

# Vigilance Algorithm Leipzig (VIGALL) Version 2.1

## Manual

Updated Version

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# 1 What is new in VIGALL 2.1

VIGALL 2.1 is the first major revision of version 2.0. The main new addition is the option to adjust the lower border of theta/delta band (VIGALL 2.0: fixed delta/theta range: 2-7 Hz; VIGALL 2.1: adjustable delta/theta range, default setting 3-7 Hz).

A crucial requirement for automatic EEG-vigilance stage classification performed by VIGALL is the absence of non-cephalic artefacts such as eye-movements artefacts. This is done by standardized pre-processing steps (see section 5) including blind source separation methods (independent component analysis, ICA). However, while ICA has been shown to separate non-cephalic artefacts as individual components, which can then be extracted from the EEG, in practice there are still cases, where this separation is not sufficiently possible, for example due to small number of observations of these artefacts or due to overall low signal strength (e.g., low voltage EEGs). Such artefacts are commonly occurring in the 2-4 Hz frequency range, VIGALL 2.1 now allows to circumvents this problem, by (partially) neglecting this frequency range for classification analyses (see Figures 1 & 2).

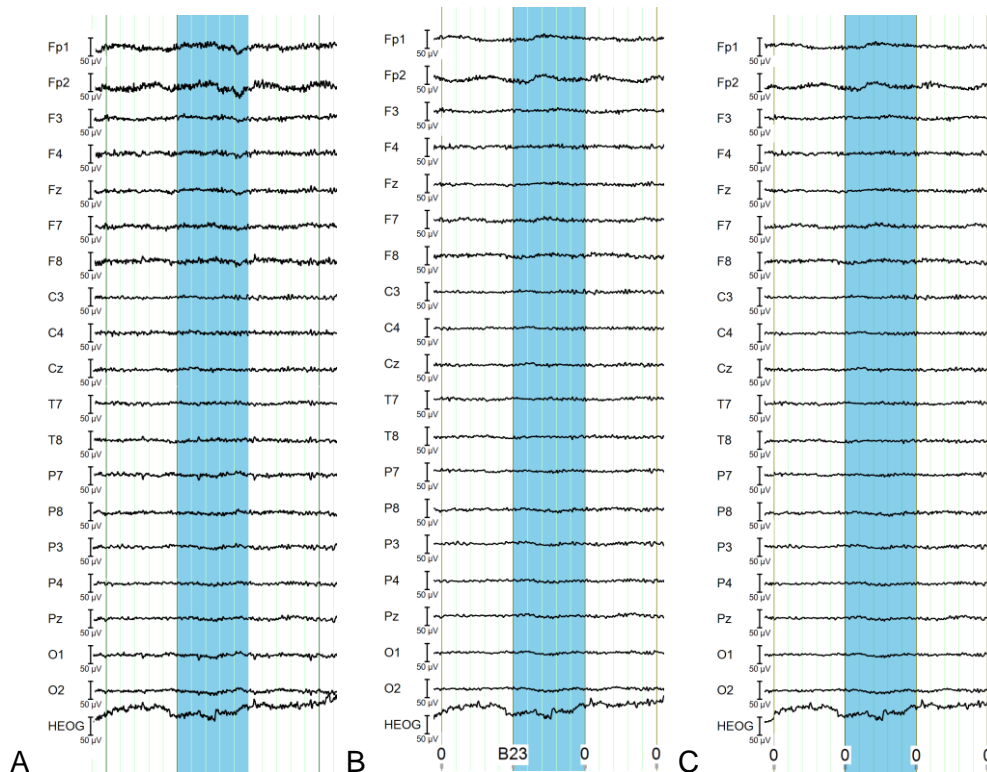


Figure 1: Eye movement artefact in low voltage EEG before ICA (A), remaining movement artefact after ICA classified as stage B23 using VIGALL 2.0 (B) and correct classification of same EEG segment as stage 0 using VIGALL 2.1 with delta/theta range 3-7 Hz (C).

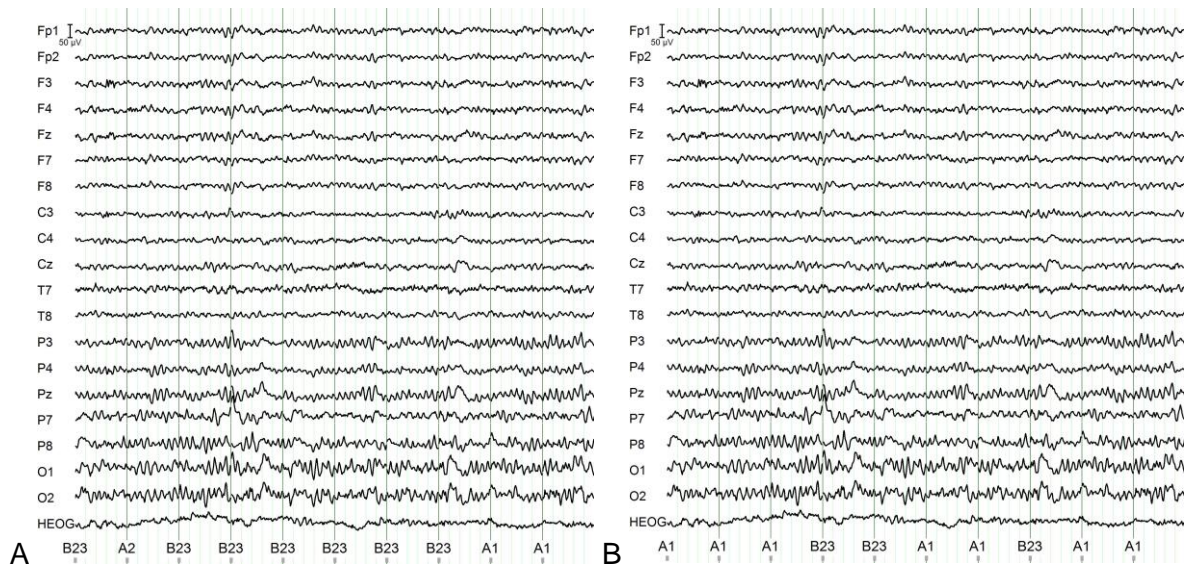


Figure 2: Implausible classification of stage B2/3 with VIGALL 2.0 despite presence of alpha (A). Classification of same EEG section using VIGALL 2.1 with delta/theta range 3-7 Hz results in more plausible A-stage classifications (B)

Furthermore, the default setting for the relative power threshold for EEG-vigilance stage A1 (alpha rhythm predominantly at occipital areas) have been modified in VIGALL 2.1. In most healthy subjects, closing the eyes will lead to alpha synchronization in occipital areas. However, with VIGALL 2.0 an over-classification of A2 stages (alpha predominantly at parietal areas) has been observed especially shortly after closing the eyes in comparison to earlier VIGALL versions<sup>1</sup>. EEGs from older subjects are particularly vulnerable to this, as the occipital alpha power decreases drastically with age<sup>2</sup>. Dominant parietal alpha is a normal variant but, according to the literature, occurs more rarely than dominant alpha at occipital areas. In VIGALL 2.1, a lowering of the relative power threshold for A1-stages is suggested (see section 6.2) resulting in shifts from A2/3- to A1-stages. The relative power threshold A1 has an impact on how many resulting segments are classified as stage A1; it is the factor by which the occipital alpha power must be higher than parietal, temporal or fronto-temporal alpha power. In VIGALL 2.0, this factor was set to 2, in VIGALL 2.1 the default value is set to 1.

<sup>1</sup> Hegerl et al. (2012). Hyperstable regulation of vigilance in patients with major depressive disorder. *The World Journal of Biological Psychiatry*. 13: 436-446; Olbrich et. al. (2012) Unstable EEG-vigilance in patients with cancer-related fatigue (CRF) in comparison to healthy controls. *The World Journal of Biological Psychiatry*. 13:146-52; Sander et al. (2010). EEG-vigilance and response to stimulants in paediatric patients with attention deficit/hyperactivity disorder. *Clinical Neurophysiology* 121: 1511-1518.

<sup>2</sup> Kropotov, J.D. (2010) *Quantitative EEG, event-related potentials and neurotherapy*. Academic Press, 2010

## 2 Assessment of brain arousal regulation via EEG-vigilance stage classification using VIGALL

### 2.1 Description

Applying EEG, different vigilance stages can not only be discerned during sleep but also during wakefulness. These *EEG-vigilance stages* indicate different *brain arousal* states. Brain arousal states fundamentally impact behaviour and brain function, and arousal has been implemented as a basic dimension of mental diseases in the Research Domain Criteria (RDoC) project of the National Institute of Mental Health<sup>3</sup>. In our daily life, brain arousal has to be precisely regulated to fulfil situational requirements. For example, brain arousal must be maintained during monotonous tasks, up-regulated in case of potential danger and reduced at bedtime. This *arousal regulation* is characterized by considerable inter-individual differences<sup>4</sup>. During a 15 to 20-minute eyes-closed resting condition, most subjects exhibit progressive declines to lower arousal levels. In contrast, other subjects continuously remain in high arousal states (hyperstable arousal regulation), while again others show rapid declines within only a few seconds (unstable arousal regulation). This trait is modulated by many individual and environmental factors, such as sleep deficits, arousal enhancing substances, effort, motivation, and disease related factors.

The recently introduced arousal regulation model of affective disorders and ADHD suggests that arousal regulation plays a pathogenetic role in depression, mania and ADHD<sup>5</sup>. For example, applying VIGALL in three independent samples, an up-regulation of arousal has been demonstrated in depressed subjects compared to controls<sup>6</sup>. Thus, arousal regulation, as assessed by VIGALL, is a promising diagnostic and prognostic biomarker for conditions with arousal disturbances, such as affective disorders, ADHD or fatigue<sup>7</sup>.

VIGALL is an Add-in to BrainVision Analyzer that enables you to objectively measure the regulation of arousal through EEG-vigilance classification. VIGALL automatically attributes

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<sup>3</sup> Cuthbert BN, Insel TR (2013). Toward the future of psychiatric diagnosis: the seven pillars of RDoC. *BMC Medicine* 11: 126.

<sup>4</sup> Huang et al (2015). Test-retest reliability of brain arousal regulation as assessed with VIGALL 2.0. *Neuropsychiatric Electrophysiology* 1: 1-13.

<sup>5</sup> Geissler et al. (2014). Hyperactivity and sensation seeking as autoregulatory attempts to stabilize brain arousal in ADHD and mania? *Attention Deficit and Hyperactivity Disorders* 6: 159-173; Hegerl U, Hensch T (2014). The vigilance regulation model of affective disorders and ADHD. *Neuroscience and Biobehavioral Reviews* 44: 45-57; Hegerl et al. (2016). Arousal Regulation in Affective Disorders. In: Frodl T (ed). *Systems Neuroscience in Depression*. Academic Press: Amsterdam, pp 341-370.

<sup>6</sup> Hegerl et al. (2012). Hyperstable regulation of vigilance in patients with major depressive disorder. *World Journal of Biological Psychiatry* 13: 436-446; Schmidt et al (2016). Impact of Serum Cytokine Levels on EEG-Measured Arousal Regulation in Patients with Major Depressive Disorder and Healthy Controls. *Neuropsychobiology* 73: 1-9; Ulke et al. (in press). Sleep disturbances and upregulation of brain arousal during daytime in depressed versus non-depressed subjects. *World Journal of Biological Psychiatry*.

<sup>7</sup> Hegerl U, Hensch T (2014). The vigilance regulation model of affective disorders and ADHD. *Neuroscience and Biobehavioral Reviews* 44: 45-57; Hegerl U, Ulke C (in press). Fatigue with up- vs. downregulated brain arousal should not be confused. *Progress in Brain Research*; Sander et al. (2010). EEG-vigilance and response to stimulants in paediatric patients with attention deficit/hyperactivity disorder. *Clinical Neurophysiology* 121: 1511-1518; Schoenknecht et al. (2010). Treatment of acute mania with modafinil monotherapy. *Biological Psychiatry* 67: e55-e57.

one of seven different EEG-vigilance stages<sup>8</sup> to short EEG segments<sup>9</sup>. The seven EEG-vigilance stages (Figure 3) indicate an arousal continuum, which – on the behavioural level – is characterized by states ranging from cognitively active wakefulness to relaxed wakefulness to increasing levels of drowsiness until sleep onset.

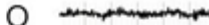













Behaviour	EEG-vigilance	EEG - characteristics	EEG - visual	
<div><div>Awake</div><div></div><div>Sleep onset</div></div>	Stage 0	Low amplitude, desynchronized non-alpha EEG in the absence of slow horizontal eye movements	<div>O</div> <div>HEOG</div>	
	Stage A	A1	Occipital dominant alpha activity	<div>F</div> <div>O</div>
		A2	Starting shifts of alpha to central and frontal cortical areas	<div>F</div> <div>O</div>
		A3	Continued frontalization of alpha	<div>F</div> <div>O</div>
	Stage B	B1	Low amplitude, desynchronized EEG with slow horizontal eye movements	<div>O</div> <div>HEOG</div>
		B2/3	Dominant delta- and theta-power	<div>F</div> <div>O</div>
	Stage C	Occurrence of K-complexes and sleep spindles	<div>F</div> <div>C</div>	

Figure 3: EEG-vigilance stages with their EEG-characteristics and the corresponding behaviour

The VIGALL classification is based on the distribution of cortical current density activity<sup>10</sup> over four distinct regions of interests (ROIs). Using data recorded from surface EEG, an estimate of the cortical current density is separately computed for the Delta/Theta and the Alpha frequency range using the LORETA method<sup>11</sup>. The alpha frequency bands can be individually adapted. Besides the surface EEG a horizontal electrooculogram (hEOG) is required for complete classification.

<sup>8</sup> This classification is based on the findings of **Loomis** [Loomis et al. (1938) Distribution of disturbance-patterns in the human electroencephalogram, with special reference to sleep. *Journal of Neurophysiology* 1:413–430], **Bente** [Bente D: Vigilanz, dissoziative Vigilanzverschiebung und Insuffizienz des Vigilanztonus. In *Begleitwirkung und Mißerfolge der psychiatrischen Pharmakotherapie*. Edited by Kranz H, Heinrich K. Stuttgart: Thieme; 1964:13-28.] and **Roth** [Roth (1961) Clinical and theoretical importance of EEG rhythms corresponding to states of lowered vigilance. *Electroencephalography and Clinical Neurophysiology* 13:395-399.].

Note that several recent studies also have endorsed this classification. For references see Sander et al. (2015). Assessment of Wakefulness and Brain Arousal Regulation in Psychiatric Research. *Neuropsychobiology* 72: 195-205.

<sup>9</sup> A segment must be at least 1 second long

<sup>10</sup> In fact, the square of the cortical current density is used as measure for cortical activity.

<sup>11</sup> Pascual-Marqui RD, Michel CM, Lehmann D. Low resolution electromagnetic tomography: a new method for localizing electrical activity in the brain. *International Journal of Psychophysiology* 1994, 18:49-65.

For each individual segment VIGALL classifies, additional data can also be presented on top of the corresponding EEG-vigilance stage. This includes the effective heart rate interval, skin conductance and body temperature. Monitoring the EEG-vigilance stages over time enables the user to determine the individual's style of arousal regulation.

Former versions of VIGALL have been validated with the Multiple Sleep Latency Test<sup>12</sup>, and simultaneous EEG-fMRI<sup>13</sup> and EEG-PET<sup>14</sup> studies. Additionally, VIGALL EEG-vigilance stages have been associated with different autonomic parameters<sup>15</sup> and arousal-sensitive evoked potentials and performance parameters<sup>16</sup>. Recently, the new version VIGALL 2.1 has been associated with heart rate and ratings of sleepiness<sup>17</sup>. Additionally, VIGALL 2.1 was able to separate depressed subjects from healthy controls<sup>18</sup>, thereby adding to prior work of VIGALL in affective disorders, OCD, ADHD and cancer related fatigue<sup>19</sup>

There are several more possible applications for the VIGALL Add-In; and the VIGALL manual at hand provides you with all the information you need to successfully implement the analysis of arousal regulation into your own research project.

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<sup>12</sup> Olbrich et al. (2015). Objective markers for sleep propensity: comparison between the Multiple Sleep Latency Test and the Vigilance Algorithm Leipzig. *J Sleep Res* 24: 450-457.

<sup>13</sup> Olbrich, et al. (2009): EEG-vigilance and BOLD effect during simultaneous EEG/fMRI measurement. *Neuroimage* 45: 319-332.

<sup>14</sup> Guenther, et al (2011): Impact of EEG-vigilance on brain glucose uptake measured with [(18)F]FDG and PET in patients with depressive episode or mild cognitive impairment. *Neuroimage* 56: 93-101.

<sup>15</sup> Olbrich et al (2011). Brain and Body: Associations Between EEG-Vigilance and the Autonomous Nervous System Activity During Rest. *Journal of Psychophysiology* 25: 190–200.

<sup>16</sup> Huang et al. (under review) Evoked Potentials and Behavioral Performance During Different States of Brain Arousal

<sup>17</sup> Jawinski et al. (submitted) Robust association between human brain arousal and daytime sleepiness.

<sup>18</sup> Ulke & Sander et al.(in press). Sleep disturbances and upregulation of brain arousal during daytime in depressed versus non-depressed elderly subjects. *World Journal of Biological Psychiatry*.

<sup>19</sup> See references in <sup>6,7</sup> and additionally: Olbrich et al (2013). EEG-vigilance regulation during the resting state in obsessive-compulsive disorder. *Clinical Neurophysiology* 124: 497-502; Olbrich et al. (2012). Unstable EEG-vigilance in patients with cancer-related fatigue (CRF) in comparison to healthy controls. *World Journal of Biological Psychiatry* 13: 146-152.

## **2.2 Limitations of VIGALL application**

VIGALL has been determined and validated with respect to non-pathological EEGs. The validity of VIGALL cannot be guaranteed in cases of pronounced pharmaco- or morbogenic modifications of the EEG. Further, at least short epochs of alpha rhythm need to be present and detectable.

Accordingly, VIGALL should not be applied in cases of:

- alpha variant rhythms (i.e. alpha centre frequencies below 8.5 or above 12.5 Hz),
- major EEG changes through drugs (e.g., anticholinergic drugs) or diseases (e.g., severe Alzheimer's disease)

In case of EEG-abnormalities (such as intermittent rhythmic delta activity), it is recommended to mark the respective segments of the EEG as artefacts to avoid misclassification as B2/3-stages or to adjust the delta range to 4-7 Hz.

Also, if too many EEG segments (>15% of the available segments) have to be excluded from analysis due to artefacts and/or EEG-abnormalities, the results may not be robust. Furthermore, VIGALL has been developed for adults and cannot simply be applied to EEGs of children under the age of 14 years or before a stable alpha rhythm has been developed.



### 3 Installation

The Vigilance Algorithm Leipzig (VIGALL) v.2.1 is implemented as an Add-In for BrainVision Analyzer 2.1. Its software requirements are:

- The BrainVision Analyzer Software 2.1
- All current updates for the Analyzer 2.1, available as downloads from <http://www.brainproducts.com/><sup>20</sup>.
- Valid license / Dongle for the BrainVision Analyzer Software.
- The current version of the VIGALL DLL, available as a download from <http://research.uni-leipzig.de/vigall/>.

If these requirements are met, the installation is simple. Just copy the file **VIGALL.dll** into Analyzer's program folder (usually C:\Vision\Analyzer2\). If your version of Windows automatically disables downloaded DLLs, you should revert this deactivation using the context menu of VIGALL.dll (right click on the file). If Analyzer is running at the time, the program needs to be restarted. After these steps, VIGALL is accessible via the menu under „Add Ins“, „VIGALL“ (see Figure 4).

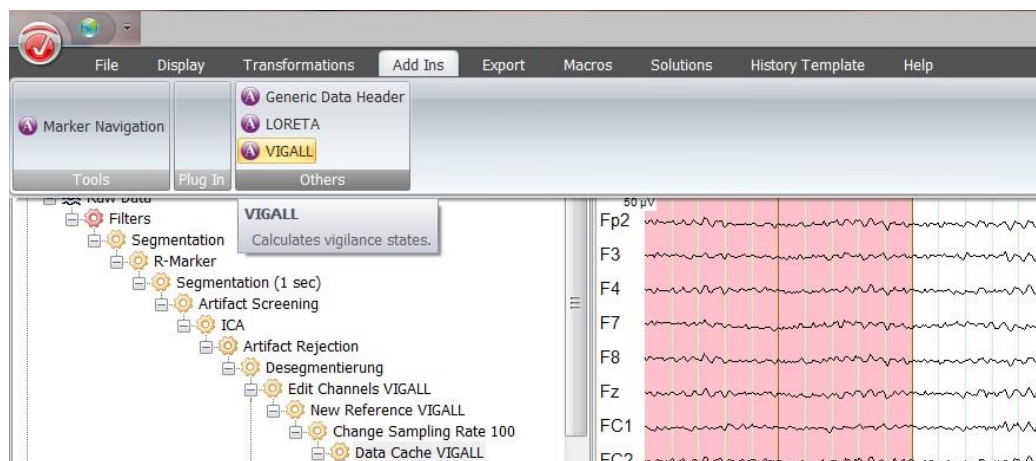


Figure 4: Selection of VIGALL in the Analyzer menu

The VIGALL Add-In is revised continuously. If you have any problems or bug reports concerning VIGALL, please make sure you are using the up-to-date, current version of both Analyzer and VIGALL.

<sup>20</sup> All procedures described in this manual are based on Analyzer-Version 2.0. The Analyzer software is continuously updated by BrainVision. Only a fully up-to-date installation of Analyzer is certain to work with VIGALL.

## **4 Recording instructions for EEG, EOG and ECG**

### **4.1 Procedure**

The standard EEG session for the assessment of brain arousal regulation will require 45- 60 minutes, including set-up and disassembly. The main part of the session is a resting-state EEG with closed eyes lasting 15-20 minutes, during which the subject is not to be talked to or disturbed, to ensure an uninterrupted time course of EEG- vigilance.

The EEG chamber should be well ventilated (if necessary turn on AC/fan shortly before starting the recording) and temperature controlled (approx. 20-24 °C). The inside of the EEG chamber should be dimly lit with lights switched on (complete darkness should be avoided).

It is recommended to assess the individual physical and mental state prior to and after the EEG by standardized questionnaires, e.g. the Karolinska or the Stanford Sleepiness Scale. The questionnaires should ascertain the actual degree of wakefulness, as well as sleep quality in the previous night and possible consumption of arousal altering substances prior to the recording.

### **4.2 Positioning of the electrodes**

The standardized placement of the electrodes is essential for the quality of the recorded EEG. At minimum, the VIGALL requires 19 electrodes placed according to the international 10-20 system. However, a configuration with 25 electrodes placed according to the extended 10-20 system is preferred. The recommended electrode configuration for the VIGALL algorithm is shown in figure 5. In case only the minimum configuration with 19 channels has been recorded, it is recommended to interpolate the missing channels when performing the data pre-processing steps described in chapter 4. Accordingly, in case more than the recommended 25 channels have been recorded, it is recommended to delete those channels (see appendix B for details).

<p><i>NOTE: Technically, it is possible to perform VIGALL analysis using alternative configurations of electrodes. In this case, however, a valid classification cannot be guaranteed.</i></p>
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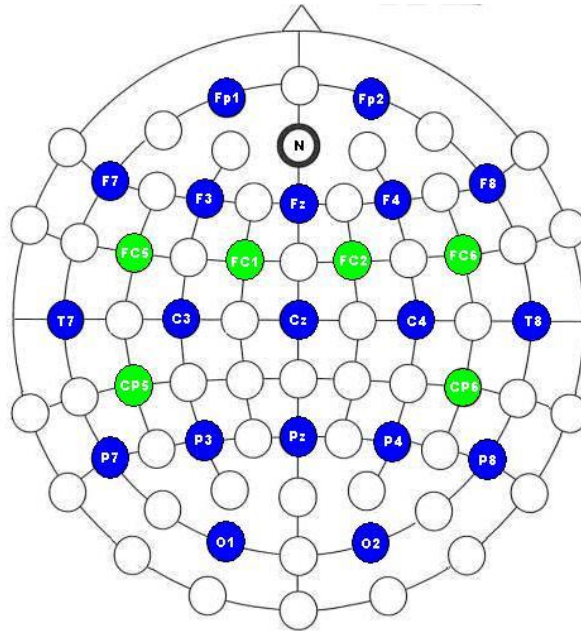


Figure 5: Schematic design of the recommended VIGALL electrodes positioning following the extended international 10-20 system. The minimum configuration with 19 channels of the standard 10-20 system is shown in black, whereas the additional 6 electrodes from the extended 10-20 system are marked in red.

In addition to the cap electrodes, four bipolar electrodes record movements of the eyes. The recording of horizontal eye movements (HEOG) is of particular importance, as the HEOG is needed for the detection slow eye movements (SEM), which allow the discrimination of low amplitude segments in active stage 0 and sub-vigilant B1-stages. HEOG should be recorded via two bipolar electrodes placed laterally to the outer canthus (see figure 6). The vertical component of eye movement (VEOG) is recorded using two bipolar electrodes, which should be placed at approximately 1cm above and beneath to the frontal pole of the right eye.



Figure 6: Electrode placement for recording vertical and horizontal eye movement

For ECG recording, limb lead 1 montage is recommended (see figure 7).

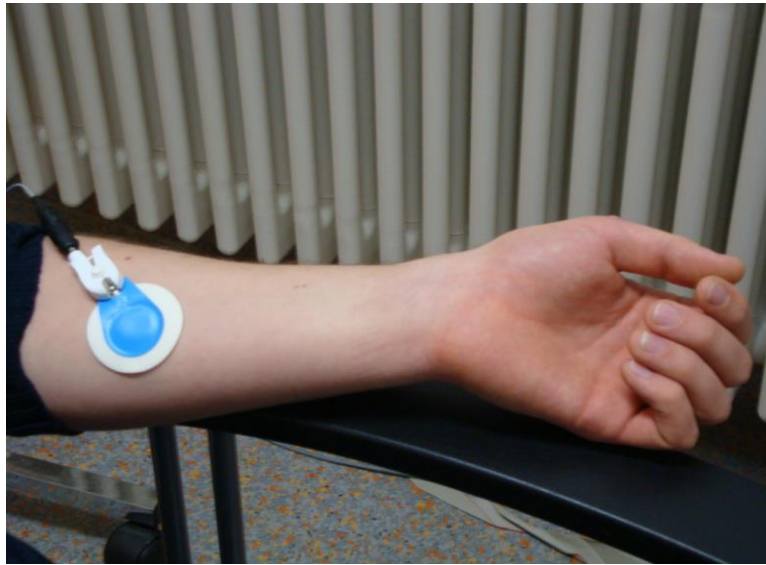


Figure 7: Electrode placement for recording the ECG

### **4.3 Impedance**

The difference between the electrode impedances should be well balanced across the scalp and as low as possible. Generally, by pre-treatment of the scalp each electrode's impedance should be kept below 10 k $\Omega$  (preferably under 5 k $\Omega$ ). Please follow your amplifiers' manual, as this general rule does not apply to all EEG-systems. Impedances should be checked and documented after placing the electrodes and, if necessary, corrected following movements of the subject and directly before the beginning of the paradigm respectively and then documented.

### **4.4 EEG-Vigilance paradigm**

During the trial the subject is in a semi-reclined position and a resting EEG under eyes-closed condition is recorded. Before starting the trial, participants are instructed that it would not matter whether they fell asleep during the recording or not. They just have to relax and keep their eyes closed. Then, when indicated, the light inside the chamber is dimmed and the door closed. The examiner should make sure that the trial is not disturbed by any noise throughout the recording.

The EEG-vigilance paradigm contains the following steps. For the interaction with the participant we recommend using the phrases printed below in *italics*, to be read out without any changes, if and when possible:

- Berger-Manoeuvre

*„We now start the trial. I want you to open or close your eyes following my instructions. If you have no more questions we will now begin our test!“*

The Berger manoeuvre starts with the instruction „Open your eyes!“ and is followed by the instruction “Close your eyes!” after 10 sec. This manoeuvre is repeated three times prior to the beginning of the resting EEG. The time at which the instructions are given should be documented in the EEG curve by means of an appropriate marker.

- Mental arithmetic task

*„Please keep your eyes closed! Now follows a mental arithmetic task. Following my instructions, please continuously subtract 6 from 100, until I ask you to give me your current result. Please keep your eyes closed during the task and please do not talk! If you do not have any more questions please start subtracting now!“*

The time of instructions is again documented in the registration curve. After 15 s the participant is requested to give their result to the investigator.

*„Please open your eyes and give me your result. [await result] Thank you.“*

The starting point of the mental arithmetic task and the instant of stating the result are again documented in the registration curve using appropriate markers.

- Instructions for resting EEG

Prior to the actual resting EEG recording the participant has the following instructions read to them whilst they are alert with their eyes open.

*„I will now record your so-called resting EEG. For this purpose I would like you to relax for the next [State duration of EEG] minutes and to move as little as possible. It doesn't matter whether you stay awake or fall asleep. It is just important that you keep your eyes closed throughout the entire resting EEG. If you don't have any more questions, please close your eyes now! “*

After that the resting-state recording begins. The starting and ending point of the recording should be documented using specific markers as well. When the time is over the subject is informed that the trial is now over.

*„The [state duration of the recording] minutes are now over. The recording is over. I will now enter the chamber and explain the next procedure to you“.*

The instructor stops the recording, opens the door to the EEG chamber, turns on the light and brings the participant to an upright sitting position. The subject is then asked to assess whether they had fallen asleep during the recording and again fill out the questionnaires regarding their current well-being.

#### **4.5 Markers**

To facilitate the EEG analysis several key elements of the recording process should be captured using markers. Furthermore, any distraction or disturbance during the resting EEG should also be indicated by markers. Appendix D contains suggested markers to be used during a vigilance EEG recording.

#### **4.6 ERP paradigms**

It is recommended to only include ERP paradigms, where subjects can be recorded with eyes closed. If recorded with eyes open, EEG characteristics of different EEG-vigilance stages can diverge (e.g. prominent alpha rhythm with closed eyes is a sign of higher arousal state, whereas it is a sign of a lower arousal state when encountered under eyes-open conditions).

## 5 Preparing the data for the Add-In

### 5.1 Resting EEG data

On the following pages you will find an overview of the recommended steps you should take when preparing data for VIGALL analysis. It comprises various transformations performed by the BrainVision Analyzer software. If you need more information on how to perform these transformations, please use the Analyzer help function and/or consult the respective chapters in the Analyzer manual.

*NOTE: It is possible to pre-process the data with different EEG software. However, as VIGALL is an add-in program for the Analyzer, the pre-processed data must be imported into Analyzer to run the VIGALL classification. If another EEG software is used, changes in the pre-processing steps and VIGALL settings may be necessary. The description below is only valid for EEG data recorded and analysed with BrainVision Recorder and Analyzer.*

#### *Preparation:*

To improve validity of the vigilance classification there are a number of data quality checks we recommend following. The implementation of these is in the responsibility of the individual performing the analysis and as such the specifics will not be covered in this manual:

- All EEGs should be screened by a clinician to make sure they are not affected by underlying pathology, alpha variant rhythms (i.e. alpha centre frequencies below 8,5 Hz or above 12,5 Hz), maturational deficits (e.g. age younger than 14 years) or major modifications of a pharmacogenic (e.g. anticholinergic drugs) or morbogenic (e.g. severe Alzheimer's disease) nature. VIGALL is not applicable to such EEGs, as its decision criteria are defined for non-pathological EEGs with normal alpha rhythm. Performing VIGALL on, e.g., low voltage EEGs might result in invalid results, as the majority of segments will be classified as 0- or B1-stages, whereas a decelerated alpha rhythm might result in misclassification of B23-stages as slow alpha could be misinterpreted as theta activity.
- Furthermore, resting periods should be checked for possible disturbances (such as noise) which violate the resting state paradigm and impede the gradual loss of arousal that would have occurred under undisturbed conditions.
- In order to perform the semi-automated analysis of a given data set, each EEG should contain consistently labelled markers. Especially the start marker, which indicates the beginning of the resting EEG, should be assigned a unique label. If no unique marker exists, a new marker indicating the beginning of the resting EEG must be set manually.

### *Pre-processing:*

Using VIGALL requires the following preparation of the selected EEG data. There are eleven pre-processing steps that need to be executed:

1. Filtering
2. Isolating the resting part by segmentation
3. Creating 1-sec segments (Segmentation)
4. Rough artefact screening
5. Marking of grapho-elements representing sleep
6. ICA execution
7. Exclusion of ICA components reflecting continuous artefacts
8. Marking of remaining artefacts
9. Reduction of sampling rate
10. Removing „New Segment“-markers (De-Segmentation)
11. Data Cache

In addition, there might be a couple of pre-processing steps necessary to meet certain specific problems in the data (e.g. interpolating disturbed channels) or you might wish to perform individual operations to meet specific requirements of your research project (e.g. R wave detection in the ECG). You will find further information regarding these procedures in the Analyzer Manual as well as the Appendix section.

#### Step 1: Filtering

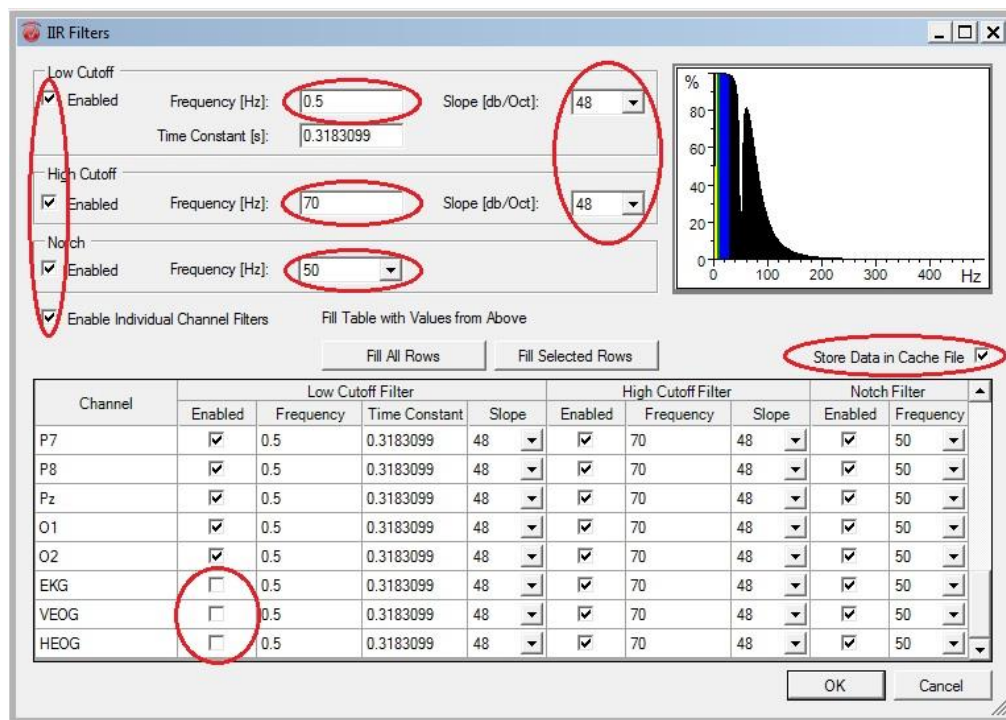
→ Transformation → IIR-Filter

Standard: 0.5-70 Hz, 50 Hz Notch, 48 dB Slope

- no Low Cut-off for non-EEG channels (e.g. ECG, HEOG, VEOG, EDA, TEMP etc.)

→ check box „Enable Individual Channel Filters“





**NOTE:** For further analysis it might be helpful to cache the filtered data (→ check box „Store Data in Cache File“).

## Step 2: Segmentation <sup>21</sup>

Beginning with the start marker the resting EEG must be isolated from the recording using the segmentation function.

**NOTE:** Depending on the design longer or shorter segments can be analysed. The existing reference data, however, refers to a resting EEG of 15 minutes duration.

**IMPORTANT:** To avoid on- and offset effects, 2s prior to the actual start and after the end of the resting period should be included into the segmentation (termed “buffer segments”).

→ Transformations → Segmentation → based on marker position → select start marker  
Select time window: Start: -2000 ms, End: 902000 ms, Duration: 904000 ms.

## Step 3: Creating 1-sec Segments

For the following artefact rejection to be based on a clear understanding of which segments are going to be excluded from classification, the resting EEG is divided into equal 1-sec segments.

→ Transformation → Segment Analysis Function → Segmentation → Divide Data in equal sized segments → Size of Segments: 1 sec

**IMPORTANT:**

„Skip bad intervals“ must NOT be checked and you should NOT request a separate data set for each segment!

<sup>21</sup> Data sets with a sampling rate over 512 Hz can be sampled down to 512 Hz or less at this point in order to speed up future steps and to reduce the size of the data set. (→ Transformation → Change Sampling Rate (Spline)). In any case it is necessary to reduce the sampling rate to 512Hz or less prior to running the VIGALL Add-In.

#### Step 4: Rough artefact screening

Normally, an EEG recording will include several artefacts, i.e. recorded activity that is not representing cortical activity (e.g. eye movements that can contaminate frontal EEG channels). Some of these artefacts can be excluded from the EEG using the independent component analysis (ICA, see step 6-7). In order for the ICA to yield clean components, segments containing strong artefacts have to be marked and not included into the ICA component calculation. However, segments containing artefacts that you wish to correct with the ICA-approach **MUST** be included into the ICA calculation. Therefore, screen the EEG for artefacts that might disturb the ICA results and mark the respective segments.

#### → Transformations → Artifact Rejection

Method: manual / mark segments instead of removing them

Channels: no input needed, if “manual” is selected method

Criterion: no input needed, if “manual” is selected as method

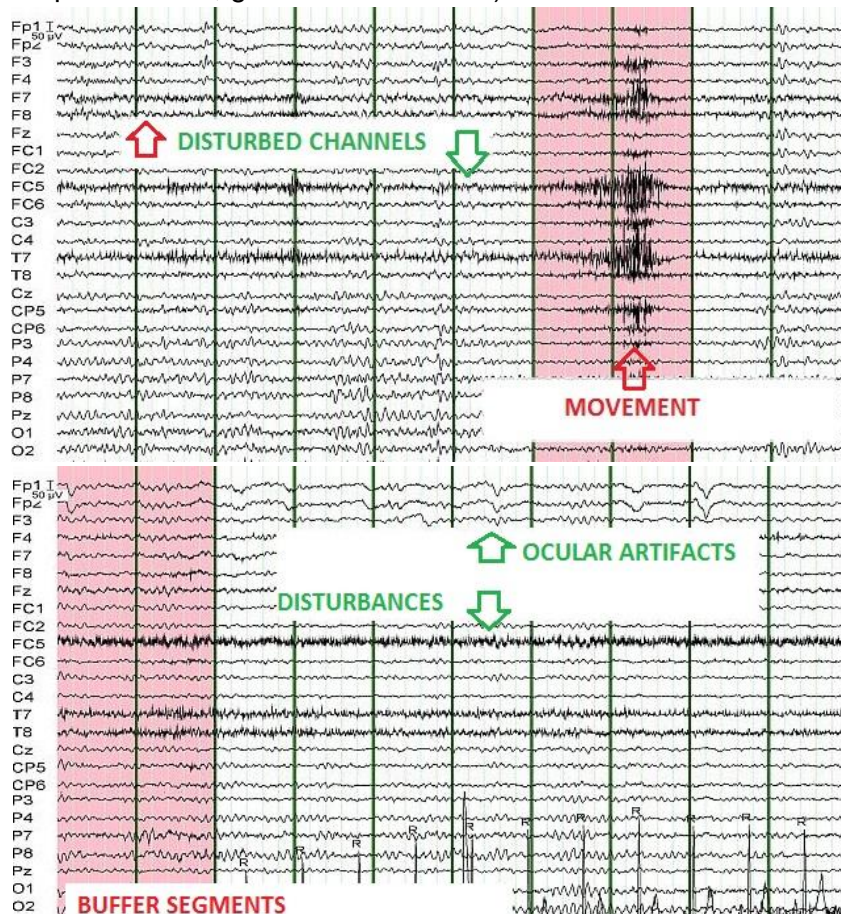
Mark:

- Segments containing heavy body movement
- Segments containing technical artefacts that only occur at a short period of the EEG

Do not mark:

- Segments containing ocular artefacts
- Segments containing disturbed channels
- Segments containing small artefacts (which will be marked later in step 8)

Examples (red = please mark, green = do not mark):



**IMPORTANT:**

- Do not mistake grapho-elements (e.g. K-complexes) for artefacts
- Take notes on continuously disturbed channels (→ step 7)

*NOTE: ICA finds ocular artefacts more reliably, if the data set contains obvious ocular movement somewhere. This might not always be the case, as test subjects are instructed to keep their eyes closed. Thus, sometimes only subliminal eye movement artefacts can be detected, which should nonetheless be corrected. Since the eyes are to be closed right before the start of the resting part, marked eye artefacts may occur during the 2 seconds preceding the start marker (buffer segments). In such cases, one should not mark these buffer segments until after the ICA has been performed.*

*IMPORTANT: While screening the EEG for artefacts please note down segments in your pre-processing protocol, in which subjects might have opened their eyes. Suspicious segments could be identified by reviewing the VEOG channel for typical eye movements. It will be hard to identify those segments after running the ICA, but they might lead to false VIGALL classifications in later stages. Do not mark them as artefacts yet, since they will improve the ICA eye component detection. Still, these segments should be marked as artefacts after the ICA (step 8).*

*NOTE: In case the EEG contains individual channels that are not evaluable due to extensive errors or lack of signal, you can choose to delete and interpolate these channels using the information of surrounding, clear channels. For further information, please see Appendix B.*

### Step 5: Marking grapho-elements representing sleep

*IMPORTANT: This step requires expertise in identifying grapho-elements representing sleep and should only be performed by experienced raters.*

**Background:**

VIGALL assigns each segment in a resting EEG to one out of six vigilance stages (0, A1, A2, A3, B1, B2/3) using characteristic EEG features. Classification of stage C is carried out afterwards if signs of sleep are present in the resting EEG, such as:

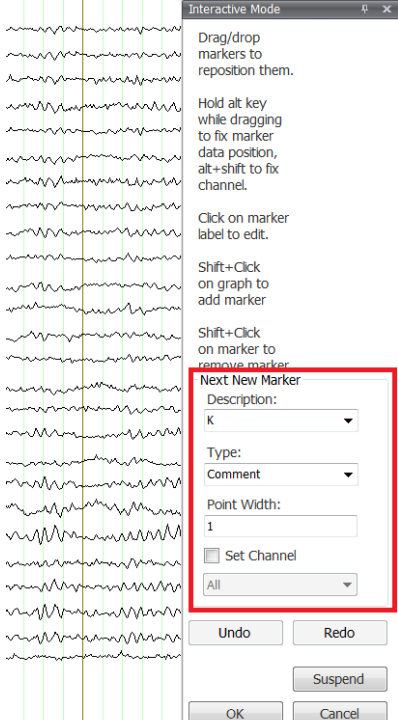
1. K-complexes
2. sleep spindles

These events must be documented in the file using suitable markers. VIGALL will subsequently classify the corresponding segment and the 30 following segments as stage C,

- unless stage A could be classified within these 30 segments (→ in this case stage A is classified and stage C classification is aborted from this segment on)
- unless another C marker is found within these 30 segments (→ in this case C classification is extended for another 30 segments).

Therefore, corresponding markers (either comment marker “C” for all grapho-elements or „K“ for K-complexes and “S” for sleep spindles, respectively) have to be written into the resting EEG before VIGALL execution.

→ Transformation → Others → Edit Markers → graphical



The configuration window will open. Configurations can be set in the „Next New Marker“-box. Choose following settings:

- Description: K or S or C
- Marker Type: Comment
- Point Width: 1

Then, C-, K- or S-markers can be placed at the appropriate location by pressing shift + left click on the graph.

After the entire EEG has been screened, confirm by clicking ,OK‘.

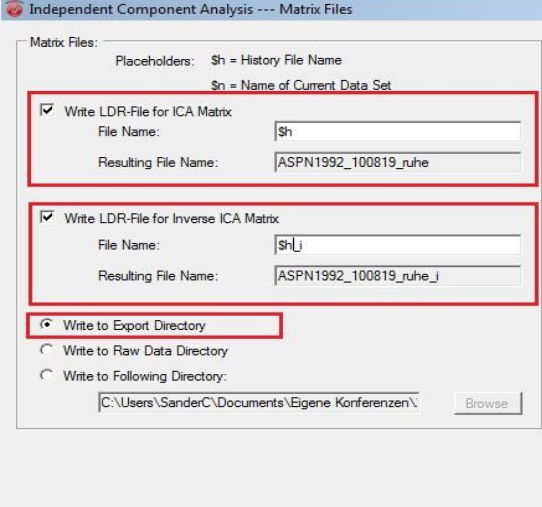
*IMPORTANT: The recommended standard operating procedure (SOP) for the classification of K complexes and sleep spindles can be found under Appendix A.*

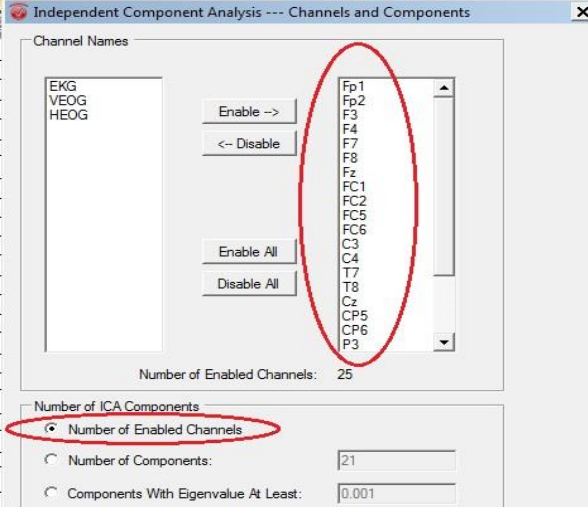
## Step 6: ICA calculation<sup>22</sup>

The purpose of the ICA is to clean up the data set. Ocular artefacts, cardioballistic (CB) artefacts and technical disturbances can be removed. In the first step the EEG signal is decomposed into independent components of which specific ones can be selected and deleted (step 7).

### → Transformation → ICA

1. Define name and export directory for Transformation Matrix and Inverse Matrix  
Choose similar, but not identical file names.
2. Enable all EEG channels and request “number of enabled channels”





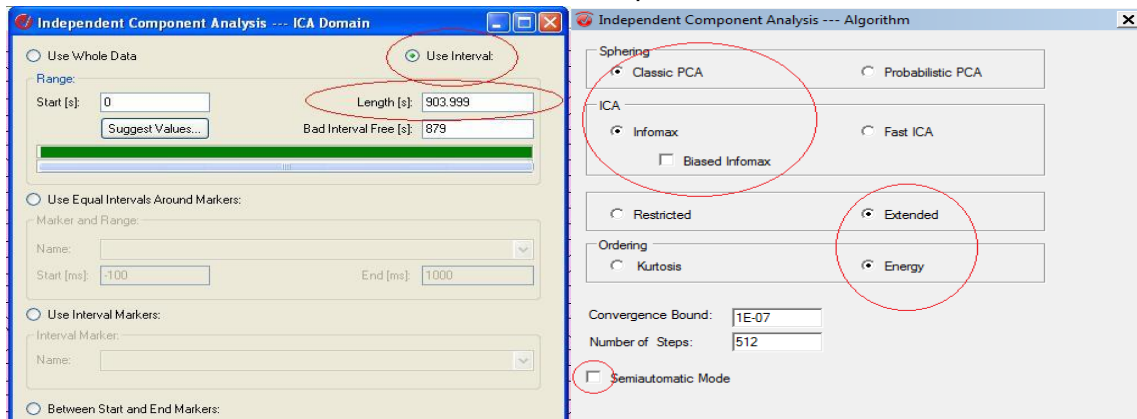
### <sup>22</sup> Data cache:

In order to reduce the calculating effort necessary for the ICA, it is strongly recommended to permanently store the data before execution:

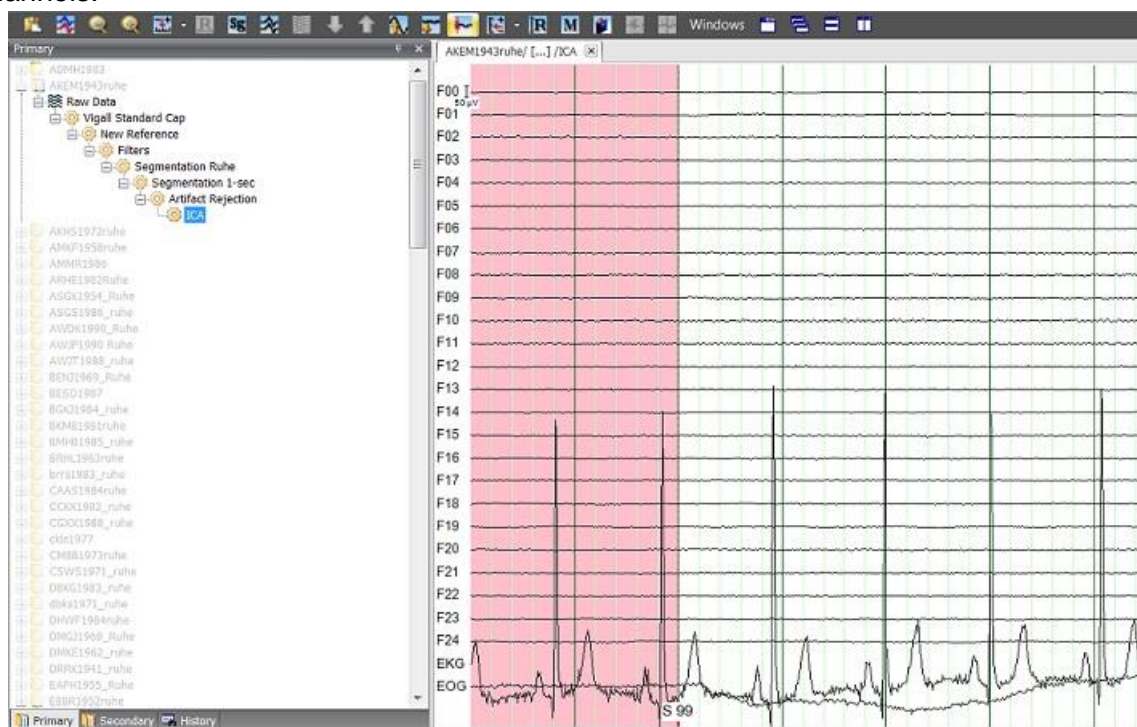
→ Transformation → Others → Data Cache → Store in persistent cache file



3. Please select which segments should be included in the ICA. Check „Use Interval“, choosing the length of the available resting EEG (15 minutes plus buffer segments equals 904 seconds) → ICA automatically excludes segments marked as artefacts from the calculation of the ICA components.
4. Select ICA algorithm: Sphering = Classic PCA, ICA = extended Infomax, Ordering = Energy
5. Do NOT select semiautomatic mode at this point!



After the ICA is finished, a new node appears showing ICA components instead of EEG channels.



**IMPORTANT:** Data recorded in common average reference is not fit for the above described ICA calculation as there is a linear dependency due to the common average reference. It is recommended to re-reference the data, e.g. to Cz-reference, before performing the ICA.

### Step 7: ICA: Removing components

Selecting the relevant disturbed components calls for a thorough approach. Each EEG assessor should have decided beforehand which artefacts they want to clean up with the ICA in order to be able to intentionally search for the corresponding components.

If component selection is made on basis of ICA solutions solely, too many components might be selected and the EEG will be overly corrected.

You will find the view to select components under:

→ Transformation → Inverse ICA

Choose „Semiautomatic Mode“.



Choose the component you want to exclude (see below) by double-clicking it in the upper right corner until it is marked red.

In the lower right area it is possible to choose between four different views that should be used in the decision process.

1. ICA Components: Gives an overview over the activity in the components → select to identify eye and CB components (see below)
2. Correction: Shows the resulting EEG after dismissing the selected components
3. Subtracted: shows the activity that will be eliminated from the EEG
4. Topographies: shows the electrode positions at which the respective component is localized.

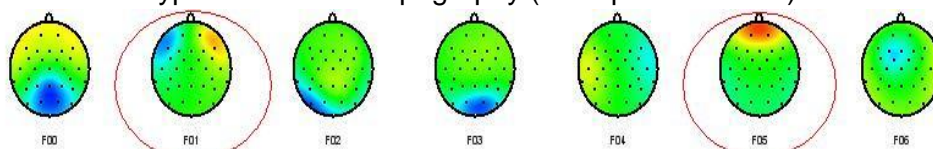
Finalize the ICA by clicking „Finish“.

### Ocular components:

Basically all data sets should be processed in the same fashion. Thus, ocular components should also be searched for in data sets, in which they are not evident on first sight, because ICA also corrects slow activity reflecting slow rolling of the eyes.

Identifying ocular components:

- Ocular components can easily be identified through their topography
- They show a clear orbito-frontal orientation. Vertical eye movement commonly is restricted to Fp1 and Fp2 (example on the right), whereas horizontal eye movement shows a typical two-sided topography (example on the left).

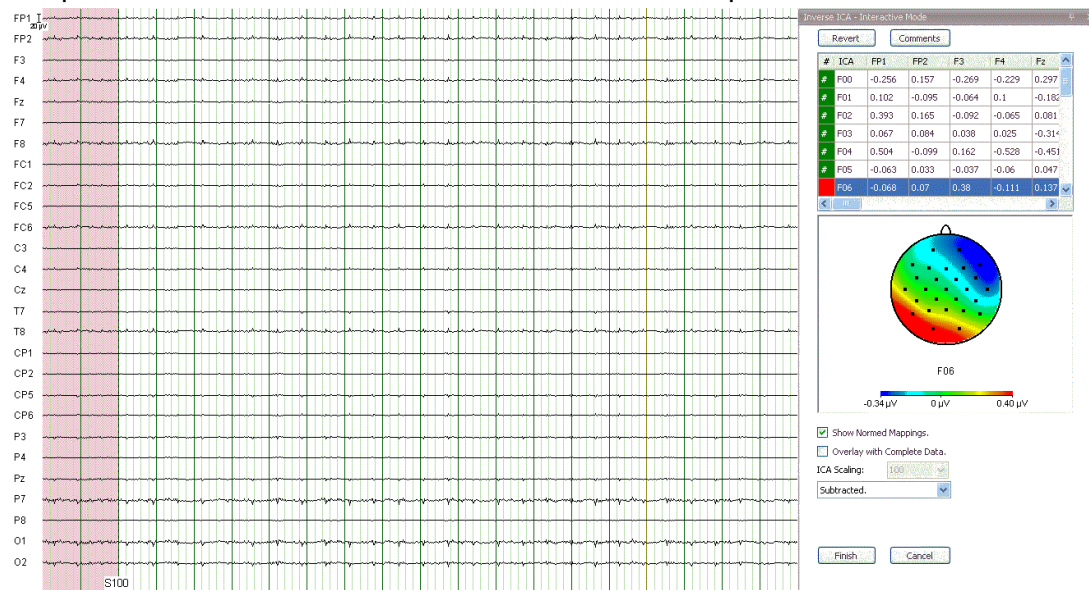


- Select „ICA Components“, to show the signal of individual components
- Select „Correction“ and „Overlay with original data“ to see the fraction of each component

*IMPORTANT: In most cases, two ocular components should be found representing horizontal and vertical eye movements respectively. However, in some cases you may find more than one potential component for horizontal or vertical eye movements as well as no component at all. Caution must be taken when the decision is made whether or not to delete suspicious components, as no component representing actual cortical activity should be deleted by mistake.*

### Cardio-ballistic components:

CB components should be searched for if the EEG shows specific CB artefacts.



### Identifying CB components:

- CB components are easily identifiable in the „ICA Components“-view, since they show spikes correlated to R waves.
- CB components typically show a characteristic topography (see above).

### Disturbed channels:

In many data sets there are channels, which contain disturbances over an extended period that cannot be marked as artefacts, since this would result in too many unusable segments. Use the ICA to remove these disturbances. In many cases this is caused by muscle artefacts that are picked up by the electrodes.

To avoid arbitrariness, plan ahead during step 4 which channels should be cleaned. Only clean up channels if absolutely necessary.

### Identifying the components:

- Select topographies and components that load onto the preselected electrodes
- In principle, components reflecting disturbances show a clear topographic attribution, i.e. they are restricted to one electrode. Look for the channels identified during step 4
- Using the views „correction“ and „subtracted“ you can check, whether elimination of a specific electrode yields the expected effect (also if mainly high frequency disturbances are eliminated)



*NOTE: The VIGALL algorithm only takes into account frequencies below ~20Hz. Any excessive activity in higher frequencies should not influence VIGALL results. Therefore, only those muscle artefacts that have negative effects in the relevant frequency range (2-20 Hz) should be approached (use Fast Fourier Transformation to check.)*

*IMPORTANT: Select the smallest possible number of components. Components reflecting temporary disturbances should not be eliminated but rather be marked as artefacts. If disturbances in a channel can only be controlled by eliminating more than two components interpolating the channel might be the better way to go.*

*NOTE: If a disturbed channel cannot be cleaned using ICA it might be useful to delete the channel from the recording, perform the ICA without this channel and recalculate it using interpolation after the ICA is finished (for details see appendix B).*

*NOTE: If no clean artefact components can be found it might be helpful to redo step 6 and mark more segments as containing artefacts.*

### **Step 8: In-depth artefact correction**

In step 4, only a superficial artefact screening had been performed, wherein prominent artefacts had been marked and several other artefacts had been left unmarked, as they should be corrected using ICA. However, since the ICA might have left some artefacts in the EEG and even might have enhanced previously unnoticed artefacts, artefact correction must now be repeated in-depth.

→ Transformations → Artifact Rejection

Method: manual / mark segments instead of removing them

Channels: no input needed, if „manual“ is selected as method

Criterion: no input needed, if „manual“ is selected as method

*IMPORTANT: The two seconds used as a buffer before and after the resting EEG must now be marked as artefacts (if not already executed during step 4).*

*In case the recording is too short mark all segments as artefacts following the end-of-resting marker*

*NOTE: Some artefacts which are very marginal (small amplitude, short duration) will doubtfully lead to a false classification (e.g. small beta disturbances superimposing a clear alpha activity → Stage A classification very likely) and therefore should not be marked too strictly. Especially disturbances in the beta frequency range and above do not necessarily affect the VIGALL classification and their removal will merely smoothen the EEG's visual appearance.*

*NOTE: After VIGALL classification the result in the corresponding segments should be reviewed. In case of an unsatisfactory classification of a segment, redo step 8, mark the respective segment as artefact and then re-classify.*

*As this is a very time consuming process, it is recommended to follow this rule of thumb:*

*Research EEGs should not comprise of more than 10% artefact and the artefacts should be spread over the full length of the recording. If an EEG contains only a few artefacts, borderline artefacts are preferably marked (to avoid the revision). If an EEG contains a lot of artefacts (and thus interpretability is at risk) segments containing small and potentially non-significant artefacts should not be marked, but revised after classification.*

*The main goal is to include as many EEGs as possible without false classification.*



#### Step 9: Removing New Segment markers

The VIGALL algorithm requires a continuous EEG, which will be divided into 1-second epochs during the VIGALL process. However, to enable an exclusion of segments containing artefacts from the vigilance classification process, EEG data had been segmented into the corresponding 1-second epochs in step 3. Therefore, this must be reversed before the VIGALL is applied. This can easily be achieved by removing all New Segment markers.

→ Transformation → Others → Edit Markers → Automatic

Choose Marker Type: New Segment

Choose Marker Type: <All Descriptions>

Delete these Markers? yes

Add this rule to the list of automatic rules to be applied.

Press button to Finish.

#### Step 10: Reducing sampling rate

The VIGALL Add-In needs a sampling rate between 100Hz and 512Hz, so if the sampling rate is higher, it has to be reduced at this point.

→ Transformation → Change Sampling Rate (Spline)

#### Step 11: Data cache

Depending on the hardware used, it might be advisable at this point to permanently store the data again by using the data cache function implemented in the Analyzer software.

→ Transformation → Others → Data Cache → Store in persistent cache file

*NOTE: The VIGALL software requires EEG data in common average reference. Therefore, if the data was recorded in another reference or has been re-referenced before the ICA it must be re-referenced against common average before the VIGALL is performed. It is recommended to perform this before the final data cache is done.*

After the data pre-processing described above, the VIGALL Add-In can be attached to the node that has been created by the most recent step. Information regarding the handling of the Add-In and the necessary settings are given in the next chapter.

## 5.2 EEG data from ERP paradigms

The VIGALL algorithm can be used to classify segments related to certain stimuli during an ERP paradigm (e.g. for classification of the EEG-vigilance stage immediately before or after presentation of a stimulus). The pre-processing steps are comparable to the steps needed for the preparation of a resting EEG; however, some adaptations have to be considered.

The main prerequisite for the VIGALL to work properly is that the EEG data is not divided into epochs but the temporal integrity of the raw data is kept intact and continuous. In the VIGALL ERP settings (see chapter 5) it can be stated which time window in relation to the stimuli is to be analysed. Therefore the pre-processing steps differ from the usual pre-processing steps in ERP analyses but are more or less comparable to the resting EEG pre-processing described above. The pre-processing steps that need to be executed are:

1. Filtering

This should be performed as in step 1 of the resting EEG pre-processing.

2. Rough artefact screening

In the resting paradigm the EEG was segmented into 1-second segments (steps 2 and 3 of the resting EEG pre-processing) and those segments containing artefacts had been marked (step 4). However, since event-related analyses will not include the whole EEG data but only specified time-windows related to specific markers, this approach might not be feasible. We recommend use of the *Raw Data Inspection* function of the Analyzer to mark sections containing clear artefacts. However, comparable to the artefact screening in the resting EEG pre-processing, the purpose of this first artefact screening is to exclude artefact epochs from the subsequent ICA, therefore all artefacts that could potentially be corrected using the ICA should NOT be marked at this stage.

If the ERP paradigm contained resting periods, it is recommended to mark them as artefacts, too, in order to exclude from further analyses.

3. Marking of grapho-elements representing sleep

In most cases, it can be assumed that subjects did not fall asleep during the ERP paradigm. Nonetheless, if there is suspicion that subjects did fall asleep those sections of the EEG must also be marked as artefacts, as a classification of C-stages is not possible when an event-related classification is to be performed. However, it can be assumed that sections where subjects fell asleep would have to be excluded from the ERP analyses anyway.

4. ICA execution and exclusion of ICA components reflecting continuous artefacts

This should be performed as in steps 6 and 7 of the resting EEG pre-processing.

5. In-depth artefact rejection

Again, this is to be handled comparable to what is described in step 8 of the resting EEG pre-processing yet again the Transformation *Raw data inspection* should be used

instead of the Transformation *Artifact rejection*. While marking sections containing artefacts, it should be taken into account which time windows in relation to which markers will be included in the VIGALL classification.

6. Reducing sampling rate and Data Cache

This should be performed as in steps 9 and 11 of the resting EEG pre-processing.

7. Assignment of unique labels for ERP stimuli.

In some cases, different stimuli might have been used in an ERP paradigm, which are represented in the EEG trace by different marker descriptions. If the EEG-vigilance stage is to be classified for more than one type of stimuli, all of them have to be labelled in the same way, as VIGALL accepts only one label. It is recommended to use the transformation *Edit Markers* → *automatic* to assign a unique marker description to all relevant markers. This unique marker description is then entered in the ERP settings of the VIGALL (see section 5.3).

## 6 Applying the VIGALL Add-In

After pre-processing, the Add-In works like a normal transformation in the Analyzer software. To use it, choose the node representing the final pre-processing step (preferably the last data-cache node).

Open VIGALL under: Add Ins → Others → VIGALL

A window will open that is divided into 3 tabs, where settings can be changed according to the specific data that is to be analysed. For most parameters it is recommended to use the default values, changes will impact the EEG-vigilance stage scoring, which is performed according to the scoring rules depicted in figure 8.

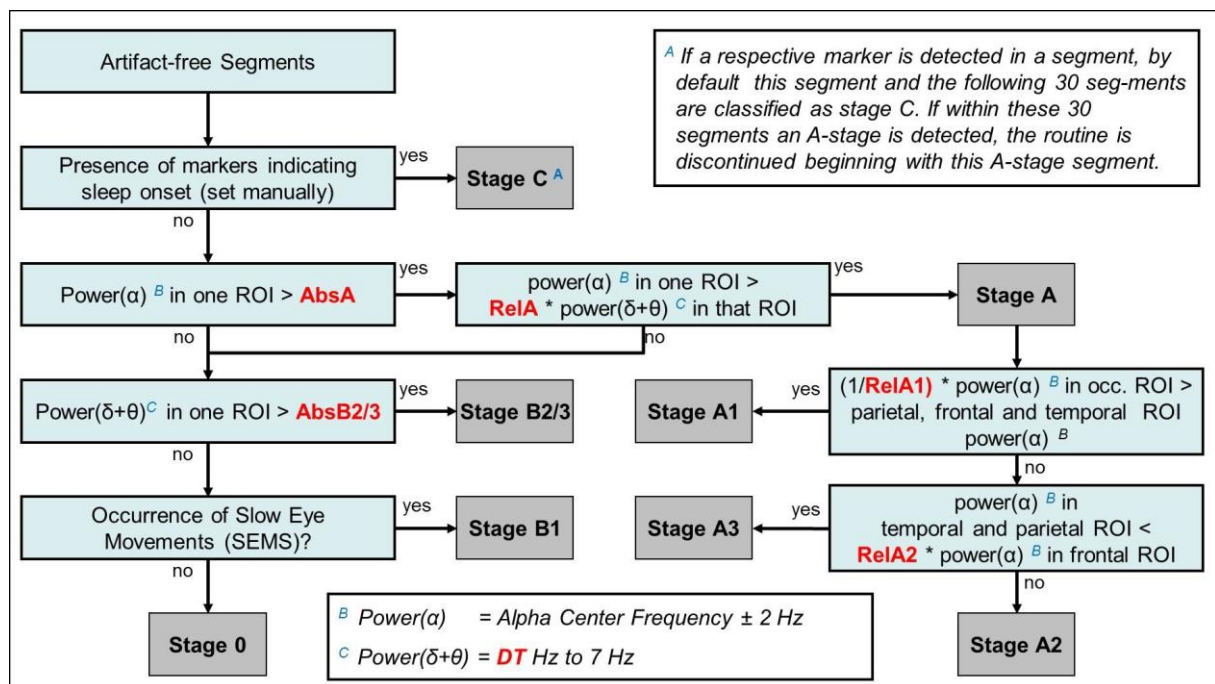


Figure 8: Scoring rules of the VIGALL algorithm (version 2.1). Red text refers to specific VIGALL parameters which will be explained in the following section.

### 6.1 Main parameters settings

On the main parameter tab (see figure 9) you find the major setting options for VIGALL. You can reset all parameters to default by clicking the 'restore original settings' button at the bottom of the VIGALL screen. The OK button starts the analysis. The Cancel button ends the Add-In and returns to BrainVision Analyzer 2.1.

**NOTE:** If you clicked OK and the procedure does not start, an error has occurred in one of the tabs. The respective input field is marked by a red exclamation mark containing more information about why the input is not admissible. If you cannot spot an exclamation mark in the current tab, check the input fields of the other tabs.

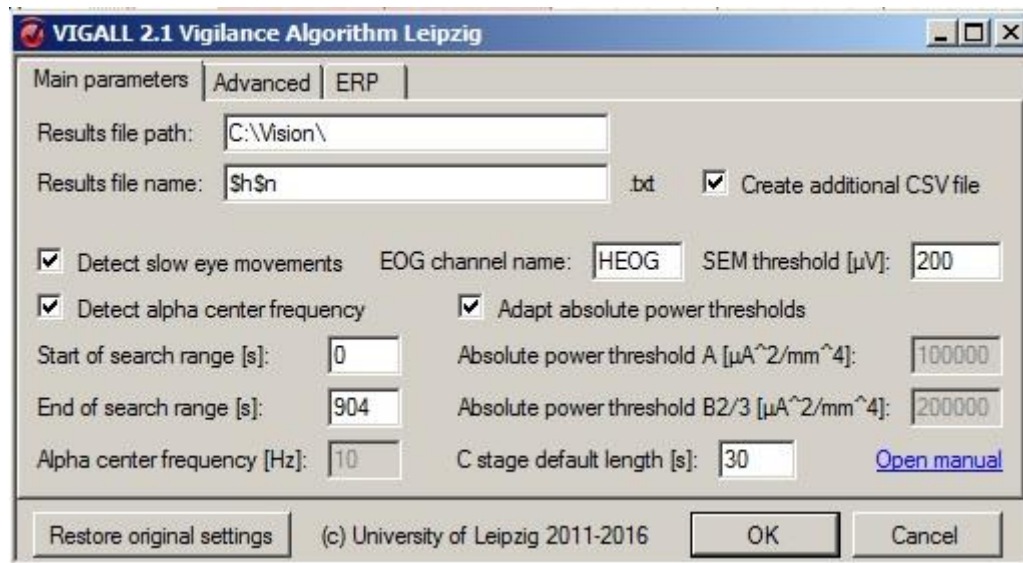


Figure 9: Main parameters tab of the VIGALL settings.

In the upper part, you can define the directory where the results file will be stored [*Results file path*] and the name of the file that will contain the results [*Results file name*]. The resulting file is automatically saved as \*.txt.

You can either type in the name directly or use the wildcards function, in which case

- *\$h* equals the history file name and
- *\$n* the active node name.

This can be useful if you want to process large data sets in an analogous manner. Check *Create additional CSV file* if you want VIGALL to create, besides the results text file, a file that contains the results in comma separated values format. This file will not contain information on the classification parameters used but facilitates analysing the results in spreadsheet or analysis software.

Check *Detect slow eye movements* (SEM) if you want VIGALL to include this information in its analysis. SEM detection is necessary for the distinction between B1 and 0 states. Therefore, by unchecking this option VIGALL will classify all respective segments as belonging to stage B1. This function requires the selection of a channel containing HEOG information [*EOG channel name*]. To complete SEM detection you have to define a *power threshold* differential. Default value is 200  $\mu$ V. (More parameters can be adapted in the advanced tab.)

The VIGALL decision criteria consider the ratio between delta/theta and alpha band activity. The delta/theta band is usually fixed between 3 and 7 Hz (see explanations in the advanced tab section below). The position of the alpha band can be adjusted for each individual. A 4 Hz window ( $\pm$  2Hz around the *alpha centre frequency*) will be used as alpha band. To have

VIGALL automatically detect the subject's alpha centre frequency check the corresponding box [*detect alpha centre frequency*]. VIGALL will divide the resting EEG in overlapping 10 second epochs for each of which the alpha peak frequency (based on the average of EEG channels O1, O2) will be detected. Segments containing artefacts or grapho-elements indicating sleep are excluded as well as segments in which delta/theta power is more prominent than alpha power (a documentation of excluded segments can be found in the VIGALL output file). From the remaining segments, VIGALL picks the one with the greatest average power in the frequency spectrum of **8 to 14 Hz**. Within this segment, it finds the 2 Hz window that contains the greatest power integral. Inside this interval, it calculates the centre of gravity frequency.

You can specify the search window by entering the starting [*start of search range (s)*] and ending [*end of search range (2)*] of an epoch in which you want VIGALL to detect the alpha centre frequency. Usually, this should be the entire range of the resting state EEG (e.g. 0 to 904 for 15min resting EEGs). Alternatively, you can manually enter the subject's alpha centre frequency [*Alpha Centre Frequency (Hz)*], after unchecking the automatic alpha detection.

*NOTE: It is recommended to review the automatically detected alpha centre frequencies, which are documented in the VIGALL output. Especially in low voltage EEGs, the detection of the alpha centre frequency may result in implausible results. The same might happen in cases of de- or accelerated alpha rhythms. In the VIGALL output, it is documented which 10-sec epoch was chosen for detection of the alpha centre frequency. If you do not agree with this choice, you could change the search window to have VIGALL consider only selected sections of the resting EEG.*

It is highly recommended to use the automatic detection of the alpha centre frequency function of the VIGALL as only this will allow adaption of the VIGALL decision criteria for A- and B2/3-stage detection to the subject's individual power distribution by checking *adapt absolute power thresholds*. Based on the individual alpha power distribution an absolute power threshold for A-stages is defined (**AbsA**). This must be surpassed by the alpha band activity of an individual EEG-segment to have it classified as an A-stage. Accordingly, a B23 threshold (**AbsB23**) is determined as 2\*AbsA which needs to be passed by delta/theta activity to have an individual segment not classified as belonging to stage A classified as B2/3-stage. Alternatively, you can enter values for AbsA and AbsB23 manually in the two corresponding fields ([*absolute power threshold A ( $\mu A^2/mm^4$ )*] and [*absolute power threshold B2/3 ( $\mu A^2/mm^4$ )*]). Although this approach is not recommended, if you chose to use it, than it is suggested to use 100.000 for AbsA and 200.000 for AbsB23.

Finally, on this tab you can define how many seconds are to be classified as stage C [C stage default length (s)] once a respective grapho-element (sleep spindle or k-complex) was marked in the EEG (see step 5 in chapter 4). The recommended default value is 30 seconds, which corresponds to the typical 30-second epochs that are scored in polysomnographic sleep recordings according to the current scoring rules in sleep medicine<sup>23</sup>.

*NOTE: On the bottom to the right, you find a link to the latest version of the VIGALL Manual. Should there be any questions that are not sufficiently answered in this version of the manual, please follow the link to check for updates that might already comprise a solution to the encountered problem. If that is not the case please contact us via the support email address given below.*

## 6.2 Advanced options settings

The advanced tab (figure 10) provides you with several further options to adapt the VIGALL classification and results.

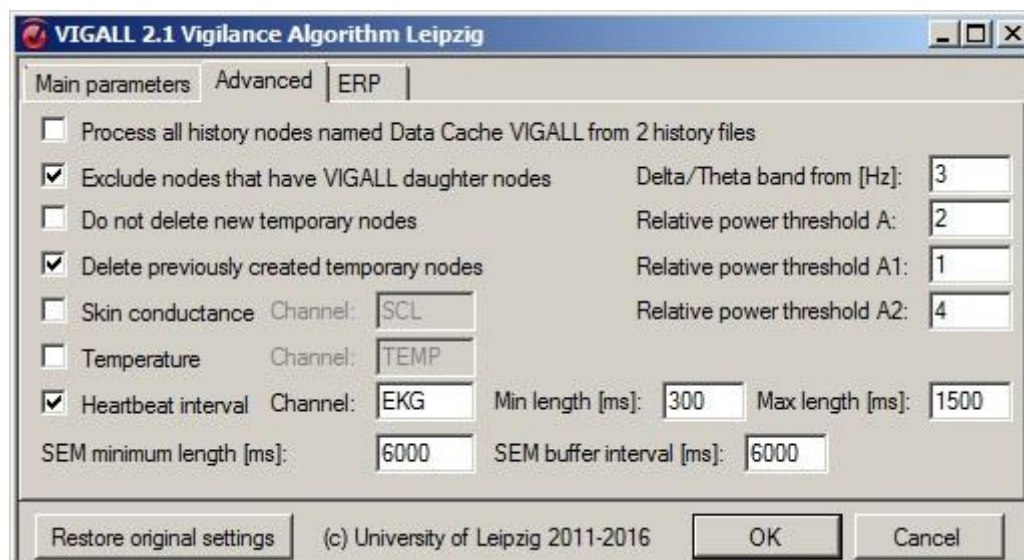


Figure 10: Advanced options tab of the VIGALL settings.

If you want to perform VIGALL analysis on a greater set of similarly named history nodes, you can use the VIGALL batch function by checking the first box on this tab [*Process all history nodes named XXX from XXX history files*]. This option works as follows: the batch will regard all nodes named identically to the one the user selects manually in one of the Analyzer's history files. In figure 9 the selected node was named "Data Cache VIGALL" and

<sup>23</sup> Iber C, Ancoli-Israel S, Chesson A, and Quan SF for the American Academy of Sleep Medicine. The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications, 1st ed.: Westchester, Illinois: American Academy of Sleep Medicine, 2007

VIGALL found such a node in 2 history files of the current data set. Please note that VIGALL effectively only considers history files for the batch that are fully expanded in the history files window. Therefore, you can select the EEGs that you want to be processed at a time by opening up the respective history files in the data set.

The next three boxes might be of interest, if the VIGALL is to be performed more than once on identical data sets. If you want to exclude all history nodes that already contain VIGALL daughter nodes from batch processing, please check *Exclude nodes that already have VIGALL daughter nodes*. They will then be left out from batch processing even if their history files are opened. The checkbox *Delete previously created temporary nodes* provides the possibility to delete temporary nodes from a previous VIGALL classification that would interfere with a repeated application of VIGALL. This might be needed, if such nodes have not been deleted during the VIGALL process, which would normally happen automatically. If you want to review the nodes created during the VIGALL classification you can check “*Do not delete new temporary nodes*”. In this case the temporary nodes filled with intermediary algorithm calculations are stored in the history node after classification.

The four options in the upper right area of the advanced tab directly influence the VIGALL classification itself, as they represent specific parameters used by VIGALL to classify the individual segments into vigilance stages. Generally the default values are recommended.

- *Delta/Theta (DT)*<sup>24</sup>: This value determines the lower border of the delta/theta band used by VIGALL, whereas the upper border is always fixed at 7 Hz. Changing this parameter has an impact on how many resulting segments are classified as stage A and stage B23. The higher the chosen value, the smaller the included delta/theta band, thus more segments will be classified as A-stages (as the ratio between delta/theta and alpha power will be more in favour of A-stages) and less segments will be classified as B2/3-stages (as delta/theta power must surpass AbsB23). See figure 8 for details. Default setting is 3 Hz<sup>25</sup>.
- *Relative power threshold A (RelA)*: This value has an impact on how many resulting segments are classified as stage A by defining the factor by which the relative Alpha power must exceed the sum of Delta and Theta power. The higher the chosen value, the less A-stages and the more B2/3-stages are classified. Default setting is 2.
- *Relative power threshold A1 (RelA1)*: This value has an impact on how many resulting segments are classified as stage A1. It defines the factor by which the occipital Alpha power must be higher than parietal, temporal or fronto-temporal Alpha power. If you

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<sup>24</sup> NOTE: If the Alpha center frequency detected in the dataset is below 9 Hz, the delta/theta and alpha band will partially overlap. Therefore, the resulting vigilance classification should be checked by a human rater.

<sup>25</sup> VIGALL2.0 did not offer the possibility to modify this parameter which had been set at 2 Hz. However, in some EEGs this was found too sensible for artifacts impacting low frequency spectra such as eye movements.



increase the value you decrease the possibility of stage A1 classification. Default setting is 1 <sup>26</sup>.

- *Relative power threshold A2 (RelA2)*: This value has an impact on how many of the remaining A-stages are assigned to the sub-stages A2 and A3. It defines the factor by which the frontal Alpha power must be higher than the parietal or temporal Alpha power. A higher value will result in less A3 stages. Recommend default setting is 4.

Apart from the vigilance classification, the VIGALL can also calculate additional information if certain non-EEG information is present in the EEG. If *skin conductance* and/or *temperature* have been recorded VIGALL can calculate their mean values for classified segments and display them in the output file. The values are calculated independently of the algorithm and do not influence the vigilance classification. Furthermore, the automated *heart rate detection* can be configured. Please note that this function is only possible if R waves have been adequately marked (see Appendix C for further information). If so, VIGALL can display estimated heartbeat intervals for classified segments in the selected channel. VIGALL marks heartbeat intervals below the selected minimum (default value: 300ms i.e. 40bpm) and above the selected maximum (default value: 1500ms i.e. 200bpm) as artefacts.

The last line in that window is the place to specify the automatic *SEM detection* that can be requested in the main parameter setting (see above). One can enter the minimum length of slow eye movements [*SEM minimum length (ms)*] to be detected (Default value is 6000 ms) and the interval before and after a SEM power differential [*SEM buffer interval (ms)*] to be influenced by positive SEM detection (Default value is 6000 ms).

### 6.3 ERP settings

If you wish to perform an event-related analysis, settings must be specified in the ERP tab (see figure 11). The event-related classification is requested by checking *Perform event-related analysis*.

---

<sup>26</sup> In VIGALL2.0 a default value of 2 was recommended for relA1. This parameter has an impact on how many segments are classified as stage A1 as it is the factor by which the occipital alpha power must be higher than parietal, temporal or fronto-temporal alpha power. In VIGALL 2.0, this often resulted in at least equal amounts of A2 and A1 within minutes after closing of the eyes, although a preponderance of A1-stages would be expected.

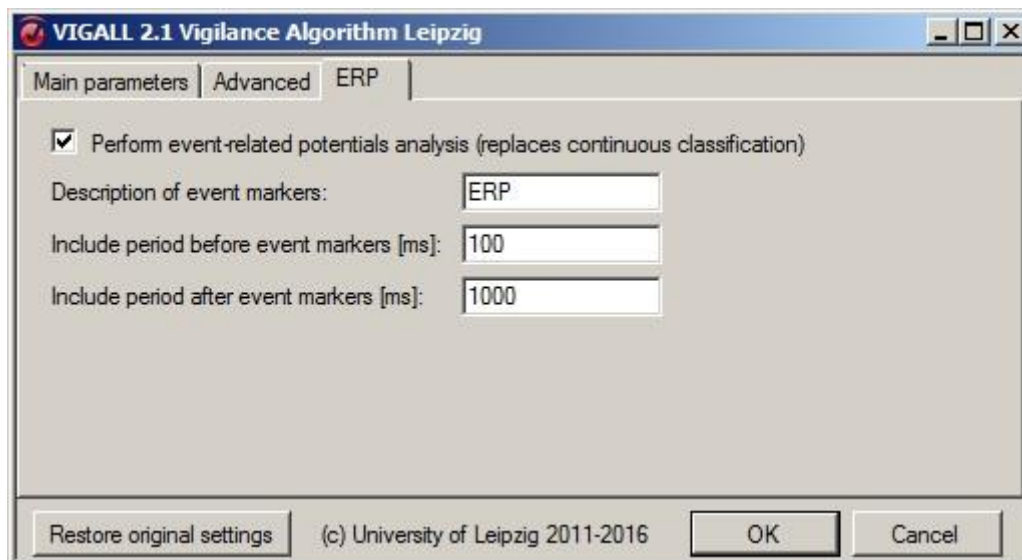


Figure 11: ERP options tab of the VIGALL settings

You have to specify the relevant markers of events where classification is to be performed. The marker type (Comment, Stimulus, ...) is disregarded, therefore only the unambiguous marker description has to be entered (see figure 12).

Furthermore, you can specify the period before and after each of the specified markers to be included in the analysis. The sum of these two intervals needs to be at least 400 ms for valid classification.

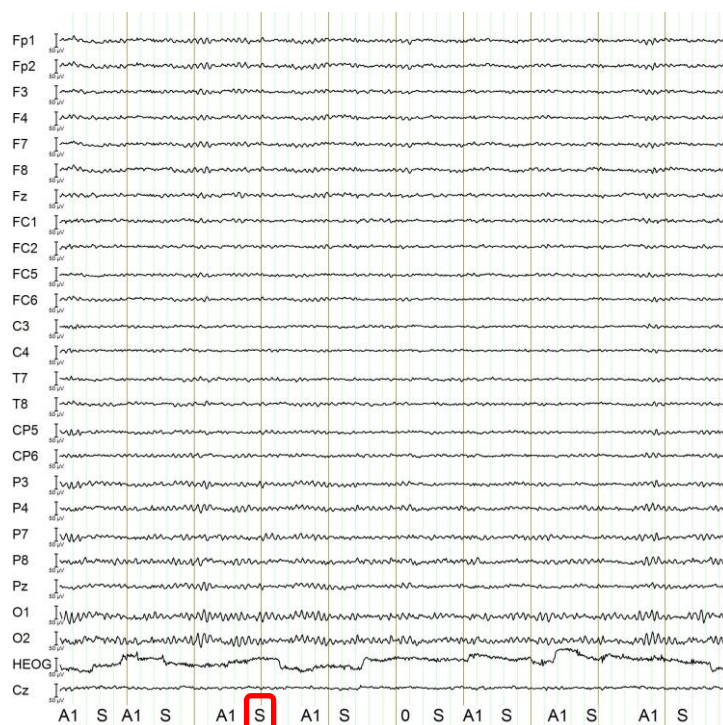


Figure 12: VIGALL ERP classification with settings of in relative to time window of 500 ms before onset of each stimulus. S represents the unique marker description for the relevant marker of events.

## 7 Results interpretation

The results of the classification are written into an output text file and, optionally, into a comma separated values file. The name of these files can be defined under the main parameters tab in the settings window (see above). Additionally, you can check the results by right-clicking on the Analyzer's resulting node and choosing Operation Info in the pop-up menu.

### 7.1 VIGALL Output

The VIGALL output text file can be opened with any text editor and offers the classification results as well as an overview of the specific settings used in the classification (see figure 13).

```
VIGALL Version: VIGALL 2.1
Output file name: Example.txt
Channels: Fp1, Fp2, F3, F4, F7, F8, Fz, FC1, FC2, FC3, FC5, FC6, C3, C4, T7, T8, C2, CP5, CP6, P3, P4, P7, P8, Pz, O1, O2, EKG,
Absolute power threshold A: 120444
Relative power threshold A: 2
Absolute power threshold B2/3: 240888
Relative power threshold A1: 1
Relative power threshold A2: 4
Default length of c stages: 30 segments Delta/Theta band starts from 3 Hz
Not transformed (out of Range, Bad Interval or C): 0-10s 1-11s 707-717s 708-718s 709-719s 710-720s 711-721s 712-722s 713-723s 714-715s 716-717s 718-719s 719-720s 720-721s 721-722s 722-723s 723-724s 724-725s 725-726s 726-727s 727-728s 728-729s 729-730s 730-731s 731-732s 732-733s 733-734s 734-735s 735-736s 736-737s 737-738s 738-739s 739-740s 740-741s 741-742s 742-743s 743-744s 744-745s 745-746s 746-747s 747-748s 748-749s 749-750s 750-751s 751-752s 752-753s 753-754s 754-755s 755-756s 756-757s 757-758s 758-759s 759-760s 760-761s 761-762s 762-763s 763-764s 764-765s 765-766s 766-767s 767-768s 768-769s 769-770s 770-771s 771-772s 772-773s 773-774s 774-775s 775-776s 776-777s 777-778s 778-779s 779-780s 780-781s 781-782s 782-783s 783-784s 784-785s 785-786s 786-787s 787-788s 788-789s 789-790s 790-791s 791-792s 792-793s 793-794s 794-795s 795-796s 796-797s 797-798s 798-799s 799-800s 800-801s 801-802s 802-803s 803-804s 804-805s 805-806s 806-807s 807-808s 808-809s 809-810s 810-811s 811-812s 812-813s 813-814s 814-815s 815-816s 816-817s 817-818s 818-819s 819-820s 820-821s 821-822s 822-823s 823-824s 824-825s 825-826s 826-827s 827-828s 828-829s 829-830s 830-831s 831-832s 832-833s 833-834s 834-835s 835-836s 836-837s 837-838s 838-839s 839-840s 840-841s 841-842s 842-843s 843-844s 844-845s 845-846s 846-847s 847-848s 848-849s 849-850s 850-851s 851-852s 852-853s 853-854s 854-855s 855-856s 856-857s 857-858s 858-859s 859-860s 860-861s 861-862s 862-863s 863-864s 864-865s 865-866s 866-867s 867-868s 868-869s 869-870s 870-871s 871-872s 872-873s 873-874s 874-875s 875-876s 876-877s 877-878s 878-879s 879-880s 880-881s 881-882s 882-883s 883-884s 884-885s 885-886s 886-887s 887-888s 888-889s 889-890s 890-891s 891-892s 892-893s 893-894s 894-895s 895-896s 896-897s 897-898s 898-899s 899-900s 900-901s 901-902s 902-903s 903-904s 904-905s 905-906s 906-907s 907-908s 908-909s 909-910s 910-911s 911-912s 912-913s 913-914s 914-915s 915-916s 916-917s 917-918s 918-919s 919-920s 920-921s 921-922s 922-923s 923-924s 924-925s 925-926s 926-927s 927-928s 928-929s 929-930s 930-931s 931-932s 932-933s 933-934s 934-935s 935-936s 936-937s 937-938s 938-939s 939-940s 940-941s 941-942s 942-943s 943-944s 944-945s 945-946s 946-947s 947-948s 948-949s 949-950s 950-951s 951-952s 952-953s 953-954s 954-955s 955-956s 956-957s 957-958s 958-959s 959-960s 960-961s 961-962s 962-963s 963-964s 964-965s 965-966s 966-967s 967-968s 968-969s 969-970s 970-971s 971-972s 972-973s 973-974s 974-975s 975-976s 976-977s 977-978s 978-979s 979-980s 980-981s 981-982s 982-983s 983-984s 984-985s 985-986s 986-987s 987-988s 988-989s 989-990s 990-991s 991-992s 992-993s 993-994s 994-995s 995-996s 996-997s 997-998s 998-999s 999-1000s
Individual Alpha Frequency: 10.9 Hz - Located at 453 - 463 s.
occAlpha: 1751844
SEM channel: HEOG - Threshold: 200 - Length: 6000 - Interval: 6000
Heart rate channel name: EKG - Minimum length: 300 ms - Heart rate Maximum length: 1500 ms
No skin conductivity calculation
No temperature calculation

Number of segments classified 0: 1
Number of segments classified A1: 477
Number of segments classified A2: 28
Number of segments classified A3: 21
Number of segments classified B1: 161
Number of segments classified B2: 118
Number of segments classified C: 88
Number of segments not classified: 10

Power values
1 X 0 0 510
2 X 0 0 705
3 A1 0 0 776 1805174 162681 0 0 257339.5 58377.22 0 0 615408.8 111571.1 0 0 32474.47 28138.49 0 1
4 A1 0 0 910 197468.9 0 0 375179 122883.6 0 0 666541.1 196849.4 0 0 51043.27 55143.88 0 1
5 A1 0 0 935 1766643 307280.1 0 0 457495.6 54237.7 0 0 734491.8 129723.5 0 0 46143.51 27265.04 0 1
6 A1 0 0 875 2435554 240635.1 0 0 473479.2 70050.45 0 0 444890.5 164353 0 0 61044.08 54675.34 0 1
7 A1 0 0 835 2586660 267024.3 0 0 473025.8 68031.86 0 0 695858.7 118889.4 0 0 53957.18 40614.75 0 1
8 A1 0 0 855 1465039 202627.9 0 0 338483.3 85547.85 0 0 500243.7 140181.6 0 0 36810.45 42089.91 0 1
9 A1 0 0 875 1807554 331367.1 0 0 488590.6 69268.38 0 0 834353.2 222041.5 0 0 60921.34 23241.16 0 1
10 A1 0 0 820 1579252 236030.6 0 0 865426.3 76499.48 0 0 896763.9 110761.4 0 0 159245.8 21171.28 0 1
11 A1 0 0 770 2926360 378054 0 0 598052.6 103068.9 0 0 1234377 189363.3 0 0 87430.88 34643.99 0 1
12 A1 0 0 805 1302134 344954.6 0 0 471042.4 96193.77 0 0 915262.8 127063.5 0 0 74810.28 41697.12 0 1
```

Figure 13: Header of the VIGALL output text-file listing the specific settings used for the VIGALL parameters.

The top states the VIGALL version and the main parameters used for classification. Use this to make sure the datasets you compare have been processed homogenously.

The second block describes the alpha centre frequency based upon which the VIGALL classification is performed, and the 10-second segment of the dataset where it was found. This may be important to evaluate, as the alpha centre frequency heavily influences classification. This block also lists 10-second segments excluded from the search.

Then there is a list of auxiliary parameters and below of them, the number of segments classified in any vigilance stage is listed. Use this to get a brief summary of vigilance-related activity in the dataset.

The main part of the file is separated into columns by spaces. Usually, only the first two columns will be relevant. If an optional CSV file was requested it will contain these information in comma separated volumes (see figure 14).

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	1 X		0	0	510																
2	2 X		0	0	705																
3	3 A1		0	0	776	1805174	162681	0	0	257339.5	58377.22	0	0	615408.8	111571.1	0	0	32474.47	28138.49	0	1
4	4 A1		0	0	910	1471611	197468.9	0	0	375179	122883.6	0	0	666541.1	196849.4	0	0	51043.27	55143.88	0	1
5	5 A1		0	0	335	1766643	307280.1	0	0	457495.6	54237.7	0	0	734491.8	129723.5	0	0	46143.51	27265.04	0	1
6	6 A1		0	0	875	2435554	240635.1	0	0	473473.2	70050.45	0	0	444890.5	164353	0	0	61044.08	54675.34	0	1
7	7 A1		0	0	835	2586660	267024.3	0	0	473025.8	68031.86	0	0	658588.7	118689.4	0	0	53957.18	40614.75	0	1
8	8 A1		0	0	855	1485039	202627.9	0	0	338483.3	85547.85	0	0	500243.7	140181.6	0	0	38810.45	42089.91	0	1
9	9 A1		0	0	875	1807554	331367.1	0	0	488590.6	63268.38	0	0	834353.2	222041.5	0	0	60321.34	23241.16	0	1
10	10 A1		0	0	820	1573252	236030.6	0	0	865426.3	76493.48	0	0	896763.9	110761.4	0	0	153245.8	21171.28	0	1
11	11 A1		0	0	770	2326360	378054	0	0	598052.6	103068.9	0	0	1234377	189363.3	0	0	87430.88	34643.99	0	1
12	12 A1		0	0	805	1302154	344954.6	0	0	471042.4	96193.77	0	0	915262.8	127063.5	0	0	74810.28	41897.12	0	1
13	13 A2		0	0	865	1019590	292049.8	0	0	313868.3	106675.3	0	0	1240429	239098.1	0	0	76326.42	45664.61	0	1
14	14 A1		0	0	870	1337730	177014.5	0	0	307905.5	68855.33	0	0	270807.3	74221.29	0	0	100550.3	21386.07	0	1
15	15 A1		0	0	810	1182564	192314.8	0	0	773841.8	81438.45	0	0	863052.2	144908.3	0	0	86425.47	30768.53	0	1
16	16 A1		0	0	845	1230436	319150.8	0	0	909587.5	132150.6	0	0	813722.4	148470.4	0	0	101765.4	23289.65	0	1
17	17 A1		0	0	830	1086306	496400.3	0	0	448539	118047	0	0	611576.2	240400.6	0	0	77683.36	34125.51	0	1
18	18 A1		0	0	855	1513187	205669.9	0	0	565310.9	53389.79	0	0	970668.7	145145.8	0	0	87039.16	52760.5	0	1
19	19 A1		0	0	810	1874835	239550.9	0	0	409879.3	112093.7	0	0	366374	118582.6	0	0	17164.37	24385.09	0	1
20	20 A1		0	0	810	1344211	264015.8	0	0	338962	120997.9	0	0	505379.8	161286.8	0	0	94685.16	51797.71	0	1
21	21 A1		0	0	740	2451093	314328.5	0	0	274734.2	139545.9	0	0	823837.9	133333.6	0	0	86087.11	32817.33	0	1
22	22 A1		0	0	710	1320753	186944	0	0	744853.7	107255	0	0	632487.1	131561.8	0	0	109163.3	49575.17	0	1
23	23 A1		0	0	740	970367	174990.3	0	0	440404.7	117137	0	0	447931	173736	0	0	36417.73	45636.26	0	1
24	24 A1		0	0	810	1341588	294486.7	0	0	514076.3	158161.1	0	0	728124.4	185152.7	0	0	130662.4	27852.69	0	1
25	25 A1		0	0	845	1512080	278563.4	0	0	595333.9	156370.5	0	0	654588.6	146149.6	0	0	58303.38	28105.25	0	1
26	26 A1		0	0	820	1380956	333822.8	0	0	637170.3	127722.9	0	0	866758.6	230618.3	0	0	124423.8	58608.43	0	1
27	27 A1		0	0	896	1089133	288817.6	0	0	448707	277315.9	0	0	777889.7	288829.6	0	0	71788.84	72452.53	0	1

Figure 14: VIGALL output csv-File. (Columns contain the following information: A: number of the segment; B: classified vigilance stage; C: average power in the skin conductivity channel (if recorded); D: average power in the temperature channel (if recorded); E: estimated heartbeat interval (in ms); F: average alpha band current density occipital ROI; G: Average delta/theta band current density occipital ROI; J: Average alpha band current density parietal ROI; K: Average delta/theta band current density parietal ROI; N: Average alpha band current density temporal ROI; O: Average delta/theta band current density temporal ROI; R: Average alpha band current density frontal ROI; S: Average delta/theta band current density frontal ROI; U: detection of slow eye movements (0=no/1=yes).

## 7.2 Further Recommendations

In order to maximize the validity of VIGALL result interpretations, we here present a few additional suggestions:

### A) 0/B1-Plausibility:

The VIGALL classifies each EEG segment independently from the surrounding segments and, in contrast to a human rater, it does not integrate context information into its classification. In some cases, this produces suspicious results in the classification of 0- or B1-stages. The separation between stage 0 and stage B1 is based on the presence or absence of slow eye movements. However, although slow eye movements are a typical sign of lowered arousal and are therefore characteristic for B1-stages, they are not always present. Therefore, it is suggested to revise segments classified as 0-stages using context information (i.e. the surrounding segments). If the surrounding segments suggest a lowered EEG-vigilance (e.g. most of the surrounding segments are classified as B2/3 or even C), a segment classified as belonging to stage 0 should be considered as B1-segment even in the

absence of slow eye movements. In most cases, such an additional revision will only result in minor changes, non-the-less the validity of VIGALL results improves if such a plausibility check is done (see appendix E).

### *B) Eligibility*

When VIGALL is used in scientific studies, we suggest using some specific quality criteria to decide whether an individual file should be included in an analysis. In general, we suggest to only include data sets with more than 85% of analysable segments (i.e. less than 15 segments excluded from analysis due to artefacts). Since in many cases a minute-by-minute analysis will be performed one should also exclude datasets with a larger number of successive artefact segments (e.g. more than 45 seconds within a minute).

### *C) Alpha Finder*

As mentioned above (see section 6.1), VIGALL automatically detects a segment with prominent alpha rhythm activity to determine the alpha centre frequency that will be used to define the location of the alpha band. However, if there is no significant alpha activity to be found within the EEG recording, this process might result in suspicious results. This is typically the case in low voltage EEGs. It is in general suggested to review the automatically detected alpha frequency but this should be done in any case if the Alpha Finder uses a very late epoch of the EEG. Normally, the alpha power declines during a resting EEG, so that the Alpha Finder in most cases uses earlier segments. Apart from the misplacement of the alpha band there is another disadvantage. Not only is the location of alpha band defined by the Alpha Finder result, the absolute power threshold for A-stages (absA) and with this for B2/3-stages (absB23) are also defined based on the alpha activity within that epoch. Therefore, when the absence of segments containing real alpha activity results in a faulty Alpha Finder result, these thresholds will subsequently be of limited value, too. Therefore, another sign of possible Alpha Finder problems is an  $\text{absA} < 25.000$ , so VIGALL results based on such low absA-values should also be revised for plausibility of the used Alpha Finder epoch.

### *D) Low Voltage:*

As mentioned above, the VIGALL is designed for use in non-pathological alpha EEGs. If the VIGALL results in more than 95% of all segments classified as stages 0 or B1, it is recommended to check whether the respective file contains a low voltage EEG, which should not be included in statistical analyses, since in this case the VIGALL is not being able to classify the full spectrum of vigilance stages.

## 8 Contact Information

### Support:

The project web site is located at <http://uni-leipzig.de/~vigall/> . If you encounter any problems, please make sure you are using the most current version of the software and this manual. Both are available at the project web site for free download.

If you have any questions not answered in this manual or the project web site, or if you would like to report a bug, please contact:

[Daniel.Boettger@medizin.uni-leipzig.de](mailto:Daniel.Boettger@medizin.uni-leipzig.de).

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<http://psychiatrie.uniklinikum-leipzig.de>

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## 10 APPENDICES

**Appendix A:** Scoring rules for sleep grapho-elements in VIGALL analyses

**Appendix B:** How to create the VIGALL standard electrode configuration

**Appendix C:** Automatic R-wave Detection

**Appendix D:** Suggested Markers

**Appendix E:** VIGALL 2.0 vs. VIGALL 2.1

## 10.1 Appendix A: Scoring rules for sleep associated grapho-elements in VIGALL analyses

### A) Requirements:

Montage: VIGALL standard cap (extended international 10-20 system)	Fp1, Fp2, F3, F4, Fz, F7, F8 FC1, FC2, FC5, FC6 Cz, C3, C4, T7, T8 CP5, CP6 Pz, P3, P4, P7, P8 O1, O2
Reference:	Common Average
Recommended scale	- 1cm on the screen = 50 $\mu$ V in EEG - 10 seconds/screen
Node	Rough Artefact Screening

### B) Definitions

#### K complexes

Definition by the American Academy of Sleep Medicine (AASM) Manual:

*K complex: A well-delineated negative sharp wave immediately followed by a positive component standing out from the background EEG, with total duration  $\geq 0.5$  seconds, usually maximal in amplitude when recorded using frontal derivations in frontal electrode sites. For an arousal to be associated with a K complex, it must commence no more than 1 second after termination of the K complex<sup>27,28</sup>.*

*NOTE: K complexes can be followed by an arousal reaction. In sleep medicine those segments are not regarded as sleep since the test subject is considered to have woken up.*

*The VIGALL analyses, however, take place in a much narrower time-frame (1 sec instead of 30 sec epochs) and therefore the state in which a K complex appears should be classified as stage C. If the K complex is followed by arousal, VIGALL stops C classification at this point and classifies subsequent arousal segments as stage A accordingly.*

*Thus, a C marker can and should be assigned to all K complexes.*

<sup>27</sup> Iber C, Ancoli-Israel S, Chesson A, and Quan SF for the American Academy of Sleep Medicine. The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications, 1st ed.: Westchester, Illinois: American Academy of Sleep Medicine, 2007

<sup>28</sup> Silber MH, Ancoli-Israel S, Bonnet MH, Chokroverty S, Grigg-Damenger MM, Hirshkowitz M, Kapen S, Keenan SA, Kryger MH, Penzel T, Pressman MR, Iber C. The Visual Scoring of Sleep in Adults. Journal of Clinical Sleep Medicine 2007, 3(2): 121-131.



## Sleep Spindles

### Definition by AASM Manual:

*Sleep Spindle: A train of distinct waves with frequency 11-16 Hz (most commonly 12-14 Hz) with a duration  $\geq 0.5$  seconds, usually maximal in amplitude over central electrode sites<sup>29</sup>.*

*NOTE: To determine the frequency, a 1-sec segment around the spindle should be analysed by FFT (mark EEG area → right-click → FFT). In EEGs with fast Alpha centre frequencies (11 - 13 Hz), spindle activity might be misdiagnosed by the alpha-adapted demodulation as Alpha activity. VIGALL then rates these segments as, for example, a stage A3 (which would lead to an end of stage C classification). To prevent that, all segments containing spindle activity must be thoroughly marked in those EEGs.*

*Spindle shape is not always perfectly recognizable in an EEG, since slower frequencies can be interwoven → filtering (high pass 11 Hz – low pass 16 Hz) will make the spindle shape pop out more distinctly (see figure A9).*

### C) VIGALL Rating Algorithm K complexes

1. EEG Trend	
<b>In preceding segments sleep-like EEG with absence of dominant Alpha activity, desynchronisation, increasing Delta/Theta activity, possibly vertex waves, sleep spindles?</b>	
YES → next step Figure A1	NO: Alternative explanation: Ocular artefact Figure A4
2. Morphology:	
<b>Rapid negativity preferably as a Sharp Wave, possibly double-peaked, followed by a slower positivity?</b>	
YES → next step Figure A2	NO: AE: incomplete K complex, Vertex wave? Figure A5
3. Time Criterion?	
<b>Min. length <math>\geq 500</math>ms Max. length <math>&lt; 1600</math> ms from start negativity to end positivity</b>	
YES → next step Figure A2	NO: AE: Vertex wave? Figures A1 and A5
4. Amplitude Criterion?	
<b><math>\geq 100\mu\text{V}</math> Peak to Peak or <math>\geq 2\times</math> EEG background amplitude in preceding 1-5s?</b>	
YES → next step Figure A2	NO: AE: incomplete K complex?

<sup>29</sup> See previous footnotes.

<b>5. Topography?</b>  <b>Maximal negativity over fronto-central areas</b> <b>(phase switch by common average over Vertex, i.e. rapid positivity and slow negativity over parieto-occipital areas)</b>	
YES → next step Figure A2	NO: AE: Delta activity, Paroxysmal Potentials? Figure A6
<b>6. Test for missing association with EOG</b>  <b>Test, if morphology in EOG visible.</b> <b>Criterion: max. amplitude of negativity</b> <b>Fz/F3/F4/F7/F8 &gt; FP1/FP2 &gt; EOG</b>	
YES → <b>K complex</b> → next step Figures A2 and A3	NO: AE: Ocular artefact? Figure A7
<b>7. associated Spindle Activity?</b>	
YES → set C-marker at negative peak of k-complex and also mark the segment containing the spindle with C-marker Figure A3	NO → set C-marker at negative peak of k-complex

*D) VIGALL Rating Algorithm Sleep Spindles*

<b>1. EEG Trend</b>  <b>In preceding segments sleep-like EEG with absence of dominant Alpha activity, desynchronisation, increasing Delta/Theta activity, possibly vertex waves, K complexes?</b>	
YES → next step Figure A1	NO: Sub-vigilant Beta Activity
<b>2. Morphology:</b>  <b>Stands out of background EEG?</b> <b>Spindle shape?</b>	
YES → next step Figure A9	NO: Beta Baseline?
<b>3. Frequency/Topography:</b>  <b>Test by using Fast Fourier Analysis</b> <b>12-15Hz over central electrodes</b> <b>11-13Hz over frontal electrodes</b>  Note: fast Alpha centre frequency must not be misinterpreted as a spindle.	
YES → next step Figure A8	NO: DD: muscle artefact, movement? Figure A7
<b>4. Time Criterion:</b>  <b>Length 500-1500ms</b>	
YES → next step	NO: DD muscle artefact Figure A7
<b>5. Amplitude Criterion:</b>  <b>≥ 10 µV</b>	
YES → <b>Sleep Spindle</b> Set Comment Marker „C“ at around the middle of the spindle.	NO: not fully defined spindle

## E) Examples



Figure A1: typical sleep EEG with vertex waves and beta spindles



Figure A2: In the process of marking a K complex



Figure A3: K-complex with superimposed fast activity



Figure A4: Ocular artefact looking similar to a K complex



Figure A5: Vertex waves



Figure A6: Delta activity



Figure A7: Ocular artefact in standard montage with superimposed muscle activity and technical disturbance in T8

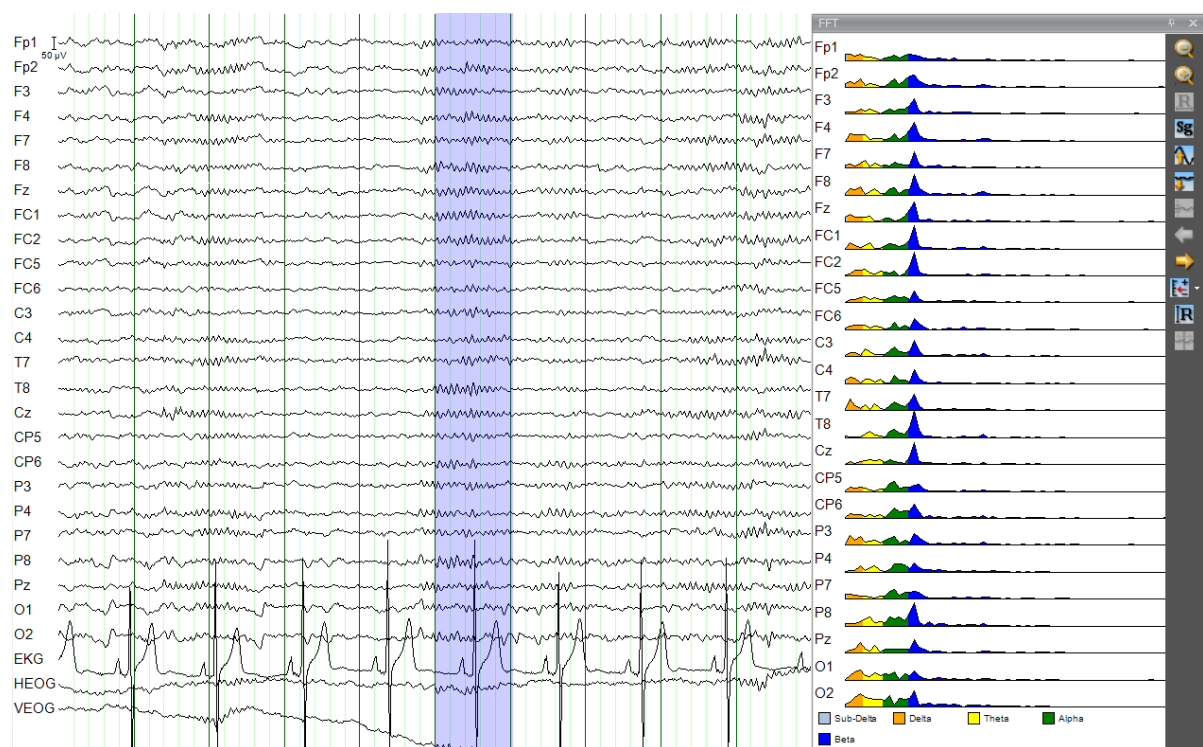


Figure A8: Checking the frequency of a spindle using FFT. An area of approx. 1 sec should be marked to yield a sufficient resolution in FFT (1 Hz steps).



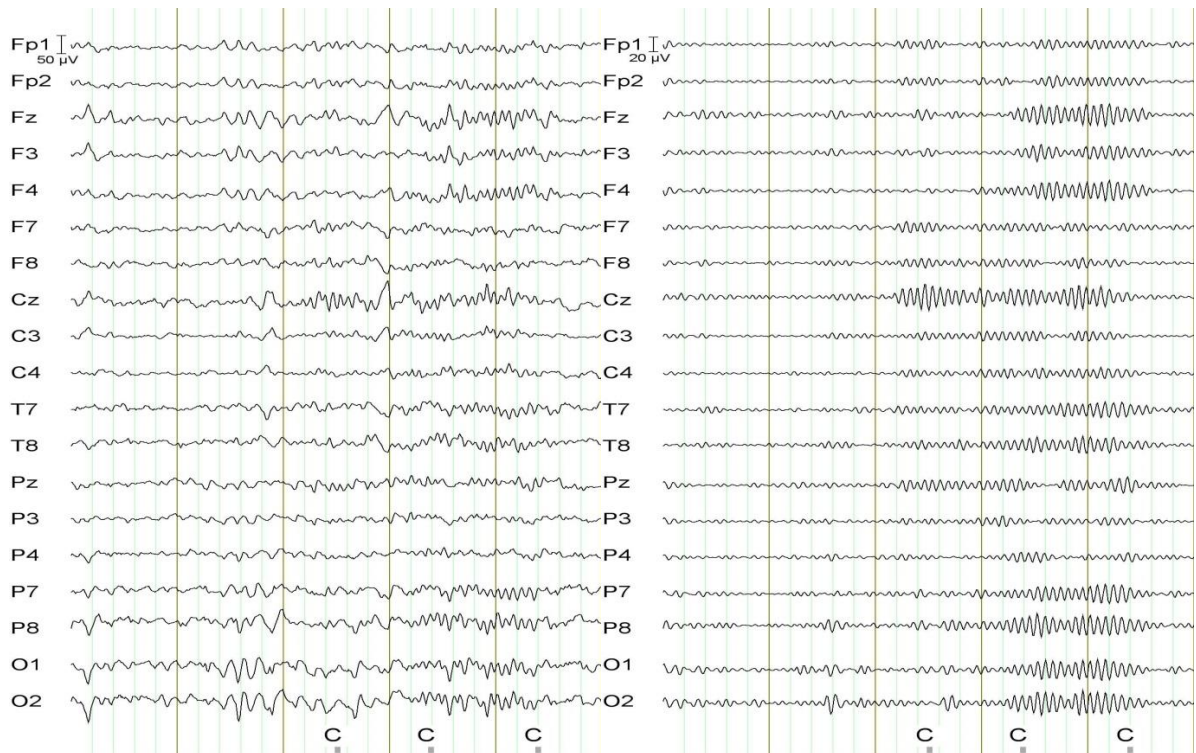


Figure A9: Comparison of an EEG segment in normal filtering (0.5 – 70 Hz + Notch Filter), in which the spindle shape is hardly recognisable (left side) with a filtering at 11 Hz high pass and 16 Hz low pass, in which the typical spindle shape is attenuated.

## 10.2 Appendix B: How to create the VIGALL standard electrode configuration

Vigilance classification using VIGALL should be based upon a defined number of channels: the VIGALL standard cap (see chapter 3). Data sets that have not been recorded using the standard VIGALL configuration should be prepared accordingly, prior to the VIGALL analysis.

The VIGALL standard cap configuration comprises 25 EEG channels of the extended international 10-20 system:

- Fp1, Fp2,
- F3, F4, Fz, F7, F8
- FC1, FC2, FC5, FC6,
- Cz, C3, C4, T7, T8,
- CP5, CP6,
- Pz, P3, P4, P7, P8,
- O1, O2

+ Non-EEG channels (e.g. VEOG, HEOG, ECG)

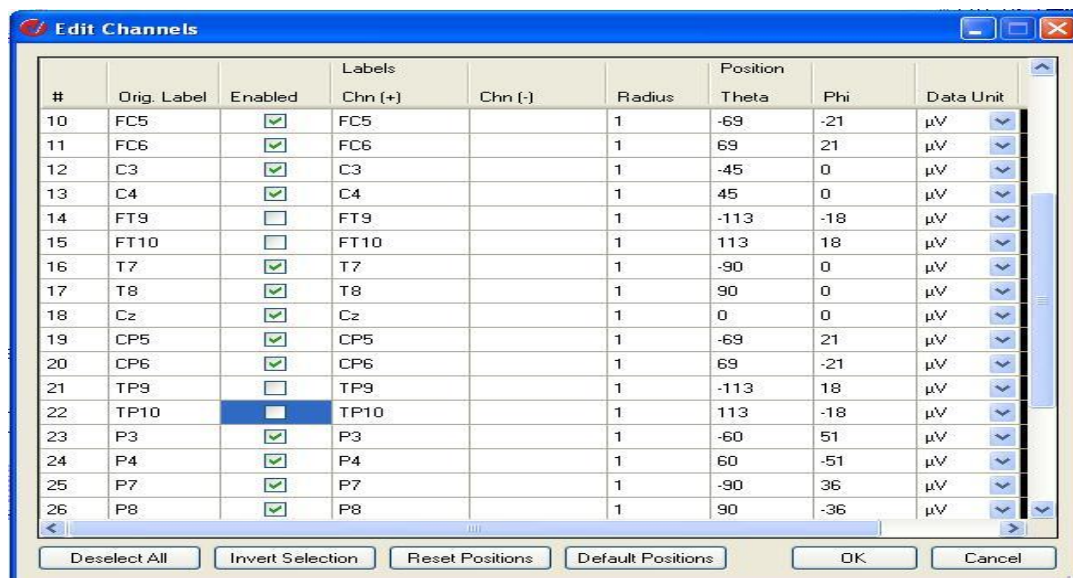
In case of identical EEG positions labelled differently, e.g. T5/P7 (Theta: -90, Phi: 36) or T3/T7 (Theta: -90, Phi: 0) both labels can be kept. However, it is important to check, whether the respective channels have indeed been recorded at the same position.

All EEG channels that do not belong to the VIGALL standard configuration have to be deleted:

→ Transformation → Edit Channels

Uncheck the channels not needed for VIGALL.

Label	Radius	Position	
		Theta	Phi
Fp1	1	-90	-72
Fp2	1	90	72
F3	1	-60	-51
F4	1	60	51
F7	1	-90	-36
F8	1	90	36
Fz	1	45	90
FC1	1	-31	-46
FC2	1	31	46
FC5	1	-69	-21
FC6	1	69	21
C3	1	-45	0
C4	1	45	0
T7	1	-90	0
T8	1	90	0
Cz	1	0	0
CP5	1	-69	21
CP6	1	69	-21
P3	1	-60	51
P4	1	60	-51
P7	1	-90	36
P8	1	90	-36
Pz	1	45	-90
O1	1	-90	72
O2	1	90	-72



**NOTE:** Non-EEG channels do not need to be deleted. Especially all channels representing EOG information (HEOG, VEOG), as well as an ECG channel with marked R waves can be kept available for further analysis.

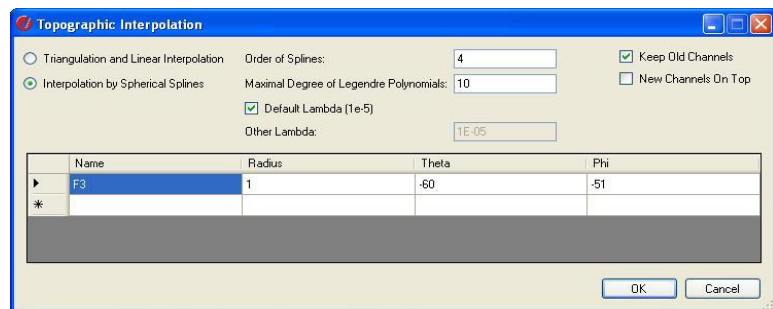


Should there be any channels missing in an individual data set that are required for the VIGALL standard configuration, they need to be generated by interpolation:

→ Transformation → Others → Topographic Interpolation

Check „Interpolation by spherical splines“ and „Keep old Channels“.

Insert the channel under „Name“



*NOTE: Interpolation of missing channels should be performed after the ICA-based correction of artefacts.*

*NOTE: The VIGALL analysis is based upon „Common Average“ reference. If an alternative reference was used for recording of the EEG or if any channels have been interpolated and/or deleted from the workspace in the previous step, the data has to be re-referenced against common average:*

→ Transformation → Channel Pre-processing → New Reference

Step 1: select all EEG channels (select „include implicit reference“!)

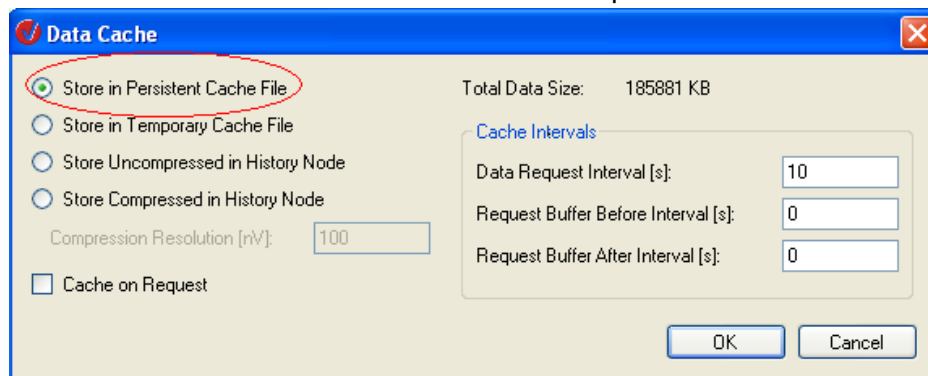
Step 2: select all EEG channels (check „keep remaining channels“)

Step 3: do not input a name for the new reference

Do NOT check „reuse old reference channel“!

Re-referencing and interpolating use up lots of cache, therefore it is recommended to store the data in a persistent cache file afterwards.

→ Transformations → Others → Data Cache → Store in persistent cache file



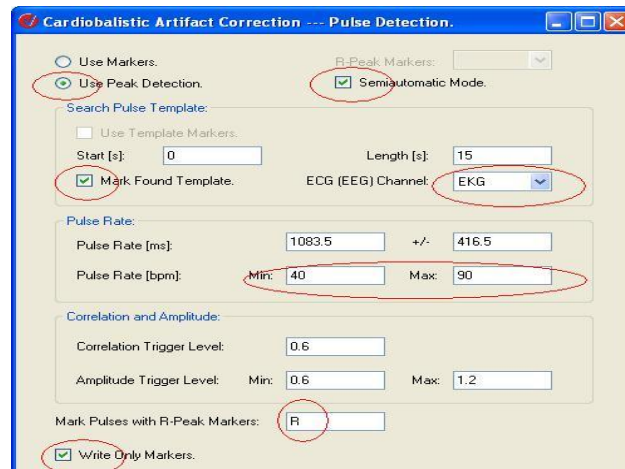
For further details regarding the procedures mentioned above, please also use the Analyzer's software manual provided by Brain Products.

### 10.3 Appendix C: Automatic R-wave detection

Analyzer → Transformations → Special Signal Processing → CB Correction

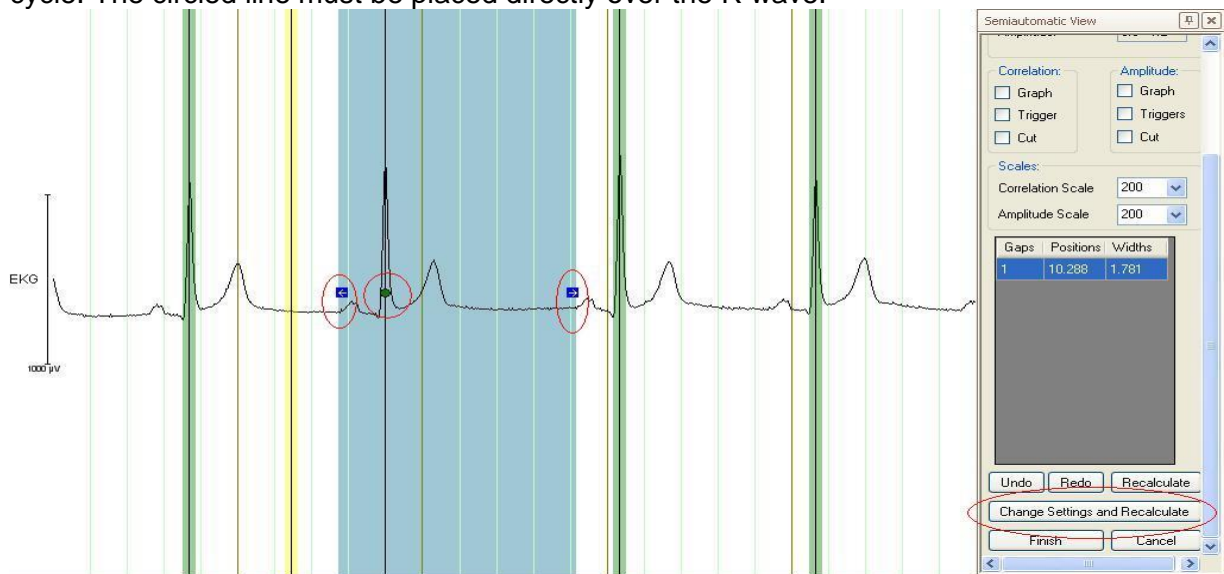
Settings:

- Use peak detection / Semiautomatic mode
- Mark found template
- ECG Channel: select the channels containing ECG information
- Pulse Rate: 40 – 90 bpm
- Correlations: use default
- Mark Pulses with: R
- Write only Markers

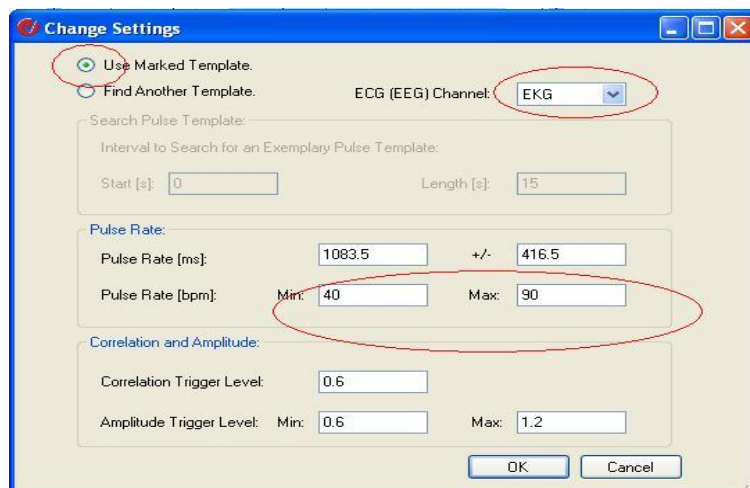


After clicking 'OK' Analyzer suggests a classification. It is recommended to create a montage containing only the ECG channel for the following steps.

Adapt the blue template in position and interval in a way that it covers a complete cardiac cycle. The circled line must be placed directly over the R wave.



→ Change Settings and Recalculate



Pulse Rate can be chosen higher if necessary (e.g. 110 instead of 90)

Analyzer recalculates the heart rate again:

1. Green marker → check position (R wave) readjust if necessary
2. Red markers → suggestions by software: move to R wave (note: if already at right position, move the marker until turning yellow and then place back at the right position)
3. No markers → click some place on the EEG graph until red marker appears, then move to right position

*IMPORTANT: Once confirmed the software considers green and yellow, but ignores red markers. Thus make sure to screen all red markers before hitting the OK button.*

*NOTE: In many cases, it can be helpful to filter the ECG channel prior to the R-wave detection. Using 5 Hz high pass and a 30 Hz low pass filters will improve the automatic detection of R-waves in many cases.*

*NOTE: For a more detailed description see Analyzer Manual under topic: Cardiobalistic Artifact Correction.*

#### 10.4 Appendix D: Suggested Markers during EEG recordings

Marker	Description	Specification
EC	Eye closure	Indicates the instruction to close eyes (e.g. during Berger's manoeuvre)
EO	Eye opening	indicate the instruction to open eyes (e.g. during Berger's manoeuvre)
MT	Mental Task	indicate the beginning of the mental activation task (mark after end of the instruction)
MR	Mental Task Result	Indicates the request for the response to the mental activation task (mark before asking the patient)
RS	Start Resting EEG	indicates the beginning of the resting EEG, should correspond to eye closure after the resting instruction
RE	End Resting EEG	marks the moment, when the patient is reawakened after the resting EEG or when resting EEG attempt is aborted
EO / EC	Eyes open / Eyes closed	Marks beginning and end of episodes with open eyes (which must be excluded from further analysis!)
Noise	noise	Indicates onset of unexpected noise outside of EEG cabin
NE	end of noise	Indicates offset of unexpected noise outside of EEG cabin
tense	tension	Indicates visible tension of the patient
move	movement	Indicates movements of the patient
gulp	gulping	Indicates gulping of the patient
speak	speaking	Indicates speaking of the patient
AI	instructions	Indicates further instructions to the patient by study personal (e.g. to calm excited patients)
IE	end of instructions	Indicates the end of additional instructions given to the patient by the study personal

## **10.5 Appendix E: Comparison of VIGALL 2.0 and 2.1 with visual scoring**

VIGALL version 2.1 extends the previous VIGALL version 2.0 by allowing users to modify the lower border of the delta/theta band used in the classification of vigilance. This option was provided due to complaints about implausible classification of B2/3-stages in the presence of visible alpha activity. This was in most cases due to segments not fulfilling the relative A-stage criterion (i.e. alpha power not exceeding delta/theta power).

To demonstrate the effect of the new classification recommendations, a comparison with a visual classification of EEG-vigilance stages was performed in 14 EEG recordings, which were chosen due to the presence of plausible and implausible A- and B2/3-stages. As usual for EEG-vigilance classifications, each 15min resting EEG was separated in 900 segments of 1sec duration and each segment was scored as belonging to one of the following stages:

- Stage 0: desynchronized EEG activity without slow horizontal eye movements
- Stage A: EEG dominated by alpha activity (no subdivision into substages A1, A2 or A3 was done)
- Stage B1: desynchronized EEG activity with slow horizontal eye movements
- Stage B2/3: EEG dominated by delta/theta activity
- Stage C: C-stages were classified according to usual VIGALL approach<sup>30</sup>

Each EEG was scored by two experienced researchers independently from one another, whose results were then compared by calculating the amount of agreement as well as a prevalence- and bias-adjusted Kappa score<sup>31</sup>. The Interrater-Agreement was 76.9%, with a PABAK-OS score of 0.71, pointing to the difficulty of scoring EEG vigilance stages manually. Therefore, each segment not classified in agreement between the two raters was revised by a third rater who determined the final classification. The resulting classification (referred to as “visual classification”) was then used for comparison with VIGALL results.

After the visual classification each EEG was classified using VIGALL versions 2.0 and 2.1 as well as version 2.1 with additional check for 0/B1-plausibility (see section 7.2). For comparison with the visual classification classifications of substages A1, A2 and A3 were combined to A-stages.

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<sup>30</sup> a segment containing sleep spindles or a k-complex is classified as belonging to stage C as well as the following 30 segments if none of the segments would be classified as A-stage (in this case, classification of stage C is stopped)

<sup>31</sup> To calculate PABAK-OS scores we used the calculator at: [www.singlecaseresearch.org/calculators/pabak-os](http://www.singlecaseresearch.org/calculators/pabak-os)

When VIGALL2.0 was compared to the visual ratings, the amount of agreement was expectedly low (64.9%), with a PABAK-OS score of only 0.56. However, VIGALL2.1 performed considerably better (71.7% agreement / PABAK-OS score = 0.65) with an additional slight increase after 0/B1-plausibility check (73.0% agreement / PABAK-OS = 0.66).

Table 1: Comparison of visual classification with VIGALL version 2.0 vs. 2.1

<b>Comparison</b>	<b>Agreement</b>	<b>PABAK-OS</b>
visual vs. VIGALL2.0	64.86 %	0,5608
visual vs. VIGALL2.1	71.65 %	0,6454
visual vs. VIGALL2.1 (0/B1)	72.96 %	0,6612

The 14 EEGs used in this comparison do not represent the vast amount of EEG recordings but had in most cases EEGs resulted in unsatisfactory VIGALL2.0-results due to the above mentioned over-classification of B2/3 stages. Thus, the visual classification constituted a higher complexity than most EEGs would do. Table 2 shows the results when those EEGs with a PABAK-OS-Score <0.70, i.e. those with too much uncertainty for visual classification, were excluded. Again, VIGALL2.1 (80.6% agreement / PABAK-OS = 0.76) performs better than VIGALL2.0 (76.1% agreement / PABAK-OS = 0.70), especially if results are revised for 0/B1-plausibility (81.4% agreement / PABAK-OS = 0.77).

Table 2: Comparison of visual classification with VIGALL version 2.0 vs. 2.1 in EEGs with sufficient interrater-reliability (PABAK-OS  $\geq$  0.7) during the visual classification.

<b>Comparison</b>	<b>Agreement</b>	<b>PABAK-OS</b>
visual vs. VIGALL2.0	76.09 %	0,7013
visual vs. VIGALL2.1	80.58 %	0,7573
visual vs. VIGALL2.1 (0/B1)	81.43 %	0,7670

### *Conclusion:*

In EEG files with a higher amount of background delta/theta activity not related to a decline in vigilance but e.g. to non-removable artefacts or maturation effects, VIGALL2.1 will result in a more valid classification. However, in most cases results obtained with VIGALL2.0 will be highly comparable to VIGALL2.1.

## 10.6 Appendix F: Comparisons of VIGALL 2.0 and VIGALL 2.1 – reanalysis of previously published data

*EEG-vigilance between 30 unmedicated depressed patients and 30 non-depressed controls.*

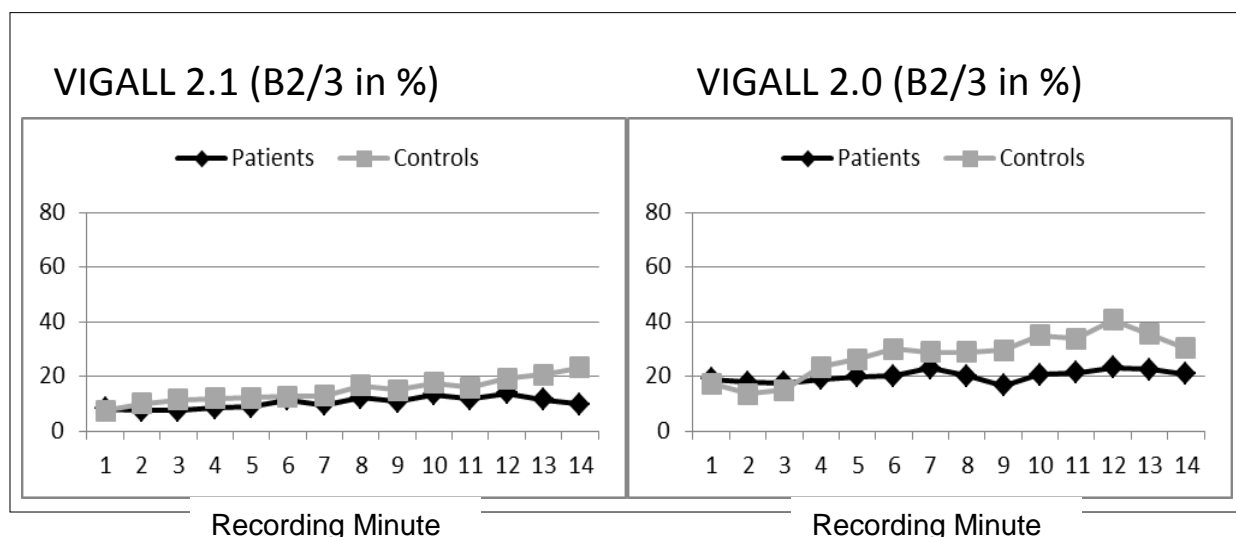
Original results using a pre-release version of VIGALL were published in:

- Hegerl, U., Wilk, K., Olbrich, S., Schoenknecht, P., Sander, C. Hyperstable regulation of vigilance in patients with major depressive disorder. *World J. Biol. Psychiatry.* 2012; 13: 436-446. doi: 10.3109/15622975.2011.579164.

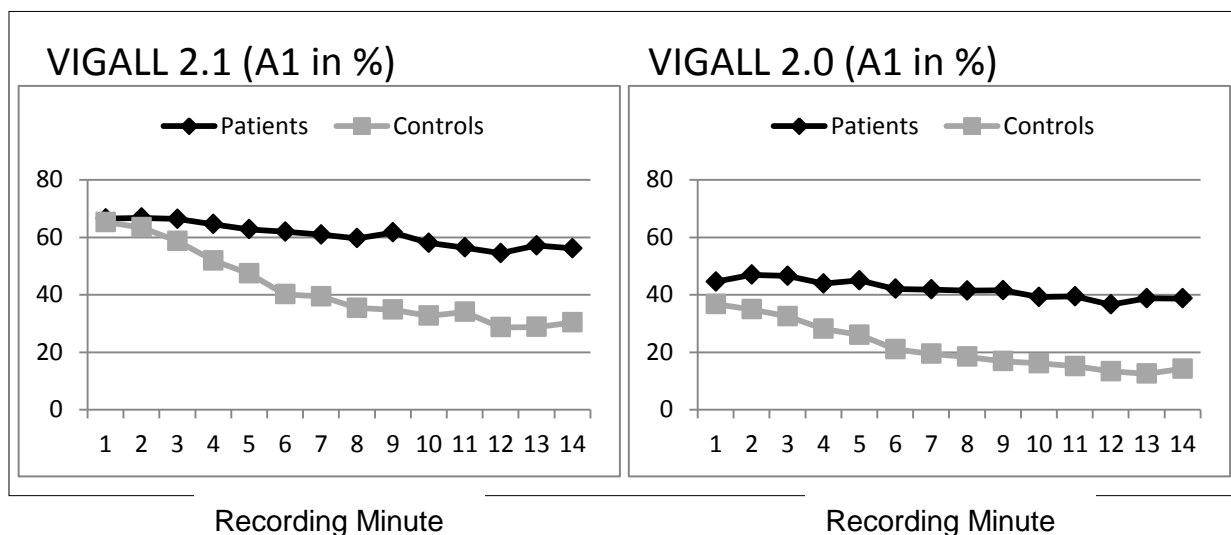
The original analyses were performed with the VIGALL 1.0. In order to compare the two recent versions of the VIGALL, all analyses between the groups were re-run with the VIGALL 2.0 and VIGALL 2.1.

Conclusions:

- 1) The lower border for the VIGALL detection range was adapted from 2Hz to 3Hz in the VIGALL 2.1. Following that, the occurrence of sub-stage B2/3 was reduced throughout the recording compared to VIGALL 2.0 (see figure below). Importantly, the proportion of sub-stage B2/3 was markedly reduced from VIGALL 2.0 to VIGALL 2.1, especially within the first minutes when such low vigilance stages are less expected. This more properly reflects the physiologically later occurrence of sub-stage B2/3:



- 2) The modifications in the settings for the relative power threshold for EEG-vigilance sub-stage A1 in the VIGALL 2.1 resulted in more prominent differences in the regulation of A1 between the two groups (see fig. below and time x group interaction in ANOVA analysis). This is in good accordance with the concept of an upregulation of brain arousal within major depression: Depressed patients and healthy controls both show an occurrence of ~65 % A1 within the first minutes. Whereas the healthy controls show an adaptive decline of sub-stage A1 over time down to 30%, the proportion of sub-stage A1 remains stable within the depressed subjects with 56% in the last minute. Further, the more prominent proportion of sub-stage A1 (compared to a higher proportion of sub-stages A2 and A3 in VIGALL 2.0) at the beginning of the recording period better reflects the preponderance of occipital alpha physiologically observed during rest:

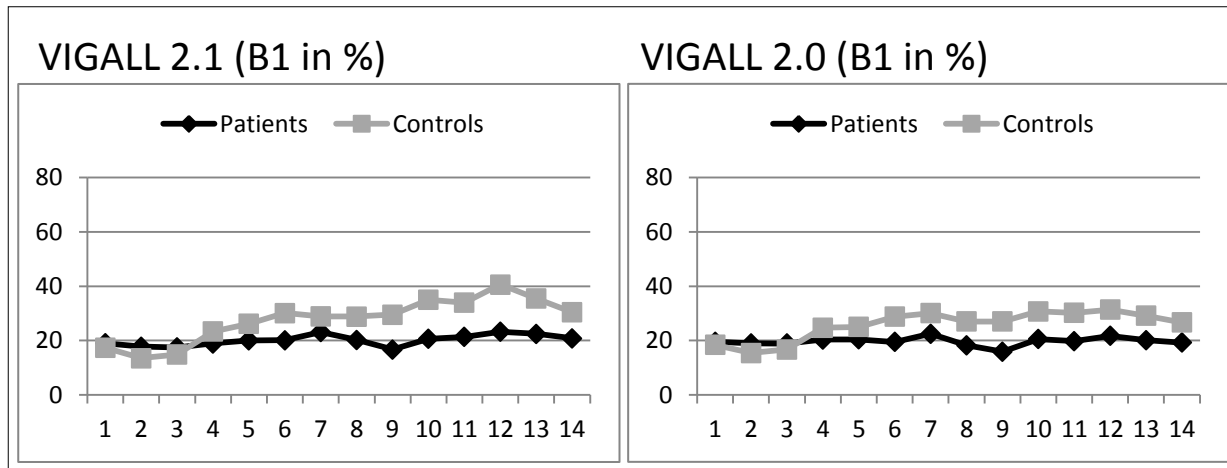


Repeated Measures ANOVA for percentage substage A1 (Minutes 1 to 14)

	VIGALL 2.1			VIGALL 2.0		
	Test statistics	p values	partial eta <sup>2</sup>	Test statistics	p values	partial eta <sup>2</sup>
<b>Time</b>	F= 22.054	<0.001	0.275	F= 15.313	<0.001	0.209
<b>Group</b>	F = 5.236	0.026	0.083	F = 7.481	0.008	0.114
<b>Time x Group</b>	F = 6.458	<0.00001	0.10	F = 3.696	0.005	0.06

- 3) Following the plausibility check on the separation of stage 0 and sub-stage B1 (see manual section 7.2), VIGALL 2.1 showed a lower proportion of stage 0 and a higher proportion of sub-stage B1. The latter was found significant for a ‘time x group’ interaction for sub-stage B1 with the VIGALL 2.1 only:

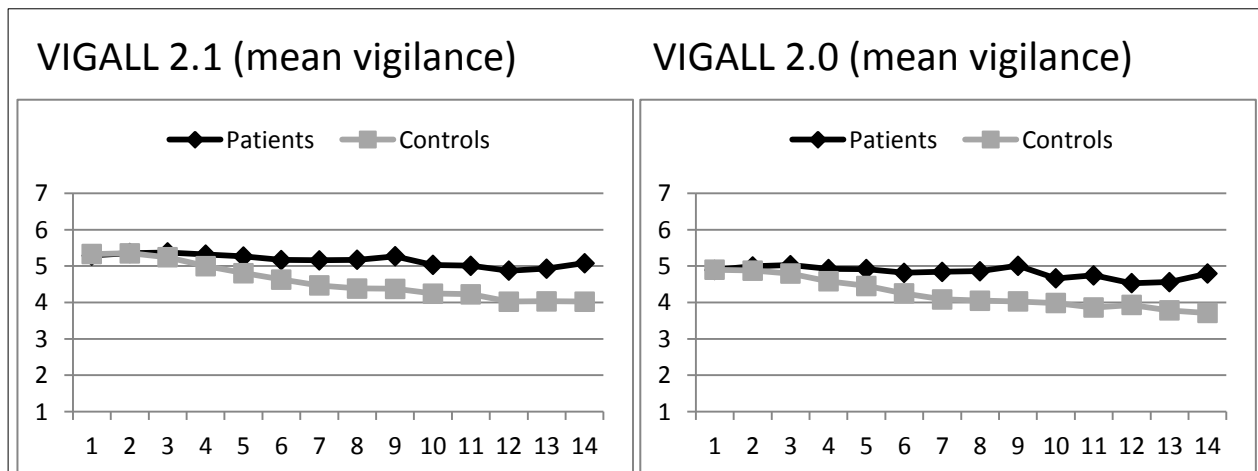




Repeated Measures ANOVA for percentage substage B1 (Minutes 1 to 14)

	VIGALL 2.1			VIGALL 2.0		
	Test statistics	p values	partial eta <sup>2</sup>	Test statistics	p values	partial eta <sup>2</sup>
<b>Time</b>	F= 5.401	<0.001	0.085	F= 2.268	0.006	0.038
<b>Group</b>	F = 1.745	0.192	0.029	F = 1.275	0.263	0.022
<b>Time x Group</b>	F = 2.623	0.028	0.043	F = 1.559	0.180	0.026

- 4) Concerning the mean vigilance, VIGALL 2.1 analyses showed better results than the VIGALL 2.0 analyses on 'group' effect (depressed vs. controls) as well as 'time x group' interactions concerning p values and partial eta<sup>2</sup> values:



Repeated Measures ANOVA for Mean Vigilance Value (Minutes 1 to 14)

	VIGALL 2.1			VIGALL 2.0		
	Test statistics	p values	partial eta <sup>2</sup>	Test statistics	p values	partial eta <sup>2</sup>
<b>Time</b>	F= 13.785	<0.001	0.192	F= 9.998	<0.001	0.147
<b>Group</b>	F = 5.776	0.019	0.091	F = 5.277	0.025	0.083
<b>Time x Group</b>	F = 4.341	0.001	0.070	F = 3.779	0.005	0.061

*Analyses of EEG-vigilance between 60 un-medicated depressed patients and 129 non-depressed controls.*

Original results on differences between groups applying VIGALL 2.0 were published in:

- Schmidt FM, Pschiesl A, Sander C, Kirkby KC, Thormann J, Minkwitz J, Chittka T, Weschenfelder J, Holdt LM, Teupser D, Hegerl U, Himmerich H. Impact of Serum Cytokine Levels on EEG-Measured Arousal Regulation in Patients with Major Depressive Disorder and Healthy Controls. *Neuropsychobiology*. 2016; 73: 1-9. doi: 10.1159/000441190

Compared with the VIGALL 2.0 analyses, the VIGALL 2.1 showed improved F and p-values for the differences in the overall occurrence of stage A and stage B between the two groups. Simultaneously, partial eta<sup>2</sup> for effect sizes improved for both stages (see upper part of the table below). The proportion of sub-stage A1 increased, whereas the proportion of sub-stage B2/3 decreased. Analyses on the mean vigilance (see lower part of the table) showed a more significant 'group' effect (depressed vs. controls) as well as 'time x group' interactions with VIGALL 2.1 than with VIGALL 2.0, both with higher partial eta<sup>2</sup> values.

Revised analyses applying VIGALL 2.1. Improvements in separating depressed vs. non-depressed subjects compared to VIGALL 2.0 are marked in bold (analyses and table adapted from Schmidt et al., 2016):

	Depressed value (± SD)	Non-depressed value (± SD)	Test statistics	p values	partial eta <sup>2</sup>
Stage 0 [%]	8.78 (± 13.23)	8.63 (± 12.14)	F=0.006	0.939	0.000
Stage A [%]	59.31 (± 29.06)	47.55 (± 28.80)	<b>F=6.771</b>	<b>0.010</b>	0.035
Substage A1 [%]	46.50 (± 27.90)	38.60 (± 26.39)	F=3.530	0.062	0.019
Substage A2 [%]	11.79 (± 16.85)	7.23 (± 12.17)	<b>F=4.435</b>	<b>0.037</b>	0.023
Substage A3 [%]	1.01 (± 1.61)	1.71 (± 3.03)	F=2.811	0.095	0.015
Stage B [%]	31.48 (± 27.14)	32.02 (± 24.27)	<b>F=6.865</b>	<b>0.010</b>	0.036
Substage B1 [%]	24.79 (± 23.43)	32.02 (± 27.14)	F=3.706	0.056	0.020
Substage B2/3 [%]	<b>6.70 (± 9.59)</b>	<b>10.69 (± 14.07)</b>	<b>F=3.975</b>	<b>0.048</b>	0.021
Stage C [%]	0.42 (± 1.47)	1.10 (± 3.77)	F=1.800	0.181	0.010

#### Repeated measures ANOVA

Time			<b>F= 56.212</b>	<b>&lt;0.001</b>	0.232
Group			<b>F = 5.419</b>	<b>0.021</b>	0.028
Time x Group			<b>F = 4.164</b>	<b>0.011</b>	0.022
Post-hoc minutes 1-3	5.34 (± 0.87)	5.27 (± 0.87)	F = 0.247	0.620	0.001
Post-hoc minutes 4-6	5.13 (± 0.89)	4.88 (± 1.09)	F = 2.352	0.127	0.012
Post-hoc minutes 7-9	4.98 (± 0.93)	4.56 (± 1.15)	<b>F = 6.243</b>	<b>0.013</b>	0.032
Post-hoc minutes 10-12	4.85 (± 1.01)	4.35 (± 1.08)	<b>F = 8.979</b>	<b>0.003</b>	0.046
Post-hoc minutes 13-15	4.74 (± 1.12)	4.73 (± 1.01)	<b>F = 5.955</b>	<b>0.016</b>	0.031