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Neuropharmacological Characterization of Extracts from *Rhodiola rosea*, *Oenothera paradoxa* and *Paullinia cupana* in Comparison to Caffeine

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Abstract

To find possible therapeutic applications involving the Central Nervous System (CNS) for herbals is a major challenge during functional food and drug discovery and development programmes. Despite the availability of numerous *in vitro* and *in vivo* tests, there is no single agreed screening procedure for pharmacological testing of herbal extracts with anticipated CNS activity. Experience gained from more than 25 years of testing has shown that two models give reasonably reliable orientation for future CNS applications: construction of an electropharmacogram based on wireless recording of field potentials from the depth of the brain of freely moving rats (Tele-Stereo-EEG) and recording of the population spike produced by pyramidal cells from hippocampal slices *in vitro*. A combination of these two methods has now been used to characterize the pharmacological profile of extracts from *Rhodiola rosea* root, *Oenothera paradoxa* seeds and *Paullinia cupana* seeds. Spectral analysis of field potentials revealed attenuation of alpha2 and beta1 waves was common for all extracts. According to previous studies, this is interpreted as activation of the dopaminergic and glutamatergic transmission. In addition, *Oenothera* and *Rhodiola* extracts attenuated delta and theta power, probably related to interference with the cholinergic and norepinephrinergic transmission, respectively. Using discriminant analysis for comparison with reference pharmaceutical and botanical drugs, *Rhodiola* projected near the position of Ginkgo extract, whereas *Oenothera* extract was projected near the position of Tramadol, an analgesic drug. Physical motion was increased only in the presence of *Paullinia* extract and caffeine. Increases of long-term potentiation were observed in the presence of *Rhodiola* extract, *Paullinia* extract and caffeine. The combined information predicts stimulant and cognitive function-enhancing activities in hu-

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mans for the *Rhodiola* extract, which could also be used as a possible caffeine-replacement, and antidepressant and analgesic activity for the *Oenothera* extract.

Keywords

Pharmacology, Field Potentials, Hippocampal Slices, *Rhodiola rosea*, *Oenothera paradoxa*, *Paullinia cupana*, Caffeine, RhodioLife®, Discriminant Analysis

1. Introduction

Therapeutic use of plants dates back thousands of years. However, knowledge on their use for specific indications is mostly based on uses in folk medicine. In order to allow for better quantitative testing and to get reproducible results, extracts are prepared from whole plants or plant parts in the hope that key pharmacologically active molecules survive this procedure in effective amounts. This is the precondition for pharmacological testing. The next problem consists in finding an appropriate *in vitro* or animal model. Since our interest is the brain as target, we focus our attention to the electric activity of the brain. Changes of local electric events in response to neurotransmitter activities govern behavior and are more accessible to study than chemical changes. Since the communication structure in the brain is based on electro-chemical processes, electric activity also contains information on underlying chemical changes induced for example by drugs. This reasoning has led to the development of a rat model allowing for continuous recording of focal field potentials from four brain regions and wireless transmission of the data for quantitative analysis by means of Fast Fourier Transformation. Testing of 40 synthetic reference drugs using this method revealed indication-specific clustering documented by linear discriminant analysis [1]. By keeping the discriminant functions fixed with regard to reference drugs in order to obtain a matrix of known pharmacological effects, it becomes feasible to classify the CNS activity of herbal extracts and extrapolate their possible therapeutic indication [2].

This approach has now been used to learn more about the CNS activities of three botanical extracts, namely from *Rhodiola rosea* root, *Oenothera paradoxa* seed and *Paullinia cupana* seed, in comparison to caffeine. Extracts from *Rhodiola* have been described to have a so-called adaptogenic action, which means that physical strength is fortified and subjects become more resilient to stress [3], however, there is also ample evidence for an antidepressant activity for *Rhodiola* [4] [5]. Recently, positive effects of *Rhodiola* on anxiety, stress, and cognition have been reported [6]. Extracts from *Paullinia* are appreciated in sports for their stimulating and endurance-enhancing actions [7] and was considered, among three other Brazilian plants, as an adaptogen [8]. Pharmacological testing of *Paullinia* extract has been only performed on the behavioural level [9]. Extracts from *Oenothera* have mainly been used in preparations aiming at treatment of skin irritations and cosmetics. Although a comprehensive review on the *Oenothera* species and its properties has been published [10], no effect on brain activity has yet been described. The present investigation was undertaken in order to classify the pharmacological action of *Oenothera paradoxa* seed extract on rat brain by producing electropharmacograms to be compared to those of reference preparations.

A second approach for the pharmacological characterization of preparations consists in elucidating the direct action on brain matter. For this purpose, we used the *in vitro* hippocampal slice preparation. By stimulation of the Schaffer Collaterals, a so-called population spike can be recorded from pyramidal cells. Theta burst stimulation results in the appearance of long-term potentiation (LTP) [11] [12], related to time and space dependent learning. The amplitude of the resulting population spike represents the number of recruited pyramidal cells. This information is well suited to supplement the EEG data, since stimulant and calming preparations can be discriminated from each other easily by either enhancing or depressing LTP, respectively.

2. Material and Methods

Rhodiola rosea extract (RhodioLife®) was provided by Polifenoles Naturales, (PoliNat), Las Palmas, Spain, containing more than 5% Rosavine. *Oenothera paradoxa* seed extract was donated by Agropharm SA, Tuszyn, Poland, containing 50% polyphenoles. *Paullinia cupana* KUNTH extract was provided by Plantextrakt GmbH & Co. KG, Vestenbergsgreuth, Germany, containing 9% - 11% caffeine and 5% - 6% catechines. All extracts

had been standardized. Caffeine was bought from Bio Trend, Cologne, Germany. All preparations were given as single dose.

2.1. Recording of Field Potentials from the Depth of the Brain *in Vivo*

The methodology of recording field potentials from the freely moving rat has been described in many earlier publications [13]. In short, maleadult Fischer rats (about 350 g) are implanted with a set containing four bipolar concentric steel electrodes connected to a plug for attachment of a wireless transmitter during the experimental phase. Recording of field potentials is performed for 4 h after an initial pre-drug baseline of 45 minutes. Transmitted data are processed by Fast Fourier Transformation (FFT) and spectral power documented for 6 frequency ranges (delta, theta, alpha1, alpha2, beta1 and beta2) within frontal cortex, hippocampus, striatum and midbrain reticular formation in hourly intervals. All 24 variables (4 electrode positions x 6 frequency ranges) are fed into a linear discriminant analysis. Statistic evaluation in comparison to control is performed by a non-parametric Wilcoxon test. While the plantextracts were administered orally by gavage, the caffeine was injected intraperitonally. Results from linear discriminant analysis was documented by depicting the results from the first three functions into space (x, y and z coordinates), from the second three functions into three colors (RGB mode like in TV).

2.2. Recording of Populations Spikes from Hippocampal Slices *in Vitro*

The methodology of preparing hippocampal slices for *in vitro* assessment has been reported earlier [13]. In short: Hippocampus slices were obtained from adult male Sprague-Dawlay rats (300 g; Charles River Wiga, Sulzbach, Germany). Rats were kept under a reversed day/night cycle for 2 weeks prior start of the experiments, to allow recording of *in vitro* activity from slices during the active phase of their circadian rhythm [14]. Animals were exsanguinated under ether anaesthesia, the brain was removed in total and the hippocampal formation was isolated under microstereoscopic vision. The midsection of the hippocampus was fixed to the table of a vibrating microtome (Rhema Labortechnik, Hofheim, Germany) using a cyanoacrylate adhesive, submerged in chilled bicarbonate-buffered saline (artificial cerebrospinal fluid (ACSF): NaCl: 124 mM, KCl: 5 mM, $CaCl_2$: 2 mM, $MgSO_4$: 2 mM, $NaHCO_3$: 26 mM, glucose: 10 mM, and cut into slices of 400 µm thickness. All slices were pre-incubated for at least 1 h in Carbogen saturated ACSF (pH 7.4) in a pre-chamber before use.Statistic evaluation in comparison to control is performed by a non-parametric Wilcoxon test.

The principles of laboratory animal care were followed in all trials and the local authorities responsible for animal care allowed the performance according to German Health Guidelines (Code: V54 19c 20 15h 01 NeuroCode Nr. 118/2014 A3/2014 by “Regierungspräsidium” Giessen, Germany). Details of the acclimatisation, housing conditions and surgery have been reported [15].

3. Results

The effect of the extract preparations and caffeine were followed *in vivo* and *in vitro* in order to learn more on their dose and concentration dependent actions on the brain. *In vivo* recording allows for determining whether active ingredients of the extracts pass the blood-brain barrier. *In vitro* recordings demonstrate the direct interaction of possible ingredients with physiological activity within the hippocampus.

3.1. Pharmacological Characterization by Recording of Field Potentials *in Vivo*

Oral administration of 15 mg/kg ***Paullinia cupana*** seed extract (corresponding to about 240 mg single dose in humans) led to a strong statistically significant attenuation of alpha2 and beta1 spectral power within all brain areas. This effect lasted for 4 h after administration. Attenuation of beta2 spectral power was considerably less, but also lasting during the whole recording period (**Figure 1**). The effect of Paullinia extract on the spectral power of these three frequency ranges was dose-dependent as documented in **Figure 2**. After administration of the highest dosage of 25 mg/kg delta spectral power was also significantly attenuated.

Oral adminstration of 50 mg/kg of ***Oenothera paradoxa*** seed extract (corresponding to about 800 mg single dose in humans) led to a statistically significant attenuation of spectral delta, theta and alpha2 spectral power in the frontal cortex. With time also alpha1 and beta1 spectral power was attenuated in comparison to baseline values. In the hippocampus and striatum clearly less attenuation was observed. In the reticular formation second

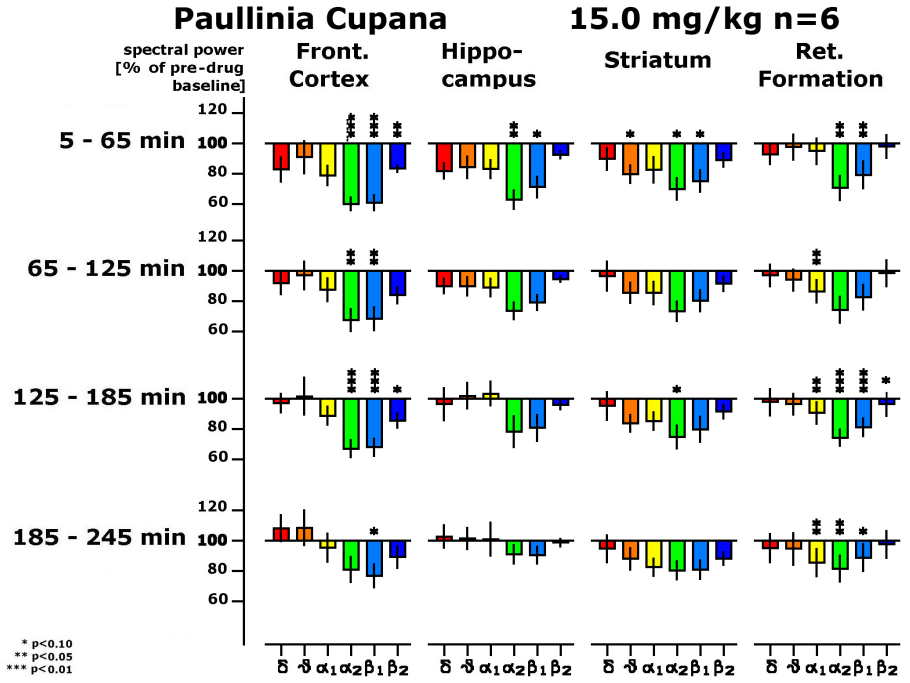

Figure 1. Time dependent changes of spectral power in four brain regions in the presence of *Paullinia* extract. Data are presented in % of the baseline values recorded for 45 minutes before oral administration by gavage. Frequency ranges are given on the abscissa: from left to right: delta (red), theta (orange), alpha1 (yellow), alpha2 (green), beta1 (turquoise) and beta2 (blue). * = $p < 0.1$; ** = $p < 0.05$; *** = $p < 0.01$.

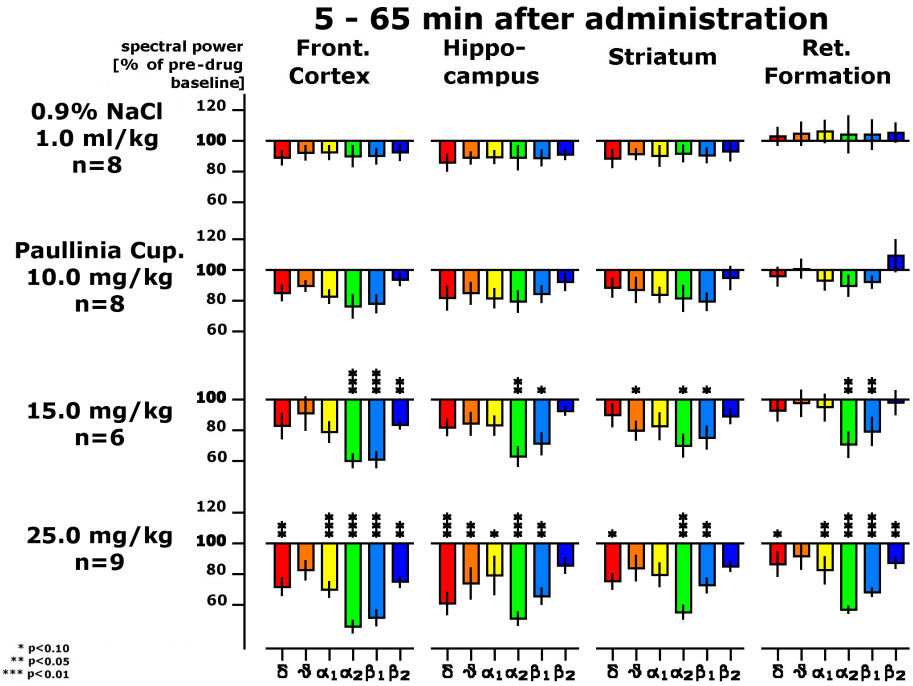

Figure 2. Dose dependent changes of spectral power in four brain regions in the presence of *Paullinia* extract. Data are presented in % of the baseline values recorded for 45 minutes before oral administration by gavage. Frequency ranges are given on the abscissa: from left to right: delta (red), theta (orange), alpha1 (yellow), alpha2 (green), beta1 (turquoise) and beta2 (blue). * = $p < 0.1$; ** = $p < 0.05$; *** = $p < 0.01$.

strongest changes were seen during later hours after administration. A time line is documented in **Figure 3**. Dose dependent changes of spectral power are documented in **Figure 4**.

Oral administration of 100 mg/kg of ***Rhodiola rosea*** root extract (corresponding to about 800 mg single dose in humans) led to significant attenuation of spectral power with respect to all frequency ranges (**Figure 5**). Most affected were alpha2 and beta1 spectral power in the frontal cortex. Second strongest changes were seen in the striatum, least changes in the reticular formation. Spectral changes lasted into the 4th hour after administration always dominated by attenuation of alpha2 and beta1 power.

Intraperitoneal injection of 2.5 mg/kg of **caffeine** (corresponding to about 40 mg single oral dose in humans contained in one cup of coffee) induced a strong attenuation of alpha2 and beta1 spectral power within all brain regions, whereas attenuation of beta1 power was strongest in frontal cortex. Clearly less attenuation was observed with respect to delta and theta spectral power. Attenuation of beta2 power decreased only in the frontal cortex. Least changes were seen in the striatum. A complete time line is given in **Figure 6**. In the hippocampus these changes lasted into the last hour after administration. Dose dependence for the first hour after injection is illustrated in **Figure 7**.

3.2. Effect of the Preparations on Motion

Motion was recorded during recording of field potentials. Whereas *Rhodiola* and *Oenothera* extract did not change motion of the freely moving rats, *Paullinia* extract and caffeine increased motion in a dose dependent and statistically significant manner in comparison to control during the first hour after administration. Data are documented in **Table 1**.

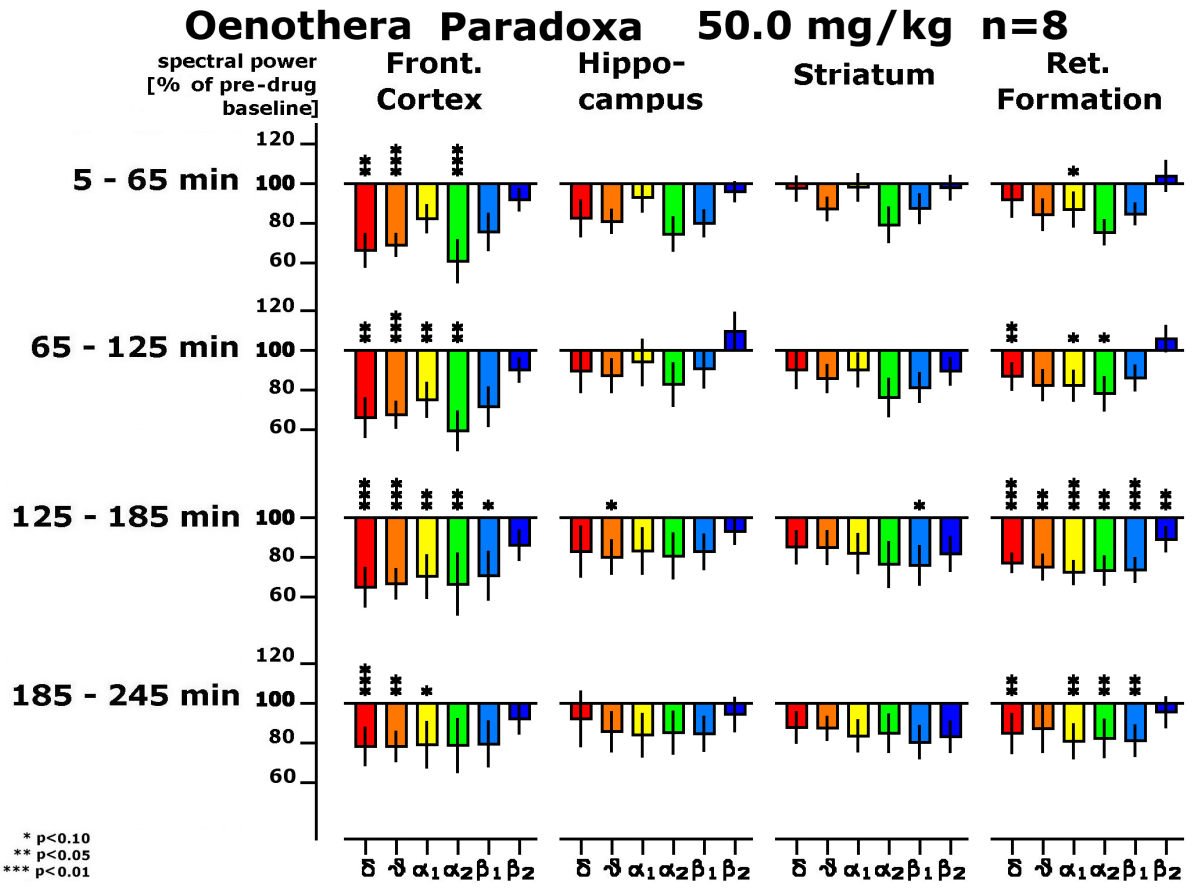

Figure 3. Time dependent changes of spectral power in four brain regions in the presence of *Oenothera* seed extract. Data are presented in % of the baseline values recorded for 45 minutes before oral administration by gavage. Frequency ranges are given on the abscissa: from left to right: delta (red), theta (orange), alpha1 (yellow), alpha2 (green), beta1 (turquoise) and beta2 (blue). * = $p < 0.1$; ** = $p < 0.05$; *** = $p < 0.01$.

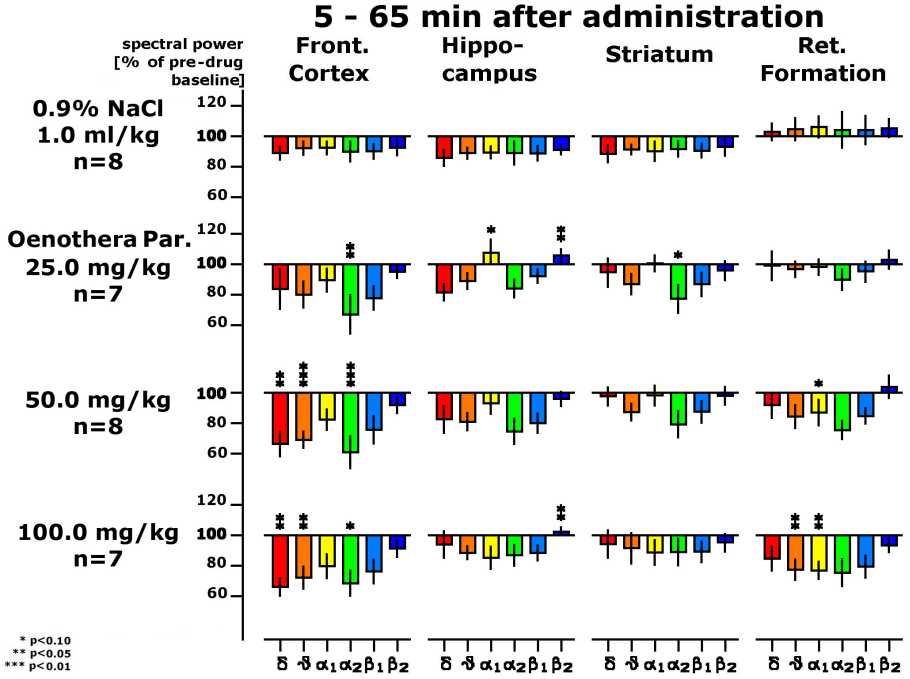

Figure 4. Dose dependent changes of spectral power in four brain regions in the presence of *Oenothera* extract. Data are presented in % of the baseline values recorded for 45 minutes before oral administration by gavage. Frequency ranges are given on the abscissa: from left to right: delta (red), theta (orange), alpha1 (yellow), alpha2 (green), beta1 (turquoise) and beta2 (blue). * = $p < 0.1$; ** = $p < 0.05$; *** = $p < 0.01$.

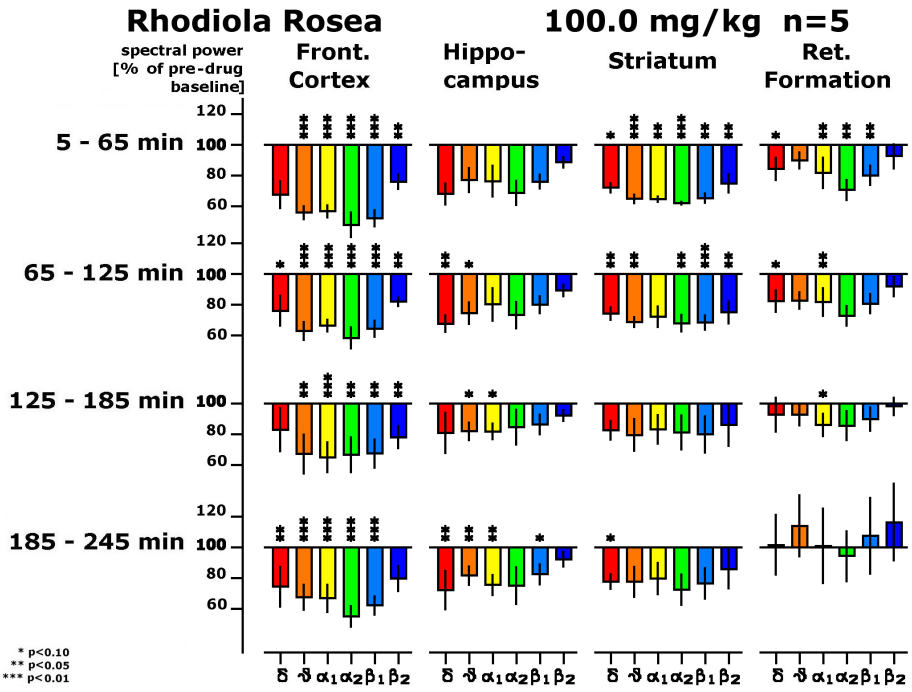

Figure 5. Time dependent changes of spectral power in four brain regions in the presence of *Rhodiola* extract. Data are presented in % of the baseline values recorded for 45 minutes before oral administration by gavage. Frequency ranges are given on the abscissa: from left to right: delta (red), theta (orange), alpha1 (yellow), alpha2 (green), beta1 (turquoise) and beta2 (blue). * = $p < 0.1$; ** = $p < 0.05$; *** = $p < 0.01$.

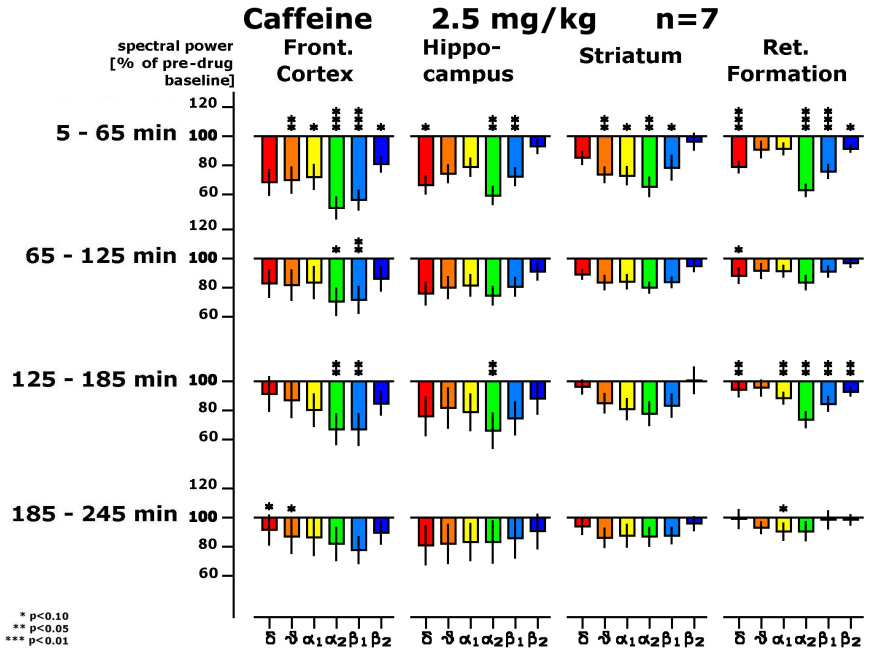

Figure 6. Time dependent changes of spectral power in four brain regions in the presence of caffeine. Data are presented in % of the baseline values recorded for 45 minutes before intraperitoneal administration (Data are taken from an earlier experiment performed under identical conditions and saved in our database). Frequency ranges are given on the abscissa: from left to right: delta (red), theta (orange), alpha1 (yellow), alpha2 (green), beta1 (turquoise) and beta2 (blue). * = $p < 0.1$; ** = $p < 0.05$; *** = $p < 0.01$.

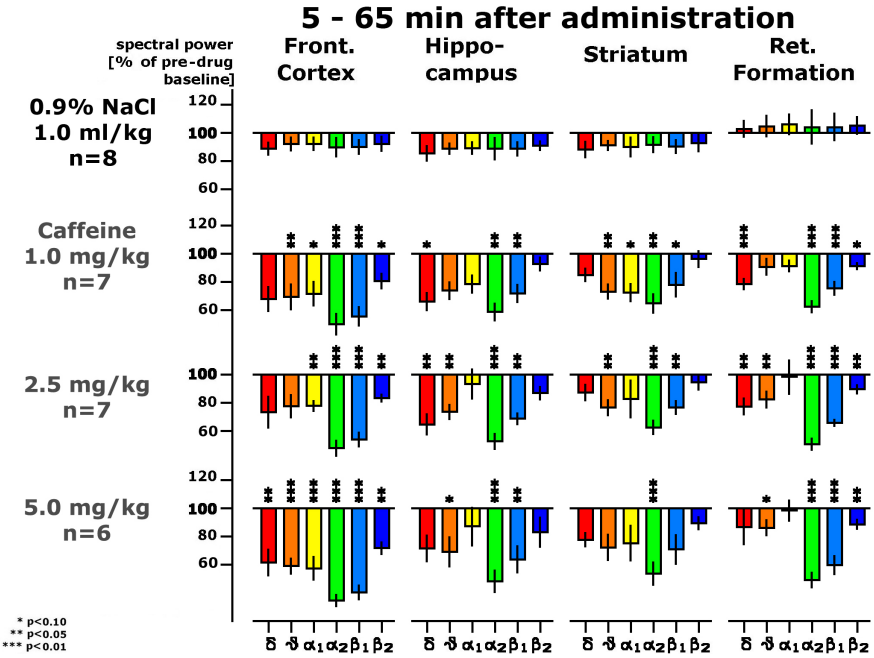

Figure 7. Dose dependent changes of spectral power in four brain regions in the presence of caffeine. Data are presented in % of the baseline values recorded for 45 minutes before intraperitoneal administration (Data are taken from an earlier experiment performed under identical conditions and saved in our database). Frequency ranges are given on the abscissa: from left to right: delta (red), theta (orange), alpha1 (yellow), alpha2 (green), beta1 (turquoise) and beta2 (blue). * = $p < 0.1$; ** = $p < 0.05$; *** = $p < 0.01$.

Table 1. Influence of herbal extracts and caffeine on motion. Motion is calculated as movement in cm/h. Statistical significance in comparison to control is given as p-value.

	Motion [cm|h] 5 - 65 min			
Saline	690.02	±	175	P<
Rhodiola Ros. 100.0 mg/kg	718.08	±	201	
Oenothera Par. 25.0 mg/kg	689.88	±	165	
50.0 mg/kg	682.05	±	249	
100.0 mg/kg	581.84	±	175	
Paullinia Cup. 10.0 mg/kg	1515.0	±	241	0.009
15.0 mg/kg	1802.0	±	241	0.014
25.0 mg/kg	2359.0	±	372	0.007
Caffeine 1.0 mg/kg	1895.0	±	270	0.003
2.5 mg/kg	1802.0	±	241	0.003
5.0 mg/kg	3133.0	±	344	0.002

3.3. Pharmacological Characterization in the Hippocampal Slice *in Vitro*

The herbal extracts and caffeine were tested in very low concentrations in the hippocampal slice *in vitro* to investigate their possible effect on long-term potentiation. The presence of a very low concentration of 10 mg/L of *Paullinia* extract already led to an increased response of the pyramidal cells after single pulse stimulation as well as after theta burst stimulation (TBS) (**Figure 8**). In the case of single stimuli amplitudes of about 2 mV were reached, after TBS amplitudes of the population spike of nearly 4 mV were documented.

The presence of a very low concentration of 5 mg/L of Oenothera extract already led to a decreased response of the pyramidal cells after single pulse stimulation as well as after theta burst stimulation (TBS; **Figure 9**). In the case of single stimuli amplitudes only about 0.5 mV were reached at a concentration of 20 mg/L, after TBS amplitudes of the population spike also of nearly 0.5 mV were documented at a concentration of 20 mg/L.

In the presence of very low concentration of 1 mg/kg of caffeine clear increases of the population spike were observed with single shock stimulation as well as with TBS. Maximum amplitudes were reached in the presence of 3 mg/kg of caffeine. Data are documented in **Figure 10**.

In the presence of *Rhodiola* extract increases of the population spike were observed during application of single stimuli as well as during theta burst stimulation. Already a concentration of 5 mg/L induced a slight increase of the amplitude of the population spike and an increase of LTP as documented in **Figure 11**. Further increases were observed by increasing the concentration up to 30 mg/L. During theta burst stimulation amplitudes of more than 4 mV were measured.

4. Discussion

In common, all three herbal extracts and caffeine predominantly attenuate alpha2 spectral power within all 4 brain areas in a statistically significant manner in comparison to control (administration of saline). Since it is known from earlier experiments performed under identical conditions that alpha2 waves change under the control of the neurotransmitter dopamine [15], it can be concluded that interference with the dopaminergic system in the brain is the main mechanism of action for all preparations. Activation of the dopaminergic system has long been assumed to play a major role during the action of stimulatory drugs including amphetamine. The second feature common to all preparations is the attenuation of beta1 spectral power. This frequency has been shown to change in a dose dependent manner in humans in the presence of a compound interacting with a glutamate receptor [16]. This indicates that all preparations are also likely interact with the glutamatergic system. One can therefore assume that dopamine and glutamate activation are related to the stimulant activities of the preparations. This interpretation is strengthened by the fact that in addition to the predominant attenuation of alpha2 and beta1 spectral power, an increase of motion is observed even at the lowest dose of *Paullinia* extract and caffeine (**Table 1**). The *Oenothera* extract and *Rhodiola* extract also induced delta and theta power decreases in

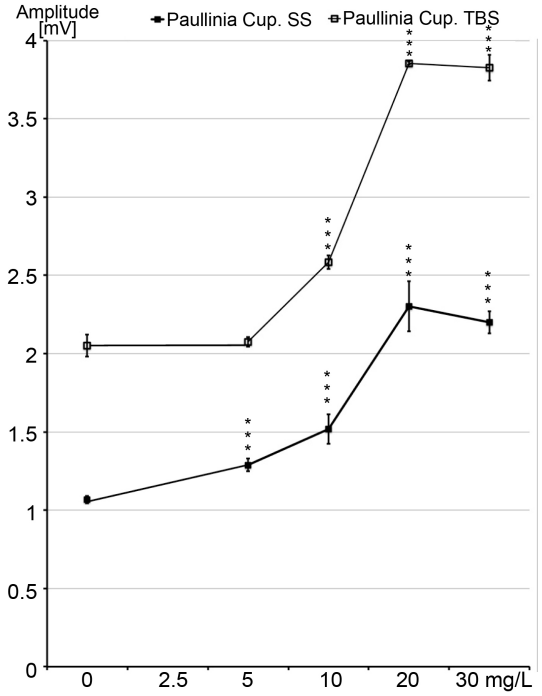

Figure 8. Concentration dependence (given as mg/L on the Abscissa) of *Paullinia* extract on the amplitude of the population spike (given as mV on the ordinate) in the hippocampal slice preparation *in vitro*.Changes of population spike amplitude in percent of pre-values after single stimuli challenge (SS) and theta burst stimulation (TBS). Data are given for n = 4 slices ± SEM. Statistical significance is documented by stars: * = $p < 0.1$; ** = $p < 0.05$; *** = $p < 0.01$.

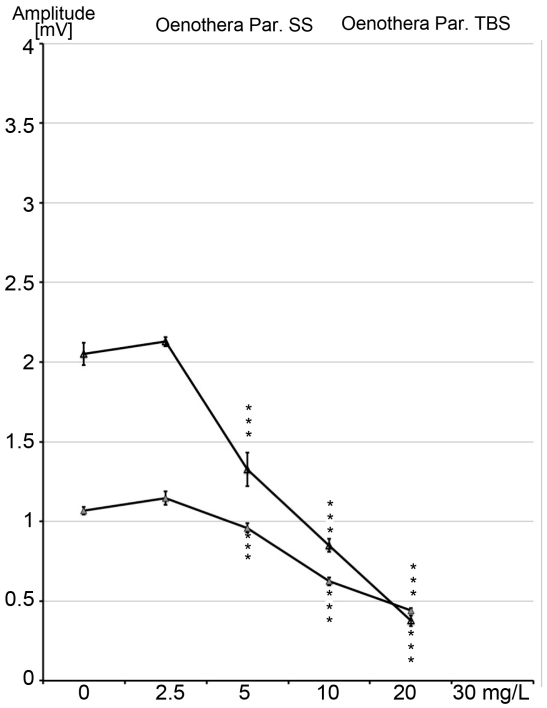

Figure 9. Concentration dependence (given as mg/L on the Abscissa) of *Oenothera* extract on the amplitude of the population spike (given as mV on the ordinate) in the hippocampal slice preparation *in vitro*. Changes of population spike amplitude in percent of pre-values after single stimuli challenge (SS) and theta burst stimulation (TBS). Data are given for n = 4 slices ± SEM. Data are given for n = 4 slices ± SEM. Statistical significance is documented by stars: * = $p < 0.1$; ** = $p < 0.05$; *** = $p < 0.01$.

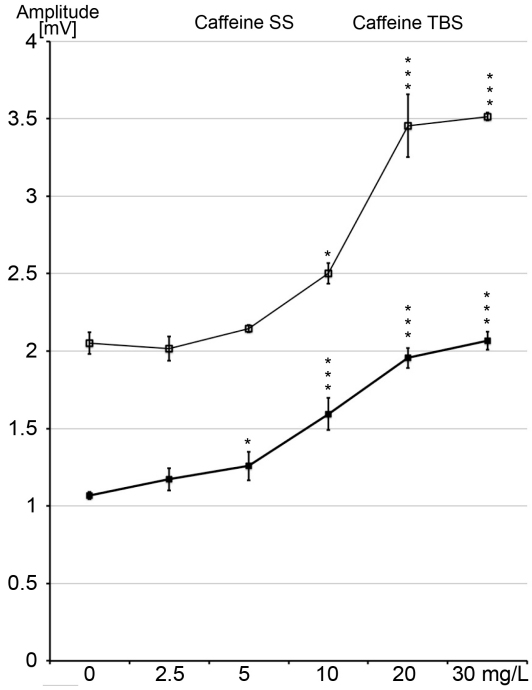

Figure 10. Concentration dependence (given as mg/L on the Abscissa) of caffeine on the amplitude of the population spike (given as mV on the ordinate) in the hippocampal slice preparation *in vitro*. Changes of population spike amplitude in percent of pre-values after single stimuli challenge (SS) and theta burst stimulation (TBS). Data are given for n = 4 slices ± SEM. Data are given for n = 4 slices ± SEM. Statistical significance is documented by stars: * = $p < 0.1$; ** = $p < 0.05$; *** = $p < 0.01$.

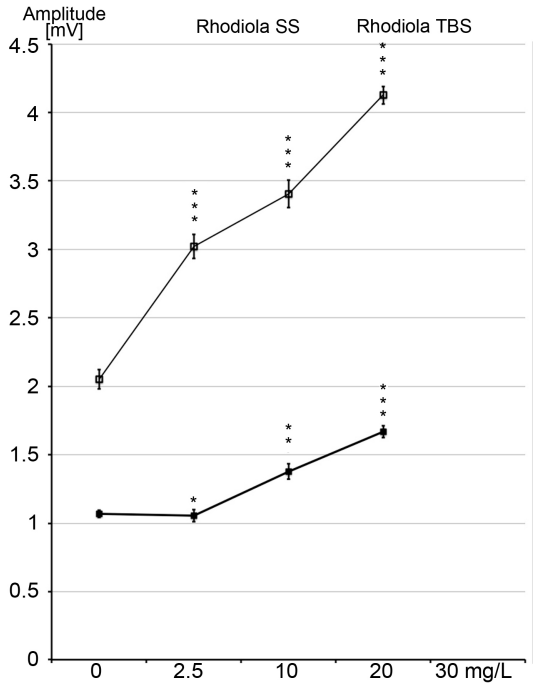

Figure 11. Concentration dependence (given as mg/L on the Abscissa) of *Rhodiola* extract on the amplitude of the population spike (given as mV on the ordinate) in the hippocampal slice preparation *in vitro*. Changes of population spike amplitude in percent of pre-values after single stimuli challenge (SS) and theta burst stimulation (TBS). Data are given for n = 4 slices ± SEM. Data are given for n = 4 slices ± SEM. Statistical significance is documented by stars: * = $p < 0.1$; ** = $p < 0.05$; *** = $p < 0.01$.

addition to the alpha2 and beta1 attenuation. Spectral delta power is under the control of acetylcholine as reported earlier [17]. Spectral theta has been reported to be related to the action of the central neurotransmitter norepinephrine, and an increase of theta power indicated sedation [18]. The concomitant influence of *Oenothera* extract and Rhodiola extract on these two transmitter systems might influence the net effect of the preparations.

In order to compare the actions of the extracts and caffeine on the brain with other reference drugs with known clinical indications, data were processed by linear discriminant analysis as reported earlier [2]. According to the projection derived from this analysis the effect of *Rhodiola* extract emerged in close proximity to the projection of the Ginkgo extract and also appeared with the same color (**Figure 12**). This is in line with the results of a clinical study where *Rhodiola* and Ginkgo extract produced similar effects with respect to psychomotor vigilance and short term working memory accuracy [19]. The effect of *Oenothera* extract also appeared in the vicinity of *Rhodiola*, but with a different color indicating a different mode of action. However, close to it, Tramadol, an opioid analgesic drug, appeared showing the same color as *Oenothera*. These discriminant analysis projections are interpreted to indicate that *Rhodiola* has CNS activities very much in common with those of Ginkgo, whereas *Oenothera* may have analgesic properties. *Paullinia* and caffeine are not too far away from each other but provide a different color and thus can be discriminated from each other quite well in spite of the fact that *Paullinia* extract also contains caffeine. An overview on the administration and time of analysis of reference drugs is given in **Table 2**.

The results obtained from the *in vitro* tests in the hippocampal slice preparation are in line with the *in vivo* results. *Rhodiola* extract, *Paullinia* extract and caffeine-all three having proven stimulatory action-increase LTP. The increase of the amplitude of the hippocampal population spike amplitude under the condition of theta burst stimulation in order to induce long term potentiation (LTP) relates to time dependent and spatial memory according to the literature (more than 1500 citations obtained for LTP and memory; for a recent review see [20] [21]). The results obtained in the presence of caffeine, *Paullinia* extract and *Rhodiola* extract are completely in

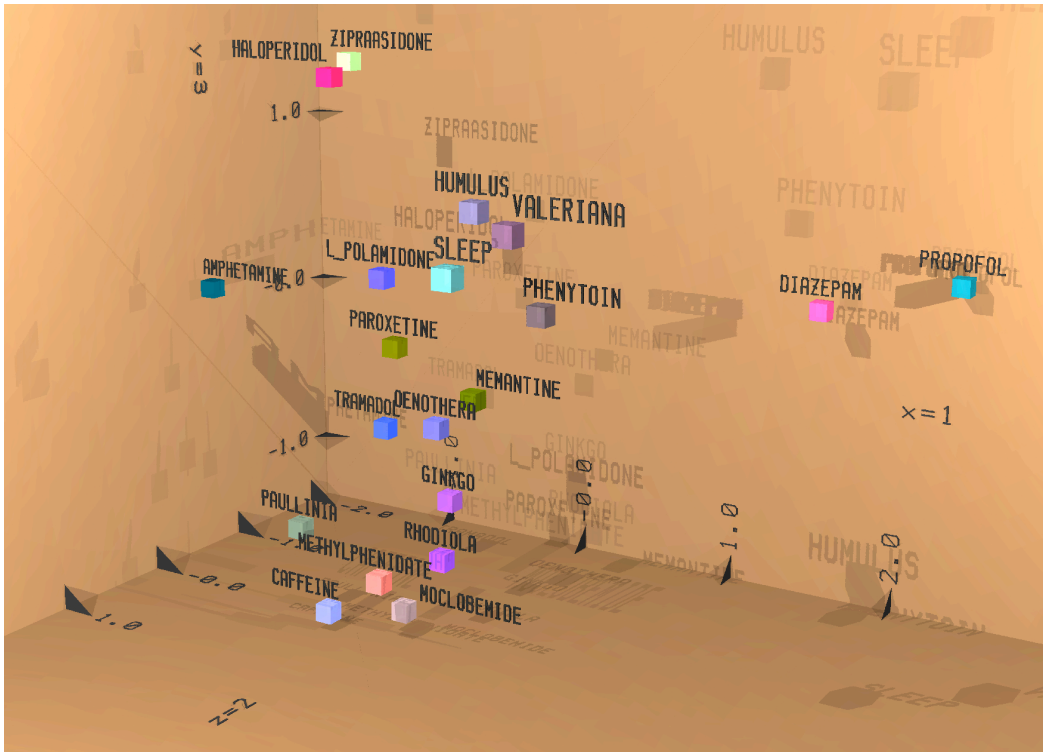

Figure 12. Result of discriminant analysis showing poly-dimensional projection of induced spectral changes in all frequency ranges and all brain regions. Results from the first three functions are depicted in space (x, y and z coordinates). Results from the next three functions are depicted as color mixture (RGB). Please note that data from *Rhodiola* extract are projected in the vicinity and with the same color as data from Gingko extract indicating a similar action, whereas *Oenothera* is projected into the vicinity of Tramadol with the same color indicating a similar therapeutic application.

Table 2. Listing of times and dosages of preparations tested. Discriminant functions were calculated using the compounds listed on the left side and fixed as a matrix. Based on these fixed functions preparations on the right side were analysed.

Substance Definition	Dose [mg/kg]	Application	Time	Substance Analysis	Dose [mg/kg]	Application	Time
Diazepam	0.50	i.p.	5 - 35 min	Caffeine	1.00	i.p.	5 - 65 min
Memantine	3.00	i.p.	5 - 35 min	Ginkgo	100.0	orally	20 - 55 min
L-Polamidon	1.00	i.p.	5 - 35 min	*Valeriana*	60.0	orally	125 - 185 min
Ziprasidone	1.00	i.p.	5 - 35 min	*Humulus*	50.0	orally	125 - 185 min
Paroxetine	1.00	i.p.	5 - 35 min	*Paullinia*	15.0	orally	5 - 65 min
Amphetamine	0.20	i.p.	5 - 35 min	*Oenothera*	50.0	orally	5 - 65 min
Propofol	60.0	i.p.	5 - 65 min	*Rhodiola*	100.0	orally	5 - 65 min
Moclobemide	5.00	i.p.	5 - 35 min				
Tramadol	5.00	i.p.	5 - 35 min				
Methylphenidate	2.50	orally	5 - 35 min				
Haloperidol	0.50	i.p.	20 - 50 min				
Phenytoin	4.00	i.p.	65 - 125 min				
Sleep			65 - 125 min				

line with the assumption that they also improve memory. The opposite result obtained in the presence of *Oenothera* extract speaks in favour of an analgesic and possibly antidepressive effect, since attenuation of the LTP has been reported also for antidepressant drugs from as early as in 1993 [22].

In summary, the combination of the two technologies—the *in vivo* recording of changes of spectral power in selected brain regions, and the *in vitro* recording of population spikes in hippocampal slices—is very well suited for a neuropharmacological classification of unknown extracts or molecules. This has been demonstrated with the extract from *Oenothera* seeds, where no action on the brain was previously known, and it can now be anticipated that this extract likely has both antidepressant and analgesic activities. Similarity to the opioid analgesic Tramadol was shown by means of changes of spectral power of field potentials, and as with antidepressant drugs *Oenothera* extract attenuates LTP. With *Rhodiola*, the extract seems to interact with the brain in a similar way as Ginkgo extract which was also able to increase LTP (to be published) and consequently can be anticipated to be used in similar clinical indications. The similarity of the *Rhodiola* extract electropharmacogram to that of caffeine, and the increased LTP *in vitro* suggest that the extract of Rhodiola may have potential as a replacement for caffeine. The effect of *Paullinia* extract can be clearly separated from the action of caffeine despite the fact that it contains about 6% caffeine. Improved cognitive performance in the presence of Paullinia extract containing approximately 12% caffeine was first reported in 2004 [23]. These authors regarded it unlikely that the low content of caffeine was responsible for the observed results. *Paullinia* extract seems to have also other pharmacologically active constituents, which might be interesting to explore [24]. A *Paullinia* extract was also tested clinically where it was suggested that the positive effects on cognitive parameters cannot be attributed to caffeine alone [25]; interestingly, no effects were observed on emotional parameters like psychological well-being, anxiety and mood [26].

5. Conclusion

In conclusion, the combination of Tele-Stereo-EEG *in vivo* and hippocampal slice preparation *in vitro* may be sufficient as a screening tool in order to gather enough pharmacological information to predict a clinical indication for herbal preparations anticipated to have CNS activity, especially when compared to a database of electropharmacograms for reference CNS-active pharmaceuticals.

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Conflict of Interest

There is no conflict in interest. JCW and TVM are employees at PoliNat (Polifenoles Naturales, Spain), who developed and provided *Rhodiola* extract. PoliNat paid for performance of the tests with this extract.

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