Scientific Research Publishing

World Journal of Neuroscience, 2017, 7, 140-171
http://www.scirp.org/journal/wjns
ISSN Online: 2162-2019
ISSN Print: 2162-2000

Effect of Zembrin® on Brain Electrical Activity in 60 Older Subjects after 6 Weeks of Daily Intake. A Prospective, Randomized, Double-Blind, Placebo-Controlled, 3-Armed Study in a Parallel Design

Wilfried Dimpfel[1], Nigel Gericke[2], Samir Suliman[3], Gwladys N. Chiegoua Dipah[3]

[1]Justus-Liebig-University, Giessen, Germany
[2]HG & H Pharmaceuticals (Pty) Ltd., Bryanston, South Africa
[3]NeuroCode AG, Wetzlar, Germany
Email: Wilfried.Dimpfel@pharma.med.uni-giessen.de

How to cite this paper: Dimpfel, W., Gericke, N., Suliman, S. and Chiegoua Dipah, G.N. (2017) Effect of Zembrin® on Brain Electrical Activity in 60 Older Subjects after 6 Weeks of Daily Intake. A Prospective, Randomized, Double-Blind, Placebo-Controlled, 3-Armed Study in a Parallel Design. *World Journal of Neuroscience*, **7**, 140-171.
https://doi.org/10.4236/wjns.2017.71011

Received: December 21, 2016
Accepted: February 6, 2017
Published: February 9, 2017

Abstract

Zembrin® is a botanical functional food and dietary supplement ingredient sold in the USA, and Canada for enhancing mood, decreasing anxiety and stress and improving cognitive function under stress. It is a proprietary extract of a cultivated selection of *Sceletium tortuosum*. The present investigation aimed at the measurement of the effect of 25 or 50 mg of Zembrin® in comparison to placebo after daily repetitive intake for 6 weeks. Sixty healthy male (n = 32) and female (n = 28) right-handed subjects between 50 and 80 years old (59.7 ± 5.43 and 56.7 ± 5.88 years, respectively) were recruited. The EEG was recorded bipolarly from 17 surface electrodes (CATEEM®) before and 1 h after intake. Six cognitive tests were performed: d2-test, memory test, calculation performance test, reaction time test, number identifying test and number connection test. Three questionnaires were included: Profile of Mood States, Hamilton Anxiety Rating Scale and a sleep questionnaire. Quantitative EEG revealed increases of delta activity during performance of the d2-test, the number identification and number connection test in the fronto-temporal brain region. Higher theta activity was seen during relaxation and performance of the d2-test after intake of 50 mg of Zembrin®. Statistically conspicuous increases of alpha1 spectral power were seen in the relaxed state. With respect to alpha2 spectral power larger increases were observed in the centro-occipital region. Discriminant analysis revealed a projection of Zembrin® data into the vicinity of the calming preparation Calmvalera tablets and a Ginkgo-Ginseng mixture. Statistically significant improvement during performance of the arithmetic calculation test and number connection test was do-

cumented. The HAM-A anxiety score revealed a statistically significant decrease (p = 0.03) after six weeks. Zembrin® showed significant activity on three levels of evidence: questionnaires, psychometry and quantitative EEG. The results indicate that in healthy people Zembrin® improves some aspects of cognitive function, decreases anxiety, and may enhance mood.

Keywords

Sceletium tortuosum, Zembrin®, EEG, Psychophysiology, Spectral Power, CATEEM®, Psychometry, Anxiety, Stress, Cognitive

1. Introduction

Since the first discovery of human electric activity by Hans Berger [1] electroencephalographic measurements were performed not only for diagnostic purposes but increasingly also during exposure to numerous mental challenges aiming at a better understanding of cognitive and emotional processes. Spectral and multivariate analysis of EEG oscillations during mental activity in man had already been reported more than 40 years ago [2]. Spectral and multivariate analysis of EEG oscillations during mental activity in man had already been reported more than 40 years ago. Subsequently, reflection of cognitive challenges in the quantitative EEG was reported to differ according to special tasks [3]. The physiology of mindfulness has been related to EEG oscillations also recently [4]. Using questionnaires a relationship between mood and EEG spectra was reported [5]. Due to the intimate relationship between psychometric testing and EEG spectral signatures it was even suggested that cognitive testing could be replaced by quantitative EEG measurements [6]. Quantitative EEG measurements in the relaxed state and during performance of cognitive tests are therefore well suited to describe functional changes of brain activity induced by intake of food [7] [8], food supplements [9] or drugs [10].

Zembrin® is a botanical functional food and dietary supplement ingredient currently sold in the USA, Canada, Brazil, Malaysia, and South Africa. It is a proprietary extract of a low-alkaloid cultivated selection of *Sceletium tortuosum*, and is used by healthy people for enhancing mood, decreasing anxiety and stress and improving cognitive function under stressful situations. Preclinical [11] as well as clinical evidence [12] [13] [14] [15] has been reported with respect to its safety, tolerability, and its efficacy in changing brain function in healthy subjects. A successful double-blind, randomized, placebo-controlled study in parallel design was performed in order to test the psychophysiological effects of Zembrin® after intake of a single dose [16]. In this study Zembrin® was shown to increase alpha waves during relaxation and delta and theta waves during cognitive challenges. Beta2 waves increased during mental performance in the presence of the higher dosage of Zembrin® in parietal, occipital and temporal brain regions.

The present investigation aimed at the objective measurement of the effect of two dosages of Zembrin® after daily repetitive intake during 6 weeks. The expe-

rimental design included 3 levels of evidence: a questionnaire, psychometric testing and recording of quantitative EEG during performance of psychometric tests. It was hoped to confirm the results from the first study on acute dosing and objectify functional effects on the brain after daily repetitive intake for 6 weeks.

2. Material and Methods

2.1. Subjects

Sixty healthy male (n = 32) and female (n = 28) right-handed subjects between 50 and 80 years old (59.7 ± 5.43 and 56.7 ± 5.88 years, respectively) and fluent in German language were recruited and gave informed consent. Their body mass index was >18.5 or <33.0. Criteria for exclusion were:

Participation in another clinical trial within the last 30 days.

Positive pregnancy test (day A) or lactating.

Cancellation of informed consent.

Psychiatric or neurologic disease including epilepsy, cerebrovascular disturbance or traumatic injury.

Important or untreated disease including severe uncontrolled diabetes, ischemia, infarct, unstable angina pectoris or uncontrolled high blood pressure.

Clinically relevant allergic symptoms.

Detection of alcohol at the time of initial examination (day SC) or on study day A and B (positive alcohol test).

Detection of drugs (positive drug test) at the time of initial examination (day SC) or drug abuse within the last 6 months.

Consumption of clinically relevant medication during last fourteen days before and during the active study period based on the notification of the subject or his case history.

Consumption of medication with primarily central action (*i.e.* psychotropic drugs or centrally acting antihypertensive).

Known intolerance/hypersensitivity (allergy) to plant derived extracts or any of the ingredients of the investigational product (anamnestic).

Presence of a rare genetic disease such as fructose intolerance, glucose-galactose malabsorption or sucrase-isomaltase deficiency (anamnestic).

Consumption of unusual quantities or misuse of coffee (more than 4 cups a day), tea (more than 4 cups a day) or tobacco (more than 20 cigarettes per day).

Smoking on day A and day B.

For safety reasons ECG was recorded in addition to a clinical examination. In addition, a pregnancy test, an alcohol test, a drug test and a blood examination was performed. For the blood and urine analysis MVZ Labordiagnostik Mittelhessen GmbH, Ursulum 1, D-35389 Giessen, Germany was responsible.

2.2. Psychometric Testing

Six cognitive test were performed before and 1 h after intake of trial prepara-

tions: d2-Test (**d2-Test**), Memory Test (**ME-Test**), Calculation Performance Test (**CPT-Test**), Reaction Time Test (**RT-Test**), Number Identifying Test (**NIT-Test**), Number Connection Test (**NCT-Test**). An overview on the time line of an experimental day is given in Figure 1.

The duration of the tests was 3 minutes in general except for the d2-test, which lasted 5 minutes. In comparison to the psychometric tests as already published [17] 3 more tests were presented: Reaction Time Test, Number Identifying Test and Number Connection Test. Result of the reaction time test is given in milliseconds. Results from all other tests are given according to the formula: total number of answers multiplied by the percentage of correct answers divided by 10,000.

2.3. EEG Recording

The EEG was recorded bipolarly from 17 surface electrodes according to the International 10/20-system [18] with Cz as physical reference electrode (**C**omputer **a**ided **t**opographical **e**lectro**e**ncephalo**m**etry: CATEEM®) using an electro cap. Before psychometric testing 6 minutes were recorded under the resting "eyes open" condition. For a detailed description of the procedure please refer to [10]. EEG data were recorded twice: before (baseline) and 1h after the intake of the medication. Between the measurements subjects spent their time in the facility's recreation room. All experiments took place at the same time of the day (starting at 7 o'clock in the morning). Quantitative evaluation was performed by using source density analysis except for the recording condition "eyes closed" [19] [20].

2.4. Questionnaires

The Profile of Mood States (POMS) is a psychological rating scale used to assess transient, distinct mood states. The POMS assessment provides a rapid method of assessing transient, fluctuating active mood states. It is an ideal instrument for measuring and monitoring treatment change in clinical, medical, and addiction counseling centers. It is also well suited to clinical drug trials because its sensitivity to change allows you to accurately document the effects of drugs on mood

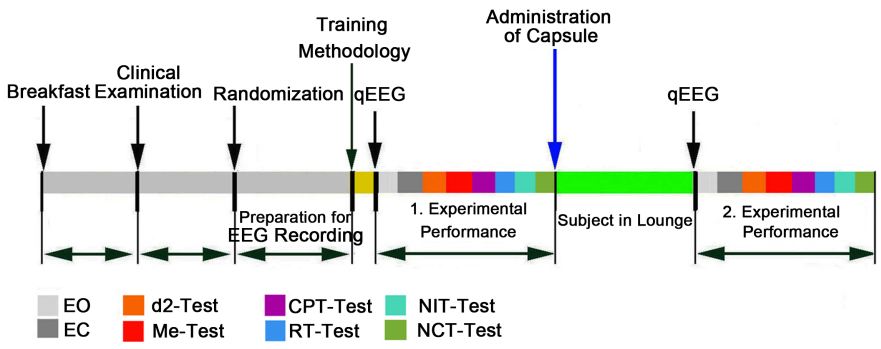

Figure 1. Time line of the experimental day A and B. Performance: Eyes open (**EO**), Eyes closed (**EC**) and different cognitive tests, d2-Test (**d2-Test**), Memory Test (**ME-Test**), Calculation Performance Test (**CPT-Test**), Reaction Time Test (**RT-Test**), Number Identifying Test (**NIT-Test**), Number Connection Test (**NCT-Test**).

state. The POMS is a standard validated psychological test formula [21] [22]. The questionnaire contains 65 words/statements that describe feelings people have. The test is required to indicate for each word or statement how one has been feeling in the past week including today.

Score 1: Dejection (Niedergeschlagenheit)

Score 2: Sullenness (Missmut)

Score 3: Fatigue (Müdigkeit)

Score 4: Thirst for action (Tatendrang)

The Hamilton Anxiety Rating Scale (HAM-A, [23]) (Hamilton Anxiety Scale, CIPS: Collegium Internationale Psychiatriae Scalarum) is a psychological questionnaire used to rate the severity of a patient's anxiety. Anxiety can refer to things such as "a mental state, a drive, a response to a particular situation, a personality trait and a psychiatric disorder". It was published by [23]. The scale consists of 14 items designed to assess the severity of a patient's anxiety. Each of the 14 items contains a number of symptoms, and each group of symptoms is rated on a scale of zero to four, with four being the most severe. All of these scores are used to compute an overarching score that indicates a person's anxiety severity. The questionnaire was performed on day A and day B.

The sleep questionnaire used was the so-called "Schlaffragebogen" B (SF-B) and is used for quantitative and qualitative description and evaluation of sleep behavior and sleep experience (CIPS: Collegium Internationale Psychiatriae Scalarum). The SF-B comprises 31 questions and refers to the past two weeks [24].

2.5. Statistical Evaluation

EEG data from the first recording session before intake of the capsules are given as absolute numbers (μV^2). For explorative statistical evaluation of efficacy against placebo the non-parametric Wilcoxon test was used. For mathematical differentiation of the different mental loads the linear discriminant analysis according to Fischer was used. Results from the first three discriminant functions were projected into space (X, Y and Z coordinates), whereas results from the fourth to sixth discriminant functions were coded into red, green and blue color, respectively, followed by an additive color mixture (so-called RGB-mode). In order to document statistically the different electric reaction of the brain to various cognitive loads, data from test were compared to the data obtained during eyes open (6 minutes) at the beginning. Comparison of 25 mg or 50 mg Zembrin® capsules versus placebo was accomplished by evaluation of the second recording of the day 60 minutes after intake. Spectral power of EEG data from the first recording (baseline) was set to 100% and electrophysiological changes produced by placebo or Zembrin® 25 mg or 50 mg capsules are depicted as %-changes thereof. Estimation of the number of subjects to be included into the study was performed by considering data from earlier experimental trials as obtained under a similar experimental design.

3. Results

3.1. Data Set Analysis/Fundamental Basis

Efficacy evaluation took place on three different levels of evidence: filling out different questionnaires (POMS, HAM-A, SF-B), performance of six psychometric tasks and recording of quantitative EEG during performance of the psychometric tasks. Sixty subjects were recruited and asked to visit the lab at two experimental days 6 weeks apart during which they had to take in daily placebo, 25 mg or 50 mg of Zembrin®. Electric brain activity was recorded under several different conditions. First recording was always done in a relaxed state with open and eyes closed. After this, 6 different mental challenges were presented during quantitative EEG recording. EEG data are documented initially as absolute spectra power (μV^2) for each electrode position (brain area) and each frequency range (delta to beta2). There were no major differences between the three groups with respect to median values of spectral power in the six frequency ranges. Absolute power values from the baseline recording with respect to all recording conditions were therefore set to 100%. Drug induced changes are documented as pre-post intake comparison in % of these baseline values for every recording condition. An overview on the absolute spectral power values during recording in the relaxed state on the first and on the last day is given in Table 1 and Table 2.

Table 1. Absolute spectral power values depicted as μV^2 for each electrode position for the placebo group, the 25 mg and 50 mg of Zembrin® groups with respect to all frequency ranges (delta to beta2). Data are given for the recording on the first day (acute). **M** = median value. **E** = indicates electrode positions according to the so-called 10/20 system [18]. **Pl** = placebo; **25 mg** = ZEMBRIN® 25 mg and **50 mg** = ZEMBRIN® 50 mg.

Absolute values of "eyes open" 0 h Acute																		
	Delta			Theta			Alpha1			Alpha2			Beta1			Beta2		
E	Pl n = 20	25 mg n = 20	50 mg n = 19	Pl n = 20	25 mg n = 20	50 mg n = 19	Pl n = 20	25 mg n = 20	50 mg n = 19	Pl n = 20	25 mg n = 20	50 mg n = 19	Pl n = 20	25 mg n = 20	50 mg n = 19	Pl n = 20	25 mg n = 20	50 mg n = 19
Cz	1.90	1.66	1.43	0.44	0.65	0.43	0.66	0.60	0.75	0.44	0.76	0.71	0.69	1.50	0.82	1.46	1.64	0.89
Fz	2.28	2.31	2.17	0.60	0.75	0.73	0.70	0.86	0.86	0.55	0.77	0.66	0.67	0.88	0.82	1.14	1.41	0.97
F3	2.74	2.49	2.83	0.65	0.64	0.66	0.73	0.79	1.07	0.53	0.73	0.89	1.37	1.45	1.38	3.41	2.26	2.17
C3	1.53	1.62	1.51	0.42	0.51	0.38	0.63	0.77	0.87	0.63	1.07	1.06	1.51	1.84	1.58	1.90	2.03	1.38
P3	1.07	1.29	1.01	0.29	0.35	0.34	0.36	0.39	0.71	0.45	0.54	0.74	0.69	0.78	0.80	0.67	0.61	0.58
Pz	1.11	1.62	1.42	0.39	0.61	0.36	0.48	0.64	0.57	0.53	0.59	0.58	0.73	1.10	0.93	0.79	0.79	0.76
P4	0.91	1.39	1.12	0.25	0.38	0.38	0.43	0.41	0.55	0.52	0.72	0.88	0.78	1.03	1.05	0.62	0.88	0.67
C4	1.78	1.51	1.31	0.52	0.62	0.38	0.68	0.70	0.83	0.76	1.41	0.92	1.35	1.93	1.61	2.10	2.50	1.62
F4	2.59	2.27	2.80	0.71	0.68	0.69	0.75	0.76	1.11	0.62	0.87	0.83	0.96	1.42	1.05	2.18	2.20	1.96
F7	7.96	7.86	8.59	1.46	1.83	1.63	1.39	1.41	2.37	1.48	1.41	2.10	2.18	2.01	2.68	4.79	2.72	4.21
T3	2.75	3.90	3.75	0.81	1.26	1.02	1.26	1.37	2.13	1.32	1.59	2.18	2.91	2.53	2.35	5.25	3.60	2.83
T5	2.20	2.92	2.31	0.67	0.94	0.72	0.93	1.11	1.71	1.21	1.29	1.67	1.73	2.26	2.02	2.40	1.75	1.62
01	2.73	3.34	3.81	0.64	0.95	0.74	0.83	0.99	0.92	0.85	1.34	1.13	1.61	1.92	1.85	2.88	2.47	2.60
02	2.50	3.08	2.69	0.77	0.84	0.66	0.80	0.93	1.31	0.82	1.14	1.13	1.72	1.75	1.63	2.46	3.13	2.11
T6	2.17	3.82	3.15	0.70	0.95	1.01	0.97	1.09	2.29	1.47	1.91	1.89	2.04	2.44	2.65	2.17	2.33	1.53
T4	3.41	4.14	3.52	0.90	0.84	0.89	1.22	1.08	1.98	1.29	1.64	2.00	2.38	2.85	2.63	3.35	3.25	2.85
F8	6.12	6.30	9.01	1.34	1.56	1.49	1.45	1.73	2.37	1.27	2.00	2.10	2.28	3.32	2.63	4.02	6.28	3.88
M	2.43	2.27	2.23	0.57	0.73	0.66	0.79	0.81	1.00	0.71	1.11	1.22	1.27	1.71	1.55	2.25	2.20	1.94

Table 2. Absolute spectral power values depicted as μV^2 for each electrode position for the placebo group, the 25 mg and 50 mg of Zembrin® groups with respect to all frequency ranges (delta to beta2). Data are given for the recording on the last day (after repetitive dosing). **M** = median value. **E** = indicates electrode positions according to the so-called 10/20 system [18]. **Pl** = placebo; **25 mg** = ZEMBRIN® 25 mg and **50 mg** = ZEMBRIN® 50 mg.

Absolute values of "eyes open" 0 h Repetitive																		
E	Delta			Theta			Alpha1			Alpha2			Beta1			Beta2		
	Pl n = 20	25 mg n = 20	50 mg n = 19	Pl n = 20	25 mg n = 20	50 mg n = 19	Pl n = 20	25 mg n = 20	50 mg n = 19	Pl n = 20	25 mg n = 20	50 mg n = 19	Pl n = 20	25 mg n = 20	50 mg n = 19	Pl n = 20	25 mg n = 20	50 mg n = 19
Cz	2.06	2.23	1.89	0.52	0.59	0.45	0.47	0.50	0.72	0.47	0.62	0.57	0.75	1.02	0.81	1.33	1.58	1.02
Fz	2.71	3.05	2.58	0.72	0.77	0.75	0.79	0.63	1.05	0.46	0.54	0.56	0.78	0.83	0.78	1.30	1.06	1.03
F3	2.35	4.37	3.37	0.59	0.78	0.90	0.69	0.81	1.35	0.53	0.57	1.01	1.29	1.22	1.07	2.54	2.14	2.17
C3	1.60	2.37	1.57	0.46	0.71	0.39	0.60	0.68	0.78	0.69	0.96	0.72	1.60	1.82	1.28	1.55	2.52	1.55
P3	1.01	1.11	1.00	0.34	0.35	0.33	0.43	0.40	0.98	0.46	0.59	0.79	0.63	0.89	0.82	0.82	0.84	0.59
Pz	1.50	1.27	1.73	0.44	0.43	0.54	0.60	0.47	0.80	0.46	0.49	0.91	0.81	0.93	0.97	0.71	0.77	0.70
P4	1.31	0.91	1.02	0.38	0.30	0.31	0.74	0.33	0.69	0.47	0.63	0.59	0.75	0.68	0.75	0.64	0.74	0.59
C4	1.84	1.75	1.28	0.53	0.44	0.45	0.81	0.68	0.96	0.74	1.09	0.98	1.84	1.76	1.33	1.97	2.02	1.25
F4	2.52	3.39	2.46	0.56	0.66	0.58	0.65	0.70	1.15	0.60	0.71	1.00	1.24	1.11	1.09	1.74	2.14	2.58
F7	12.17	10.72	10.44	1.72	1.77	1.67	1.90	1.70	2.49	1.50	1.49	2.32	2.45	2.33	2.63	3.47	3.88	3.77
T3	3.07	4.13	4.73	0.67	0.92	0.91	0.88	1.00	2.06	1.14	1.95	2.07	2.43	2.57	3.25	3.28	2.93	3.16
T5	2.71	2.83	2.00	0.84	0.73	0.62	1.29	1.06	1.53	1.25	1.19	1.29	1.54	1.79	1.24	1.79	1.54	1.56
01	2.84	4.34	3.08	0.73	1.02	0.76	0.86	0.89	0.95	0.83	1.13	0.87	1.35	2.59	1.28	2.00	3.60	1.86
02	2.63	3.01	2.36	0.80	0.80	0.74	1.02	0.89	1.00	0.85	1.15	0.90	2.29	1.25	1.97	2.14	2.11	2.06
T6	3.43	2.78	2.72	1.04	0.68	0.82	1.11	1.02	2.79	1.49	1.39	2.07	2.55	2.20	2.54	1.66	1.78	1.54
T4	3.15	3.40	3.17	0.78	0.95	0.77	1.12	1.48	1.64	1.11	1.42	1.43	2.65	3.51	2.35	2.67	4.61	3.27
F8	7.64	7.66	8.40	1.20	1.46	1.39	1.26	1.34	2.29	1.01	1.58	2.13	1.82	3.03	2.61	3.72	4.83	3.54
M	2.65	2.96	2.61	0.64	0.71	0.68	0.82	0.73	1.20	0.72	1.00	1.07	1.49	1.74	1.28	1.94	2.19	1.66

Analysis of the spectral power in all participating subjects before intake of trial preparations revealed significant test-specific changes with respect to single brain regions (electrode positions) and defined frequency ranges in comparison to the recording during the relaxed state. For sake of easier overview two functionally related brain areas are defined as regions of interest (ROI): the fronto-temporal area represented by electrode positions $F_{z,3,4,7,8}$ T_{3456} and the centro-parieto-occipital area represented by electrode positions $C_{3,4}$ $P_{z,3,4}$ $O_{1,2}$. With respect to delta power increases were observed during performance of all tests, in comparison to the relaxed state, which were statistically significant in comparison to the relaxed state except for the reaction time test (RT) and number connection test (NCT). Theta waves increased during performance of the d2-concentration test (d2-test), the number identification and number connection test in a significant manner in comparison to the relaxed state. Alpha1 frequencies were attenuated in the memory test, the calculation test and both number identification and connection tests. Alpha2 waves were attenuated during performance

of all tests except for the d2-test. Beta waves changed only slightly. Details and statistical significances are given in Table 3 for the fronto-temporal region of interest. Changes in the centro-parieto-occipital area were a little bit different. Increases in the slow wave frequencies delta and theta are not so prominent, but attenuation of alpha and beta1 waves was highly significantly stronger in comparison to data recorded during the relaxed state. Details are documented in Table 4.

3.2. Efficacy of Zembrin® in the Relaxed State (EO)

Zembrin® was administered as a single 25 mg or 50 mg dose on the first experimental day and then continued daily for six weeks. Measurements took place on the first day (day A) and one day after the continuous intake for 6 weeks (day B). Results of the placebo group are depicted in Figure 2 (first day). Concomitant performance of psychometric tests and EEG recordings revealed quantitative differences between placebo and the two dosages of Zembrin® with respect to all recording conditions.

Effects of the lower dosage of 25 mg Zembrin® in comparison to placebo revealed statistically significant increases of spectral delta and theta power in central brain regions and at electrode position T_4 during the "eyes open" recording condition on the first recording day (Figure 2). On the last day, only some decreases of slow waves (C_3 and F_8) and increases of beta power emerged (not shown). The higher dosage of 50 mg of Zembrin® induced statistically significant

Table 3. Changes of spectral power during tests in comparison to the recording condition "eyes open" in relaxed state at fronto-temporal electrode positions (F_{z3478} T_{3456}). Eyes open (EO), d2-concentration test (d2-Test), memory test (ME-Test), arithmetic calculation test (CPT-Test), reaction time test (RT-Test), number identification test (NIT), number connection test (NCT). P-values calculated according to sign test.

	F_{z3478} T_{3456}					
[μV²] n = 59	Delta	Theta	Alpha1	Alpha2	Beta1	Beta2
Eyes open	**3.00**	**0.83**	**1.04**	**1.29**	**2.02**	**2.70**
d2-Test	3.79	0.96	1.14	1.08	2.08	3.86
p =	0.006	0.004				
ME-Test	4.09	0.88	0.91	1.03	1.88	2.64
p =	0.000005		0.000001	0.0002	0.02	
CPT-Test	3.99	0.91	0.98	1.06	1.65	2.44
p =	0.003		0.002	0.04	0.06	
RT-Test	3.22	0.82	1.03	0.96	1.87	2.76
p =		0.02		0.00007		
NIT-Test	3.96	0.96	0.98	1.07	2.00	2.79
p =	0.002		0.02	0.04		
NCT-Test	3.63	0.93	1.00	1.10	1.99	3.05
p =	0.008	0.07	0.036			0.04

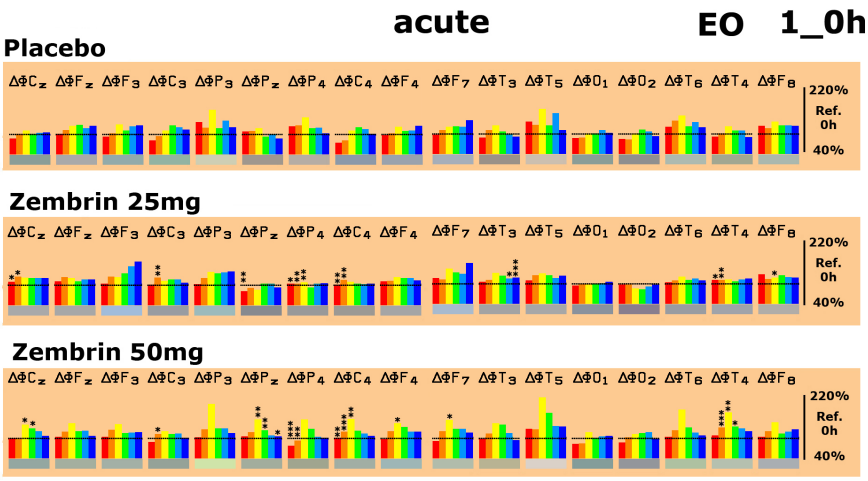

Figure 2. Effects of Zembrin® on spectral frequencies in different regions of the brain on the first recording day (acute). Changes of spectral power are depicted in percent of the pre-drug baseline recording on the ordinate. Electrode positions are labeled as C for central, F for frontal, P for parietal, T for temporal and O for occipital. Even numbers describe data from the right hemisphere, uneven numbers from the left hemisphere. Red bars: delta, orange: theta, yellow: alpha1, green: alpha2, turquoise: beta1, blue: beta2 spectral power. Error probability is marked by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

Table 4. Changes of spectral power during tests in comparison to the recording condition "eyes open" in relaxed state at centro-parietal-occipital electrode positions ($C_{34}P_{z34}O_{12}$). Eyes open (EO), d2-concentration test (d2-Test), memory test (ME-Test), arithmetic calculation test (CPT-Test), reaction time test (RT-Test), number identification test (NIT), number connection test (NCT). *P*-values calculated according to sign test.

	$C_{34}P_{z34}O_{12}$					
[μV²] n = 59	Delta	Theta	Alpha1	Alpha2	Beta1	Beta2
Eyes open	**1.73**	**0.52**	**0.74**	**0.77**	**1.44**	**1.73**
d2-Test	2.07	0.53	0.51	0.48	0.85	1.62
p =	0.000005		0.00002	0.00000001	0.000005	
ME-Test	1.76	0.48	0.52	0.55	1.01	1.52
p =		0.00002	0.00000001	0.0000003	0.000001	0.009
CPT-Test	1.79	0.45	0.44	0.49	0.86	1.38
p =	0.07	0.00002	0.00000001	0.00000001	0.00000001	
RT-Test	1.54	0.43	0.49	0.57	1.06	1.61
p =	0.07	0.00000001	0.00000001	0.00007	0.00007	
NIT-Test	1.70	0.51	0.48	0.51	0.95	1.39
p =			0.00002	0.0000003	0.0006	0.07
NCT-Test	1.98	0.51	0.47	0.47	0.69	1.20
p =	0.0006		0.000001	0.00000001	0.00007	0.004

increases of theta frequencies at C_4 and T_4 as well as increases of alpha1 frequencies in 6 different brain regions. On the last day (day B), dominant increases of theta activity were observed mostly in fronto-temporal brain areas. In addition, alpha wave increases were seen not only in fronto-temporal areas but also in the parietal region at electrode positions P_3 (not shown).

3.3. Efficacy of Zembrin® during Performance of the d2-Concentration Test (d2)

During performance of the d2-concentration test (d2) a dose dependent effect was observed at the first day of recording. Whereas the lower dosage of 25 mg hardly induced spectral changes, the higher dosage induced statistically significant increases of local delta, theta and alpha1 spectral power in comparison to placebo, mainly in frontal and temporal brain areas (electrode positions F_3, F_4, T_3 and T_4). Complete data with respect to all brain areas are documented in Figure 3. Differences in comparison to placebo at the last day of recording were less pronounced with respect to delta and theta power. However, with respect to alpha1 waves a statistically conspicuous increase was observed in central and parietal brain areas in the presence of the higher dosage (not shown). A significant increase of alpha2 spectral power was only seen within the right hemisphere at the central and temporal area. There was also some indication of a temporal increase of beta waves at the last day (not shown).

3.4. Efficacy of Zembrin® during Performance of the Memory Test (ME)

During performance of the memory test (ME) on the first day of recording statistically significant focal increases of delta power emerged in frontal (F_z and F_7) and temporal areas (T_3 and T_4) already in the presence of the lower dosage. In the presence of the higher dosage a trend to increases of central delta power was seen. Within the right temporal lobe (T_4) and centrally (C_4) statistically significant increases of alpha1 and alpha2 spectral power emerged besides a conspi-

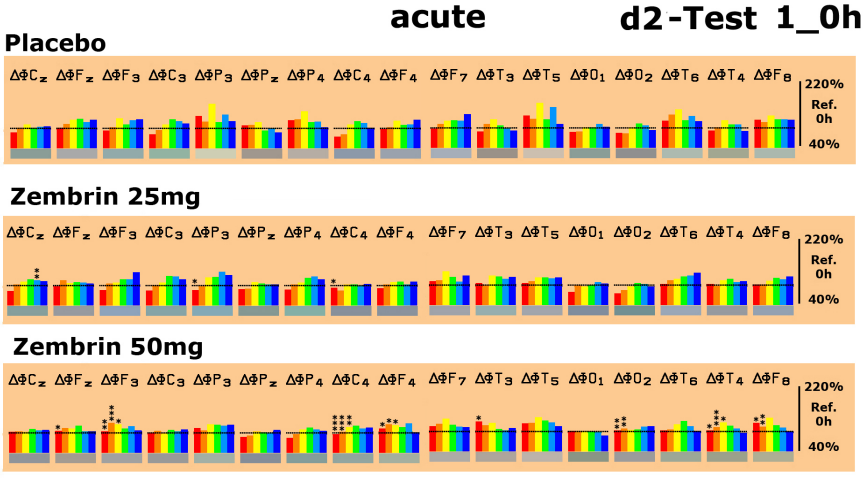

Figure 3. Effect of Zembrin® in comparison to placebo on spectral frequencies in different regions of the brain on the first recording day during performance of the d2-test (d2). Changes of spectral power are depicted in percent of the pre-drug baseline recording on the ordinate. Electrode positions are labeled as C for central, F for frontal, P for parietal, T for temporal and O for occipital. Even numbers describe data from the right hemisphere, uneven numbers from the left hemisphere. Red bars: delta, orange: theta, yellow: alpha1, green: alpha2, turquoise: beta1, blue: beta2 spectral power. Error probability is marked by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

cuous increase of beta power in the temporal lobe. Complete data with respect to all brain areas are documented in Figure 4. At the last day (day B) of recording no spectral changes were observed in the presence of the lower dosage of Zembrin®. In the presence of the higher dosage some statistically conspicuous increases of alpha1 power were detected in the central, temporal and occipital region in comparison to placebo (not shown).

3.5. Efficacy of Zembrin® during Performance of the Arithmetic Calculation Test (CPT)

During performance of the arithmetic calculation test (CPT) hardly any change of spectral power was detected in the presence of the lower dose of Zembrin® on the first day of recording. In the presence of the higher dose a statistically significant increase of all frequencies was recognized at the right central electrode position C_4 and of alpha2 and beta at C_3. Statistically significant increases of alpha1 and alpha2 spectral power were seen in central and right temporal regions. Likewise, statistically significant increases of beta1 and beta2 power in central, temporal and occipital brain areas were recognized. Complete data with respect to all brain areas are documented in Figure 5. On the last day of recording after repetitive dosing no statistically significant changes of spectral power were found. Increases of alpha power were observed in central and parietal brain areas, but did not reach statistical significance (not shown).

3.6. Efficacy of Zembrin® during Performance of the Reaction Time Test (RT)

During performance of the reaction time test (RT) on the first day (day A) of

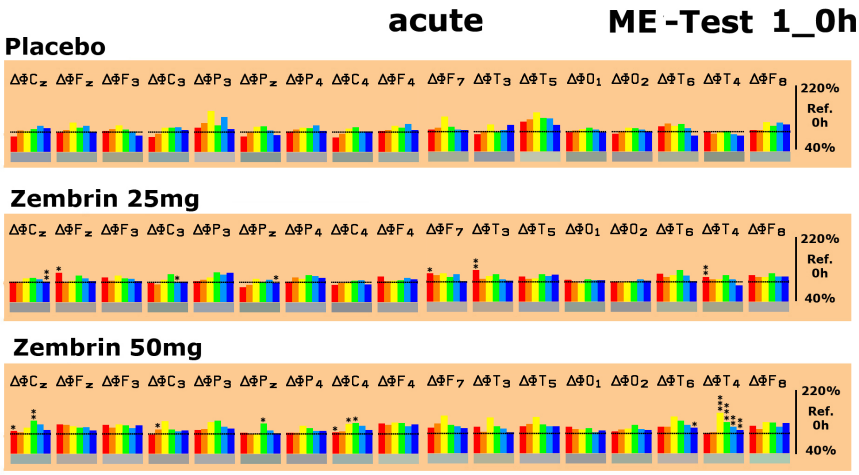

Figure 4. Effect of Zembrin® in comparison to placebo on spectral frequencies in different regions of the brain on the first recording day during performance of the memory-test (ME). Changes of spectral power are depicted in percent of the pre-drug baseline recording on the ordinate. Electrode positions are labeled as C for central, F for frontal, P for parietal, T for temporal and O for occipital. Even numbers describe data from the right hemisphere, uneven numbers from the left hemisphere. Red bars: delta, orange: theta, yellow: alpha1, green: alpha2, turquoise: beta1, blue: beta2 spectral power. Error probability is marked by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

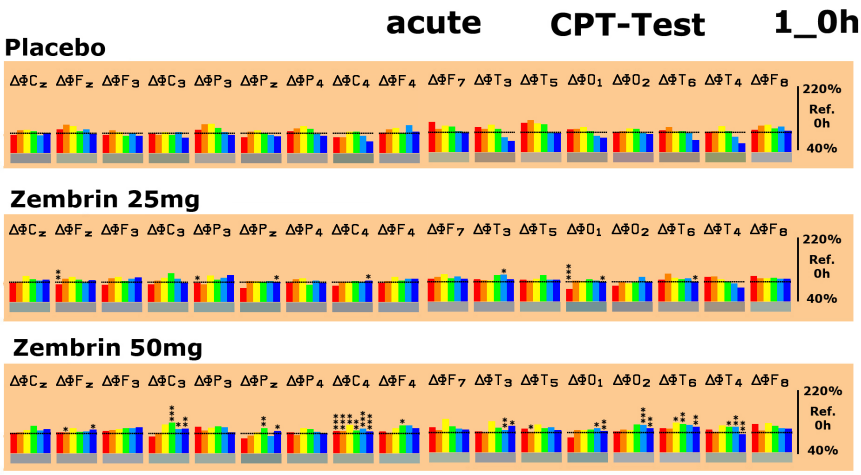

Figure 5. Effect of Zembrin® in comparison to placebo on spectral frequencies in different regions of the brain on the first recording day during performance of the arithmetic calculation test (CPT). Changes of spectral power are depicted in percent of the pre-drug baseline recording on the ordinate. Electrode positions are labeled as C for central, F for frontal, P for parietal, T for temporal and O for occipital. Even numbers describe data from the right hemisphere, uneven numbers from the left hemisphere. Red bars: delta, orange: theta, yellow: alpha1, green: alpha2, turquoise: beta1, blue: beta2 spectral power. Error probability is marked by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

recording no spectral changes were observed after intake of the lower dose. However, after intake of the higher dose highly significant focal increases of delta and theta power were documented for the right central electrode position C_4, similar to changes as observed during arithmetic calculation (CPT). In addition, a tendency to increases of alpha1 and alpha2 power was recognized in fronto-temporal brain areas (alpha1 statistically significant in the right temporal lobe at T_4 and T_6, alpha2 at positions T_3 and T_6). Complete data with respect to all brain areas are documented in **Figure 6**. After the repetitive dosing, only marginal spectral changes were observed during performance of the reaction time test. Some statistically conspicuous increases of beta power were seen in the presence of both dosages (not shown).

3.7. Efficacy of Zembrin® during Performance of the Number Identification Test (NIT)

During performance of the number identification test (NIT) statistically conspicuous or significant increases of delta power were recognized in all temporal brain areas on the first day of recording in the presence of the lower dosage. After intake of the higher dosage some increases of theta spectral power reached statistical significance in comparison to placebo. These changes were accompanied by a highly significant increase of alpha2 power in the temporal lobe at electrode position T_6 and frontally at F_7. Finally, a trend of beta wave increase became visible. Complete data with respect to all brain areas are documented in **Figure 7**. After 6 weeks of daily intake of Zembrin® no major changes of spectral power except for some decreases of power in the alpha and beta range were seen (not shown).

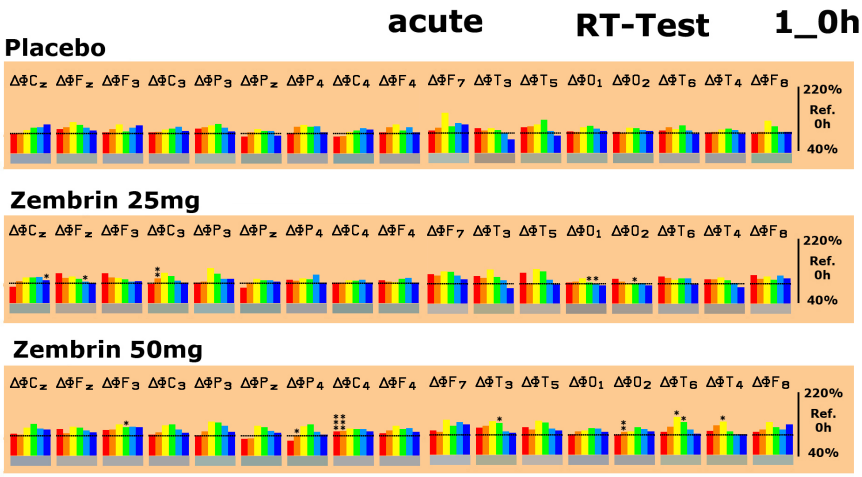

Figure 6. Effect of Zembrin® in comparison to placebo on spectral frequencies in different regions of the brain on the first recording day during performance of the reaction time test (RT). Changes of spectral power are depicted in percent of the pre-drug baseline recording on the ordinate. Electrode positions are labeled as C for central, F for frontal, P for parietal, T for temporal and O for occipital. Even numbers describe data from the right hemisphere, uneven numbers from the left hemisphere. Red bars: delta, orange: theta, yellow: alpha1, green: alpha2, turquoise: beta1, blue: beta2 spectral power. Error probability is marked by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

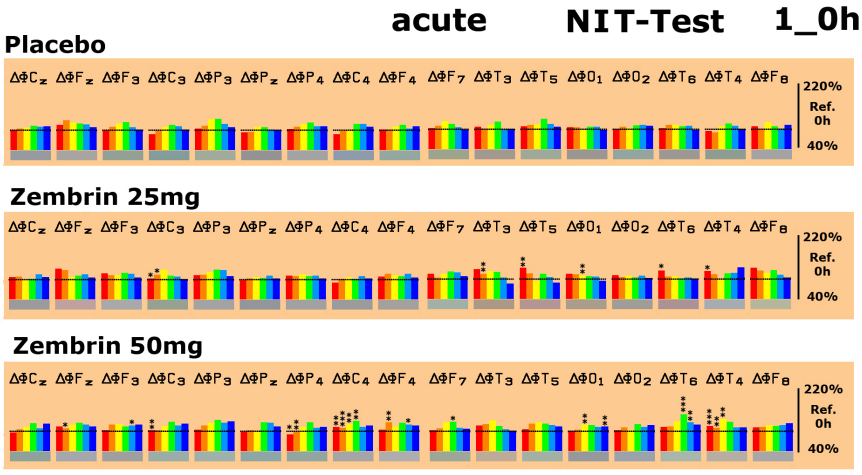

Figure 7. Effect of Zembrin® in comparison to placebo on spectral frequencies in different regions of the brain on the first recording day during performance of the number identification test (NIT). Changes of spectral power are depicted in percent of the pre-drug baseline recording on the ordinate. Electrode positions are labeled as C for central, F for frontal, P for parietal, T for temporal and O for occipital. Even numbers describe data from the right hemisphere, uneven numbers from the left hemisphere. Red bars: delta, orange: theta, yellow: alpha1, green: alpha2, turquoise: beta1, blue: beta2 spectral power. Error probability is marked by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

3.8. Efficacy of Zembrin® during Performance of the Number Connection Test (NCT)

During performance of the number connection test (NCT) on the first day of recording statistically significant increases of spectral power were observed in

the presence of the lower dosage only with respect to frontal theta power (electrode positions F_7 and F_8). However, in the presence of the higher dosage statistically significant increases of alpha1 and alpha2 power were recognized in frontal (F_3 and F_4) central (C_3 and C_4), temporal ($T_{4,5,6}$) and occipital brain areas ($O_{1,2}$). With respect to beta1 power only less prominent increases were seen. Complete data with respect to all brain areas are documented in Figure 8. After 6 weeks of daily intake, fronto-temporal increases of alpha1 power were still visible but did not reach statistical significance in comparison to placebo. Significant increases of theta power were confined to electrode positions C_3 and T_3 (not shown).

3.9. Efficacy of Zembrin® in Brain Regions of Interest

Since different brain areas are functionally connected, analysis of the efficacy of Zembrin® was calculated for two regions of interest: the fronto-temporal area and the centro-occipital area. There is an obvious dose dependence with respect to administration of Zembrin®, since the lower dose induced a statistically significant increase of delta waves only during performance of the number identification test on the first day. Statistically significant increases of spectral power in the presence of the higher dose were observed during several tests on both days of recording. There was a highly significant increase of delta and theta power during performance of the d2-test on the first day of recording ($p < 0.01$ and $p < 0.05$, respectively). During the number connection test, highly significant increases of alpha1 and alpha2 spectral power were recognized on the first day of recording. On the last day of recording, a highly significant increase of

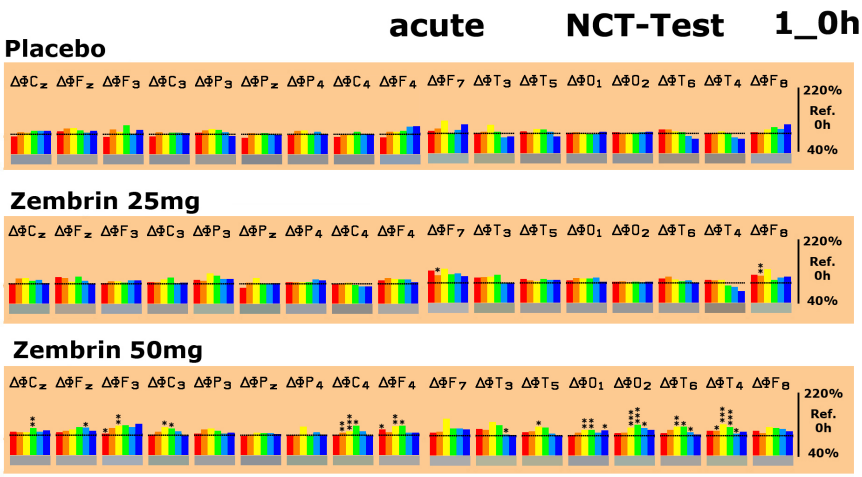

Figure 8. Effect of Zembrin® in comparison to placebo on spectral frequencies in different regions of the brain on the first recording day during performance of the number connection test (NCT). Changes of spectral power are depicted in percent of the pre-drug baseline recording on the ordinate. Electrode positions are labeled as C for central, F for frontal, P for parietal, T for temporal and O for occipital. Even numbers describe data from the right hemisphere, uneven numbers from the left hemisphere. Red bars: delta, orange: theta, yellow: alpha1, green: alpha2, turquoise: beta1, blue: beta2 spectral power. Error probability is marked by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

theta power emerged. With respect to the d2-test now some increase of theta power and a highly significant increase of alpha1 power were seen. During the memory test and the number connection test a significant increase of alpha1 power was documented. An overview for the fronto-temporal area is given in Figure 9.

In the centro-occipital region the lower dose of 25 mg induced statistically significant increases of delta and theta power during relaxation on the first recording day. The higher dose of 50 mg induced conspicuous (delta and alpha1) or statistically significant (theta) increases of spectral power. The lower dose induced a statistically conspicuous increase of beta2 and the higher dose highly significant increases of beta1 and beta2 besides a conspicuous increase of alpha1 power during performance of the arithmetic calculation test. During performance of the reaction time test both dosages led to statistically significant increases of theta power. Performance of the number identification test induced increase of delta, alpha1 and alpha2 waves. Performance of the number connection test induced a highly significant increase of alpha1, significant increase of alpha2 and a conspicuous increase of beta1 power. In the presence of the higher dose most of the recording conditions showed changes of spectral power on the first day, less on the last day. Details are given in Figure 10.

3.10. Efficacy of Zembrin® with Respect to Repetitive Dosing

The question now arose if the daily repetitive dosing of Zembrin® had induced long lasting changes of spectral power in any of the brain regions after 6 weeks. In order to test this possibility, data recorded at the baseline of the first day were set to 100% and spectral power at baseline of the last day was expressed as % of the results of the first day. Results showed that both dosages of Zembrin® had induced long lasting changes of brain responses during all test conditions. For example, during the relaxed state delta and theta power at electrode position P_4, P_z and T_6 were lower after six weeks daily intake of 25 mg Zembrin® in a significant manner. After intake of 50 mg Zembrin® delta and theta power were significantly higher at F_3 and C_3 (Figure 11). During performance of the d2-test the lower dosage significant higher delta activity was induced in comparison to the first day of recording. Larger increases of delta and theta spectral power were observed at electrode positions F_3, C_3 and F_8 in comparison to the original recordings on the first day after daily intake of 50 mg of Zembrin® (Figure 12). After intake of the lower dosage of Zembrin® for 6 weeks a long- lasting change of the brain's response during performance of the memory test was observed as a statistically significant increase of delta spectral power at electrode position T_4. Increases of delta, theta power were seen during performance of the memory test after repetitive intake of the higher dosage in comparison to baseline recording on the first day in frontal (F_3), central (C_3) and occipital (O_1) electrode positions (Figure 13). During performance of the arithmetic calculation test no long-lasting changes of excitability except for delta power at T_4 were seen. Statistically significant general increases of power of all frequencies were observed at baseline recording on the last day after repetitive intake of the higher dosage of Zembrin®

Figure 9. Effect of Zembrin® (25 mg = yellow bars; 50 mg = red bars) on spectral power in all frequency ranges during recording condition "eyes open" and during performance of tests in comparison to placebo (grey bars) **at fronto-temporal electrode positions (F_{z3478} T_{3456}) on day A (first recording) and day B (last recording)**. Statistical significance according non-parametric Wilcoxon test is indicated by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

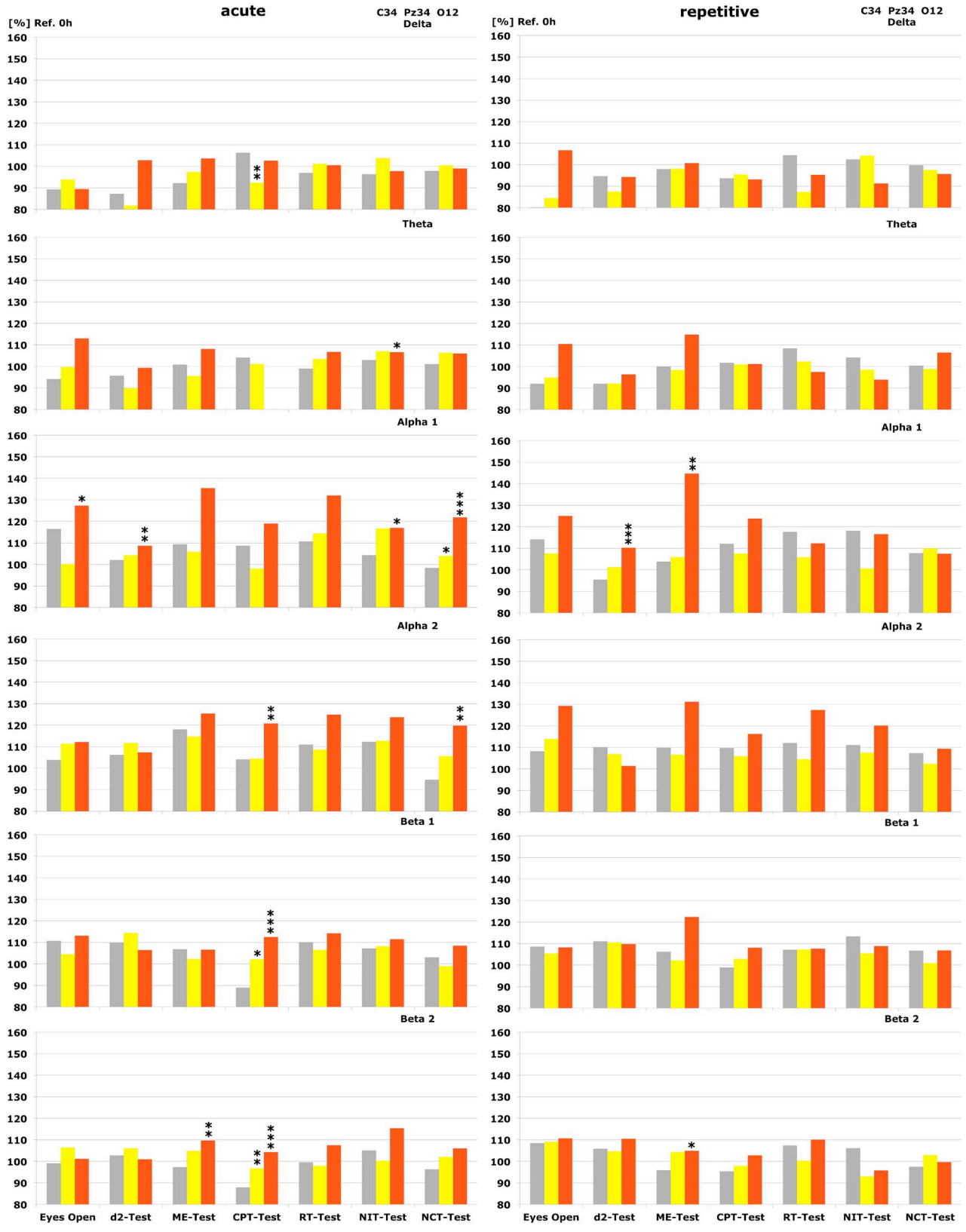

Figure 10. Effect of Zembrin® (25 mg = yellow bars; 50 mg = red bars) on spectral power in all frequency ranges during recording condition "eyes open" and during performance of tests in comparison to placebo (grey bars) at **centro-occipital electrode positions ($C_{z,3,4}$ $O_{1,2}$) on day A (first recording) and day B (last recording)**. Statistical significance according non-parametric Wilcoxon test is indicated by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

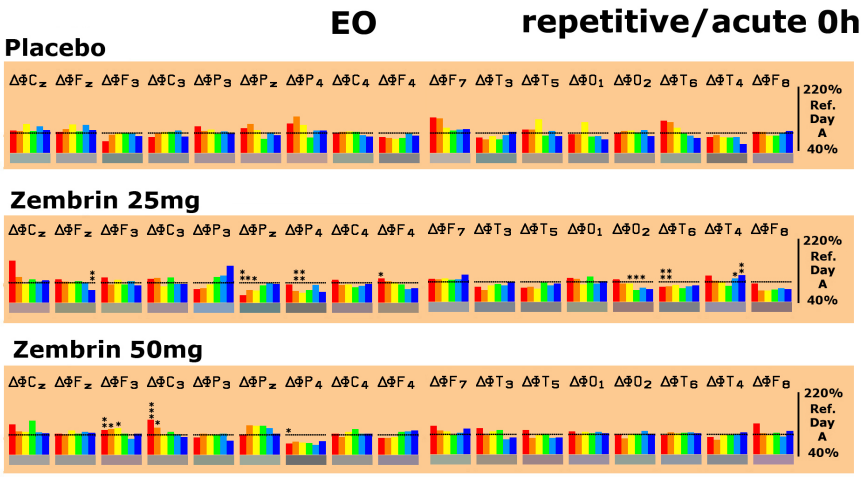

Figure 11. Documentation of permanent changes induced by Zembrin® in comparison to placebo on spectral frequencies in different regions of the brain in percent of the results of the first recording day during relaxation. Electrode positions are labeled as C for central, F for frontal, P for parietal, T for temporal and O for occipital. Even numbers describe data from the right hemisphere, uneven numbers from the left hemisphere. Red bars: delta, orange: theta, yellow: alpha1, green: alpha2, turquoise: beta1, blue: beta2 spectral power. Error probability according to non-parametric Wilcoxon test is marked by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

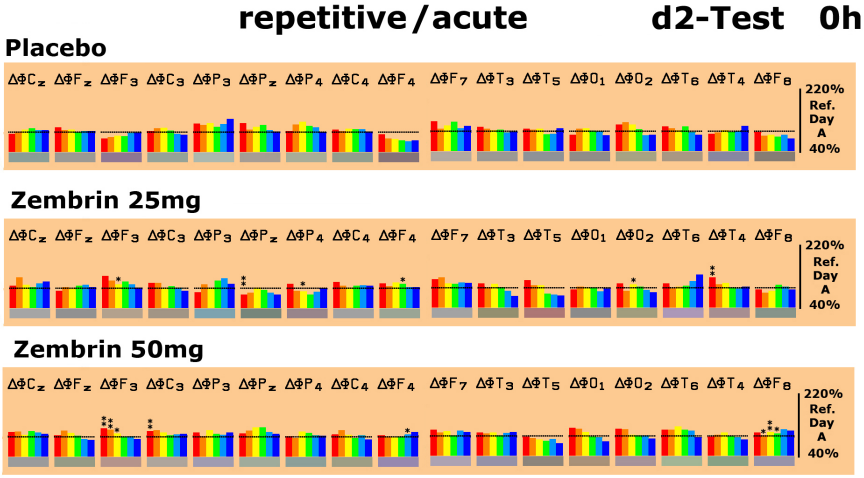

Figure 12. Documentation of permanent changes induced by Zembrin® in comparison to placebo on spectral frequencies in different regions of the brain in percent of the results of the first recording day during performance of the d2-test. Electrode positions are labeled as C for central, F for frontal, P for parietal, T for temporal and O for occipital. Even numbers describe data from the right hemisphere, uneven numbers from the left hemisphere. Red bars: delta, orange: theta, yellow: alpha1, green: alpha2, turquoise: beta1, blue: beta2 spectral power. Error probability according to non-parametric Wilcoxon test is marked by stars: * = $p < 0.10$; ** = $p < 0.05$.

at frontal (F_3), central (C_3) and temporal (T_6) areas (Figure 14). In addition, beta2 power was higher in central (C_z and C_4) and frontal areas (F_4 and F_8) than on the first day of recording during this test. During performance of the reaction time test no major changes were observed after repetitive intake of the lower

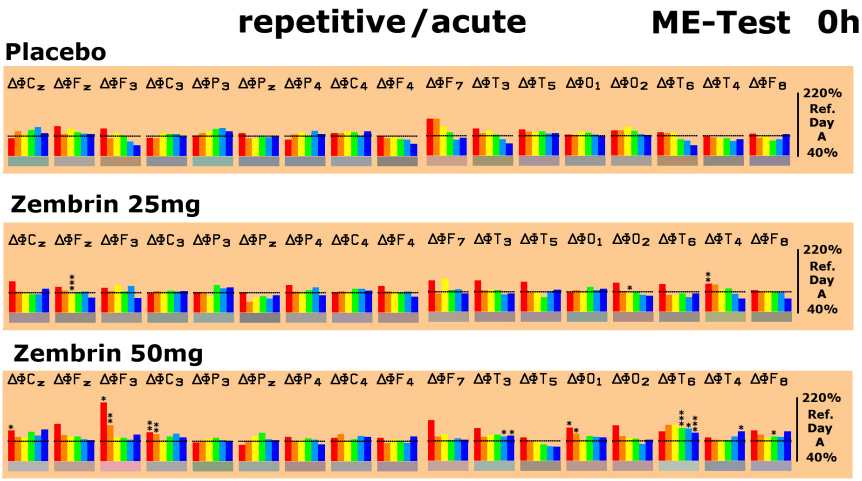

Figure 13. Documentation of permanent changes induced by Zembrin® in comparison to placebo on spectral frequencies in different regions of the brain in percent of the results of the first recording day during performance of the memory-test. Electrode positions are labeled as C for central, F for frontal, P for parietal, T for temporal and O for occipital. Even numbers describe data from the right hemisphere, uneven numbers from the left hemisphere. Red bars: delta, orange: theta, yellow: alpha1, green: alpha2, turquoise: beta1, blue: beta2 spectral power. Error probability according to non-parametric Wilcoxon test is marked by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

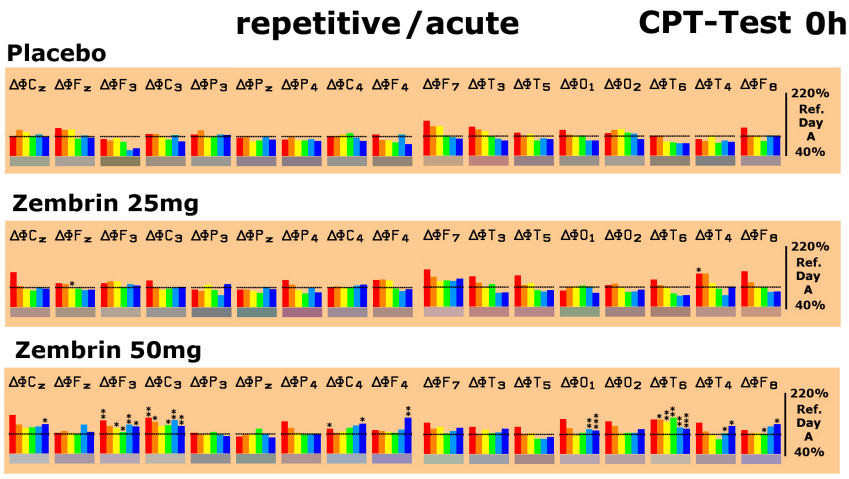

Figure 14. Documentation of permanent changes induced by Zembrin® in comparison to placebo on spectral frequencies in different regions of the brain in percent of the results of the first recording day during performance of the arithmetic calculation test. Electrode positions are labeled as C for central, F for frontal, P for parietal, T for temporal and O for occipital. Even numbers describe data from the right hemisphere, uneven numbers from the left hemisphere. Red bars: delta, orange: theta, yellow: alpha1, green: alpha2, turquoise: beta1, blue: beta2 spectral power. Error probability according to non-parametric Wilcoxon test is marked by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

dosage. Statistically significantly higher alpha1 and alpha2 power became visible after intake of the higher dosage frontally (F_3) and temporally (T_6) (Figure 15). Strongest differences with respect to spectral power of the first day were observed during performance of the number identification and number connection

tests. During performance of these tests significantly higher spectral power was seen within all frequency ranges at several brain regions after daily intake of 50 mg of Zembrin® (Figure 16 and Figure 17).

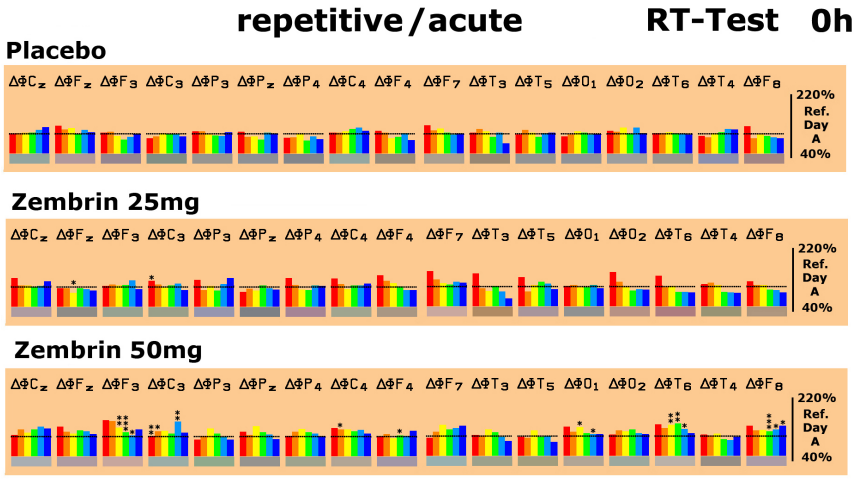

Figure 15. Documentation of permanent changes induced by Zembrin® in comparison to placebo on spectral frequencies in different regions of the brain in percent of the results of the first recording day during performance of the reaction time test. Electrode positions are labeled as C for central, F for frontal, P for parietal, T for temporal and O for occipital. Even numbers describe data from the right hemisphere, uneven numbers from the left hemisphere. Red bars: delta, orange: theta, yellow: alpha1, green: alpha2, turquoise: beta1, blue: beta2 spectral power. Error probability according to non-parametric Wilcoxon test is marked by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

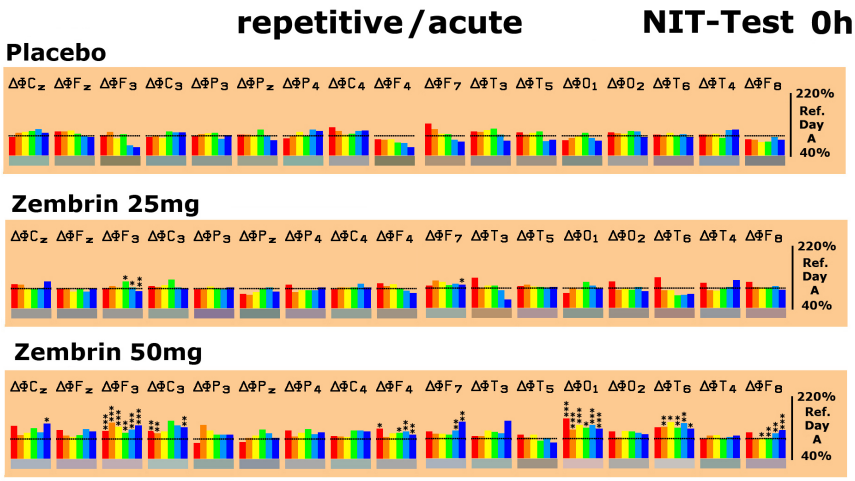

Figure 16. Documentation of permanent changes induced by Zembrin® in comparison to placebo on spectral frequencies in different regions of the brain in percent of the results of the first recording day during performance of the number identification test. Electrode positions are labeled as C for central, F for frontal, P for parietal, T for temporal and O for occipital. Even numbers describe data from the right hemisphere, uneven numbers from the left hemisphere. Red bars: delta, orange: theta, yellow: alpha1, green: alpha2, turquoise: beta1, blue: beta2 spectral power. Error probability according to non-parametric Wilcoxon test is marked by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

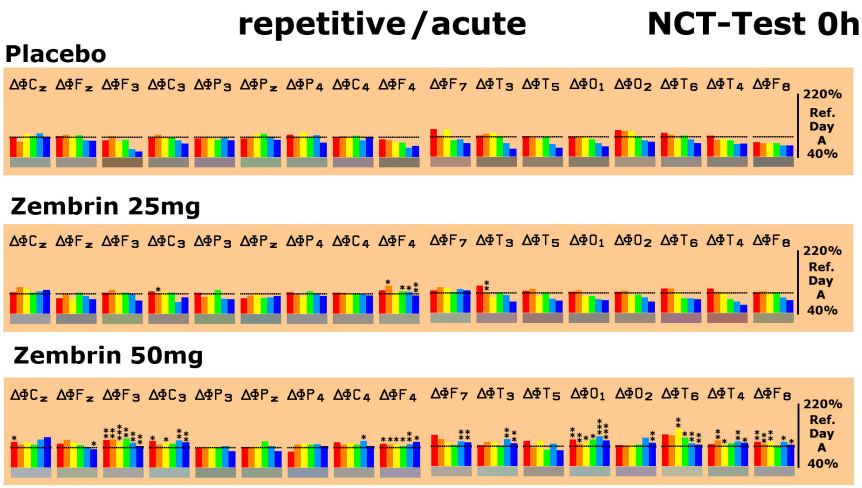

Figure 17. Documentation of permanent changes induced by Zembrin® in comparison to placebo on spectral frequencies in different regions of the brain in percent of the results of the first recording day during performance of the number connection test. Electrode positions are labeled as C for central, F for frontal, P for parietal, T for temporal and O for occipital. Even numbers describe data from the right hemisphere, uneven numbers from the left hemisphere. Red bars: delta, orange: theta, yellow: alpha1, green: alpha2, turquoise: beta1, blue: beta2 spectral power. Error probability according to non-parametric Wilcoxon test is marked by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

3.11. Efficacy of Zembrin® in Brain Regions of Interest after Repetitive Intake

In order to see if 6 weeks of Zembrin® intake resulted in long lasting changes of the EEG spectra, frequency changes were compared by setting data from the first day to 100% for monitoring the difference. In the fronto-temporal region conspicuous increases of theta and alpha1 power were registered during performance of the d2-test. During performance of the memory test statistically significantly more beta2 spectral power was produced in comparison to the first day recording. The same increase was seen during performance of the arithmetic calculation test, where also alpha1 and beta1 power showed higher values. During performance of the reaction time test increases of alpha1, alpha2 and beta1 power increases were observed. Delta, theta and both beta frequencies went up in a statistically significant manner during performance of the number identification test, whereas all frequencies showed a statistically significant increase of spectral power during performance of the number connection test. An overview is depicted in Figure 18.

In the centro-occipital region also different responses of the brain to various challenges were detected in comparison to the begin of the study. Obvious delta power increases were seen during all tests except for the d2-test. Theta frequencies were stronger enhanced during the memory test, the reaction time test and number identification test. Highly statistically significant higher power values were registered during performance of the reaction time and number identification test. Beta1 and beta2 power values were larger during arithmetic calculation and both number tests. Details are given in Figure 18.

Figure 18. Documentation of changes of electric baseline patterns in particular brain regions of interest (ROI) after 6 weeks of repetitive dosing of placebo (grey bars), 25 mg of Zembrin® (yellow bars) or 50 mg of Zembrin® (red bars) Ordinate: spectral power in % of the recordings of the first day before intake at the experimental day. Left side: fronto-temporal region, right side: centro-occipital region. Changes are given for all frequency ranges: delta, theta, alpha1, alpha2, beta1 and beta2 waves. Statistical differences to placebo are marked by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$ according to Non-parametric Wilcoxon test.

3.12. Efficacy of Zembrin® Documented by Discriminant Analysis

Discriminant analysis is a statistical tool to separate large data sets from each other. In the past, data from several studies dealing with the effects of clinically used pharmaceutical drugs and botanical extracts were fed into this type of analysis based on 102 different EEG parameters (17 brain areas times 6 frequency ranges). Through this data set, a matrix of discriminant functions was built, on which data from new investigative products can be compared. If preparations cluster together in space (x, y and z coordinates), a similar CNS clinical indication can be assumed. If the color is also similar a related mechanism of action is very probable. Data obtained after intake of Zembrin® were projected into the vicinity of products with proven calming action including Calmvalera and a Ginkgo-Ginseng preparation tested earlier. At some distance (blue, more in the back) the antidepressant drug fluoxetine and the calming preparation Neurexan (red) were projected, however, with a different color. The effect of Zembrin® in comparison with other drugs and preparations is documented in Figure 19.

3.13. Efficacy of Zembrin® during Psychometric Testing

Performance of psychometric testing revealed statistically significant improvement in two different tests: **CPT** = arithmetic calculation test and **NCT** = number connection test (Table 5). Performance of the difficult arithmetic calculation test was statistically significant better in the presence of both dosages of Zem-

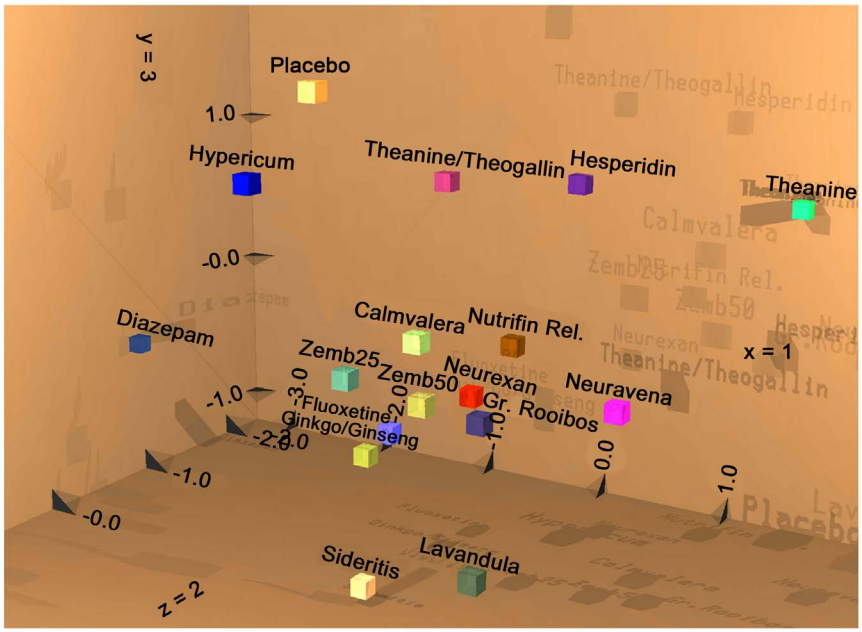

Figure 19. Result of discriminant analysis based on all brain regions and frequencies (102 parameters). Results from the first three discriminant functions are depicted with the space coordinates x, y and z. Results from the next three discriminant functions are depicted as RGB colour mixture like in TV technology. **Pl** = placebo; **Zemb25** = 25 mg ZEMBRIN®, **Zemb50** = 50 mg ZEMBRIN®. If preparations cluster together in space (x, y and z coordinates) a similar clinical indication can be assumed. If the color is also similar, a related mechanism of action is very probable. This is for Ginkgo/Ginseng the case.

Table 5. Mental performance (definition see under methods, reaction time is given in ms) in cognitive tests 1 h after intake of 25 mg Zembrin® (Zem25), 50 mg Zembrin® (Zem 50) or placebo. SD = standard deviation. *p*-values are documented according to two-sided T-test for independent samples.

Mental performance in cognitive tests on day A and day B 1 h after intake							
		Day A (acute)			Day B (repetitive)		
		Performance of d2 Test (d2)					
		Placebo	Zem25	Zem50	Placebo	Zem25	Zem50
1 h	Mean	11.02	11.57	11.11	12.18	12.46	12.38
	SD	±3.02	±2.95	±2.69	±3.61	±2.82	±2.67
		Performance of Memory Test (ME)					
		Placebo	Zem25	Zem50	Placebo	Zem25	Zem50
1 h	Mean	6.51	7.05	6.60	7.10	6.51	7.73
	SD	±1.93	±2.51	±2.42	±2.51	±2.18	±1.93
		Performance of Arithmetic Calculation (CPT)					
		Placebo	Zem25	Zem50	Placebo	Zem25	Zem50
	Mean	2.41	4.09	3.86	2.67	3.26	3.19
1 h	SD	±1.68	±2.54	±2.14	±2.34	±2.49	±2.82
			$p < 0.02$	$p < 0.03$			
		Performance of Reaction Time Test (RT)					
		Placebo	Zem25	Zem50	Placebo	Zem25	Zem50
1 h	Mean	420.73	419.29	420.96	408.75	405.96	412.53
	SD	±39.53	±37.83	±48.54	±30.78	±41.76	±37.65
		Performance of Number Identifying Test (NIT)					
		Placebo	Zem25	Zem50	Placebo	Zem25	Zem50
1 h	Mean	28.42	29.80	28.60	29.59	30.28	29.96
	SD	±.97	±1.76	±3.74	±2.24	±2.23	±1.75
		Performance of Number Connection Test (NCT)					
		Placebo	Zem25	Zem50	Placebo	Zem25	Zem50
	Mean	125.43	162.06	137.32	140.08	162.51	138.59
1 h	SD	±54.45	±31.49	±42.22	±48.68	±29.96	±32.49
			$p < 0.01$			$p < 0.09$	

brin® on the first day (day A), whereas improvement in the number connection test (NCT) was only significant in the presence of the lower dosage, however, on both recording days. However, both performance values were also considerably higher after repetitive dosing, but did not reach statistical significance.

A comparison of all tests between the baseline of the first day and the baseline of the last day was calculated in order to see if the intake of Zembrin during 6 weeks had resulted in better performance.

This was the case with respect to nearly all tests but statistical significance in comparison to placebo was only reached with respect to the lower dose for the number identifying test, and for the d2-test after intake of the higher dose. However, improvement was also seen with respect to the number connection test in the placebo group. Complete data are given in Table 6.

Table 6. Mental performance (definition see under methods; reaction time is given in ms) in cognitive tests before intake of 25 mg Zembrin® (Zem25), 50 mg Zembrin® (Zem 50) or placebo at beginning of the trial and after 6 weeks of daily intake. SD = standard deviation. *p*-value in NCT is documented according to two-sided T-test for independent samples.

Performance of cognitive tests on day A and day B before intake (0h)				
Performance of d2 Test (d2)				
		Placebo	**Zem25**	**Zem50**
Day A (acute)	**Mean**	**10.12**	**10.60**	**10.41**
	SD	3.07	2.98	3.05
Day B (repetitive)	**Mean**	**11.50**	**11.85**	**11.91**
	SD	3.45	2.81	2.31
				$p < 0.10$
Performance of Memory Test (ME)				
		Placebo	**Zem25**	**Zem50**
Day A (acute)	**Mean**	**5.90**	**6.82**	**6.22**
	SD	2.59	3.25	2.82
Day B (repetitive)	**Mean**	**6.14**	**7.25**	**6.48**
	SD	2.90	2.51	2.40
Performance of Arithmetic Calculation Test (CPT)				
		Placebo	**Zem25**	**Zem50**
Day A (acute)	**Mean**	**2.17**	**3.33**	**2.97**
	SD	1.75	2.86	2.93
Day B (repetitive)	**Mean**	**2.63**	**3.40**	**2.53**
	SD	2.02	2.94	1.76
Performance of Reaction Time Test (RT)				
		Placebo	**Zem25**	**Zem50**
Day A (acute)	**Mean**	**431.15**	**432.52**	**422.36**
	SD	32.32	29.77	47.44
Day B (repetitive)	**Mean**	**419.57**	**417.30**	**421.90**
	SD	40.21	39.99	35.70
Performance of Number Identifying Test (NIT)				
		Placebo	**Zem25**	**Zem50**
Day A (acute)	**Mean**	**29.10**	**28.63**	**28.23**
	SD	1.91	2.97	4.18
Day B (repetitive)	**Mean**	**28.32**	**30.64**	**28.89**
	SD	6.09	0.89	2.79
			$p < 0.01$	
Performance of Number Connection Test (NCT)				
		Placebo	**Zem25**	**Zem50**
Day A (acute)	**Mean**	**115.88**	**139.90**	**110.66**
	SD	43.33	27.97	42.12
Day B (repetitive)	**Mean**	**137.60**	**150.27**	**127.82**
	SD	36.53	29.21	31.20
		$p < 0.10$		

3.14. Efficacy of Zembrin® as Determined by Filling out Questionnaires

Three questionnaires were filled out after intake of the three preparations: The Profile of Mood States (POMS), The Hamilton Anxiety Scale (HAM-A) and the "Schlaffragebogen" SF-B. Results obtained by the POMS revealed improvement with respect to all score values, but statistical conspicuousness was only reached with respect to thirst of action (S4) with the lower dosage on day A and with respect to sullenness (S2) and fatigue (S3) on day B with the higher dosage. Details are given in Table 7.

Evaluation of the HAM-A questionnaire revealed lower scores at day B in the presence of both dosages. However, a statistically significant lower score on day B was only recognized for the higher dosage ($p < 0.03$). Details are given in Table 8.

Results from the "Schlaffragebogen SF-B" did not reveal differences with respect to score values before or after intake of both dosages of Zembrin®. Averaged score values are given in Table 9.

4. Discussion

Quantitative EEG measurements have been extensively used in the past to characterize drug action on the brain [10] [17]. Interpretation of the major neurotransmitter activities underlying frequency changes has become possible through preclinical experiments using several selective neurotransmitter agonists and antagonists. For example, delta waves have been shown to reflect actions of acetylcholine, theta waves actions of norepinephrine. Pharmacological intervention with the serotonergic system resulted in changes of alpha1 frequencies [25], whereas dopaminergic activity could be recognized by changes of alpha2 waves [26]. Finally, changes in the glutamatergic transmitter system were reflected in changes of beta1 activity [11] and GABA-ergic modulation was recognized by changes in beta2 frequencies [27].

Table 7. Result from POMS score questionnaire. S1 = Dejection (Niedergeschlagenheit), S2 = Sullenness (Missmut), S3 = Fatigue (Müdigkeit), S4 = Thirst for action (Tatendrang). Zem25 = 25 mg Zembrin®, Zem50 = 50 mg Zembrin®.

		POMS-questionnaire							
		Day A (acute)				Day B (repetitive)			
		S1 Dejection	S2 Sullenness	S3 Fatigue	S4 Thirst for action	S1 Dejection	S2 Sullenness	S3 Fatigue	S4 Thirst for action
Placebo	**Mean**	**0.35**	**0.48**	**1.61**	**2.54**	**0.39**	**0.39**	**2.06**	**2.64**
n = 20	SD	0.64	0.90	1.30	1.43	0.78	0.60	1.60	1.52
Zem25	**Mean**	**0.19**	**0.26**	**1.16**	**3.19**	**0.10**	**0.21**	**1.59**	**2.84**
n = 20	SD	0.28	0.46	1.05	1.07	0.19	0.43	1.50	1.19
Placebo_Zem25					**$p < 0.10$**				
Zem50	**Mean**	**0.24**	**0.23**	**1.20**	**2.85**	**0.20**	**0.14**	**1.29**	**2.47**
n = 19	SD	0.32	0.36	1.11	1.14	0.31	0.20	1.04	1.45
Placebo_Zem50							**$p < 0.08$**	**$p < 0.09$**	

Table 8. Comparison of HAMA scores (Hamilton Anxiety Scale) on the first and last day after repetitive intake. Zem25 = 25 mg Zembrin®, Zem50 = 50 mg Zembrin®. Difference between day A and day B: * = $p < 0.03$.

HAMA (Hamilton Anxiety Scale)						
	Day A (acute)			Day B (repetitive)		
	Placebo	Zem25	Zem50	Placebo	Zem25	Zem50
Mean	**10.80**	**7.90**	**11.05 ***	**9.30**	**6.65**	**6.37 ***
SD	5.63	4.27	7.40	6.73	5.55	5.12

Table 9. Results from Schlaffragebogen SF-B (questionnaire). Zem25 = 25 mg Zembrin®, Zem50 = 50 mg Zembrin®.

SF-B (Schlaffragebogen B)						
	Day A (acute)			Day B (repetitive)		
	Placebo	Zem25	Zem50	Placebo	Zem25	Zem50
Mean	**3.49**	**3.64**	**3.60**	**3.63**	**3.63**	**3.57**
SD	0.77	0.44	0.58	0.75	0.64	0.47

After finding evidence for an acute dose dependent action of Zembrin® on electric brain activity in an earlier study [16], the present experimental series aimed at collecting evidence on its efficacy after daily repetitive dosing for 6 weeks. EEG's were recorded during several conditions and gave similar starting values in terms of absolute power in the relaxed state. Performance of different cognitive tests revealed that electric activity changed according to the particular test with high statistical significance in comparison to the relaxed state. Efficacy of Zembrin® was observed with respect to all frequencies.

In the fronto-temporal region (this brain region has been recognized to be very important for memory retrieval [28], increases of delta activity during performance of the d2-test, the number identification and number connection test were observed in the presence of Zembrin®. These additional increases of delta waves in comparison to baseline indicate a more positive intense brain activity and mental activation. Based on the evidence that delta waves reflect the activity of the cholinergic transmitter system, Zembrin® seems to modulate this activity. Acetylcholine is used in electric circuits related to cognitive processing. Higher theta activity was seen in this brain area during relaxation and performance of the d2-test on both recording days after intake of the higher dosage of Zembrin®. Frontal increases of theta waves are regularly observed during concentration and higher attention related to memory [29] and reflect consciousness [30]. Additional increases after intake of Zembrin® may therefore be interpreted as an increase of attention. The marked influence of these neurochemical systems also on prefrontal memory processes has been widely described [31]. Statistically conspicuous increases of alpha1 spectral power as seen in the relaxed state after intake of Zembrin® must be regarded as a state of increased calmness. Biochemically this corresponds to increases of serotonergic activity possibly also reflect-

ing improvement of mood. It is interesting that one of the reported mechanisms of action of Zembrin® is serotonin reuptake inhibition [32]. With respect to alpha2 spectral power, which has been related to cognitive and memory performance [33], larger statistically significant increases were observed during performance of the memory test and number connection test in the fronto-temporal region. Alpha2 waves are under the control of dopamine [26]. A relatively selective role for prefrontal dopamine in spatial memory was reported more than three decades ago [34]. Increases of alpha2 waves were observed also in the centro-occipital region. These waves are normally attenuated within this brain region during strong mental activity. Less attenuation of alpha2 activity may therefore indicate a less stressful experience of the tests. In the fronto-temporal region a statistically conspicuous increase was only observed during performance of the number identifying test. No major changes of spectral beta power were recognized except for the performance of the arithmetic calculation test (CPT) in the centro-occipital brain region. Here, both dosages of Zembrin® exerted statistically conspicuous and significantly different increases of spectral power in comparison to placebo. However, this effect was probably due to unusual low power during the placebo condition and do not reflect a true effect of Zembrin® (**Figure 10**).

Discriminant analysis revealed a clear separation not only between placebo and verum, but also with respect to different recording conditions on both recording days. This can be taken as evidence that the lower dosage also exerted enough efficacy to be separated by this kind of analysis (not shown). A second approach using the discriminant analysis for comparison with other CNS drug and preparation profiles revealed a projection of the Zembrin® data into the vicinity of calming preparations like Calmvalera. The activity interpreted from the discriminant analysis projection is consistent with the increases of alpha1 spectral power observed during different recording conditions. Zembrin® also projected in the vicinity of a cognition enhancing mixture of the botanicals Ginkgo/Ginseng. From this, a positive effect on cognition may be interpreted for Zembrin®, supported by the detailed frequency analysis on single dose activity.

Regarding the effect of daily intake of Zembrin® during 6 weeks, the data of the two baselines recorded on the first and last day before intake of the preparations were compared. Data from the first day were set to 100% as reference. As documented, spectral power had not changed over time with respect to the relaxed state, but consisted in test dependent increases of spectral power in many brain regions. The strongest changes were seen with respect to beta power when performing the arithmetic calculation (CPT), number identification (NIT) and number connection test (NCT). This indicates that the repetitive intake of Zembrin® has led to a measurable different electric response of the brain in the presence of mental loads, meaning the reactivity of the brain had changed. Due to this, change of responses to various challenges on this second experimental day are somewhat different in comparison to the recording of the first day. With respect to the two previously specified regions of interest, differences between the

two experimental days were statistically conspicuous or significant during performance of all tests, but not in the relaxed state (eyes open). Very strong differences are noted between the first and last recordings taken before intake of Zembrin®. These differences were also documented with respect to beta power during several different tests (**Figure 18**). This suggests that daily intake has also modulated glutamatergic and GABA-ergic neurotransmission. This interpretation is in line with preclinical ex vivo testing in the hippocampal slice preparation, where Zembrin® was able to attenuate the amplitude of the population spike by interfering with glutamatergic transmission (to be published). Similar effects in this model have been seen with all calming and antidepressant drugs. Positive effects of Zembrin® on cognition may therefore be indirect and based on its calming or anxiolytic action, as it is well known that stress negatively affects executive function. Another explanation with respect to the positive action of Zembrin® on cognitive function parameters reported in a previous clinical study [15] may be inhibition of phosphodiesterase 4, reports earlier [32].

Results from psychometric testing revealed improvements from both dosages during performance of the arithmetic calculation test (CPT) on the first experimental day and in the presence of the lower dosage during performance of the number connection test (NCT) on both experimental days. Evaluation from the POMS questionnaire revealed improvements of all scores, but no statistical significance was reached, probably due to high scatter of score values. Sullenness and fatigue scores decreased statistically conspicuously ($p < 0.09$) on the last experimental day. With respect to the HAM-A anxiety scale a comparison of the score obtained after repetitive intake of 50 mg of Zembrin® with the starting score on the first experimental day revealed a statistically significant decrease of the score ($p = 0.03$). This behavioral result is consistent with the changes seen in brain electric activity in response to mental loads after repetitive dosing.

5. Conclusion

In summary, this double-blind, randomized, placebo-controlled clinical study revealed a dose dependent effect of Zembrin® on three levels of evidence: at the level of questionnaires, at the level of psychometry, and the level of quantitative EEG. The overall results support the use of Zembrin® as a functional food with potential to improve mental health and enhance well-being with respect to cognitive function, anxiety, stress and mood. Facing an ever-increasing level of stress and anxiety (for example to lose the job) or a decline of cognitive function in the elderly, the results of this clinical study open the door to better cope with daily life. A future controlled clinical study on Zembrin® should be considered in subjects suffering from exam-related anxiety, to investigate activity on the cognitive- function, perceived stress and anxiety.

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