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Psychophysiological Effects of a Combination of Sideritis and Bacopa Extract (memoLoges®) in 32 Subjects Suffering from Mild Cognitive Impairment. A Double-Blind, Randomized, Placebo-Controlled, 2-Armed Study with Parallel Design

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Abstract

Mild cognitive impairment (MCI) can be regarded as a non-demented transitional stage during the development of Alzheimer's disease. Early recognition of this stage might increase the chance of prevention by early treatment. Within a pilot study, two plant-derived preparations and mixtures thereof were tested successfully in subjects suffering from MCI. A combination of *Sideritis scardica* and *Bacopa monnieri* extract (memoLoges®) was chosen now for a repetitive dosing during 4 weeks. Thirty-two subjects aged 50 to 80 years and suffering from MCI (having a DemTect questionnaire score between 8 and 13) were recruited for intake of 2 capsules of the preparation per day. Quantitative EEG recording during relaxation and concomitant performance of three 5 minutes lasting psychometric tests (d2-concentration test, arithmetic calculation test and memory test) was achieved at the first day and one day after the last repetitive intake. Seventeen channels of EEG and one channel EOG (for artefact rejection) were recorded. After frequency analysis (FFT) current source density was calculated as reported earlier. One, two and three hours after intake of the herbal extract or placebo the whole procedure was repeated. Brain imaging was achieved by conversion of numerical values of spectral EEG power into spectral colors and additive color mixture according to RGB as used in TV settings. Intake of memoLoges® induced a trend of improvement of performance in psychometric testing (all three tests). During relaxation quantitative as-

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sessment of EEG data revealed attenuation of delta and theta spectral power in frontal brain as likewise reported in the presence of the Alzheimer drug rivastigmine, bringing the spectrum back to "normality". During mental work memoLoges® induced statistically significant increases of beta power. Since MCI subjects produce less beta power in comparison to healthy subjects, this increase must likewise be seen as a positive effect pointing to a healthier spectrum.

Keywords

***Sideritis scardica*, *Bacopa monnieri*, EEG Source Density, Spectral Power, Mild Cognitive Impairment, memoLoges®, Psychophysiology, CATEEM®**

1. Introduction

"Mild Cognitive Impairment (MCI) describes the cognitive state of non-demented individuals who report memory deficits, which should preferably be corroborated by an informant, and measurable by objective testing" [1]. Objective testing has been performed on two levels: performance of psychometric tests and concomitant recording of quantitative EEG [2]. In this earlier publication, the d2-concentration test had turned out to be more sensitive than an arithmetic calculation or memory test. Performance in this test correlated very well with the so-called DemTect score, which was used as inclusion criterion. Others have also recorded quantitative EEG during extensive neuropsychological testing in order to objectify brain dysfunction [3]. Successful identification of an early EEG-based biomarker of mild cognitive impairment has also been claimed during the performance of an attention task [4]. It has been stated in the literature, that the use of biomarker data can supplement clinical characterization and identification of MCI and dementia pathologies [5]. There is converging evidence, that MCI is an early stage of Alzheimer's disease preceding it for up to 10 years [6]. Objective measurement of such functional disturbances might therefore open the possibility of an early treatment in order to prevent the progression into Alzheimer's disease, and it is also the precondition for proofing the efficacy of any treatment.

The present investigation aimed at such an option of early treatment. After successful characterization of a new combination of extract from *Sideritis scardica* in combination with *Bacopa extract* within a single dose clinical trial, there was a need for repetitive dosing during longer time. In this case, we decided to follow a daily intake for 4 weeks. Main inclusion criterion was a score in the DemTect questionnaire between 8 and 13, which has been validated to represent mild cognitive impairment [7].

2. Methods

2.1. Subjects

Twenty-eight male and four female subjects (average age: 58.63 ± 5.79 y) were recruited by advertisements in newspapers. Thirty-two subjects-selected by the questionnaire "DemTect" [8] having a score between 8 and 13—were included into the study. Within a parallel design they performed a daily intake of the herbal extract combination or placebo in a double-blind and randomized manner. Besides placebo they obtained two capsules containing 120 mg *Bacopa monnieri extract* + 380 mg *Sideritis scardica* extract plus 20 mg of Vitamin B6, 500 µg of Vitamin B12, 400 µg folic acid, 18 mg of Vitamin B5 and 1,5 mg Zinc as active ingredients per day for 4 weeks. Placebo capsules did not contain active ingredients, mainly cellulose (provided by Dr. Loges GmbH, Winsen, Germany). The following inclusion criteria were followed: male or female subjects aged 50 to 80 years suffering from mild cognitive impairment, giving informed consent, negative ethanol test. Exclusion criteria consisted in:

- Suffering from acute or chronic disease.
- Intake of centrally acting medication.
- Intolerability against herbal preparations.
- Intake of unusual high amounts of coffee or nicotine.
- Participation at another clinical study within the last 30 days.
- Positive alcohol testing.
- Intake of green tea, St. John's wort, red rooibos tea, ginseng extract or ginkgo extract.

- DemTect questionnaire score 14 or higher.
- Revocation of informed consent.

The time line of experimental days is given in **Figure 1**.

2.2. EEG Recording

Basically, the method of quantitative EEG recording in combination with performance of psychometric challenges was followed as published [9]. EEG equipment was from MEWICON CATEEM-Tec. GmbH, Schwarzenberg am Böhmerwald, Austria. In short, three mental tests were performed concomitantly with the EEG recording for 5 minutes: a d2-concentration test (d2-test), an arithmetic calculation test (CPT) and a memory-test (ME). Seventeen channels of EEG and one channel EOG (for artifact rejection) were recorded. After frequency analysis (FFT) current source density was calculated as reported earlier [10] [11]. Baseline values (μV^2) before the administration were set to 100% for further data processing. One, two and three hours after intake of the herbal extract or placebo the whole procedure was repeated. Results are given in % of this baseline values for each measurement. Brain Imaging was achieved by conversion of numerical values of spectral EEG power into spectral colors and additive color mixture according to RGB as used in TV settings and represent a true result of the measurement [12]. The maps are constructed by nonlinear LaGrange interpolation and mathematically correspond to a 64 channel EEG.

2.3. Statistics

Due to the parallel design of the study the non-parametric Wilcoxon test was applied to separate the effects of memoLoges® from placebo. In order to differentiate results with respect to effectiveness during relaxation or performance of psychometric tests, data using all 102 parameters (data from 17 electrode positions times 6 frequency ranges) were fed into linear discriminant analysis according to Fischer. Results from the first three discriminant functions were depicted in space (x, y and z coordinates). Results from the 4th to 6th discriminant functions were transformed into color according to the RGB mode (like in TV).

3. Results

3.1. Results of Psychometric Performance

As described under methods three psychometric tests were performed during the EEG recordings before and after intake of the 2 capsules. Performance was individually very heterogeneous. Results obtained during performance of the d2-concentration test from single individuals 3 hours after intake are depicted in **Figure 2**. Clear improvements within the placebo group were reached by 8 subjects, whereas in the active group 10 subjects performed better after 4 weeks intake. During performance of the arithmetic calculation test 7 subjects in each group showed improvements, however—on average—the active group showed higher improvements. Data from single subjects are documented in **Figure 3**. A similar picture arose during performance of the memory test,

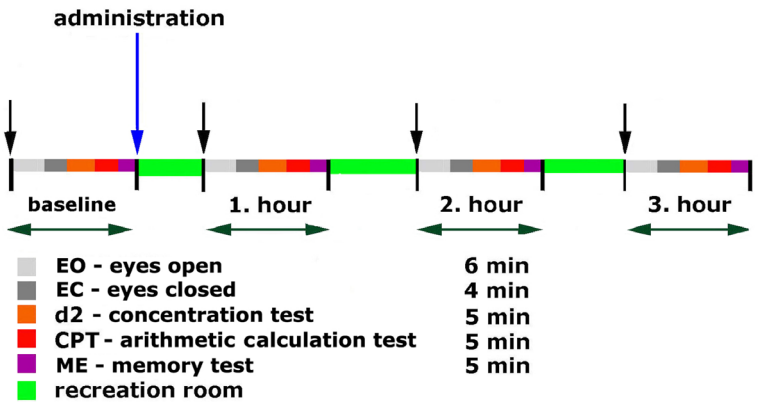

Figure 1. Time line of consecutive actions on the two experimental days at the beginning and next day after 4 weeks of daily intake of trial preparations.

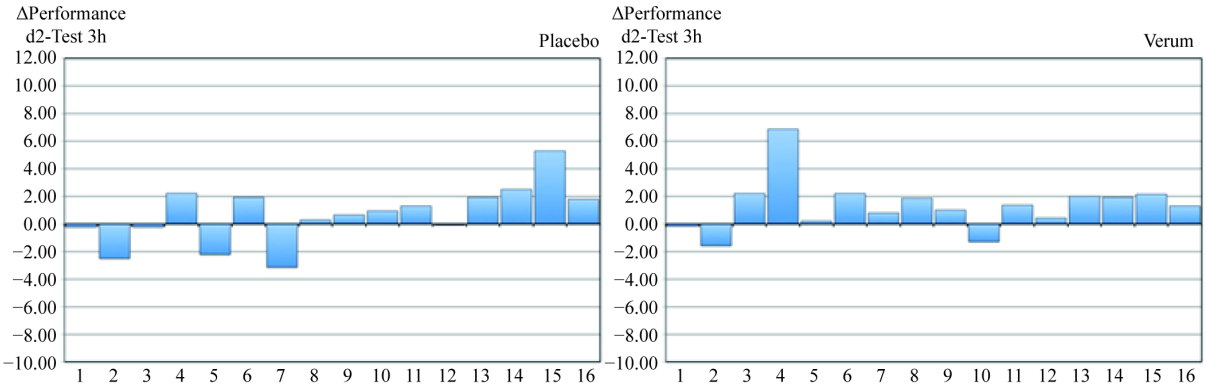

Figure 2. Differences in psychometric performance between day A and B during the d2-concentration test in single subjects after intake of placebo (left side) or memoLoges® (right side).

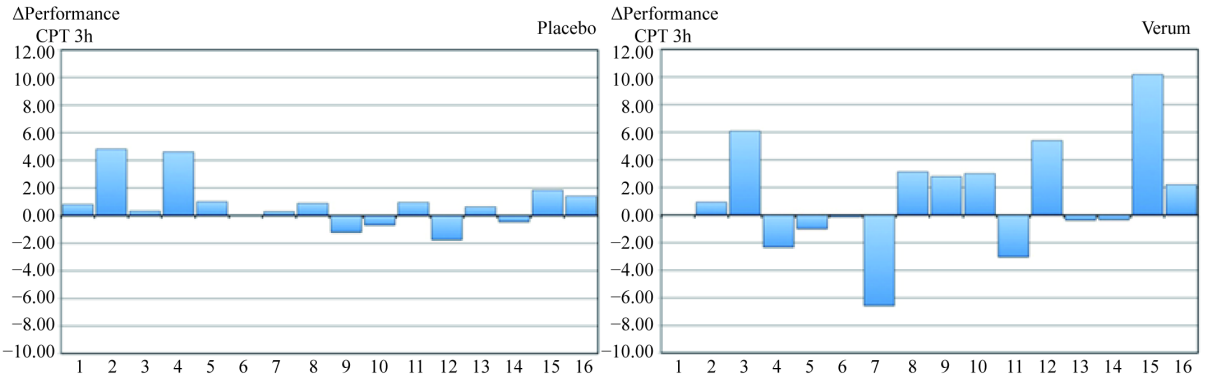

Figure 3. Differences in psychometric performance between day A and B during the arithmetic calculation test in single subjects after intake of placebo (left side) or memoLoges® (right side).

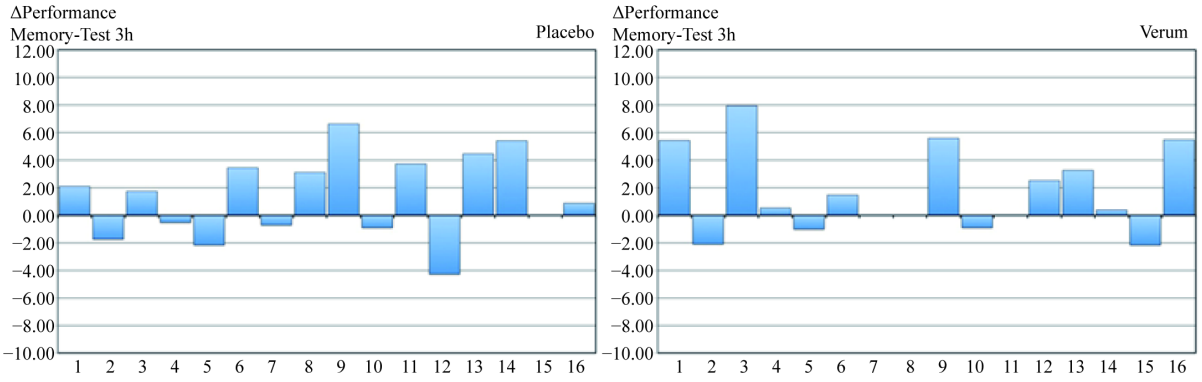

Figure 4. Differences in psychometric performance between day A and B during the memory test in single subjects after intake of placebo (left side) or memoLoges® (right side).

where 8 subjects in the placebo group showed improved performance, 7 subjects in the active group, however with stronger individual performance within these responders (**Figure 4**). Average performances are given in **Table 1**. However, there was no statistically significant difference between the two groups.

3.2. Quantitative EEG Data

3.2.1. Spectral Frequency Power during Relaxation (Eyes Open)

Absolute spectral baseline power was comparable in both groups with respect to slow and middle frequencies at

Table 1. Comparison of psychometric performance between day A (beginning of intake) and day B (next day after 4 weeks intake). Data are given as difference in mean score values (marked fat): Average values (AV) and standard deviation (SD) as well as standard error of the mean (SEM). Statistically no significant differences between placebo and memoLoges® were detected. D2 = d2 concentration test; CPT = arithmetic calculation test; ME = memory test.

	d2-Performance 3h Difference day B-A	
	Placebo	Verum
AV	**0.65**	**1.33**
SD	2.11	1.89
SEM	1.58	1.24
	CPT-Performance 3h Difference day B-A	
	Placebo	Verum
AV	**0.84**	**1.25**
SD	1.78	3.96
SEM	1.20	3.00
	ME-Performance 3h Difference day B-A	
	Placebo	Verum
AV	**1.32**	**1.65**
SD	3.01	3.06
SEM	2.50	2.54

day A. However, there were some differences with respect to beta power. Alpha2, Beta1 and Beta2 power were clearly higher in the placebo group after 4 weeks ($p < 0.05$ for all three frequencies, details in **Table 2**).

On day B after 4 weeks of daily intake of the preparations the differences between the placebo-group were still present with respect to the fast frequencies. Data are documented in **Table 3**. These values are set to 100% and serve for determination of the change of spectral power in the presence of either placebo or extracts. Changes of spectral power will be documented in % of this baseline value.

3.2.2. Documentation of Neurophysiological Testing

The surface of the brain is anatomically divided into several regions, which are involved during performance of different tasks. With regard to consciousness mainly the frontal brain seems to be involved, represented by electrode positions $F_{3,4,7,8}$. Electric circuits dealing with memory processes are located within the temporal lobe represented by electrode positions $T_{3,4,5,6}$. Associative processes are found more in central and even more in parietal regions represented by electrode positions $C_{3,4}$ and $P_{3,4}$. According to this neuroanatomical features performance of different psychometric tests leads to changes of the frequency pattern in various parts of the brain, which can be defined as regions of interest (ROI). In order to document the effectiveness of the preparations spectral power during baseline recording is set to 100% and changes in the presence of preparations are calculated as percent thereof. Data were recorded during relaxation (with eyes open) and three task related conditions (under mental load).

Comparison between placebo and verum is performed for each electrode position separately and documented as a bargraph. In addition, data from both groups are also depicted as source density color maps containing non-linear interpolated data and thus corresponding mathematically to a 64 channel EEG. Since color coding represents a true result and not a so-called “false” color coding, resulting maps can be interpreted more clearly since they represent linear changes and not a “staircase”.

3.3. Effectiveness of Memologes® during Relaxation (Eyes Open) on Day A and B

The first condition for recording of the EEG consisted in a state of relaxation with open eyes before and after intake of 2 capsules of placebo or verum. Results are calculated as spectral power in % of the baseline values

Table 2. Documentation of absolute spectral power values as median in μV^2 on day A. Starting values of absolute EEG spectral power in relaxed condition with eyes open. Data are given in μV^2 for each electrode position and each frequency range. Verum: 120 mg of Bacopa extract plus 380 mg of Sideritis extract. E = electrode position, Med = median over all electrode positions.

Absolute Spectral Power during relaxation (eyes open) 0 h before acute intake at day A												
	Delta		Theta		Alpha1		Alpha2		Beta1		Beta2	
Subjects	**n = 16**	**n = 16**	**n = 16**	**n = 16**	**n = 16**	**n = 16**	**n = 16**	**n = 16**	**n = 16**	**n = 16**	**n = 16**	**n = 16**
Electrode	**Placebo**	**Verum**	**Placebo**	**Verum**	**Placebo**	**Verum**	**Placebo**	**Verum**	**Placebo**	**Verum**	**Placebo**	**Verum**
Cz	2.17	1.64	0.55	0.42	0.88	0.38	0.63	0.36	0.76	0.42	1.03	0.57
Fz	2.91	2.47	0.84	0.67	1.00	0.66	0.83	0.41	0.85	0.46	1.12	0.65
F3	2.71	2.59	0.82	0.69	0.87	0.69	0.79	0.65	1.20	0.97	2.67	1.63
C3	1.89	1.35	0.54	0.40	0.78	0.52	1.03	0.74	1.58	1.28	2.07	1.38
P3	1.55	0.90	0.38	0.25	0.65	0.32	0.60	0.37	0.76	0.60	0.56	0.46
Pz	1.75	1.44	0.44	0.36	0.78	0.56	0.64	0.50	0.57	0.62	0.50	0.46
P4	1.07	1.19	0.29	0.33	0.62	0.47	0.58	0.42	0.54	0.47	0.41	0.35
C4	1.84	1.49	0.42	0.39	0.96	0.43	1.14	0.65	1.60	0.74	1.38	0.83
F4	2.59	2.26	0.74	0.67	0.77	0.93	0.71	0.64	1.33	0.82	2.10	1.16
F7	8.47	8.37	1.60	1.45	1.82	1.83	1.73	1.24	2.93	1.81	4.55	2.93
T3	3.26	3.70	0.98	0.84	1.31	1.65	1.69	1.32	2.33	1.64	3.90	2.55
T5	2.85	2.32	0.99	0.94	1.84	1.33	1.38	1.00	1.66	1.37	1.62	1.18
O1	3.26	2.77	0.79	0.66	0.92	0.69	1.05	0.69	1.12	1.13	2.12	1.26
O2	3.38	3.20	0.73	0.68	0.99	0.82	1.48	0.82	1.72	0.92	1.60	1.11
T6	2.78	2.19	0.90	0.71	1.42	1.43	1.48	1.38	1.84	1.69	1.37	1.08
T4	3.11	3.22	0.83	0.77	1.52	1.40	1.54	1.31	2.75	1.42	2.76	1.98
F8	6.72	6.58	1.27	1.48	1.61	1.79	1.60	1.43	2.35	1.83	3.68	2.24
Med	**2.61**	**2.29**	**0.64**	**0.69**	**0.85**	**0.86**	**0.99**	**0.76**	**1.35**	**0.97**	**1.80**	**1.11**

Table 3. Documentation of absolute spectral power values as median in μV^2 on day B. Starting values of absolute EEG spectral power in relaxed condition with eyes open. Data are given in μV^2 for each electrode position and each frequency range. Verum: 120 mg of Bacopa extract plus 380 mg of Sideritis extract. E = electrode position, Med = median over all electrode positions.

Absolute Spectral Power during relaxation (eyes open) 0 h after daily repetitive intake for 4 weeks												
	Delta		**Theta**		**Alpha1**		**Alpha2**		**Beta1**		**Beta2**	
Subjects	**n = 16**	**n = 16**	**n = 16**	**n = 16**	**n = 16**	**n = 16**	**n = 16**	**n = 16**	**n = 16**	**n = 16**	**n = 16**	**n = 16**
Electrode	**Placebo**	**Verum**	**Placebo**	**Verum**	**Placebo**	**Verum**	**Placebo**	**Verum**	**Placebo**	**Verum**	**Placebo**	**Verum**
Cz	2.02	1.46	0.63	0.43	1.04	0.66	0.84	0.50	1.15	0.59	1.21	0.70
Fz	2.54	2.66	0.70	0.78	1.01	0.92	1.05	0.49	0.99	0.55	1.74	0.67
F3	2.43	2.27	0.65	0.79	1.06	0.85	0.79	0.56	1.52	1.15	3.02	1.37
C3	1.45	1.59	0.42	0.42	0.93	0.68	1.20	0.83	1.60	1.35	2.04	1.39
P3	1.59	0.89	0.38	0.28	0.87	0.56	0.75	0.50	0.86	0.59	0.75	0.54
Pz	2.06	1.65	0.42	0.37	1.68	0.63	0.80	0.48	0.98	0.59	0.64	0.51
P4	1.63	1.03	0.39	0.38	0.97	0.50	0.58	0.46	0.73	0.45	0.54	0.37
C4	1.56	1.36	0.48	0.42	0.95	0.67	1.50	0.51	1.92	0.88	1.69	0.81
F4	2.10	2.01	0.65	0.75	0.80	0.94	0.98	0.53	1.59	0.74	3.14	1.21
F7	8.08	8.08	1.48	1.47	2.26	2.08	1.92	1.43	2.32	1.85	4.85	3.15
T3	3.52	4.38	0.89	1.03	2.55	2.36	2.09	1.62	2.78	2.05	3.39	2.80
T5	2.73	2.10	0.97	0.68	1.52	0.95	1.57	1.11	1.62	1.18	1.64	1.37
O1	3.17	2.21	0.73	0.60	0.92	0.76	1.27	0.67	1.47	1.18	2.56	1.31
O2	3.10	2.13	0.80	0.67	1.33	0.87	1.99	0.69	1.71	1.12	2.57	1.15
T6	2.91	2.27	0.86	0.80	1.27	1.75	1.78	1.23	2.22	1.46	1.48	1.02
T4	3.96	2.89	0.96	0.81	3.09	1.34	1.80	1.43	2.87	1.83	5.54	2.06
F8	7.48	6.45	1.38	1.27	2.28	1.42	1.99	1.19	2.95	1.79	4.83	2.28
Med	**2.68**	**2.15**	**0.65**	**0.66**	**1.06**	**0.91**	**1.42**	**0.69**	**1.54**	**1.00**	**2.23**	**1.14**

before intake and documented as bar graph and map. Main differences between placebo and memoLoges® consisted in an increase of alpha1 power within several brain regions. As documented in **Figure 5(a)** this increase (yellow bars) was statistically significantly different from control in the presence of memoLoges®. This change of the frequency pattern is also seen as yellow spot in the map (**Figure 5(b)**, upper image).

MemoLoges® (2 capsules) was taken daily for 4 weeks before repetition of the measurements according to an identical scheme one day thereafter. First recording condition was in the relaxed state with open eyes. Like observed during the recording on day A again increases of alpha1 spectral power emerged, which–however–did not reach wide statistical significance in comparison to control. Increases of beta power was seen mainly in fronto-temporal areas. This led to a dominance of blue color in the map 3 hours after intake. Changes with respect to all single electrode positions are depicted in **Figure 5(a)**, lower image.

Documentation of the results with respect to three main regions of interest revealed time dependent effects of memoLoges® in comparison to control. Time lines of spectral changes are depicted in **Figure 6** for frontal brain (represented by electrode positions $F_{3,4,7,8)}$, temporal lobe (represented by electrode positions $T_{3,4,5,6)}$ and centro-parietal areas (represented by electrode positions $C_{3,4}$ and $P_{3,4)}$. Delta spectral power is attenuated in all three regions of interest, whereas alpha1 spectral power has increased in the temporal lobe and centro-parietally.

Regarding the time line of effectiveness after 4 weeks of daily intake significant attenuation of theta power in the frontal lobe was observed like in the first recording, thus confirming this effect of memoLoges®. Increase of centro-parietal alpha1 and alpha2 power did not reach statistical significance compared to placebo. Also stable increase of beta2 power likewise missed statistical significance. An overview on all time dependent changes is depicted in **Figure 7**.

3.4. Effectiveness of memoLoges® during Performance of the d2-Test on Day A and B

During performance of the d2-test a statistically highly significant attenuation of the spectral power within the centro-parietal region was observed in the presence of memoLoges® on the first day of intake. This was also observed in the temporal lobe (electrode position T_6).

In addition, a tendency of higher spectral power in the delta range was observed on the first day, whereas attenuation was seen with respect to theta and alpha1 spectral power in the presence of memoLoges®. This attenuation became statistically significant only during the 3rd hour after intake (**Figure 8(a)**). In the parietal lobe a statistically highly significant attenuation with respect to all frequencies. Alpha2 waves were also attenuated in the frontal lobe and temporal lobe during the first 2 hours.

After 4 weeks of daily intake performance of the d2-test led to enhanced delta activity in the frontal brain and also within the left temporal lobe (electrode position T_5). In centro-parietal areas reduction of alpha waves was observed, which partially reached statistical significance. Regarding the maps no clear difference between memoLoges® and placebo was recognized. Effects on single areas (electrode positions) are documented in **Figure 9**.

Regarding time dependent effectiveness during performance of the d2-test a highly significant increase of delta power was observed in the temporal lobe during the 1st and 2nd hour after intake, in the frontal lobe only during the 1st hour. However, opposite to the first recording at the beginning of the experiment beta power was attenuated to a significant degree. Details are given in **Figure 9**. Regarding the time line of effectiveness after 4 weeks of daily intake statistically significant attenuation of beta spectral power was observed in centro-parietal brain regions in comparison to placebo (**Figure 10**).

3.5. Effectiveness of memoLoges® during Performance of the Arithmetic Calculation Test on Day A and B

During performance of the arithmetic calculation test a general increase of spectral beta power was observed. This led to a predominance of blue color within the map as documented in **Figure 11**. However no statistical significance emerged.

Furthermore, in the centro-parietal region a significant decrease of theta power was observed. In the temporal lobe a statistically significant increase of alpha2, beta1 and beta2 spectral power was seen. An overview on changes during performance of this test is given in **Figure 12**.

Performance of the arithmetic calculation test after 4 weeks of daily intake of memoLoges® or placebo no major differences were recognized between the two preparations except for some attenuation of alpha activity. Maps also differed only slightly. Details can be taken from **Figure 12**.

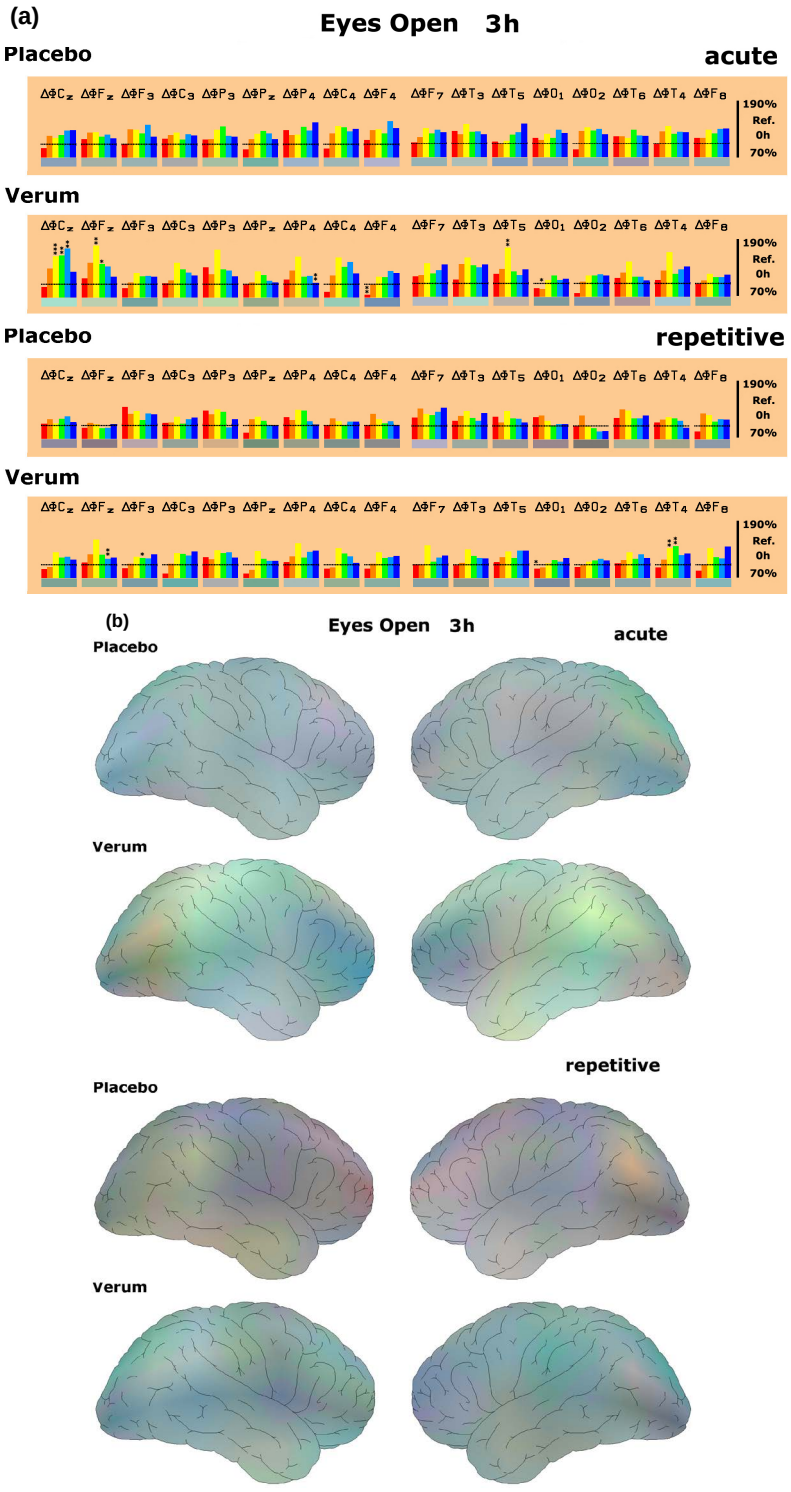

Figure 5. Documentation of the effect of memoLoges® in comparison to placebo during relaxation (eyes open) as change in % of the baseline spectral power during the 3rd hour after intake. Changes of spectral power are documented for each electrode position: C = central, F = frontal, P = parietal, T = temporal and O = occipital. Frequency windows are coded into colors: red = delta, orange = theta, yellow = alpha1, green = alpha2, turquoise = beta1 and blue = beta2. Statistical probabilities according to non-parametric Wilcoxon tests are marked by stars: *=p < 0.1; **=p < 0.05; ***=p < 0.01. Maps were constructed by coding single frequencies into spectral colors and additive color mixture according to the so-called RGB mode (like in television). Acute data: upper image; repetitive data: lower image.

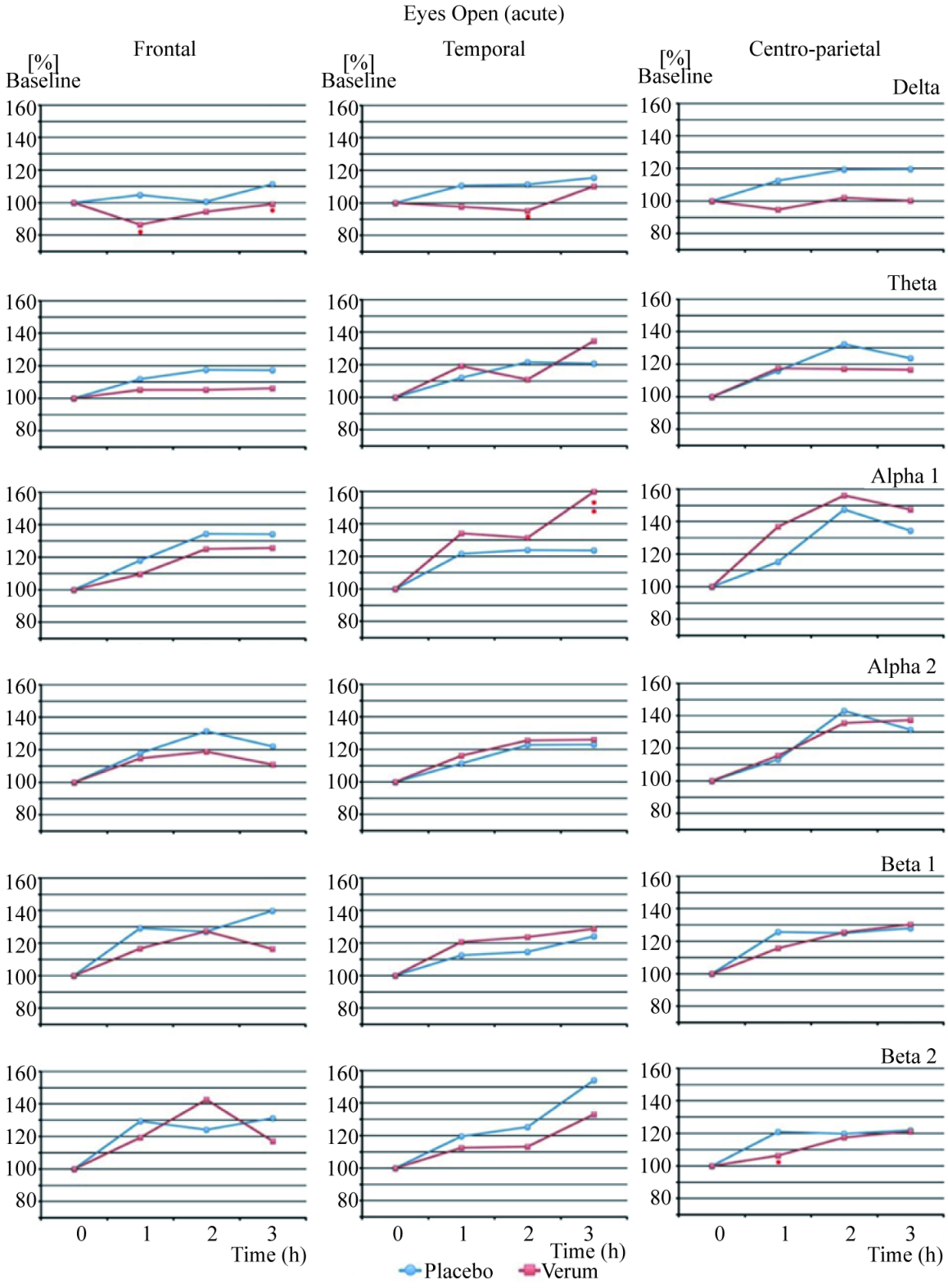

Figure 6. Time line of the effectiveness of memoLoges® (red line) in comparison to control (blue line) in three regions of interest under the recording condition "eyes open" (day A). Changes are given in % of the baseline recording on the ordinate. Time after intake (day A) is given on the abscissa. Statistical probabilities according to non-parametric Wilcoxon tests are marked by stars: *=p < 0.1; **=p < 0.05; ***=p < 0.01.

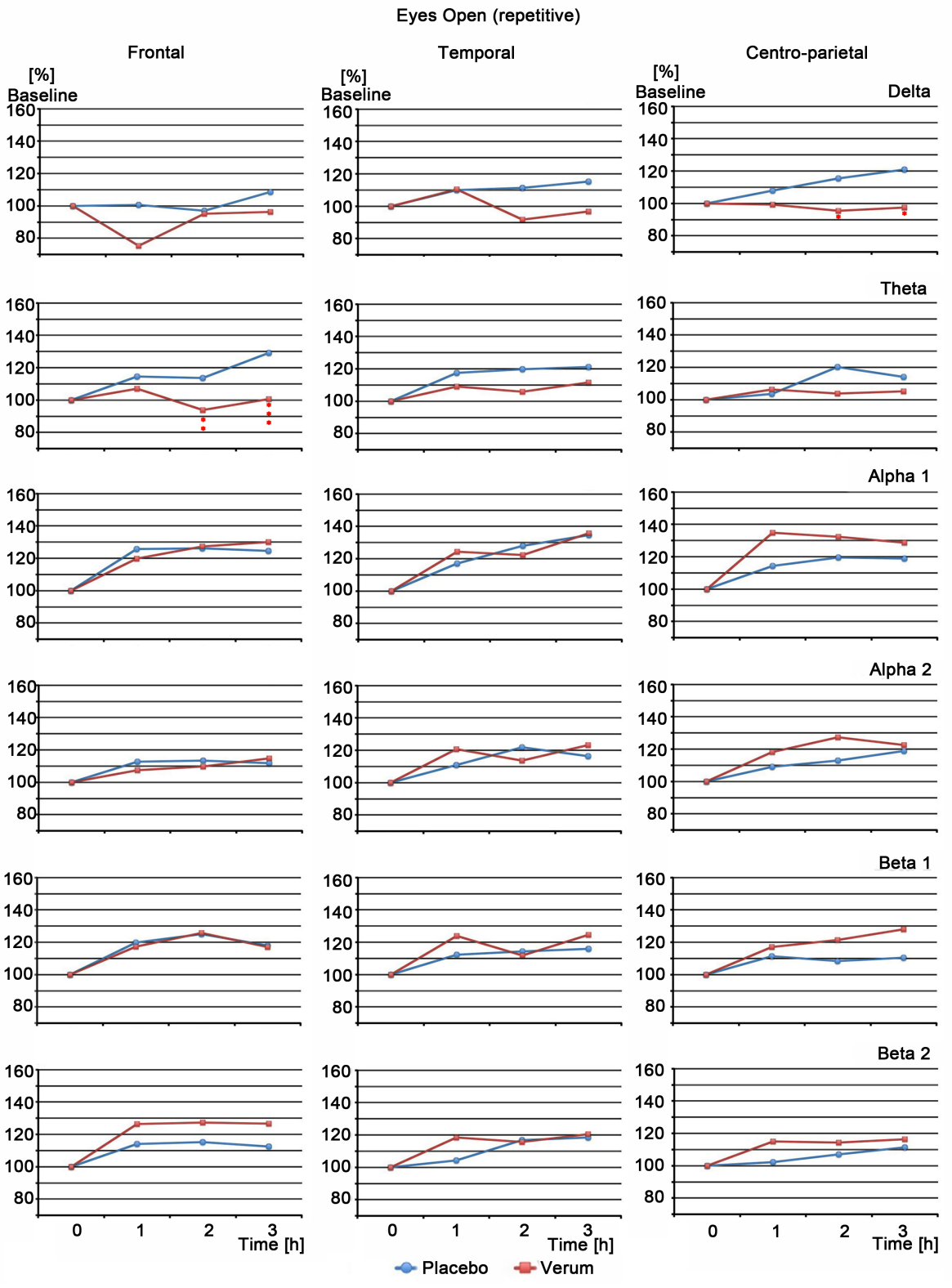

Figure 7. Time line of the effectiveness of memoLoges® (red line) in comparison to control (blue line) in three regions of interest during relaxed state with open eyes after 4 weeks of daily intake. Changes are given in % of the baseline recording on the ordinate. Time after intake is given on the abscissa. Statistical probabilities according to non-parametric Wilcoxon tests are marked by stars: $^{*}=p < 0.1$; $^{**}=p < 0.05$; $^{***}=p < 0.01$.

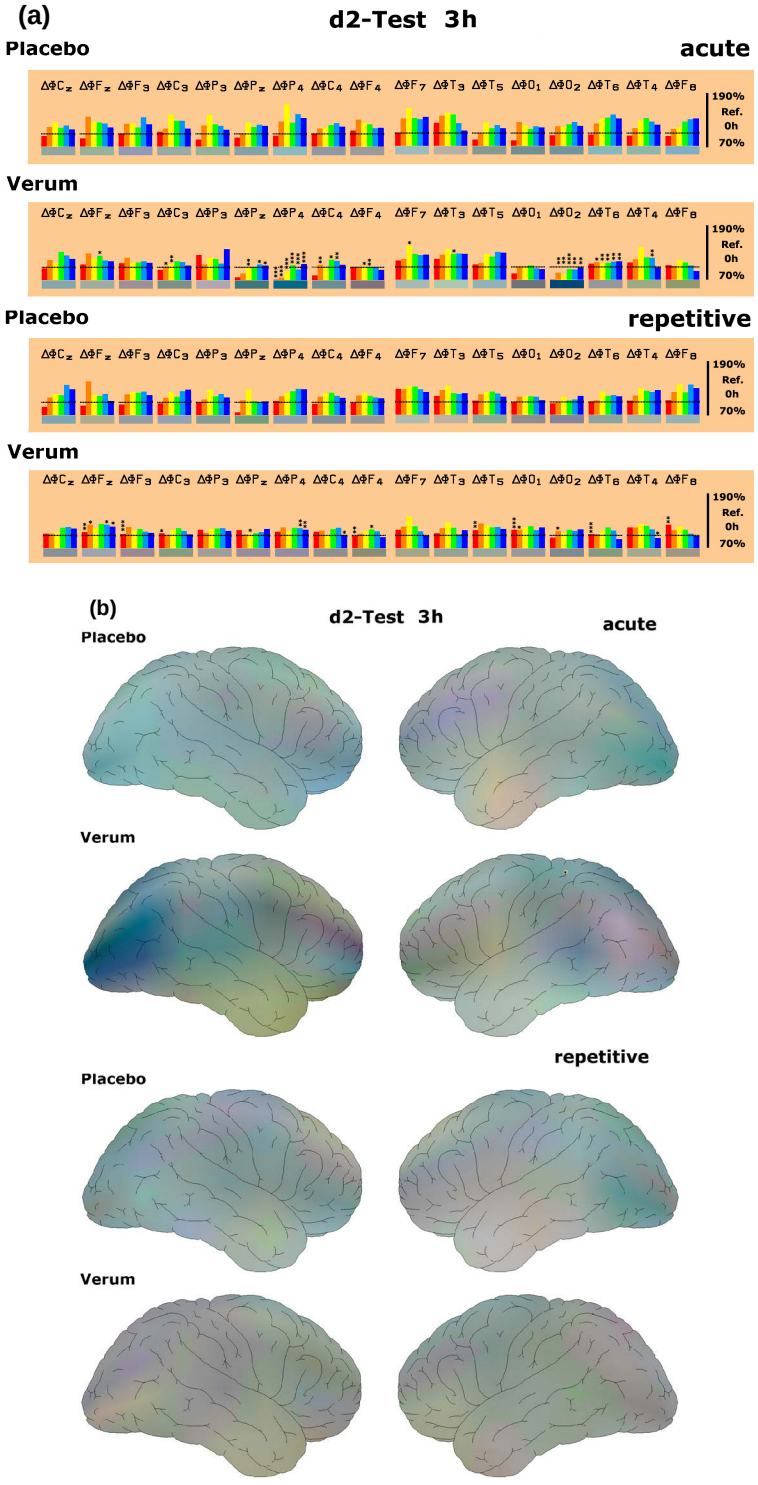

Figure 8. Documentation of the effect of memoLoges® in comparison to placebo during performance of the d2-test as change in % of the baseline spectral power during the 3rd hour after intake. Changes of spectral power are documented for each electrode position: C = central, F = frontal, P = parietal, T = temporal and O = occipital. Frequency windows are coded into colors: red = delta, orange = theta, yellow = alpha1, green = alpha2, turquoise = beta1 and blue = beta2. Statistical probabilities according to non-parametric Wilcoxon tests are marked by stars: *=p < 0.1; **=p < 0.05; ***=p < 0.01. Maps were constructed by coding single frequencies into spectral colors and additive color mixture according to the so-called RGB mode (like in television). Acute effects: upper image; repetitive effects: lower image.

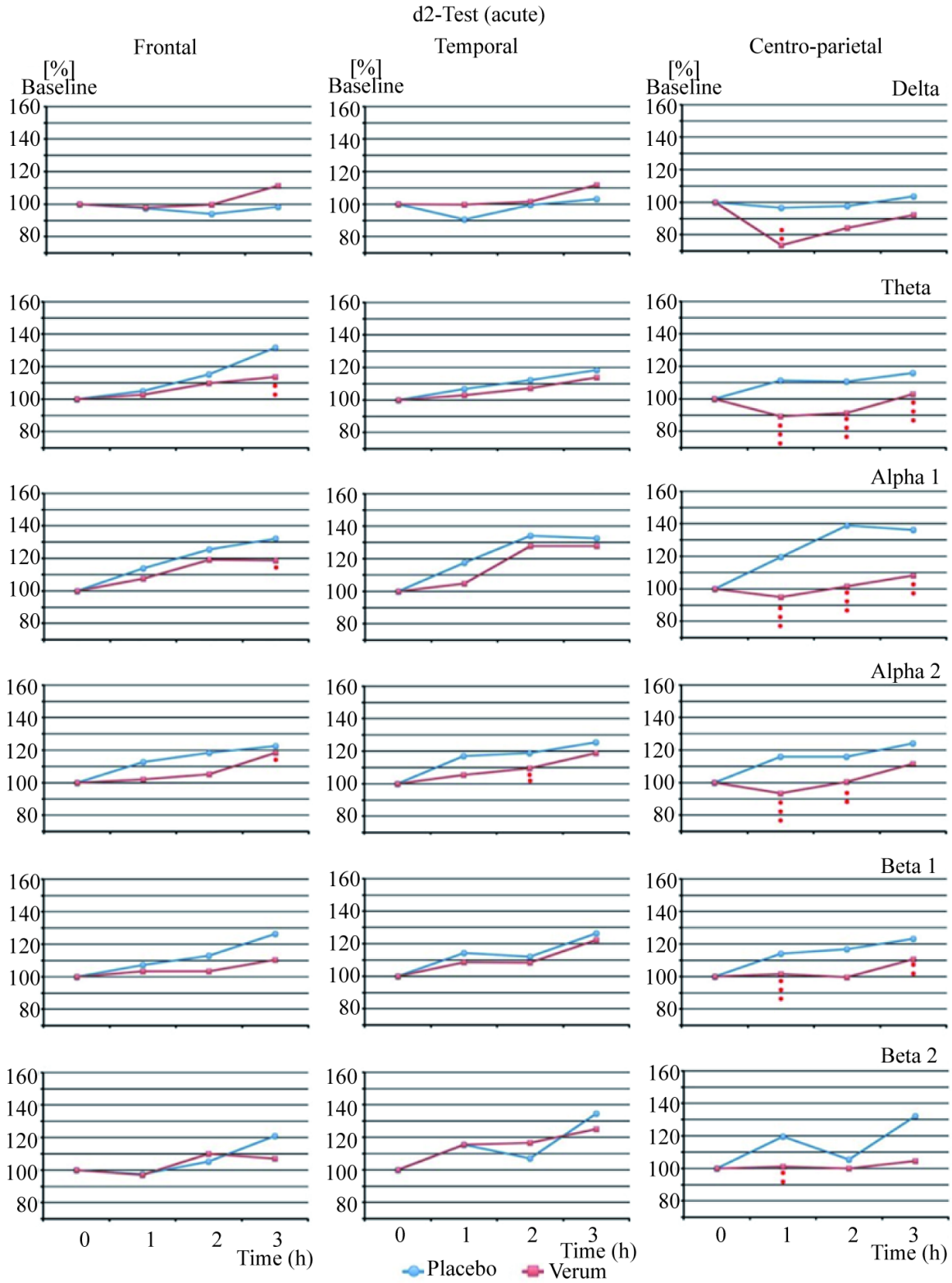

Figure 9. Time line of the effectiveness of memoLoges® (red line) in comparison to control (blue line) in three regions of interest during performance of the d2-test (day A). Changes are given in % of the baseline recording on the ordinate. Time after intake is given on the abscissa. Statistical probabilities according to non-parametric Wilcoxon tests are marked by stars: *=p < 0.1; **=p < 0.05; ***=p < 0.01.

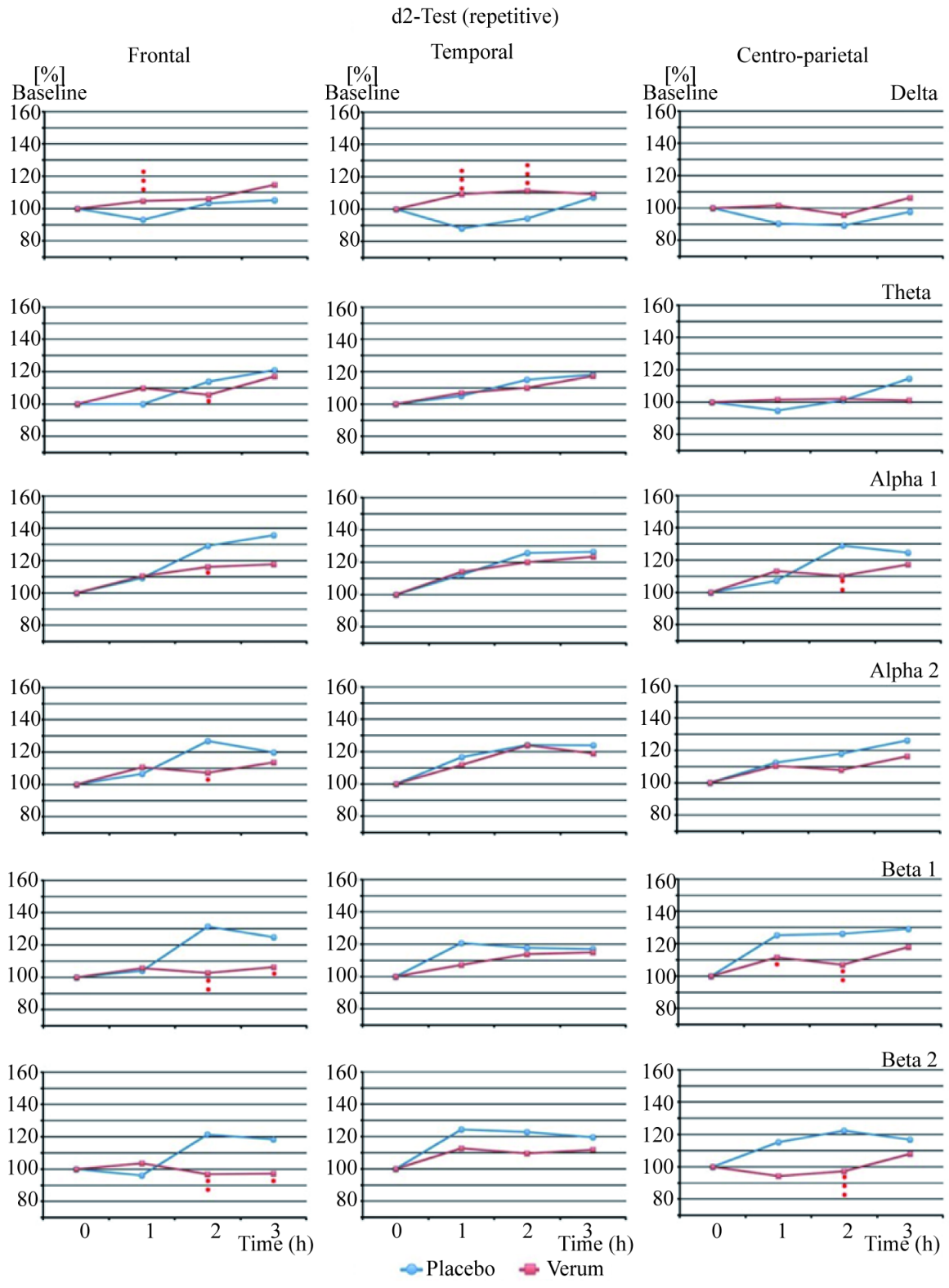

Figure 10. Time line of the effectiveness of memoLoges® (red line) in comparison to control (blue line) in three regions of interest during performance of the d2-test after 4 weeks of daily intake. Changes are given in % of the baseline recording on the ordinate. Time after intake is given on the abscissa. Statistical probabilities according to non-parametric Wilcoxon tests are marked by stars: $^{*}p < 0.1$; $^{**}p < 0.05$; $^{***}p < 0.01$.

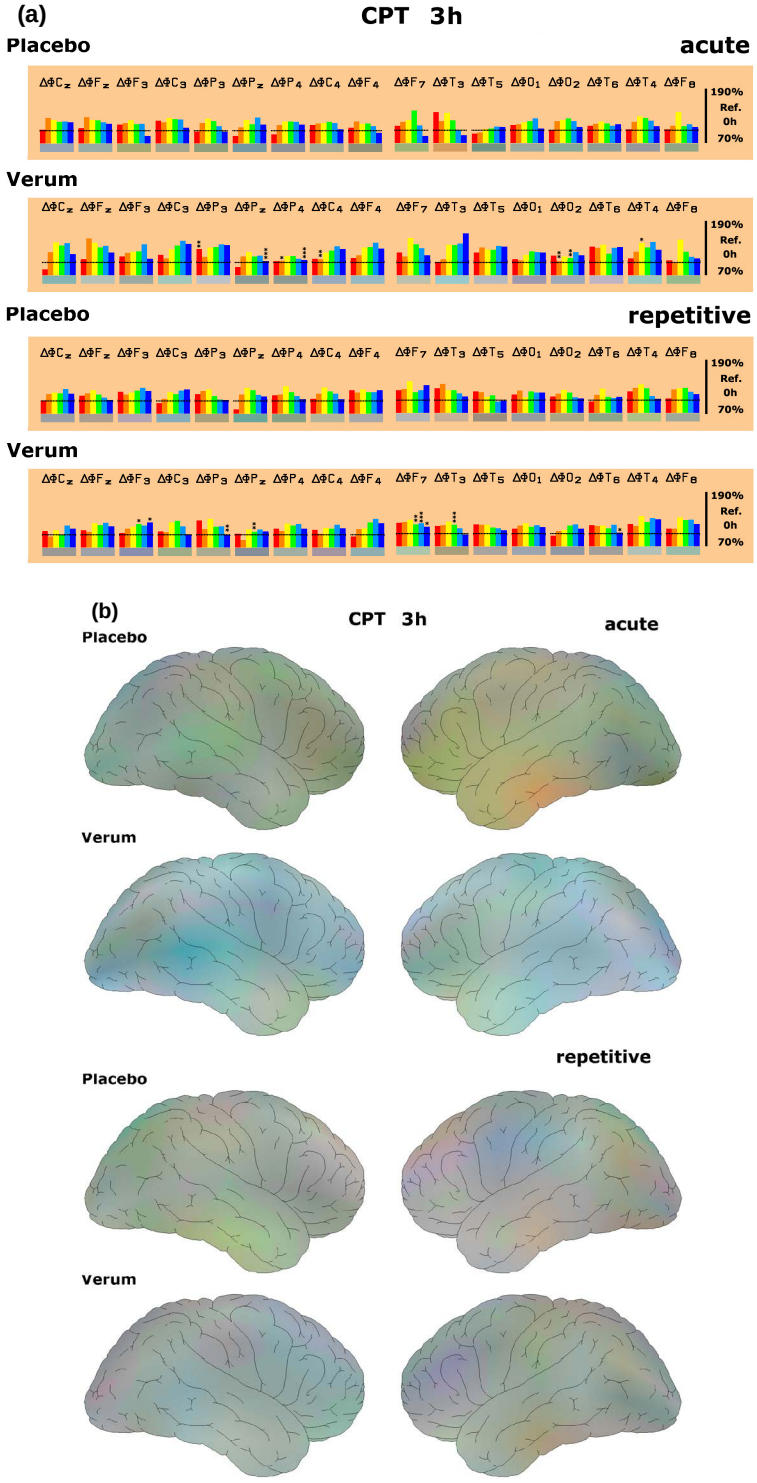

Figure 11. Documentation of the effect of memoLoges® in comparison to placebo during performance of the arithmetic calculation test (CPT) as change in % of the baseline spectral power during the 3rd hour after intake. Changes of spectral power are documented for each electrode position: C = central, F = frontal, P = parietal, T = temporal and O = occipital. Frequency windows are coded into colors: red = delta, orange = theta, yellow = alpha1, green = alpha2, turquoise = beta1 and blue = beta2. Statistical probabilities according to non-parametric Wilcoxon tests are marked by stars: *=$p < 0.1$; **=$p < 0.05$; ***=$p < 0.01$. Maps were constructed by coding single frequencies into spectral colors and additive color mixture according to the so-called RGB mode (like in television). Acute effects: upper image; repetitive effects: lower image.

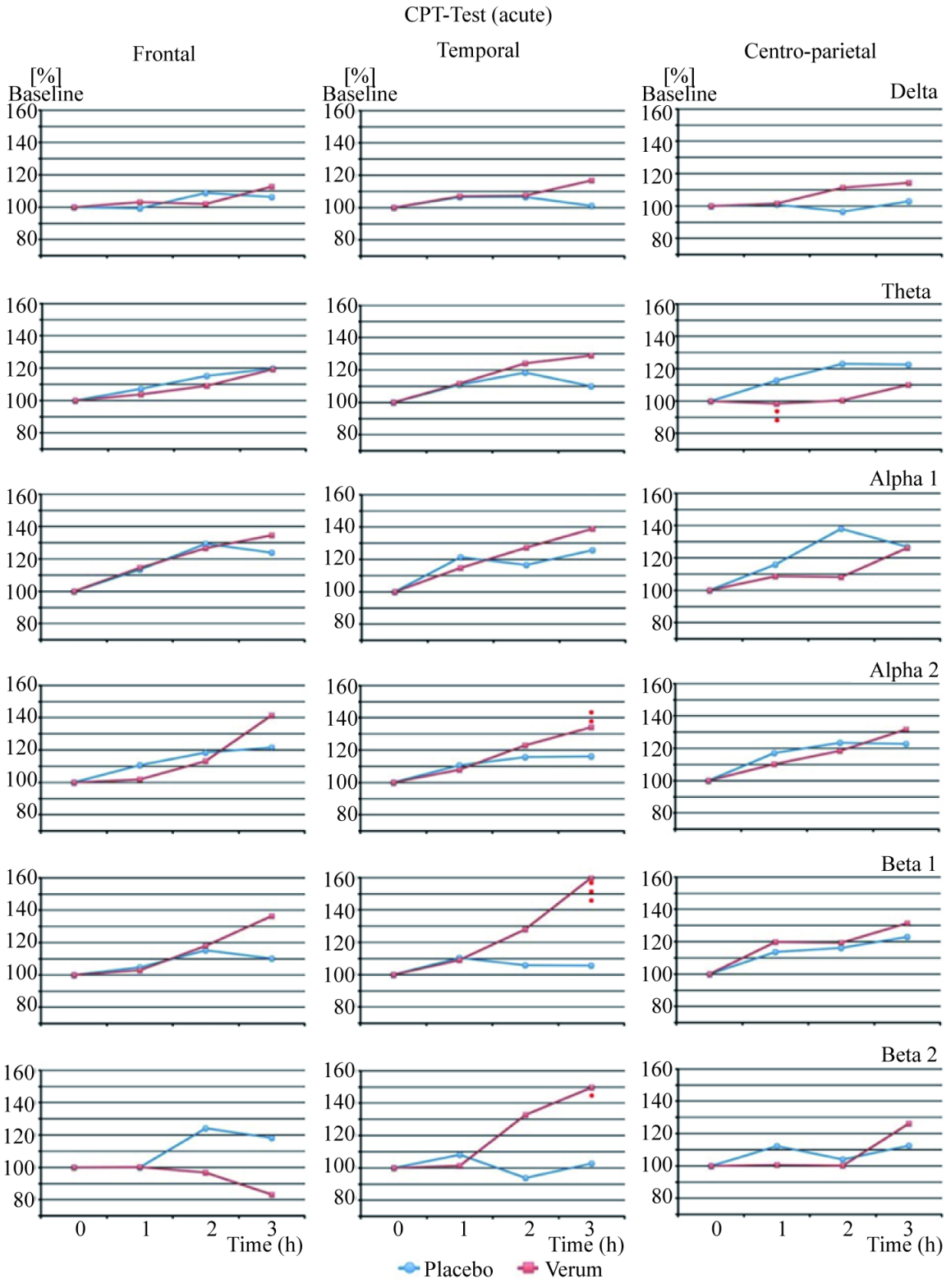

Figure 12. Time line of the effectiveness of memoLoges® (red line) in comparison to control (blue line) in three regions of interest during performance of the arithmetic calculation test (day A). Changes are given in % of the baseline recording on the ordinate. Time after intake is given on the abscissa. Statistical probabilities according to non-parametric Wilcoxon tests are marked by stars: $^{*}=p < 0.1$; $^{**}=p < 0.05$; $^{***}=p < 0.01$.

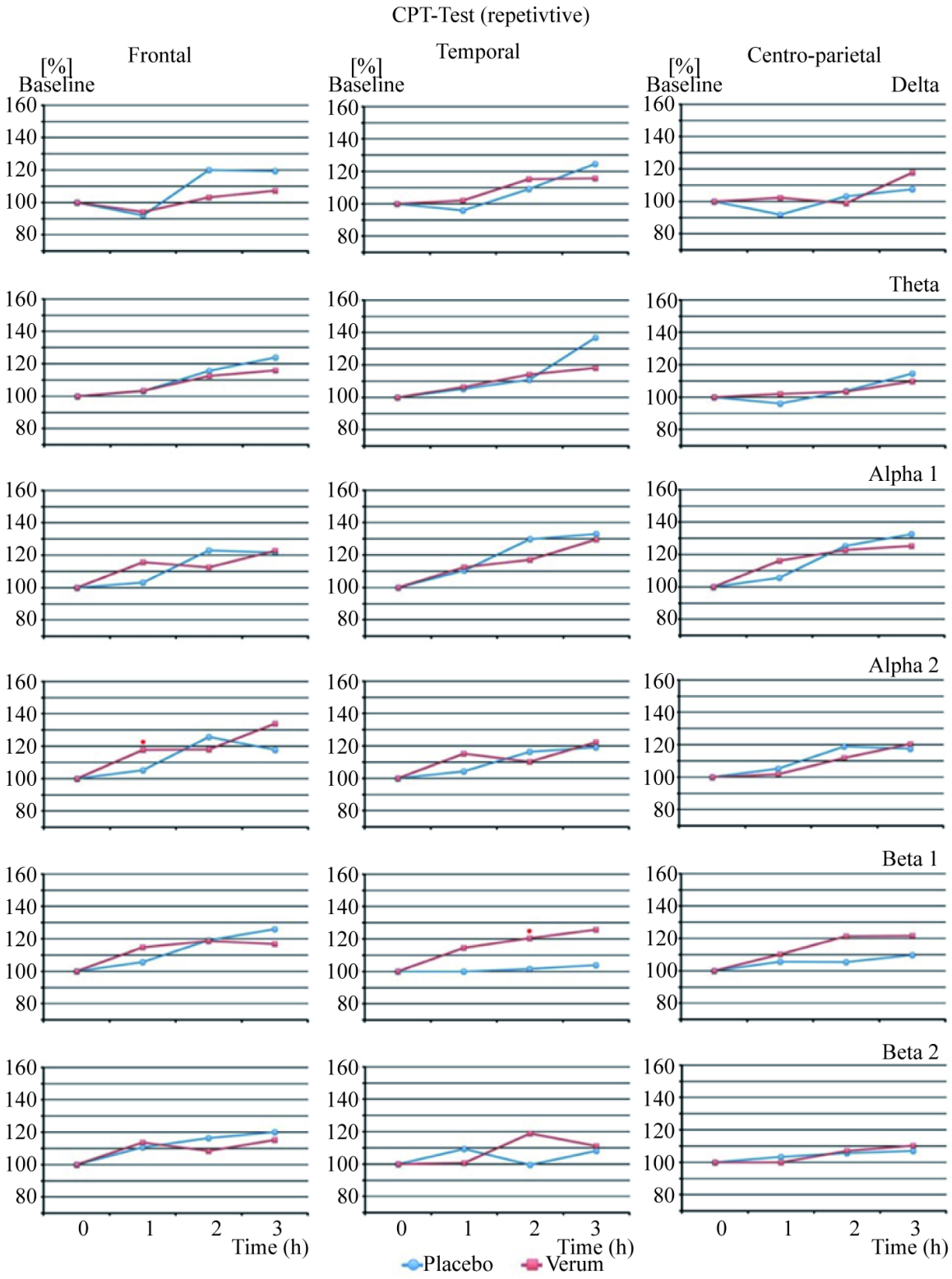

Figure 13. Time line of the effectiveness of memoLoges® (red line) in comparison to control (blue line) in three regions of interest during performance of the arithmetic calculation test after 4 weeks of daily intake. Changes are given in % of the baseline recording on the ordinate. Time after intake is given on the abscissa. Statistical probabilities according to non-parametric Wilcoxon tests are marked by stars: $^{*}=p < 0.1$; $^{**}=p < 0.05$; $^{***}=p < 0.01$.

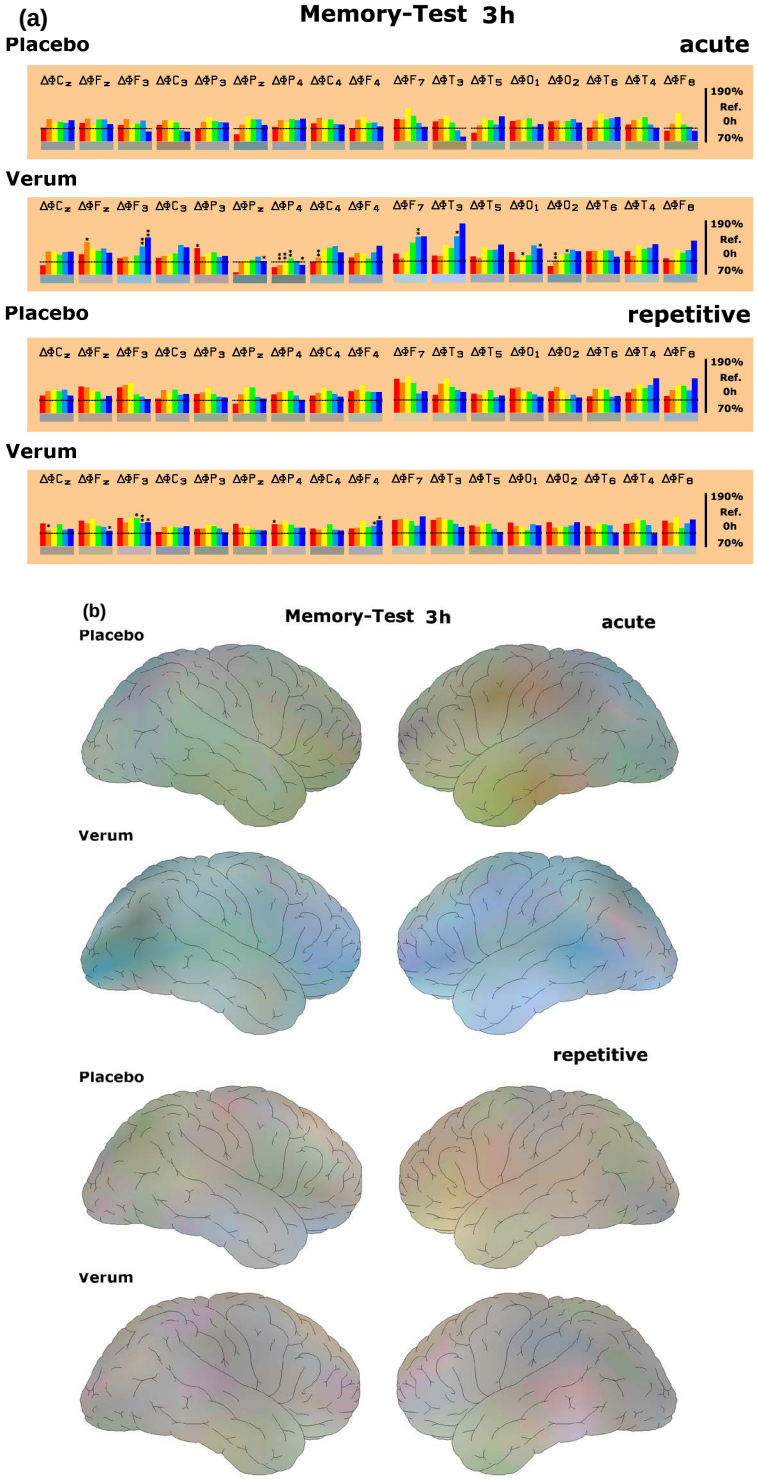

Figure 14. Documentation of the effect of memoLoges® in comparison to placebo during performance of the Memory test (ME) as change in % of the baseline spectral power during the 3rd hour after intake. Changes of spectral power are documented for each electrode position: C = central, F = frontal, P = parietal, T = temporal and O = occipital. Frequency windows are coded into colors: red = delta, orange = theta, yellow = alpha1, green = alpha2, turquoise = beta1 and blue = beta2. Statistical probabilities according to non-parametric Wilcoxon tests are marked by stars: *=p < 0.1; **=p < 0.05; ***=p < 0.01. Maps were constructed by coding single frequencies into spectral colors and additive color mixture according to the so-called RGB mode (like in television). Acute effects: upper image; repetitive effects: lower image.

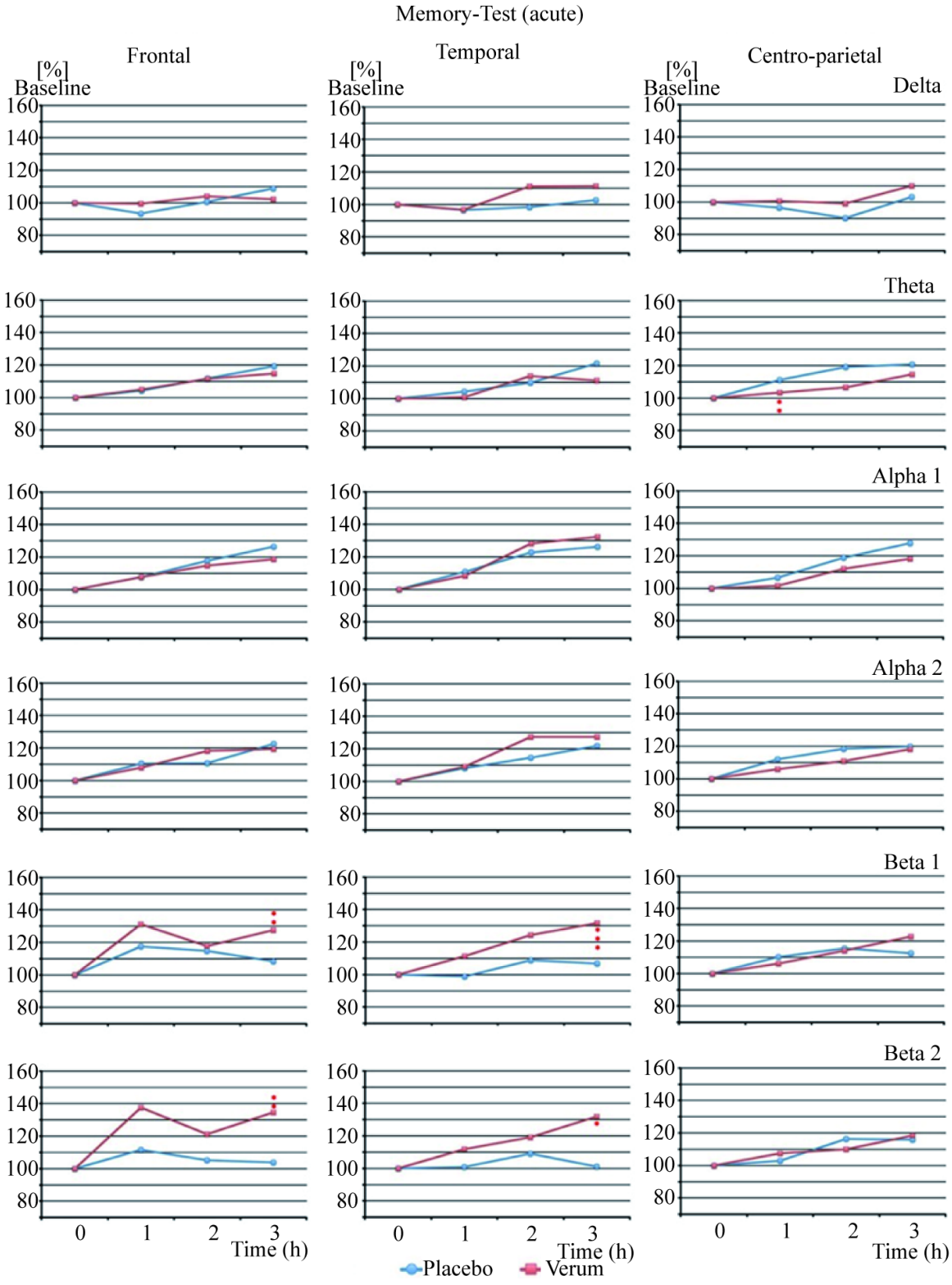

Figure 15. Time line of the effectiveness of memoLoges® (red line) in comparison to control (blue line) in three regions of interest during performance of the memory test (day A). Changes are given in % of the baseline recording on the ordinate. Time after intake is given on the abscissa. Statistical probabilities according to non-parametric Wilcoxon tests are marked by stars: $^{*}p < 0.1$; $^{**}p < 0.05$; $^{***}p < 0.01$.

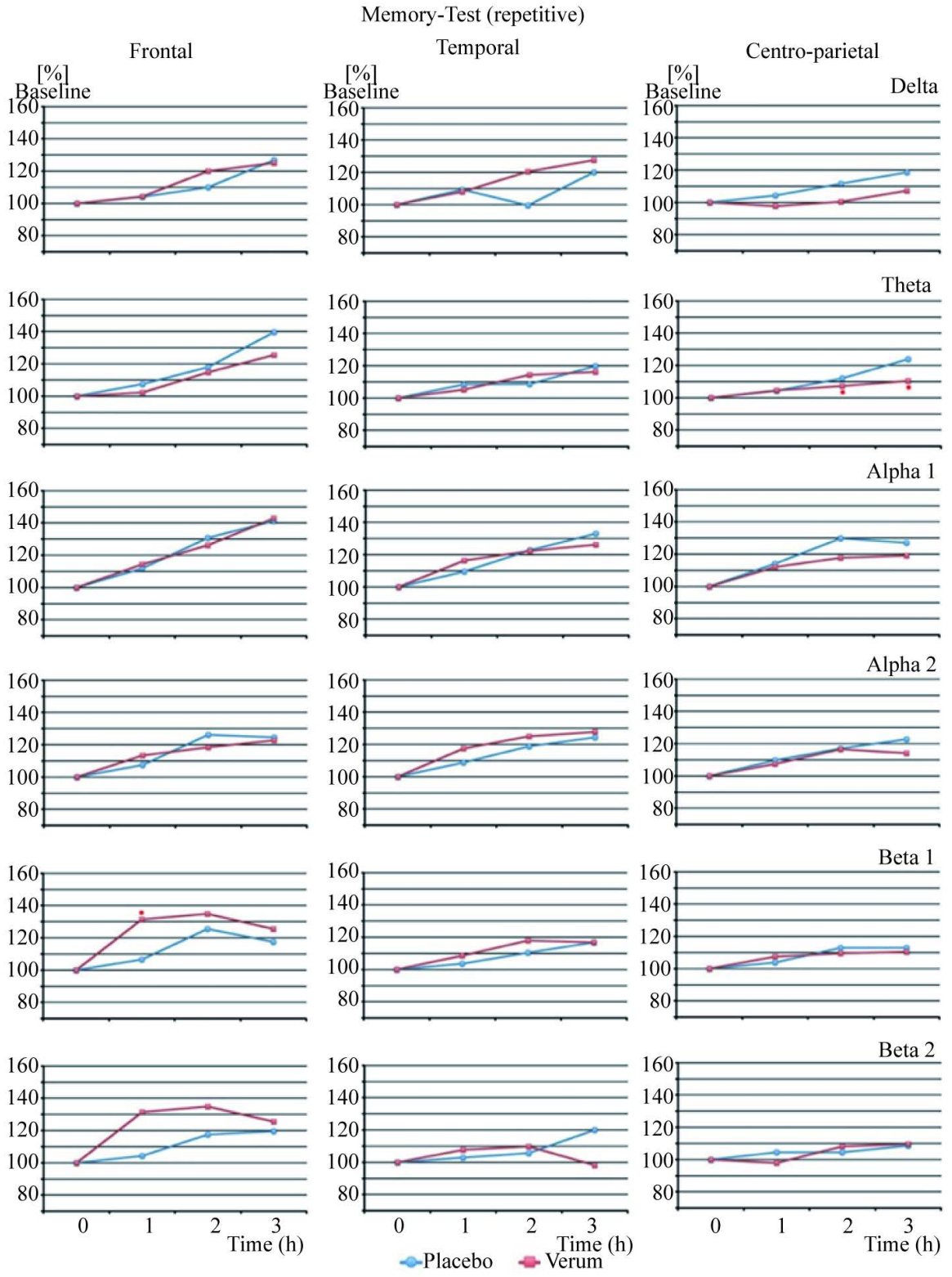

Figure 16. Time line of the effectiveness of memoLoges® (red line) in comparison to control (blue line) in three regions of interest during performance of the memory-test after 4 weeks of daily intake. Changes are given in % of the baseline recording on the ordinate. Time after intake is given on the abscissa. Statistical probabilities according to non-parametric Wilcoxon tests are marked by stars: $^{*}p < 0.1$; $^{**}p < 0.05$; $^{***}p < 0.01$.

Regarding the time line of effectiveness after 4 weeks daily intake only some but constant increase of beta power in the temporal lobe were seen. An overview on all data is given in **Figure 13**.

3.6. Effectiveness of memoLoges® during Performance of the Memory Test on Day A

Performance of the memory test induced—similar to the arithmetic calculation test-increases of spectral beta power predominantly in the frontal and temporal lobe in a statistically significant manner. In the parietal lobe statistically significant attenuation occurred, whereas in the mid-frontal area an increase of theta power reached statistical significance. Details are given in **Figure 14**. With respect to the map this led to predominant blue color.

After 4 weeks of daily intake of the preparations performance of the memory test induced a significant increase of beta waves within the frontal brain. Maps calculated for the third hour after intake differed only slightly showing a little bit more blue coloring. Details are given in **Figure 15**.

Regarding the time line strongest increases of beta1 and beta2 power were observed only in the frontal and temporal lobe, absolutely not in the centro-parietal region. This increase became statistically significant during the 3rd hour after intake. However, in the centro-parietal area less of theta power was produced in comparison to control. An overview on time dependent effects is given in **Figure 15**.

The increase beta activity in the frontal brain, which became statistically significantly different from control during the third hour after intake had also been seen during the original recording at the beginning of the experiment. An overview is given in **Figure 16**.

3.7. Results from Discrimination Analysis

In order to use all information provided by quantitative EEG analysis all 102 parameters (17 electrode positions times 6 frequency ranges) were fed into discriminant analysis according to Fischer. Results from the first three discriminant functions are projected using the x, y and z coordinates. Results from the 4th to 6th function are coded using additive color mixture according the RGB (red-green-blue) mode like in TV. As documented in **Figure 17** results from the 4 different recording conditions are very well separated from each other. In addition, the effect of memoLoges® is also very well separated from placebo data. Thus, discriminant analysis is well suited to show statistically the overall effect of memoLoges® in comparison to placebo in subjects suffering from mild cognitive impairment.

3.8. Safety

For safety reasons ECG and pulse were recorded at each measurement period. No deviations from normality were recognized. MemoLoges® was tolerated very well by all subjects without any side effect.

4. Discussion

Interpretation of EEG data depends on the recording condition. One discriminates between recording in the relaxed state (eyes open or closed) or during performance of mental challenges. In the relaxed state it has been shown earlier that subjects suffering from mild cognitive impairment have higher delta and theta spectral power in the frontal brain [2]. This higher spectral power of these slow waves prevents further increases during mental performance due to a ceiling effect (during performance of psychometric tests massive increases of focal delta and theta power were observed in healthy subjects [13]. Attenuation of these waves during relaxation as observed in the presence of memoLoges® in comparison to placebo can therefore be regarded as a positive effect, since it allows for stronger increases during mental loads. This result is in line with data reported with respect to the efficacy of rivastigmine in Alzheimer disease. Spectral analysis of EEG data showed a significant power decrease in the delta and theta frequency bands, *i.e.* a shift of the power spectrum towards "normalization" [14]. Concomitantly, alpha1 spectral power had increased in the presence of memoLoges® during relaxation. This indicates a higher degree of relaxation as reported earlier [9].

Interpretation of EEG data during audio-visual challenges is completely different as has been shown recently [15]. Dependent on the type of mental challenge different brain areas react in a different manner with respect to frequency changes. During psychometric performance increases of delta and theta power were observed in frontal and temporal brain regions, whereas central regions showed attenuation of alpha waves in healthy subjects. In subjects suffering from mild cognitive impairment more spectral slow wave power was produced in the presence of memoLoges® during performance of the d2-test, because basic spectral power in these frequencies during relaxation had been lowered and by it allowing more production of slow waves during the mental challenge.

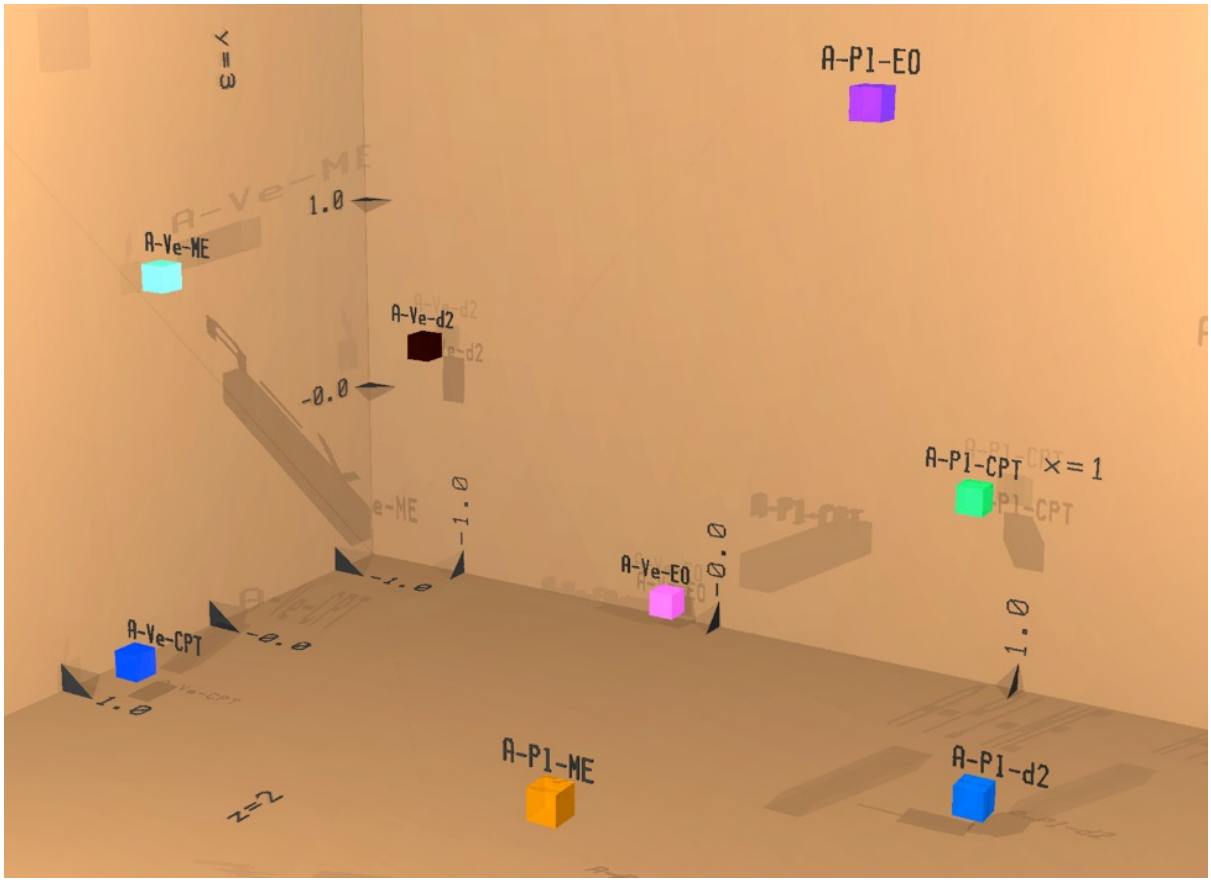

Figure 17. Result of discriminant analysis 3 hours after intake of memoLoges®. Projection of the result of the first three discriminant functions is achieved using the x, y and z coordinates. Result from the 4th to 6th function is coded using additive color mixture according the RGB (red-green-blue) mode like in TV. Data from the day A are depicted. A = day A, Ve = Verum, Pl = Placebo. EO = Eyes open; d2 = d2-test; CPT = arithmetic calculation test; ME = Memory test. Please note: not only different recording conditions but also effects of memoLoges® and placebo are well discriminated from each other.

Concomitantly, memoLoges® induced a highly statistically significant attenuation of alpha waves in the centro-parietal region. This additional decrease of alpha waves during performance of the d2-test in the presence of memoLoges® indicates a recovery, because central attenuation of alpha waves is an important feature during mental performance [15]. Exactly this was also observed in this study during performance of the arithmetic calculation test in the presence of memoLoges®.

Coming back to the frontal and temporal areas, the damage of temporo-frontal area of the brain has been shown to result in retrograde memory deficits [16]. Furthermore, it has been shown, that mental challenges induce higher spectral power in the beta range. However, subjects suffering from mild cognitive impairment produced clearly less beta power during for example the d2-test [2]. Therefore, the statistically significant increase of beta power as observed in the presence of memoLoges® in comparison to placebo in this study during mental performance (arithmetic calculation test and memory test) must be regarded as a sign of restoration to normal functioning.

Beta1 waves are under the control of glutamate as shown in humans by a correlation in the presence of different dosages of a glutamate agonist [9]. The statistically significant effects of memoLoges® therefore point to an involvement of this transmitter with respect to the mechanism of action. An in vitro analysis of the major ingredient of memoLoges®, namely an extract of *Sideritis scardica*, revealed an increase of long term potentiation, which is related to time and space dependent memory. Data (to be published) showed the involvement of the AMPA receptor mediated electric activity. These data corroborate the view that memoLoges® acts via a glutamatergic mechanism as indicated by changes in spectral beta activity.

After repetitive dosing a similar picture of frequency changes emerged. Acute dosing one day after the 4

weeks intake revealed again attenuation of delta power during relaxation and increase during performance of the d2-test as well as an increase of beta1 power in frontal and temporal brain during performance of the arithmetic calculation test. Beta power also increased in frontal brain during the memory test. Generally, the effects were somewhat weaker, possibly indicating a return to more normal brain functioning. Results of an earlier pilot study with *Sideritis scardica* and *Bacopa monnieri* extracts in the presence of single dosages were confirmed [17].

5. Conclusion

Intake of memoLoges® induced a trend of improvement of performance in psychometric testing (all three tests) in subjects suffering from mild cognitive impairment. Quantitative assessment of EEG data revealed attenuation of delta and theta spectral power in frontal brain as likewise observed in the presence of the Alzheimer drug rivastigmine, bringing the spectrum back to "normality". Since MCI subjects produce less beta power in comparison to healthy subjects, significant increases of beta power in the presence of memoLoges® must likewise be seen as a positive effect. Data are in line with a recent discovery, that Sideritis extract as the main ingredient in memoLoges® induced an increase of long term potentiation in the hippocampus slice preparation, which is related to time and space dependent memory.

Acknowledgements

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