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Resting EEG measures of brain arousal in a multisite study of major depression

Short title: EEG measures of brain arousal in the multisite EMBARC project

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Abstract

Several studies have found upregulated brain arousal during 15-min EEG recordings at rest in depressed patients. However, studies based on shorter EEG recording intervals are lacking. Here we aimed to compare measures of brain arousal obtained from 2-min EEGs at rest under eyes-closed conditions in depressed patients and healthy controls in a multisite project—**E**stablishing **M**oderators and **B**iosignatures of **A**ntidepressant **R**esponse for Clinical **C**are (EMBARC). We expected that depressed patients would show stable and elevated brain arousal relative to controls. Eighty-seven depressed patients and 36 healthy controls from four research sites in the United States were included in the analyses. The Vigilance Algorithm Leipzig (VIGALL) was used for the fully automatic classification of EEG-vigilance stages (indicating arousal states) of 1-sec EEG-segments; VIGALL-derived measures of brain arousal were calculated. We found that depressed patients scored higher on arousal stability (Z = -2.163, p = .015) and A-Stages (dominant alpha activity; p = .027) but lower on B1-Stages (low voltage non-alpha activity, p = .008) compared to healthy controls. No significant group differences were observed in Stage B2/3. In summary, we were able to demonstrate stable and elevated brain arousal during brief 2-min recordings at rest in depressed patients. Results set the stage for examining the value of these measures for predicting clinical response to antidepressants in the entire EMBARC sample, and evaluating whether an upregulated brain arousal is particularly characteristic for responders to antidepressants.

Keywords

EMBARC, Electroencephalogram, VIGALL 2.1, Major Depressive Disorder, Brain Arousal Regulation, EEG-vigilance

Introduction

Resting EEG-measures as predictive and diagnostic biomarkers

Major depressive disorder (MDD) is a highly prevalent and chronic disorder, and a leading cause of disability worldwide (1). Considering its immense contribution to the overall global burden of disease, the delayed onset of the effects of antidepressants (AD) and an AD non-response rate of up to 50% (2), a robust and simple method for predicting AD treatment response would be very valuable. Electroencephalogram (EEG)-derived neurophysiological measures are promising biomarkers for predicting AD treatment response and for discriminating between MDD patients and healthy subjects (see (3-5) for review). They are highly heritable (6), widely available and they provide direct information on brain activity with a temporal resolution in the millisecond range (7).

Among the most investigated findings in studies examining resting EEG-measures as AD response predictors are changes in the alpha band: several studies found greater resting state EEG alpha power in depressed patients who respond to antidepressants relative to non-responders (4, 8-11), mainly at posterior scalp locations. The posterior location was confirmed in a study analyzing spectra from reference-free current source density waveforms (8), which represent more closely the underlying neuronal generators (12, 13). For decades, elevated alpha activity during rest has consistently been found in MDD patients in comparison to controls (see (3) for review). Importantly, prominent resting EEG characteristics, such as posterior EEG alpha oscillations, are highly stable over long time intervals (> 12 years) in adults, thereby meeting the requirement of a trait biomarker (11, 14, 15).

More recently, Hegerl and colleagues developed the Vigilance Algorithm Leipzig (VIGALL; http://www.uni-leipzig.de/vigall/), which classifies consecutive 1-sec segments of eyes-closed resting EEG into different EEG-vigilance stages, indicating states of brain arousal. On a behavioral level, several states of arousal can be discerned during the waking state (16, 17), ranging from high

wakefulness to sleep onset (18). VIGALL allows the objective assessment of the dynamics of brain arousal within multichannel EEG recordings using low-resolution electromagnetic tomography (LORETA; (19, 20)) for its automatic stage classification. It was broadly validated with simultaneous EEG-FDG-PET (21), as well as EEG-fMRI-studies (22), and by relating EEG-vigilance stages to parameters of the autonomous nervous system (23-26). A recent genome-wide association analysis (GWA) with arousal regulation (as assessed with VIGALL) revealed the involvement of a transmembrane protein, which has also been linked to depression in other GWAs (27). Evidence of elevated and more stable brain arousal regulation in depressed individuals compared to healthy controls, based on 15-min eyes-closed EEGs, was found (28) and replicated at two different testing sites (29, 30).

A relative new paradigm in biomarker research is a multimarker strategy to improve the discriminative power and to achieve sufficient prediction accuracy in order to personalize treatment. For example, in the context of the multi-site, placebo-controlled randomized clinical trial—**E**stablishing **M**oderators and **B**iosignatures of **A**ntidepressant **R**esponse for **C**linical Care (EMBARC;(31, 32))—the value of multiple biomarkers for differential prediction of response to antidepressants (AD) are systematically examined to develop biosignatures (31, 32), which consist of a combination of markers with combined predictive value (31). Prior to examining brain arousal regulation as a marker for response prediction in the EMBARC study, the current feasibility study was conducted.

Rationale and aim of the feasibility study

Within the EMBARC project, a new standardized processing procedure had been developed to ensure data compatibility between EEG acquisition sites (32). This procedure implemented a standardized EEG procedure manual, data interpolation of different EEG recording setups to a

common montage and sample rate, and a single standardized processing pipeline at all test sites (see Figure 1 in (32)). Test-retest reliability of EEG-derived measures following the standardized procedures was demonstrated to be good-to-excellent (32). The assessment of brain arousal in the resting EEG data of the EMBARC study (four 2-min periods, half with eyes open, half with eyes closed) presented several challenges for VIGALL assessment. For example, the duration of each eyes-closed period was only 2-min, as opposed to the 15-20 min recording period, usually used for EEG-vigilance analyses (18). In addition, the EMBARC standardized processing procedure differed from the VIGALL standardized processing procedure (e.g., concerning artefact correction). To evaluate whether automatic staging of EEG-vigilance in this dataset is feasible this initial study was conducted in a subsample of the EMBARC study before addressing the main study question of AD-response prediction in a separate report.

To this end, we examined whether the upregulated brain arousal previously demonstrated in depressed patients as compared to healthy adults using 15-min resting EEG data (28-30) could be replicated in 2-min EEG recordings at rest under eyes-closed conditions. We expected that depressed patients would show a more stable regulation and higher level of brain arousal than healthy controls.

Materials and Methods

Study participants

The sample characteristics of all randomized depressed participants (N = 309) is described elsewhere (31). In this feasibility study, a subsample of 96 patients with MDD (among the first 100 batch with usable EEG data) constituted the patient sample from four testing sites: Columbia University Medical Center in New York (CU; n = 22), Massachusetts General Hospital in Boston (MG; n = 11), University of Texas Southwestern Medical Center in Dallas (TX; n = 41; and

University of Michigan in Ann Arbor (UM; n = 22). Main inclusion criteria were age between 18 and 65 (m/f), chronic (episode duration > 2 years) or recurrent (≥ 2 recurrences) non-psychotic MDD (according to DSM-IV) with an early onset (before age 30), fluency in English, and provision of written informed consent. Main exclusion criteria included diagnosis of bipolar disorder or schizophrenia (current or lifetime), other Axis I or II diagnoses (except for nicotine/caffeine dependence), meeting DSM-IV criteria for substance abuse in the last 6 months (except for nicotine). Of the 96 participants in the subsample used for the current analysis, data from eight depressive participants were eliminated due to bad EEG quality (≥ 70% of artefactual epochs in the first eyes-closed period), thus leaving data of 87 patients for the EEG-vigilance analyses.

The control sample for this feasibility study consisted of a total of 38 healthy adults (24 female, mean age of 37.6, age range 18 - 65 (31, 32), including study participants from CU (n = 10), MG (n = 9), TX (n = 10), and UM (n = 9). Recruitment and screening methods are described elsewhere (32). Main inclusion criteria included age between 18 and 65, Quick Inventory of Depressive Symptomatology Self-Report (QIDS-SR; (33)) score < 8, fluency in English, provision of written informed consent. Main exclusion criteria included diagnosis of major depression, bipolar disorder or schizophrenia (current or lifetime), current Axis I or II diagnoses (except for nicotine/caffeine dependence), meeting DSM-IV criteria for substance abuse in the last 6 months (except for nicotine). Between testing sites, there was no significant difference in mean age or gender ratio; a more detailed description of inclusion and exclusion criteria is provided by Tenke et al. (32). Data from two control subjects were eliminated due to bad EEG quality (≥ 70% of artefactual epochs in the first eyes-closed period), thus leaving data of 36 controls for the EEG-vigilance analyses.

Questionnaires

The Hamilton Rating Scale for Depression 17-item (HAMD-17; (34)) was administered to assess the severity of depressive symptoms. The sum score ranges between 0 and 52 whereby scores of 0

- 7 are considered as being normal, 8 - 16 indicate mild depression, 17 - 23 moderate depression and scores over 24 suggest severe depression (35). The Edinburgh Handedness Inventory (EHI; (36)) laterality quotient (LQ; -100 to +100 max left to max right-handed) was used to assess handedness.

Resting EEG acquisition procedure

All four test-sites followed the EEG Procedure Manual to ensure standardized test administration (32). Experimenters at each lab were certified by the Columbia lab for EEG cap placement and task instruction via video conference. EEG acquisition at the four testing sites was conducted using different equipment, extensively outlined in (32). Continuous EEG data were recorded while participants sat quietly during four 2-min periods in fixed order: eyes-open (block 1), eyes-closed (block 2), eyes-closed (block 3), eyes-open (block 4). During the recording, participants were instructed to remain still, inhibit blinks or eye movements and, during the eyes-open condition, fixate a central cross on a monitor (32). For the purpose of this study, only block 2 was examined (i.e., the first eyes-closed condition).

Preprocessing pipeline for resting EEG

The preprocessing strategies to obtain comparable data of the four testing sites have been described by Tenke et al. (32). Figure 1 presents the flowchart of the (a) standardized preprocessing pipeline for resting EEG of the EMBARC study and (b) the procedure preceding automatic EEG-vigilance stage classification.

First, after data format conversion and import into Brain Vision Analyzer, a marker (“USE”) was placed in every second 2-sec epoch of block 2. Thereafter, 1000 ms after “USE” were

segmented to obtain sequential non-overlapping 1-sec segments. Due to the preprocessed and segmented state of the resting EEG data, we refrained from preprocessing steps usually applied to raw data before applying VIGALL to comply with the standardized EMBARC protocol.

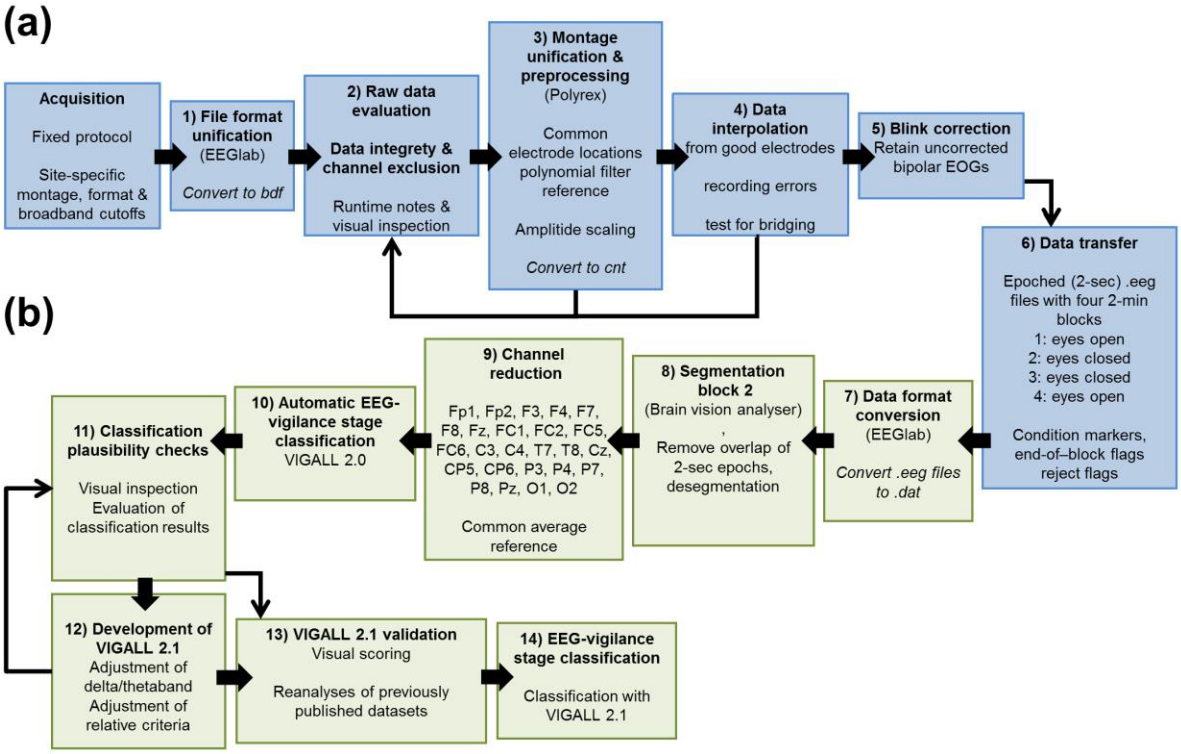

Figure 1. Flowcharts of the preprocessing pipeline (a) for continuous EEG of the EMBARC study, reproduced with permission from publisher (32) and (b) preceding the EEG-vigilance staging with VIGALL 2.1.

We refrained from using independent component analysis (ICA; (37-39)) because of its potentially reduced efficacy for selection of independent components due to the cleaned and blink-removed data. We also refrained from marking of grapho-elements (e.g., K-complexes), because epochs exceeding a 100 µV threshold on any channel (including the EOG channels) had been automatically rejected to remove any epochs containing eye blinks. Notwithstanding, all single trials were screened for sleep spindles by an experienced rater, but none were identified.

Adaptation to VIGALL algorithm

In a first step, plausibility checks of the automatic EEG-vigilance stage classification were conducted, resulting in methodological adjustments to VIGALL and the release of VIGALL 2.1. The necessity arose from the fact that initial EEG-vigilance staging with an earlier version of the algorithm (VIGALL 2.0) yielded an incorrect classification of segments containing traces of eye movement artefacts as EEG-vigilance Stage B2/3, which is characterized by dominant delta or theta power in the EEG (cf. Table 1). Since non-cephalic artefacts often occur in the 2 - 4 Hz frequency range (40), and given the absence of sleep-spindles in this dataset, we circumvented this problem in a new version of VIGALL, which allows the manual adjustment of the delta/theta range (18), by omitting the delta range. The decision criteria of the algorithm are presented in Figure 2. The software was written by one of the authors, is licensed under GPL3 and available at https://github.com/danielboettger/VIGALL/.

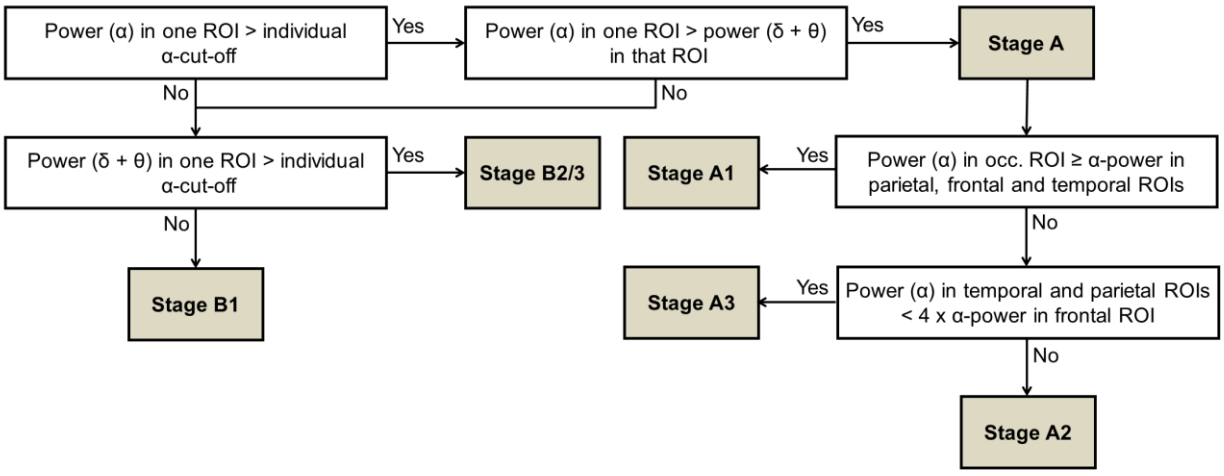

Figure 2. Decision criteria of the Vigilance Algorithm Leipzig (VIGALL) used in the current study. Classification of vigilance stages is based on power in four regions of interest (ROI; frontal, parietal, temporal and occipital lobes). For these ROIs, current density power is calculated using low-resolution electromagnetic tomography (LORETA, (19)) for the alpha and delta/theta band. Prior to classification, alpha frequency and amplitude level is individually adapted, based on a 10-sec epoch with prominent alpha activity (default range 7.5 – 12.5 Hz). For the respective epoch, the individual center of gravity for the alpha frequency and mean power in the occipital ROI are calculated. Based on this frequency, the alpha range (individual frequency ±2 Hz) is determined. Occipital alpha power is used to determine the individual alpha threshold as cut-off value in the classification of A and B2/3 stages.

EEG-vigilance staging and arousal parameterization

Using VIGALL2.1, the consecutive 1-sec EEG segments were classified into five different EEG-vigilance Stages: A1, A2, A3, B1 and B2/3 (C was not observed; cf. Table 1), based on frequency bands and source localization with LORETA.

Table 1. Assessment of EEG-vigilance stages by applying VIGALL in the current study

VIGALL stages	Stage Scoring	EEG-characteristics
A1	6	predominant occipital alpha activity
A2	5	shifts of alpha to central and frontal cortical areas
A3	4	continued frontalization of alpha
B1	3	low amplitude, desynchronized non-alpha EEG with or without slow eye movements
B2/3	2	dominant delta- and theta-power
C*	1	occurrence of grapho-elements indicating sleep onset

VIGALL=Vigilance Algorithm Leipzig; EEG=electroencephalogram; *not observed

To note, as no continuous EOG data were available, we could not discriminate stage B1 from stage 0, since this is done by detecting slow horizontal eye movements (SEM). Thus, stages B1 and 0 were combined as B1 as it is suggested when SEMs cannot be assessed (18). The VIGALL 2.1 classification results were written to a text file and imported into an Excel template. Next, brain arousal parameters were calculated with Visual Basic for Applications (VBA) macros in Microsoft Excel and using SPSS-syntax in SPSS. Each 1-sec staged EEG-segment was assigned a score ranging from 6 (A1) to 2 (B2/3; cf. Table 1).

Arousal regulation. To quantify the extent of arousal decline (i.e., arousal regulation), we calculated an arousal stability index based on 1-min intervals (interval 1: segments 1-60, interval 2: segments 2-61 etc.). Scoring criteria are presented in Table 2; high score corresponds to a stable arousal regulation.

Table 2. Scoring criteria of the Arousal Stability Index

Scoring criteria	score
≥ 2/3 of all segments classified as A1 (min 1 or 2)	6
≥ 2/3 of all segments classified as A1-3 (min 1 or 2)	5
≥ 1/3 of segments in min 2 classified as B1	4
≥ 1/3 of segments in min 1 classified as B1	3
≥ 1/3 of segments in min 2 classified as B2/3	2
≥ 1/3 of segments in min 1 classified as B2/3	1

Arousal level. The absolute amount and the percentage (amount * 100/total number of non-artefact segments) of EEG-vigilance staged segments (A, B1 and B2/3) were calculated for block 2 (i.e., the first eyes-closed condition). To calculate mean EEG-vigilance across block 2, we computed and averaged the mean of all scored 1-sec segments without considering artefactual segments as well as the mean of scored 1-sec segments for each of the eight 15-sec intervals.

Statistical analyses

Statistical analyses were performed in SPSS Statistics 24.0 (IBM corp.; Armonk, NY, USA). To assess whether groups differed concerning gender, race, handedness, age, education and severity of depressive symptomatology we conducted independent chi-squared test (gender, handedness, race), and analyses of variance (continuous demographic variables). To assess group differences concerning arousal regulation (i.e., arousal stability index), arousal level (i.e., the relative amount of EEG-vigilance stages A, B1, and B2/3, mean EEG-vigilance) we conducted Mann-Whitney *U* tests due to non-normality of the data. For post-hoc analysis of mean EEG-vigilance, we limited the number of artefactual segments in each of the eight 15-sec intervals. Thereby, subjects with 80% artefactual segments or more in any 15-sec interval were excluded, leaving 66 depressed patients and 28 healthy controls. The one-tailed significance level was set to $p \leq .05$.

Results

Characteristics of sample

Table 3 presents the demographic characteristics and the HAMD-17 scores at baseline of the 36 healthy controls and the 87 participants with MDD. Of the 36 healthy controls (mean age: 37.0 [years] ± 2.4 SE), the majority (58.3%) were female and Caucasian (69.4%); mean HAMD-17 scores of < 1 (range 0 - 3) were in the normal range. Of the 87 depressed patients (mean age: 39.0

[years] ± 2.4 SE), the majority (64.4%) were female and Caucasian (65.5%); mean HAMD-17 scores of 18.7 (range 11 - 32) indicated a mild to severe depressive symptomatology in depressed participants. Groups did not differ in gender, race, handedness, age, EHI score, and education ($F_{1,122} < 1.0$, n.s). Groups differed in depression severity ($F_{1,119} = 533.94$, $p < .001$).

Table 3. Characteristics of 36 healthy controls and 87 depressed patients

Characteristics	Healthy controls (N=36)			Depressed patients (N=87)			Comparison		
	N	*%*		*N*	*%*		*Chi-Square p (*Fisher's exact p)*		
Sex							.529		
Female	21	58.3		56	64.4				
Male	15	41.6		31	35.6				
Race							.883		
White	25	69.4		57	65.5				
Black or African American	7	19.4		22	25.3				
Asian	2	5.6		3	3.4				
American Indian/Alaska native	0	-		1	1.2				
More than one race	2	5.6		4	4.6				
Handedness (EHI)							.296*		
Left-handed	5	13.9		6	6.9				
Right-handed	31	86.1		81	93.1				
	Mean	*SE*	*Range*	*Mean*	*SE*	*Range*	*F*	*df*	*p*
Age (years)	37.0	2.4	18-65	39.0	1.5	18-65	0.52	122	.474
Education (years)	15.2	0.4	10-20	15.1	0.3	9-21	0.04	122	.839
EHI score	67.5	4.7	-100-100	74.7	4.7	-100-100	0.60	122	.441
17-item Hamilton Depression Rating Scale	0.7	0.2	0-3	18.7	0.5	11-32	533.94	119	<.001

Annotations: EHI = Edinburgh Handedness Inventory

Between-group comparisons of EEG measures of brain arousal

Examples of the individual time course of EEG-vigilance stages across block 2 are presented in Figure 3. The time course of the mean EEG-vigilance over the 2-min EEG (eight 15-sec intervals) and the frequency distribution of the arousal stability scores in depressed patients and controls are presented in Figure 4. Between-group comparisons of arousal stability scores, mean EEG-vigilance and relative amount of EEG-vigilance Stages A, B1 and B2/3 are presented in Table 4.

In general, arousal stability, mean vigilance and Stage A vigilance scores were greater in MDD patients than healthy controls. However, vigilance scores in Stage B1 were greater in healthy

controls than MDD patients, and no significant group differences were observed in Stage B2/3 (cf. Table 4).

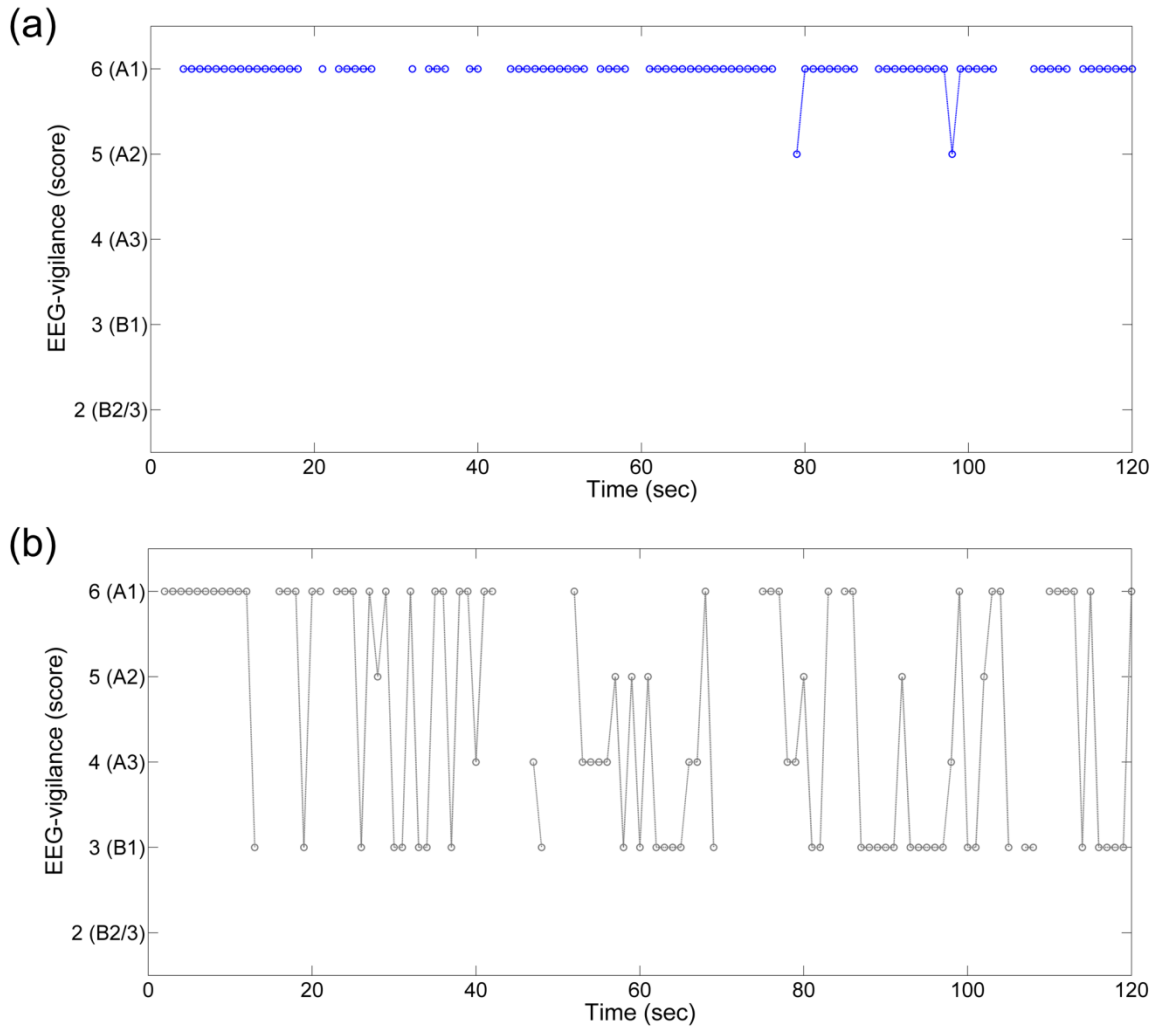

Figure 3. Time course of scored EEG-vigilance over 120 consecutive 1-sec segments in a (a) patient with major depressive disorder and (b) healthy control subject. To obtain EEG-vigilance scores, consecutive 1-sec-EEG segments were classified using the Vigilance Algorithm Leipzig into five different EEG-vigilance stages: A1, A2, A3, B1, B2/3 (based on frequency bands and source localization with LORETA). Each staged segment was assigned a number ranging from 6 (highest stage A1) to 2 (lowest stage B2/3).

Concerning the arousal stability score, the between-group analyses revealed significant results with moderate effect size (Cohen's *d:* 0.461; $p \leq .015$). Hereby, depressed patients remained longer in A-stages, as compared to healthy controls.

Table 4. Group comparisons (MDD patients vs healthy controls) of EEG-measures of brain arousal—arousal stability index, mean EEG-vigilance and relative amount of EEG-vigilance Stages A, B1 and B2/3—based on 2-min EEG periods under eyes closed condition (block 2)

	MDD patients means±SD (*N=87*)	Healthy controls means±SD (*N=36*)	Test-value[a] *Z*	Cohen's *d*	*p*-value (one-tailed)
Stability index, score	5.0±1.3	4.4±1.3	-2.165	0.461	.015 *
Mean vigilance, score	5.1±0.8	4.8±1.0	-1.373	0.333	.085 (*)
Stage A, %	83.3±24.3	69.1±34.3	-1.933	0.485	.027 *
Stage B1, %	13.4±22.8	29.0±33.8	-2.491	0.551	.006 **
Stage B2/3, %	3.3±8.7	2.0±5.6	-0.630	0.181	.269 n.s.
Artefacts[b], %	24.5±14.7	25.1±15.3	-	-	.924 n.s.

[a]Mann Whitney *U* test. Cohen's *d* was calculated using G-power (41). [b]Number of artefactual segments*100/total number of segments. To note, the relative amount of vigilance stages was calculated without considering artefacts: amount*100/total number of non-artefactual segments.
(*) p ≤ .10, * p ≤ .05, ** p ≤ .01

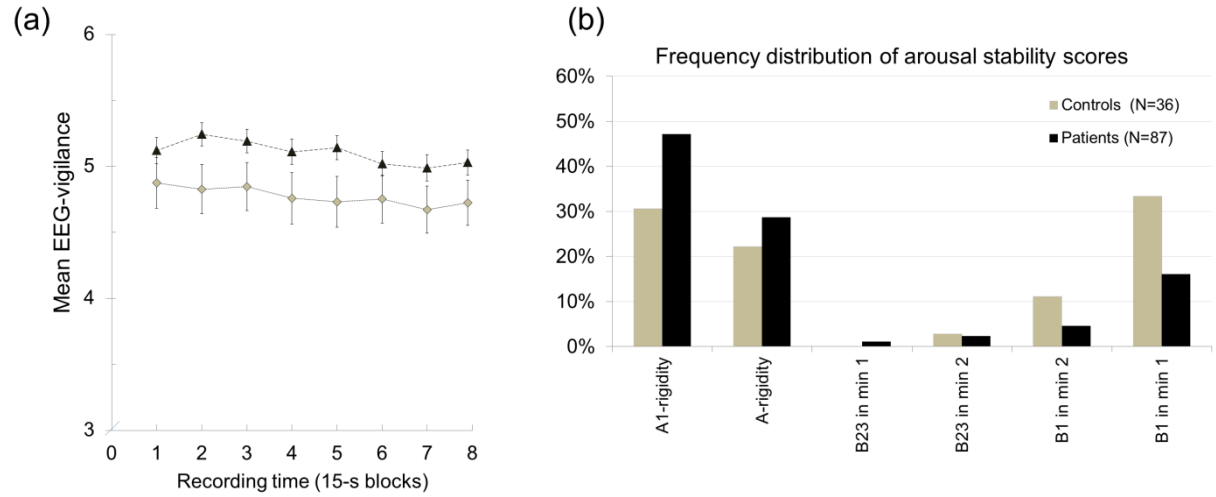

Figure 4. Time course of (a) mean EEG-vigilance of eight 15-sec intervals and (b) frequency distribution of the arousal stability scores in depressed patients (N=87) and healthy controls (N=36). Error bars indicate ± 1SE.

Concerning the relative amount of EEG-vigilance stages, MDD patients had significantly larger amount of EEG-vigilance Stage A (p = .027) with a moderate effect size (Cohen's d = 0.485) and had significantly smaller amount of EEG-vigilance Stage B1 ($p \leq .008$), with moderate effect size (Cohen's d = 0.551).

Concerning the mean EEG-vigilance of the entire block 2, no significant differences could be obtained (Cohen's d = 0.333, p = .085), albeit a trend was observed. Post-hoc analysis of mean vigilance did, however, reveal a significant effect (Z = -1.889, p = .029), when a successive artefact criterion was applied (see Methods). To note, comparing both groups concerning the number of

artefactual segments per 15-sec interval no significant differences were found between groups (before and after limiting the number of artefactual segments) in the entire 2-min recording period or in any of the eight 15-sec intervals.

Discussion

The present study used VIGALL 2.1 (18) to compare EEG measures of brain arousal obtained from 2-min eyes-closed recordings in depressed patients and healthy controls in the multisite EMBARC study. As expected, MDD patients showed a more stable arousal regulation, as evidenced by a higher arousal stability score, as well as relatively more A Stages (alpha activity) and less B1 Stages (low voltage, non-alpha activity) than healthy controls. However, there were no group differences during B2/3 Stages (indicating drowsiness) and the 2-min mean EEG-vigilance score was marginally significant.

EEG-measures of brain arousal – regulation

Our results are in line with previous studies reporting evidence of a hyperstable arousal regulation in unmedicated depressed patients during a 15-min resting EEG (28), wherein depressed patients had a longer latency to Stages A2, A3 and B2/3 and less switches between main stages A, B and C, as well as significantly less frequent switches between EEG-vigilance substages, relative to healthy controls. These effects were already present in the first two minutes (28), albeit more pronounced toward the end of the 15-min recording. Conversely, although Schmidt et al. (30) found a significant group x time interaction between unmedicated depressed patients and healthy controls using the means of EEG-vigilance of five 3-min intervals as a within-subjects factor time on task, significant group differences of mean EEG-vigilance did not occur before the third 3-min interval (min 7 - 9) (30). This may indicate that a longer EEG-recording (i.e., 15-min vs 2-min recording

period) may ensure more robust findings and may be more suitable for clinical practice than short 2-min recordings.

EEG-measures of brain arousal – level

Mean EEG-vigilance. The mean vigilance over the 2-min recording period was greater in depressed patients than healthy controls, but this finding was less robust than between-group differences of arousal stability score. Still, when restricting the number of artefacts in consecutive eight 15-sec intervals, group differences of mean EEG-vigilance reached the level of significance in support of this observation. One reason for the higher vulnerability of mean EEG-vigilance to the unequal distribution of artefact segments in the 2-min recording period could be due to missing segments at the end or the beginning of the recording, which may create a bias in producing results that are falsely low or high. For example, given that eyelid closure results in alpha synchronization in most people (42), dominant artefact contamination in the seconnd minute of recording could result in a falsely high EEG-vigilance score, given that artefacts were not taken into account for the calculation of the 2-min mean vigilance score. Thus, for the assessment of the mean EEG-vigilance, a successively applied artefact criterion is crucial.

EEG-vigilance stages A, B1 and B2/3. Several studies have found higher occurrence of EEG-vigilance Stage A and lower occurrence of B1-stages in depressed patients, relative to controls, in 15-20-min resting EEGs (28-30). Despite the relative short eyes-closed period of 2-min analysed in the current study, we obtained similar findings, i.e., a pattern of increased alpha and decreased desynchronized non-alpha EEG in MDD patients. Of note, group differences of Stages A and B1 were significant, but not group differences of Stage B2/3. We attribute this to its overall rare occurrence (<3.5%) within the short recording time. Our findings are consistent with previous

studies that demonstrated increased alpha power in MDD patients (43, 44) in comparison to healthy controls (reviewed in (3)).

Limitations

Limitations of the current study include a lack of control for sleep quality or duration during the night preceding the EEG recording, although participants had been instructed to get adequate sleep before testing. Despite these limitations, the present findings are in remarkable agreement with those of prior reports (28, 45).

Conclusion

We were able to replicate the finding of a more stable regulation and elevated level of brain arousal in MDD patients during short 2-min EEG recordings at rest using an EEG-based algorithm for automatic EEG-vigilance stage classification. For the first time, we applied a fully automatic version of VIGALL in a multisite study which uses standardized procedures across testing sites that differ from VIGALL's standard operating procedure, suggesting a broader applicability of this algorithm. Accordingly, an evaluation of these EEG measures of brain arousal as a putative predictor of AD response is warranted as the logical next step in keeping with the aims of the EMBARC study.

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Declaration of conflicting interests

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Author contributions

CU and CET served as co-first authors and contributed equally to the work. CU contributed to the design of the feasibility study, VIGALL 2.1 development, analysis, interpretation, drafted the manuscript and agrees to be accountable for all aspects of the EEG-vigilance analyses ensuring integrity and accuracy; CET contributed to conception, design, acquisition, analysis, interpretation and drafting of manuscript; JK contributed to conception, design, analysis, interpretation and drafting of manuscript and agrees to be accountable for all other aspects of the work ensuring integrity and accuracy. CS contributed to design of the feasibility study, VIGALL 2.1 development, analysis, interpretation, critically revised manuscript, gave final approval; DB contributed to VIGALL 2.1 development, coding, critically revised manuscript, gave final approval; LYXW contributed to statistical analysis, critically revised manuscript, gave final approval; JEA contributed to acquisition, critically revised manuscript, gave final approval; MF, PJM, PJD, MJM, MHT, MMW, and DAP contributed to conception, design, acquisition, critically revised the

manuscript, gave final approval; UH contributed to the conception and design of the feasibility study, VIGALL 2.1 development, analysis, interpretation, and drafting of the manuscript; GEB contributed to conception, design, acquisition, interpretation and drafting of manuscript.

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