2. SYNOPSIS

Sponsor:	Hevert-Arzneimittel GmbH & Co. KG In der Weiherwiese 1, D-55569 Nussbaum, Germany
Study title:	Effectiveness of CALMVALERA HEVERT as measured by quantitative EEG in 24 subjects during audio-visual cognitive and emotional challenges. A double-blind, randomized, placebo-controlled, 2-armed, Phase IV study in parallel design.
Investigators:	Samir Suliman, Dr. med. Klaus Koch
Study centre:	NeuroCode AG, Sportparkstr. 9, D - 35578 Wetzlar, Germany MVZ Labordiagnostik Mittelhessen GmbH, Ursulum 1,D-35396 Gießen, Germany
Study period:	First subject included: 01.10.2015 Last subject completed: 03.11.2015
Test preparation, dose mode of administration and batch number:	CALMVALERA HEVERT - oral - 6 tablets Ch.-no 002510 Placebo – oral – 6 tablets Ch.-no 002510
Duration of treatment:	Single administration
Clinical phase:	IV
Demographic data:	Age 18-40 years, Overall n=24, Mean 24.88 year, SD 4.21 Male n=10, Mean 25.20 year, SD 3.74 Female n=14, Mean 24.64 year, SD 4.63
Objective:	Anxiolytic effects of CALMVALERA HEVERT tablets shall be tested in subjects suffering from test anxiety after single intake by aid of a newly developed, validated method consisting of a combination of Eye-Tracking (following glances) with Neurocode-Tracking (quantitative EEG with a time resolution of 364 ms) termed "EnkephaloVision".

Criteria for evaluation

Primary Objective

Primary outcome measures: Comparison of verum and placebo is performed on electric power in 17 different brain regions using six frequency ranges defined as target parameters in the presence of different stress inducing cognitive tests and emotional video scenes (i.e. quiz, memory test, Stroop test). With respect to cognition and emotion changes in frontal and temporal spectral beta power are evaluated.

Secondary Objective

Secondary outcome measures are tolerability and a correlation between questionnaire score (HAMA) and spectral EEG power with regard to alpha frequency ranges.

Methodology:

Quantitative-topographical EEG by measurement of current source density during different cognitive and emotional challenges (Dimpfel et. al., 1996).
Combination of Neurocode-Tracking (quantitative-topographic EEG with epoch length of 364 ms) with Eye-Tracking (following eye gazes) termed "EnkephaloVision (Dimpfel and Hofmann 2011, Dimpfel 2014, Dimpfel and Morys 2014).

Diagnosis and main criteria for inclusion:

Healthy male and female subjects.
Age between 18 and 40 years (both included).
Test Anxiety questionnaire "PAF" (for pre-selection of subjects) - values above T > 60 are regarded as conclusive.
Subject must be capable of giving informed consent.
Acceptance of written consent to participate in the study after instruction in written and oral form (informed consent).

Criteria for exclusion:

Acute or chronic disease with an impact on the study, which becomes obvious by case history or clinical examination.
Clinically relevant pathological findings from clinical and laboratory findings.
Presence of clinically relevant pathological EEG features or artifact-free portion of the screening EEG <30%.
Clinically relevant allergic symptoms.
Detection of alcohol at the time of initial examination (day SC) or on study day A (positive alcohol test) or by case history. Detection of drugs (positive drug test) at the time of initial examination (day SC).
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 antihypertensives). Known intolerance / hypersensitivity (allergy) to plant derived extracts (Cimicifuga, Cocculos, Passiflora, Valeriana etc. ....) 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).
BMI (Body Mass Index) < 18 or > 32.
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.
Participation in another clinical trial within the last 60 days.
Positive pregnancy test (day A).
Lactation.
Bad compliance.
Cancellation of informed consent.

Safety: ECG, pregnancy test, alcohol test, drug test, blood test, clinical examination.

Statistical methods: EEG data from the first recording session before intake of the capsules are given as absolute numbers (μV^2). For explorative statistical evaluation the nonparametric 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 colour, respectively, followed by an additive colour mixture (so-called RGB-mode). In order to document statistically the different electric reactions of the brain to various cognitive and emotional loads, data from each part of the presentation were divided by the data obtained during recording in a relaxed state with eyes open (1 minute) at the beginning. Comparison of CALMVALERA HEVERT tablets versus placebo was accomplished by evaluation of the second recording of the day 90 minutes after intake. Data from the first recording (baseline) were set to 100% and electrophysiological changes produced by placebo or CALMVALERA HEVERT tablets were depicted as %-changes thereof.

Results: The effectiveness of 6 tablets of CALMVALERA HEVERT was tested by using a new methodology called "EnkephaloVision", which consists of a combination of a fast quantitative EEG analysis termed "Neurocode-Tracking" and Eye-Tracking. Twenty-four subjects suffering from examination anxiety - selected by a questionnaire - were exposed to a series of mental tasks and emotional audio-visual video-clips before and 90 minutes after intake of 6 tablets of CALMVALERA HEVERT. A correlation between the HAMA questionnaire score values and measurement of absolute spectral EEG power was detected in the alpha1 and alpha2 range in a special fronto-central brain area represented by electrode positions Cz and $F_{3,4}$ (Figure I). Quantitative analysis of the frequency changes induced by CALMVALERA HEVERT with respect to different brain regions revealed a statistically significant increase of apha1 and alpha2 spectral power at electrode positions F_3, C_3, P_3, T_3 and O_1

already under the recording condition "eyes open as depicted in Figure II and in the presence of several challenges. Remarkably, these electrode

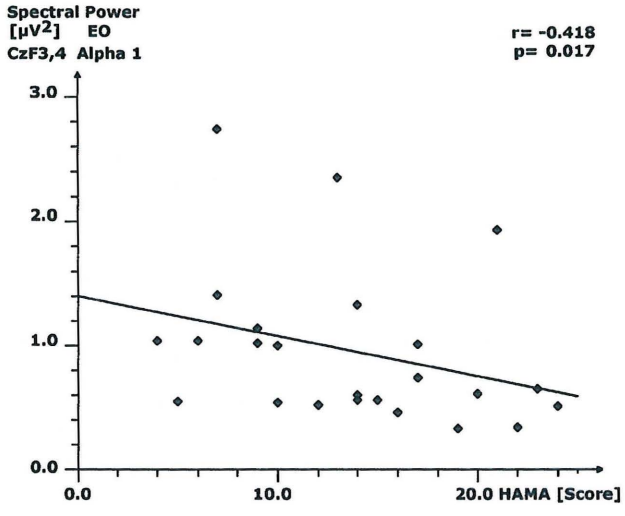

Figure I Correlation between anxiety questionnaire (HAMA) and spectral alpha1 power during the recording condition "eyes open" before drug intake. Spectral power was averaged including electrodes C_z, F_3 and F_4.

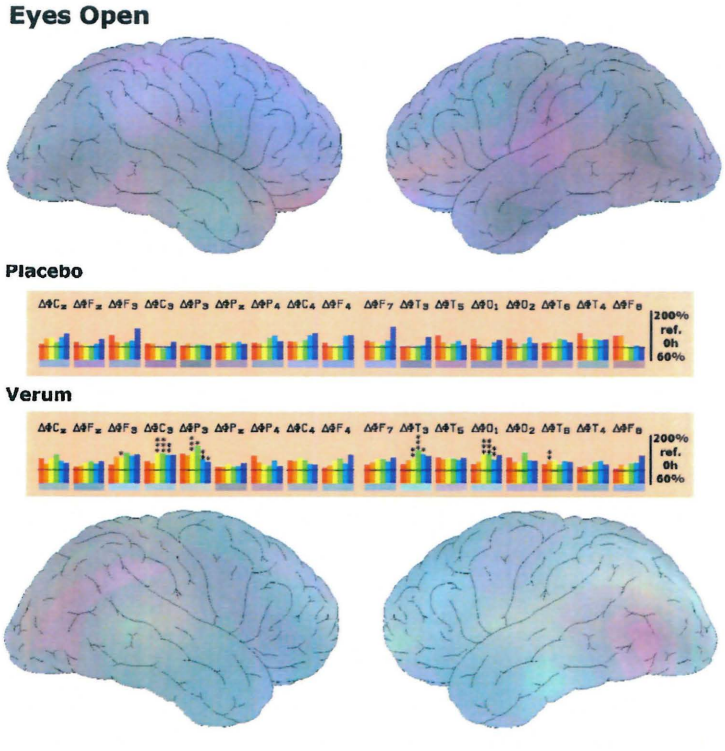

Figure II Effect of Placebo or Verum (CALMVALERA HEVERT) on spectral frequency power for each brain region represented by 17 electrode positions. Delta waves are coded into red, theta into orange, alpha1 into yellow, alpha2 into green, beta1 into turquoise, beta2 into blue. Data are given as % of baseline (ref) before intake. Statistical significance (Wilcoxon-Test) in comparison to placebo is indicated by stars: *=p<0.10; **=p<0.05; ***=p<0.01.

positions represent all areas of the left hemisphere (uneven numbers). There are also minor increases on the right hemisphere but nearly none became statistically significant. Statistically significant increases of spectral alpha 1 and alpha2 power were observed during 5 out of 10 of the challenges nearly only in the left hemisphere (Figure III for alpha1).

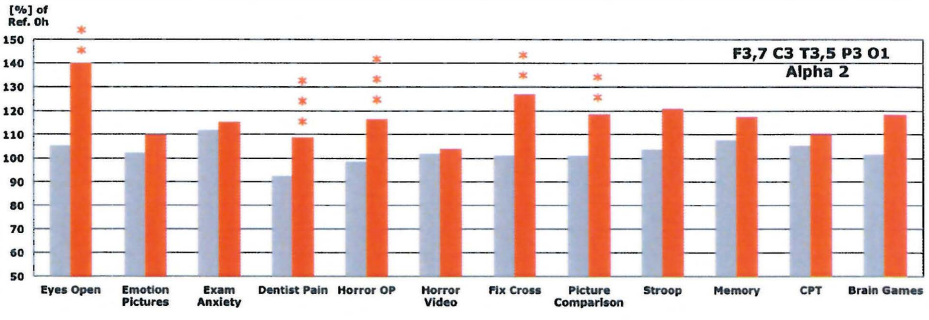

Figure III Increases of spectral alpha1 power 90 min after intake of 6 tablets of CALMVALERA HEVERT. Statistical significance (Wilcoxon-Test) in comparison to placebo is indicated by stars: **=p<0.05; ***=p<0.01.

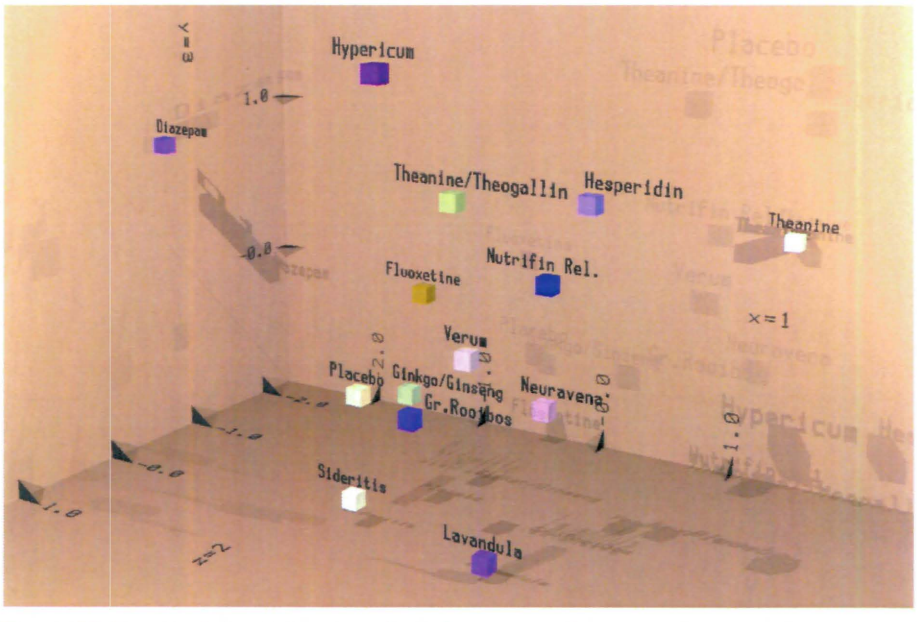

Figure IV Result of discriminant analysis based on all brain regions and frequencies 90 minutes after intake. Results from the first three discriminant functions are depicted with the space coordinates x, y and z. Results from the next three discriminant functions are depicted as RGB colour mixture like in TV technology. 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.

Comparing now the changes of spectral power in the presence of CALMVALERA HEVERT tablets with the changes as observed in the

presence of Placebo, clearly higher production of alpha power was observed in most of the challenges. Mainly left-brain areas were involved.

Finally, all 102 parameters (17 electrode positions x 6 frequency ranges) were fed into a linear discriminant analysis for comparison with other drugs. As documented in Figure IV the effect of CALMVALERA HEVERT tablets (Verum) was projected at a considerable distance to the effect of its placebo (Figure IV) proving its fast effectiveness within 90 min after intake.

CONCLUSION

Intake of 6 tablets of CALMVALERA HEVERT did not disturb processing of cognitive or emotional challenges in terms of delta-theta or beta electric activity. However, a statistical significant increase of alpha1 and alpha2 activity was observed in the presence of CALMVALERA HEVERT during most of the challenges, especially within the left hemisphere. Prevalence of alpha activity is regarded as a safe indicator of higher relaxation. Since alpha1 waves are under the control of serotonin and alpha2 waves under the control of dopamine, these two neurotransmitters seem to be down regulated in the presence of the drug. This down-regulation also corresponds to calming behaviour. Thus, the new methodology of "EnkephaloVision" not only objectively unravelled the calming action of CALMVALERA HEVERT, but also gave some hints on the mode of action by inducing attenuation of catecholaminergic neurotransmission.