

Proof of Effectiveness of PASCOFLAIR® in Subjects Suffering from Examination Anxiety Using Quantitative EEG in Combination with Eye-Tracking (EnkephaloVision). A Double-Blind, Randomized, Placebo Controlled, 2-Armed, Phase IV Study in Parallel Design

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Abstract

The pharmaco-dynamic action of plant-derived drugs still remains a big challenge in the field of pharmacology. This applies especially for the discovering of the possible mechanism of action. With respect to the brain, surrogate parameters may be very helpful. Non-invasive pharmaco-EEG has been proven to provide valid information on drug effects in general. Fast dynamic EEG recording with epoch length of 364 ms has now been achieved in combination with Eye-Tracking in order to elucidate single scenes during cognitive and emotional challenges (EnkephaloVision). The present investigation deals with the psychophysiological characterization of the effect of a plant-derived preparation marketed in Germany under the name of PASCOFLAIR® containing 425 mg of passionflower extract in subjects suffering from examination anxiety. Forty healthy volunteer subjects (17 men and 23 woman), with ages varying between 18 and 40 years (26 ± 6.726 for men and 25 ± 5.397 for women) were included into the study when having a score higher than 60 in the PAF (Prüfung-sangstfragebogen). Spectral power analysis of pre-drug data with respect to beta waves revealed a significant correlation to the score of the PAF. Increases of delta, theta and beta waves as induced by 4 cognitive and 4 emotional challenges were attenuated in the presence of 2 tablets of PASCOFLAIR® already 45 minutes after intake in a statistically significant manner in comparison to placebo. Regarding attenuation of beta1 and beta2 spectral power, effects on glutamatergic and GABAergic

neurotransmission, respectively, can be suspected according to our knowledge of frequency—transmitter relations. Accordingly, this attenuation must be interpreted as ability to counteract examination induced stress symptoms. Results confirm and extend earlier experimental data showing a calming action of PASCOFLAIR®.

Keywords

EEG, Source Density, Spectral Power, PASCOFLAIR®, Psychophysiology, Cognition, Emotion, Stress, CATEEM®, Neurocode-Tracking, Eye-Tracking, Enkephalovision

1. Introduction

PASCOFLAIR® is a herbal drug marketed in Germany for treatment of restlessness and nervousness as well as for better coping with stress. Its pharmacodynamic active ingredient is an extract of passionflower. Efficient calming down has been reported in the past without interfering with cognitive performance in healthy subjects [1]. Also many other herbal preparations have been tested using neurophysiological techniques [2] [3]. There is convincing evidence that emotional states directly relate to brain electric activity in terms of spectral frequency changes [4]. The present investigation aimed at the possibility that PASCOFLAIR® might be able to help subjects suffering from examination anxiety. In order to test this, a new design of a clinical study was used, namely “EnkephaloVision”. This new approach consists of the combination of quantitative EEG recording providing very high time resolution of epochs of 364 ms (Neurocode-Tracking) with conventional Eye-Tracking. This method has already been used successfully for characterization of other herbal extracts [5] [6].

According to our experience, when presenting videos with emotional content, reproducible changes of brain electric activity have been recorded. Especially, horror clips induced strong transient increases of spectral beta power. This led to the hypothesis, that spectral beta power might be a good indicator of stressful reactions. Therefore, 40 subjects suffering from examination (or test) anxiety were exposed visually to 4 cognitive and 4 emotional challenges by asking for task performance and watching video clips of short duration. Video presentations are well suited to induce emotional stress (called emphatic stress) as proven by significant physiological increases of cortisol levels [7].

2. Methods

The double-blind, randomized, placebo-controlled, two-armed study was conducted from May to August 2015 in the research labs of NeuroCode AG, Wetzlar, Germany. The medical investigation was approved by the ethics committee of the Hessen State Medical Association (Frankfurt, Germany) and government authorities (BfArM, Bonn, Germany) before initiation. Subjects signed an informed consent, which contained detailed information about the study. They were randomized to receive either placebo or verum according a randomization schedule, that was generated by a statistician. The

investigator, the medical staff, the study assistants and subjects were kept blind to the allocation. Verum and placebo tablets were undistinguishable and both were blistered, packed and labeled identically. The study was performed under the EudraCT-Nr. 2014-003369-50. Approval of the ethic committee (Landesärztekammer Hessen, Frankfurt, Germany) was on 17.12.2014. The study was performed in accordance with the current version of the declaration of Helsinki (52nd WMA General Assembly, Edinburgh, Scotland, October 2000). The trial was conducted in agreement with the International Conference on Harmonization (ICH) guidelines on Good Clinical Practice (GCP).

2.1. Subjects

Forty healthy volunteer subjects (17 men and 23 woman), with ages varying between 18 and 40 years (26 ± 6.726 for men and 25 ± 5.397 for women) were recruited by advertisement and filling out a questionnaire dealing with examination anxiety, called PAF-questionnaire (Prüfungsangstfragebogen PAF-S, Hogrefe Verlag, Göttingen, Germany [8]). Subjects having a score between 61 and 80 were included in the study and they underwent a medical examination before entering the trial. Results from several questionnaires did not reveal greater differences between the two groups. Results are listed in **Table 1**.

According to the literature reproducible changes of brain electric activity are described as the resultant of showing videos with emotional content by subjects [9]. These changes occurred especially by presenting horror clips, which induced strong transient increases of spectral beta activity in the brain. This led to the hypothesis, that spectral beta power might be a good indicator of stressful reactions as already reported earlier [10].

This hypothesis was also verified in this study by all emotional challenges. For illustration two representative scenes from a video were chosen (video clip from “examination anxiety” and “at the dentist”). As we can see in **Figure 1** and **Figure 2**, both scenes showed a high beta activity in the temporal lobe (blue color in the bar graph or blue spot in the map) as an indicator of stress.

Table 1. Comparison between groups with respect to questionnaires: PAF = Prüfungsangstfragebogen (exam anxiety), HAMA = Hamilton Anxiety Scale, dental treatment anxiety, SD = standard deviation, SEM = standard error of the mean, n = number of subjects, verum = PASCOFLAIR® 425 mg.

Questionnaires		All subjects n = 40	Placebo n = 20	Verum n = 20
PAF	Mean	65.95	65.50	66.40
	SD	4.03	4.07	4.04
	SEM	0.64	0.91	0.90
HAMA	Mean	15.13	15.50	14.75
	SD	6.57	7.55	5.59
	SEM	1.04	1.69	1.25
Dental treatment anxiety	Mean	5.00	4.80	5.20
	SD	3.27	3.44	3.16
	SEM	0.52	0.77	0.71

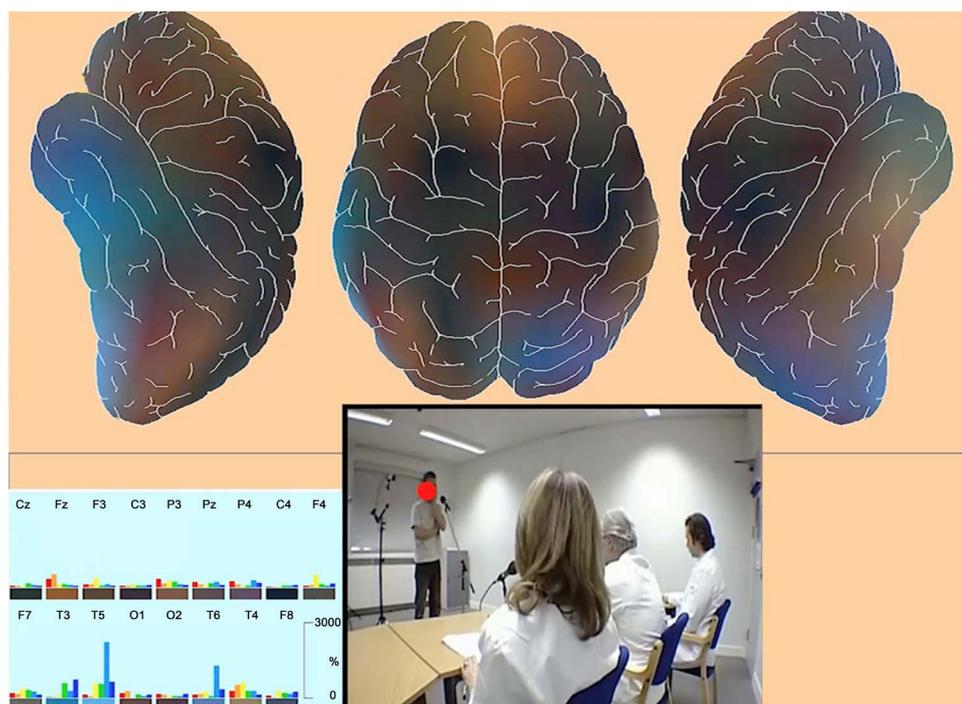


Figure 1. Representative scene of 364 ms duration taken from the video clip “examination anxiety” showing high beta activity in the temporal lobe (blue color on electrode positions T_5 and T_6 in the bar graph and blue spot on the map) indicating enhanced stress. Red spot in inserted image represents eye glance on examination candidate.

The exclusion criteria were: hypersensitivity to the ingredients of the investigational product, acute or chronic disease, use of antidepressants, psychotropic drugs or centrally acting antihypertensives, use of clinically relevant medication during last fourteen days before and during the study period, presence of clinically relevant pathological EEG features or artifact-free portion of the screening EEG less than 30%, participation in another clinical trial within the last 60 days, positive alcohol or drug test, history of alcohol or drug abuse, body mass index less than 18 or more than 32, misuse of coffee or tea (more than 4 cups a day) or tobacco (more than 20 cigarettes a day), use of beverages containing caffeine preceding the EEG recording, smoking on the study day (day A), excursions of safety parameters, bad compliance, cancellation of informed consent, positive pregnancy test or nursing.

2.2. EEG-Recording

In this Study the Neurocode-Tracking software was used for EEG-recording on the experimental day (day A). This is a 17 channel EEG recording combined with Eye-Tracking. A detailed description of EEG-recording has been published earlier with a new modified software for analysis of shorter times epochs of 364 ms duration [11] (neo-CATEEM®, supplied by MEWICON CATEEM-Tec GmbH, 4164 Schwarzenberg am Böhmerwald, Austria). In short: For the new approach EEG frequency ranges had to be adjusted slightly to give the following exact ranges. Delta: 1.375 - 4.125 Hz; Theta:

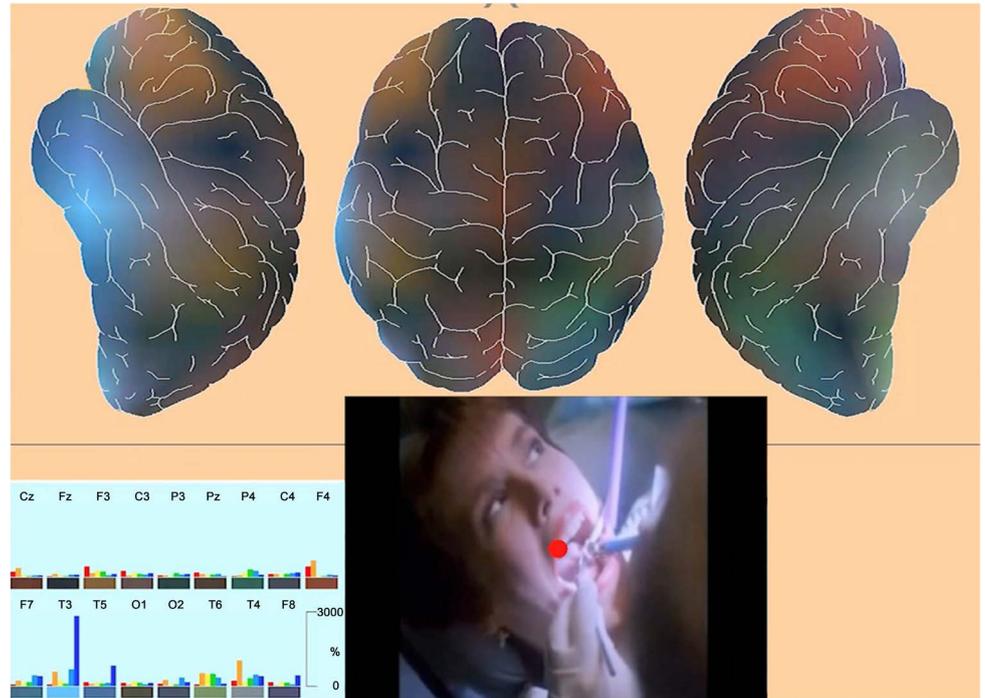


Figure 2. Representative scene taken from the video clip “at the dentist” showing high beta activity in the temporal lobe (blue color on electrode positions T₃ and T₅ in the bar graph and blue spot on the map) indicating enhanced stress. Red spot in inserted image represents eye glance on mouth under treatment.

4.125 - 6.875 Hz; Alpha1: 6.875 - 9.625 Hz; Alpha2: 9.625 - 12.375 Hz; Beta1: 12.375 - 17.875 Hz; Beta2: 17.875 - 34.375 Hz. This adjustment is a precondition for the new fast dynamic frequency analysis (Neurocode-Tracking) because under this condition each frequency range from delta up to alpha2 contains only one frequency (middle frequency within the particular frequency band). Data were analyzed in current source density mode. All other features remained identical to the classic analysis used now for nearly 20 years. Maps were constructed by transforming spectral power values into spectral colours followed by additive colour mixture (RGB-mode like in TV).

EEG-recording occurred at two different times: before and 45 min after intake of the study medication (*i.e.* placebo/verum). Subjects fulfilling all inclusion criteria were invited to come with empty stomach on the experimental day (day A). After having a breakfast, they underwent clinical examination and were assigned at random to one of the two study medications (*i.e.* placebo or verum). They also answered a simple questionnaire, called HAMA [12] (Hamilton Anxiety Scale, CIPS: Collegium Internationale Psychiatriae Salarum) with questions about their general health (anamnesis) and their anxiety. Before recording of the first EEG (first presentation), subjects were informed and made familiar to the Neurocode-Tracking. Each subject spent around 43 min in the facility’s break room after the medication intake.

EEG recording was performed in the presence of a video clip, which contained several different cognitive and emotional challenges in series (Table 2). At the beginning,

Table 2. Audio-visual challenges during EEG recordings at experimental day (day A).

Neurocode-Tracking in Combination with Eye-Tracking Audio-visual Presentation		
	Elements	Time
1	Instructions of volunteers in the Neurocode-Tracking method	5 min. 00 s
2	Gong (for synchronization Neurocode-Tracking (qEEG and Eye-Tracking))	21 s
3	Eyes open (Black Screen)	1 min. 00 s
4	Fix Cross (Eyes open)	1 min. 00 s
5	Exam Anxiety-Video	1 min. 55 s
6	Memory Test (n = 4 tasks) including instructions	1 min. 35 s
7	Stroop Test (n = 1 foil)	1 min. 00 s
8	Picture Comparison (n = 1 foil) with 8 errors	1 min. 00 s
9	Brain Teaser (n = 10 tasks)	5 min. 03 s
10	Horror-Video	1 min. 00 s
11	Dentist Pain-Video	52 s
12	Emergency-OP-Video	1 min. 11 s
	Total Time	21 min. 12 s

after a gong for synchronization purposes, a fixation cross was presented for 1 minute as reference. The total video was presented twice: During baseline recording and 45 min after intake of two 425 mg tablets PASCOFLAIR® or placebo tablets on the examination day (day A). A time line is given in **Figure 3**.

2.3. Statistical Analyses

EEG data from the first recording session before intake of the tablets are given as absolute values (μV^2). These Data were set to 100% for statistical evaluation using the non-parametric Wilcoxon test and electrophysiological changes produced by the investigational products (*i.e.* placebo and verum) were depicted as %-changes thereof. In order to document statistically the different electric reaction of the brain to various cognitive and emotional loads, data from each part of the presentation were divided by the data obtained during fixation of the cross on the monitor (1 minute) at the beginning. Comparison of PASCOFLAIR® versus placebo was accomplished by evaluation of the second recording of the day 45 minutes (0.75 h) after intake.

Further evaluations were done using the linear discriminant analysis according to Fischer. 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 an additive color mixture (so-called RGB-mode). Sample size was defined according to several earlier studies performed under identical conditions.

2.4. Extract

PASCOFLAIR® is a coated tablet which contains 425 mg dry extract of *Passiflora L.*

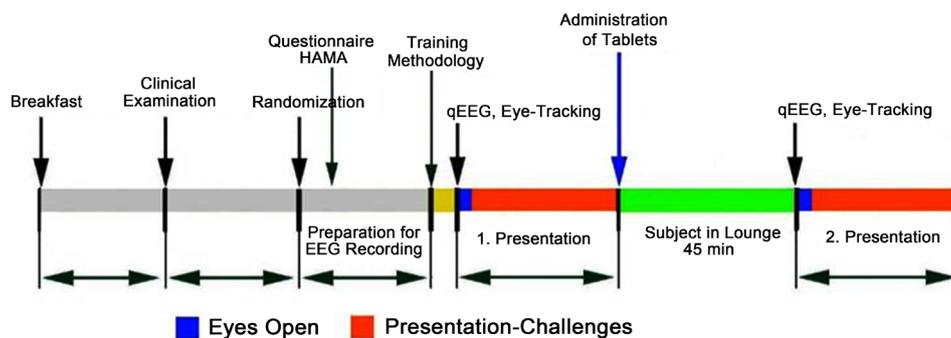


Figure 3. Time line of the experimental day (day A).

(aerial parts) corresponding to 2.125 - 2.975 g of passionflower. Ethanol 50% (v/v) is used as extract medium. PASCOFLAIR® 425 mg is used in herbal medicine for the treatment of anxiety, restlessness, sleeplessness and mental stress. The manufacture of the placebo tablets was based on the verum except for the active ingredient, that was replaced by a mixture of microcrystalline cellulose, cellulose powder, silica, highly dispersed and stearic acid. The investigational products (PASCOFLAIR® 425 mg and placebo) were carried out by Pascoe pharmazeutische Präparate GmbH, Giessen, Germany.

2.5. Efficacy and Safety Parameters

For safety assessments, physical examinations, clinical laboratory tests and a 12-lead electrocardiogram (ECG) were performed before (screening day) and after drug intake (final examination day). Samples for blood chemistry (including creatinine, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (Gamma-GT)), hematology (including platelet count, red blood cell (RBC) count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH)) and urine analysis (including specific gravity, power of hydrogen (pH), glucose, protein, blood and ketones and microscopic examination) were collected in our institute and the analyses were performed in a central laboratory. Vital signs (pulse rate and blood pressure), temperature and adverse events were recorded at each visit.

3. Results

3.1. Frequency Changes in the Presence of Cognitive and Emotional Challenges

Forty subjects were recruited for this study on the base of filling out a questionnaire dealing with examination anxiety. Concomitant presentation of a series of mental and emotional challenges during recording of the EEG was performed before and 45 minutes after intake of 2 tablets of PASCOFLAIR®. The absolute starting values of spectral power in the six frequency ranges (delta to beta2) are given in **Table 2**. These values do not differ from each other in a statistically significant way suggesting that both groups-

placebo and verum-have a similar starting position. This allows for a pre-post comparison of drug action. These absolute values are set to 100% for further analysis and drug induced changes are documented in % of these starting values.

In order to relate the result from the questionnaire with EEG parameters a correlation was searched based on the first recordings before administration of the drug. The result indicates that absolute spectral beta1 and beta2 power from all 40 subjects during the recording condition “eyes open” in a relaxed state before drug administration correlated with the score values of the questionnaire. Since increases of beta power mainly emerge in fronto-temporal brain areas, spectral power was calculated for the combination of all frontal and temporal electrodes for correlation purposes. **Figure 4** document the results of this analysis showing a correlation coefficient of $r = 0.32$ ($p < 0.031$) for beta1 and of $r = 0.33$ ($p < 0.024$) for beta2 spectral power. According to the correlations found, increases of beta power were the main target to look for uncovering drug effects related to exam anxiety and stress.

Next step in the analysis was to find out if the different cognitive and visually presented emotional challenges lead to increases of delta, theta and spectral beta power among other changes before the intake of verum/placebo. For this purpose, data from each challenge were averaged over the presentation time in order to follow each of the brain regions with respect to the pattern of spectral power changes with respect to each frequency. As documented in **Table 3** all challenges except for the stroop test and the clips “examination anxiety” and “dentist pain” induced statistically significant beta power besides massive increases of delta and theta spectral power (except for emotional challenges!).

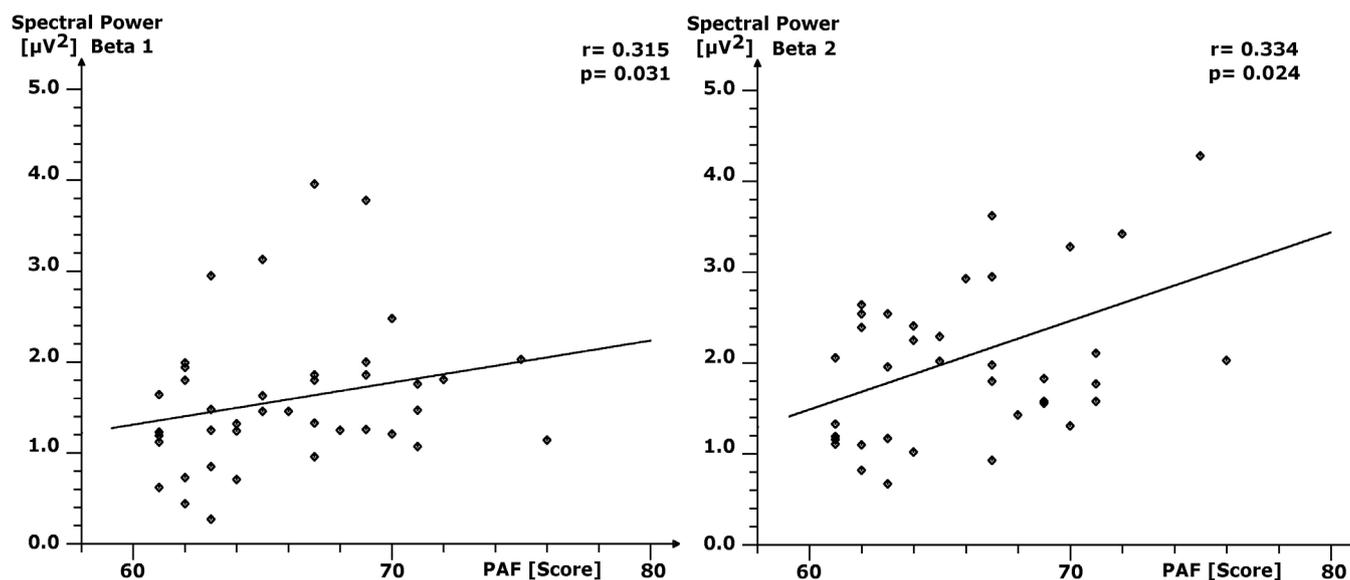


Figure 4. Correlation between PAF examination anxiety questionnaire (score on abscissa) and spectral beta1 power (left), beta2 power (right) during the recording condition “eyes open” before drug intake. Spectral power was averaged including all frontal and temporal electrodes. Ordinate: spectral power, Abscissa: score of questionnaire PAF. Correlation coefficient beta1 ($r = 0.315$; $p = 0.031$) and beta2 ($r = 0.334$; $p = 0.024$).

Table 3. Spectral power before intake of verum/placebo with respect to 17 locations under the recording condition “eyes open”. Comparison of the absolute starting values of both groups of subjects. Data are given as μV^2 within each frequency range (delta-beta2). Med = median value. Electrode positions according to the so-called 10/20 system [13]. Verum = PASCOFLAIR®, n = number of subjects.

Absolute Spectral Power during relaxation (eyes open) 0h before acute intake at day A												
Subjects	Delta		Theta		Alpha1		Alpha2		Beta1		Beta2	
	n = 20	n = 20										
Electrode	Placebo	Verum										
Cz	1.46	1.49	1.25	1.13	1.06	0.97	0.74	0.70	0.71	0.54	0.91	0.96
Fz	2.37	2.31	1.57	1.65	1.04	1.32	0.67	0.79	0.75	0.67	1.02	0.93
F3	1.74	2.23	1.09	1.49	0.82	1.12	0.62	0.83	0.81	0.88	2.02	1.49
C3	0.98	1.04	0.84	0.86	0.89	0.81	1.16	0.73	1.10	0.95	1.27	1.54
P3	0.73	0.72	0.52	0.58	0.47	0.57	0.46	0.65	0.45	0.62	0.50	0.69
Pz	1.15	0.92	0.79	0.77	0.65	0.95	0.65	1.25	0.84	0.79	0.71	0.64
P4	0.77	0.86	0.52	0.59	0.50	0.91	0.54	0.81	0.73	0.71	0.80	0.71
C4	0.98	1.19	0.74	0.86	0.56	0.94	0.58	0.96	0.89	0.91	1.13	1.24
F4	1.83	1.91	1.34	1.41	1.23	1.06	0.86	0.80	0.88	0.89	1.43	1.53
F7	5.58	6.41	3.28	3.03	1.82	2.01	1.32	1.77	1.69	1.59	2.33	2.38
T3	2.47	2.82	1.89	2.10	1.28	1.63	1.42	1.36	1.85	1.73	1.93	2.41
T5	2.17	1.77	1.58	1.61	1.30	1.36	1.20	1.43	1.64	1.52	1.37	1.53
O1	1.93	2.16	1.34	1.37	1.42	1.10	1.39	1.42	1.36	1.39	1.81	2.05
O2	1.99	2.31	1.39	1.45	0.91	1.75	0.91	2.12	1.28	1.64	1.82	1.76
T6	2.11	2.17	1.43	1.69	1.60	2.11	2.42	3.27	2.22	1.97	1.52	1.87
T4	3.05	2.58	2.00	1.70	1.23	1.37	1.45	1.49	2.01	2.35	2.91	3.92
F8	5.62	5.06	3.08	2.74	1.72	1.92	1.38	2.01	1.78	1.61	3.23	2.82
Med	1.81	2.00	1.24	1.43	1.18	1.12	0.81	1.10	1.15	1.18	1.35	1.48

3.2. Pharmacodynamic Effects of PASCOFLAIR®

After establishment of spectral beta power as related to emotional stress, this parameter was looked at when comparing spectral changes induced by the placebo or the verum group. For example, a comparison of the same single scenery before and after intake of PASCOFLAIR® revealed a reduction of beta power at several electrode positions when considered for all 20 subjects per group (blue color in the bar graph of **Figure 5(a)** and **Figure 5(b)**). The reduction is also well documented by means of the maps, where less blue color dominates.

Regarding the effect of PASCOFLAIR® on different brain regions under the recording condition “eyes open” a general decrease of beta power (blue) is recognized. However, only beta1 at P₃ and beta2 at T₄ were statistically conspicuous ($p < 0.10$). In the occipital region alpha2 increases were observed, mostly at the electrode position O₂. Complete data are given in **Figure 6**. When subjects were asked to fix a cross on the screen for one minute, intake of PASCOFLAIR® led to a reduction of beta power in fronto-temporal areas (statistically conspicuous or significant at F₃, T₃, T₄ and F₈) as well as at P₃. Data are also documented in **Figure 6**.

Performance of a memory test also led to decreases of beta power in the presence of PASCOFLAIR®. With regard to spectral beta power, frontal as well as parieto-occipital regions (electrode positions P₃ and O₁) showed a diminution in a statistically conspicuous or significant manner (statistically conspicuous or significant at F₃, F₇ and T₅). Under this recording condition also delta and theta power were attenuated significantly

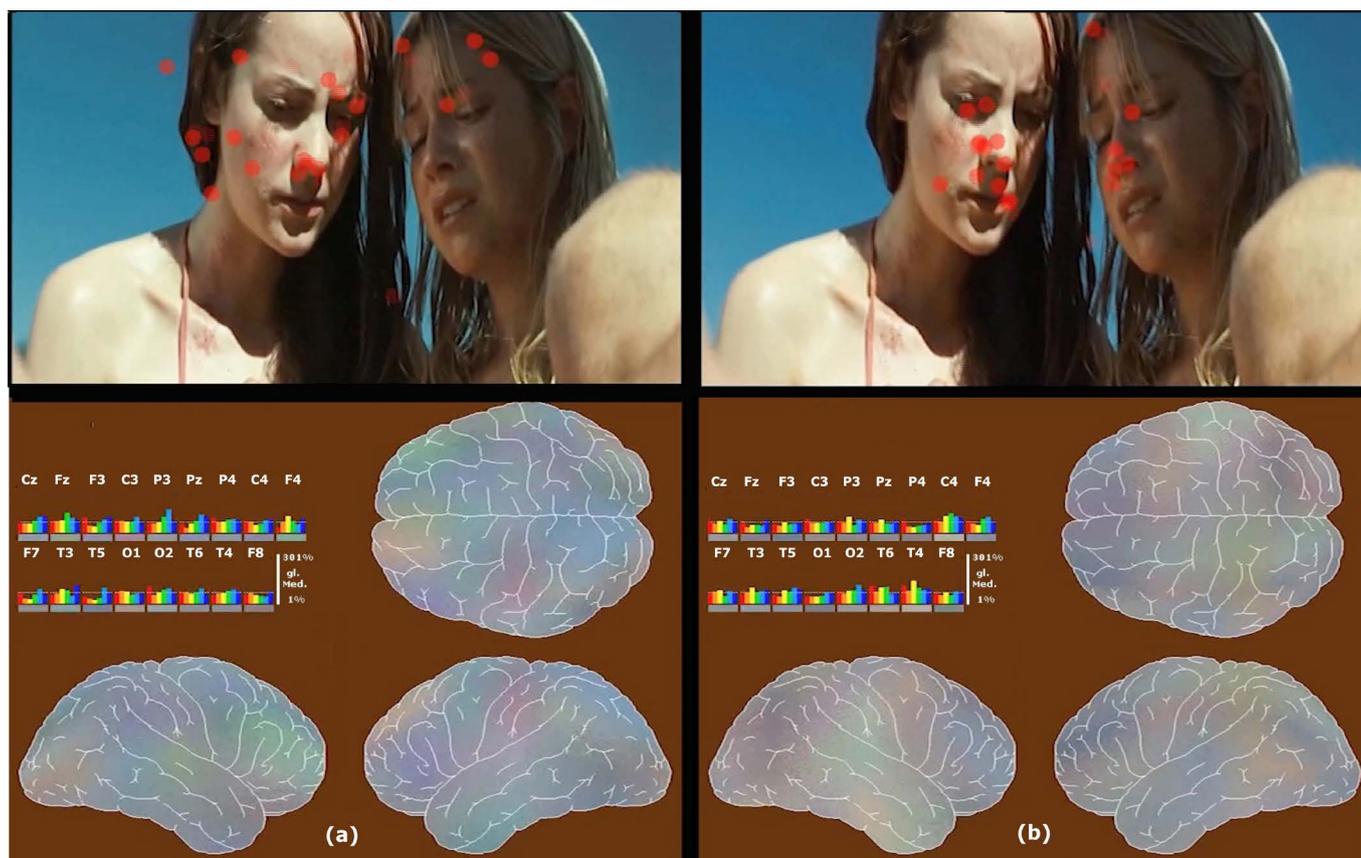


Figure 5. Comparison of an arbitrarily chosen but representative single scene of 364 ms duration before (a) and after intake of PASCOFLAIR® (b). Data taken from the emergency video clip were averaged for all 20 subjects. Eye-Track results are depicted in the upper image, EEG data are depicted in the lower image. Frequency changes are documented in the bar graph (ordinate: global median power from 1 to 301 %). Delta waves are coded into red, theta into orange, alpha1 into yellow, alpha2 into green, beta1 into turquoise, beta2 into blue.

by PASCOFLAIR® as depicted in **Figure 6**. During performance of the Stroop test again a fronto-temporal reduction of beta power was discovered (statistically conspicuous or significant at F₃, F₇ and T₆) again accompanied by the parietal region represented by electrode position P₃. Complete set of data is documented in **Figure 6**.

The recording condition in the presence of the video clip “exam anxiety” revealed reduction of beta power in fronto-temporal areas (statistically conspicuous or significant at electrode positions F₃, F₇, T₃, T₅ and T₄). Complete data are documented in **Figure 7**. The recording condition “emergency-OP” also provided evidence for reduction of beta power mainly in fronto-temporal areas (statistically conspicuous or significant at electrode positions F₄, T₆ and T₄). Here also diminution of delta waves emerged, which were statistically conspicuous or significant at F_z, P₃, F₇, T₃ and T₅. Data are shown in **Figure 7**.

Remarkably, 7 challenges showed a statistically conspicuous lower production of beta power at the left parietal lobe (electrode position P₃) besides the general fronto-temporal areas 45 minutes after intake of PASCOFLAIR®. There was virtually no effect in central brain areas. An overview on statistically relevant power decreases at different

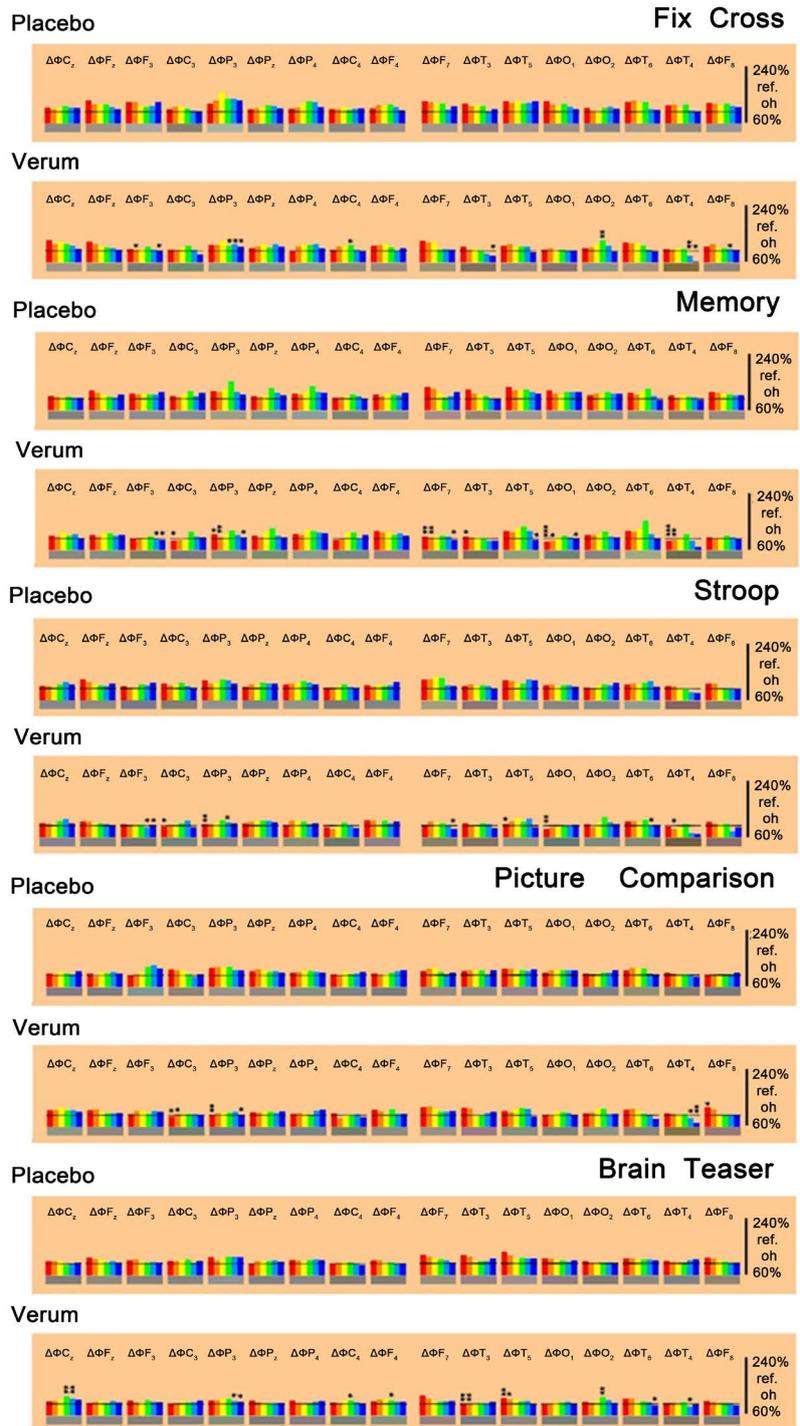


Figure 6. Spectral power differences (predrug-postdrug) within the window of 60% to 240% (ordinate) in the presence of placebo or verum in all brain regions represented by the electrode positions. C = central, P = parietal, O = occipital, F = frontal, T = temporal. Even numbers are located in the right hemisphere, uneven numbers in the left one. Frequencies: red = delta, orange = theta, yellow = alpha1, green = alpha2, turquoise = beta1 und blue = beta2. Statistical significance (Wilcoxon-Test) between placebo and verum is indicated by stars. *= $p < 0.10$; **= $p < 0.05$; ***= $p < 0.01$.

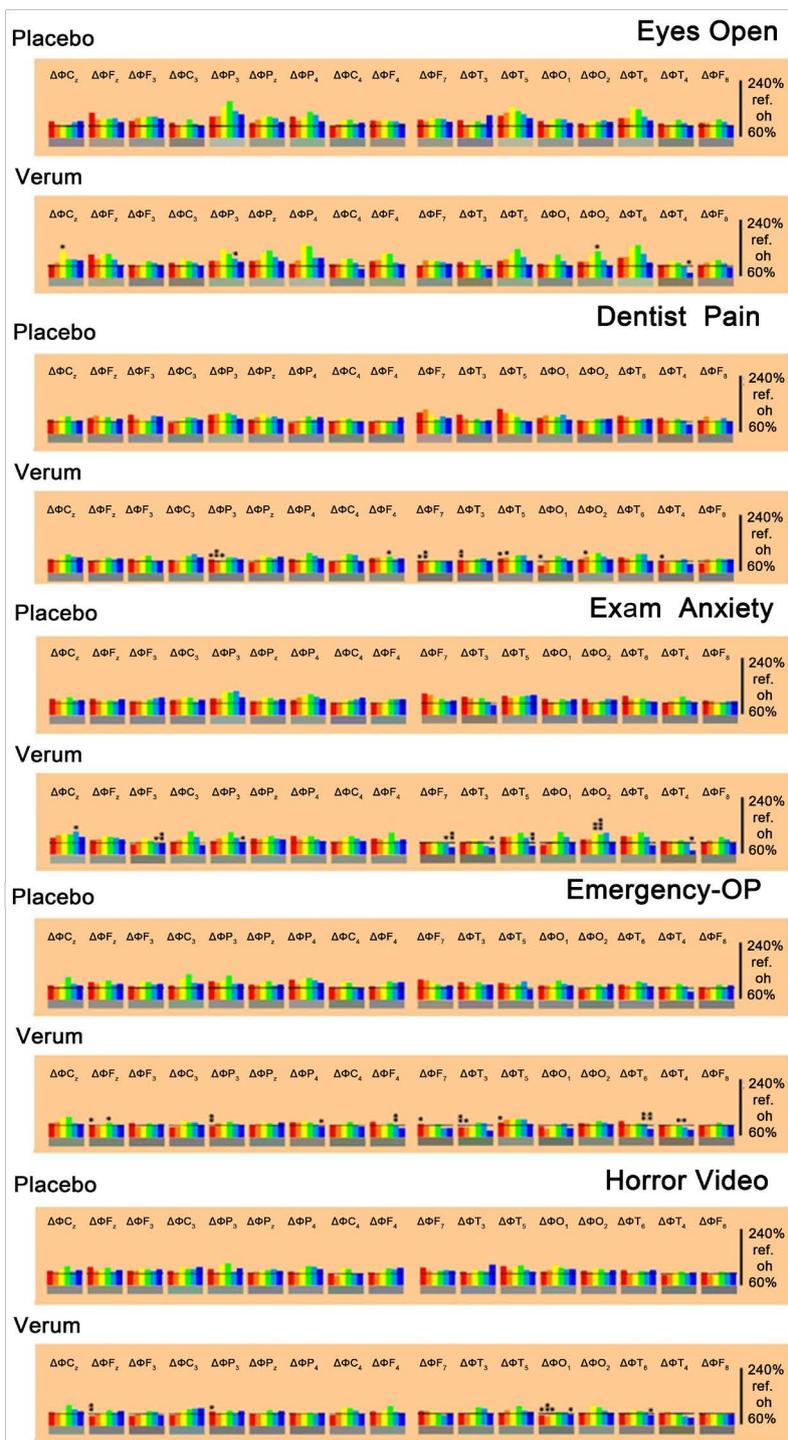


Figure 7. Spectral power differences (predrug-postdrug) within the window of 60% to 240% (ordinate) in the presence of placebo or verum in all brain regions represented by the electrode positions. C = central, P = parietal, O = occipital, F = frontal, T = temporal. Even numbers are located in the right hemisphere, uneven numbers in the left one. Frequencies: red = delta, orange = theta, yellow = alpha1, green = alpha2, turquoise = beta1 und blue = beta2. Statistical significance (wilcoxon-test) between placebo and verum is indicated by stars. * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

electrode positions is given in **Table 4**. Decreases of delta and theta power occur mainly on the left hemisphere with the exception of T₄ and F₈. Decreases in beta1 and beta2 likewise are observed mainly in the left hemisphere except for T₄ and F₈. Further exceptions are for beta1 at electrode position F₄ and for beta2 at electrode position P₄.

Table 4. Overview on statistically conspicuous ($p < 0.10$) or significant ($p < 0.05$ or less) decreases of spectral power in delta (upper left), theta (upper right), beta1 (lower left) and beta2 (lower right) images. FX = Fix Cross, ME = Memory Test, ST = Stroop test, PC = picture comparison, BT = brain teaser, EO = eyes open, PD = dentist pain, EA = exam anxiety, E-OP = emergency-OP, HV = horror video, E = electrode positions.

Delta E	FX	ME	ST	PC	BT	EO	DP	EA	E-OP	HV	Theta E	FX	ME	ST	PC	BT	EO	DP	EA	E-OP	HV	
Cz											Cz											
Fz									X	X	Fz											
F3											F3	X										
C3		X	X	X						X	C3				X							
P3			X	X			X		X		P3		X							X		
Pz											Pz											
P4											P4											
C4											C4											
F4											F4											
F7		X					X				F7		X							X		
T3		X			X		X		X		T3				X						X	
T5			X		X		X		X		T5				X					X		
O1		X	X				X			X	O1		X									X
O2											O2									X		
T6											T6											
T4		X					X				T4		X	X								
F8				X							F8											
Beta1 E	FX	ME	ST	PC	BT	EO	DP	EA	E-OP	HV	Beta2 E	FX	ME	ST	PC	BT	EO	DP	EA	E-OP	HV	
Cz								X			Cz											
Fz											Fz											
F3		X	X					X			F3	X	X	X	X					X		
C3											C3											
P3	X		X		X	X					P3	X	X			X				X		
Pz											Pz											
P4											P4										X	
C4											C4											
F4									X		F4											
F7								X			F7		X	X						X		
T3											T3	X								X		
T5			X								T5		X							X		
O1											O1		X									X
O2											O2											
T6									X		T6					X					X	X
T4	X			X		X			X		T4	X			X		X		X			
F8	X										F8											

With respect to data averaged by including all electrodes a general lower production of beta1 and beta2 waves is seen. In the case of the beta2 waves a statistically conspicuous lower production was seen during the two video-clips dealing with examination anxiety and an emergency surgery (OP). Data for beta2 wave reduction for all electrodes are given in **Table 4**. With respect to beta2 waves a statistically conspicuous lower production was seen when watching the emergency clip, and a statistically significant lower production during watching the examination video clip. A similar effect was also seen with regard to the parieto-occipital area, where also during viewing the emergency clip statistically significantly less beta2 waves were produced besides the statistically conspicuous reduction when watching the horror video. Interestingly, delta and theta attenuation was observed very often during watching the video on dentist performance, but no attenuation of beta waves was observed under this condition. In general, changes of spectral power varied according to the test. For example, during watching the examination video, no attenuation of delta or theta activity was observed, however, attenuation of beta1 and beta2 power was seen in fronto-temporal areas and P₃.

3.3. Discriminant Analysis

Finally, all 102 parameters (17 electrode positions × 6 frequency ranges) were fed into a linear discriminant analysis for comparison with other drugs. As documented in **Figure 8** the effect of PASCOFLAIR® (verum) was projected at a considerable distance to the effect of its placebo proving its fast effectiveness within 45 min after intake.

3.4. Performance in Psychometric Tests

Analysis of the mental performance during the cognitive testing did not reveal any difference with respect to drug intake. Results are documented in **Table 5**.

4. Discussion

To follow the pharmaco-dynamic action of plant-derived drugs still remains a big challenge in the field of pharmacology. This applies especially for the discovering the possible

Table 5. Mental performance in three cognitive tests: Picture comparison, Memory test and brain teaser before and after intake of 2 tablets PASCOFLAIR® (verum) or placebo. Data are given in % of maximally reachable success (=100%). SD = standard deviation, SEM = standard error of the mean, h = hour.

	Performance of Picture Comparison			Performance of Memory Test			Performance of Brain Teaser		
		Placebo	Verum		Placebo	Verum		Placebo	Verum
0h	Mean	58.13	61.88	Mean	87.25	90.00	Mean	72.50	70.00
	SD	13.00	14.89	SD	17.43	18.85	SD	19.97	21.03
	SEM	2.91	3.33	SEM	3.90	4.22	SEM	4.47	4.70
0.75h	Mean	66.88	69.38	Mean	85.00	83.50	Mean	75.00	73.50
	SD	11.67	13.13	SD	17.01	26.11	SD	13.57	13.87
	SEM	2.61	2.94	SEM	3.80	5.84	SEM	3.04	3.10

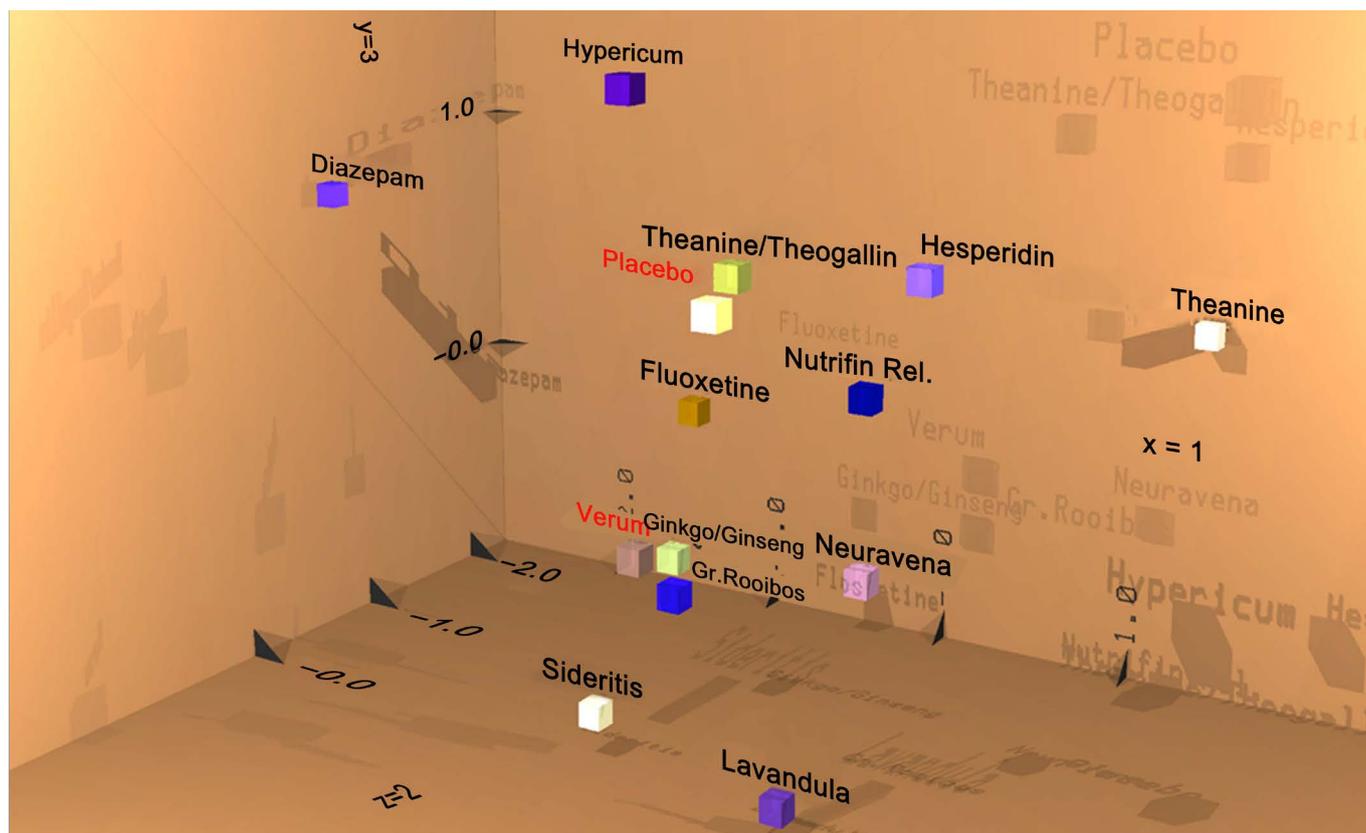


Figure 8. Result of discriminant analysis based on all brain regions and frequencies 45 minutes after intake. Results from the first three discriminant functions are depicted with the space coordinates x , y and z . Results from the next three discriminant functions are depicted as RGB colour mixture like in TV technology. Results from placebo and verum are marked red. Difference to baseline is taken for each drug. Diazepam and fluoxetine are looked at 1 hour after intake, hypericum after 2 hours, all other preparations 3 hours after intake. Please note that there is no preparation in the direct vicinity of PASCOFLAIR® (verum is far in the back, when looking into the three-dimensional space!).

mechanism of action. With respect to the brain, surrogate parameters may be very helpful and possibly are able to replace directly psychometric measurements [14].

Another surrogate parameter obtained from nuclear magnetic resonance measurements known as BOLD response has been related to electrophysiological signatures based on EEG frequencies [15]. These authors brought up evidence, that beta1 power extracted from the posterior cingulate cortex correlated positively with the activity in the right supplementary cortex and that beta2 power extracted from the precuneus correlated positively with activity in the left frontal pole. In contrast to the indirect character of the BOLD signal, electroencephalography (EEG) is a direct measure of neuronal activity, and provides an effective means of measuring neuronal firing [16] [17].

A first important result in the present study was obtained by searching for a relationship between the questionnaire “examination anxiety” called “PAF” in this report and quantitative EEG changes in terms of spectral power. For the median over all electrodes of beta1 as well as of beta2 power a statistically significant correlation was found.

As already detected in the correlation analysis mainly fronto-temporal brain areas were involved plus the left parietal brain area represented by the electrode position P₃. Increases in these areas have also been described in the presence of anxiety shortly before treatment at a dentist (Inaugural dissertation by Johanna Reuter, to be published). Increase of beta waves in the temporal and frontal lobe of the brain has already been seen in earlier experiments during watching horror videos (Dimpfel, unpublished). In a study on students it was observed that in subjects with high motivation and anxiety level the relative delta activity increased in right frontal brain areas, whereas in student with medium motivation immediately before an examination the relative beta2 power increases in right frontal brain areas [18].

This suggested a closer look at changes of delta and beta waves in order to proof the efficacy of PASCOFLAIR®. A further precondition for this goal consisted in the question if the selected audio-visual challenges result in an increase of spectral beta power in comparison to just starring at a fixation cross on the screen. This was clearly the case, by it confirming that beta power should be selected as the target of quantitative EEG analysis in order to proof the efficacy of PASCOFLAIR®. In addition to these changes of beta spectral power, statistically significant increases of delta and theta power during most of the different challenges indicated a great mental activation (Table 6) and

Table 6. Overview on changes in spectral power induced by all challenges with respect to all frequencies (beta power shadowed). Data are given in μV^2 (bold) for the fronto-temporal brain area (combination of all frontal and temporal electrodes) $n = 40$ (baseline 0 h). Statistical significance (sign-test) in comparison to watching a fixation cross on the screen is indicated as “p<” beneath the spectral power.

E-Combination [μV^2] F ₂₃₄₇₈ T ₃₄₅₆	Delta	Theta	Alpha1	Alpha2	Beta1	Beta2
Fix Cross	2.37	1.80	1.49	1.25	1.33	1.93
Memory	3.19	1.95	1.28	1.21	1.49	2.13
	0.000001	0.017	0.154		0.039	0.017
Stroop	2.83	1.95	1.18	0.89	1.36	1.70
	0.0002	0.039	0.006	0.000001		
Picture Comparison	3.54	2.14	1.38	1.04	1.45	2.36
	0.000001	0.00004		0.081	0.039	0.0007
Brain Teaser	3.22	2.21	1.38	1.10	1.59	2.27
	0.000001	0.0002			0.017	0.039
Eyes Open	2.47	1.71	1.43	1.38	1.46	2.00
Dentist Pain	2.95	1.76	1.21	1.00	1.41	2.20
	0.081		0.002	0.006		0.038
Exam Anxiety	2.73	1.76	1.21	1.14	1.39	1.97
	0.002		0.017	0.0002		
Emergency-OP	2.81	1.87	1.24	1.12	1.57	2.19
	0.002		0.039	0.081	0.039	0.039
Horror Video	2.79	1.78	1.22	1.00	1.46	2.02
	0.017		0.039	0.006	0.039	0.081

confirm results already published during similar or identical presentations [9]. Comparing now the changes of spectral power in the presence of PASCOFLAIR® with the changes as observed in the presence of placebo, a reduction of delta and theta power and clearly lower increases of beta power were observed in nearly all challenges (s. overview in Table 4). The reduction of spectral power increases of beta waves can therefore be taken surely as a surrogate parameter for the anti-stress and calming effect of PASCOFLAIR®. The fact that always the same fronto-temporal electrodes and P₃ showed more or less statistically conspicuous or significant reductions in the beta range provides safe evidence that subjects experienced less stress and anxiety leading to higher focusing on the presented tasks. Reduction of anxiety in the presence of Pascoflair® has been reported in ambulatory surgery patients [19].

According to earlier investigations changes in beta1 power are related to glutamatergic neurotransmission, whereas changes in beta2 frequencies are related to GABAergic transmission [6]. But there is also evidence from earlier experiments, that beta1 waves are under the control of GABA [20]. However, it is generally accepted, that spectral beta power is related to GABAergic transmission [21].

Due to the influence of PASCOFLAIR® on beta activity its mechanism of action can be assumed to be related to changes of glutamatergic and GABAergic transmission, respectively. Modulation of the γ -Aminobutyric Acid (GABA) system by *Passiflora incarnata* has been recognized earlier based on in vitro experiments [22]. The current results confirm this hypothesis, however, point to an involvement of the glutamatergic system as well. Interestingly, psychometric performance indicated no negative influence on cognitive function. Finally, discriminant analysis confirmed statistically the quick and efficient effectiveness of PASCOFLAIR® in relation to other drugs or herbal preparations confirming and extending the data from the earlier publication. The fast begin of effectiveness defined as cerebral bioavailability of PASCOFLAIR® (within 30 minutes) has been detected already earlier [1]. In this earlier publication 3 tablets were administered. In the present investigation only 2 tablets were given and fast effectiveness confirmed at 45 minutes after intake. A further difference to the earlier experimental series consisted in the fact that now subjects suffering from examination anxiety were tested.

5. Conclusion

Psychophysiological characterization of the effect of PASCOFLAIR® in subjects suffering from examination anxiety revealed attenuation of delta, theta and beta spectral power during cognitive and emotional challenges in several brain regions. In view of a significant correlation between beta power and the score of exam anxiety questionnaire PAF (Prüfungsangstfragebogen) this attenuation must be interpreted as ability to counteract examination induced stress symptoms. Results confirm and extend earlier experimental data showing a calming action of PASCOFLAIR®.

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