Effect of the Putative Lithium Mimetic Ebselen on Brain *Myo*-Inositol, Sleep, and Emotional Processing in Humans

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Lithium remains the gold standard in treating bipolar disorder but has unwanted toxicity and side effects. We previously reported that ebselen inhibits inositol monophosphatase (IMPase) and exhibits lithium-like effects in animal models through lowering of inositol. Ebselen has been tested in clinical trials for other disorders, enabling us to determine for the first time the effect of a blood–brain barrier-penetrant IMPase inhibitor on human central nervous system (CNS) function. We now report that in a double-blind, placebo-controlled trial with healthy participants, acute oral ebselen reduced brain *myo*-inositol in the anterior cingulate cortex, consistent with CNS target engagement. Ebselen decreased slow-wave sleep and affected emotional processing by increasing recognition of some emotions, decreasing latency time in the acoustic startle paradigm, and decreasing the reinforcement of rewarding stimuli. In summary, ebselen affects the phosphoinositide cycle and has CNS effects on surrogate markers that may be relevant to the treatment of bipolar disorder that can be tested in future clinical trials.

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INTRODUCTION

Bipolar disorder affects 1–3% of the world population and is one of the biggest disease burdens in industrialized countries (Lopez *et al*, 2006). Lithium is the most effective treatment for bipolar disorder, as it controls both mania and depression and decreases suicide (BALANCE investigators and collaborators *et al*, 2010), but the therapeutic window is small, and it has several unwanted side effects (McKnight *et al*, 2012), some medically serious. Other drugs such as anticonvulsants and antipsychotics used to treat bipolar disorder are not as effective in stabilizing mood and preventing suicide as lithium (McKnight *et al*, 2012). Indeed, no agent has been specifically developed as a mood stabilizer for bipolar disorder on the basis of an understanding of the illness or mechanism of action of effective treatments (Conn and Roth, 2008).

Rational design of a mood stabilizer could be pursued based on the mechanism of action of lithium, but its therapeutic target remains unknown (Quiroz *et al*, 2004). Based on clinically relevant lithium concentrations (0.6–1.2 mM), the two most likely targets are glycogen synthase kinase 3 and inositol monophosphatase (IMPase) (Agam *et al*, 2009; O'Brien and Klein, 2009). Evidence for and against both targets comes from animal studies employing genetic and pharmacological manipulations (Agam *et al*, 2009; O'Brien and Klein, 2009). However, as certain animal models are reported to be poor predictors of clinical efficacy (Conn and Roth, 2008; Harmer *et al*, 2011), both therapeutic mechanism and target validation must be evaluated ultimately in humans. In this regard, surrogate efficacy markers in healthy volunteers are emerging as a promising bridge to efficacy studies (Harmer *et al*, 2011).

Berridge's 'inositol depletion hypothesis' has provided an attractive mechanistic explanation for the action of lithium and a rationale for testing it by predicting a lowering in *myo*-inositol levels (Berridge *et al*, 1989). This hypothesis proposes that lithium normalizes signaling in overactive neurons by inhibiting IMPase and depleting *myo*-inositol. As inositol is poorly blood–brain barrier penetrant, brain cells depend largely on recycling and *de novo* synthesis rather than uptake, making the central nervous system (CNS) uniquely sensitive to IMPase inhibition (Berridge *et al*, 1989). The location of IMPase in inositol metabolism enables it to exert control of several diverging downstream pathways including cytosolic calcium, protein kinase C, protein kinase B, and phosphatidylinositol 3-kinase (Figure 1a).

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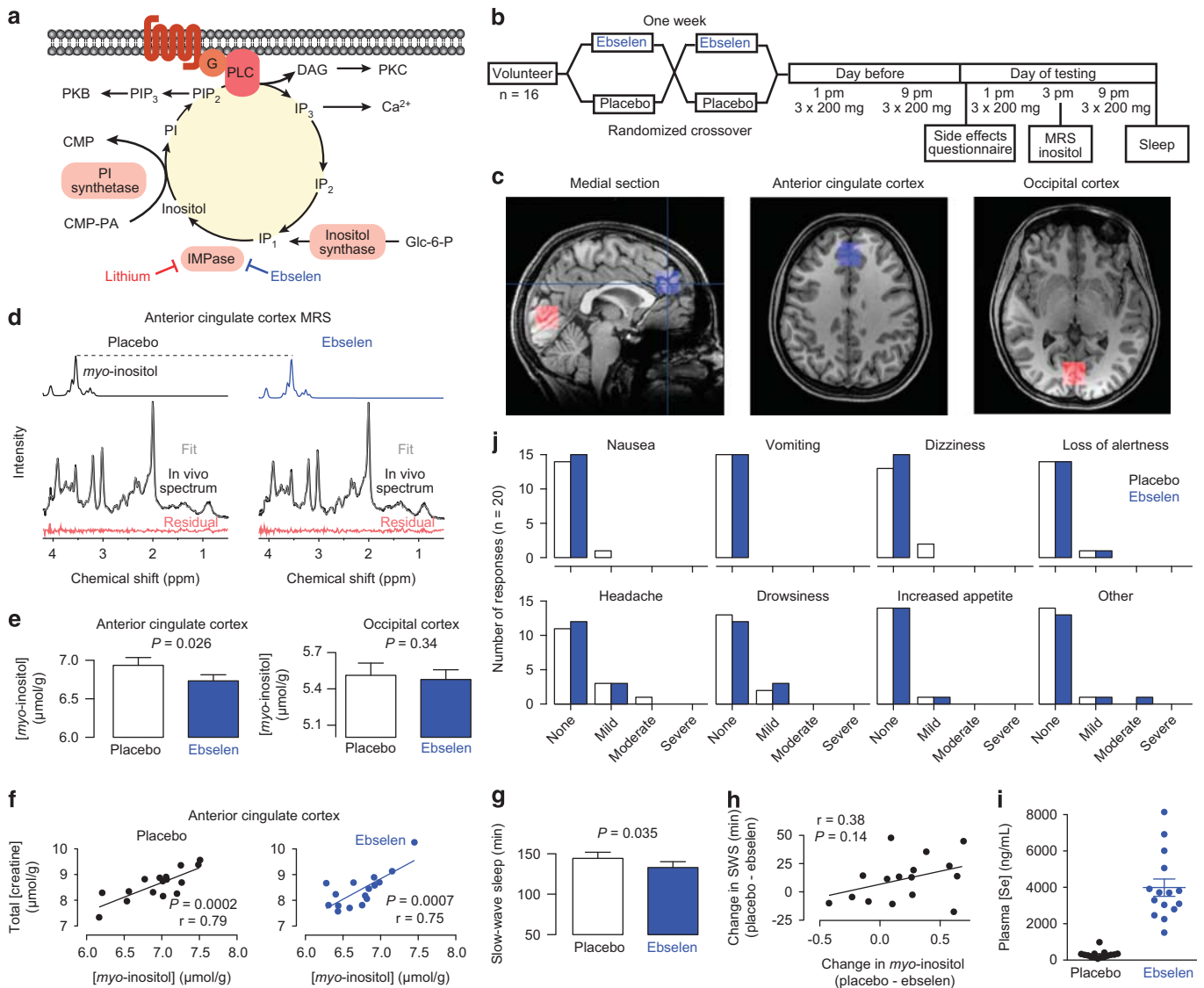
Figure 1 Ebselen is orally bioavailable, well tolerated, and decreases *myo*-inositol in the anterior cingulate cortex in humans. (a) Simplified diagram showing the central role of IMPase in the inositol cycle and its effect on several signaling pathways. PA, phosphatidic acid; PI, phosphatidylinositol; PK, protein kinase. (b) Schematic of experimental design. (c) Brain slice images from magnetic resonance imaging (MRI) showing locations of spectroscopic voxels in the anterior cingulate cortex and occipital cortex. (d) Proton magnetic resonance spectroscopy (MRS) spectrum traces illustrating the quantification of *myo*-inositol in the anterior cingulate cortex. A color-coded label specifies each trace. (e) Effect of ebselen on *myo*-inositol in the anterior cingulate cortex and the occipital cortex. (f) Correlation plots between *myo*-inositol and total creatine (creatine plus creatine phosphate). (g) Ebselen decreases slow-wave sleep. (h) Correlation plot between ebselen-induced changes in *myo*-inositol and slow-wave sleep (SWS). (i) Plasma levels of ebselen and its metabolites based on quantification of selenium. (j) Comparison of the frequency of side effects reported by participants after taking ebselen or placebo. Bar charts (e and g) show the mean ± SEM ($n = 16$) with actual p calculated by a paired two-way t-test. Scatter plots (f and h) show Pearson's correlation coefficient (r) with actual p calculated as a two-way test ($n = 16$).

The hypothesis has been tested by measuring *myo*-inositol levels in animals and humans, but with mixed results possibly because of a combination of compensatory changes during the ramp up to pharmaceutical concentrations in the CNS and lithium inhibition of multiple targets (Davanzo *et al*, 2003; Moore *et al*, 1999; Patel *et al*, 2008; Sharma *et al*, 1992; Silverstone *et al*, 2005; Silverstone and McGrath, 2009). The only known selective IMPase inhibitor, L-690,330, developed by Merck, Sharpe, and Dohme, affects the inositol cycle in mouse brain but required high doses (mmol/kg) because of its high polarity and lack of membrane permeability (Atack *et al*, 1993).

Evaluation of IMPase in humans has not been possible because of the lack of a bioavailable, blood–brain barrier-penetrant inhibitor. Recently, we reported that ebselen inhibited IMPase and caused lithium-like neuropharmacological effects in mice (Singh *et al*, 2013). Ebselen is a bioavailable antioxidant drug that has been tested in humans for other diseases but has not been marketed (Lynch and Kil, 2009; Parnham and Sies, 2013; Yamaguchi *et al*, 1998) and can therefore be 'rescued' for a new disorder (Cavalla, 2009). We have now used ebselen to determine the effect of IMPase inhibition on CNS function in humans.

MATERIALS AND METHODS

Study Oversight

The studies were overseen by the Oxford University Clinical Trials and Research Governance team and sponsored by the University of Oxford. Ethical approval was granted from the National Research Ethics Service Committee South Central. Informed consent was obtained from each participant. A safety-monitoring group collated adverse events.

Study Design and Participants

The sleep and inositol study involved 16 healthy participants (6 females and 10 males) in a randomized, double-blind, placebo-controlled, crossover design (Figure 1b). Ebselen capsules and identical matching placebo (containing Avicel microcrystalline cellulose) capsules were purchased from Shasun Pharmaceuticals. Ebselen was administered as 3×200 mg capsules. The day before the test day, the first dose was administered (by the participant themselves) at 1300 h and then again 2 h before bed. The following morning, within 20 min of waking, participants completed the Leeds Sleep Evaluation Questionnaire (Parrott and Hindmarch, 1980) and side-effects profile using a subjective 4-point rating scale. Participants administered the third dose at 1300 h and were scanned at the Oxford Centre for Clinical Magnetic Resonance Research (OCMR), University of Oxford, at 1500 h. A blood sample was collected at 1615 h and the participants were then fitted with the ambulatory Embla Titanium sleep recorder (Natus Neurology, Middleton, WI) and returned home to sleep as usual. The final dose was administered 2 h before sleeping. The following morning, participants completed the Leeds Sleep Evaluation Questionnaire and side-effects profile. The study was repeated a week later with the alternative treatment. On completion, participants were asked to guess on which occasion they had taken the ebselen and placebo.

The emotional processing study involved 40 healthy participants, 20 per group, in a randomized, double-blind, placebo-controlled, parallel-group design (Figure 2b). The group demographics and results from baseline screening questionnaires are given in Table 1. The day before the test day, ~ 15 min before the administration of the first dose of ebselen/placebo (3×200 mg) at ~ 0930 h, participants were asked to complete a set of baseline questionnaires: positive and negative affective schedule (Watson et al, 1988), the Befindlichkeit mood and energy questionnaire (Von Zerssen, 1986), the Beck depression inventory, visual analog scales (Bond and Lader, 1974), and a state-anxiety questionnaire (Spielberger et al, 1970). Participants were asked to take the second dose (3×200 mg) at ~ 2130 h. On the test day, participants took the final dose (3×200 mg) at 0915 h in the department. At 1115 h, the participants again completed the above questionnaires as well as one for side effects (a 4-point rating scale) to reveal any drug-related effects. Testing began at 1130 h and was carried out in the same order and by the same researcher, in all cases.

Participant Exclusion Criteria

The exclusion criteria were as follows: history of any axis I psychiatric disorder (determined at screening using the Standard Clinical Interview for Diagnostic and Statistical Manual for Mental Health Disorders-fourth edition); pregnancy or lactation; current usage of any other prescription medication, except the oral contraceptive; any other medical condition; and heavy smokers. Specific exclusion criteria for the sleep and inositol study included fulfilling safety requirements to allow admittance to the scanner, any current or past sleep disorder and claustrophobia, and, for the emotional processing study, prior exposure to the battery of psychological tests, dyslexia, and poor spoken or written English. Participants were asked to abstain from alcohol while participating in the study.

Emotional Processing Tasks

Full details of the tasks are provided in the Supplementary Information Subjects and Methods. The first task was the Auditory Verbal Learning Task (Rey, 1964), interspersed with the Reward and Punishment Learning Task (described in detail below). This was followed by the Emotional Testing Battery conducted as described previously (Harmer et al, 2009; Murphy et al, 2008) that constituted the Facial Emotion Recognition Task, the Emotional Categorization, the Dot Probe Task, the Emotional Recall, and the Emotional Recognition Task. The final task was the Emotion Potentiated Startle Task (Lang et al, 1993). The testing was usually complete in ~ 2 h and participants were given breaks at certain designated times. At the end of the study the participants were asked to report whether they thought they were on placebo or ebselen.

Reward and Punishment Learning Task

This computer-based cognitive task is a within-subject assessment of probabilistic learning from negative and positive feedback. It is divided into two separate reward and punishment conditions of 100 trials each. Every participant undertakes both conditions, and the order of presentation of conditions is randomized. In each condition, the 100 trials are divided into blocks of 25 trials (4 blocks of 25 trials per condition). In the reward condition, the person gains points for making the most winning choice, and in the punishment condition, the person does not lose points for making the least losing choice. On each trial, the subject is presented with four decks of cards, each with probabilities of 50%, 60%, 70%, and 80%, respectively, of gaining reward (reward condition) or of generating punishment (punishment condition). One deck of the four will have the most optimal deck of cards. This deck will be the most winning in the reward condition, and the least losing in the punishment condition. Three other nonoptimal decks of cards were presented with the optimal deck. In each trial, the participant is asked to choose one of the four decks with the expectation that over time, the participant will learn which of the four decks is the most optimal deck.

In the reward condition, the participant starts with a score of 0 and is asked to choose the 'best' deck, which in this case is the deck with 80% probability of gain. The choice of the optimal deck yields in positive feedback by the addition of 10 points, otherwise nothing. The participant is expected to continue selecting the optimal stimulus after learning it, despite the lack of positive feedback in some of the trials.
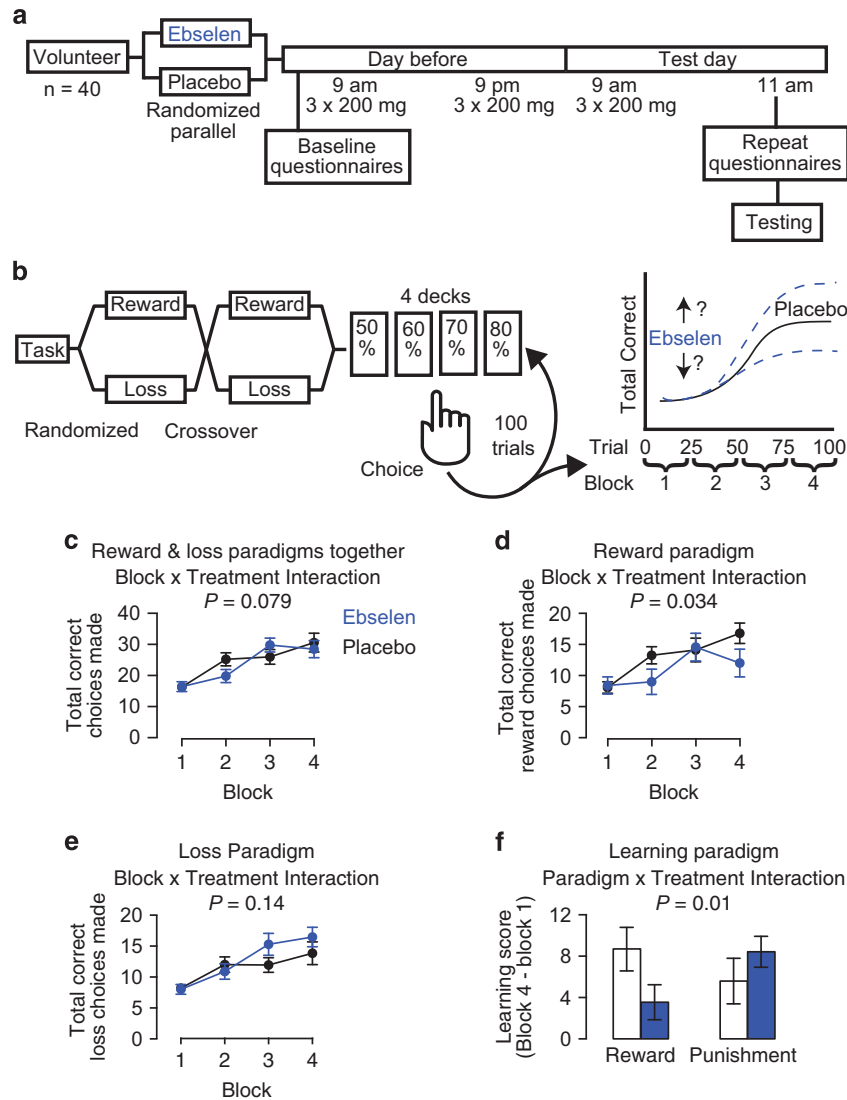
Figure 2 Ebselen affects learning influenced by either reward or punishment. (a) Schematic of experimental design. (b) Schematic of the reward and punishment learning task. The task involves picking one card at a time from four decks with two conditions, 'pick the best deck' or 'avoid the worst deck', and through trial and error the participant learns which of the four decks of virtual cards provides the most points in a paradigm of reward or punishment. Ebselen could increase or decrease learning relative to the placebo learning response. (c–e) Effect of ebselen on total correct choices made over time under conditions of both reward and punishment (c), as well as under the reward (d) and punishment (e) condition separately. (f) Effect of ebselen on total learning. Statistical comparisons were made with a two-way repeated measures analysis of variance with actual p shown ($n = 20$).

In the punishment condition, the participant is asked to avoid the 'worst deck' or the deck with the highest probability of punishment. They start with a score of 1000 and if the deck chosen yields punishment, they lose points. The optimal deck in this condition is the 50% probability of loss. Again, the participant is expected to continue selecting the optimal deck after learning it, despite the negative feedback in some of the trials.

The optimal choices for reward (best deck = 80% probability of gain) and for loss (best deck = 50% probability of loss) are rewarded one point each and added to calculate the total optimal choice accuracy. Learning is calculated by subtracting the total optimal choices made in block 1 from the total optimal choices made in block 4 for each condition.

Sleep Architecture

On each study night, polysomnograms were recorded as each participant slept at home, using the Embla titanium recording system (Natus Neurology Incorporated). Participants retired and rose at their usual time, and this was kept constant for all study nights and all preceding nights. The industry standard montage was used that comprised six electroencephalogram channels (C3-M2, C4-M1, O1-M2, O2-M1, F3-M2, and F4-M1), two electrooculogram channels (E1-M2 and E2-M2), and submentalis electromyogram channels using three electrodes. Polysomnograms were staged in 30-s epochs using the Embla RemLogic sleep diagnostic software (Natus Neurology). RemLogic adheres to the American Academy of Sleep Medicine scoring criteria. In

Table 1 Demographics of the Two Participant Groups (Placebo and Ebselen) and the Information from the Screening Questionnaires

Screening questionnaires	Placebo group, mean ± SEM (N)	Ebselen group, mean ± SEM (N)	P-value
Age	21.95 ± 0.70 (20)	23.25 ± 0.96 (20)	0.28
Sex	8 Males, 12 females	7 Males, 13 females	—
BMI	21.95 ± 0.36 (20)	22.20 ± 0.45 (20)	0.66
National adult reading test	122.9 ± 0.49 (20)	123.1 ± 0.42 (19)	0.71
Trait anxiety	31.80 ± 1.13 (20)	33.65 ± 1.52 (20)	0.34
State anxiety	50.10 ± 0.61 (20)	50.32 ± 0.52 (19)	0.79
Eysenck personality questionnaire	32.25 ± 1.19 (20)	31.05 ± 1.47 (20)	0.53
Positive and negative affect schedule			
Positive	28.40 ± 1.50 (20)	28.75 ± 1.78 (20)	0.88
Negative	12.60 ± 0.67	11.95 ± 0.44	0.42
Befindlichkeit questionnaire			
Mood	17.15 ± 3.07 (20)	13.25 ± 2.17 (20)	0.31
Energy	5.90 ± 1.13 (20)	4.35 ± 1.16 (20)	0.35
Beck depression inventory	3.34 ± 0.68 (19)	4.20 ± 0.89 (20)	0.47

addition, an experienced sleep physiologist who was blind to the treatment status analyzed the polysomnograms.

Proton Magnetic Resonance Spectroscopy

Spectra were measured using the Siemens Trio 3-Tesla whole body MRI scanner and a 32-channel coil at the OCMR (University of Oxford). A high-resolution T1-weighted MP RAGE (Brant-Zawadzki et al, 1992) image was acquired for accurate MRS voxel placement and subsequent structural analyses (TR = 2040 ms, TE = 4.7 ms, flip angle = 8°, 192 transverse slices, 1-mm isotropic voxels). B0 shimming was achieved using a GRESHIM (Shah et al, 2009), available as a work-in-progress package on the Siemens system. Spectra were measured with a semiadiabatic localization by adiabatic selective refocusing (SEMI-LASER) sequence (TE = 28 ms; TR = 4s; 64 averages) with variable power radiofrequency pulses with optimized relaxation delays (VAPOR) water suppression and outer volume saturation (Deelchand et al, 2014). Unsuppressed water spectra acquired from the same voxel were used to remove residual eddy current effects and to reconstruct the phased array spectra (Natt et al, 2005). Data were acquired in single-shot mode, that is, a single free induction decay was saved separately. Single-shot spectra were frequency and phase corrected before averaging over 64 scans.

Metabolites were quantified with LCModel (Provencher, 1993) using the unsuppressed water signal as reference. T1-weighted structural images were brain extracted and tissue-type segmented using the Brain Extraction Tool of FMRIB Software Library (FSL) (Smith, 2002) and Automated Segmentation Tool of FMRIB (Zhang et al, 2011). The percentage of gray matter, white matter, and cerebrospinal fluid within the MRS voxel was calculated from the resulting images and used to correct metabolite concentrations for cerebrospinal fluid fraction.

Statistics

We used two-tailed t-test to compare two means and analysis of variance to compare more than two means followed by Bonferroni *post hoc* test or t-tests corrected for multiple comparisons. Where possible, actual p-values are provided rather than a cutoff to declare significance or not. For data in which an underlying relationship was evident, we used regression and global nonlinear curve fitting as this provides more statistical power (Motulsky and Christopoulos, 2003).

RESULTS

Ebselen Reduces *Myo*-Inositol in the Anterior Cingulate Cortex

In studies using mice, ebselen decreased *myo*-inositol in brain extracts (Singh et al, 2013). To determine whether ebselen inhibits IMPase *in vivo* in humans, we used proton magnetic resonance spectroscopy (MRS) to quantify *myo*-inositol in healthy participants. In a double-blind, randomized crossover experimental design (Figure 1b and Table 1), ebselen reduced *myo*-inositol in the anterior cingulate cortex but not the occipital cortex (Figure 1c–e). In addition to *myo*-inositol, we also used MRS to quantify several other metabolites, and although some trends were observed such as an increase in combined glutamine and glutamate and a decrease in ascorbate, creatine, and choline, no changes were significant after correction for multiple comparisons (Supplementary Figure S1). Total creatine (creatine plus phosphocreatine) levels positively correlated with *myo*-inositol in both the placebo and ebselen groups (Figure 1f).

Ebselen Decreases Slow-Wave Sleep

Many psychotropic drugs used to treat mood disorders, including lithium and antidepressants, have defined effects on sleep (Friston et al, 1989; Sharpley et al, 1994). Lithium extends slow-wave sleep, also termed stage N3 sleep, through a mechanism thought to involve the 5-HT$_2$ receptors (Friston et al, 1989), possibly through inhibition of IMPase. To determine the effects of ebselen on sleep architecture, we monitored sleep in the same healthy participants as in the MRS study (Figure 1b). Ebselen decreased slow-wave sleep (Figure 1g) by reducing the number of slow-wave sleep episodes ($p = 0.058$) rather than the duration of each slow-wave sleep episode (Supplementary Table S1). Ebselen produced no other significant effects on sleep (Supplementary Table S1), and the decrease in slow-wave sleep did not affect either the total sleep time (Supplementary Table S1) or total sleep quality, as reported by the Leeds Sleep Evaluation Questionnaire (Parrott and Hindmarch, 1980) (Supplementary Figure S2). No correlation between the effect of ebselen on slow-wave sleep and *myo*-inositol was observed ($r = 0.38$, $p = 0.14$; Figure 1h). Taken together, these data show that ebselen decreases slow-wave sleep.

Oral Ebselen is Bioavailable and Well Tolerated

Plasma selenium concentrations have been shown to correlate with plasma levels of ebselen (Fischer et al, 1988; Lynch and Kil, 2009). Using selenium as a surrogate measure of ebselen and its metabolites, we found higher selenium in plasma of the participants taking ebselen than placebo (Figure 1i), demonstrating both compliance with dosing and the bioavailability of oral ebselen in humans, as reported previously (Fischer et al, 1989; Lynch and Kil, 2009; Yamaguchi et al, 1998). In our parallel arm experimental design (Figure 1b), participants who received ebselen then placebo 1 week later would have a predicted remaining amount of ebselen of 0.006% of the initial dose calculated from a reduction of ~ 14 half-lives (Lynch and Kil, 2009), which should be a sufficient washout time. Plasma selenium levels correlated with the magnitude of decrease in slow-wave sleep ($p = 0.017$; $r = 0.36$), but not with myo-inositol levels ($p = 0.70$). The participants consistently reported the same low number and low intensity of side effects with ebselen as the placebo (Figure 1j), consistent with the acceptable tolerability of ebselen as reported previously (Lynch and Kil, 2009; Parnham and Sies, 2013; Yamaguchi et al, 1998). Although mood questionnaires were administered, ebselen did not induce any changes relative to the placebo (Table 1), but this is not surprising because the dose was acute and even validated antidepressants taken acutely do not alter mood ratings (Harmer et al, 2011). Moreover, participants were healthy volunteers with low levels of negative affect and therefore may be relatively insensitive to change in subjective state.

Ebselen Decreases Reward Stimulus Reinforcement

Many neuropsychiatric disorders, including mania, are associated with an increase in poor decision making because of increased impulsivity and a hypersensitivity to reward (Whitton et al, 2015). As probing such functions invokes tasks that implicitly measure optimal choices made over time, we used a randomized, parallel-arm, experimental design (Figure 2a). To determine the effect of ebselen on decision making and learning, we used a task that tests the ability to distinguish between stimuli (decks of cards) with different probabilistic values of gain or loss, and the influence of learning from positive and negative reinforcement (Figure 2b). The task is divided into two conditions: the reward condition and the punishment condition. Each time the person has to chose one out of four decks of cards where they are likely to gain the most, that is, add points in the reward paradigm or not lose points in the punishment paradigm.

Ebselen had no overall discernable effect on the total correct choices made, when both the reward and loss paradigms were combined together (Figure 2c). However, when the correct choices made for rewarding stimulus and punishing stimulus were analyzed separately, ebselen significantly decreased the total correct reward choices made ($p = 0.034$; Figure 2d), although no significant increase in total correct loss choices made ($p = 0.14$; Figure 2e) was observed. This interaction was further corroborated through a learning measure that showed a significant interaction between the reward and loss paradigms and ebselen

($p = 0.01$), whereby ebselen decreases reward reinforcement and increases punishment reinforcement ($p = 0.01$; Figure 2f). Taken together, these data show that ebselen decreases learning through reward reinforcement.

Ebselen Increases Recognition of Disgust and Happiness in Emotional Processing Tasks

In mood disorders there are alterations in neural circuits regulating emotional processing, emotional regulation, and reward processing (Phillips and Swartz, 2014). Moreover, many psychotropic drugs affect tasks involving these processes (Phillips and Swartz, 2014), and certain emotional processing tasks in healthy participants respond to drugs in a manner that is predictive of clinical effect, suggesting such tasks might provide insight into the ultimate efficacy of new drugs (Harmer et al, 2011). To determine whether ebselen alters emotional processing, we used a battery of computerized tasks designed to tap basic emotional processing and mood responses to positive and negative emotional material. As the tasks have significant learning effects we used naive participants in a randomized parallel experimental design (Figure 2a).

In the facial emotion recognition task, participants are shown randomized pictures of varying degrees (40 for each emotion, 4 per degree) of the emotions anger, disgust, happiness, fear, sadness, and surprise, as well as 10 neutral faces (Figure 3a). Participants given ebselen showed a significant difference in the way they perceived some of these emotional faces ($p = 0.045$; Figure 3b). Ebselen did not affect miscalculations ($p = 0.10$) or sensitivity ($p = 0.078$; Figure 3b). Looking at the recognition of faces over different intensities, clear trends were evident with degrees of emotion (Figure 3c). As statistical power can be gained by fitting data to relationships that are not accounted for in analysis of variance, we applied a curve-fitting analysis using a four-parameter logistic equation (Motulsky and Christopoulos, 2003). Based on this analysis ebselen increased the recognition of anger ($p = 0.018$), disgust ($p < 0.0001$), and happiness ($p = 0.003$), but only disgust and happiness are deemed significant if a Bonferroni correction for multiple comparisons ($p < 0.008$) is applied. No differences were found in the recognition of sadness, fear, and surprise between the two groups (Figure 3b and c). Importantly, there was no difference in reaction time observed between the participants on placebo and those on ebselen (Figure 3b). Taken together, these results demonstrate that ebselen affected emotional processes in healthy participants. Ebselen did not affect performance in the Dot Probe (attentional bias), the emotional memory tasks (Supplementary Figure S3), or the Auditory Verbal Learning Task (Supplementary Figure S4).

Ebselen Decreases Latency in the Emotion Potentiated Acoustic Startle Task

Another way of investigating emotional processing is through the effect of emotion on the startle response, one of the most basic and primitive sensory responses to threatening external stimuli (Perry, 1999). Startle is largely modulated by the brain stem, though the emotional modulation might involve the limbic areas (Perry, 1999).
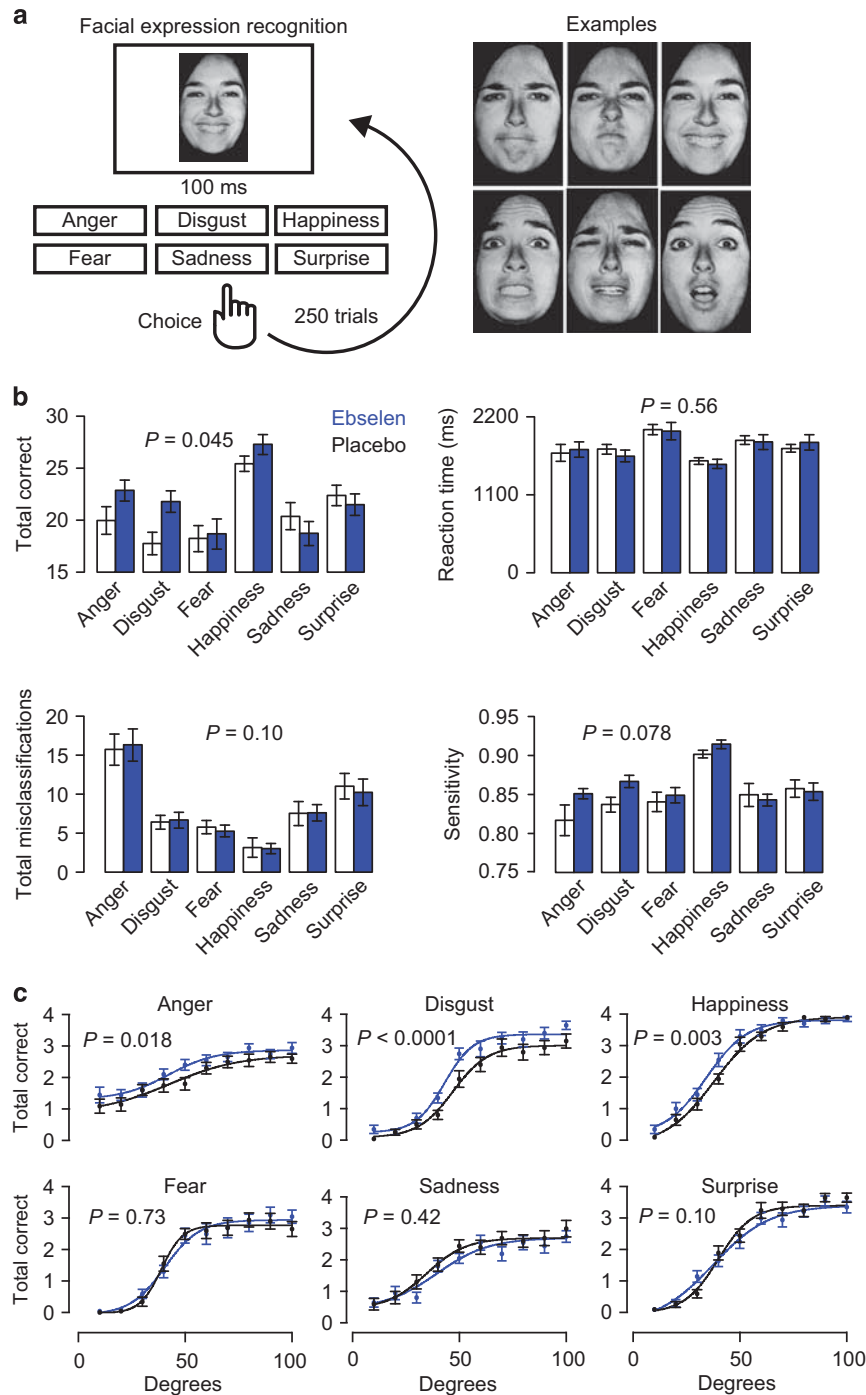
Figure 3 Ebselen increases the recognition of certain emotions but not others in facial expressions. (a) Schematic of the facial emotional expression recognition task. (b) Effect of ebselen on parameters related to the ability to recognize facial emotion. (c) Effect of ebselen on the ability to correctly recognize varying degrees of facial emotional expression. All data are plotted as mean ± SEM with $n = 20$. Statistical comparisons were performed with a two-way repeated measures analysis of variance. Statistical comparisons for (c) were performed by fitting a four-parameter equation (top, bottom, half-maximum and slope) and comparing whether the data sets were better fit with a common curve or independent curves. Actual p-values are shown in the panels.

To determine whether ebselen affected an emotional potentiated startle response, we monitored the effect of viewing pictures that elicit positive, negative, or neutral emotions on the amplitude and latency of the eye-blink component of the startle response (Figure 4a) (Lang et al, 1993). The participants and study design were the same as in the reward–loss and emotional processing tasks (Figure 2a). Ebselen did not affect the amplitude of response ($p = 0.51$; Figure 4b). The valence of the emotion (pleasant, neutral, and unpleasant) affected the amplitude of the response ($p = 0.009$; Figure 4b), consistent with previous reports (Murphy et al, 2008), and validates the experimental setup.
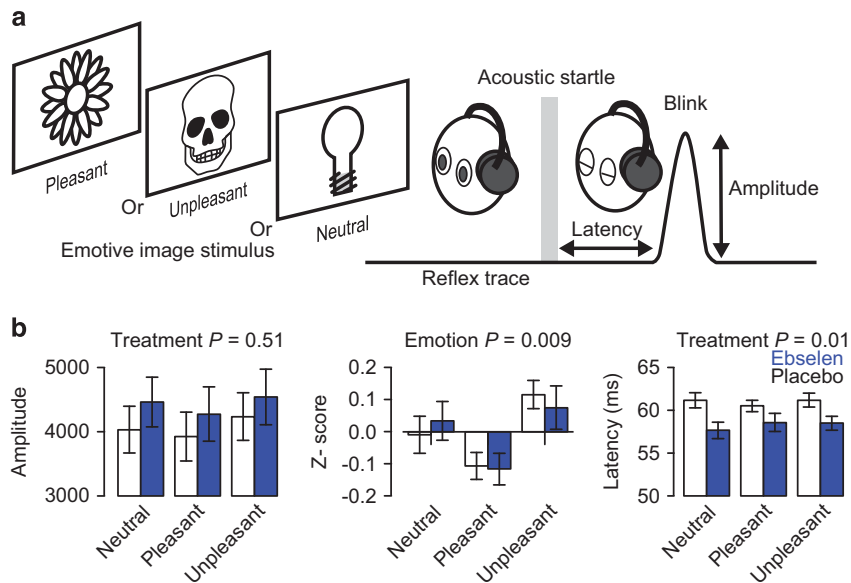
Figure 4 Ebselen decreases the latency in the emotion potentiated startle task. (a) Schematic of the startle task. An emotional image shown for 13 s modifies the latency and amplitude of an eye blink response elicited by an acoustic startle (100 dB, 100 ms). (b) Effect of ebselen on the emotionally modified startle reflex. Statistical comparisons were performed with a two-way analysis of variance with the p-values for interaction, treatment, and emotion, respectively: Z-score: 0.081, 0.72, and 0.009; amplitude: 0.79, 0.51, and 0.011; and latency: 0.44, 0.01, and 0.77. Each graph is labeled with the most salient p-value for its parameter.

Interestingly, ebselen decreased the latency of response ($p = 0.012$; Figure 4b) to the acoustic stimulus and the decrease in latency associated with ebselen was independent of the emotional valence of the picture (treatment × emotion interaction, $p = 0.443$).

DISCUSSION

We conducted an experimental medicine study of ebselen in humans with two interrelated goals: first, to determine whether it is warranted to progress the development of ebselen as a possible treatment for bipolar disorder by determining the effect of ebselen on CNS function, and, second, to gain insight into the mechanism of action of ebselen as a putative lithium mimetic. With regard to our first goal, ebselen is well tolerated and exhibited effects on emotional processing, thereby providing a validation step between animal models and trials for clinical efficacy, justifying continued study of ebselen as a potential treatment for bipolar disorder. Importantly, with regard to side effects and toxicity, none have been reported to date for ebselen at the administered doses (Lynch and Kil, 2009; Parnham and Sies, 2013; Yamaguchi et al, 1998), in marked contrast to lithium (McKnight et al, 2012). With regard to our second goal, the data are consistent with ebselen reducing *myo*-inositol *in vivo*, thereby indicating target engagement with IMPase.

We identified ebselen through a drug repurposing or rescue approach in which we screened for IMPase inhibitors (Singh et al, 2013). Underlying this approach is that all small molecules exhibit polypharmacology, meaning they affect multiple targets. Ebselen's polypharmacology (Parnham and Sies, 2013) is a double-edged sword with regard to our goals. As a possible treatment for bipolar disorder, ebselen's

polypharmacology (Parnham and Sies, 2013) is desired as it inhibits two targets shared with lithium and suggested to be therapeutically relevant including IMPase (Berridge et al, 1989; Quiroz et al, 2004) and protein kinase C (Zarate and Manji, 2009). Moreover, ebselen is an antioxidant, inhibits several of the proinflammatory enzymes, and, like lithium, is a neuroprotectant (Parnham and Sies, 2013). In terms of a novel drug for treating bipolar disorder, the most important things are safety and efficacy. Safety seems excellent based on all animal and human studies to date including the current one. Efficacy for bipolar disorder can be tested, and our results show that this is worthwhile as ebselen has effects in emotional processing tasks that are surrogate markers for efficacy (Harmer et al, 2011).

With regard to our second goal of gaining insight into the therapeutic target and mechanism of lithium, ebselen's polypharmacology complicates definitive conclusions regarding mechanism. All drugs have multiple targets, but insight can be gained by analyzing targets that are shared or unique. Ebselen is ∼20-fold selective for inhibition of IMPase over glycogen synthase kinase-3β (Singh et al, 2013). The main contenders for the therapeutic target of lithium are glycogen synthase kinase-3β and IMPase (Quiroz et al, 2004). As lithium and ebselen affect several targets but IMPase is the known shared target, the most parsimonious explanation for similar effects of lithium and ebselen is inhibition of IMPase. Moreover, using similar logic, it is likely that the side effects and acute toxicity of lithium (McKnight et al, 2012) and its effects on slow-wave sleep (Friston et al, 1989; Sharpley et al, 1994) originate not through IMPase inhibition but through one of its other targets (Quiroz et al, 2004).

That we observed changes in *myo*-inositol in the anterior cingulate cortex but not the occipital cortex is consistent with the relevance of these regions with regard to bipolar disorder

and changes in these regions in response to lithium (Davanzo et al, 2003; Moore et al, 1999; Sharma et al, 1992). Indeed, in studies with lithium where changes in myo-inositol were observed, changes were in the frontal lobes, which are associated with emotional regulation and linked to mood disorders, and not in the occipital, parietal, or temporal regions (Moore et al, 1999). Indeed, this is why we chose to make our measurements in these two regions. As myo-inositol decreases precede and may be predictive of clinical improvement in bipolar disorder (Davanzo et al, 2003; Moore et al, 1999), ebselen may have clinical utility. In comparison, previous studies with lithium in which myo-inositol levels were measured in humans, both in bipolar patients and healthy participants, have yielded mixed results (Davanzo et al, 2003; Moore et al, 1999; Patel et al, 2008; Sharma et al, 1992; Silverstone et al, 2005; Silverstone and McGrath, 2009). Ebselen may have a more immediate and pronounced effect compared with lithium, as it does not have to be slowly titrated to achieve therapeutic concentrations while avoiding toxicity. In addition, we expressed myo-inositol in absolute terms (standardized to cerebrospinal fluid fraction in the voxel), avoiding the influence of possible changes in other metabolites such as creatine that can change with treatment (Silverstone et al, 2005; Silverstone and McGrath, 2009). The magnitude of the decrease in myo-inositol is modest, but steady-state levels might equate to much larger changes in actual metabolic flux. In addition, although we tested a very specific hypothesis relating to whether ebselen was inhibiting IMPase and causing a reduction in brain myo-inositol, alternative explanations exist and could be tested in future experiments such as ebselen inducing a change in kidney function that affected plasma osmolality that would then result in a change in brain myo-inositol where it functions as an osmolyte (Häussinger et al, 1994).

A well-known difficulty in screening novel drugs for their effects on mood is that even with drugs of validated efficacy, it can take several weeks for the effects to manifest (Harmer et al, 2011). This has stimulated an interest in identifying surrogate markers that would show detectable and quantifiable changes after an acute dosing. Even if no mechanistic explanation exists, a reliable surrogate marker can serve as a valuable step in screening compounds, as it would be fast and inexpensive relative to a clinical trial designed to probe efficacy in a given disorder. A particularly promising area for surrogate markers that respond to drugs with validated long-term efficacy in the treatment of mood disorder but show a rapid, reproducible, and quantifiable response is emotional processing (Harmer et al, 2004, 2009, 2011; Murphy et al, 2008; Rock et al, 2010). These tests are designed to tap into the neuronal circuits postulated to be altered in patients with mood disorder and manifest as negative emotional bias in tasks such as recognizing the mood in faces (Harmer et al, 2011). For example, depressed patients demonstrate negative emotional bias in the facial recognition task by being less likely to recognize happiness and more likely to recognize sadness. The utility of these tasks is potentially great because even in healthy subjects, drugs can predictably alter emotional bias and which correlates with the known long-term efficacy of the drugs in treating depression (Harmer et al, 2011).

Ebselen affected emotional processing in healthy participants. The effect sizes are in the moderate range based on Cohen's d and consistent with the effect size induced by other drugs used in the treatment of mood disorder (Harmer et al, 2004, 2009, 2011; Murphy et al, 2008; Rock et al, 2010). These results provide good evidence to suggest that ebselen is having effects on pathways known to be modulated by drugs currently in use to treat mood disorders. Several of the observed responses to ebselen can be taken as promising in relation to using it in the treatment of bipolar disorder. For example, that ebselen decreases learning through reward reinforcement may be relevant to bipolar disorder given that patients, especially in the manic or hypomanic phases, have an increased tendency to make impulsive decisions, gamble, and are hyperresponsive to rewarding stimuli (Whitton et al, 2015). Ebselen may increase the caution with which a choice is being made that might reduce reckless behavior in bipolar patients or patients with increased impulsivity. Similarly, ebselen increases the recognition of happy faces (positive expression) as well as disgusted faces (negative expression). Antidepressants also show significant effects in this task, but typically increase the relative processing of positive rather than negative facial expressions (Harmer et al, 2004). The effect of ebselen to increase recognition of disgust is intriguing because previous studies with the same task revealed the same trend in medicated euthymic bipolar patients (Harmer et al, 2002). Moreover, untreated students at risk of mood disorders are less able to identify 'disgust' accurately (Rock et al, 2010).

A crisis exists in drug development for mental health with industry abandoning research and development on psychiatric disorders because of the lack of both mechanistic understanding and validated targets (Conn and Roth, 2008). This has opened opportunities for academics to pursue strategies such as finding new uses for old drugs termed repurposing for marketed drugs or rescuing for abandoned drugs (Cavalla, 2009; Conn and Roth, 2008). The major promise of these strategies is that they can facilitate the rapid translation of results into humans because the compounds have cleared the early hurdles of drug development. However, development of a marketed drug for a new disease is often prevented by commercial, regulatory, and reimbursement challenges (Cavalla, 2009). In contrast, development of a nonmarketed drug like ebselen for bipolar disorder is more attractive because of enforceable market exclusivity based on a 'use' patent (Cavalla, 2009).

CONCLUSIONS

Ebselen decreases myo-inositol in brain regions associated with emotional processing consistent with inhibition of IMPase as proposed by the inositol depletion hypothesis (Berridge et al, 1989). These results highlight the potential for developing more selective inhibitors of IMPase or other targets in the phosphoinositol cycle. Ebselen affected performance in several emotional processing tasks. As these tasks are surrogate markers for affective disorders and correlate with efficacy of known mood-modulating drugs (Harmer et al, 2011), it is warranted to test ebselen for efficacy in bipolar disorder.

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AUTHOR CONTRIBUTIONS

GCC, SRV, TS, and PJC conceived the study. NS and ALS managed the project. ALS performed and analyzed the sleep studies. UEE performed the MRS studies. CM helped with recruitment. MMH and MAG developed the reward and punishment task. CJH designed the emotional processing studies that were performed and analyzed by NS. NS compiled and analyzed the data with input from all authors. NS and GCC wrote the manuscript with input from all the authors.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)

SUPPLEMENTARY INFORMATION

Effect of the putative lithium mimetic ebselen on brain *myo*-inositol, sleep and emotional processing in humans

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SUPPLEMENTARY SUBJECTS AND METHODS

Facial expression recognition task

The facial recognition task is designed to test the participant's ability to recognize different emotional expressions from picture stimuli, as well as distinguish between the degrees of those emotional expressions. The task features 6 basic emotions: anger, disgust, fear, happiness, sadness and surprise, which are taken from the Pictures of Facial Affect series[75]. Additionally, the pictures have been morphed by taking a variable percentage of the shape and texture differences between the two standard images 0% (neutral) and 100% (full emotion) in 10% steps, as described previously[76]. Four examples of each of the different emotions were presented. Each face was also given in a neutral expression, giving a total of 250 stimuli presentations, that is, 40 for each of the six emotions (6 x 40 = 240) plus 10 neutral expressions.

The order of face stimuli presentation on a computer screen was randomized, and each stimulus was presented for 500 ms followed by a blank screen. Participants had to indicate which emotional expression was presented by pressing the appropriately labeled key on a keyboard, and were instructed to respond as quickly and accurately as possible. Only once a response had been registered, was the next stimuli presented. For each emotion, accuracy (total correct out of 40) and response time (ms) were recorded.

Statistical significance was determined using the two way, repeated measures analysis of variance, the two-tailed, unpaired Student's t-test, or by a global curve fitting, as appropriate.

Emotion potentiated startle task

For this task, participants were instructed that pictures would be shown on the screen, of which some may be quite gruesome and all they had to do was watch the pictures[44]. They were further

instructed to wear headphones and that there would occasionally be a loud burst of white noise, which they should ignore. The lights were switched off for the task, and there was an experimenter present in the room, just in case the participant did not want to carry on with the task. In order to habituate participants they were shown an introductory set of nine neutral pictures during which they received nine startle probes, just prior to the task itself.

Picture stimuli from the International Affective Picture System were used and presented for 13 s. There were three experimental blocks of trials, 21 pictures per block presented in a random order. The pictures were designed to elicit positive, negative or neutral emotions. The negative pictures and the positive pictures were rated to be of equal arousal but difference valence, that is, the negative pictures have the same intrinsic aversiveness as the positive pictures have intrinsic attractiveness. The neutral pictures were rated to be low on arousal and average on valence[45].

The eye-blink component of the startle response was recorded from the orbicularis oculi using surface electro-myography startle response system, San Diego Instruments, San Diego, CA, USA). Acoustic probes were 50 ms, 100-decibel bursts of white noise with a nearly instantaneous rise time delivered binaurally through headphones (generated through the noise generator and amplifier component of the electro-myography-SR system, San Diego Instruments). Probes were delivered at 1.5, 4.5 and 7.5 s after picture onset. Within each block of 21 pictures, probes were delivered during 5 of each trial type (neutral, positive and negative). To limit expectation of the noise, two trials per valence did not contain any startle probe, and three probes per block were delivered in the intertrial interval.

Electro-myography signals were sampled at a rate of 1,000 samples per s, and the signal was filtered between 1 and 300 Hz and then smoothed with a filter window of 5 ms and rectified.

Eye blink magnitudes (in µV) were calculated as the peak amplitude of the eye blink reflex 20–120 ms after probe onset relative to baseline (average electro-myography signal for 20 ms time frame after probe onset). Eye-blink reflexes with excessive noise, that is, higher baseline levels of activity compared to identified peaks, were excluded. An experimenter who was blind to the treatment group allocation evaluated trials. The quantified peak was identified as the one with the highest amplitude between 30–80 ms after the probe onset. Eye-blink magnitudes were analyzed both as raw data and also z (or t)- transformed within participants to allow direct comparison of the acoustic startle response during neutral, positive and negative pictorial stimuli presentation. The numbers for the two groups were $n = 19$ for the placebo group, and $n = 17$ for the ebselen group. Other participants were excluded if the signal to noise was too low, or they did not complete the task. On average, at least 25% of the total trials had to be quantified for inclusion in data analysis. Statistical significance was determined using the two-way, repeated measures analysis of variance.

Auditory Verbal Learning Task

The testing was carried out as published by Rey[65,66]. Each participant was read out a list of 15 unrelated common nouns (List A) and was asked to recall as many of the words as possible, which were noted by the researcher. This process was repeated four more times (total five times). After five free recalls, another list of 15 nouns, called the interference list (List B), was read out and the participant was asked to recall as many words as possible. Immediately after this, the participant was asked to recall List A again (short delay recall). After an approximately 12 min gap (during which the reward and punishment learning task was carried out) the participants were asked once again to recall as many words as they could from List A (long delay recall). Lastly, a list of 50 nouns, containing all of the 15 words from List A, was read out and the

participant was asked to say if the words were present in List A or not (Recognition). The total correct entries were calculated for each recall, as well as the incorrect words (intrusions) and repeated words (repetitions). Statistical significance was determined using the two-way, repeated measures analysis of variance or the two-tailed, unpaired Student's t-test, as appropriate.

Emotional categorization: recall and recognition task

In this task, participants had to characterize personality related words, as likable (positive) or unlikable (negative). Sixty personality characteristic words, selected to be disagreeable (e.g., domineering, hostile) or agreeable (e.g., cheerful, generous), taken from Anderson[77], were presented on a computer screen for 500 ms. Participants were instructed to indicate, as quickly and accurately as possible, whether they would 'like' or 'dislike' to be described as the personality characteristic displayed on the screen, by pressing the appropriate key. The classification and response times for the correct choices was recorded[47]. For the recall section of the task, the participants were asked write down all the words that they could remember, within one min. This part of the task took place 10 min later and was interspersed with the dot-probe task.

Following the recall task, recognition memory was tested by asking the participants to respond to the personality characteristic words presented on the screen, by responding 'Yes', if they thought that the word was presented in the recognition part of the task, or 'No', if it was not. The words presented contained all the sixty words that were presented earlier in addition to 60 matched distractors (30 positive and 30 negative), presented in randomized order. Participants were not told about the Recall and Recognition aspects of the tasks prior to undertaking it. Statistical significance was determined using the two-way, repeated measures analysis of variance.

Dot probe task

This task is designed to measure the participant's vigilance or attention to respond to a stimulus, when a positive or negative emotional expression precedes the response[47]. Pairs of photographs of 20 individuals were taken from the JACFEE/JACNeuF sets of facial expressions[78]. Each face pair comprised one emotional and one neutral expression of the same individual or two neutral expressions of the same individual. Half of the emotional faces were fearful and the other half were happy. Thus, there were three types of face pair: neutral–neutral, fearful–neutral and happy–neutral.

On each trial, one of the faces appeared above and the other below the central fixation position. The emotional faces appeared in the top and bottom location with equal frequency. In the unmasked condition, the face pair was presented for 100 ms, and then, a probe appeared in the location of one of the preceding faces. The probe was two dots presented either vertically (:) or horizontally (··). Participants were required to report the orientation of the dots by pressing a labeled key on a keyboard. Participants were asked to respond as quickly and as accurately as possible. The sequence of events was the same in the masked condition, except the face pair was displayed for 16 ms and followed by a mask (constructed from a jumbled face), which was displayed for 84 ms.

On half of the emotional–neutral face trials, the probe appeared in the same position as the emotional face, and on the other half, the probe appeared in the same position as the neutral face. There were 192 trials in total (masked: 32 happy–neutral, 32 fear–neutral, 32 neutral–neutral; unmasked: 32 happy–neutral, 32 fear–neutral, 32 neutral– neutral). There were 8 blocks of unmasked trials (12 trials per block) and 8 blocks of masked trials (12 trials per block), which were presented in an alternating order.

Incorrect trials were excluded from the data analysis. Attentional vigilance scores were calculated for each participant by subtracting the mean reaction time from trials when probes appeared in the same position as the emotional face from trials when probes appeared in the opposite position to the emotional face (incongruent trials minus congruent trials). Statistical significance was determined using the two-way, repeated measures analysis of variance.
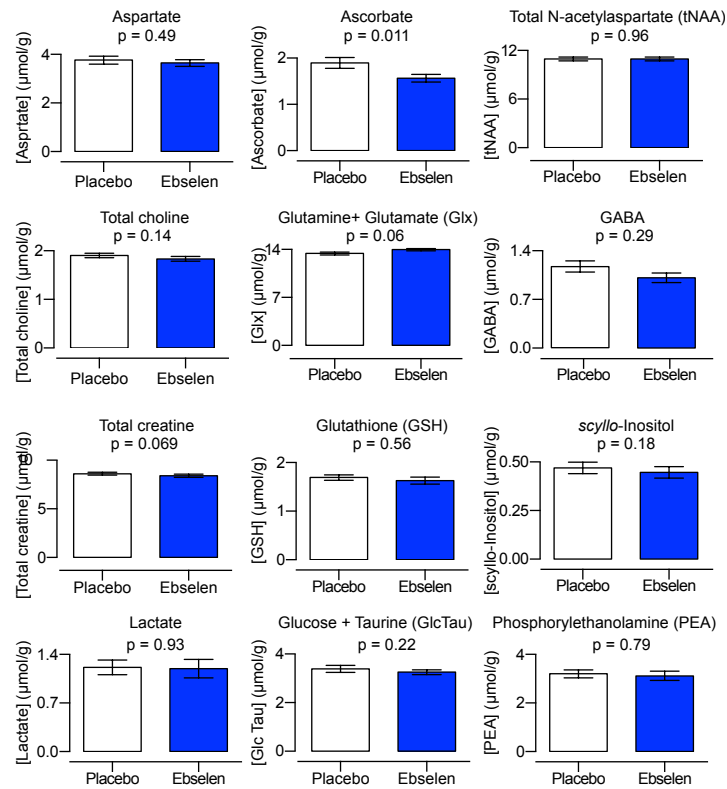
Supplementary Figure 1   Effect of ebselen on the other measured metabolites in the anterior cingulate cortex. *P*-values are calculated using the two-tailed, paired, Student's t-test. The *P*-values shown here are not corrected for Type-1 error. In all cases n = 16, except in case of ascorbate (n = 15), lactate (n = 12) and GABA (n = 7).
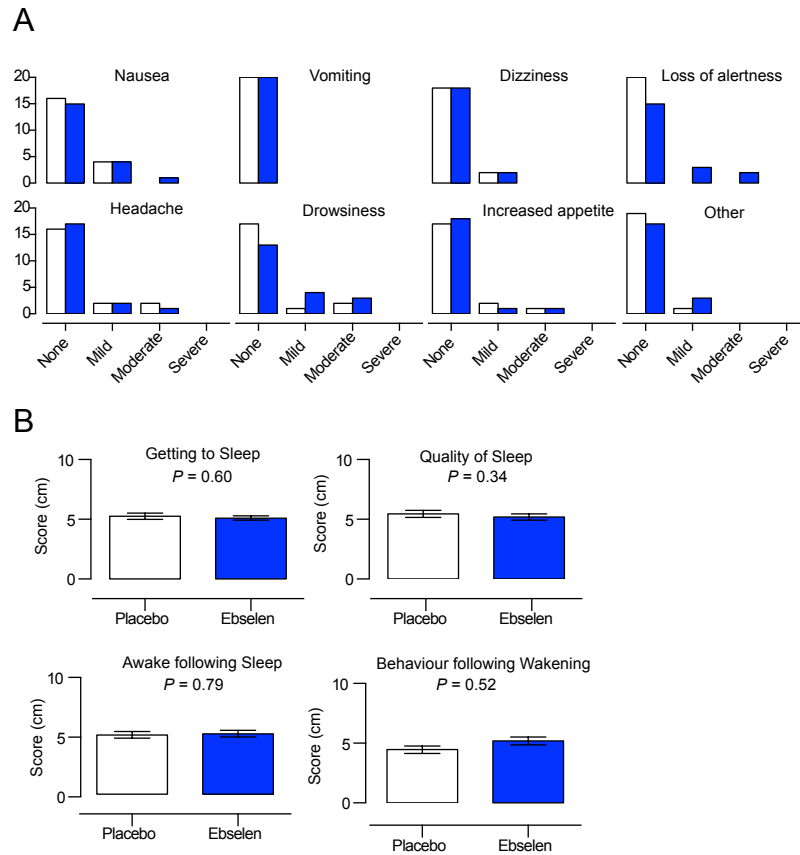
Supplementary Figure 2   Summaries of questionnaires (**A**) Comparison of the frequency of side effects reported by participants in the emotional processing tasks after taking three doses of ebselen or placebo (n = 20/group). (**B**) Comparison of the quality of sleep as reported by the participants in the Leeds Sleep Evaluation Questionnaire after taking ebselen and placebo (n = 16). There were to statistically significant differences found on all four parameters assessed, i.e., getting to sleep, quality of sleep, awake following sleep and behavior following wakening, using the two-tailed, paired, Student's t-test.
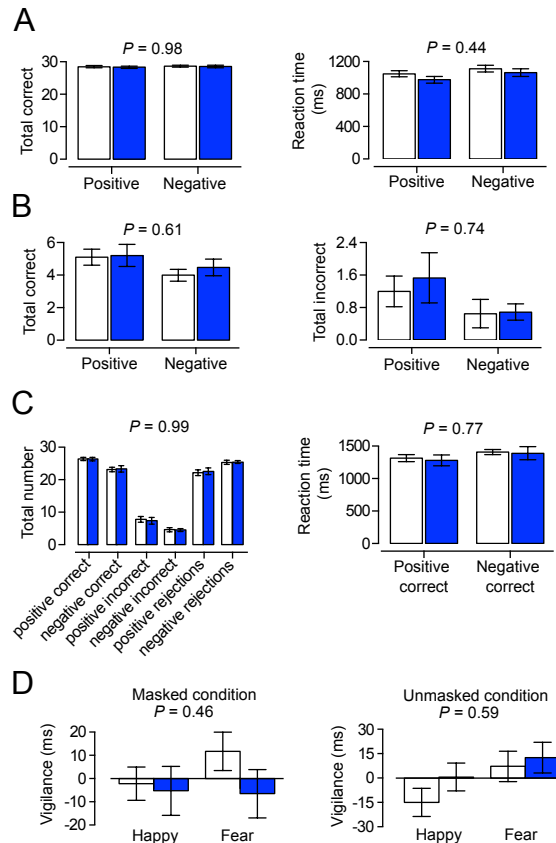
Supplementary Figure 3   Additional emotional processing tasks. (**A**) Emotional Categorization Task. Participants on ebselen showed no statistically significant difference in the ability to categorize positive and negative emotional words, nor in the reaction time to categorize them, compared to participants on placebo. (**B**) Emotional Recall Task. Participants on ebselen, when compared to participants on placebo, showed no statistically significant difference in the recall of the positive and negative words shown previously in the emotional categorization task, as shown by the figure on the left hand side. Additionally, the figure on the right hand side shows that there were no significant differences present in the recall of emotional words not present in the categorization task. (**C**) Emotional Recognition Task. Participants on ebselen showed no significant differences in the recognition of the positively and negatively valenced emotional words presented previously in the categorization task. Also, there were no significant differences in the inaccuracy of the recognition of these words, between the participants on ebselen and placebo (figure on the left hand side). The figure on the right shows that there was no significant difference in reaction times in either accurate recognition of the positively or the negatively valenced words. (**D**) Dot Probe task. No significant differences were observed in vigilance to either the happy or fear conditions, in the masked (left) and unmasked (right) instances (n = 20). In all cases, n = 20/group and the *P*-value was determined using the two-way, repeated measures, analysis of variance statistical test.
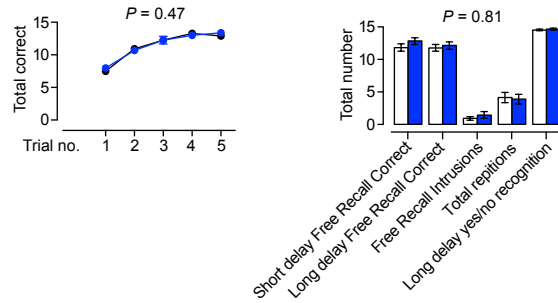
Supplementary Figure 4 The Auditory Verbal Learning Task. No significant differences were seen in any of the parameters measured in this task. There was no difference in learning over time, as shown in the figure on the left, between participants on ebselen or placebo. Additionally, there were no differences in recalls, intrusions, repetitions or recognition of the words as shown in the figure on the right (n = 19). *P*-values were calculated using the two-way, repeated measures, analysis of variance statistical test.

Supplementary Table 1 Sleep Parameters

Sleep Parameters	Placebo (Mean ± SEM)	Ebselen (Mean ± SEM)	P value
Total Sleep Time (min)	414.8 ± 12.1	421.3 ± 12.2	0.48
Sleep Efficiency (%)	88.56 ± 1.9	89.5 ± 1.8	0.49
Wake After Sleep Onset (min)	34.66 ± 7.4	30.63 ± 7.8	0.64
Stage N1 (min)	42.34 ± 4.0	46.63 ± 3.3	0.24
Stage N2 (min)	120.6 ± 9.0	126.1 ± 8.7	0.56
Stage N3 or slow wave sleep (SWS) (min)	144.6 ± 7.3	133.1 ± 7.3	0.035 *
Stage N3 (SWS) (%)	35.38 ± 2.2	31.77 ± 1.8	0.027*
Number Of SWS periods	7.25 ± 0.37	6.38 ± 0.41	0.058
Rapid Eye Movement (REM) (min)	107.1 ± 5.8	115.5 ± 8.7	0.26
REM Latency (min)	71.53 ± 5.0	60.53 ± 3.4	0.076