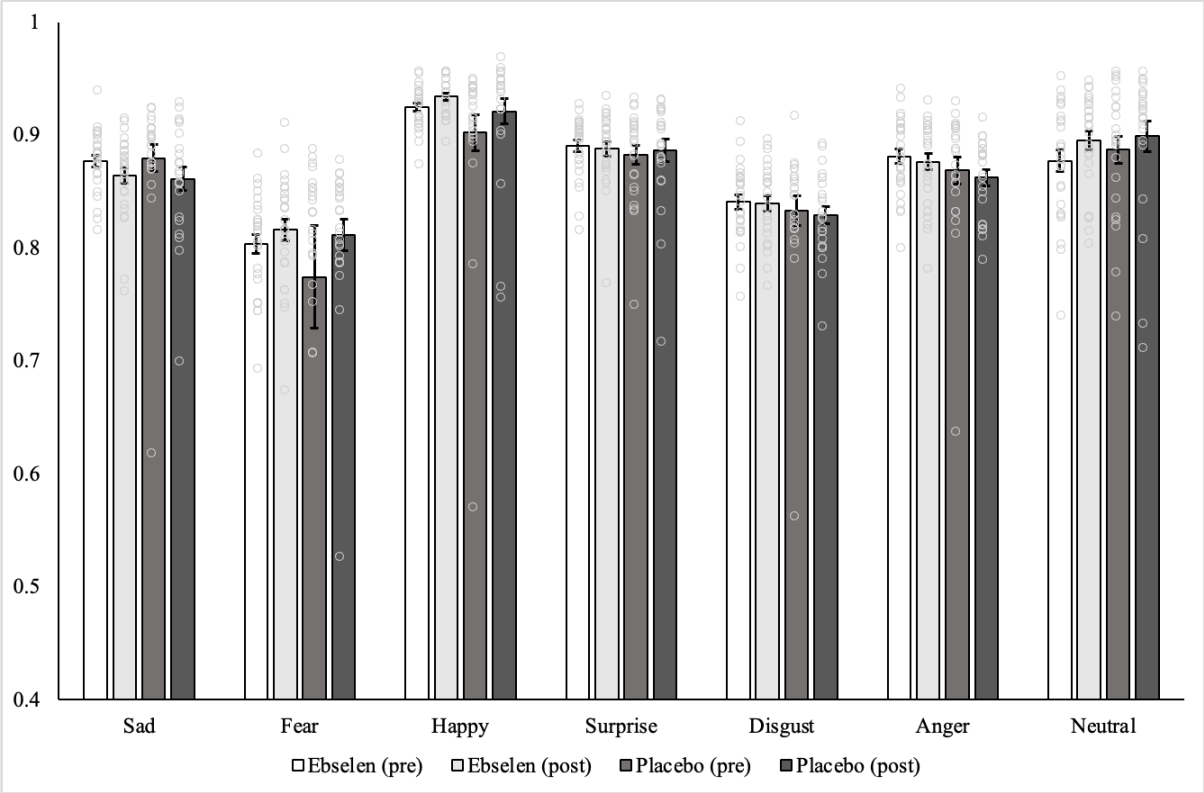


Figure 3.3. Means (± SEMs) d prime in the FERT. There were no significant effects of treatment (ebselen versus placebo) in the ANOVA. No significant differences between treatment groups in the post-hoc analysis (n=51).

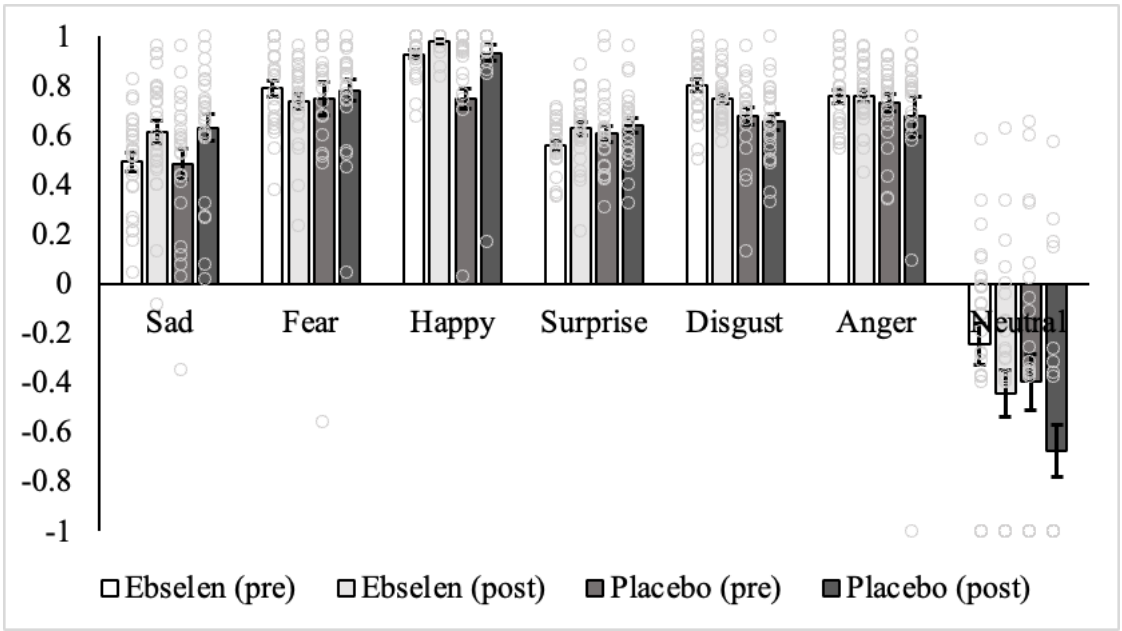


Figure 3.4. Means (± SEMs) beta in the FERT. There were no significant effects of treatment (ebselen versus placebo) in the ANOVA. No significant differences between treatment groups in the post-hoc analysis (n=51).

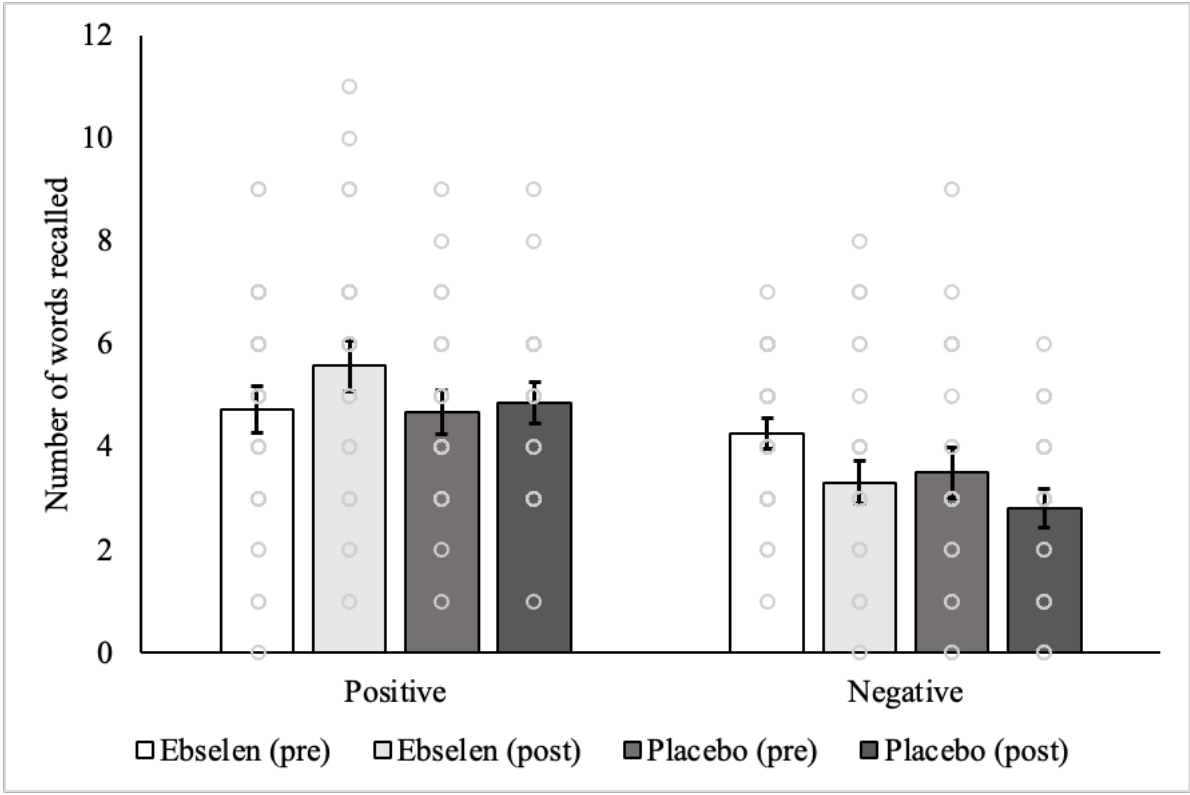


Figure 3.5. Means (± SEMs) count accuracy in the EREC task. There were no significant effects of treatment (ebselen versus placebo) in the ANOVA. No significant differences between treatment groups in the post hoc analysis (n=48).

Table 3.5. Analysis of covariance (ANCOVA) for all emotional testing battery parameters using baseline values as covariates.

Valence		Unadjusted	Adjusted

	ANCOVA statistics	Ebselen (mean + SD)	Placebo (mean + SD)	Ebselen (mean + SE)	Placebo (mean + SE)
FERT					
Accuracy (percentage)					
Sad	$F(1,48) = 0.36$, $p = 0.55$, partial $\eta^2 = 0.01$	58.52 ± 13.56	58.02 ± 15.07	59.25 ± 2.34	57.20 ± 2.48
Fear	$F(1,48) = 1.40$, $p = 0.24$, partial $\eta^2 = 0.03$	39.44 ± 12.64	36.88 ± 13.17	39.77 ± 1.88	36.51 ± 2.00
Happy	$F(1,48) = 0.06$, $p = 0.81$, partial $\eta^2 = 0.001$	73.89 ± 6.63	70.94 ± 17.67	72.22 ± 1.70	72.82 ± 1.80
Surprise	$F(1,48) = 1.06$, $p = 0.31$, partial $\eta^2 = 0.02$	65.00 ± 12.03	65.10 ± 13.36	63.71 ± 1.88	66.55 ± 1.99
Disgust	$F(1,48) = 0.13$, $p = 0.72$, partial $\eta^2 = 0.003$	45.93 ± 11.79	47.29 ± 9.78	46.95 ± 1.55	46.14 ± 1.65
Anger	$F(1,48) = 2.32$, $p = 0.13$, partial $\eta^2 = 0.05$	57.87 ± 13.33	50.94 ± 17.57	56.79 ± 2.08	52.15 ± 2.21

Neutral	$F(1,48) = 0.01$, $p = 0.92$, partial $\eta^2 = 0.00$	88.15 ± 14.15	85.83 ± 27.17	87.30 ± 3.26	86.79 ± 3.46
Reaction time (seconds)					
Sad	$F(1,48) = 0.03$, $p = 0.86$, partial $\eta^2 = 0.001$	1.58 ± 0.41	1.58 ± 0.38	1.57 ± 0.05	1.59 ± 0.05
Fear		1.82 ± 0.36	1.86 ± 0.53		
Happy	$F(1,48) = 2.45$, $p = 0.12$, partial $\eta^2 = 0.05$	1.63 ± 0.40	1.57 ± 0.46	1.68 ± 0.05	1.54 ± 0.05
Surprise		1.63 ± 0.39	1.64 ± 0.50		
Disgust	$F(1,48) = 0.02$, $p = 0.89$, partial $\eta^2 = 0.000$	1.72 ± 0.36	1.69 ± 0.52	1.70 ± 0.06	1.71 ± 0.07
Anger		1.74 ± 0.39	1.74 ± 0.55		
Neutral		1.44 ± 0.54	1.29 ± 0.44		
Misclassification (percentage)					
Sad	$F(1,48) = 0.01$, $p = 0.94$, partial $\eta^2 = 0.00$	6.23 ± 4.70	6.45 ± 6.75	6.37 ± 0.71	6.29 ± 0.76
Fear	$F(1,48) = 2.64$, $p = 0.11$, partial $\eta^2 = 0.05$	3.92 ± 3.39	2.88 ± 2.82	3.92 ± 0.44	2.87 ± 0.47
Happy		0.19 ± 0.46	1.23 ± 4.53		

Surprise	$F(1,48) = 0.25$, $p = 0.62$, partial $\eta^2 = 0.01$	5.22 ± 2.22	5.18 ± 2.88	5.06 ± 0.42	5.36 ± 0.45
Disgust	$F(1,48) = 3.61$, $p = 0.06$, partial $\eta^2 = 0.07$	3.63 ± 1.69	5.71 ± 3.47	3.91 ± 0.52	5.40 ± 0.55
Anger		3.32 ± 1.97	3.55 ± 3.12		
Neutral	$F(1,48) = 1.11$, $p = 0.30$, partial $\eta^2 = 0.02$	24.03 ± 6.77	23.85 + 8.41	23.24 ± 0.97	24.74 ± 1.03
d’					
Sad	$F(1,48) = 0.25$, $p = 0.62$, partial $\eta^2 = 0.01$	0.86 ± 0.04	0.86 ± 0.05	0.87 ± 0.01	0.86 ± 0.01
Fear		0.82 ± 0.05	0.81 ± 0.07		
Happy	$F(1,48) = 0.004$, $p = 0.95$, partial $\eta^2 = 0.000$	0.93 ± 0.02	0.92 ± 0.05	0.93 ± 0.01	0.93 ± 0.01
Surprise		0.89 ± 0.03	0.89 ± 0.05		
Disgust		0.84 ± 0.03	0.83 ± 0.04		
Anger	$F(1,47) = 2.11$, $p = 0.15$, partial $\eta^2 = 0.04$	0.88 ± 0.04	0.86 ± 0.04	0.88 ± 0.01	0.86 ± 0.01

Neutral	F(1,47) = 0.01, p = 0.94, partial η^2 = 0.000	0.90 ± 0.04	0.90 ± 0.07	0.90 ± 0.01	0.90 ± 0.01
Beta					
Sad	F(1,48) = 0.19, p = 0.67, partial η^2 = 0.004	0.62 ± 0.24	0.63 ± 0.26	0.61 ± 0.04	0.64 ± 0.04
Fear	F(1,47) = 4.64, p = 0.04*, partial η^2 = 0.09	0.74 ± 0.16*	0.78 ± 0.22*	0.73 ± 0.02*	0.80 ± 0.02*
Happy		0.98 ± 0.04	0.93 ± 0.17		
Surprise	F(1,48) = 0.19, p = 0.66, partial η^2 = 0.004	0.63 ± 0.14	0.64 ± 0.15	0.64 ± 0.02	0.62 ± 0.03
Disgust	F(1,48) = 2.39, p = 0.13, partial η^2 = 0.05	0.75 ± 0.10	0.66 ± 0.16	0.73 ± 0.03	0.67 ± 0.03
Anger		0.76 ± 0.13	0.68 ± 0.40		
Neutral	F(1,48) = 1.64, p = 0.21, partial η^2 = 0.03	-0.45 ± 0.49	-0.67 ± 0.51	-0.47 ± 0.09	- 0.64 ± 0.10
ECAT					
Accuracy (percentage)					

Positive	$F(1,48) = 1.33$, $p = 0.25$, partial $\eta^2 = 0.03$	95.37 ± 8.43	94.17 ± 6.37	95.72 ± 1.16	93.77 ± 1.23
Negative	$F(1,48) = 0.00$, $p = 0.99$, partial $\eta^2 = 0.000$	97.78 ± 4.67	95.42 ± 11.97	96.68 ± 1.19	96.65 ± 1.27
Reaction time (seconds)					
Positive	$F(1,48) = 0.09$, $p = 0.77$, partial $\eta^2 = 0.002$	0.99 ± 0.26	1.01 ± 0.25	1.01 ± 0.04	0.99 ± 0.04
Negative		1.02 ± 0.21	1.05 ± 0.23		
EREC					
Correct recall (count)					
Positive	$F(1,45) = 1.40$, $p = 0.24$, partial $\eta^2 = 0.03$	5.58 ± 2.50	4.86 ± 1.83	5.57 ± 0.40	4.88 ± 0.43
Negative	$F(1,45) = 0.09$, $p = 0.76$, partial $\eta^2 = 0.02$	3.31 ± 2.13	2.82 ± 1.76	3.16 ± 0.36	3.00 ± 0.39
Incorrect recall (count)					
Positive	$F(1,45) = 1.43$, $p = 0.24$, partial $\eta^2 = 0.03$	2.77 ± 1.86	2.05 ± 1.84	2.68 ± 0.30	2.15 ± 0.33

Negative	$F(1,45) = 3.04$, $p = 0.09$, partial $\eta^2 = 0.06$	1.96 ± 2.38	1.05 ± 1.33	1.98 ± 0.37	1.03 ± 0.40
FDOT (attentional vigilance score)					
Happy					
Unmasked	$F(1,48) = 0.002$, $p = 0.96$, partial $\eta^2 = 0.000$	-0.03 ± 0.04	-0.03 ± 0.06	-0.03 ± 0.01	-0.03 ± 0.01
Masked	$F(1,48) = 2.63$, $p = 0.11$, partial $\eta^2 = 0.05$	-0.003 ± 0.04	-0.003 ± 0.05	-0.004 ± 0.01	-0.02 ± 0.01
Fear					
Unmasked	$F(1,48) = 0.83$, $p = 0.37$, partial $\eta^2 = 0.02$	0.01 ± 0.06	0.03 ± 0.05	0.01 ± 0.01	0.03 ± 0.01
Masked	$F(1,48) = 5.82$, $p = 0.02$, partial $\eta^2 = 0.11$	0.03 ± 0.06	-0.007 ± 0.04	0.03 ± 0.01	-0.01 ± 0.01
EMEM					
Accuracy (percentage)					
Positive	$F(1,48) = 0.32$, $p = 0.58$, partial $\eta^2 = 0.01$	78.32 ± 10.85	78.35 ± 9.76	79.05 ± 1.84	77.53 ± 1.95

Negative	$F(1,48) = 0.002$, $p = 0.96$, partial $\eta^2 = 0.000$	76.06 ± 11.85	78.15 ± 11.94	76.97 ± 2.19	77.12 ± 2.33
Reaction time (seconds)					
Positive		1.11 ± 0.22	1.14 ± 0.21		
Negative	$F(1,48) = 0.01$, $p = 0.94$, partial $\eta^2 = 0.000$	1.12 ± 0.21	1.18 ± 0.22	1.15 ± 0.03	1.15 ± 0.03
Misclassification (percentage)					
Positive	$F(1,48) = 0.32$, $p = 0.58$, partial $\eta^2 = 0.01$	21.68 ± 10.85	21.65 ± 9.76	20.95 ± 1.84	22.47 ± 1.95
Negative	$F(1,48) = 0.002$, $p = 0.96$, partial $\eta^2 = 0.000$	23.94 ± 11.85	21.85 ± 11.94	23.03 ± 2.19	22.88 ± 2.33
d’					
Positive	$F(1,48) = 0.10$, $p = 0.75$, partial $\eta^2 = 0.002$	0.84 ± 0.09	0.86 ± 0.05	0.85 ± 0.01	0.86 ± 0.02
Negative	$F(1,48) = 0.05$, $p = 0.82$, partial $\eta^2 = 0.001$	0.88 ± 0.07	0.91 ± 0.07	0.89 ± 0.01	0.90 ± 0.01
Beta					

Positive	F(1,48) = 0.004, p = 0.95, partial η^2 = 0.000	0.07 ± 0.43	0.06 ± 0.34	0.07 ± 0.07	0.06 ± 0.08
Negative	F(1,48) = 0.39, p = 0.53, partial η^2 = 0.01	0.32 ± 0.37	0.42 ± 0.42	0.34 ± 0.07	0.40 ± 0.08

*Indicates a significant value of p<0.05

Only the mean and standard deviation values of the second visit were reported when there were violations of assumptions for regression of coefficient slope.

3.3.2 Effects on clinical measures of depression and anxiety

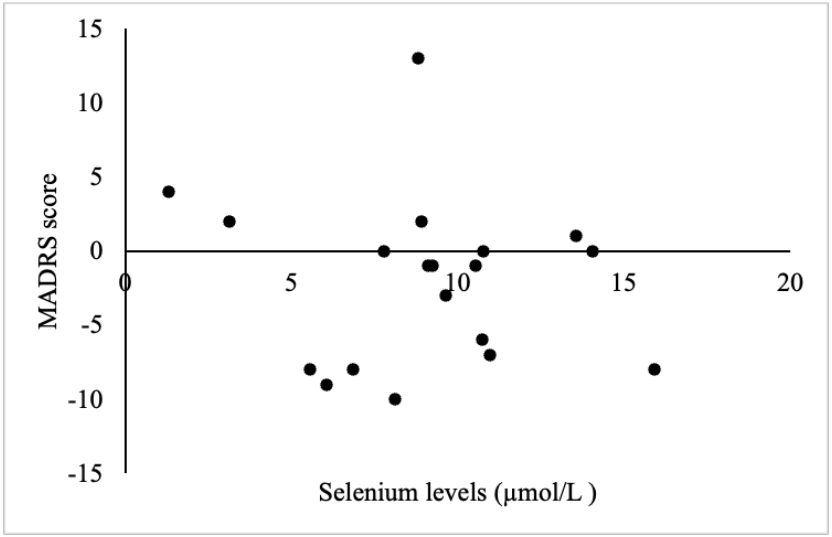
No significant differences were observed between groups in clinical measures of depression, MADRS (t(49) = 0.175, p = 0.86) and QIDS-SR-16 (t(49) = 0.789, p = 0.43). A smaller decrease in the ebselen group compared to the placebo group was found numerically in GAD-7 scores, but the findings were of borderline statistical significance (t(49) = 1.889, p = 0.07). When the groups were analysed separately in a paired t-test, the placebo (t(23) = -4.207, p < 0 .001) but not the ebselen (t(26) = -1.129, p = 0.269) group showed a significantly lower reduction in the GAD-7 score between baseline and post-treatment visits.

3.3.3 Correlation between selenium levels and clinical scores

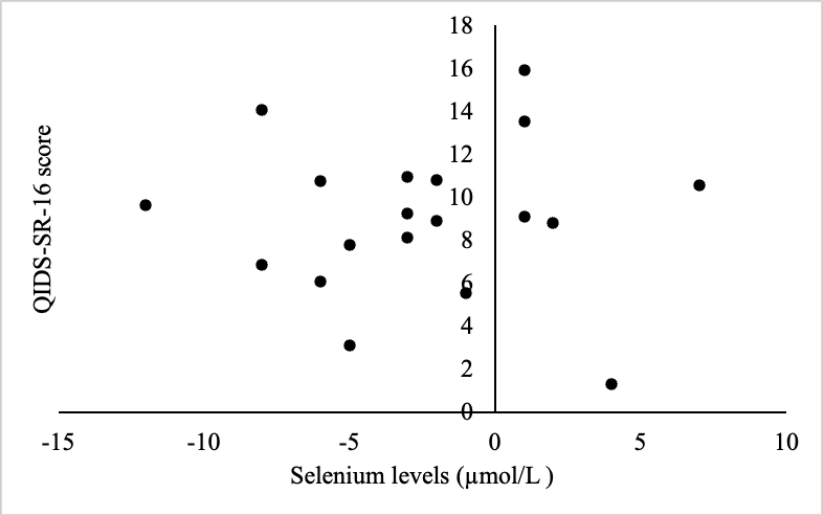
Out of 27 participants who received ebselen, the blood samples were only available for 22 participants (due to technical difficulties in the case of three participants and two participants did not wish to provide blood samples). The mean value of selenium levels was 9.07 ± 0.72 µmol/L (normal values: 2.99–3.99 µmol/L). Selenium levels were raised in all

patients randomised to ebselen, suggesting good compliance. There was no significant correlation between selenium levels and MADRS ($r(17) = -0.12$, $p = 0.62$), QIDS-SR-16 ($r(17) = -0.004$, $p = 0.99$), and GAD-7 change scores in the study ($r(17) = 0.25$, $p = 0.31$) (Figure 3.6).

A.



B.



C.

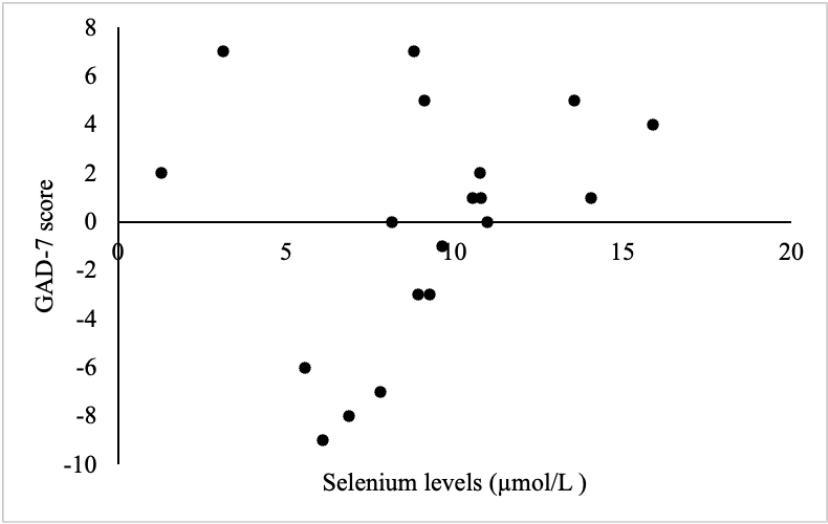


Figure 3.6. Correlations between selenium levels and changes in MARDS (A), QIDS-SR-16 (B), and GAD-7 (C) scores.

3.3.4 Adverse effects associated with ebselen

The adverse effects between ebselen and placebo groups were comparable (Figure 3.7). Headache ($p = 0.34$) and loss of alertness ($p = 0.20$) were reported more often in the ebselen group, but there were no significant differences with the placebo group. No participants stopped the treatment due to adverse effects. All adverse effects were mild and moderate, and no serious adverse effects were reported.

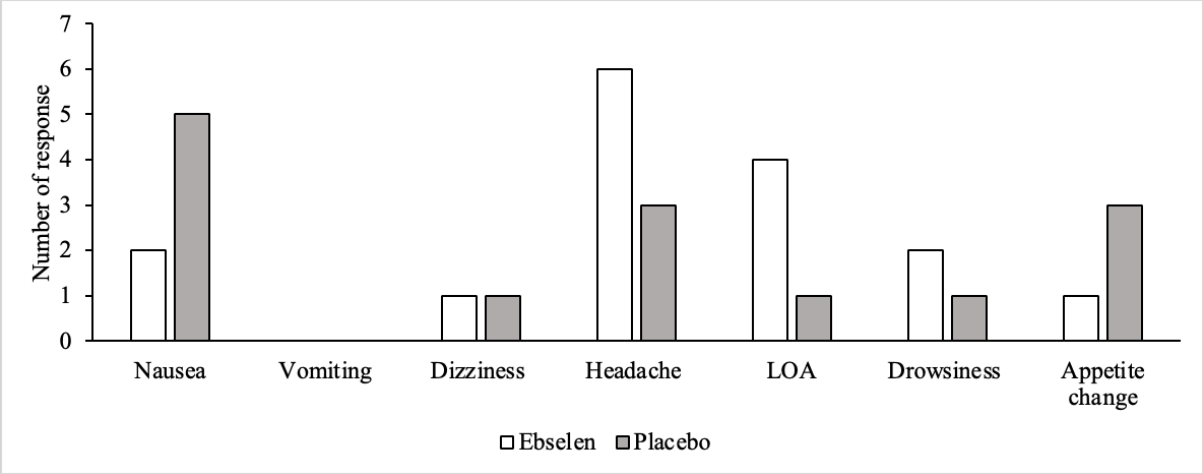


Figure 3.7. Frequency of adverse effects reported by the participants following ebselen or placebo treatment (n=51).

3.4 Discussion

There were no significant treatment effects of ebselen on FERT accuracy, which was the primary outcome of the study. Similarly, no significant treatment interactions were observed in other ETB parameters. Controlling the baseline values as covariates, a significant treatment interaction was only observed in beta values for fear in the FERT. There was a significantly lower beta value for fear in ebselen than in placebo groups, indicating a bias towards fearful responding. Interestingly, parallel to this biased response towards fearful emotion, over the seven days of the study, a trend to a lower reduction in GAD-7 scores was observed in the ebselen group relative to placebo-treated participants. This raises the possibility that ebselen may have mild anxiogenic properties in this patient group (see below) which offset the small reduction in anxiety symptomatology expected in the first week of a placebo-controlled study with a high level of clinical support.

There were no significant differences between treatment groups in clinical measures of depression on the MADRS or QIDS-SR-16. The effect of lithium addition to antidepressant medication is usually regarded as requiring a number of weeks to be fully expressed clinically. However, in some controlled augmentation trials, beneficial effects of lithium addition on depression scores have been apparent after one week (for example, see Baumann et al. (1996)). There are also uncontrolled observations of rapid antidepressant responses to lithium addition in depressed patients (de Montigny et al., 1983). However, there was no evidence in this study for such an effect of ebselen.

The first study of the effect of ebselen on emotional processing in healthy volunteers demonstrated that ebselen given at a dose of 1800 mg over 24 hours increased the accuracy of recognizing happiness and disgust facial expressions (Singh et al., 2016). Similarly, another study conducted using a dose of 3600 mg over a similar period in healthy volunteers reported that ebselen significantly increased the accuracy of identifying a positive emotion (happiness) (Masaki, Sharpley, Cooper, et al., 2016). However, the effects of ebselen in shifting positive biases, as reported previously in healthy volunteers, were not replicated in the current study.

A possible explanation for these inconsistent findings might be due to the concomitant antidepressant medication which our participants were taking prior to ebselen/placebo addition. These antidepressant medications might have already caused the maximum possible shift in pharmacologically-induced positive bias in emotional face recognition; therefore adding ebselen might not have been able to increase this positive bias further. It is also possible that ebselen may work via a different antidepressant mechanism in MRD patients, for example, on reward processes, thereby causing negligible treatment effects in the emotional processing models used in the current study. A controlled comparison of ebselen and placebo in unmedicated depressed patients may provide additional insight into the impact of ebselen on

emotional processing in the depressed population. Another useful comparison is between MRD patients and healthy controls, which could inform us whether the antidepressant treatment, albeit ineffective, might have normalised the negative emotional processing typical of the depressed state. This will be addressed in Chapter 4.

As noted above, the ebselen group showed significantly more false alarms towards fearful facial stimuli than the placebo group. This finding was consistent with the effects of ebselen on the GAD-7 anxiety measure, suggesting a possible anxiogenic-inducing property of this drug in depressed patients on antidepressants. The effects of ebselen towards measures of signal detection d' and beta were not evaluated in the previous studies involving healthy volunteers, so prior findings could not be used to compare or draw a conclusion. Acute treatment with standard antidepressants, such as citalopram, fluoxetine, and sertraline, has been shown to induce short-term anxiogenic effects in preclinical and clinical studies, perhaps mediated by indirect activation of 5-HT2C receptors (Bagdy et al., 2001; Birkett et al., 2011; Sinclair et al., 2009). If this is the case, the potential anxiogenic effects of ebselen might also be short-lived and expected to resolve in long term treatment. The negligible findings of ebselen effects in other tasks of ETB parameters (ECAT, EREC, EMEM, FDOT) corroborated the findings in healthy volunteers (Singh et al., 2016).

3.5 Conclusion

Ebselen, as an add-on treatment, did not alter emotional processing in clinically depressed patients on antidepressants. This might be due to normalised or maximised positive bias shifts in emotional processing by preceding antidepressant treatment. Another reason is ebselen might not act therapeutically through changing emotional processing bias in this population. The next chapter addresses the differences in emotional processing between the

depressed patients taking antidepressants at baseline (before the ebselen treatment) in the above study and age- and sex-matched healthy volunteers.

CHAPTER 4

4. Emotional Processing in Patients with Medication-Resistant Depression and Healthy Controls

4.1 Introduction

To explore the baseline emotional processing of MRD patients before treatment allocation to ebselen or placebo, an additional study was conducted. The reason for this assessment is to determine whether current antidepressant treatment, although clinically ineffective, might nevertheless normalise negative biases in emotional processing in depressed patients. Besides, the comparison also allows us to detect any residual negative biases in the patient's group. This may help address the reasons for the maintenance of MDD in clinically depressed patients on currently ineffective antidepressants.

Many studies reported that acute antidepressant treatment with various antidepressants produces a range of positive emotional shifts in both healthy participants and depressed patients. The shift can favour more positive biases, including faster RT for categorizing positive words, higher recall rates of positive words and more accurate happy facial expression recognitions (Harmer et al., 2003; Harmer et al., 2009; Harmer et al., 2004). Also, reduced negative biases, characterised by the reduction in recognizing negative facial expressions, is another form of emotional bias shift reported with conventional antidepressant treatments (Harmer et al., 2004).

The evidence of emotional processing biases in medicated patients who are not responsive to antidepressant therapy is scarce. A previous study comparing three groups of healthy volunteers and unmedicated and medicated depressed patients to assess selective

attention reported that healthy volunteers and medicated depressed patients had higher selective attention towards positive visual stimuli compared to unmedicated depressed patients. The differences between medicated depressed patients and healthy volunteers were negligible. The findings indicated that emotional processing in selective attention towards positively- or negatively-valanced visual stimuli might have been normalised by antidepressant treatment (Wells et al., 2014). However, the above study did not stratify the medicated depressed patients into responsive or remitted and treatment-resistant.

A systematic review and meta-analysis assessing facial emotional recognition in depressed patients compared to healthy controls reported significant deficits in recognizing all basic emotions except sad. Further analysis found no significant effects of treatment status (medicated vs unmedicated) on happiness or sadness (Dalili et al., 2015). Again, the effects of the response status of medicated patients on facial recognition were not evaluated. Response status is an essential confounding factor for antidepressant effects on emotional processing. Also, other emotional processing domains were not evaluated. Given the lack of studies addressing the differences between healthy volunteers and clinically depressed patients with inadequate response to standard antidepressant therapies, this study aimed to fill a gap of knowledge in the literature. Evaluations of other emotional processing domains can also give more insight into the state of emotional processing in this subset of patients.

4.2 Method

The complete description has been given in Chapter 2 (refer to subsection 2.2. Study 1B). Healthy controls were required to be free from any current or history of major psychiatric disorders and were screened using SCID-5 and HAM-D. Generally, depressed patients not responsive to current antidepressants were compared with healthy volunteers at baseline in

terms of emotional processing on the ETB battery. Secondary outcomes included MADRS, QIDS-SR-16, and GAD-7. Descriptive data are presented as the means and standard error of means and analysed using an independent *t*-test for the group comparisons. A categorical variable of sex was presented as count and percentage and analysed using a chi-squared test to compare between groups. We utilised a two-way ANOVA with emotion or valence as a within-subject factor and group (patients vs healthy volunteers) as a between-subject factor for all ETB tasks except the FDOT in which three-way (group x valence x condition) and two-way ANOVAs were utilised. A significant interaction was followed up with post-hoc independent and paired *t*-tests. Correlations between clinical measures of depression (HAM-D, MADRS, QIDS-SR-16) and anxiety (GAD-7) and ETB parameters were conducted using a Pearson's product-moment correlation if there was a significant two-way ANOVA. A p-value of <0.05 was considered statistically significant.

4.3 Results

Fifty-four patient who were still clinically depressed and not responsive to their current antidepressant treatments and 45 healthy volunteers were included in the final analysis. There were no significant differences in age ($t(97) = -1.097$, $p = 0.275$) or sex ($p = 0.635$ (χ^2)) between the two groups. As expected, patients had significantly higher scores on the HAM-D ($t(59.6) = 30.96$, $p < 0.001$), MADRS ($t(60) = 25.15$, $p < 0.001$), QIDS-SR-16 ($t(84.4) = 18.24$, $p < 0.001$), and GAD-7 ($t(61) = 12.81$, $p < 0.001$) (Table 4.1).

Table 4.1. Characteristics and clinical measures of participants included in the study (n=99).

Variables	Patients (n=54)	Healthy volunteers (n=45)
Age (years)	38.4 $\pm$ 14.20	41.7 $\pm$ 14.27

Sex	Female: 36 (66.7%); Male: 18 (33.3%)	Female: 32 (71.1%); Male: 18 (28.9%)
HAM-D	19.5 ± 4.3	0.7 ± 1.0
MADRS	27.0 ± 7.3	1.3 ± 1.7
QIDS-SR-16	18.4 ± 5.2	3.3 ± 2.8
GAD-7	10.1 ± 5.0	0.9 ± 1.3

Continuous variables are presented as means + standard error of means and categorical variables are presented as counts and percentages.

4.3.1 Differences in emotional processing between patients with MRD and healthy volunteers

There were no significant group interactions in the FERT task for all parameters, including accuracy ($F_{4.3,404.7} = 1.30$, $p = 0.266$, partial $\eta^2 = 0.014$), RT ($F_{1.7,155.9} = 0.39$, $p = 0.636$, partial $\eta^2 = 0.004$), misclassification ($F_{2.6,239.7} = 1.16$, $p = 0.322$, partial $\eta^2 = 0.012$), d' ($F_{4.1,378.1} = 1.42$, $p = 0.225$, partial $\eta^2 = 0.015$), and beta ($F_{2.2,207.1} = 0.53$, $p = 0.609$, partial $\eta^2 = 0.006$).

There was a significant group interaction in the ECAT RT ($F_{1,97} = 6.44$, $p = 0.013$, partial $\eta^2 = 0.062$). A follow-up paired *t*-test showed quicker responses towards positive (0.916 ± 0.044) than negative valence words (1.089 ± 0.056) in healthy controls ($t(44) = -2.936$, $p = 0.005$) (Figure 4.1). However, no significant differences in RT for categorizing positive (1.013 ± 0.031) and negative self-referent words (1.033 ± 0.026) were observed in the patient group ($t(53) = -0.835$, $p = 0.407$). A follow-up independent *t*-test showed a trend towards quicker responses towards positive valence words ($t(97) = 1.817$, $p = 0.072$) in healthy controls (0.916 ± 0.295) than patients with MRD (1.013 ± 0.231). No significant differences between groups

in response time for categorizing negative valence words ($t(62.7) = -0.903$, $p = 0.370$). No significant group interaction in the ECAT accuracy was observed ($F_{1,97} = 1.68$, $p = 0.198$, partial $\eta^2 = 0.017$).

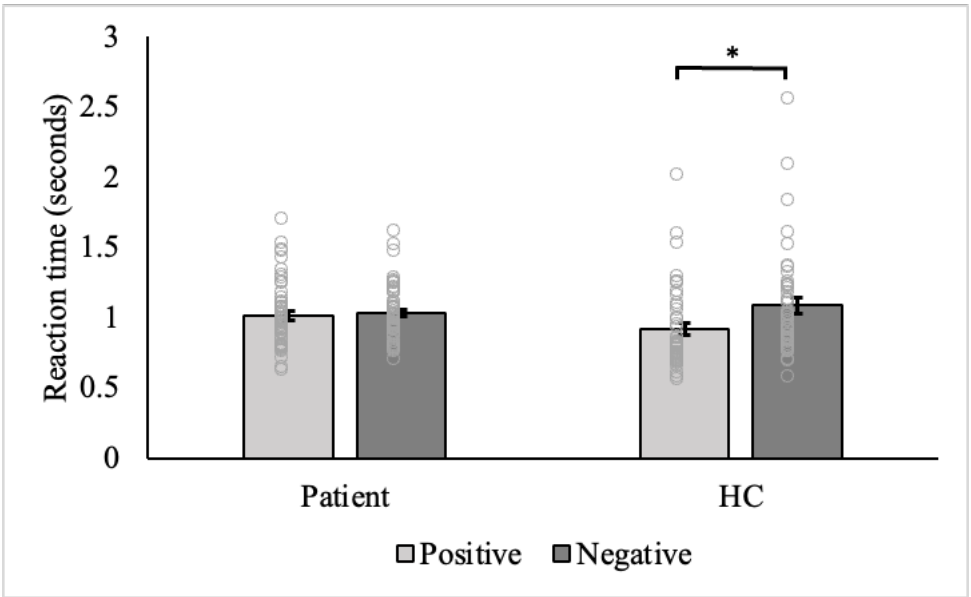


Figure 4.1. Means (± SEMs) reaction time in the ECAT task. There was a significant two-way ANOVA (F1,97 = 6.44, p = 0.013, partial η2 = 0.017). A follow-up paired *t*-test showed healthy controls were faster to respond towards positive self-referent words (0.916 ± 0.044) than negative self-referent words (1.089 ± 0.056; t(44) = -2.936, p = 0.005*).

There was a significant group interaction in the EREC accuracy ($F_{1,91} = 7.90$, $p = 0.006$, partial $\eta^2 = 0.080$). A follow-up independent *t*-test showed higher accuracy in recalling positive valence words ($t(91) = -2.332$, $p = 0.022$) in healthy volunteers (5.84 ± 0.376) than in patients (4.71 ± 0.314). The number of negative valence word recalled was higher in patients than in healthy controls but did not reach statistical significance ($t(91) = 0.745$, $p = 0.458$). A follow-up independent *t*-test showed significantly higher positive than negative valence word recalls in healthy volunteers ($t(44) = 6.159$, $p = <0.001$) and patients ($t(47) = 2.208$, $p = 0.032$). No

significant group interaction in the incorrect word recall in the EREC task was observed ($F_{1,91}$ = 0.51, p = 0.479, partial η^2 = 0.006).

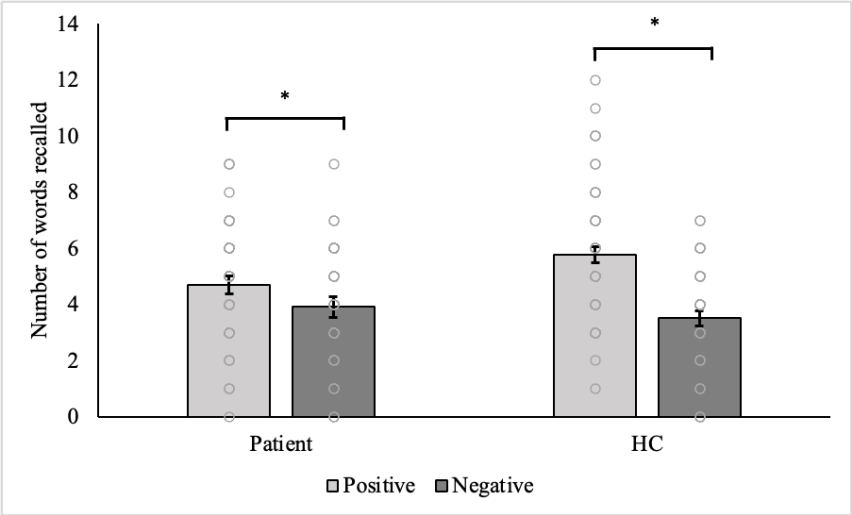


Figure 4.2. Means (± SEMs) number of positive and negative self-referent words recalled in the EREC task. There was a significant two-way ANOVA ($F_{1,91}$ = 7.90, p = 0.006, partial η^2 = 0.080). A follow-up paired *t*-test showed higher accuracy in recalling positive than negative self-referent words in healthy volunteers (*t*(44) = 6.159, p = <0.001) and patients (*t*(47) = 2.208, p = 0.032).

There were no significant group interactions for the EMEM task parameters, including accuracy ($F_{1,97}$ = 1.46, p = 0.230, partial η^2 = 0.015), RT ($F_{1,97}$ = 0.004, p = 0.951, partial η^2 = 0.000), misclassification ($F_{1,97}$ = 1.46, p = 0.230, partial η^2 = 0.015), d' ($F_{1,97}$ = 0.469, p = 0.495, partial η^2 = 0.005), and beta ($F_{1,97}$ = 0.31, p = 0.578, partial η^2 = 0.003).

In the FDOT task, there were no significant interactions in the three-way ($F_{1,97}$ = 0.80, p = 0.373, partial η^2 = 0.008) and two-way ANOVA between valence and group ($F_{1,97}$ = 0.64, p = 0.428, partial η^2 = 0.007), condition and group ($F_{1,97}$ = 0.05, p = 0.822, partial η^2 = 0.001), and valence and condition ($F_{1,97}$ = 2.82, p = 0.097, partial η^2 = 0.028).

4.3.2 Correlations between clinical measures and emotional testing battery parameters

A significant positive correlation was found between MADRS scores and the ECAT RT for categorising positive words ($r(54) = 0.30$, $p = 0.03$) (Figure 4.3, Table 4.2). Similarly, a positive correlation was found between MADRS scores and the ECAT RT for categorising negative self-referent words in patients, but the result did not reach a statistical significance ($r(54) = 0.23$, $p = 0.095$) (Figure 4.3). There was a trend towards a positive correlation between HAM-D scores and the ECAT RT for categorising positive self-referent words in patients ($r(54) = 0.23$, $p = 0.094$) (Figure 4.4).

No significant correlations were observed between clinical measures of HAM-D, MADRS, QIDS-SR-16, and GAD-7 and the EREC task accuracy (Table 4.2). However, we found a trend towards a negative correlation between GAD-7 scores and the accuracy of recalling positive self-referent words in patients ($r(48) = -0.25$, $p = 0.09$) (Figure 4.5).

Table 4.2. Correlations between clinical measures of HAM-D, MADRS, QIDS-SR-16, and GAD-7 and reaction times of the ECAT and accuracy of the EREC tasks.

	HAM-D	MADRS	QIDS-SR-16	GAD-7
ECAT reaction time				
Healthy controls				
Positive	$r(45) = -0.09$, $p = 0.55$	$r(45) = -0.17$, $p = 0.27$	$r(45) = 0.02$, $p = 0.90$	$r(45) = -0.12$, $p = 0.42$

Negative	$r(45) = 0.09$, $p = 0.56$	$r(45) = -0.12$, $p = 0.44$	$r(45) = 0.04$, $p = 0.80$	$r(45) = 0.14$, $p = 0.35$
Patients				
Positive	$r(54) = 0.23$, $p = 0.09$	$r(54) = 0.30$, $p = 0.03$*	$r(54) = 0.14$, $p = 0.30$	$r(54) = 0.17$, $p = 0.22$
Negative	$r(54) = 0.06$, $p = 0.64$	$r(54) = 0.23$, $p = 0.095$	$r(54) = 0.13$, $p = 0.34$	$r(54) = -0.003$, $p = 0.99$
EREC accuracy				
Healthy controls				
Positive	$r(45) = 0.05$, $p = 0.77$	$r(45) = 0.01$, $p = 0.97$	$r(45) = -0.09$, $p = 0.56$	$r(45) = -0.11$, $p = 0.47$
Negative	$r(45) = -0.06$, $p = 0.70$	$r(45) = 0.02$, $p = 0.88$	$r(45) = -0.08$, $p = 0.62$	$r(45) = -0.04$, $p = 0.81$
Patients				
Positive	$r(48) = -0.09$, $p = 0.54$	$r(48) = -0.07$, $p = 0.62$	$r(48) = 0.01$, $p = 0.93$	$r(48) = -0.25$, $p = 0.09$
Negative	$r(48) = 0.02$, $p = 0.90$	$r(48) = -0.12$, $p = 0.40$	$r(48) = 0.07$, $p = 0.63$	$r(48) = -0.01$, $p = 0.95$

*p-value < 0.05

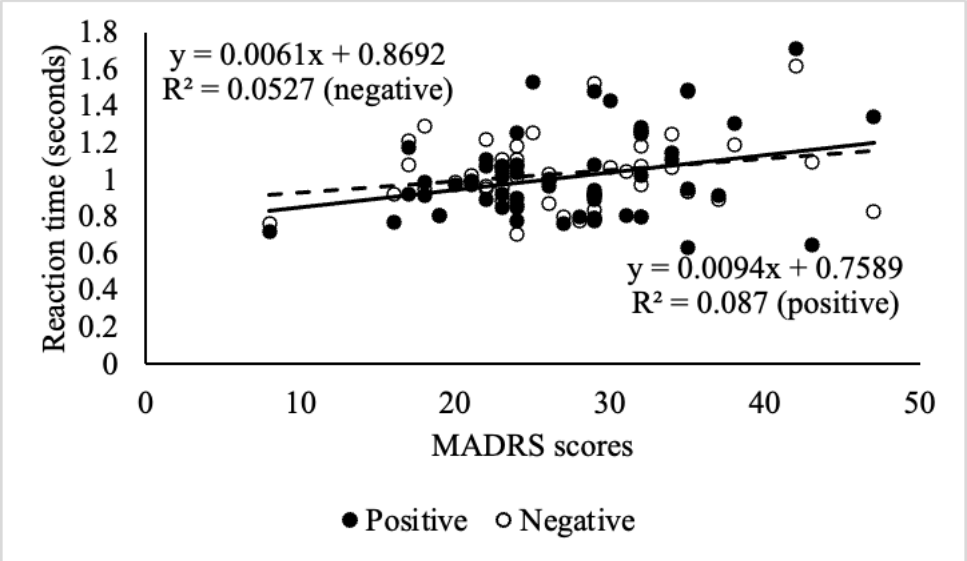


Figure 4.3. Correlation between MADRS scores and reaction time for categorizing positive ($r(54) = 0.30$, p = 0.03*) and negative ($r(54) = 0.23$, p = 0.095) self-referent words in the patient group.

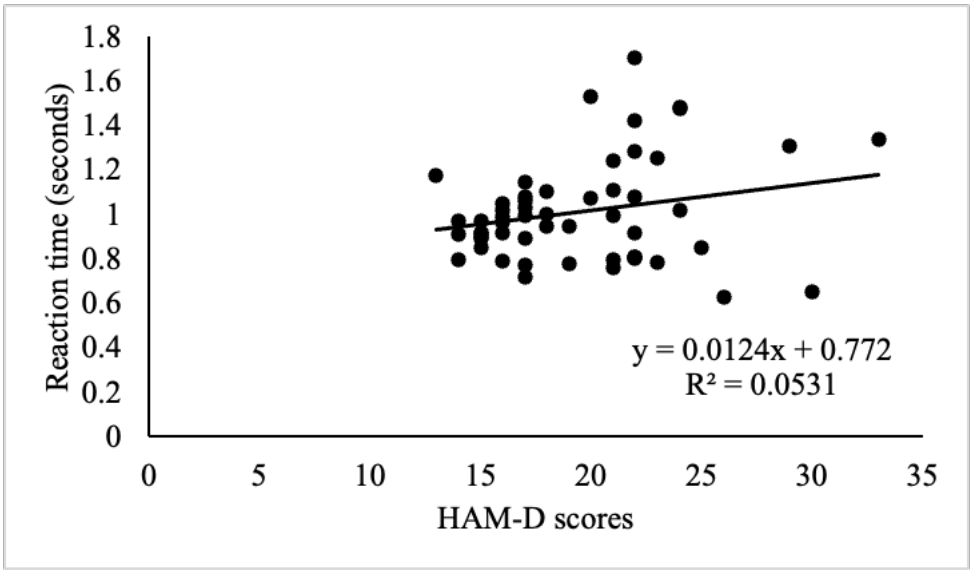


Figure 4.4. Correlation between HAM-D scores and reaction time for categorizing positive self-referent words ($r(54) = 0.23$, p = 0.09) in the patient group.

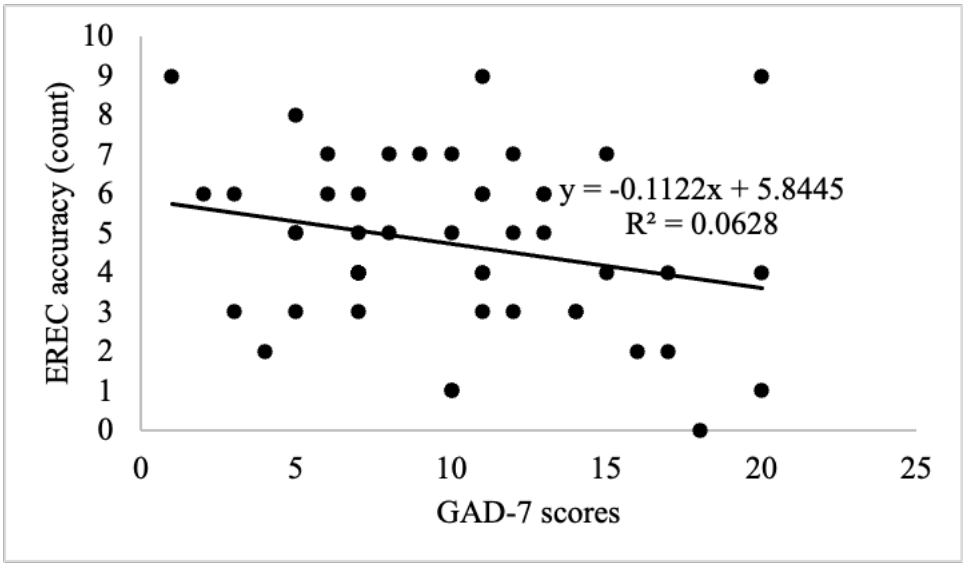


Figure 4.5. Correlation between GAD-7 scores and EREC accuracy (counts) for categorizing positive self-referent words ($r(48) = -0.25$, $p = 0.09$) in the patient group.

4.4 Discussion

Healthy volunteers had significantly quicker responses towards positive than negative self-referent words. However, no such difference was found in the MRD patient group. There were also significant differences between patients and healthy volunteers in recalling self-referent words, with healthy volunteers recalling more positive words than patients. The number of negative words recalled was numerically lower in healthy volunteers than in patients, but no statistical significance in this difference was observed. No significant differences were observed in the FERT, FDOT, and EMEM tasks. The outcomes of the current study indicate that some negative biases in depressed patients on clinically ineffective antidepressants still exist. Indeed, it is possible that such persistent negative biases play a role in maintaining the depressive disorder (Pringle & Harmer, 2015).

A study evaluating the effects of a single dose of reboxetine (a noradrenergic reuptake inhibitor) in depressed patients reported significant effects of reboxetine in reducing RT for

categorizing positive self-referent words and increasing recall of positive self-referent words and recognizing happy facial expressions (Harmer et al., 2009). Repeated administration of the SSRI citalopram and reboxetine for seven days in healthy participants also resulted in a positive bias shift, evidenced by reduced accuracy of identifying negative faces and increased positive self-referent word recall relative to negative words (Harmer et al., 2004). However, negative biases on emotional categorization and memory of self-referent words are still apparent in our patient population, suggesting that ineffective antidepressant treatment might not reverse the negative biases seen in these domains of emotional processing.

Interestingly, our study did not find any significant differences between medicated depressed patients and controls in the emotional facial recognition task, which corroborated the study of Wells et al. (2015). Therefore, current antidepressant treatments appeared to normalise the processing of emotional visual stimuli, even when clinical therapeutic responses were not obtained. Previous studies proposed the potential role of the emotional testing battery in predicting later clinical response of antidepressants. Two studies reported that the early changes in the FERT (1–2 weeks) predicted clinical responses at 4–6 weeks (Browning et al., 2019; Tranter et al., 2009). Intriguingly, our current findings found no significant differences in facial emotional recognition between depressed patients with inadequate responses towards antidepressants and healthy controls. The reason for this apparent discrepancy is not clear from this present data. It is possible that responders to antidepressant medication might have earlier, more rapid changes in emotional bias than non-responders.

Previous studies of ebselen in healthy volunteers reported that effects on emotional processing bias were apparent in the recognition of the facial expression domain (Masaki, Sharpley, Cooper, et al., 2016; Singh et al., 2016). Ebselen did not affect the other components of the ETB including emotional categorization of and memory of self-referent words and

attentional vigilance (Singh et al., 2016). It is possible that these latter domains of emotional processing are not sensitive to ebselen in either healthy participants or depressed patients.

There was a significant positive correlation between the clinical measure of MADRS and the ECAT RT for positive self-referent words and a trend towards significance for negative words in the same direction. Another correlation with a trend towards a positive correlation found was between HAM-D and the ECAT RT for positive self-referent words. The correlation patterns between clinical measures of depression and the ECAT RT might indicate a general psychomotor slowing in patients with depression.

Our findings indicate that negative biases in self-referential emotional judgement and memory persist in clinically depressed patients taking antidepressant medication. The outcomes suggest that emotional encoding and memory continues to be a sensitive measure of emotional processing bias in the presence of clinical depression and ongoing antidepressant treatment. Neurobiological changes contributing to negative memory biases in depressed patients might be attributable to suppressed hippocampal neurogenesis, inhibition of midbrain dopaminergic neurons, and over-sensitization of the amygdala (Dillon & Pizzagalli, 2018). Although antidepressants have been reported to reverse structural changes in the hippocampus (Boldrini et al., 2013), the evidence regarding the differential effects of responder status is still scarce.

The study has several limitations. We did not have measures of emotional processing in the depressed patients prior to their antidepressant treatment. Therefore, we cannot exclude the possibility that these depressed patients may have lacked a negative biases in facial expression recognition even before they received antidepressants. Further, the case-control design of the study only allowed us to assess the association but not the causality of the persistent negative biases in the MRD participants. Therefore, a longitudinal study evaluating

emotional processing at several time points, including at baseline, is recommended to assess the causality of negative emotional processing biases in depressed patients with inadequate responses towards their current antidepressant treatment. Perhaps such patients are less likely to respond to conventional antidepressants. The sample size was relatively small and the study may, therefore, have lacked the power to detect particularly subtle differences between patients and healthy controls in some aspects of emotional processing. A larger sample size in a future study will allow for subgroup analysis of the effects of individual or specific classes of antidepressants on emotional processing. For instance, SSRIs and SNRIs were reported to have superior positive effects in tasks of memory recall than tricyclic antidepressants (Rosenblat et al., 2015).

4.5 Conclusion

Negative biases in facial expression recognition in depressed patients appear to be normalised by current antidepressant treatment even when the therapeutic response is lacking. However, negative biases in emotional categorisation and emotional memory seems to persist. Targeting these persistent negative biases could represent potential treatment avenues for developing psychological and pharmacological approaches for patients with difficult-to-treat depression. One example of such an agent is the rapidly acting antidepressant, ketamine, which modulates neurotransmission in the medial prefrontal cortex, thereby reducing previously acquired negative biases (Stuart et al., 2015).

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CHAPTER 5

5. The Effects of Ebselen on Brain Neurochemistry on Medication-Resistant Depression Patients

5.1 Introduction

The inositol depletion hypothesis is one of the most well-known proposed mechanisms suggested to underpin lithium's therapeutic action (Berridge et al., 1989a). The hypothesis originated from a pre-clinical model which reported that lithium reduced brain inositol levels to about one-third while increasing inositol-1-phosphate, a source of inositol in the PI second messenger cycle. The reduction in inositol level by lithium is partly attributable to its inhibitory effect on the IMPase enzyme. Lithium displaces magnesium near the active catalytic site of IMPase, preventing phosphate release from inositol-1-phosphate and thus reducing inositol production (Harwood, 2005). A reduced inositol pool impairs various second messenger systems which utilise the PI-signalling pathway. Serotonergic receptors 5-HT2A and 5-HT2C are linked to the PI-signalling pathway (Antoniadou et al., 2015; Antoniadou et al., 2018). Repeated lithium treatment has been shown to abolish behavioural and molecular responses to challenge with the 5-HT2A receptor agonist DOI in a rodent model (Antoniadou et al., 2018).

While lithium is effective, due to its toxicity, which mandates careful drug monitoring, there is a need to find an equally effective but less toxic drug to replace lithium. Ideally, an alternative drug must have similar mechanisms of action to lithium but with fewer side effects. Singh and colleagues identified ebselen as an IMPase inhibitor (IC_{50} 1.5 μM)

with superior potency to lithium (IC_{50} 0.8 mM) (Singh et al., 2013). Further, in pre-clinical studies, ebselen crossed the blood-brain barrier to reduce brain inositol levels by around 30%, an effect quantitatively similar to that of lithium. Like lithium, ebselen treatment dampens 5-HT2A/2C receptor responses by inhibiting the PI-signalling pathway (Antoniadou et al., 2015; Antoniadou et al., 2018; Singh et al., 2013). Attenuations by ebselen of DOI-induced head twitch response and DOI-induced mRNA expression of *Arc*, *c-fos*, and *Erg2* in limbic and cortical areas support its inhibitory effects on 5-HT2A receptor mediated responses (Antoniadou et al., 2018; Singh et al., 2013). In addition to effects on 5-HT2A receptors, ebselen reduces the molecular effects of the 5-HT2C receptor agonist Ro 60-0175, as evidenced by attenuation of *Arc* and *c-fos* expression in cortical regions (Antoniadou et al., 2015).

Convincing outcomes in the pre-clinical studies lead to a translational effort to investigate the neurochemical effects of ebselen in humans. Two previous studies in healthy volunteers using proton MRS reported significant effects of ebselen to reduce myo-inositol levels in the ACC using two different dosing regimens (Masaki, Sharpley, Godlewska, et al., 2016; Singh et al., 2016).

In contrast, the effect of ebselen to lower MRS-measured glx (a combined measure of glutamate and glutamine) in ACC was apparent only with higher doses of ebselen (3600 mg over 24 hours) and a more powerful magnetic field of 7 tesla (Masaki, Sharpley, Godlewska, et al., 2016). The absence of similar changes in myo-inositol or glx levels in the occipital cortex suggests region-specific effects of ebselen (Masaki, Sharpley, Godlewska, et al., 2016; Singh et al., 2016). Interestingly, a positron emission tomography (PET) study in a rodent model reported a reduction in cortical synaptic glutamate availability following

ebselen treatment, potentially due to ebselen's inhibitory effects on glutaminase (Mota et al., 2020).

Given the significant effects of ebselen on these neurometabolites in healthy volunteers, an investigation in a clinical population is needed to demonstrate that ebselen produces target engagement in such populations. Brain neurochemistry in clinically depressed patients who are currently on their antidepressants may be different from healthy volunteers. Elucidating the mechanism of action of ebselen and defining appropriate dosing is important before clinical trials are conducted. Thus, the objective of the study was to investigate the effects of ebselen on myo-inositol, glx, and glutamate levels, and their correlations with serum selenium levels in depressed patients who were currently on antidepressant therapy but with an inadequate clinical response.

5.2 Methods

The detailed description of the MRS methodology can be found in Chapter 2. Generally, clinically depressed patients on their current antidepressants were randomised to ebselen 600 mg twice daily or matching placebo for seven days. Baseline and post-treatment proton MRS were conducted. The voxel of interest was ACC (Figure 5.1). LCModel was used to generate concentrations and spectra of myo-inositol, glx, and glutamate (Figure 5.2). Blood samples were taken at post-treatment visits, but only those who received ebselen had their selenium levels measured after we unblinded the treatment.

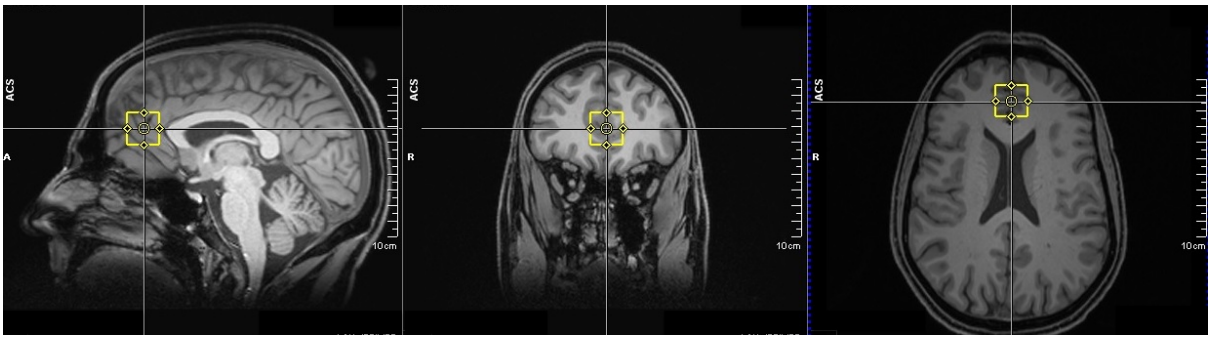


Figure 5.1. The voxel placements in the ACC from one of the participants in one of the visits.

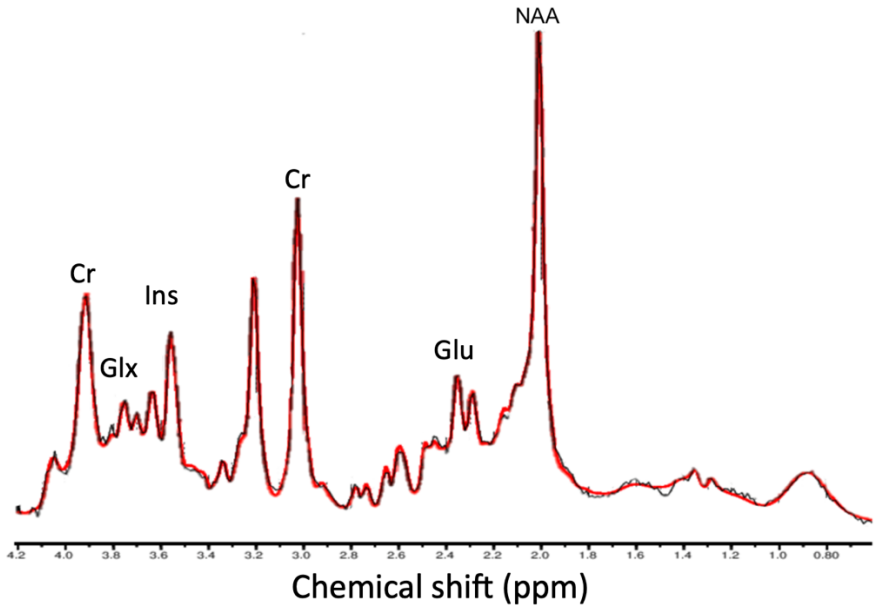


Figure 5.2. The representative spectrum obtained from the ACC of one of the participants in one of the visits. Cr: creatine; Glx: glutamate + glutamine; Glu: glutamate; Ins: myo-inositol; NAA: N-acetyl aspartate.

5.2.1 Statistical analysis

Categorical variables are presented in counts and percentages and analysed using a chi-squared test. Continuous variables are presented in the means and SEMs, and analysed

using an independent *t*-test for demographic data. The treatment effect of ebselen was analysed using a two-way ANOVA with time as a within-subject factor and treatment group as a between-subject factor. A significant treatment interaction was followed up with a post-hoc paired *t*-test analysis. The correlations between selenium levels and individual metabolite (myo-inositol, glx, glutamate) changes were tested using Pearson's product-moment correlation. A p-value of <0.05 was considered significant.

5.3 Results

Forty-eight participants completed the scans of both visits but five were excluded due to significant deviations from the expected spectra in one or both visits. The remaining 43 participants aged 20–66 (36.4 $\pm$ 1.9 years) were included in the final analysis (Table 5.1). There were no significant differences between groups in age ($t(41) = 0.347$, $p = 0.73$), sex ($p = 0.377$ (χ^2)), and number of failed antidepressant treatments ($p = 0.686$ (χ^2)). The means and SEMs of signal-to-noise ratio (SNR) were 43.4 $\pm$ 1.4 and 43.12 $\pm$ 1.9 for the first and second visits, respectively. The means and SEMs of linewidth for the first visit was 8.71 $\pm$ 0.30, and 8.79 + 0.33 for the second visit. The FWHM for the baseline and post-treatment visits were 0.05 $\pm$ 0.003 and 0.05 $\pm$ 0.002, respectively. There were no significant differences between these measures (all p-values > 0.05).

5.3.1 Effects of ebselen on brain neurochemistry

There was no significant interaction between time and treatment for the absolute concentration of total creatine ($F_{1,41} = 0.98$, $p = 0.328$, partial $\eta^2 = 0.023$), indicating that ebselen treatment did not affect the total creatine, which was used as a reference for

neurometabolites. There was a significant interaction between time and treatment for myo-inositol ($F_{1,41}$ = 4.32, p = 0.044, partial η^2 = 0.095), glx ($F_{1,41}$ = 6.79, p = 0.013, partial η^2 = 0.142), and glutamate ($F_{1,41}$ = 5.75, p = 0.021, partial η^2 = 0.123), in a two-way ANOVA. The post-hoc paired *t*-test showed significantly lower myo-inositol and glx levels in the ebselen group following treatment (myo-inositol: (*t*(23) = -2.274, p = 0.033; glx: *t*(23) = -3.591, p = 0.002), but no significant changes in the placebo group (myo-inositol: *t*(18) = 0.766, p = 0.454; glx: *t*(18) = 1.144, p = 0.268) (Figure 5.3). There was a reduction in glutamate level in the ebselen group, but the result did not reach statistical significance (*t*(23) = -1.982, p = 0.060).

Table 5.1. Characteristics of participants included in the study (n=43).

	Ebselen (n=24)	Placebo (n=19)
Age (years)	37.0 ± 2.4	35.7 ± 3.2
Sex	Female: 17 (39.5%); male: 7 (16.3%)	Female: 11 (25.6%); male: 8 (18.6%)
Number of failed antidepressant treatments (1-2 vs >2)	1–2 = 15 (34.9%); >2 = 9 (20.9%)	1–2 = 13 (30.2%); >2 = 6 (14.0%)
Changes in neurometabolites concentration following seven days treatment period		
Myo-inositol (ratio)	-0.32 ± 0.14*	0.12 ± 0.16
Glx (ratio)	-1.03 ± 0.29*	0.81 ± 0.71
Glutamate (ratio)	-0.55 ± 0.28	0.55 ± 0.38
Total creatine (μmol/g)	0.10 ± 0.13	-0.09 ± 0.16

Continuous variables are presented as means ± standard error of means and categorical variables are presented as counts and percentages. The concentrations of myo-inositol, glx, and glutamate used total creatine as a reference and were corrected for white and grey matter and cerebrospinal fluid. Total creatine is a sum of creatine and phosphocreatine concentration and is presented in absolute concentration (* p-value <0.05).

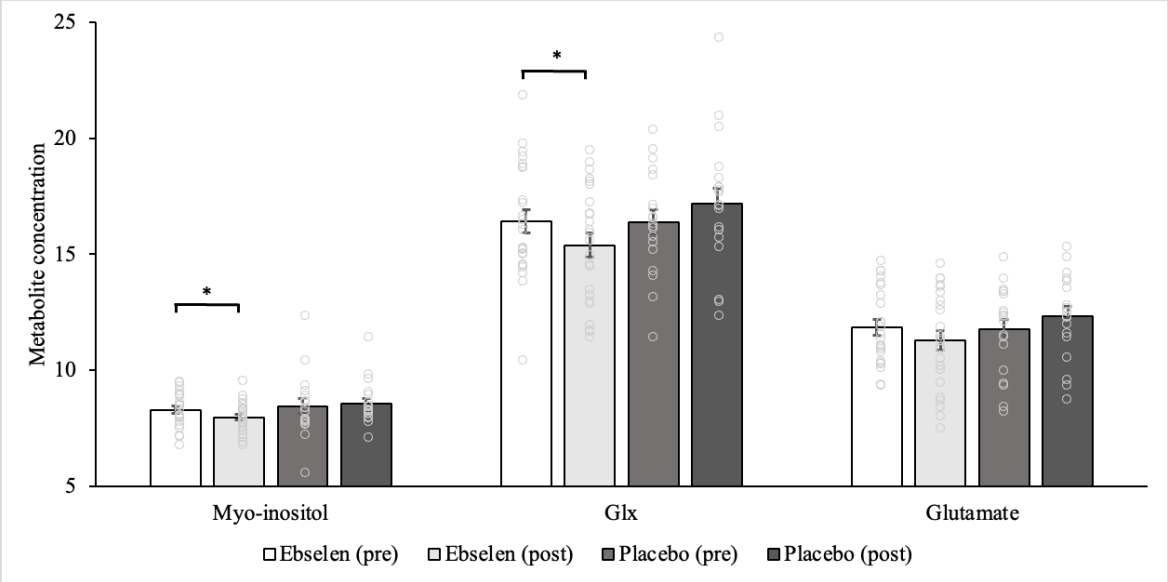


Figure 5.3. Concentrations of myo-inositol, glx, and glutamate (means ± SEMs) referenced to total creatine and phosphocreatine and corrected for white and grey matter and cerebrospinal fluid. *indicates the p-value of <0.05.

5.3.2 Correlation between selenium levels and brain neurochemistry

Blood samples were obtained from 22 out of 27 participants in the ebselen arm (due to technical difficulties in the case of three participants, and two participants did not wish to give blood). Three more samples from participants whose MRS spectra showed a significant

deviation were also excluded. A trend towards an inverse association was observed between selenium and a change in myo-inositol levels ($r(17) = -0.38$, $p = 0.11$), indicating that the higher the selenium levels, the greater the decrease in changes in myo-inositol levels. No significant correlations were observed between selenium and changes in glx ($r(17) = -0.07$, $p = 0.78$) and glutamate levels ($r(17) = -0.04$, $p = 0.89$).

5.4 Discussion

We found significant effects of ebselen in patients with MRD to lower myo-inositol and glx levels and a trend towards glutamate reduction in the ACC. However, no significant correlation was found between selenium levels and change in the levels of these three metabolites.

The reduction in myo-inositol levels in ACC by ebselen in this study corroborated findings in the two previous studies in healthy volunteers. In the first study, participants received either three doses of ebselen 600 mg or matching placebo given at a12-hour interval before a 3T MRS scan. The study reported a significant decrease in myo-inositol levels in the ACC but not in the occipital cortex (Singh et al., 2016). Similarly, another randomised, placebo-controlled study which employed higher doses of ebselen (three doses of 1200 mg given over 24 hours or matching placebo) with a 7T MRS scan reported similar findings (Masaki, Sharpley, Godlewska, et al., 2016). The region-specific inhibition of myo-inositol in a brain region linked to emotional processing, the ACC, might partly explain the antidepressant potential of ebselen observed in healthy volunteers, although we did not observe evidence of such an emotional processing effect in our current study in depressed patients.

The similar reductions in myo-inositol levels (about 4%) by ebselen in the two previous subacute dosing studies in healthy volunteers guided the dose used in our study of depressed patients (600 mg twice daily). Intriguingly, we found a similar reduction in myo-inositol levels (3.9%) by ebselen given in this dose over seven days. The decrease in myo-inositol might be explained by allosteric inhibition of IMPase by ebselen, as reported in the pre-clinical studies (Fenn et al., 2020; Singh et al., 2013). IMPase is an enzyme essential to convert inositol monophosphate to inositol in the PI-recycling pathway. IMPase inhibition reduces inositol levels, a substrate of PIP_2. Serotonergic receptors 5-HT2A and 5-HT2C are G-protein-coupled receptors linked to phospholipase C, which utilise the PI cycle (Antoniadou et al., 2015; Antoniadou et al., 2018). Upon receptor activation, phospholipase C catalyses the conversion of PIP_2 to DAG and IP_3. Reduced PIP_2 levels secondary to decreased inositol levels impair the synthesis of secondary messengers DAG and IP_3, inhibiting the PI-signalling pathway (Ramli et al., 2022). Impaired PI-signalling pathway may reduce the sensitivity of post-synaptic 5-HT2 serotonergic receptors, leading to disruption of a cortical feedback mechanism, resulting in enhanced serotonin synthesis and release from the presynaptic 5-HT neurons, thereby increasing post-synaptic serotonin neurotransmission at non-5-HT2 receptors (Antoniadou et al., 2018; Ramli et al., 2022). This could be the basis of a possible antidepressant action of ebselen in patients with depression.

It should be noted, however, that the effects of lithium itself on myo-inositol levels in human brain measured by MRS have been inconsistent. For example, Silverstone et al. (1999) found no effect of seven days' lithium treatment in healthy participants to alter basal myo-inositol levels but this treatment regime did decrease the turnover of myo-inositol metabolites following amphetamine challenge, suggesting an inhibitory effect on the PI cycle. Results of lithium treatment in patients with bipolar disorder are also inconsistent.

However, Davanzo et al. (2001) reported that seven days' lithium treatment in children with bipolar disorder lowered myo-inositol levels in ACC.

The reduction in glx following ebselen treatment is another finding observed in the current study that replicates the findings observed in healthy volunteers. Glx is a composite of glutamate and glutamine, and its reduction might be attributable to a decrease in both metabolites. A PET study in a rodent model reported that ebselen treatment reduced glutamate availability in the synapse (Mota et al., 2020). Decreased synaptic glutamate levels can be due to reduced glutamate synthesis and diminished glutamate release from synaptic vesicles (Mota et al., 2020; Porciúncula et al., 2001). Glutaminase is an enzyme essential for glutamate synthesis, and ebselen can inhibit glutaminase, resulting in decreased glutamate production. Hypothetically, the inhibition should cause the pooling of glutamine. Therefore, the expected value of glx should remain unchanged (Masaki, Sharpley, Godlewska, et al., 2016). However, glutamate contributes about 80% of glx, and a decrease in glutamate can have a significant impact on glx levels (Moriguchi et al., 2019). Also, as glutamine is a metabolite of glutamate, reduced glutamate release might eventually decrease glutamine production. Another potential factor is the inhibitory effect of ebselen on glutamate dehydrogenase (GDH), an enzyme which catalyzes the conversion of glutamate to glutamine (Yu et al., 2017).

Glutamatergic dysfunction, characterized by elevated brain glutamate levels, is a possible factor in the pathophysiology of depression, as excessive glutamate is toxic to the brain. While meta-analyses of MRS cortical glutamate in depressed patients tend to show lowered levels of glx, this may be attributable to concurrent antidepressant medication (Moriguchi et al., 2019). A recent proton MRS study comparing unmedicated chronically depressed MDD patients (mean duration of nine years) and healthy volunteers reported that

the glx and glutamate levels in the ventromedial prefrontal cortex/ACC region were higher in MDD patients than in healthy controls (Kantrowitz et al., 2021). Thus, antidepressant drugs may lower glx in cortex and it is of interest that ebselen apparently produces a similar effect.

No significant correlations were observed between selenium levels and changes in levels of neurometabolites of myo-inositol, glx, and glutamate. A previous study in healthy volunteers receiving ebselen also reported no significant correlation between selenium and change in myo-inositol levels (Singh et al., 2016). Phase I and II studies investigating the pharmacokinetic profile of ebselen reported positive correlations between blood ebselen (combined ebselen and its metabolites) and selenium levels, which justified the use of selenium levels in the current study as a surrogate marker of ebselen (Kil et al., 2017; Lynch & Kil, 2009). However, a possible explanation for the non-significant correlations between selenium levels and decrease in ACC myo-inositol might be due to differences in inhibitory effects on IMPase between ebselen and its metabolites, all of which might be captured by the selenium assay. Compared to the parent compound, these ebselen metabolites might have reduced or enhanced activity towards specific molecular targets.

The penetrability of ebselen across the blood-brain barrier is another factor to consider. A pre-clinical study reported that only 21% of ebselen reached the brain one hour after intravenous infusion of ebselen (Imai et al., 2001). The bioavailability of ebselen administered orally might be lower than the intravenous administration, so lower levels of ebselen crossing the blood-brain barrier is expected. Although the brain penetrability of ebselen has been confirmed in the pre-clinical study by measuring the inhibitory effect of ebselen on IMPase and inositol, this study utilised a brain hemisphere for quantification (Singh et al., 2013). We measured the myo-inositol levels in one specific region of ACC in

a clinical population. Ebselen might not be equally distributed between various brain regions, so the effects of ebselen on IMPase or glutaminase might differ between brain regions.

The study has several limitations. Proton MRS quantifies neurometabolites in the specific region as a whole (ACC in this study). Measuring neurometabolite levels in more specific compartments can give further insight into the mechanism of action of ebselen. For instance, we are particularly interested in myo-inositol levels in the post-synaptic neuron as it is an essential component of the PI-signalling pathway. However, high concentrations of myo-inositol are located elsewhere, including in astrocytes, where inositol functions as an osmolyte; inositol in this location will also be quantified by MRS. Regarding glutamate, we are interested in neuronally active glutamate in the synapse rather than the stored vesicular glutamate or that in intermediate metabolism. One potential neuroimaging technique that is applicable for future study in the clinical population of depressed patients is PET. The imaging technique can be used to assess the effect of ebselen on metabotropic glutamate subtype-5 receptor (mGluR5) binding. As previously stated, a PET study in a rodent model reported an increased mGluR5 radiotracer binding, suggesting decreased glutamate levels in the synapse (Mota et al., 2020). The translation of this finding to humans is yet to be carried out.

Also, the use of proton MRS at 3T in the current study does not allow precise quantification of glutamate and glutamine individually. Using a higher magnetic field MRS scan at 7T for example, allows the reliable separation of glx peak into glutamate and glutamine. Another potential limitation is the treatment duration. The treatment duration for the current study was one week, and longer-term effects of ebselen on neurometabolite levels remain to be elucidated. For example, an open-label study in adolescents with bipolar

depression reported that myo-inositol levels were significantly higher at day 42 compared to day seven of lithium treatment (Patel et al., 2006). Nevertheless, the current study gives some perspectives on the mechanistic aspects of ebselen in clinically depressed patients on concurrent antidepressant treatment.

5.5 Conclusion

Ebselen reduced myo-inositol and glx levels, potentially due to IMPase and glutaminase and GDH inhibition. This study replicated the neurochemical effects of ebselen treatment seen in healthy volunteers and in preclinical studies. This suggests that the dose of ebselen used in the present study (600 mg twice daily), was able to produce biochemical changes consistent with the target engagement that we sought to produce in MRD patients.

CHAPTER 6

6. The Effects of Ebselen on Resting-State Functional Connectivity in Medication-Resistant Depression Patients

6.1 Introduction

Altered resting-state functional connectivity in various networks has been reported in MDD patients (Javaheripour et al., 2021). A meta-analysis study assessing resting-state functional connectivity in MDD patients reported changes in functional connectivity, including hypoconnectivity within a network of frontoparietal (FPN) regions and between FPN and dorsal attention network (DAN) and hyperconnectivity within the default mode network (DMN) and between DMN and FPN (Kaiser et al., 2015). The deficits in external-oriented attention and excessive focus on internally-oriented attention may underlie negative rumination, which prevails in MDD.

Antidepressant treatments have been shown to normalise some resting state networks (RSNs). For instance, a meta-analysis study reported reduced functional connectivity of the DMN in medicated depressed patients compared to unmedicated depressed patients (Yan et al., 2019). A longitudinal study assessing the treatment effects of antidepressants in depressed patients found a normalisation of the posterior subnetwork of the DMN after 12 weeks of treatment (Li et al., 2013). Interestingly, studies investigating the potential use of baseline resting-state functional connectivity to predict future responder status reported distinct characteristics in terms of primary areas of abnormalities (Lui et al., 2011; Martens et al., 2022).

The previous chapters indicated that ebselen had no effects on emotional processing but produced reductions in key neurometabolites related to second messenger signalling and

glutamatergic neurotransmission. Investigating the effects of ebselen on resting-state functional connectivity might give some insight into the possible explanation for the contrasting findings in previous chapters. To date, there are no data in the literature on the effects of ebselen on resting-state functional connectivity. The current chapter aimed to investigate the effects of seven days' treatment with ebselen on changes in resting-state functional connectivity in MRD patients.

6.2 Methods

A detailed description of the resting-state functional connectivity methodology can be found in Chapter 2. Generally, clinically depressed patients on their current antidepressants were randomised to ebselen 600 mg twice daily or matching placebo for seven days. Baseline and post-treatment resting state fMRIs were conducted. Pre-processing of individual resting state MRI data was conducted, which included brain extraction of structural and functional data, correction for any movement, unwarping the EPI data using the field map data, co-registration of the EPI data to the structural data, and normalization of the data into MNI standard space using linear and non-linear registrations. We also extracted grey matter volume using FSLVBM and included it as a covariate for analysis to account for interindividual variability in structural data.

Two types of analysis were conducted; seed-based functional connectivity analysis and ICA. The former method was the region of interest-driven, while the latter was data-driven. Therefore, performing both these methods gave more perspectives on the effect of ebselen treatment on changes in resting-state functional connectivity relative to placebo. Pregenual ACC was chosen as a seed region to investigate any functional connectivity with other regions. Also, changes in resting-state functional connectivity between this region and

other brain regions were used to correlate with changes in neurometabolite concentrations of myo-inositol, glx, and glutamate. The ICA was another analysis conducted using MELODIC to identify any possible networks that were affected by ebselen treatment. The permutation-based method (5000 permutations) with Threshold-Free-Cluster-Enhancement and a family-wise-error was used to test the difference between groups. The significant values for the seed-based analysis were only corrected for the number of *t*-tests conducted (two tests as there were two contrasts): corrected p-value = 1 – 0.05/2 = 0.975, as we only analysed one seed region. The significant values for ICA were corrected for multiple comparisons using the following formula: corrected p-value = 1 – 0.05/(total number of identified RSNs x 2 *t*-tests) = 0.999.

6.3 Results

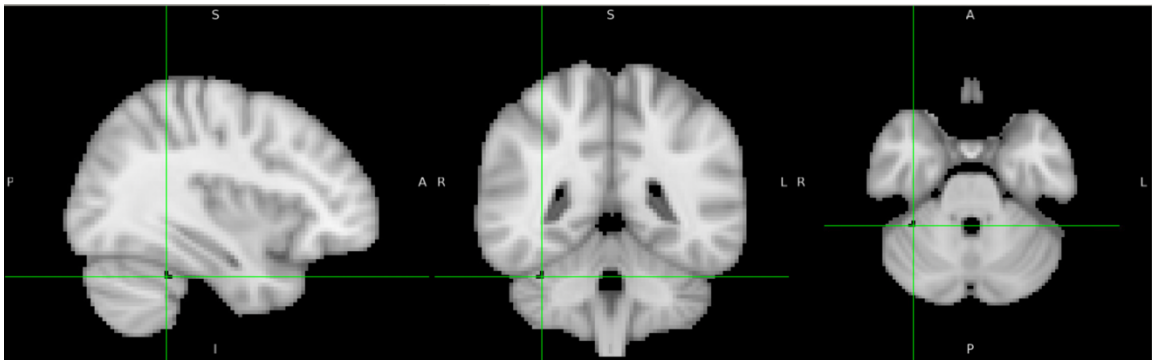
Forty-five participants aged 18–66 (37.1 $\pm$ 2.0 years) were included in the final analysis. There were no significant differences between groups in age ($t(43) = 0.007$, $p = 0.994$), sex ($p = 0.371$ (χ^2)), number of failed antidepressant treatments ($p = 0.944$ (χ^2)), and baseline HAM-D ($t(43) = 0.213$, $p = 0.833$).

6.3.1 Seed-based analysis of changes in resting-state functional connectivity between pregenual ACC and other brain regions and its correlations with neurometabolite changes in ACC

There were greater changes in resting-state functional connectivity between pregenual ACC and cerebellum in the ebselen group compared to the placebo group (the cerebellum cluster consisted of only four significant voxels). However, the difference in changes in resting-state functional connectivity became negligible after corrections for multiple comparisons (Figure 6.1). No correlations were observed between groups in changes of resting-state functional connectivity between the pregenual ACC and other brain

regions and changes in neurometabolite concentrations of myo-inositol, glx, and glutamate in ACC.

A.



B.

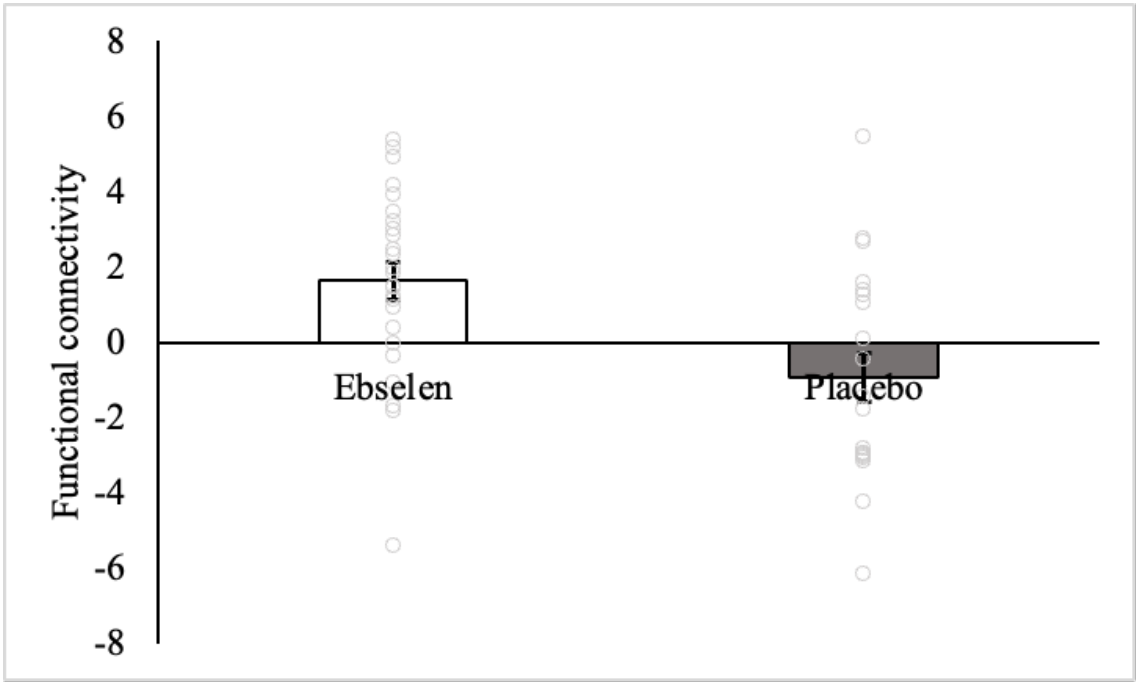


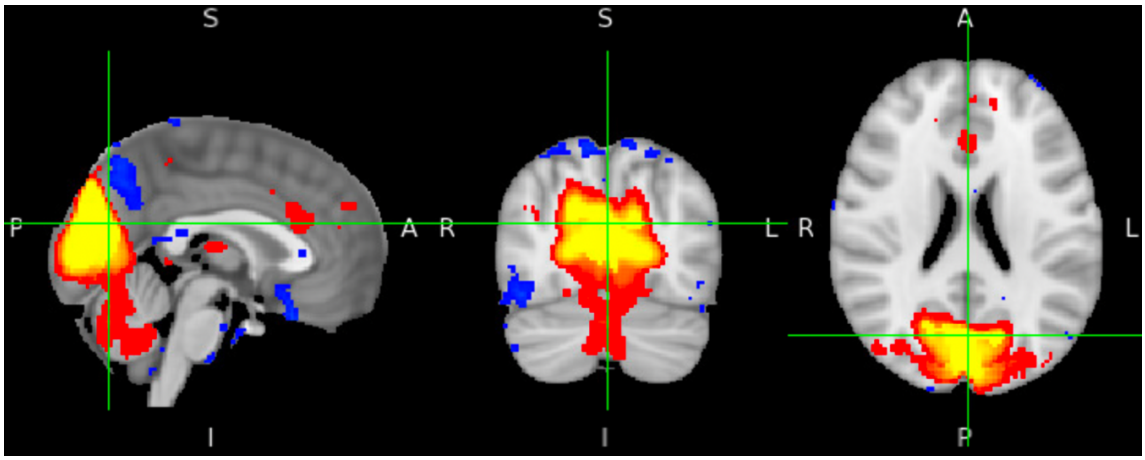
Figure 6.1. Changes in resting-state functional connectivity between pregenual ACC and cerebellum. A. The green colour area in the cerebellum represents the cluster (four voxels in total) for which the ebselen group had significantly higher changes in resting-state functional connectivity with pregenual ACC compared to the placebo (the most significant voxel location, the cursor coordinate: MNI 27, 41, 21). B. Parameter estimates of the

changes in resting-state functional connectivity between pregenual ACC and cerebellum in ebselen and placebo groups at a threshold of 0.95 (uncorrected for two *t*-tests).

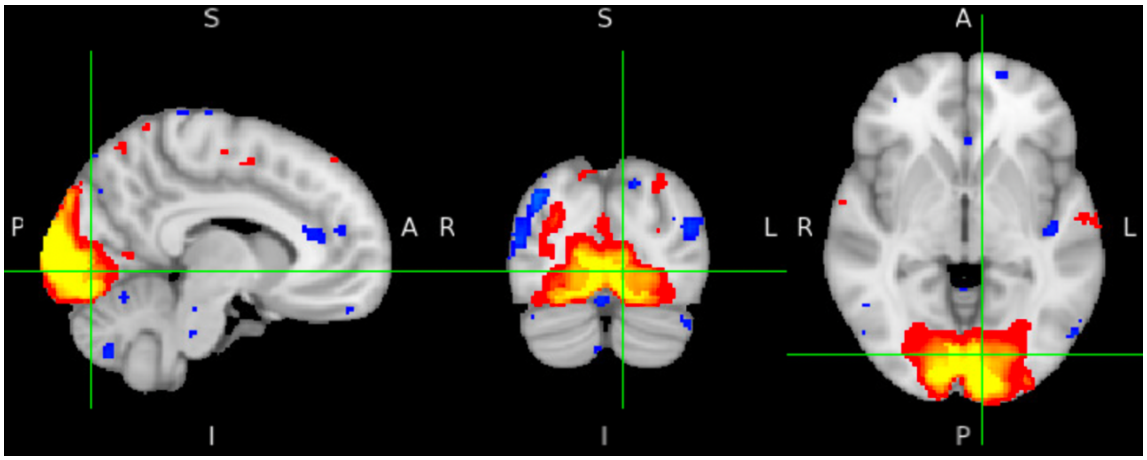
6.3.2 Independent Component Analysis (ICA)

Each participant's datasets were quality-checked. We excluded three participants who had movement > 2.4 mm in translation along x, y, or z planes or 3° (equivalent to 0.052 radians) rotation in any direction (pitch, roll, or yaw) (Sankar et al., 2021). Out of 25 ICs extracted from the temporal concatenation, 19 components were identified as RSNs of interest corresponding to previously identified canonical RSNs (Martens et al., 2022; Smith et al., 2009; Yeo et al., 2011). Six other components were noise (physiological and head movement), which FIX failed to eliminate. All the identified RSNs are presented in Figure 6.1.

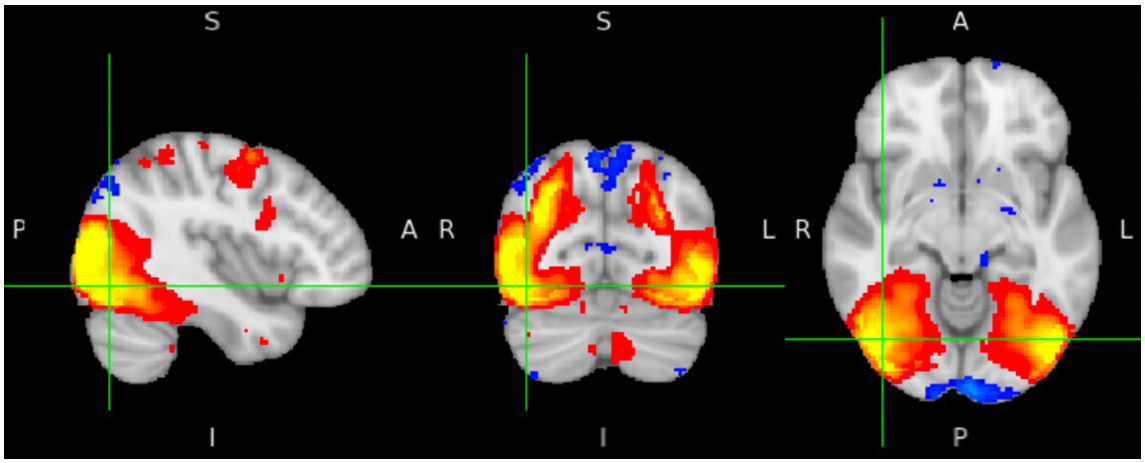
Visual networks



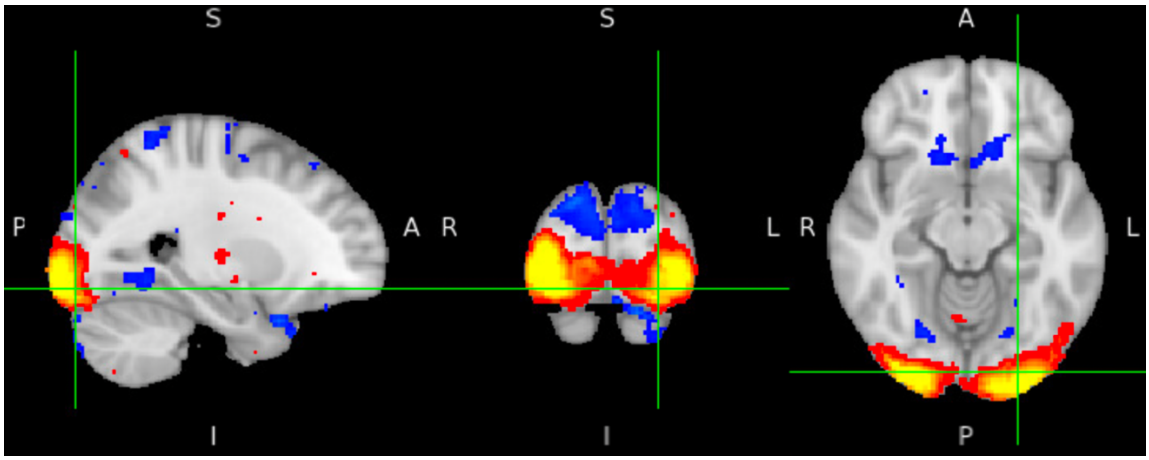
Visual: MNI 0, -71, 22



Visual medial: MNI -8, -81, -3

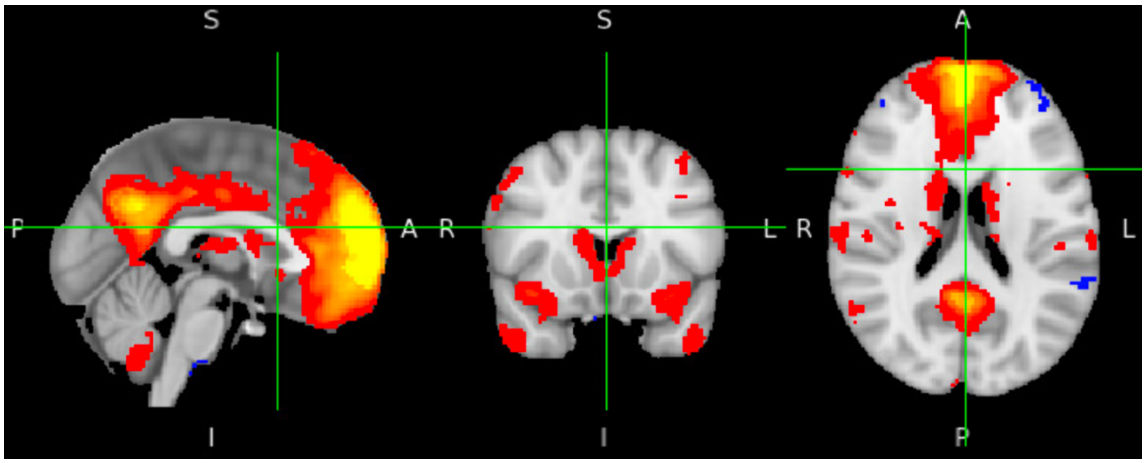


Visual lateral: MNI 41, -72, -9

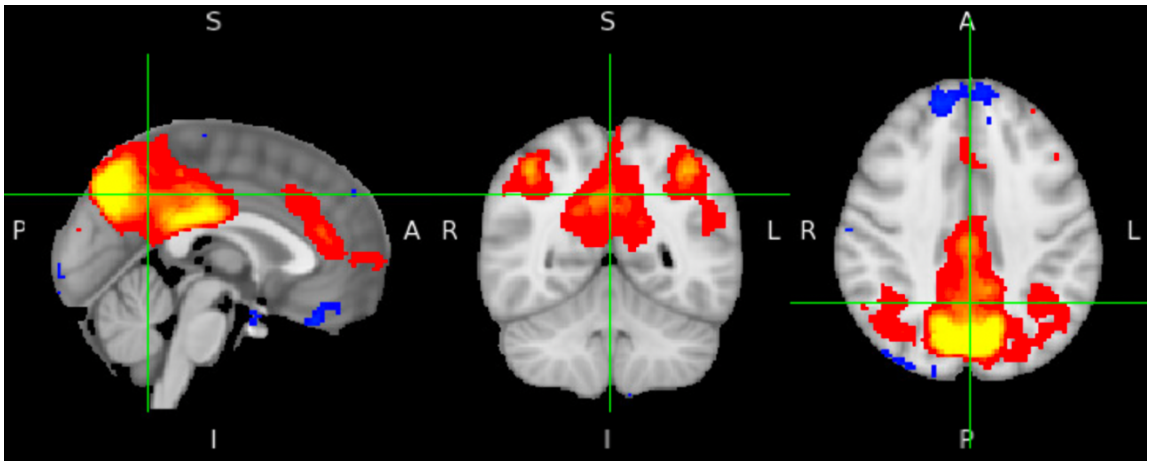


Visual lateral: MNI -25, -90, -12

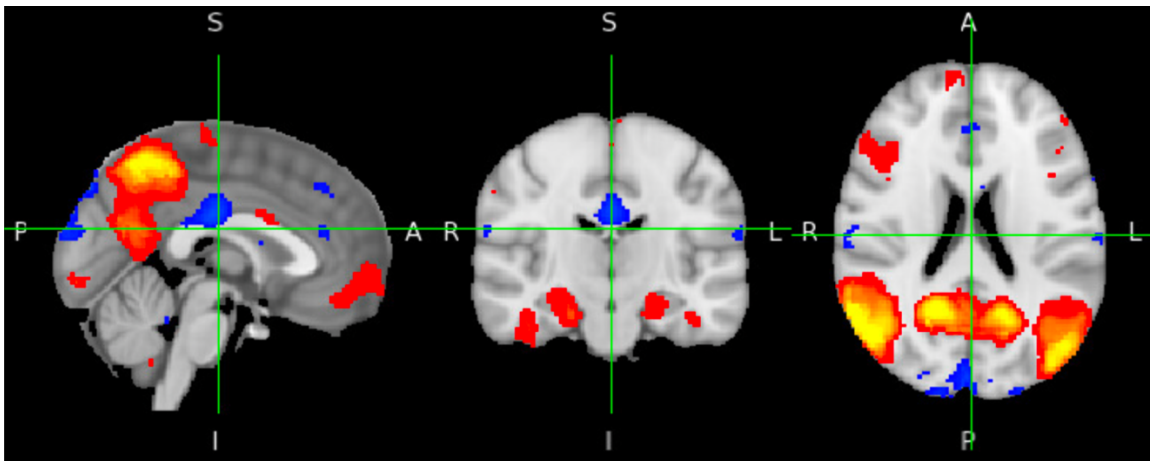
Default mode networks (DMN)



DMN: MNI 0, 14, 20

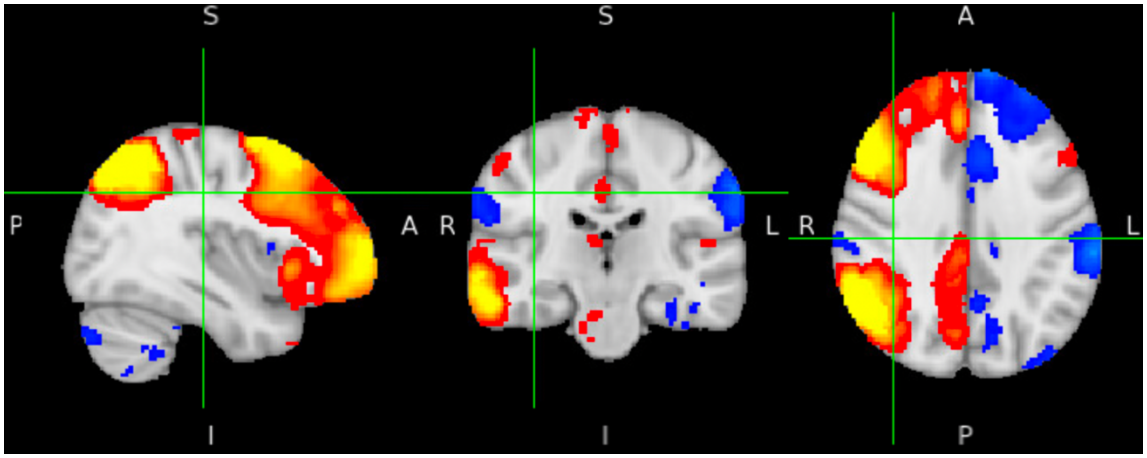


Precuneus: MNI 0, -53, 38

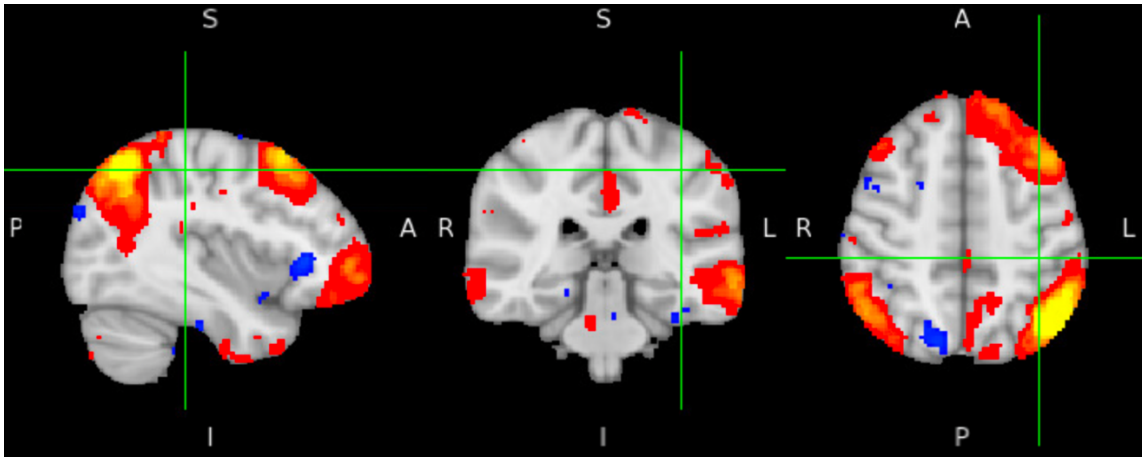


Posterior: MNI 0, -18, 21

Frontoparietal networks (FPN)

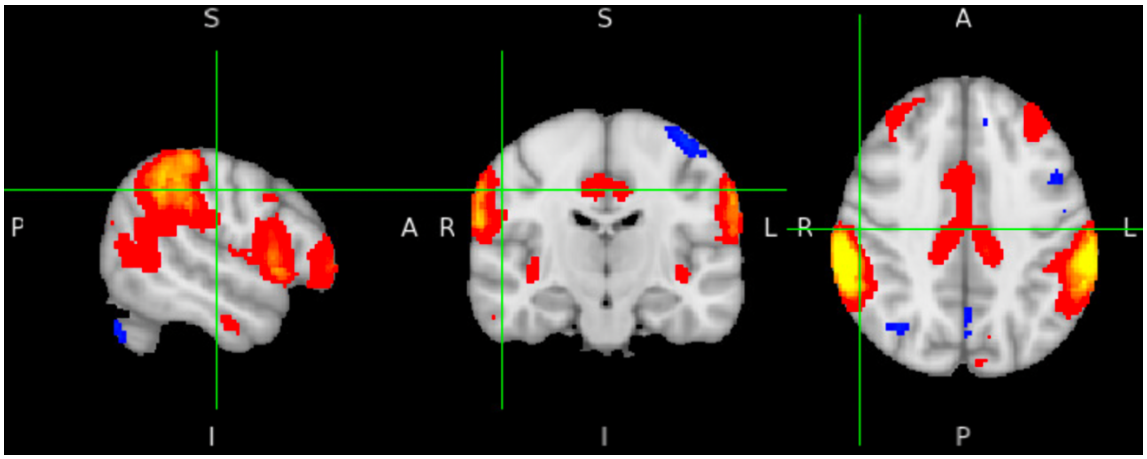


Right: MNI 38, -23, 36

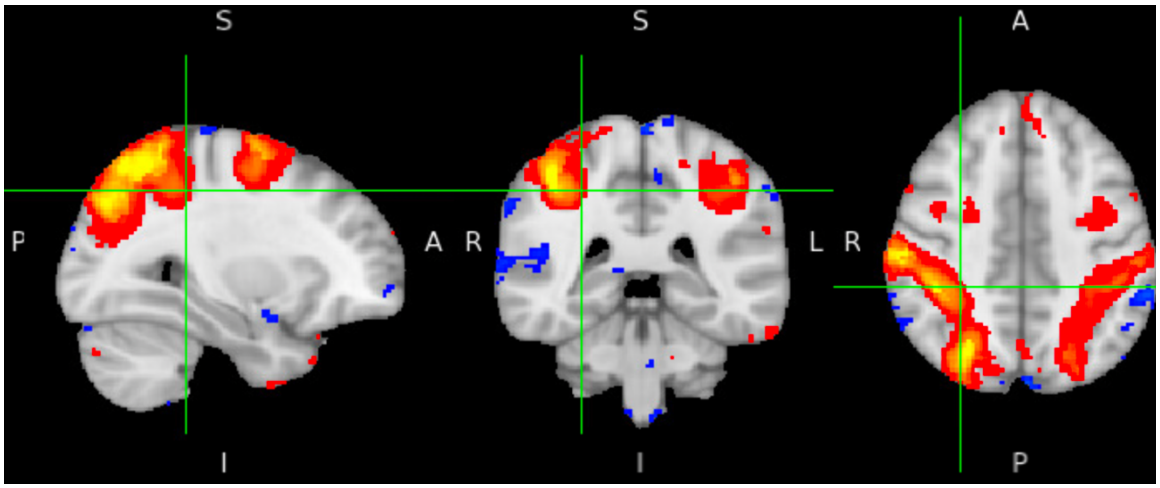


Left: MNI -38, -32, 49

Parietal networks

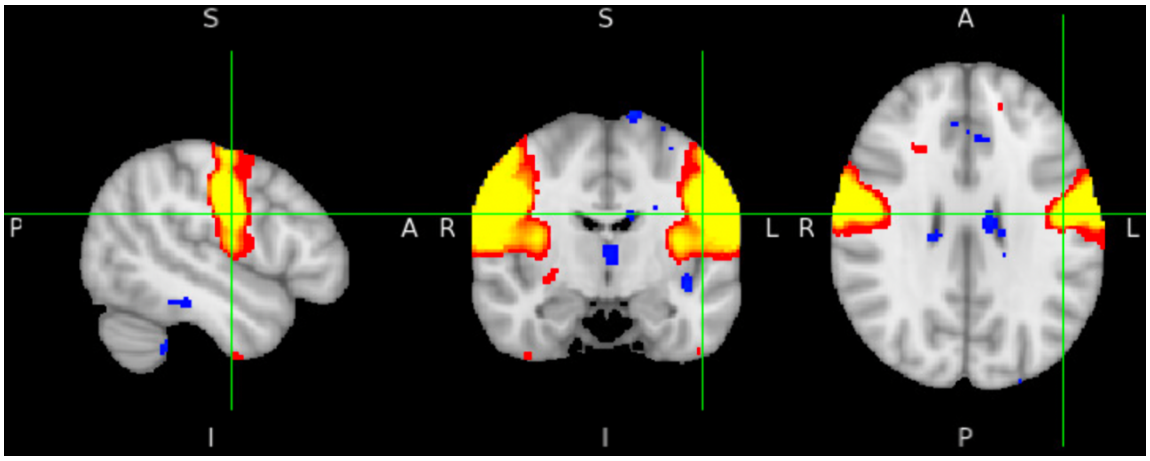


MNI 54, -17, 39

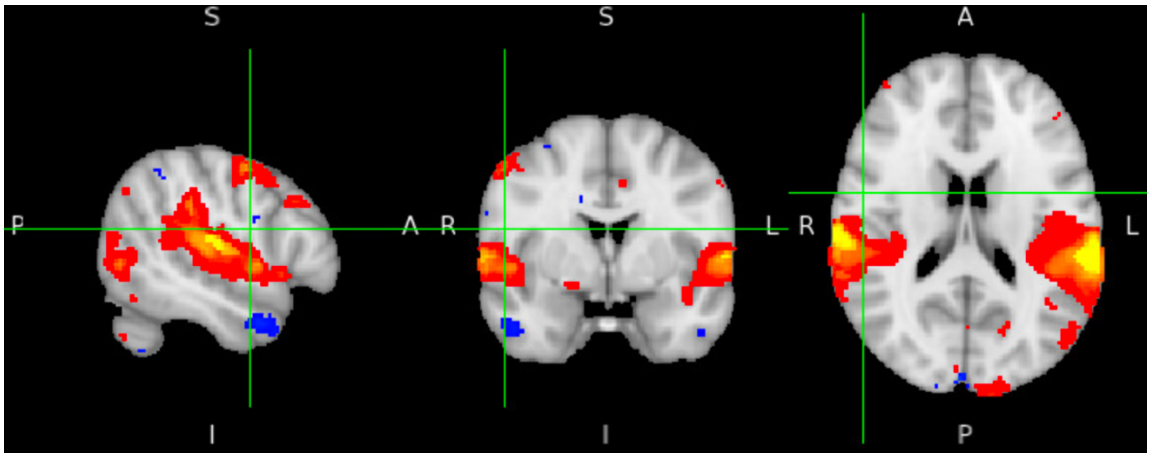


MNI 30, -38, 44

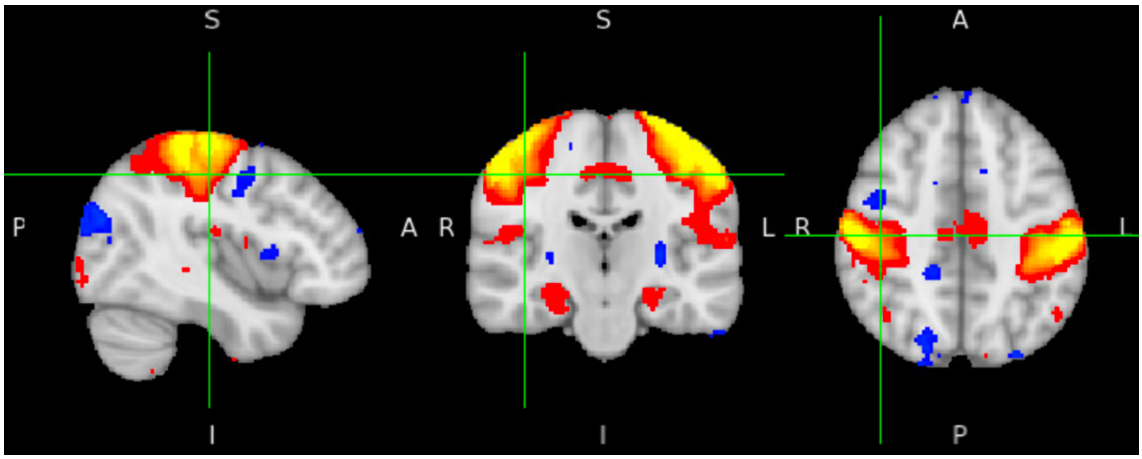
Sensorimotor/auditory networks



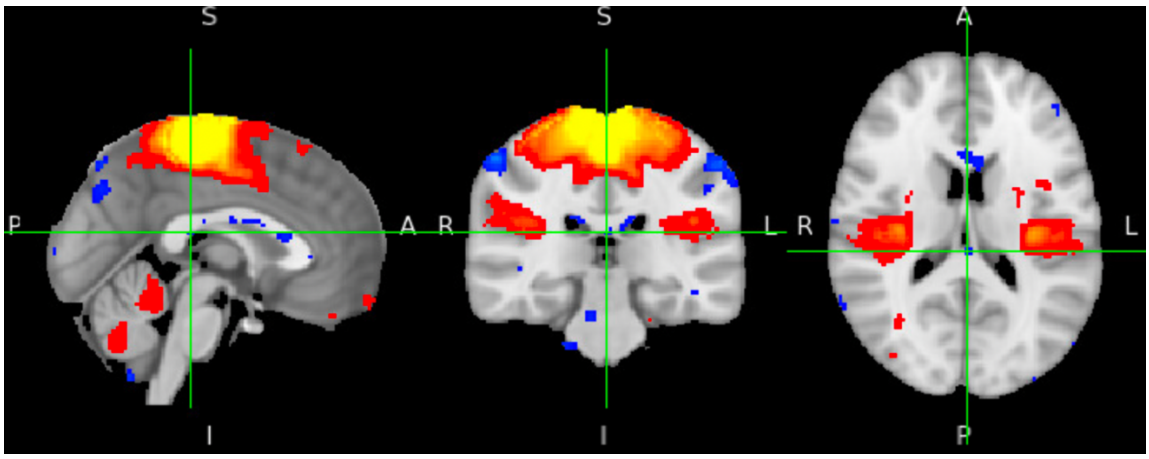
Sensorimotor: MNI -48, -9, 27



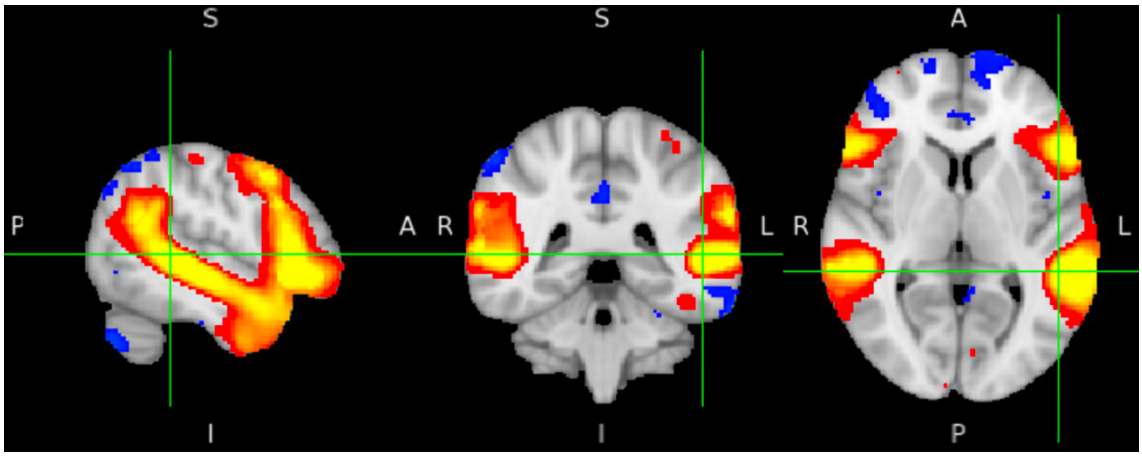
Sensorimotor/Auditory: MNI 53, 0, 18



Motor Parietal: MNI 42, -21, 46

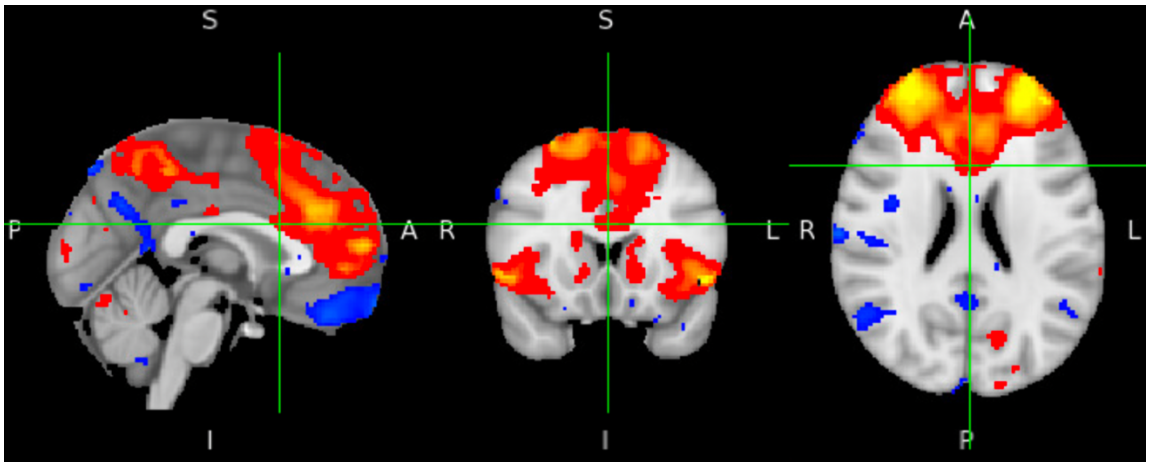


Motor Superior: MNI 0, -29, 16



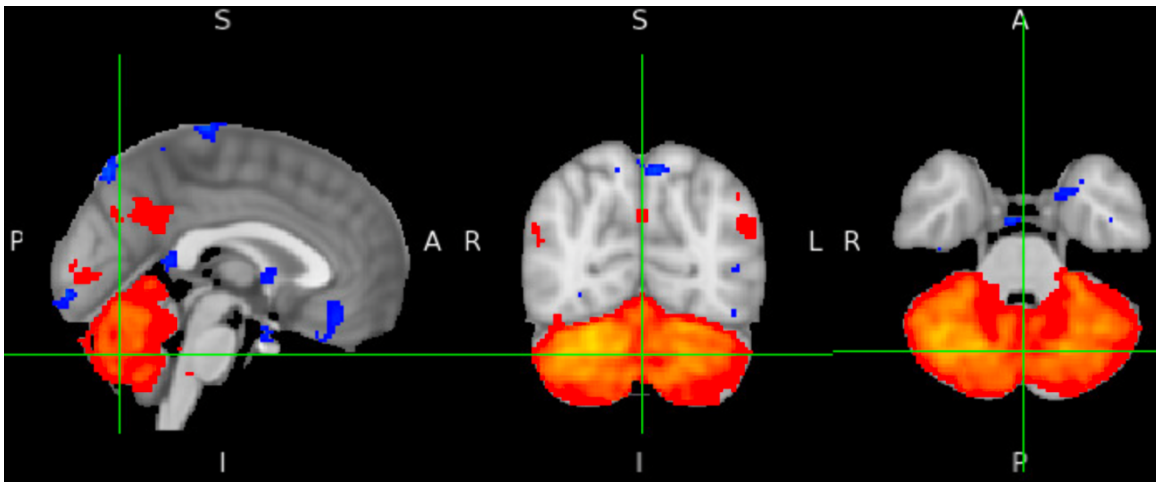
Frontotemporal / Auditory: MNI -50, -40, 5

Executive Cognitive Network



MNI 0, 16, 23

Cerebellar network



MNI 0, -69, -35

Figure 6.2. Identified resting state networks from group independent component analysis (n=45). Threshold value of 2.3.

Further analysis found significant treatment effects within visual networks and between visual networks and sensorimotor networks (Figure 6.3, Table 6.1). However, the effects became negligible after correction with multiple RSNs (corrected p-value = 0.999, 19 RSNs x 2 *t*-tests comparisons).

Table 6.1. Differences between ebselen and placebo groups in changes in resting-state functional connectivity within visual networks and between visual and sensorimotor networks (n=45).

Resting state network	Number of voxels identified	Maximum value in the cluster	Coordinate of the voxel with the maximum value (x, y, z)	Coordinate of the centre of gravity (x, y, z)	Treatment effects
Visual area	1	0.951	37, 20, 41	37, 20, 41	Ebselen < Placebo
Somatosensory area (cluster 1)	30	0.992	63, 51, 61	63.1, 50.9, 60.9	Ebselen > Placebo
Somatosensory area (cluster 2)	25	0.975	65, 52, 67	64.2, 53.8, 67.3	Ebselen > Placebo

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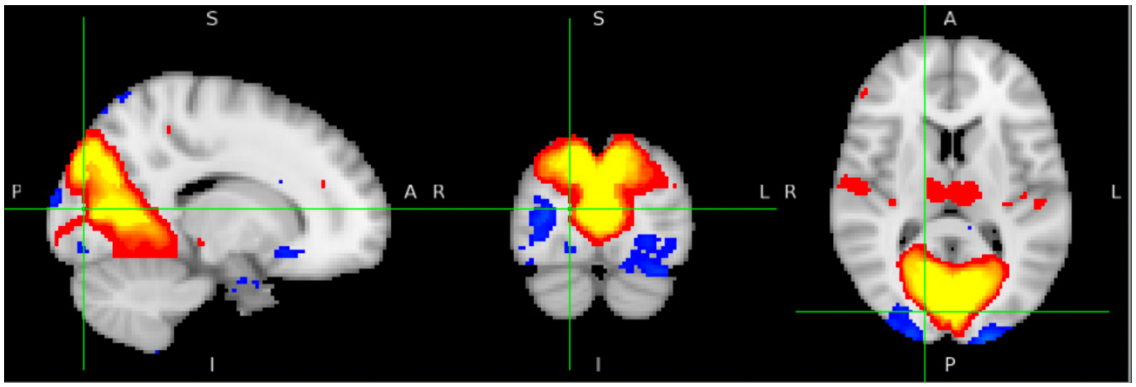

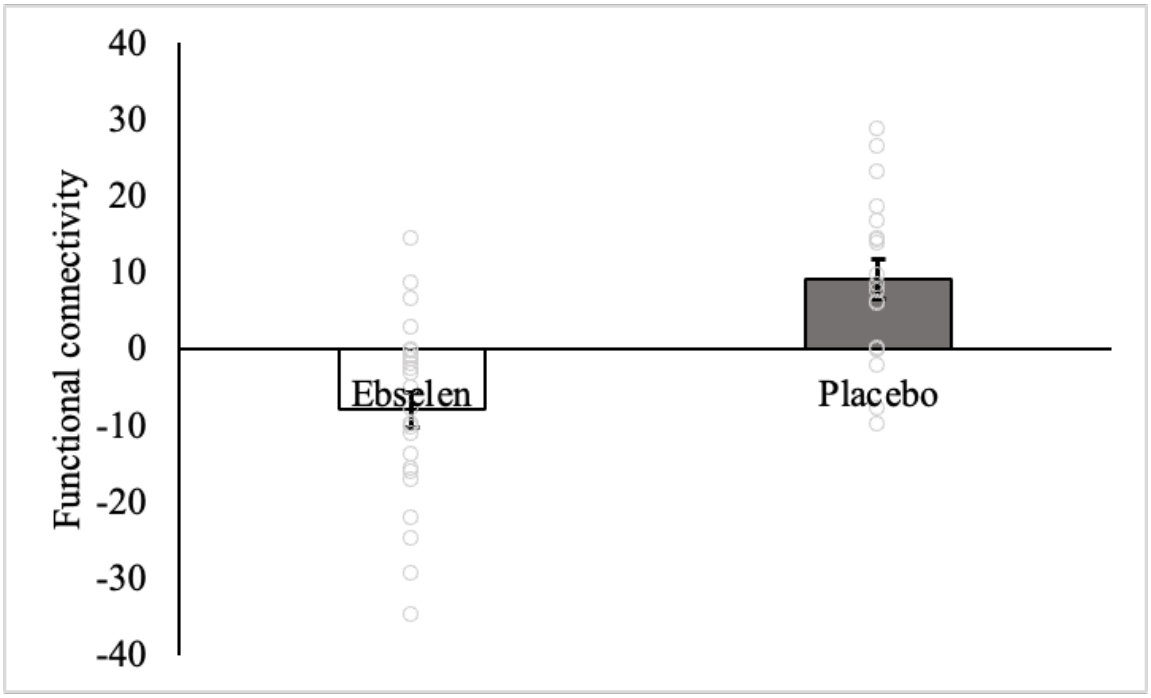
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Functional connectivity
Ebselen
Placebo

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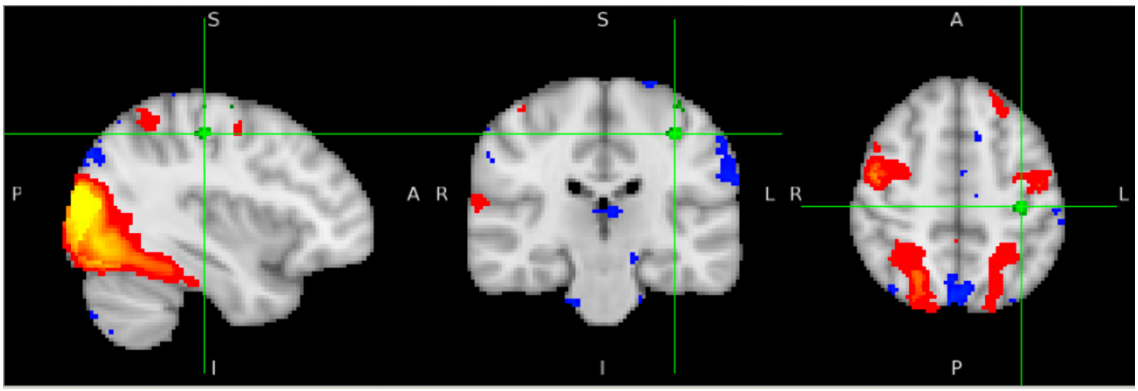
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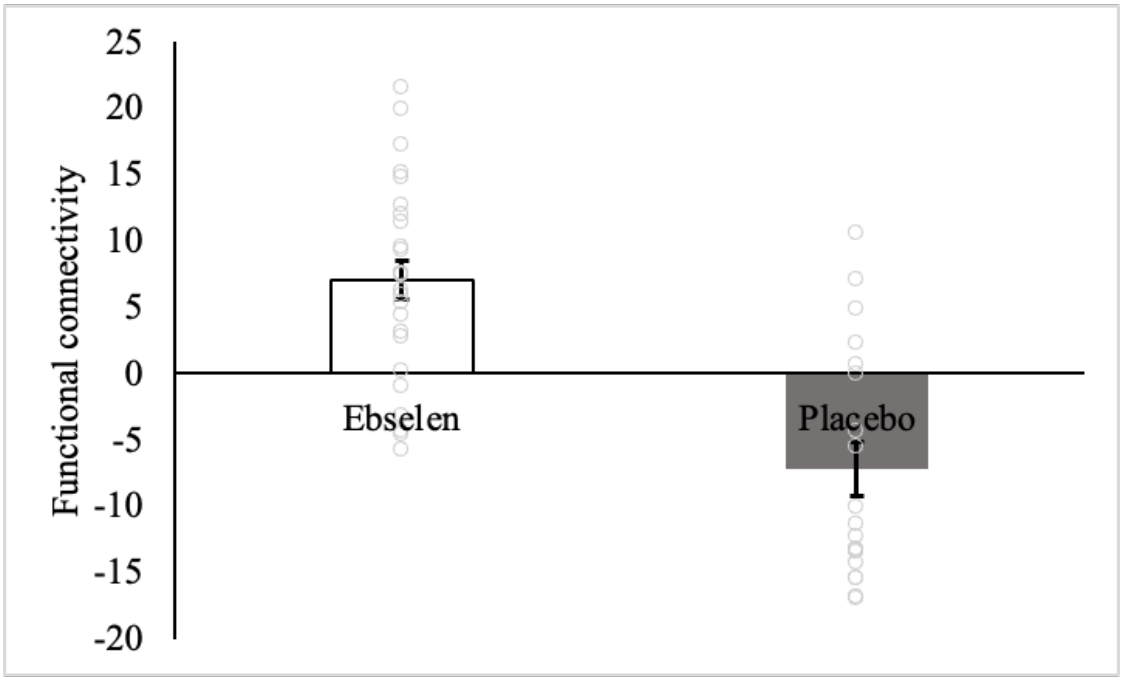
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Functional connectivity
Ebselen
Placebo

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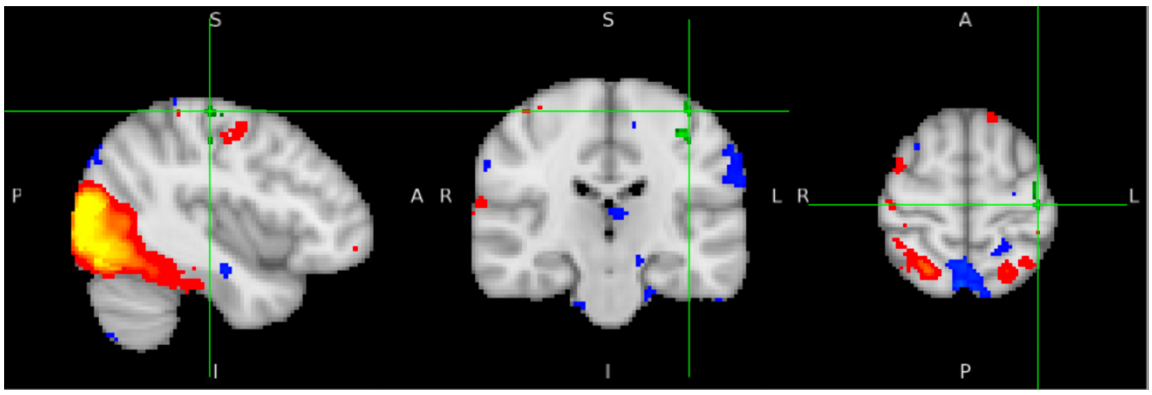
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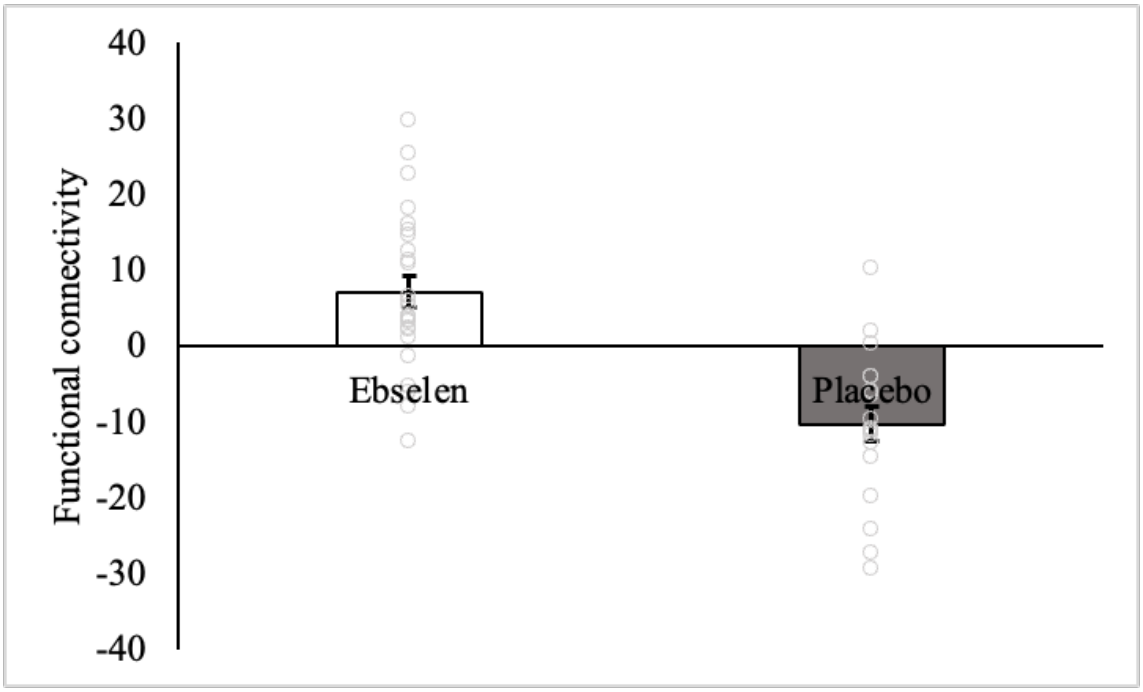

Figure 6.3. Changes in resting-state functional connectivity within the visual network and between visual and sensorimotor networks. A. Greater changes in resting-state functional connectivity in placebo compared to ebselen within the visual networks before adjustment for multiple resting state networks with a threshold of 0.95. The cursor indicates one significant voxel location (green colour, MNI 37, 20, 41) situated within visual networks. B. Greater changes in resting-state functional connectivity in ebselen compared to placebo between visual and sensorimotor networks (a cluster of 30 significant voxels, green colour, cursor position, MNI 63, 51, 61). C. Greater changes in resting-state functional connectivity in ebselen compared to placebo between visual and sensorimotor networks (a cluster of 25 significant voxels, green, cursor position, MNI 65, 52, 67). Note: Red-yellow: regions with correlated BOLD signal time-course; blue: regions with anti-correlated BOLD signal time-course; green: group differences.

6.4 Discussion

Relative to placebo, seven days' ebselen treatment did not have any significant effect on changes in resting-state functional connectivity between pregenual ACC and other brain regions. There was no correlation between changes in resting-state functional connectivity between the pregenual ACC and other brain regions and changes in neurometabolite concentrations of myo-inositol, glx, and glutamate in ACC. Furthermore, the ICA found no significant treatment effects after correction for multiple comparisons.

Visual and sensorimotor networks were the only RSNs identified in the group ICA to be affected by ebselen treatment before adjustment for multiple RSN comparisons. Relative to placebo treatment, we found that ebselen produced increased changes in functional connectivity between visual and sensorimotor networks before correction for multiple RSNs. This is of interest in view of the study of Javaheripour and colleagues (2021) who reported that in 1546 patients with MDD, there was lower functional connectivity between visual and somatosensory motor areas. In the context of the study, if a longer treatment duration of ebselen could intensify the effect seen in the present study, ebselen might have the potential to normalize the altered functional connectivity between primary visual sensory and sensorimotor regions.

There was a minor treatment effect within visual networks, with the ebselen group showing significantly lower changes in resting-state functional connectivity than the placebo group. However, this effect did not survive multiple comparisons. The visual network is one of RSNs typically affected in MDD. A previous study investigating the effects of 8-week antidepressant treatment with SSRI escitalopram and SNRI duloxetine in first-episode drug-naïve MDD patients reported extensive reductions in various networks (Li et al., 2021).

Interestingly, the most prominent effect was observed in the visual network, while the DMN was the least affected by antidepressants.

This might partly explain the reason for the null observation of ebselen treatment in the FERT, as current antidepressant therapy in our population, although ineffective, might have produced a substantial effect on primary visual processing areas, which are connected to other critical areas relevant to facial emotional processing. An alternative explanation is that medication is not an essential factor for changes in visual networks, as reported by another study (Javaheripour et al., 2021). However, generalization of the previous findings in our study is limited by different study populations, response status (88 % of Li et al. study achieved remission, and Javaheripour et al. ((2021)) only considered treatment status in their analysis, not responder status) and baseline resting state characteristics (no significant differences between patients at baseline and healthy volunteers in resting-state functional connectivity in the Li et al. study).

A previous meta-analysis study reported significant hyperconnectivity within DMN and between DMN and regions known to be associated with self-referential thinking, such as hippocampus, middle temporal gyrus, and medial prefrontal cortex (Kaiser et al., 2015). However, the current study found negligible differences between the placebo and ebselen treatment groups on this measure. One potential explanation for the finding could be due to the short duration of ebselen treatment, which might not be sufficient to induce a substantial effect on DMN.

A previous longitudinal study involving unmedicated depressed patients all of whom responded to 12-weeks' antidepressant treatment reported a normalisation of the posterior subnetwork of the DMN (Li et al., 2013). However, antidepressant effects on functional connectivity in non-responders were not evaluated in the study, as all patients achieved

adequate treatment responses. In our study, the baseline resting-state functional connectivity in depressed patients with inadequate responses to standard antidepressants relative to healthy volunteers is not known, as we did not conduct this comparison. Further, the effect of a longer duration of ebselen treatment (for example, 8 – 12 weeks) on the DMN is yet to be determined. If a longer treatment duration could significantly normalise or reduce the elevated functional connectivity of DMN, in MDD, ebselen could have a potential role in modulating the DMN in depressed patients with inadequate responses towards their current antidepressants. Of course, it is not known whether such an action would lead to a useful therapeutic response.

Another reason for the lack of differences between ebselen and placebo on changes in resting-state functional connectivity in our study, might be the medication-resistant status of our cohort, which could have distinct characteristics compared to a group of depressed patients responsive to standard antidepressant treatment. Martens and colleagues (2022) identified distinct baseline patterns in resting-state functional connectivity between responders and non-responders who received 6-weeks' escitalopram treatment. Compared to responders, non-responders were characterised by lower baseline functional connectivity between the right FPN and three other regions (bilateral precuneus; left pre- and post-central gyri; and somatosensory association cortex). However, no post-treatment fMRI was conducted. Thus, changes in functional connectivity (e.g. normalisation, alteration) after antidepressant treatment in responders and non-responders could not be determined. Also, the participants were medication-free at baseline and had received only one specific SSRI in their study, and our participants were on various antidepressants before ebselen administration.

Alternatively, short-term ebselen treatment might not produce a significant effect on DMN. In our study, current antidepressant treatments, while not therapeutically effective, might have altered the functional connectivity in this network. In a multicentre study, Javaheripour and colleagues (2021) reported that medicated MDD patients had significantly lower functional connectivity in various networks, including DMN, compared to unmedicated MDD patients. A systematic review and meta-analysis reported that medication status was an essential modulating factor in reducing resting-state functional connectivity in DMN (Yan et al., 2019). In this study, Yan and colleagues found that medicated patients with recurrent depression had significantly reduced DMN functional connectivity compared to depressed patients not on antidepressants. Intriguingly, no significant differences were observed between first-episode depressed patients on treatment (response status was not reported) and healthy volunteers, as well as patients with recurrent depression on treatment, indicating that antidepressants might reduce or normalise functional connectivity in the DMN.

However, one of many caveats for this interpretation of the current study is that our population might be different from the population in the above meta-analysis. We only included depressed patients with inadequate responses towards their current antidepressants, but the responder status was unknown in other studies (Javaheripour et al., 2021; Yan et al., 2019). However, Yan et al. (2019) found a significant positive correlation between a clinical measure of depression (HAM-D) and resting-state functional connectivity of DMN in medicated patients with recurrent MDD (Yan et al., 2019), suggesting that treatment responsiveness might be an important determining factor for a successful reduction in functional connectivity.

The pregenual ACC was chosen as seed region in the study as it is involved in processing information related to emotional cues, and previous studies in healthy volunteers reported significant effects of ebselen on brain neurochemistry in this brain region. In the current study, patients receiving ebselen had significantly higher changes in resting-state functional connectivity between the seed region pregenual ACC and the cerebellum than the placebo group. However, the effect became negligible after adjustment for multiple comparisons. If the effect had remained significant after adjustment, ebselen could potentially modulates the cerebellar-cortical circuit. The cerebellum is indirectly connected to ACC through ventromedial thalamic neurons. A recent preclinical study using a novel multimodal approach found a link between cerebellar-ACC functional connectivity and social interaction (Hur et al., 2024). The outcomes proposed that the cerebellum is not only essential for locomotor behaviour , but is also involved in the expression of social behaviour, which involves cognition and emotional regulation.

Nevertheless, the reason for the insignificant effects of ebselen on resting-state functional connectivity between pregenual ACC and other regions in the seed-based analysis is potentially due to distinct characteristics of resting-state functional connectivity between responder and non-responders to antidepressant medication. Lui and colleagues (2011) observed altered baseline functional connectivity in prefrontal and thalamus areas in depressed patients who were eventually classified as treatment-resistant depression. In contrast, decreased connectivity in limbic system and ACC, characterised depressed patients who were responsive to antidepressant therapy. The lack of effect of ebselen on resting state measures might also, of course, reflect its inability to produce neuropsychological changes characteristics of antidepressant action.

The study has several limitations. The lack of a healthy control group did not allow the exploration of the existing abnormal RSNs that might persist after unsuccessful antidepressant treatments. The addition of another cohort of unmedicated depressed patients could also give some insights into the possible RSNs which might have been altered by current antidepressants even though ineffective in producing a therapeutic response. Although a previous study reported a significant change in resting-state functional connectivity in the ventral precuneus and posterior cingulate cortex after ten days of SSRI treatment (citalopram and escitalopram), the study population concerned were healthy volunteers rather than depressed patients who may differ in their neural responses to antidepressant medication (Kraus et al., 2014). Also, non-responder patients have different characteristics from responders, and the duration of treatment might be an essential factor in modulating abnormal connectivity. Seven days' treatment with ebselen might not be sufficient to induce such changes. Extending the duration of ebselen treatment might be a potential direction for future studies. Another interesting development in the resting-state fMRI field is the method known as chronnectome, which allows measurements of dynamic changes during the scan rather than the current conception of the static functional connectivity between brain regions (Lv et al., 2018).

6.5 Conclusion

Acute treatment with ebselen in comparison to placebo did not produce any substantial changes in resting-state functional connectivity in depressed patients on current ineffective antidepressants. This lack of effect was apparent despite changes in relevant neurochemistry in the ACC. Overall, the lack of effect of ebselen in changes in resting-state functional

connectivity is consistent with its absence of indications of therapeutic activity in MRD patients.

CHAPTER 7

7. General Discussion and Conclusion

7.1 Overview

MDD is a debilitating disorder often requiring complex management. Although standard antidepressants have been reported to be effective, the remission rate remains low. About one-third to two-thirds of MDD patients still have significant clinical depressive symptoms after sufficient duration and dose of two antidepressant therapies (McIntyre et al., 2023; Pigott et al., 2023). Augmentation with another class of drug, such as atypical antipsychotics and lithium, is one of the further treatment strategies. Although effective, atypical antipsychotic use is associated with many adverse effects, including troublesome metabolic problems, which increase the risk of discontinuation (Nelson & Papakostas, 2009).

Lithium is another augmenting agent in MDD patients with insufficient responses towards standard antidepressants. Interestingly, additional benefits of lithium in reducing the risk of deliberate self-harm, suicide, and general mortality, make it a suitable augmenting agent, particularly in a subset of patients with self-harming behaviour and suicides (Cipriani et al., 2013; Nuñez et al., 2022). Lithium has been used safely by millions of patients with psychiatric disorders and is generally not toxic if a proper monitoring plan is implemented to maintain lithium levels in a safe and effective range. However, a complicated monitoring requirement may discourage lithium use by prescribers and patients. Thus, identifying a drug with similar mechanism and efficacy to lithium, but without or at least fewer adverse effects than lithium could be a useful treatment development.

Previous studies reported that, like lithium, ebselen, an organoselenium compound, inhibited IMPase, an enzyme in the PI-signalling pathways utilised by many serotonergic receptors. The inhibition was associated with molecular and behavioural changes that corresponded to antidepressant effects in animal models (Antoniadou et al., 2015; Antoniadou et al., 2018; Singh et al., 2013). In human experimental models, acute ebselen treatment reduced myo-inositol levels in a brain region critically involved in emotional regulation, that is, the ACC, potentially through IMPase inhibition (Masaki, Sharpley, Cooper, et al., 2016; Singh et al., 2016). Further, using emotional processing as a surrogate biomarker to investigate the potential of a drug with antidepressant properties, acute ebselen administration shifted affective bias towards more positive processing (Masaki, Sharpley, Godlewska, et al., 2016; Singh et al., 2016).

Given the potential use of ebselen as an augmenting agent in MDD patients with insufficient response to their current antidepressants, this thesis aimed to investigate the effects of ebselen given for seven days on emotional processing, clinical measures of depression and anxiety, brain neurochemistry, and resting-state functional connectivity in MRD patients. The outcomes of the study are important to determine whether formal clinical trials of ebselen as an augmenting agent in MRD or TRD patients are warranted.

7.2 Summary of the chapters

Chapter 1 is the introductory chapter which describes the current pharmacological strategies in the management of MDD patients and their challenges, followed by the justifications of conducting the current study.

Chapter 2 describes the designs of the study and details of methods used to assess the effects of ebselen on various outcomes, including the ETB for assessing emotional processing, blood selenium levels as the surrogate marker of ebselen (and its metabolite levels), clinical measures of MADRS, QIDS-SR-16, and GAD-7, proton MRS to measure neurometabolites of myo-inositol, glx, and glutamate, and resting state fMRI to evaluate neural functional connectivity at resting state.

Chapter 3 aimed to investigate the effects of seven days' treatment ebselen on emotional processing in MRD patients. No significant effects of ebselen were observed in any emotional processing parameters of FERT, ECAT, FDOT, EREC, and EMEM, except for beta fear of the FERT. This indicated that ebselen increased false alarms towards facial expressions of fear. There were no significant differences between groups in clinical measures of depression. However, a significant reduction in GAD-7 was observed in patients receiving placebo, but no significant differences in this scale were observed in the ebselen group between baseline and day 7. The findings suggest a potential anxiogenic effect of ebselen when administered for a short term. A similar effect can be seen with some antidepressants such as SSRIs.

As expected blood selenium levels were raised in each depressed participant randomised to ebselen, indicating good compliance with treatment in the ebselen group. However, no significant correlations were found between blood selenium levels and clinical measures of depression and anxiety. Ebselen was well tolerated with no drop-outs due to side effects. The adverse effects were comparable between ebselen and placebo groups. Based on the emotional processing experimental medicine outcomes, we concluded that currently, the evidence does not support a role for ebselen as an add-on antidepressant in MDD patients.

To investigate their baseline emotional processing state before the MRD patients were randomised to their treatment group, we recruited healthy volunteers to assess any residual negative biases in emotional processing in MRD patients compared to healthy volunteers. The study was discussed in Chapter 4. We found no significant differences between groups in facial expression recognition, suggesting that current antidepressant therapy, although ineffective, might have normalised emotional processing related to visual stimuli. Interestingly, we found that negative biases in emotional processing concerning emotional categorization (encoding) and in recall memory apparently persist in this population. This suggests that negative biases in emotional encoding and emotional memory continue in patients who remain depressed despite receiving adequate antidepressant medication. This might make such memory biases, a potential target for psychological or pharmacological treatment. Interestingly, ketamine, which is helpful in patients with TRD, has been shown in preclinical studies to reduce negative biases for previously acquired memories (Hinchcliffe et al., 2024). As observed in the previous studies in healthy volunteers, ebselen did not have any significant effects in these latter emotional processing domains. This, together with the prior normalisation of emotional processing towards positively- and negatively-valenced visual stimuli, might explain the reason for the negligible effects of ebselen in emotional processing in MRD patients.

Of course, it possible that ebselen might have effects on other neuropsychological mechanisms that are relevant to the treatment of depression, such as cognition, decision-making, and reward learning. In healthy volunteers, ebselen was reported to reduce impulsivity and reinforcement learning, and increase reward seeking (Masaki, Sharpley, Cooper, et al., 2016; Singh et al., 2016).

Interestingly, in MRD patients, there was a positive correlation between MADRS scores and the ECAT RT for categorising positive self-referent words in patients, as well as trends towards significant correlations between MADRS scores and the ECAT RT for categorising negative self-referent words and between HAM-D scores and the ECAT RT for categorising positive self-referent words, consistent with a general psychomotor slowing in the depressed patients.

Chapter 5 detailed the effects of ebselen on brain neurochemistry. A 7-day ebselen treatment significantly lowered myo-inositol and glx levels in the ACC, as reported previously in healthy volunteers. The postulated mechanism of action for a reduction in myo-inositol levels is IMPase inhibition. Inhibitions of glutaminase and GDH might explain a reduction in glx levels. There was a reduction in glutamate levels, but the post-hoc differences did not reach statistical significance. This observation is of value in showing that the dosing regime of ebselen in the current study produced engagement of the primary therapeutic target, IMPase. However, there were no significant correlations between blood selenium levels and neurometabolite levels in the ACC.

Chapter 6 assessed the effects of ebselen on changes in resting-state functional connectivity. Relative to placebo, there was a significant increase in changes in resting-state functional connectivity between pregenual ACC (seed region) and the cerebellum in ebselen in the seed-based analysis, but the effect was attenuated after adjustment for multiple comparisons. Also, no significant correlations between changes in resting-state functional connectivity (between pregenual ACC and other brain regions) and changes in neurometabolite concentrations in ACC were observed in either group. A possible explanation for the outcomes might be attributable to the negligible effects of ebselen on emotional processing in MRD patients. Other possible reasons are current antidepressant

use and a duration of ebselen treatment was too short to induce substantial changes in functional connectivity. ICA identified significant effects of ebselen on the primary visual sensory areas. Relative to placebo, there were diminished changes in resting-state functional connectivity within the visual networks and increased changes in resting-state functional connectivity between visual and sensorimotor networks following ebselen treatment. However, the effects subsequently became non-significant when adjustments for multiple comparisons were conducted. There may be numerous reasons for the negligible effects of ebselen, including short-term treatment duration, distinct characteristics of our cohort (medication-resistant patients compared to treatment-responsive patients), and current antidepressant treatment.

7.3 Generalizability

The thesis investigated the effects of a 7-day ebselen addition on neuropsychological, neurobiological, and clinical parameters in depressed patients with inadequate responses towards their current (diverse) antidepressants. Compared to previous studies in healthy volunteers, the results from the thesis can be expanded to a clinical population of patients with MRD. However, the generalizability is limited to MRD patients with mild to moderate depression severity as the majority of the study population had mild to moderate depressive symptoms based on clinician-rated questionnaires (84.3% at baseline (HAM-D) and 68.6% at the first study visit (MADRS). Further, the lack of depression subtype information limits our inference on which depressive subtypes might be sensitive to the effects of ebselen on the parameters tested.

7.4 Strength of the study

This is the first study to investigate the effects of ebselen in a clinical population of depressed patients with inadequate responses towards their current antidepressants. The inclusion of patients who failed to respond satisfactorily to diverse antidepressants might represent the real-world scenario compared to studies that focussed on a failed specific drug or class of antidepressants before the augmentation drug began. The use of emotional processing as a surrogate marker of ebselen's antidepressant potential in MRD patients in this thesis allowed us to make a go/no-go decision on the future direction of the research, which we think should be directed to bipolar mood disorder. Further, there was no previous study investigate the effects of ebselen on resting-state functional connectivity. The study confirmed the ability of ebselen to lower levels of myo-inositol and glx in the brain.

7.5 What was known before?

- Ebselen reduced brain myo-inositol levels and inhibited IMPase in *in vivo* and *in vitro* studies, respectively.
- Ebselen reproduced many molecular and behavioural effects of lithium in preclinical models.
- Ebselen induced positive biases in emotional processing biases among healthy volunteers.
- Ebselen reduced inositol, glx, and glutamate levels in ACC in healthy volunteers.

7.6 What this thesis adds?

- Assessment of ebselen's effects on surrogate markers of emotional processing in a target psychiatric population of depressed patients with inadequate responses towards their current antidepressants in which no significant treatment effects were observed between ebselen and placebo given for seven days. This suggests that ebselen is unlikely to be useful as an add-on treatment in patients with TRD.

- Robust findings of ebselen's effects in reducing myo-inositol and glx in ACC in depressed patients. This adds to evidence from two previous studies in healthy volunteers.

- The evaluation of ebselen's effects on changes in resting state functional connectivity, which had not been published before. Negligible effects of ebselen on resting state functional connectivity were observed after correction for multiple comparisons.

- Negative biases in emotional encoding and memory in patients with MRD compared to healthy volunteers. This suggests that negative biases in emotional memory could prove a useful target for psychological and pharmacological treatments.

7.7 Limitations and suggestions

Table 7.1 Limitations of the current study and suggestions for future studies.

No	Limitations	Suggestions
1.	The sample size was small. a. The power to detect the differences between groups in specific domains of emotional processing might be limited. b. Subgroup analysis of specific classes of antidepressants on emotional processing could not be carried out.	To increase sample size in the future study.
2.	Concomitant antidepressant treatment might nullify the effect of ebselen to positively bias responses to facial expressions in the ETB.	Carry out a similar study with ebselen in unmedicated depressed patients.
3.	Lack of other neuropsychological battery that are relevant to the treatment of depression, such as cognition, decision-making, and reward learning.	To conduct a similar study in MRD or TRD patients to evaluate the effects of ebselen on other neuropsychological domains - cognition, decision-making, and reward learning.

4.	The proton MRS quantified neurometabolite concentrations in the whole voxel. So, the concentrations obtained did not reflect the changes in the specific area of interest - the post-synaptic neuron for myo-inositol, and synapse for glutamate.	To assess the effect of ebselen on mGluR5 binding using PET imaging.
5.	Lower precision for quantifying separate peaks of glutamate and glutamine by proton MRS at 3T.	To replicate the study in the same population using a higher magnetic field of MRS at 7T.
6.	A short treatment duration of seven days. Longer duration of ebselen treatment might have different effects on brain neurochemicals, resting-state functional connectivity, and clinical measures.	I. To assess the effects of longer treatment duration on brain neurochemistry (e.g. myo-inositol levels) in MRD or TRD patients. For instance, neurometabolite concentrations can be assessed at baseline and days 7 and 42 of ebselen treatment. A previous lithium study in adolescents with bipolar depression reported an

		upregulation of myo-inositol level in the prefrontal cortex levels at 42 days compared to seven days of lithium treatment (Patel et al., 2006). II. To assess the longer treatment duration effects on resting-state functional connectivity. III. To assess the longer treatment duration effects on clinical measures of anxiety and depression
7.	Resting state measurements of fMRI has limitations.	Task-based fMRI can give insights into particular brain areas activated while performing specific assignments for example reward learning.
8.	Static measurements of resting-state functional connectivity.	The chronnectome method can give more insight into the dynamic changes of resting-state functional connectivity during the scan.

7.8 Conclusion

Relative to placebo, seven days' treatment with ebselen did not alter emotional processing in patients with inadequate response to their current antidepressant treatment. Therefore, the study did not support the potential use of ebselen as an augmenting agent in MRD patients based on the cognitive neuropsychological model of antidepressant drug action. Unfortunately, there are no studies, as far as I am aware, that have studied emotional processing changes in the course of pharmacological augmentation treatments, such as quetiapine, in depressed patients. Interestingly, a placebo-controlled study of seven days' quetiapine treatment in healthy volunteers reported no significant effects on the emotional processing domains of ETB (Rock et al., 2016). The findings of the effects of repeated lithium administration (five days) on emotional processing in healthy volunteers using the same tasks as the studies in this thesis are not yet available (refer to https://doi.org/10.17605/OSF.IO/W2MXQ). Overall, therefore, it is not possible to put the negative findings on emotional processing of ebselen in the current study in the context of other augmentation approaches of known efficacy.

Also, ebselen treatment significantly reduced myo-inositol and glx levels, potentially due to IMPase and glutaminase and GDH inhibition. The finding suggests that the dose of ebselen used in the study was able to produce the biochemical changes to confirm target engagement in depressed patients.

There is a short-term clinical trial indicating that ebselen at the current dose was able to decrease symptoms of mania in patients with bipolar disorder (Sharpley et al., 2020). This suggests that it may be worthwhile pursuing the potential benefit of ebselen in bipolar patients both as a treatment for acute mania and perhaps for the longer-term prophylaxis of

mood disorder episodes. If effective, ebselen would have the advantage over lithium of good tolerability and the absence of need for regular blood monitoring

While lithium is generally regarded as an effective treatment in TRD patients, the evidence base is not extensive (Rafeyan et al, 2020), and in the STAR*D study, the efficacy of lithium in TRD patients was modest (15.9% remission) and numerically less than that of tri-iodothyronine augmentation (24.7% remission) (Nierenberg et al., 2006). In this respect, the results of a large pragmatic study (LQD) comparing lithium and quetiapine in TRD patients should be available soon and will be of great interest (Marwood et al., 2017). If lithium is shown to be less effective than quetiapine in this analysis, it will reinforce the suggestion that future work with ebselen should focus on bipolar disorder where lithium still has a major role to play in management, particularly as a mood stabiliser (Geddes et al., 2010).

8. Reference

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