

Zynamite® (*Mangifera indica* Leaf Extract) and Caffeine Act in a Synergistic Manner on Electrophysiological Parameters of Rat Central Nervous System

Wilfried Dimpfel^{1*}, Julia Wiebe², Nigel Gericke², Leonie Schombert³

¹Justus-Liebig-University Giessen c/o NeuroCode AG, Wetzlar, Germany

²Nektium S.L., Las Palmas, Spain

³NeuroCode AG, Wetzlar, Germany

Email: *wilfried.dimpfel@pharma.med.uni-giessen.de

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Abstract

Zynamite®, a special extract from *Mangifera indica*, exerted stimulatory properties on the central nervous system during a pilot study. The question arose if Zynamite® would have a similar action on the central nervous system as caffeine. Two well established animal models were used: a) quantitation of spectral power of field potentials in the freely moving rat and b) induction of long term potentiation (LTP) in the hippocampus slice preparation ex vivo after one week of daily administration. In the presence of 25 mg/kg of Zynamite®, predominantly alpha2 and beta1 spectral frequencies were attenuated in all brain areas during the first hour after administration. Exactly this pattern of frequency changes had been observed in earlier studies with i.p. administration of caffeine. Discriminant analysis confirmed this similarity by projection of Zynamite® and 0.5 mg/kg caffeine into close neighborhood and showing identical colours, which points to a similar mechanism of action in this analysis. In addition, when Zynamite® was combined with very low doses of caffeine synergistic effects were observed. Since alpha2 waves are under the control of dopamine, activation of this neurotransmitter system might be responsible for the stimulating property of Zynamite®. These results are corroborated by the results from the ex vivo study using the hippocampus slice in vitro to follow changes in excitability in the presence of 0.5 mg/kg of caffeine, 25 mg/kg of Zynamite® or their combination in comparison to Placebo after daily administration for one week. Both caffeine and Zynamite® increased LTP. LTP relates to space and time dependent memory. From these studies it is evident that both caffeine and Zynamite® act in similar ways on brain elec-

trical activity, and have potential to improve cognitive function. Bioactive compounds of Zynamite® clearly pass the blood brain barrier to act on the central nervous system. Due to the demonstrated similarity of action, Zynamite® has potential as a CNS-activating nutraceutical that could be used to replace caffeine.

Keywords

Zynamite®, *Mangifera indica*, Caffeine, Rat, Field Potential, Hippocampus Slice, Synergy

1. Introduction

Mangifera indica is commonly known as “Mango” and belongs to the family of Anacardiaceae. It was domesticated in India about 2000 BC (Wikipedia). The leaves contain a high amount of Mangiferin, a pharmacologically active hydroxylated xanthone C-glycoside. Extract of the leaves have shown antibiotic properties and have diverse uses in traditional Ayurveda medicine [1].

This investigation deals with Zynamite®, a special Mango leaf extract containing 60% of Mangiferin. Since a recent pilot clinical trial in 16 healthy subjects has shown brain stimulatory activity with no cardiovascular changes, the question arose whether Zynamite® could replace caffeine without the side effects known for intake of high doses of caffeine. The present series of experiments focus on the comparison of the effect of Zynamite®, caffeine and a combination thereof to placebo 1) with respect to quantitation of spectral power of field potentials in the freely moving rat and 2) with respect to induction of long term potentiation in the hippocampus slice preparation *ex vivo* after one week of daily administration.

Recording of field potentials from freely moving rats and their wireless transmission has been used now for more than 30 years [2]. Frequency analysis of signals from four brain regions (frontal cortex, hippocampus, striatum and reticular formation) by Fast Fourier transformation (FFT) allowed to differentiate clinically used drugs with respect to their indication when using linear discriminant analysis [3]. This method has been demonstrated to be able to characterize CNS-active herbal preparations with respect to their potential clinical use [4].

The hippocampal slice preparation is a validated model for direct analysis of interaction of substances with living neuronal tissue [5] [6]. Due to the preservation of the three-dimensional structure of the hippocampal tissue, substance effects on the excitability of pyramidal cells can be studied in a unique manner. The stimulation of Schaffer Collaterals leads to release of glutamate resulting in excitation of the postsynaptic pyramidal cells. The result of the electrical stimulation is recorded as so-called population spike (pop-spike) representing the number of recruited pyramidal cells. An interesting result using this model was for example the demonstration of the ability of memantine, a pharmaceutical

used in the treatment of dementia, to increase the population spike amplitude in response to single stimuli and theta burst stimulation to increase long term potentiation [7].

2. Material and Methods

2.1. Field Potential Analysis in Freely Moving Rats

EEG signals were recorded from frontal cortex, hippocampus, striatum and reticular formation of freely moving rats from inside a totally copper shielded room. Signals were wirelessly transmitted by a radio-telemetric system (Rhema Labortechnik, Hofheim, Germany, using 40 Megahertz as carrier frequency) and were amplified and processed as described earlier to give power spectra with a resolution of 0.25 Hz [8]. In short, after automatic artefact rejection signals were collected in sweeps of 4 s duration and Fast Fourier transformed using a Hanning window. Sampling frequency was 512 Hz. Four values were averaged to give a final sampling frequency of 128 Hz, well above the Nyquist frequency. The resulting electrical power spectra were divided into 8 specially defined frequency ranges (delta: 1.50 - 4.00 Hz; theta: 4.25 - 6.75 Hz; alpha1: 7.00 - 9.50 Hz; alpha2: 9.75 - 12.25 Hz; beta1a: 12.50 - 15.00 Hz; beta1b: 15.25 - 17.75 Hz; beta2: 18.00 - 34.25 Hz; gamma: 34.50 - 81.00 Hz). Spectra were averaged in steps of 3 minutes each and displayed on-line. In an off-line procedure spectra were averaged to give longer periods for further analysis and data presentation. All dosages of caffeine and its combination with Zynamite® were tested by oral acute administration. Dosage of Zynamite was chosen on the base of earlier experiments. Solutions were prepared fresh for each experimental day and administered orally by gavage after 45 minutes of pre-drug Vehicle recording. Vehicle was water. Extracts are listed in **Table 1**.

Nine adult male Fisher 344 rats (5 months of age and day-night converted, weight about 350 - 400 g, provided by Charles River Laboratories, D-97633, Sulzfeld) remained from a preceding study with allowance from the local legal authority "Regierungspräsidium Giessen" and were used in this experimental series. Animals were day-night reversed (12h/12h). The principles of good laboratory animal care were followed in all trials. Animals were implanted with

Table 1. Test drugs-Listing of experimental extracts and vehicle.

Test drugs	Dose	From
Zynamite®	25 mg/kg	Nektium Pharma S.L.
Zynamite® + Caffeine EP USP Natural Anhydrous	25 mg/kg + 0.25 mg/kg	Nektium Pharma S.L.
Zynamite® + Caffeine EP USP Natural Anhydrous	25 mg/kg + 0.50 mg/kg	Nektium Pharma S.L.
Caffeine EP USP Natural Anhydrous	0.5 mg/kg	Nektium Pharma S.L.
VEHICLE 0.9% NaCl	1.0 ml/kg	Braun Melsungen

electrodes into the brain and were given two weeks for recovery from surgery. After this, the wireless transmitter was plugged in for adaptation and control experiments. Unfortunately, 3 animals dropped the implant after performance of the placebo series. After testing the two combinations one further animal dropped the implant thus allowing to test the final single dose of caffeine in only 5 animals. During the recording rats were not restricted and could move freely but did not have food available (chewing would have produced too many artefacts).

Rats were implanted as reported earlier [8]. A crossover design with at least three days of drug holidays in between the administrations was used. Controls were performed by oral administration of 1.0 ml/kg of vehicle (0.9% NaCl) and the mixture Zynamite® (25 mg/kg) and Caffeine (0.25 and 0.50 mg/kg) as well as Caffeine (0.50 mg/kg). Data were averaged from 5 - 9 animals. Data are expressed as mean values \pm S.E.M. Statistics were calculated by means of the Wilcoxon, Mann, Whitney U-test. The animals were dosed orally at a constant volume of 1.0 ml/kg per kg body weight. The dosage administered to each animal was determined every day by the weight of that animal at the time of administration. Wilcoxon, Mann, Whitney U-test was used throughout all experimental data for comparison to results obtained by vehicle administration at the particular timing.

2.2. *Ex Vivo* Analysis of Population Spikes in the Hippocampal Slice Preparation

Hippocampus slices were prepared as reported earlier [8]. In short, after daily administration of trial medication for one week 4 animals were sacrificed under deep anesthesia and the hippocampus was quickly taken out and chopped by a vibrating razor blade to give 6 slices of about 400 μ thickness per animal. After adaptation to artificial cerebrospinal fluid for one hour, slices were transferred to an incubation chamber [9] and superfused with cerebrospinal fluid at 35°C [10]. Stimulation of the Schaffer Collaterals by single stimuli or theta burst stimuli resulted in the induction of a population spike or long-term potentiation, respectively [5]. Amplitudes of the population spikes were recorded after stimulation every 10 minutes to prevent potentiation mechanisms. Each animal had been treated for one week with each one of the preparations. Slices were taken one day after the last treatment. Excitability of the hippocampus slices was checked after administration of 25 mg/kg of Zynamite®, a combination with 0.5 mg/kg of caffeine and 0.5 mg/kg of caffeine alone. Rats were treated with each of the preparations daily for one week before the hippocampus was taken out at the next day. An example of the amplitude of the population spike after single shock stimulation and theta burst stimulation is given in **Figure 1** for the animal treated with placebo (upper part of the image) and the combination of Zynamite® with caffeine (lower part of the image). Stimulation of the Schaffer Collaterals was performed only every 10 minutes in order to prevent physiological potentiation mechanisms.

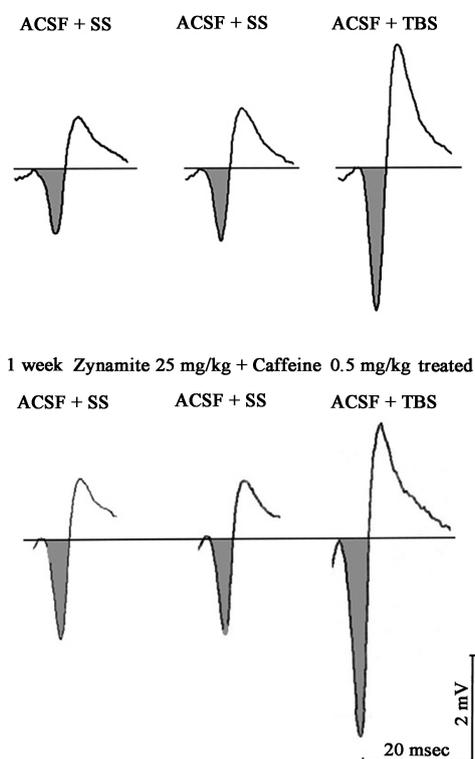


Figure 1. Documentation of original signals (one slice) showing the effects of using single stimuli (SS) or theta burst stimulation (TBS) in the presence of artificial cerebro-spinal fluid (ACSF), Placebo 1 ml/kg or combination from Zynamite® (25 mg/kg) and Caffeine (0.5 mg/kg). The amplitude is calculated from baseline to the down reflection of the signal (shadowed). Scales: Time is given in milliseconds (ms), amplitude in millivolts (mV).

3. Results

3.1. Field Potential Analysis

Oral administration of the vehicle (0.9% NaCl) only resulted in very minor changes of spectral power within the four brain areas. During the second and third to the fifth hour tiny decreases of power emerged in the frontal cortex and hippocampus. With respect to gamma power some tiny increases were observed. A complete time course is given in **Figure 2**.

Oral administration of 25 mg/kg of Zynamite® resulted in a statistically significant attenuation of all classic frequencies during the first hour after administration. Strongest effects were documented with respect to alpha2 and beta1 spectral frequencies. Mainly frontal cortex and striatum were involved. During the third hour after administration significant increases of delta power were seen ($p < 0.05$). Within the striatum a non-significant attenuation of alpha1 and beta power was recognized throughout the whole recording period of 5 hours (**Figure 3**).

Oral administration of Caffeine (0.50 mg/kg) resulted in a statistically conspicuous attenuation ($p < 0.1$) of delta power in the frontal cortex. In the hippocampus statistically significant attenuation of delta, theta and beta power was

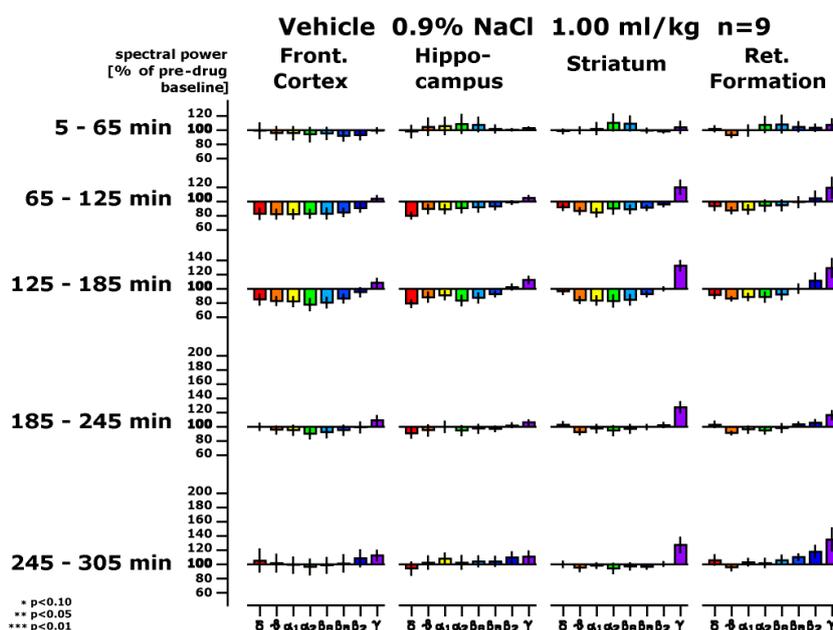


Figure 2. Effect of Vehicle: Time dependence of changes of spectral power (Ordinate) in % of the 45 min lasting pre-drug baseline values in four brain regions of the freely moving rat in the presence of Vehicle (0.9% NaCl 1.0 ml/kg). Frequency ranges are depicted as coloured bar graphs on the abscissa representing delta (red), theta (orange), alpha1 (yellow), alpha2 (green), beta1a (light blue) and beta1b (dark blue) and gamma spectral power (violet) from left to right within the four brain areas as mentioned on top of the graph.

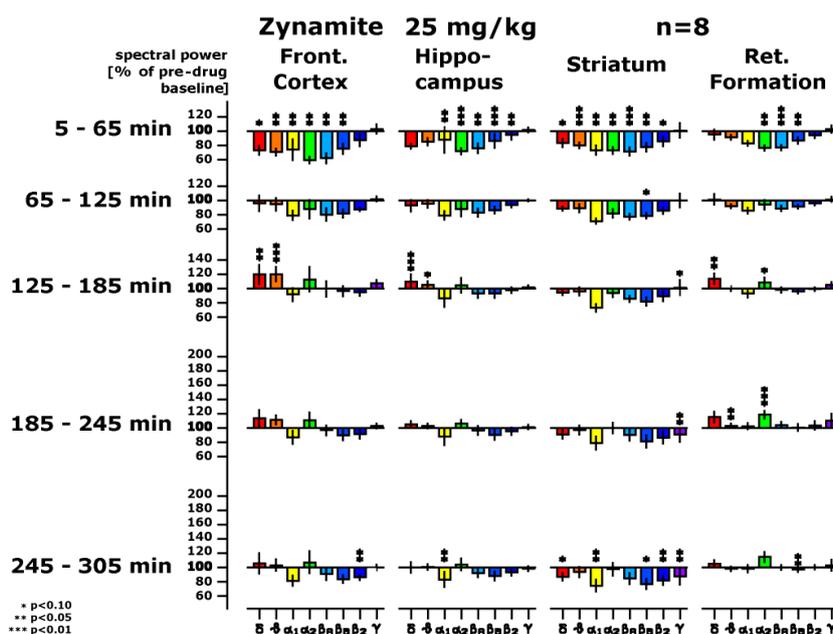


Figure 3. Frequency changes in the presence of 25 mg/kg Zynamite®. Frequency ranges are depicted as coloured bar graphs on the abscissa representing delta (red), theta (orange), alpha1 (yellow), alpha2 (green), beta1a (light blue) and beta1b (dark blue) and gamma spectral power (violet) from left to right within the four brain areas as mentioned on top of the graph. Statistical significance in comparison to control (vehicle) is documented by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

recognized. In the striatum a minor but statistically conspicuous decrease of delta waves was seen. From the second hour after administration on, increases of delta power emerged for the rest of the recording period in the frontal cortex and reticular formation. During the last hour increases of alpha2 power were observed except for the striatum. A whole time course is given in **Figure 4**.

Oral administration of the combination of Zynamite® (25 mg/kg) + Caffeine (0.25 mg/kg) resulted in a statistically significant attenuation of all frequencies except for delta spectral power in comparison to placebo during the first hour after administration. Strongest attenuation was seen with respect to alpha1 and beta1 power in all brain regions. The alpha1 attenuation lasted into the second hour after administration, within the striatum into the last hour. During the third hour an increase of delta power emerged in the frontal cortex and hippocampus and somewhat less in the reticular formation. At the same time significant increases of alpha2 power were documented during the last 2 hours after administration. A total time course is given in **Figure 5**.

Oral administration of the mixture of Zynamite® (25 mg/kg) with Caffeine (0.50 mg/kg) resulted in a statistically significant attenuation of spectral power with respect to all frequencies except for gamma power in comparison to placebo during the first two hours after administration. Effects were strongest in frontal cortex and hippocampus. The attenuation lasted until the third hour after administration, in the hippocampus and striatum until the end of the recording period. A total time course is given in **Figure 6**. When directly comparing the

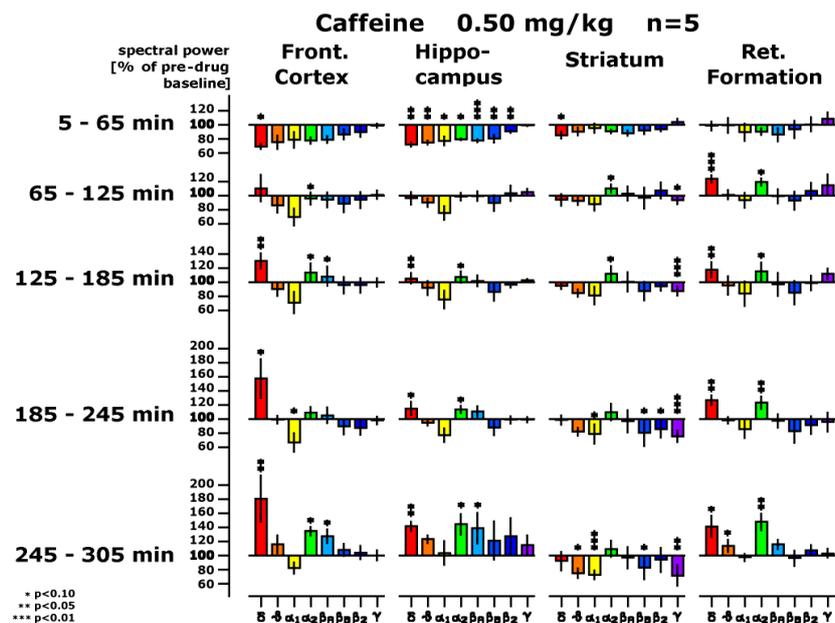


Figure 4. Frequency changes in the presence of 0.5 mg/kg caffeine. Frequency ranges are depicted as coloured bar graphs on the abscissa representing delta (red), theta (orange), alpha1 (yellow), alpha2 (green), beta1a (light blue) and beta1b (dark blue) and gamma spectral power (violet) from left to right within the four brain areas as mentioned on top of the graph. Statistical significance in comparison to control (vehicle) is documented by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

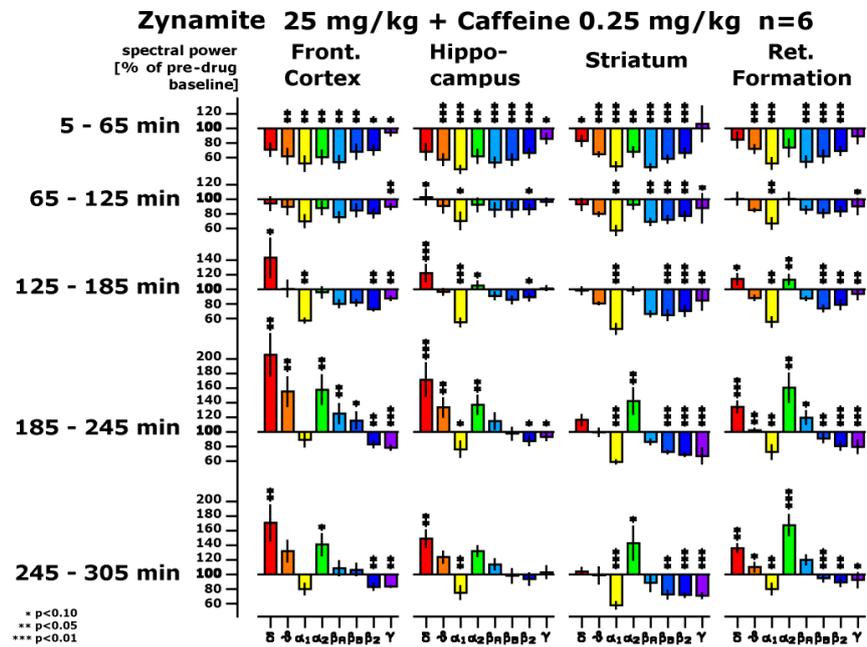


Figure 5. Frequency changes in the presence Zynamite® (25 mg/kg) in combination with 0.25 mg/kg caffeine. Frequency ranges are depicted as coloured bar graphs on the abscissa representing delta (red), theta (orange), alpha1 (yellow), alpha2 (green), beta1a (light blue) and beta1b (dark blue) and gamma spectral power (violet) from left to right within the four brain areas as mentioned on top of the graph. Statistical significance in comparison to control (vehicle) is documented by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

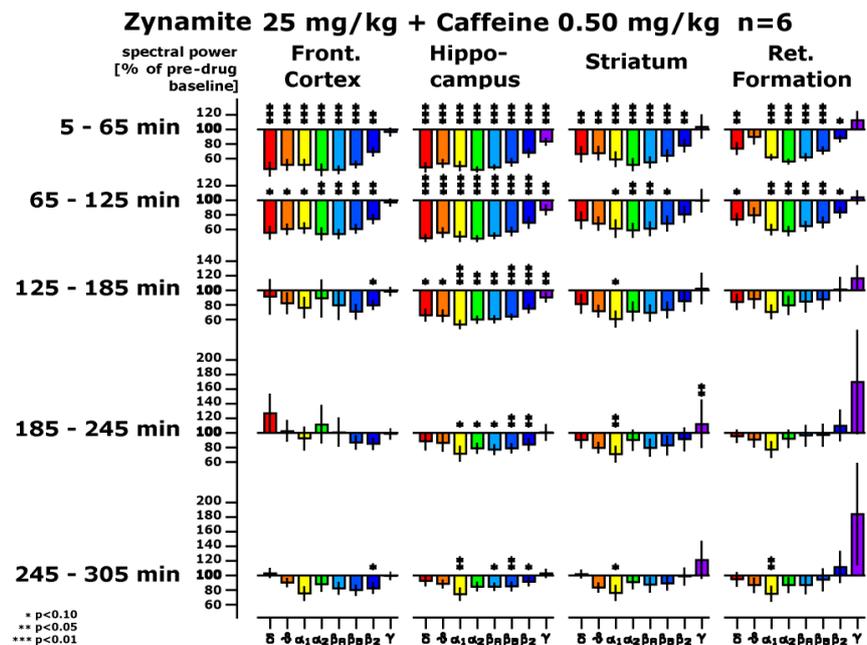


Figure 6. Frequency changes in the presence of Zynamite® (25 mg/kg) in combination with 0.5 mg/kg caffeine. Frequency ranges are depicted as coloured bar graphs on the abscissa representing delta (red), theta (orange), alpha1 (yellow), alpha2 (green), beta1a (light blue) and beta1b (dark blue) and gamma spectral power (violet) from left to right within the four brain areas as mentioned on top of the graph. Statistical significance in comparison to control (vehicle) is documented by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

effects of Zynamite® and caffeine during the first hour after administration a close similarity between both becomes visible. Both combinations induce a much stronger attenuation of nearly all frequencies. Strongest difference to the mono-preparation consists in a large decrease of alpha1 spectral frequencies indicating a different mechanism of action (Figure 7). This alpha1 attenuation also dominates the effect of Zynamite® and caffeine during the second hour after administration. Likewise, the combination of Zynamite® with the low dose of caffeine shows a strong attenuation with respect to alpha1 waves (Figure 8). A massive attenuation of all frequencies is observed in the presence of the combination of Zynamite® (25 mg/kg) with the higher dose of caffeine (0.5 mg/kg). This indicates a synergistic action, which can be demonstrated even more clearly when looking at the frequency changes in the hippocampus during the first 2 hours after administration (Figure 9).

A valuable methodology for comparison of frequency changes induced by different preparations has been described in numerous publications. Feeding all 24 variables (4 brain areas times 6 frequency ranges) into linear discriminant analysis the similarity of the action of Zynamite® and a low dose of caffeine is confirmed by projection of both in close neighborhood and showing the identical blue colour (Figure 10). The result of the combination is shifted more towards the plots for Paullinia and Methylphenidate indicating a different mechanism of

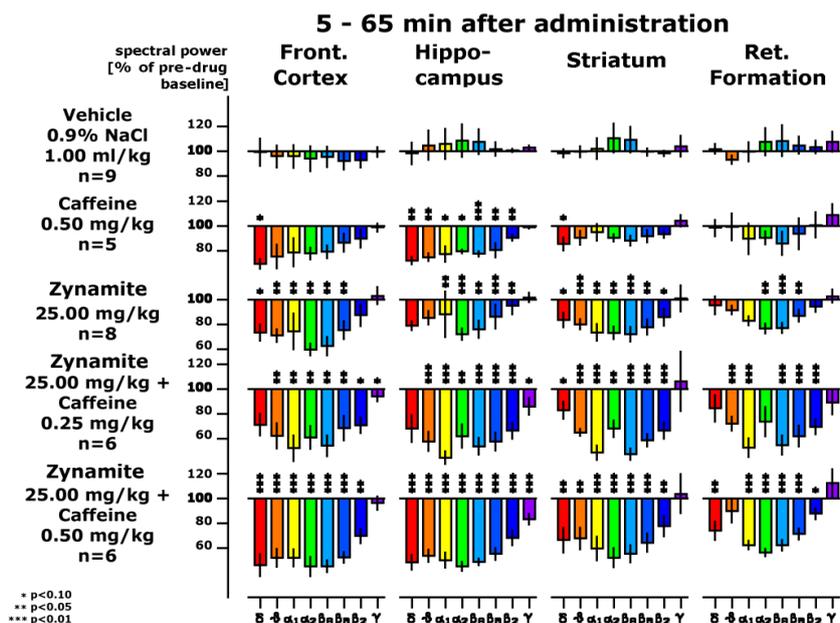


Figure 7. Direct comparison of the frequency changes observed in the presence of Zynamite® and caffeine alone and in the presence of the combinations **during the first hour** after administration. Frequency ranges are depicted as coloured bar graphs on the abscissa representing delta (red), theta (orange), alpha1 (yellow), alpha2 (green), beta1a (light blue) and beta1b (dark blue) and gamma spectral power (violet) from left to right within the four brain areas as mentioned on top of the graph. Statistical significance in comparison to control (vehicle) is documented by stars: * = p < 0.10; ** = p < 0.05; *** = p < 0.01. Please note different scaling with respect to ordinate in comparison to previous images.

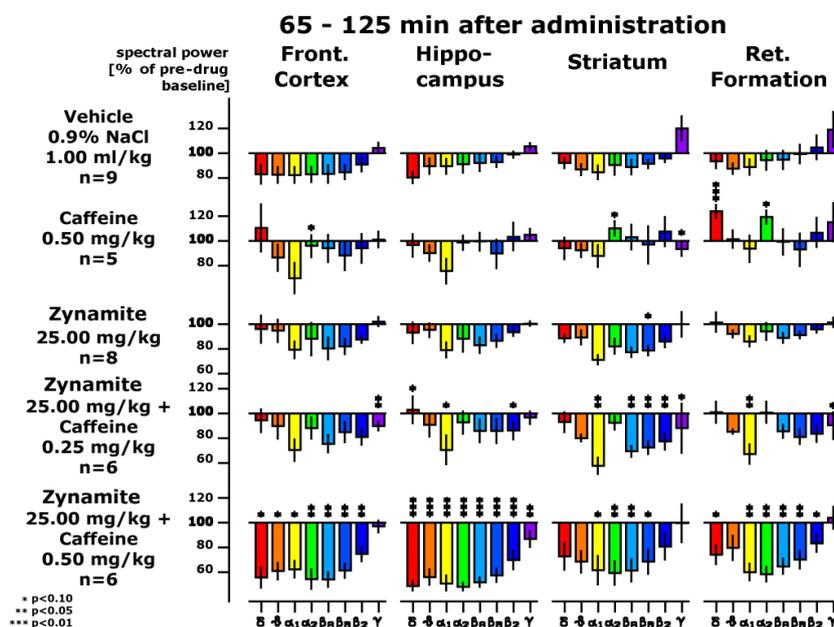


Figure 8. Direct comparison of the frequency changes observed in the presence of Zynamite® and caffeine alone and in the presence of the combinations **during the second hour** after administration. Frequency ranges are depicted as coloured bar graphs on the abscissa representing delta (red), theta (orange), alpha1 (yellow), alpha2 (green), beta1a (light blue) and beta1b (dark blue) and gamma spectral power (violet) from left to right within the four brain areas as mentioned on top of the graph. Statistical significance in comparison to control (vehicle) is documented by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$. Please note different scaling with respect to ordinate in comparison to previous images.

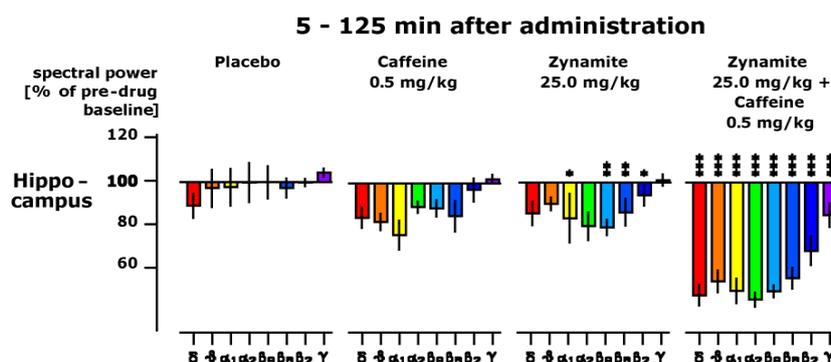


Figure 9. Documentation of synergy with respect to Caffeine and Zynamite® alone and their combination during the first two hours after administration in the hippocampus. Frequency ranges are depicted as coloured bar graphs on the abscissa representing delta (red), theta (orange), alpha1 (yellow), alpha2 (green), beta1a (light blue) and beta1b (dark blue) and gamma spectral power (violet) from left to right within the four brain areas as mentioned on top of the graph. Statistical significance in comparison to control (vehicle) is documented by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$. Please note different scaling with respect to ordinate in comparison to previous images.

action for the combination than for the mono-preparations. Details of administration of the preparation are listed in **Table 2**.

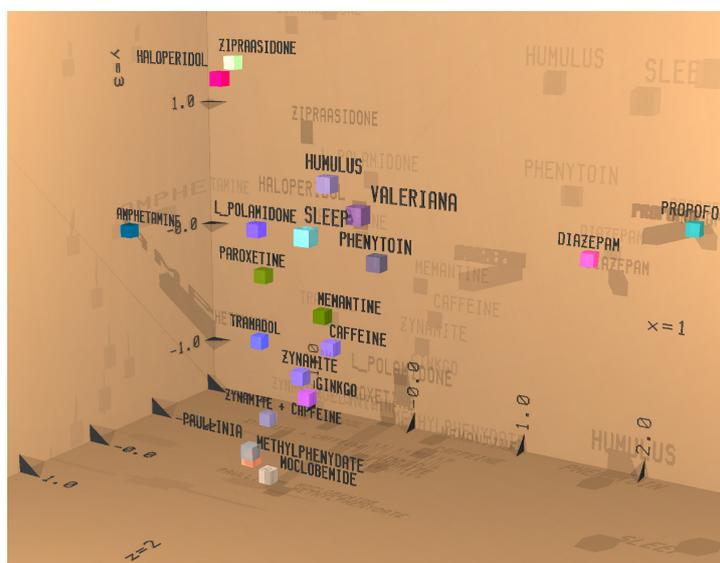


Figure 10. Documentation of the result of linear discriminant analysis of field potential data. Results of the first three discriminant functions are projected using spatial x, y and z-axis. Results from the next three functions are coded in colour using additive colour mixture according RGB like in TV.

Table 2. Listing of reference compounds documented in the discriminant analysis with respect to dosage and the type of administration. “i.p.” = intraperitoneal administration.

Substance Definition	Dose [mg/kg]	Application	Time	Substance Analysis	Dose [mg/kg]	Application	Time
Diazepam	0.50	i.p.	5 - 35 min	Ginkgo	100.00	orally	5 - 65 min
Memantine	3.00	i.p.	5 - 35 min	Paullinia	15.00	orally	5 - 35 min
L-Polamidon	1.00	i.p.	5 - 35 min	Valeriana	60.00	orally	125 - 185 min
Ziprasidone	1.00	i.p.	5 - 35 min	Humulus	50.00	orally	125 - 185 min
Paroxetine	1.00	i.p.	5 - 35 min				
(+) Amphetamine	0.20	i.p.	5 - 35 min				
Propofol	60.00	i.p.	5 - 65 min	Zynamite	25.00	orally	5 - 65 min
Moclobemide	5.00	i.p.	5 - 35 min	Zynamite + Caffeine	25.00 + 0.25	orally	5 - 65 min
Tramadol	5.00	i.p.	5 - 35 min				
Methylphenidate	2.50	orally	5 - 35 min				
Haloperidol	0.50	i.p.	5 - 35 min				
Caffeine	0.50	orally	5 - 65 min				
Phenytoin	4.00	i.p.	65 - 125 min				
Sleep			65 - 125 min				

Analysis of motion only revealed a statistically significant transient increase in the presence of the combination of Zynamite® with the lower dose of caffeine during the first hour. Caffeine alone and the combination of Zynamite® with the higher dose of caffeine induced higher motion during the first hour after administration, but did not reach statistical significance (Table 3).

3.2. Ex Vivo Analysis by Means of Hippocampus Slice Preparation

Time courses of the recordings from the 4 animals revealed very stable results over 2 hours. Population spike amplitudes after administration of 0.5 mg/kg of caffeine revealed no difference to placebo during single shock stimulation (blue line in Figure 11), but revealed clearly higher amplitudes during theta burst stimulation (green line in Figure 11).

The daily administration of 25 mg/kg of Zynamite® resulted in definitely higher amplitudes of the population spike already during single shock stimulation as documented in Figure 11. Theta burst stimulation induced not only massive higher amplitudes than after Placebo administration, but also clearly higher amplitudes in comparison to administration of caffeine (red line in Figure 11).

Finally, recording of population spike amplitudes after daily administration of the combination of 25 mg/kg Zynamite® with 0.5 mg/kg of caffeine revealed highest values during single shock stimulation as well during theta burst stimulation (dark blue line in Figure 11). The mean average values of three measurements (shadowed area in Figure 11) are documented with statistical significances in Figure 12.

Regarding now the numerical differences of the population spike amplitudes during single shock stimulation an over-additive effect becomes visible when comparing the effect of caffeine and Zynamite® alone with the combination. With respect to theta burst stimulation at least an additive effect is obvious (Table 4).

Table 3. Results of motion analysis.

Time [min]	Motion [cm h]			
	Vehicle 0.9% NaCl 1 ml/kg n = 9	Caffeine 0.50 mg/kg n = 5	Zynamite 25.0 mg/kg + Caffeine 0.25 mg/kg n = 6	Zynamite 25.0 mg/kg + Caffeine 0.50 mg/kg n = 6
-45 - 0	626.67 ± 120	874.45 ± 299	854.52 ± 142	650.78 ± 235
5 - 65	676.49 ± 117	1049.00 ± 112	1170.00 ± 160	1060.00 ± 239
65 - 125	794.39 ± 97	798.41 ± 83	952.00 ± 240	994.55 ± 218
125 - 185	1051.10 ± 81	682.94 ± 143	677.73 ± 128	1040.00 ± 205
185 - 245	913.30 ± 89	843.34 ± 154	603.47 ± 154	847.19 ± 198
245 - 305	695.38 ± 80	629.49 ± 115	696.78 ± 98	870.84 ± 224

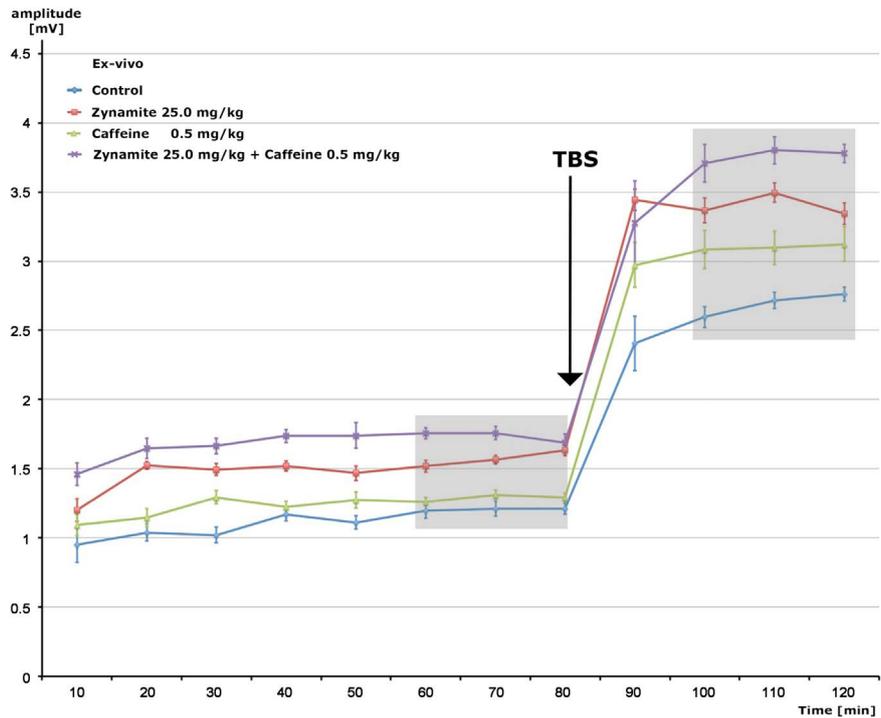


Figure 11. Time dependent effects of Control (Placebo), Caffeine (0.5 mg/kg), Zynamite® (25.0 mg/kg), and a combination of both (Zynamite® 25.0 mg/kg + Caffeine 0.5 mg/kg) on pyramidal cell activity in terms of changes of population spike amplitudes (as voltage on the ordinate). Results as obtained after performance of single stimuli (10 - 80 min) or after burst stimuli (90 - 120 min). Data are given as mean ± S.E.M. of n = 6 slices from 1 animal. Values from shadowed areas are averaged to give one value as documented in **Figure 12**.

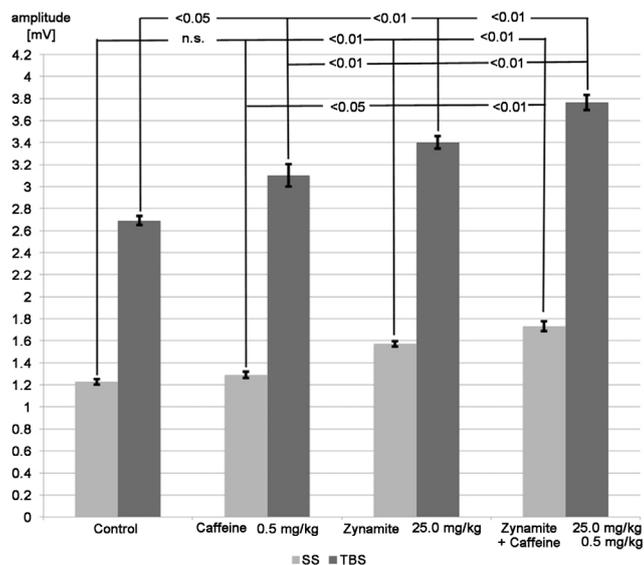


Figure 12. Effects of Control (Placebo), Caffeine (0.5 mg/kg), Zynamite® (25.0 mg/kg) and a combination of both (Zynamite® 25.0 mg/kg + Caffeine 0.5 mg/kg) on pyramidal cell activity in terms of changes of population spike amplitudes (as voltage on the ordinate). Results as obtained after performance of single stimuli (60 - 80 min) or after burst stimuli (90 - 120 min). Data are given as mean ± S.E.M. of n = 6 slices from 1 animal.

Table 4. Documentation of mean numeric differences between the 4 preparations in the presence of single stimuli and theta burst stimuli. Please note over-additive effect during single stimuli.

Mean \pm SEM [μ V]	SS 60 - 80 min	Diff. [μ V]	TBS 100 - 120 min	Diff. [μ V]
Control	-1226.48 \pm 23.85		-2691.17 \pm 39.78	
Caffeine 0.5 mg/kg	-1289.17 \pm 27.43	62.69	-3101.22 \pm 100.84	410.05
Zynamite 25.0 mg/kg	-1571.89 \pm 24.46	345.41	-3401.67 \pm 57.47	710.50
Zynamite 25.0 mg/kg + Caffeine 0.5 mg/kg	-1733.72 \pm 46.30	507.24	-3762.39 \pm 67.25	1071.22

4. Discussion

The animal model “Tele-Stereo-EEG” [2] has been used to characterize more than 200 preparations with respect to changes of the frequency content of field potentials recorded from different regions of the depth of the brain, namely frontal cortex, hippocampus, striatum and reticular formation. Feeding the data into linear discriminant analysis revealed that classical CNS-active drugs with well-established clinical indications group together according to the categories of clinical application [3].

The result of the present investigation revealed that the administration of Zynamite® or caffeine alone induced statistically significant attenuation of spectral power during the first hour after administration. In the presence of Zynamite® predominantly alpha2 and beta1 spectral frequencies in all brain areas were attenuated. Exactly this pattern of frequency changes with respect to dominating attenuation of alpha2 and beta1 frequencies was observed after intraperitoneal injection of higher dosages of 1 mg/kg, 2.5 mg/kg or 5 mg/kg of caffeine [11]. Thus, there is a great similarity between the frequency changes induced by Zynamite® with those seen in the presence of caffeine. Further, discriminant analysis confirms this similarity by projection the results of both preparations in close proximity to each other and showing identical colours, which points to a similar stimulant category of CNS activity.

Since it has been shown, that alpha2 waves are predominantly under the control of dopamine, activation of this neurotransmitter system might be responsible for the stimulating property of Zynamite®. Beta1 waves seem to be regulated by the neurotransmitter glutamate [12]. Changes of the glutamatergic system are observed during performance of cognitive tasks. Interestingly, Mangiferin, the major bioactive ingredient in Zynamite® has been reported to improve long-term object memory in rats [13]. Bioavailability of Mangiferin has been shown to be increased after oral administration of a polyherbal preparation in comparison to administration of isolated pure Mangiferin, and that Mangiferin was bound to various tissues except for the brain [14]. This raises the question if Mangiferin alone is responsible for the activating effect of Zynamite® or if co-ingredients

from the remaining 40% of the herbal formulation also contain pharmacologically active principles. The reproducible and stable attenuation of alpha1 spectral power during the rest of the recording time is interpreted as a higher state of wakefulness. Alpha1 waves are under the control of serotonin as reported earlier [15].

However, when Zynamite® was combined with very low doses of caffeine more than mere additive effects were observed. This can be recognized best by focusing on the frequency changes in the striatum and reticular formation (Figure 7). It is obviously that a potentiation mechanism has taken place. The same feature is observed when looking at the second hour after administration. In particular, when looking at the results obtained in the striatum and reticular formation, the highly significant attenuation of alpha1 waves cannot be explained by a simple additive effect. We emphasize that the combination of Zynamite® with a dosage of caffeine fifty times lower results in a tremendous attenuation of all frequencies except for spectral gamma power in all brain regions beginning in the second hour after administration. Thus, if most caffeine is replaced by Zynamite® but at least 2% of caffeine is retained, this large increase in stimulation can be achieved.

The results of the field potential approach are corroborated by the results from the *ex vivo* trial using the hippocampus slice *in vitro* to follow excitability changes in the presence of 0.5 mg/kg of caffeine, 25 mg/kg of Zynamite® or their combination in comparison to Placebo after daily administration for one week. This approach was undertaken in order to demonstrate that CNS-active bioactive compounds from Zynamite® are able to pass the blood brain barrier and exert their action by changing the excitability state of the hippocampus after daily administration of the four preparations. The results are in favour of such an action since Zynamite® alone and in combination with caffeine induces increases of the amplitude of the population spike in the presence of single shock stimulation and theta burst stimulation, whereas caffeine alone only increases the population spike during theta burst stimulation leading to long-term potentiation. Long-term potentiation relates to space and time dependent memory [16]. From this it is evident, that caffeine as well as Zynamite® act in a similar way and have potential to improve cognitive functions. Based on the similarity of their action with respect to long-term potentiation, a replacement of caffeine by Zynamite® makes sense.

5. Conclusion

In conclusion, *in vivo* administration of Zynamite® or caffeine displayed a similar action on the central nervous system with respect to changing the frequency pattern of brain regional field potentials and *ex vivo* by changing the excitability of the hippocampus of rats. A combination of both revealed over-additive or potentiating effects in both animal models pointing to a synergy between Zynamite® and caffeine. Zynamite® has potential as a CNS-activating nutraceutical that could be used to replace caffeine.

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Conflict of Interest

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