NeuroPharm study

EEG wakefulness regulation as a biomarker in MDD

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NeuroPharm study: EEG wakefulness regulation as a biomarker in MDD

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ABSTRACT

While several electroencephalogram (EEG)-based biomarkers have been proposed as diagnostic or predictive tools in major depressive disorder (MDD), there is a clear lack of replication studies in this field. Markers that link clinical features such as disturbed wakefulness regulation in MDD with neurophysiological patterns are particularly promising candidates for e.g., EEG-informed choices of antidepressive treatment. We investigate if we in an independent MDD sample can replicate abnormal findings of EEG-vigilance regulation during rest and as a predictor for antidepressive treatment response. EEG-resting state was recorded in 91 patients and 35 healthy controls from the NeuroPharm trial. EEG-vigilance was assessed using the Vigilance Algorithm Leipzig (VIGALL). We compared the vigilance regulation during rest between patients and healthy controls and between remitters/non-responders after eight weeks of SSRI/SNRI treatment using two different sets of response criteria (NeuroPharm and iSPOT-D). We replicated previous findings showing hyperstable EEG-wakefulness regulation in patients in comparison to healthy subjects. Responders defined by the iSPOT-D criteria showed a higher propensity toward low vigilance stages in comparison to patients with no response at pretreatment, however, this did not apply when using the NeuroPharm criteria. EEG-wakefulness regulation patterns normalized toward patterns of healthy controls after 8 weeks of treatment. This replication study supports the diagnostic value of EEG-vigilance regulation and its usefulness as a biomarker for the choice of treatment in MDD.

1. Introduction

Despite concerted efforts, there is still a lack of validated objective biomarkers in major depressive disorder (MDD) (Kennis et al., 2020) although this would be useful not only for differential diagnostic purposes but also for predicting pharmacological treatment response (Olbrich and Conradi, 2016). Neurophysiological methods have gained attention as they provide an affordable framework that reflects the functional aspects of the nervous system at a high-resolution timescale (Olbrich and Arns, 2013; Widge et al., 2018). In particular, electroencephalogram (EEG) is a non-invasive measurement of neuronal activity with the potential to provide clinically relevant biomarkers (Grzenda and Widge, 2020; Olbrich and Arns, 2013; Rolle et al., 2020).

Dysregulation of sleep and wakefulness is part of the diagnostic criteria for MDD (Nutt et al., 2008; Seifritz, 2001): Patients often feel tired and fatigued during the day but have difficulties falling asleep and wake up early which leads to a vicious cycle of sleep disturbances and tiredness. Polysomnographic measurements have with some success been used to differentiate patients and healthy controls (Thase, 2006), but there is only limited value for those markers to predict antidepressive drug response (Steiger and Pawlowski, 2019) and is not sensitive to important aspects of the sleep-wake dysregulation in MDD.

Abbreviations: GAD-10, generalized anxiety disorder-10 score; HDRS, Hamilton Depression Rating Scale.
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The Vigilance Algorithm Leipzig (VIGALL) has been developed to identify and analyze different functional brain states from full wakefulness to sleep onset with closed eyes by using EEG and electrooculogram data (Hegerl et al., 2012; Olbrich et al., 2015). The outcome of the algorithm has been compared with other imaging modalities (Guenther et al., 2011; Olbrich et al., 2009) and clinical data (Jawinski et al., 2015).

According to the VIGALL framework of EEG wakefulness regulation, MDD patients tend to show a hyperstable wakefulness regulation with a less propensity toward relaxation and sleep stages in comparison to healthy subjects (Hegerl and Hensch, 2014; Olbrich et al., 2012). For the full clinical utility of VIGALL, it is essential to demonstrate its prognostic power for treatment outcomes. So far, two studies have investigated this. Data from the International Study to Predict Optimised Treatment - in Depression (iSPOT-D) (Williams et al., 2011) showed that patients with a good response to selective serotonin reuptake inhibitors (SSRIs) had a faster decline of wakefulness during a 2-min resting state than non-responders (Olbrich et al., 2016). Contradictory, a study by Schmidt et al. (2017) showed that patients with a pronounced hyperstable wakefulness regulation during 15 min of rest were more likely to respond to SSRI treatment.

Since treatment response seems to be a clinically more relevant topic than the improvement of diagnostic features (Olbrich and Conradi, 2016), the main aim of this study was to replicate previous findings when considering the prediction of treatment outcome. This is especially important in the light of missing replications in this field (Widge et al., 2018). Therefore, this study investigated the VIGALL outcomes in an independent cohort of patients with MDD from the NeuroPharm trial (Köhler-Forsberg et al., 2020), adjusting all possible parameters according to the previous iSPOT-D study (Olbrich et al., 2016), including channel number, EEG-parameters and used clinical outcome measures.

We wanted to see if we could replicate 1) the predictive properties of the VIGALL algorithm with respect to treatment outcome for SSRIs and SNRIs as reported from the iSPOT-D study (Olbrich et al., 2016) and 2) the findings of a hyperstable EEG wakefulness regulation in MDD in comparison to healthy controls. It was hypothesized that 1) responders will show a less stable EEG wakefulness regulation over time as assessed with the VIGALL algorithm and 2) patients suffering from MDD will show more high vigilance stages and a less propensity toward sleep stages in comparison to healthy controls. In an exploratory analysis, we also assess the treatment effect on VIGALL parameters.

2. Materials and methods

NeuroPharm is a non-randomized, open-label clinical trial in an outpatient setting. A completed consortium diagram and the flowchart of the study protocol are available elsewhere (Köhler-Forsberg et al., 2020, also see Fig. 1). The current VIGALL analysis was not included in the study protocol thus it is an add-on analysis to the NeuroPharm trial.

2.1. Subjects

One-hundred medication-free MDD outpatients were recruited and their diagnosis was ascertained by a Mini-International Neuropsychiatric Interview (Sheehan et al., 1998) and confirmed by a certified psychiatrist. Details of inclusion and exclusion criteria are described elsewhere (Köhler-Forsberg et al., 2020). Thirty-five healthy subjects were included as controls (See Table 1 for demographic features). All participants provided written informed consent prior to participation. Ethical approval was obtained by the National Committee on Health Research Ethics (protocol: H-15017713).

After the pretreatment visit, patients were treated first line with the

![Fig. 1. The flowchart of the study design. See the dashed frame for the definition of treatment responses in NeuroPharm. Medication-free patients diagnosed with MDD were included and allocated to baseline intervention. The treatment responses were measured as the percentage change in HDRS6 score from baseline to week 4 or 8 (ΔHDRS6). Three categorical treatment outcomes were defined: Remitters included patients with ΔHDRS6 > 50% at week 4 and <5 points on the HDRS6 scale at week 8; non-responders included patients with ΔHDRS6 < 25% at week 4 and ΔHDRS6 < 50% at week 8 were considered non-responders. Patients who did not fulfill either remitter or non-responder criteria were classified as intermediate responders.](image-url)
Table 1
Sociodemographic characteristics for pretreatment visit and clinical outcome at week 8.

<table>
<thead>
<tr>
<th>Clinical outcome</th>
<th>Pretreatment visit</th>
<th>Clinical outcome at week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy controls</td>
<td>MDD (91)</td>
</tr>
<tr>
<td>(n)</td>
<td>(35)</td>
<td>(42)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>10/25</td>
<td>25/66</td>
</tr>
<tr>
<td>Age (Mean ± SD)</td>
<td>29.0 ± 9.7</td>
<td>27.4 ± 8.3</td>
</tr>
<tr>
<td>Education</td>
<td>16.0 ± 1.4</td>
<td>14.9 ± 2.2</td>
</tr>
<tr>
<td>GAD&lt;sub&gt;x&lt;/sub&gt;</td>
<td>26.9 ± 9.7</td>
<td>23.4 ± 7.9</td>
</tr>
<tr>
<td>Pretreatment HDRS</td>
<td>22.9 ± 3.4</td>
<td>23.0 ± 3.5</td>
</tr>
<tr>
<td>Week 8 HDRS</td>
<td>(12.4 ± 1.7&lt;sup&gt;d&lt;/sup&gt;)</td>
<td>7.0 ± 3.3</td>
</tr>
</tbody>
</table>

Notes.
<sup>a</sup> HDRS<sub>17</sub> scores are shown in both pretreatment and week 8 HDRS<sub>17</sub> scores.
<sup>b</sup> HDRS<sub>6</sub> scores are shown in both pretreatment and week 8 HDRS<sub>6</sub> scores.
<sup>c</sup> Healthy controls had a significantly higher education score compared to MDD (t (98) = −2.607, p = .011).
<sup>d</sup> Responders were defined by at least 50% improvement of depressive symptoms assessed by HDRS<sub>17</sub> score. HDRS<sub>17</sub> scores are shown in both pretreatment and week 8 HDRS<sub>17</sub> scores.
<sup>e</sup> Intermediate responders were not included in the primary analysis; thus, no statistical testing was performed with this group of patients.
<sup>f</sup> Between-group comparisons showed that remitters had significantly lower HDRS<sub>6</sub> score at week 8 compared to non-responders (t (34) = −12.71, p < .001); responders had significantly lower HDRS<sub>17</sub> score at week 8 compared to non-responders (t (77) = −11.90, p < .001). No significant effect was found for other demographic characteristics (p values > .05).

SSRI escitalopram at flexible doses of 5–20 mg/day. Doses were adjusted depending on effects and side effects by trained physicians at each visit at weeks 1, 2, 4, 8, and 12. Hamilton Depression Rating Scale 6 items (HDRS<sub>6</sub>) was used for assessment of antidepressant response since it has been shown to be more sensitive to treatment outcome (Bech et al., 2006, 2010; Østergaard et al., 2016). Patients with no response to escitalopram after 4 weeks, or with intolerable side effects were offered second line treatment of SNRI duloxetine, with a dose ranging of 120 mg/day (n = 15).

EEG recordings were obtained at pretreatment visits (unmedicated) for all included participants, and 40 of the patients were recorded again after 8 weeks of treatment. A total of 79 patients (see Fig. 1 for the exclusion reason) and 35 healthy controls were included in the pre-protocol analyses for the pretreatment data. In order to explore the drug effect, three patients suspected of non-compliance because of drug levels below ≤5 nM at week 8 were excluded from the post-hoc analysis. One patient was excluded from the follow-up analysis due to a technical error. Therefore, EEG data from 39 patients’ data was available for further analyses at 8 weeks follow-up.

2.2. Clinical measures and treatment response

The treatment responses of NeuroPharm were measured as a percentage change in HDRS<sub>6</sub> score from baseline to week 4 or 8 (ΔHDRS<sub>6</sub>). Three categorical treatment outcomes were defined (Fig. 1): Remitters included patients with ΔHDRS<sub>6</sub> > 50% at week 4 and <5 points on the HDRS<sub>6</sub> scale at week 6; non-responders included patients with ΔHDRS<sub>6</sub> < 25% at week 4 and ΔHDRS<sub>6</sub> < 50% at week 8 were considered non-responders. Patients who did not fulfill either remitter or non-remitter criteria were classified as intermediate responders (Fig. 1).

To allow comparisons with already existing studies, the criterion defined by the NeuroPharm consortium (Köhler-Forsberg et al., 2020) and the one used for the iSPOT-D data (Olbrich et al., 2016) were reported in this add-on analysis to the NeuroPharm Trial. When treatment response of iSPOT-D was assessed, using the NeuroPharm data, remission was defined as a score of 7 at week 8 on HDRS<sub>17</sub> else patients were defined as non-remitter. Responder was defined as > 50% decrease in HDRS<sub>17</sub> (from pretreatment to week 8) else patients were defined as non-responders.

2.3. EEG recordings

Resting EEG was recorded during four 3 min periods with a counterbalanced order of COCO (O for eyes open, C for eyes closed) or COCO between subjects (details of recording condition are detailed elsewhere: Ip et al., submitted). EEG data was acquired from a 256-channel HydroCel Sensor Net system (EGI, Inc., Eugene, OR) at 1000 Hz sampling rate and referenced to the vertex electrode. Impedances were kept below 50 kΩ.

2.4. EEG processing and classification of EEG-vigilance

Corrupted channels were interpolated using spline interpolation (Perrin et al., 1989). Twenty-five VIGALL EEG channels were selected from our high-density net according to the VIGALL manual (VIGALL 2.1 manual; https://research.uni-leipzig.de/vigall/). HEOG was recorded from two electrodes at the outer canthus of the right and left eye and VEOG was recorded from the infraorbital and supraorbital regions of the right eye, keeping it as close as possible to the VIGALL manual. The EEG with the applied VIGALL montage was down-sampled to 250 Hz and re-referenced offline to an average reference. The data was cut into 1 s epochs for visual artefact inspection such as movement and electrical artifacts and was further processed in Brain Vision Analyzer 2.0 (Brain Products GmbH, Glitching, Germany). Artefact segments were marked, but not removed to retain the full-time series for each subject. A zero-phase digital IIR Butterworth bandpass filter with cut-off frequencies 0.5 and 70 Hz, and a 50 Hz notch filter was applied to the EEG-data after eye-movement artifacts correction (independent component analysis approach). Electrooculogram channels were band-pass filtered with cut-off frequencies below 0.01 and above 70 Hz to retain slow eye movements.

Vigilance was assessed from the eye-closed resting EEG data by using the algorithm-based Vigilance Algorithm Leipzig (VIGALL 2.0, 11, 12). Slow eye movements (SEMs) criteria were set to 100 μV with a 6-s window length to detect any drowsiness in the recording (Jodicke et al., 2013; Santamaría and Chiappa, 1987). Each 1 s epoch was automatically classified into the following arousing states, resulting in a vigilance time-course: stage 0 (highest arousal), A1, A2, A3, B1, B2/3, C (lowest arousals, sleep onset, classified visually by slow grapho-elements from an experienced rater), according to the classification from (Bente, 1976; Roth, 1961; Santamaria and Chiappa, 1987). The arousal continuum was classified mainly based on the distribution of alpha cortical current density over four distinct regions of interests (ROIs): frontal, central, temporal, and occipital. In a typical case, non-alpha activity with the absence of SEMs would first appear after closing eyes (stage 0). The alpha activity would then dominate gradually from occipital (A1) to
central and frontal (A2), and to mainly centralized at the frontal area (A3) along with the relaxation of the participant. Subsequently, the alpha activity would disappear and be replaced by low amplitude activity with SEM (B1) then dominate by delta and theta activity (B2/3) (for details refer to VIGALL 2.1 manual). Since no subject showed stage C segments and the prevalence of stages A2 and A3 have been quite low in previous studies, we followed the usual procedure of pooling two A stages (A2/3), resulting in five different vigilance stages (0, A1, A2/3, B1, B2/3) and were assigned numerically with a range from 6 (stage 0) to 2 (stage B2/3). To align with previous studies, the median vigilance of each 1 min-block of eyes closed condition (in a total of 6 blocks) was calculated for its ordinal-scale feature (Olbrich et al., 2016). Further, the slope was calculated using linear regression of the median vigilance at each vigilance stage within each 1 min-block for each participant (vigilance slope). The vigilance slopes were reported for each 3 min eyes closed recording. Mean and standard deviations of the slopes were reported from the vigilance slope of all participants. A positive slope indicates an increase in alertness; a negative slope indicates a decrease in alertness. Percentages of the different vigilance stages within each 1 min-block were calculated after subtraction of the number of artefact segments.

2.5. Statistics

Repeated measures analyses of variance (ANOVA) were performed separately for each included VIGALL outcomes as within-subject factors including median vigilance of each block (6 blocks), and vigilance slope (estimated from 2 separate eyes closed recordings each had 3 recording blocks: 1st and 2nd recordings). When assessing the percentages of different vigilance stages, separate ANOVA models were performed for each vigilance stage across 6 recording blocks to simplify the complexity of statistical models. Since we aimed at replicating prior results in terms of the prediction of treatment outcome (Olbrich et al., 2016), here we assessed the group effects between patients with different clinical outcome including the criteria of iSPOT-D (Olbrich et al., 2016) and of NeuroPharm (Köhler-Forsberg et al., 2020). In all models, group effects were included as between-subject effects to determine whether there were temporal dynamic changes in EEG vigilance patterns 1) between pretreatment MDD and healthy controls, 2) in pretreatment vigilance between responders and non-responders (iSPOT-D criteria); between remitters and non-remitters (iSPOT-D criteria); between remitters and non-remitters (iSPOT-D criteria); 3) between pretreatment and after 8 weeks of treatment (follow-up), and whether these differences were distinct in responders and non-responders (iSPOT-D criteria); in remitters and non-remitters (iSPOT-D criteria); in remitters and non-remitters (iSPOT-D criteria); and of NeuroPharm criteria. Age was included as covariate. Significant interactions were examined by analysis of simple effects. A separate F-test was used to test group differences for each pair of clinical outcomes. The corresponding p-values (denoted p) were not adjusted for multiple comparisons (here 4) meaning that the type I error was only controlled for a given clinical outcome and categorization. For the models of median vigilance and vigilance slope, the significant level was set at p < .05. Bonferroni correction was performed when testing the group differences per vigilance stage (in a total of five vigilance stages). Therefore, the significant level was set at p < .01 for the analysis of vigilance stage and block. To be consistent, Bonferroni’s correction was then used to adjust for multiple comparisons and post hoc analyses to ensure that the type I error was controlled for a given clinical outcome. The corresponding p-values are then denoted \( p_{\text{adjusted}} \). We further examined the normalization effect on vigilance measures after 8 weeks of pharmacological intervention. When assessing the normalization effect, p values from the exploratory analysis were not adjusted for multiple comparisons and were denoted \( p_{\text{uncorrected}} \). Degrees of freedom were corrected by Greenhouse-Geisser correction when necessary. In order to assess the discriminative power, the analysis of Receiver Operating Curve (ROC) was performed for any successful discriminant on the clinical outcome. Group differences in sex, age, educational scores, pretreatment generalized anxiety disorder-10 score (GAD10) (Bech et al., 2005), pretreatment HDRS scores, and HDRS scores at week 8 were tested by simple t statistic or using the \( z^2 \) statistic (sex). Two-sided \( p \) values were chosen for all tests. To investigate the vigilance effect on clinical outcome, a correlation analysis on \( \Delta \text{HDRS} \) scores (both \( \Delta \text{HDRS}_0 \) and \( \Delta \text{HDRS}_T \)) and vigilance slope was performed.

3. Results

The details and the results of each statistical model were given in the supplementary materials (S.2-S.5).

3.1. Sociodemographic characteristics

Patients with MDD and healthy controls did not differ in age and sex (all \( p \) values > .32, Table 1), but the healthy controls had a higher education score compared to patients (t (98) = -2.607, \( p = .011 \)). Pretreatment HDRS score did not differ significantly between responders and non-responders (iSPOT-D criteria) nor between remitters and non-remitters (iSPOT-D criteria) nor between remitters and non-responders (NeuroPharm criteria) (\( p \) values > .23). No significant difference was found on GAD10 measures (\( p \) values > .05, Table 1).

3.2. Pretreatment MDD patients vs. healthy controls

A repeated ANOVA with age as covariate yielded a significant group effect of median slope (\( F(1, 123) = 4.59, p = .034, \text{Tables 2 and S3} \)). Post hoc analysis using Bonferroni’s correction revealed that prior to treatment MDD patients had less pronounced propensities for sleep stages in comparison to healthy controls (0.02 vs. -.014, \( p_{\text{adjusted}} = .034, \text{Fig. 2 left panel} \)). No significant group effect was found for median EEG-vigilance or percentages of each vigilance stage at each block (\( p_{\text{adjusted}} \) values > .10, S.2 & S.3).

3.3. VIGALL as predictor for clinical outcome

When assessing the main outcomes of this study (prediction of treatment outcome), there was no significant difference in median vigilance, percentages of each vigilance stage or vigilance slope between remitters and non-remitters when using the iSPOT-D criteria (\( p_{\text{adjusted}} > .12 \)) or nor remitters and non-responders prior to treatment when using the NeuroPharm criteria (\( p_{\text{adjusted}} > .51, \text{S.3} \)). However, when assessing responders and non-responders using the iSPOT-D criteria to replicate previous findings (Olbrich et al., 2016), we observed a significant interaction between vigilance slope and group (\( F(1, 76) = 4.16, p = .045, \text{Tables 2 and S3} \)). The analyses of simple effects using Bonferroni’s correction showed that responders had a higher propensity toward sleep stages compared to non-responders in the first 3 min recording (1st recording: -.011 vs. 0.13, \( p_{\text{adjusted}} = .042, \text{Table 2} \)), but not the second 3 min recording (2nd recording: \( p_{\text{adjusted}} = .70, \text{Fig. 2 right panel} \)). Post-hoc analysis indicated that excluding patients with low serum concentrations slightly affected the results, but the trend remained (\( F(1, 73) = 3.86, p = .053; 1\text{st recording: } -0.11 \text{ vs. } 0.14, p_{\text{adjusted}} = .044 \)). However, receiver operator characteristics yielded an area under the curve of only 0.6 (\( p = .12 \)). No significant difference was found for median vigilance or percentages of each vigilance stage (\( p \) values > .32, S.2 & S.3). The associations between \( \Delta \text{HDRS}_0 \) change and vigilance slope were \( r(80) = -.16 \) with \( p = .158 \) for the 1st and \( r(80) = .03 \), with \( p = .81 \) for the 2nd recording. We did not find any significant correlation when correlating \( \Delta \text{HDRS}_{17} \) scores at week 8 and vigilance slope (1st recording: \( r(80) = -.12, p = .286; 2\text{nd recording: } r(80) = .06, p = .650 \)).

3.4. Treatment effects on VIGALL parameters

By pooling all the follow-up data (n = 39) regardless of their
Fig. 2. Mean median EEG vigilance and the corresponding error bar (represents ±1 standard error) were depicted in the figure. Left panel: Vigilance slope between pretreatment MDD patients and healthy controls. Pretreatment MDD patients had a steeper vigilance slope compared to healthy controls. Right panel: Responders defined by iSPOT-D criteria had a higher propensity toward sleep stages compared to non-responders in the first recording, but not the second recording.

Table 2: EEG-vigilance parameters at pretreatment visit and week 8.

<table>
<thead>
<tr>
<th>Clinical criteria</th>
<th>Pretreatment visit</th>
<th>Clinical outcome at week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy controls</td>
<td>MDD (91)</td>
</tr>
<tr>
<td>Clinical outcome (n)</td>
<td>(35)</td>
<td>(42)</td>
</tr>
<tr>
<td>Stage 0 (%; Mean ± SD)</td>
<td>13.7 ± 22.6</td>
<td>15.3 ± 24.3</td>
</tr>
<tr>
<td>Stage A1</td>
<td>32.6 ± 32.9</td>
<td>36.3 ± 35.3</td>
</tr>
<tr>
<td>Stage A23</td>
<td>10.1 ± 16.2</td>
<td>14.1 ± 21.4</td>
</tr>
<tr>
<td>Stage B1</td>
<td>34.8 ± 30.6</td>
<td>26.4 ± 29.1</td>
</tr>
<tr>
<td>Stage B23</td>
<td>8.8 ± 14.6</td>
<td>7.8 ± 15.6</td>
</tr>
<tr>
<td>Median vigilance (Mean ± SD)</td>
<td>4.07 ± 1.1</td>
<td>4.27 ± 1.1</td>
</tr>
<tr>
<td>Vigilance slope at 1st recording</td>
<td>−0.26 ± 0.43</td>
<td>−0.02 ±</td>
</tr>
<tr>
<td>(Mean ± SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vigilance slope at 2nd recording</td>
<td>−0.01 ± 0.51</td>
<td>0.05 ±</td>
</tr>
</tbody>
</table>

Notes.  
<sup>a</sup> HDRS<sub>17</sub> scores are shown in both pretreatment and week 8 HDRS<sub>17</sub> scores.  
<sup>b</sup> HDRS<sub>8</sub> scores are shown in both pretreatment and week 8 HDRS<sub>8</sub> scores.  
<sup>c</sup> Intermediate responders were not included in the primary analysis; thus, no statistical testing was performed with this group of patients.  
<sup>d</sup> p < .05, age was included as covariate in all models.

treatment responses, we found a significant main effect of recording time (pretreatment vs. follow-up) at vigilance stage B1 (F (1, 37) = 10.13, p<sub>adjusted</sub> = .015, S.4). The analyses of simple effects demonstrated that MDD patients had a higher amount of low vigilance stage B1 (23% vs. 35%, p<sub>adjusted</sub> = .003, Table 3) at follow-up in comparison to pretreatment (Fig. 3). The effect was not found for other vigilance stages (p<sub>adjusted</sub> values < .50, Fig. 3 & S4). Moreover, there was a trend of decreased median vigilance in MDD patients when comparing follow-up versus pretreatment (4.06 vs. 4.32, p<sub>adjusted</sub> = .08, Tables 3 and S5), suggested by the borderline significant effect of recording time (F (1, 38) = 3.24, p = .08). Post-hoc analysis after excluding patients with low serum concentrations did not change the direction of the results (recording time at vigilance stage B1: F (1, 35) = 10.29, p<sub>adjusted</sub> = .015; median vigilance: F (1, 36) = 4.12, p = .049). No significant result was found for vigilance slope (p > .79, S.5).

We further investigated the treatment effects on patients with different clinical outcomes. As shown in Table 3, there seem to be numerical trends at stage B1 in patients with different clinical outcomes. However, a repeated ANOVA with age as covariate did not indicate a two-way interaction between recording time (pretreatment and follow-up) and group when assessing iSPOT-D’s nor NeuroPharm’s criteria in any vigilance stage (p<sub>adjusted</sub> values > .25, S.4). However, a significant interaction between recording time and group was found when assessing median vigilance (F (1, 36) = 4.35, p = .04, S.5). The analysis of simple effects showed that responders (iSPOT-D criteria) had a lower median vigilance at follow-up (4.41 vs. 3.93, p<sub>adjusted</sub> = .010) in comparison to pretreatment. Post-hoc analysis after excluding patients with low serum concentrations did not change the results.

3.5. Exploratory analysis: normalization effect on VIGALL parameters after 8 weeks of treatment

Since treatment effects for vigilance stage B1 were observed for all MDD patients and median vigilance for responders (iSPOT-D criteria), we decided to further examine if there was any normalization effect towards patterns of EEG-vigilance of healthy controls in these patients. T statistics for recording time and comparing patients with healthy controls at stage B1 revealed that pretreatment MDD (n = 39) had a lower amount of vigilance stage B1 compared to healthy controls (mean difference = −13%, t (72) = −2.09, p<sub>uncorrected</sub> = .040, 95% CI [-24%, −0.6%], Fig. 3). This difference had disappeared after 8 weeks of treatment (follow-up MDD vs. HC: mean difference = −0.7%, t (72) =...
Treatment effects on EEG-vigilance parameters (pretreatment vs. follow-up).

<table>
<thead>
<tr>
<th>Clinical outcome (n)</th>
<th>No clinical criteria applied</th>
<th>iSPOT-D&lt;sup&gt;a&lt;/sup&gt;</th>
<th>NeuroPharm&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDD (39)</td>
<td>Pretreatment Follow-up</td>
<td>Pretreatment Follow-up</td>
<td>Pretreatment Follow-up</td>
</tr>
<tr>
<td></td>
<td>15.6 ± 4.3 ± 9.7 ± 12.6 ±</td>
<td>15.3 ± 4.3 ± 5.4 ± 9.7 ±</td>
<td>15.6 ± 4.3 ± 17.1 ±</td>
</tr>
<tr>
<td></td>
<td>26.2 ± 10.7 ± 24.3 ± 24.9 ±</td>
<td>28.9 ± 24.3 ± 12.2 ± 24.9 ±</td>
<td>24.9 ± 16.8 ± 25.2 ± 18.2 ±</td>
</tr>
<tr>
<td>Stage 0 (%, Mean ± SD)</td>
<td>40.2 ± 9.7 ± 36.3 ± 34.3 ±</td>
<td>43.9 ± 34.9 ± 39.9 ± 39.9 ±</td>
<td>44.6 ± 34.9 ± 40.4 ± 34.0 ±</td>
</tr>
<tr>
<td>Stage A1</td>
<td>24.6 ± 17.2 ± 28.9 ± 24.3 ±</td>
<td>39.9 ± 39.9 ± 21.9 ± 23.9 ±</td>
<td>38.1 ± 36.0 ± 36.6 ± 37.0 ±</td>
</tr>
<tr>
<td>Stage A23</td>
<td>10.8 ± 8.2 ± 12.2 ± 5.4 ± 9.7 ±</td>
<td>22.9 ± 22.9 ± 21.9 ± 22.9 ±</td>
<td>9.5 ± 2.5 ± 13.8 ± 10.2 ±</td>
</tr>
<tr>
<td>Stage B1</td>
<td>18.2 ± 15.5 ± 22.9 ± 22.9 ±</td>
<td>21.9 ± 21.9 ± 25.3 ± 25.3 ±</td>
<td>15.1 ± 6.7 ± 21.2 ± 19.2 ±</td>
</tr>
<tr>
<td>Stage B23</td>
<td>22.7 ± 34.5 ± 23.2 ± 39.6 ±</td>
<td>21.9 ± 21.9 ± 25.3 ± 25.3 ±</td>
<td>14.4 ± 34.9 ± 28.3 ± 40.5 ±</td>
</tr>
<tr>
<td>Median vigilance</td>
<td>19.4 ± 18.6 ± 20.8 ± 23.9 ±</td>
<td>25.4 ± 25.4 ± 23.9 ± 23.9 ±</td>
<td>19.8 ± 29.8 ± 30.3 ± 32.7 ±</td>
</tr>
<tr>
<td>(Mean ± SD)</td>
<td>10.3 ± 9.5 ± 14.4 ± 12.8 ±</td>
<td>14.0 ± 14.0 ± 12.8 ± 12.8 ±</td>
<td>23.8 ± 17.9 ± 32.7 ± 32.7 ±</td>
</tr>
<tr>
<td>Vigilance slope at 1st recording (Mean ± SD)</td>
<td>4.3 ± 1.2 ± 4.4 ± 3.9 ±</td>
<td>4.5 ± 1.2 ± 4.4 ± 4.0 ±</td>
<td>3.9 ± 4.3 ± 3.9 ± 4.1 ±</td>
</tr>
<tr>
<td>0.56 ± 0.45 ± 0.49 ± 0.55 ±</td>
<td>0.21 ± 0.21 ± 0.04 ± 0.04 ±</td>
<td>0.02 ± 0.02 ± 0.04 ± 0.04 ±</td>
<td>0.07 ± 0.07 ± 0.07 ± 0.07 ±</td>
</tr>
<tr>
<td>0.04 ± 0.07 ± 0.06 ± 0.12 ±</td>
<td>0 ± 0 ± −0.02 ± 0 ±</td>
<td>0.05 ± 0.05 ± 0.08 ± 0 ±</td>
<td>0 ± −0.07 ± 0 ± 0 ±</td>
</tr>
<tr>
<td>* 0.04 ± 0.07 ± 0.06 ± 0.12 ±</td>
<td>0 ± 0 ± −0.02 ± 0 ±</td>
<td>0.05 ± 0.05 ± 0.08 ± 0 ±</td>
<td>0 ± −0.07 ± 0 ± 0 ±</td>
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<tr>
<td>* 0.04 ± 0.07 ± 0.06 ± 0.12 ±</td>
<td>0 ± 0 ± −0.02 ± 0 ±</td>
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<td>* 0.04 ± 0.07 ± 0.06 ± 0.12 ±</td>
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<td>* 0.04 ± 0.07 ± 0.06 ± 0.12 ±</td>
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<td>0.05 ± 0.05 ± 0.08 ± 0 ±</td>
<td>0 ± −0.07 ± 0 ± 0 ±</td>
</tr>
</tbody>
</table>

Notes.

<sup>a</sup> HDRS<sub>28</sub> scores are shown in both pretreatment and week 8 HDRS<sub>28</sub> scores.
<sup>b</sup> HDRS<sub>17</sub> scores are shown in both pretreatment and week 8 HDRS<sub>17</sub> scores.
<sup>c</sup> Intermediate responders were not included in the primary analysis; thus, no statistical testing was performed with this group of patients.
<sup>d</sup> p < .05.

![Graph showing treatment effects on different vigilance stages](image_url)

**Fig. 3.** Treatment effects on different vigilance stages on MDD patients. The mean percentage and the corresponding error bar (represents ±1 standard error) were depicted in the figure. After 8 weeks of treatment, MDD patients had a significant higher percentage of stage B1 compared to pretreatment visits. Moreover, depressed patients at follow-up showed a normalization effect towards the vigilance pattern of healthy controls (HC) at stage B1.

−0.11, \( p_{uncorrected} = .910, 95\% CI [-14\%, 12\%] \). No normalization effect was found for the median vigilance (\( p_{uncorrected} \) values > .13).

### 4. Discussion

We here investigated putative biomarkers based on EEG-vigilance and replicated them in an independent sample of MDD patients. We
examined MDD patients’ attenuated wakefulness regulation toward sleep onset during rest – which is a discriminative feature of depressed patients – and compared it with that of healthy subjects. In addition, we studied the distinction in vigilance response between responders and non-responders to SSRI/SNRI treatment. Although recent research on EEG showed the potential of using EEG biomarkers as predictive tools in depression (Arns et al., 2016; Olbrich et al., 2016; Pizzagalli et al., 2018; Rolle et al., 2020; Wu et al., 2020), its clinical use has so far been limited (Widge et al., 2018). One major reason has been the lack of replication in independent datasets of any of the putative markers (Widge et al., 2018). Hence our findings and replications may help foster the use of EEG-vigilance biomarkers in clinical practice.

4.1. EEG-vigilance in major depression

The vigilance framework (Hegerl and Hensch, 2014) proposes that patients suffering from MDD show a more rigid wakefulness regulation during rest. This means that their functional brain states, as assessed with EEG, stay at high wakefulness levels during their resting state, as shown in previous studies (Hegerl et al., 2012; Olbrich et al., 2012). This condition results in difficulties falling asleep and high inner tensions usually associated with depressed patients. On the behavioral level, patients may try to counteract the hyperstable vigilance by avoiding any arousing activities (Hegerl and Hensch, 2014). EEG-vigilance measurements allow the assessment the time courses of this dynamic wakefulness regulation. Previous studies showed higher wakefulness levels in unmedicated patients who also demonstrated more high vigilance stages than healthy controls (Hegerl et al., 2012; Olbrich et al., 2012; Schmidt et al., 2017). The data from the NeuroPharm study showed no differences in the amount of similar EEG-vigilance stages; however, the data showed hyperstable vigilance profile for patients with MDD after accounting for changes in EEG-vigilance regulation.

Thus, the presented study showed that MDD patients were less likely to have decreasing vigilance over time compared to healthy controls. While this is not a straightforward replication, as previous studies focused on the percentage of vigilance stages, our findings still indicate an overall higher vigilance in patients. Notably, the present study used a relatively short EEG resting state segment compared to previous studies, thus proving that differences in vigilance regulation can be assessed using a resting state as short as 3 min.

4.2. EEG vigilance and treatment response

To date, two studies have investigated the predictive value of EEG-vigilance regulation for pharmacological antidepressant treatment response in MDD (Olbrich et al., 2016; Schmidt et al., 2017). In a large cohort of 599 patients, one study found an association between the slope of EEG-vigilance, i.e. the change of vigilance over time, and the response to SSRI treatment using EEG with a 2 min resting state (Olbrich et al., 2016). In another study with a smaller cohort of 65 patients, hyperstable EEG-vigilance regulation was found to be associated with a better response to different types of antidepressive drugs when using a much longer EEG vigilance resting state session of 15 min (Schmidt et al., 2017). The response in the study of Schmidt et al. (2017) was defined as a 50% decline of HDRS scores after 28 days whereas the NeuroPharm study assessed clinical response after 56 days. In the present study, we replicate the findings from the iSPOT-D study (Olbrich et al., 2016), showing that a faster decline towards lower vigilance stages is linked to a significantly better outcome following SSRI treatment. Importantly, the finding was only replicated using the iSPOT-D treatment response criteria, and not the NeuroPharm criteria. This highlights the importance of using standardized outcome measures when comparing studies. As stated in Widge et al. (2018), replication needs to carefully reproduce the methods used in the studies being replicated. Thus, we used the EEG-montages, number of channels, and sampling rate to calculate the vigilance slope the same way as it was done in the precedent study (Olbrich et al., 2016). The only difference between the presented study and the iSPOT-D trial can be found in the usage of different amplifiers. Since the results are comparable, this hints toward possible generalizability of the usage of EEG-vigilance measures across different recording platforms. Furthermore, this is the first independent replication of treatment prediction using EEG-vigilance measures in a retrospective analysis; and the next step would be a prospective study showing the efficacy of a EEG-informed treatment choice in a randomized controlled design.

4.3. Treatment effect on EEG vigilance

The present study showed that patients with depression demonstrated lowered vigilance and an increase of low vigilance stage after 8 weeks of treatment. This aligns with a previous study where researchers have reported acute treatment effects on brain arousal stages (Schmidt et al., 2017). Specifically, Schmidt et al. (2017) have reported decreases in high vigilance stages, including stage 0 and stage A2, and an increase in stage B1 after 2 weeks of pharmacological treatments. Even though the decreases in amount of vigilance stages was not statistically significant in the present study, descriptively we observed a decrease especially in the high vigilance stages of 0, A1, A2/3 (Table 3 & Fig. 3). Our findings further suggests that this trend in treatment effect is sustained after 8 weeks and thus strengthens the putative association between reduction of arousal regulation and improvement in depressive symptoms. Furthermore, when comparing the EEG-vigilance profiles of MDD patients after 8 weeks of treatment to that of healthy controls, we found that patients’ wakefulness regulation appeared to be normalized. It is noteworthy that the normalization of EEG wakefulness regulation was mainly restricted to stage B1, a brain state associated with high inner slow eye movement and characterized by desynchronized and low-amplitude EEG activity (Santamaria and Chiappa, 1987). This stage reflects the transition between the alpha-dominated period after closing one’s eyes and the occurrence of slow wave activity just before sleep onset. The increasing amount of B1 stage in responders after 8 weeks of treatment may reflect the normalization of their wakefulness regulation. Thus, it might be hypothesized that stage B1 is a gate-keeping brain state and that patients suffering from MDD might have difficulties passing through these desynchronized stages to achieve recreational rest.

In summary, our findings support that the wakefulness regulation in MDD patients shows hyperstable patterns with less drifts toward lowered vigilance during rest. The degree of rigidity seems to be associated with the response to pharmacological treatment: patients with the most hyperstable vigilance regulation seem to profit less from this kind of treatment. In addition, the wakefulness regulation of MDD patients appears to normalize after pharmacological treatment. In a clinical context, these observations could be useful to 1) initiate treatments other than SSRI in subjects with hyperstable EEG-vigilance regulation, 2) augment treatment early in the course of intervention in patients with hyperstable EEG-vigilance regulation, 3) provide patients with unstable EEG-vigilance regulation a treatment with SSRIs in a shared decision making process and 4) stratify patients in future drug development programs.

4.4. Limitations

There are some limitations of the study should be noted. The NeuroPharm Trial was not designed for replication of prior work therefore the sample size might not be adequate to have the same power as in previous studies (please refer to our supplementary materials S.1). However, the effect size of vigilance slope was compatible when using the same clinical criteria as in previous study (Olbrich et al., 2016). We were able to replicate that vigilance slope can be used as a predictor for SSRI treatment response when using the iSPOT-D criteria. Furthermore, the NeuroPharm study is a naturalistic study without any placebo arm, meaning that the eventual drug effects on vigilance measures cannot be
assessed. However, a number of Pharmaco-EEG studies have shown an increase in the \( \theta \) and a decrease in \( \alpha \) band on drug-free MDD patients who were administered with buspirone, an agonist of 5-HT1A receptor (Anderer et al., 2000; McAllister-Williams et al., 2007; McAllister-Williams and Massey, 2003). In a four-way crossover study on the effects of desipramine and two regimens of duloxetine on healthy males compared to placebo, results showed that duloxetine prolonged the onset latency of rapid eye movement sleep. Intriguingly, the drug effects on vigilance measures have been done in preclinical studies in which SSRI treatment decreases the firing rate of the locus coeruleus (LC) and thus decreases vigilance stages (Hegerl and Hensch, 2014). Moreover, investigated group differences in EEG-vigilance across several VIGALL parameters and patients with different clinical criteria might raise the issue of multiple testing. We see repeated tests over categorization as a sensitivity analysis. Bonferroni’s correction was chosen for this purpose to ensure the type I error was controlled when testing the VIGALL parameters with a given clinical outcome. The unadjusted \( p \)-value in the supplementary materials (S.1 and S.3) enables the interested reader to perform further adjustments for multiple comparisons. Another potential limitation is that the recordings of EEG-resting state were limited to 2 × 3 min whereas in Schmidt et al. (2017) recordings were longer. However, 2 × 3 min recording appears to be sufficient since it was long enough to replicate the differential properties of EEG wakefulness regulation for healthy controls and patients.

5. Conclusions

Based on the EEG data collected from the NeuroPharm trial, our study independently confirms the previously reported usefulness of EEG-vigilance biomarkers for predicting outcome to SSRI treatment in MDD. This is an important step towards possible clinical applications of the aforementioned method. Future research should include 1) identification of treatment approaches that work better for patients with a hyperstable EEG-vigilance regulation and 2) a larger prospective randomized controlled trial with a treatment group followed the usual treatment procedure and a treatment group informed by EEG-vigilance regulation.

Author statement

C. Ip: Acquiring, analyzing & interpreting data, Drafting & revising the manuscript, Approving final content of the manuscript. M. Ganz: Interpreting & analyzing data, Drafting the manuscript, Approving final content of the manuscript. V. Dam: Acquiring data, analyzing data and art work, Revising the manuscript, Approving final content of the manuscript. B. Ozenne: Analyzing & interpreting data, Revising the manuscript and response letter to reviewers, Approving final content of the manuscript. A. Rüesch: Analyzing & interpreting data, Revising the manuscript, Approving final content of the manuscript. K. Köhler-Forsberg: Acquiring data, Revising the manuscript, Approving final content of the manuscript. K. Jørgensen: Acquiring data, conception and design, Revising the manuscript, Approving final content of the manuscript. V. Frøkjær: Acquiring data, conception and design, Revising the manuscript, Approving final content of the manuscript. B. Sogaard: Contributing to conception, Revising the manuscript, Approving final content of the manuscript. S. Christensen: Contributing to conception, Revising the manuscript, Approving final content of the manuscript. G. Knudsen: Conception and design, Revising the manuscript, Approving final content of the manuscript. S. Olbrich: Analyzing, conception and design & interpreting data, Drafting & revising the manuscript, Approving final content of the manuscript.

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Declaration of competing interest

All authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2021.06.021.

References


