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Electropharmacogram of Sceletium tortuosum extract based on spectral local field power in conscious freely moving rats

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ABSTRACT

Ethnopharmacological relevance: The endemic succulent South African plant, *Sceletium tortuosum* (L.) N.E. Br. (synonym *Mesembryanthemum tortuosum* L.), of the family Mesembryathemaceae, has an ancient oral tradition history of use by San and Khoikhoi people as an integral part of the indigenous culture and *materia medica*. A special standardized extract of *Sceletium tortuosum* (Zembrin®) has been developed and tested pre-clinically in rats, and clinically in healthy subjects.

Aim of the study: The present investigation aimed at the construction of electropharmacograms of Zembrin® in the presence of three dosages (2.5, 5.0 and 10.0 mg/kg), and comparative electropharmacograms and discriminatory analyses for other herbal extracts, citicoline and rolipram.

Material and methods: Seventeen adult Fischer rats were each implanted with a set consisting of four bipolar concentric steel electrodes fixed by dental cement and three screws driven into the scalp. After two weeks of recovery from surgery the animals were adapted to oral administration by gavage and to experimental conditions (45 min pre-drug period and 5 h of recording after a rest of 5 min for calming down). Data were transmitted wirelessly and processed using a Fast Fourier Transformation (FFT). Spectral power was evaluated for 8 frequency ranges, namely delta, theta, alpha1, alpha2, beta1a, beta1b, beta2 and gamma power.

Results: Zembrin® dose dependently attenuated all frequency ranges, to varying degrees. The most prominent was the statistically significant reduction in alpha2 and beta1a waves, correlated with activation of the dopaminergic and glutamatergic transmitter systems respectively. This feature is common to all synthetic and herbal stimulants tested to date. The second strongest effects were reduction in both the delta and the theta frequency ranges, correlated with changes in the cholinergic and norepinephrine systems respectively, a pattern seen in preparations prescribed for neurodegenerative diseases. Theta wave reduction in common with the delta, alpha2 and beta1 attenuation has been noted for analgesic drugs. Attenuation of alpha1 waves emerged during the highest dosage in all brain areas, a feature seen in all antidepressants.

Discussion: The electropharmacogram of Zembrin® was compared to the electropharmacograms of herbal extracts archived in our database. Extracts of *Oenothera biennis* and *Cimicifuga racemosa* gave a very similar electropharmacograms to that of Zembrin®, and extracts of *Ginkgo biloba* and *Rhodiola rosea* gave rather similar electropharmacograms to Zembrin®. Linear discriminant analysis confirmed these similarities and demonstrated that all three dosages of Zembrin® plotted in close neighbourhood to each other. Citocoline, a synthetic compound originally developed for cognitive enhancement, had a similar electropharmacogram to Zembrin®. Similarity to the electropharmacograms of the synthetic phosphodiesterase-4 inhibitor, rolipram, suggests Zembrin® has antidepressant and cognitive function enhancing potential.

Conclusion: The combined results from the electropharmacograms and comparative discriminatory analyses suggest that Zembrin® has dose dependent activity, with potential applications as a cognitive function enhancer, as an antidepressant, and as an analgesic.

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1. Introduction

1.1. Ethnobotanical background

The endemic succulent South African plant, *Sceletium tortuosum* (L.) N.E. Br. (synonym *Mesembryanthemum tortuosum* L.), of the family Mesembryathemaceae, has an ancient oral tradition history of use by San and Khoikhoi people as an integral part of the indigenous culture and *materia medica*. The use of the plant by indigenous people was first documented by the first Governor of the Cape Colony, Simon van der Stel in 1685, this report was accompanied by a remarkably detailed painting of the plant (Waterhouse et al., 1979).

Sceletium tortuosum has been traditionally used by hunter-gatherers and pastoralists for endurance on hunting forays, and for coping with the stress of living in the challenging arid environments of Bushmanland, Namaqualand and the Karoo. The indigenous Nama name for *Sceletium tortuosum* is *Kanna*, or *Channa,* while the name given to the plant by the /Xam San people was*! Kwain*. The Dutch colonists called *Sceletium tortuosum Kaauwgoed* or *Kauwgood* (Pappe, 1868; Smith, 1966) which translates as "chewing stuff'.

Dried Sceletium plant material was once widely traded in South Africa among indigenous people and in more recent historical times it was sold through rural trading stores. An intriguing ethnobotanical record from 1873 alludes to a memory-enhancing activity for Sceletium. Manuscript MSS BC151 006 from the Manuscript and Archives Department of the University of Cape Town documents the information on *Kaauwgoed* related by the /Xam San informant /Kabbo to the German linguist Wilhelm Bleek: *"It is chewed by Bushmen, and gives strength to their limbs; and takes away pain, and make*[s] *their memory strong".*

Over the last two decades increasing numbers of botanical supplements, health teas and beverages containing *Sceletium tortuosum* have become available through farm stalls, health food shops, pharmacies and via internet sales. The products are typically used for promoting a sense of calm and wellbeing, relieving stress, elevating mood and reducing anxiety (Gericke and Viljoen, 2008), and by students to enhance concentration during exam times.

During ethnobotanical documentation of *Sceletium tortuosum* in 1995, elderly folk from the Namaqualand hamlets of Paulshoek and Nourivier were interviewed who had chewed Sceletium daily for the past thirty to forty years of their lives (Gericke and Viljoen, 2008). One of us (NG) noted that these elderly people were in surprisingly good general health and seemed more lucid than was typical their age-peers from other rural areas in South Africa where Sceletium is not available, and speculated that long-term Sceletium use may have neuro-protective properties.

Veterinarian studies in Japan demonstrated that *Sceletium tortuosum* reduced cage stress and travel stress in companion animal cats, and decreased the excessive crying and nocturnal barking of aged cats and dogs with a clinical diagnosis of dementia (Hirabayashi et al., 2002, 2004, 2005), while the first clinical case-reports on *Sceletium tortuosum* described rapid onset of anxiolytic and antidepressant activity, and mentioned anecdotal reports on the use of Sceletium in South Africa for the management of drug addiction at a community treatment centre (Gericke, 2001).

A proprietary standardized and characterized extract of cultivated *Sceletium tortuosum* (Zembrin®), a dual 5-HT uptake inhibitor and PDE4 inhibitor (Harvey et al., 2011), was shown to be safe and well tolerated in pre-clinical and clinical studies (Murbach et al., 2014; Nell et al., 2013). The acute effects of Extract *Sceletium tortuosum* (Zembrin®) administration in 16 healthy young adults was studied in a pharmaco-fMRI study focused on anxiety-related activity in the amygdala and its connected neurocircuitry. Amygdala reactivity to fearful faces under low perceptual load conditions was attenuated after a single 25.0 mg dose of Zembrin®. Follow-up connectivity analysis on the emotion matching task demonstrated that amygdala–hypothalamus coupling was also reduced. These results demonstrated for the first time the attenuating effects of *Sceletium tortuosum* on the threat circuitry of the human brain and provide supporting evidence that the dual 5-HT reuptake inhibition and PDE4 inhibition of Zembrin® might have anxiolytic potential by attenuating the subcortical threat response (Terburg et al., 2013).

The neurocognitive effects of extract *Sceletium tortuosum* (Zembrin®) was tested for the first time in a group of adults ($n=21$, mean age 54.6 years) using the CNS Vital Signs® battery of tests. Zembrin® at 25.0 mg daily dosage significantly improved executive function and cognitive set flexibility compared with the placebo group. It was concluded that the promising cognitive enhancing effects of Zembrin® supported PDE-4 inhibition as a mechanism of action, and PDE4-cAMP-PCREB cascade, suggesting potential application in managing early Alzheimer's Disease (Chiu et al., 2014).

1.2. Neurophysiological background

Drugs exert their action within the organism by interaction with targets defined biochemically (receptors, enzymes, channels transporters et cetera), large protein molecules sometimes also sitting at the outer surface of cells. With respect to the central nervous system neurotransmitter receptors represent main targets of drugs. Interaction of drugs with these molecules induces a signalling cascade, which finally ends up with the control of ion channel conductance. Since the electric activity of single neurons depends on the set of momentarily active ion channels, communication between neurons is governed by channel activity. From here, it is obvious that field potentials contain the information of larger local networks of electrically active neurons, by it reflecting the interaction of drugs with their targets within the concert of neurotransmission.

Frequency analysis of the field potentials in the presence of drugs can be depicted as the so-called electropharmacogram, which has been widely used to characterize drug actions on rat (Christian et al., 2015) and human brains (Alonso et al., 2015). Interpretation of the results is made with respect to the likely underlying neurotransmitter activity responsible for changes in the selected frequency ranges, and aims at advancing an understanding of possible clinical applications in humans. The relationship between EEG delta waves and cholinergic neurotransmission was first recognised in 2005 (Dimpfel, 2005), while it was recognized that theta waves are influenced by drugs acting at the biochemically defined norepinephrine alpha2 receptor (Dimpfel and Schober, 2001). Presynaptic interaction with this receptor leads to drowsiness and sleep, and increases of theta waves have been used as part of a formula describing depth of sleep in humans. Dopaminergic activity is reflected by changes in alpha2 frequencies (Dimpfel, 2008).

1.3. Aim of the present study

The convergence of prior evidence (ethnobotanical, in-vitro mechanism of action, companion animal studies, and clinical research) suggests that Zembrin® has cognitive-enhancing potential, and the present study was undertaken as part of a translational research initiative to characterize the effect of Zembrin® in a rat pharmaco-EEG (local field potentials). It was anticipated that additional insights into Zembrin's® activity on the brain could be obtained from changes over time in the local field potential (LFP) of defined frequency ranges, recorded from four areas of the rat

brain. The resulting electopharmocograms of Zembrin® could then be compared with a databased reference collection of botanicals and supplements, as well as to the pharmacogram of the archetypal research phosphodiesterase-4 inhibitor (PDE4 inhibitor), rolipram.

2. Material and methods

2.1. Local field potentials (LFP)

Local field potentials 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 MHz as carrier frequency) and were amplified and processed as described earlier to give power spectra with 0.25 Hz resolution (Dimpfel et al., 1986). 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 6 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 min each and displayed on-line. In an off-line procedure spectra were averaged to give longer periods for further analysis and data presentation.

2.2. Test Item

All dosages of the compound Zembrin® (2.5 mg/kg, 5.0 mg/kg and 10.0 mg/kg) were tested by oral administration by gavage. The "Tele-Stereo-EEG" animal model consisting of continuous recording of intracerebral field potentials was used in combination with a video tracking system for detection of changes in motility (GJB Datentechnik GmbH, D-98704 Langewiesen, Germany). This system recognized locomotion as well as stereotyped behaviour by following a contrast difference of the black transmitter on the head of the animal in comparison to its environment. The system has been validated in a previous study with different dosages of caffeine.

Solutions were prepared fresh for each experimental day and administered orally by gavage after 45 min of pre-drug Vehicle recording. Vehicle was 0.9% NaCl. Zembrin® (Batch 110325) from HG&H Pharmaceuticals (Pty) Ltd, South Africa was tested in three dosages (2.5, 5.0 and 10.0 mg/kg).

2.3. Animals and management

Eighteen adult Fisher 344 rats (11 month of age or 15 month of age) and day-night converted, weight about 420 g, provided by Charles River Laboratories, D-97633, Sulzfeld) were used in two experimental series. Animals were implanted with electrodes into the brain and were given two weeks for recovery from surgery. After this, the transmitter was plugged in for adaptation and control experiments. During the recording rats were not restricted and could move freely but did not have food available (chewing would have produced too many artefacts). 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 (Dimpfel, 2013).

2.4. Treatment groups

A crossover design with at least one week of drug holidays in between the administrations was used. Controls consisted of orally administration of 1 ml/kg of vehicle for Zembrin® 2.5 mg/kg, 5.0 mg/kg and 10.0 mg/kg. After a pre-drug period of 45 min for pre-drug recording, drug effects were observed continuously on the screen (artefact control) for 300 min subdivided into 15 min periods after a lag time of 5 min for calming of animals after orally administration. Changes of electric power (μV^2) are expressed as % of the 45 min lasting absolute pre-drug spectral power values within each frequency band. Data were averaged from 16 animals for each series since there were some missing data in different rats in each of the series for technical reasons. Data are expressed as mean values $\pm$ S.E.M. Statistics were calculated by means of the Wilcoxon, Mann, Whitney *U*-test.

2.5. Route and means of administration

Dose levels of Zembrin® (2.5 mg/kg, 5.0 mg/kg and 10.0 mg/kg) were calculated starting from clinical dosage based on the proposal of Shannon et al. (2007). The animals were dosed orally by a gavage at a constant volume of 1 ml dosing solution per kg of body weight. The dosage administered to each animal was determined each day by the weight of that animal at the time of administration.

2.6. Statistical analysis

Wilcoxon, Mann, Whitney *U*-test was used throughout all experimental data for comparison to results obtained by vehicle injection at the particular timing. For comparison of data to reference compounds tested earlier under identical conditions linear discriminant analysis according to Fischer was used. A total of the classic 24 variables (six frequency ranges times 4 brain areas) were used for analysis. Please note, that this analysis does not contain gamma activity for historical reasons (gamma activity was not recorded earlier!) Firstly, projection of the results from reference compounds plus physiological sleep was performed using the three spatial coordinates for the first three discriminant-axes. Secondly, coding of the result of the fourth to sixth discriminant analysis into red, green and blue was followed by an additive colour mixture in analogy to the so-called RGB mode (as used in TV). A reference matrix of earlier tested drug actions is kept constant (preparations labelled as "**Substance Definition**")) for classification of unknown preparations (preparations labelled as "**Substance Analysis**").

3. Results

3.1. Effect of vehicle

Orally administration of the vehicle did only result in very minor changes of spectral power within the four brain areas. During the third to the fifth hour a tiny increase of delta and alpha2 power emerged only in the frontal cortex. A complete time line is given in Fig. 1.

3.2. Electropharmacogram of Zembrin®

Oral administration of the lowest dosage of Zembrin® (2.5 mg/kg) resulted in a highly statistically significant attenuation of spectral power in all frequencies except for gamma within the

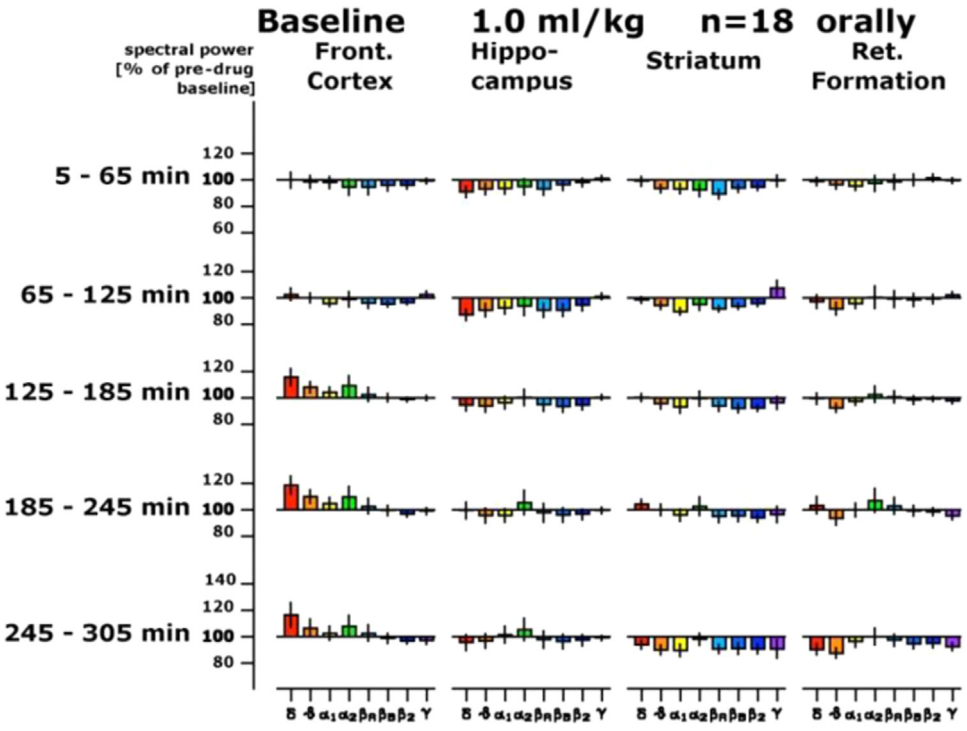

Fig. 1. Effect of Vehicle: time dependence of changes of spectral power (Ordinate) in % of the 45 min lasting pre-drug baseline values in four brain regions of the freely moving rat in the presence of Vehicle (1 ml/kg). Frequency ranges are depicted as coloured bar graphs on the abscissa representing 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. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

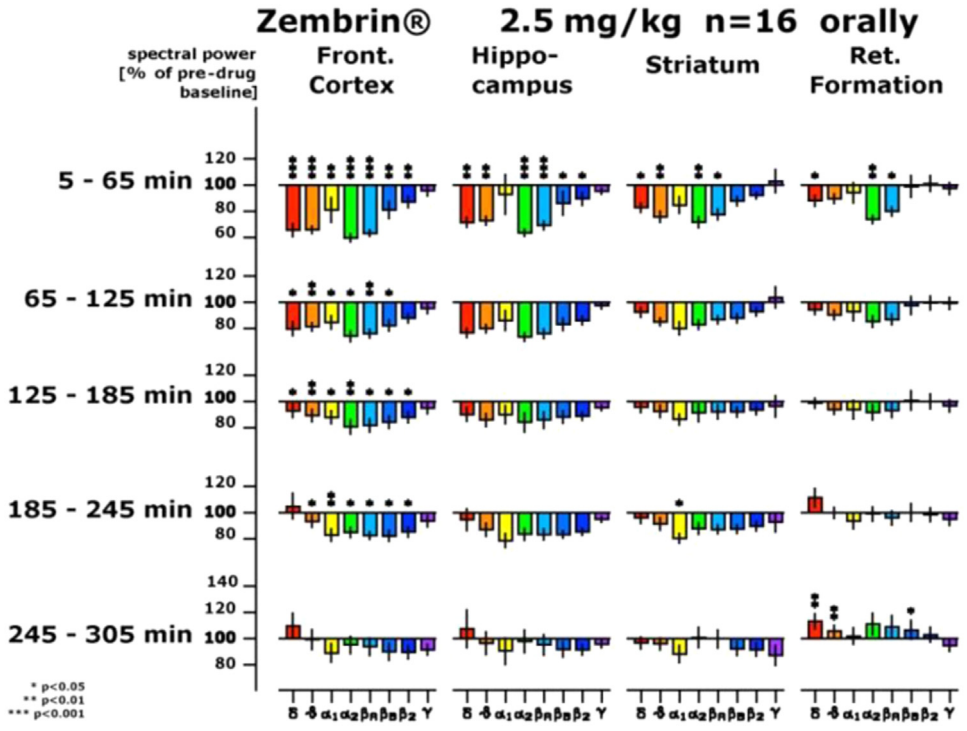

Fig. 2. Effects of Zembrin® (2.5 mg/kg): for other details see legend to Fig. 1. Statistical significance in comparison to control (vehicle) is documented by asterisks: $^{*}=p<0.05$; $^{**}=p<0.01$; $^{***}=p<0.001$.

frontal cortex during the first hour. In the hippocampus delta, theta, alpha2 and beta1a spectral power was significantly attenuated. Within the striatum, only delta and beta power were significantly reduced. In the reticular formation delta, alpha2 and beta1a power was diminished. Almost identical changes were recorded during the second hour after administration, however, statistical significance was only reached in recordings from the frontal cortex. During the third hour, statistically significant attenuation of all frequencies was seen in the frontal cortex, an effect lasting to the 4th hour. A complete time line is given in Fig. 2.

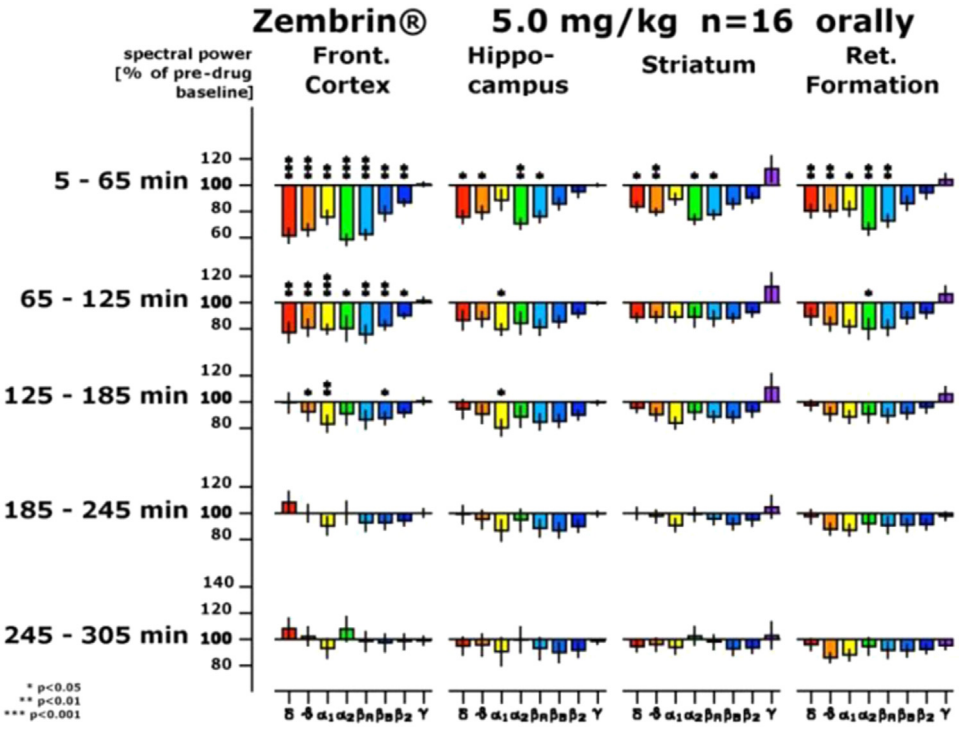

Fig. 3. Effects of Zembrin® (5.0 mg/kg): For other details see legend to Fig. 1. Statistical significance in comparison to control (vehicle) is documented by asterisks: $^{*}=p<0.05$; $^{**}=p<0.01$; $^{***}=p<0.001$.

Administration of the middle dosage of Zembrin® (5.0 mg/kg) resulted in a statistically significant attenuation of spectral power within all frequencies except for gamma in the frontal cortex during the first hour providing a very similar pattern of changes as observed for the lower dosage. However, changes in the hippocampus were less statistically significant despite the same pattern was produced. In general, changes of the frequency pattern were dominated by attenuation of alpha2 and beta1a frequencies. The changes were nearly identical during the second hour. But changes emerged in the gamma range in form of increases, not observed in the presence of the lowest dosage. But they did not reach statistical significance. After this time only a significant decrease of alpha1 power was seen in the cortex and hippocampus. A complete time line is given in Fig. 3.

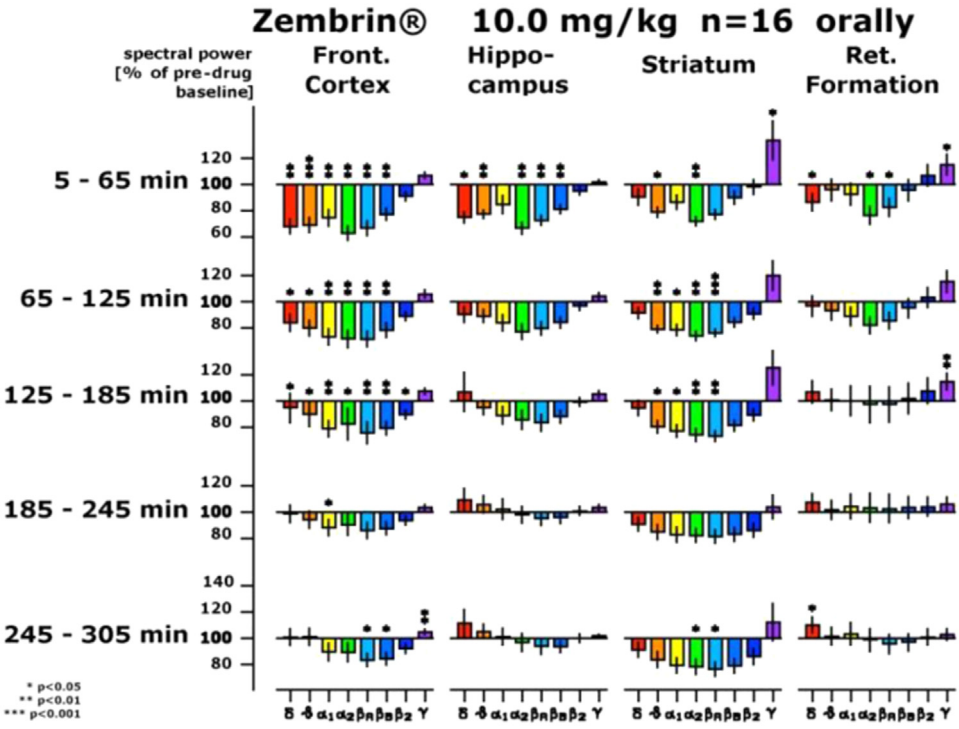

Fig. 4. Effects of Zembrin® (10.0 mg/kg): for other details see legend to Fig. 1. Statistical significance in comparison to control (vehicle) is documented by asterisks: $^{*}=p<0.05$; $^{**}=p<0.01$; $^{***}=p<0.001$.

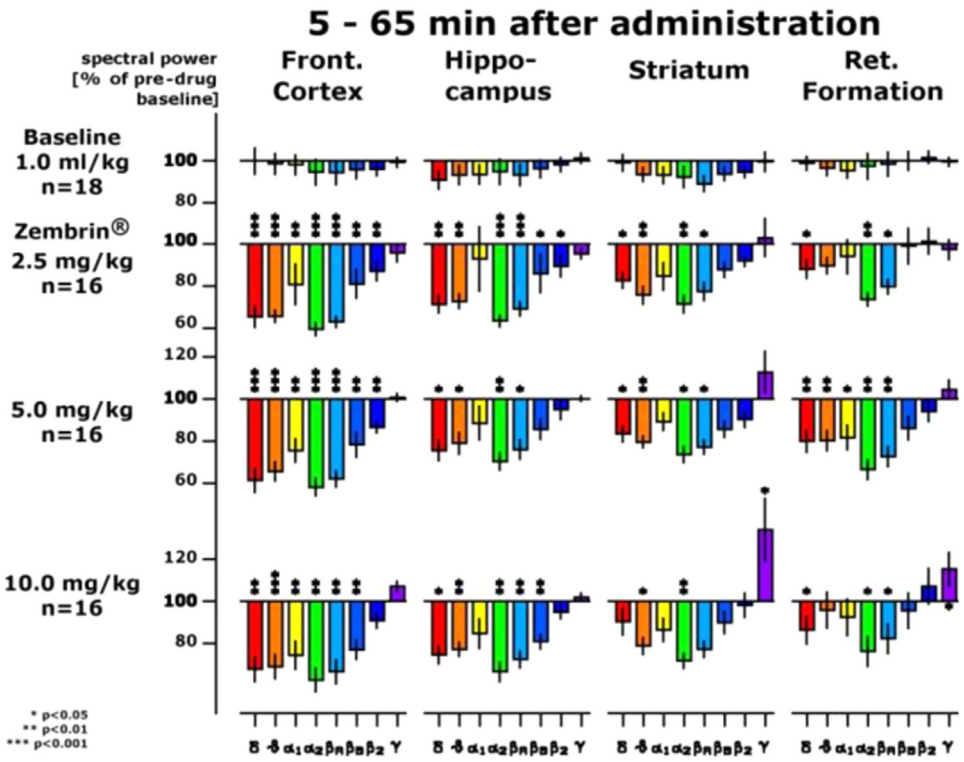

Fig. 5. Dose dependent Effect of Zembrin® (5–65 min): For other details see legend to Fig. 1. Statistical significance in comparison to control (vehicle) is documented by asterisks: $^{*}=p<0.05$; $^{**}=p<0.01$; $^{***}=p<0.001$.

Administration of the highest dosage of Zembrin® (10.0 mg/kg) resulted in a statistically significant attenuation of spectral power within all frequencies except for beta2 and gamma in the frontal cortex during the first hour, providing a very similar pattern of changes as observed for the lower dosages. Gamma spectral power was statistically significantly increased in the striatum and reticular formation during the first hour as a new feature. The other changes in the striatum and reticular formation were similar so those observed in the lower dosages. A complete time line is given in Fig. 4.

The dose dependent effects of Zembrin® on the electropharmacogram are shown in Fig. 5, for the 3 doses studied. From this comparison it becomes obvious that the overall pattern of changes is stable and reproducible. With respect to alpha1 frequency, attenuation is clearly dose dependent with higher reduction at higher dosage. With respect to gamma frequency an increase is clearly dose dependent.

3.3. *Effect of Zembrin® with respect to motion*

Motion in the presence of Zembrin® did not change except for the 4th hour in the presence of 5.0 mg/kg. However, comparing this change to changes in the baseline (saline) value, no real difference can be assumed. During this time a statistically significant reduction was observed (Table 1). However, there was quite a scatter of data making it difficult to draw firm conclusions.

Table 1
Effects of Zembrin® on motion. Changes in motion (cm/h) given for the whole time course of 5 h after drug administration in hourly intervals. Mean average values are given ± S.E.M. Statistical comparison to the results with control (Vehicle) were determined using the Wilcoxon, Mann, Whitney *U*-test (*p* values are given on the right side).

	Motion [cm/h]				
Time [min]	**NaCl 0.9% 1 ml/kg *n*=18**	**Zembrin® 2.5 mg/kg *n*=16**	**Zembrin® 5.0 mg/kg *n*=16**		**Zembrin® 10.0 mg/kg *n*=16**
−45 to 0	788.56 ± 107	638.88 ± 102	640.04 ± 99		640.96 ± 76
5–65	838.48 ± 77	724.66 ± 64	710.29 ± 78		779.11 ± 80
65–125	803.59 ± 108	823.59 ± 72	742.60 ± 73		784.52 ± 111
125–185	751.87 ± 85	781.96 ± 73	654.78 ± 107		808.81 ± 121
185–245	769.43 ± 79	642.39 ± 82	555.93 ± 89	0.056	643.73 ± 97
245–305	632.58 ± 77	621.63 ± 107	574.39 ± 81		618.89 ± 121

4. Discussion

Construction of electropharmacograms has been successfully used during drug discovery (Dimpfel, 2015). Based on numerous experimental results it has been concluded that preparations inducing a similar pattern of changes of spectral power have similar clinical therapeutic indications (Dimpfel, 2003). Zembrin® dose dependently induced attenuation of all frequency ranges to a varying degree. The most prominent was the statistically significant reduction in alpha2 and beta1a waves, correlated with activation of the dopaminergic and glutamatergic transmitter systems respectively. This feature is common to all synthetic and herbal stimulants tested to date. The second strongest effects were reduction in both the delta and the theta frequency ranges, correlated with changes in the cholinergic and norepinephrine systems respectively, a pattern seen in preparations prescribed for neurodegenerative diseases. Theta wave reduction in common with the delta, alpha2 and beta1 attenuation has been noted for analgesic drugs. Attenuation of alpha1 waves emerged during the highest dosage in all brain areas, a feature seen in all antidepressants. The dose dependent increase of gamma activity has

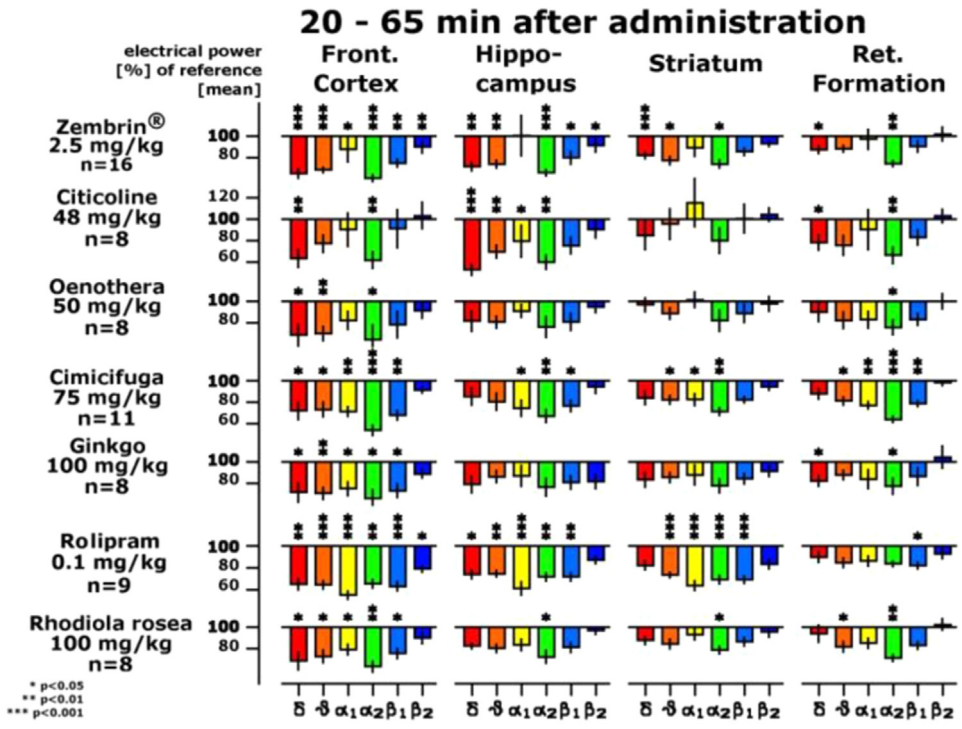

Fig. 6. Citicoline, rolipram and botanicals with similar effects to Zembrin® (20–65 min): for other details see legend to Fig. 1. For historical reasons (content of database) only 6 frequency ranges are documented. Statistical significance in comparison to control (vehicle) is documented by asterisks: $^{*}=p<0.05$; $^{**}=p<0.01$; $^{***}=p<0.001$.

been clearly related to motion in a previous publication (Dimpfel and Schombert, 2015), however no conclusions could be made as direct measurement of motion resulted in too high variability, while gamma activity had already appeared simply with the first motion of the head.

Our database was interrogated by a special program designed to detect similar actions on brain spectral power by comparing the relationship of spectral frequency changes for each substance to each other. (Please note that the patterns documented in Fig. 6 do not contain gamma activity since gamma activity has only been analyzed for in more recently studies). With respect to botanicals or herbal preparations a special extract of *Oenothera biennis* containing 50% polyphenols was recognized as the most similar herbal preparation to the middle dosage of Zembrin®. Extracts of *Cimicifuga racemosa* and *Ginkgo biloba* showed a quite similar profile of changes to those for Zembrin. The electropharmacogram for *Ginkgo biloba* extract was similar to that for Zembrin®, with the difference consisting of attenuation of alpha1 spectral power in Ginkgo, an effect not apparent in the profile for Zembrin® at lower doses. A close similarity to the action of Zembrin® was also observed for an extract of *Rhodiola rosea*. Rhodiola has been classified as a so-called adaptogenic botanical, which helps one cope with physical and emotional stress. The clinical efficacy of a *Rhodiola rosea* extract has been recently characterized using a combination of fast dynamic EEG analysis (Neurocode-Tracking) in combination with Eye-Tracking (now called "Enkephalovision") (Dimpfel, 2014).

Finally, the synthetic preparations citicoline, originally developed to enhance cognitive function, and rolipram, originally developed as an antidepressant drug, showed a similar pattern of frequency changes to that for Zembrin®. Both rolipram and Zembrin® are PDE4 inhibitors, and were expected to have similar electropharmacograms, rolipram prominently attenuated alpha1 spectral power, an activity correlated with serotonergic transmission, whereas Zembrin® only attenuated alpha 1 spectral power after administration of the highest dosage. A comparison of Zembrin® to reference extracts, citicoline and rolipram is documented in Fig. 6 including the dosages administered. One can conclude from this comparison that the antidepressant activity of Zembrin® may only take place at higher dosages.

These results are confirmed by discriminant analysis of the data. The three dosages of Zembrin® are projected together in close vicinity and show the same colour, as documented in Fig. 7. The comparison to other herbals as well as to citicoline and rolipram revealed that the nearest botanical preparation is an Oenothera extract also showing the same colour as Zembrin®. Other herbal preparations, which are clearly difficult to discriminate from Zembrin® are *Cimicifuga racemosa* extract and *Gingko biloba* extract having a similar colour as seen for the lowest dosage of

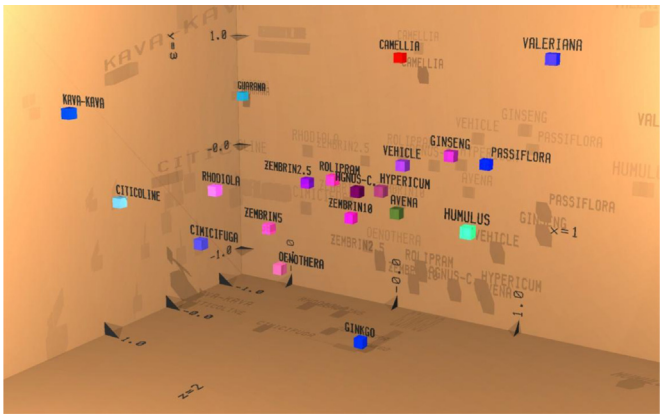

Fig. 7. Discriminant analysis of electropharmacograms. Comparison of the electropharmacogram of orally given **Zembrin2.5** (**2.5 mg/kg**); **Zembrin5** (**5.0 mg/kg**) and **Zembrin10** (**10.0 mg/kg**). All preparations were administered orally. Patterns of reference drugs providing similar spectral frequency changes according to the results of the first three discriminant functions are positioned in close neighbourhood (see *x*, *y* and *z* coordinates). Close similarity with respect to relative position **and** colour indicates similar spectral changes (Dimpfel, 2013). Data from the first recording period after administration (see Table 2). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Listing of herbal and synthetic reference preparations used for the discriminant analysis. Doses and time of recording are given. Reference compounds are listed with dosage given and time of recording after administration. Zembrin® was given orally. A reference matrix of earlier tested drug actions is kept constant (preparations labelled as "**Substance Definition**") for classification of unknown preparations (preparations labelled as "**Substance Analysis**").

Substance Definition	Dose [mg/kg]	Appli-cation	Time (min)	Substance Analysis	Dose [mg/kg]	Appli-cation	Time (min)
Kava–Kava	200	Orally	20–65	Avena	100	Orally	20–65
Guarana	25	Orally	20–65	Ginseng	100	Orally	20–65
Humulus	50	Orally	125–185	Passiflora	100	Orally	20–65
Valeriana	100	Orally	125–185	Oenothera	50	Orally	20–65
Ginkgo	100	Orally	20–65	Cimicifuga	75	Orally	20–65
Agnus-Castus	50	Orally	20–65	Camellia sin.	25	Orally	20–65
Rhodiola	100	Orally	20–65	Citicoline	48	Orally	20–65
Hypericum	250	Orally	20–65	Rolipram	0.1	Orally	20–65
				Vehicle	0.0	Orally	20–65
				Zembrin2.5	2.5	Orally	20–65
				Zembrin5	5.0	Orally	20–65
				Zembrin10	10.0	Orally	20–65

Zembrin®. The recording times and dosages for these preparations are given in Table 2.

This statistical result indicates that Zembrin® can be expected to have similar CNS-related clinical properties in common with *Gingko biloba* and *Cimicifuga racemosa* extract. With respect to the similarity to *Gingko biloba,* positive cognitive and antidepressant effects can be expected in terms of clinical use. A similar pattern of electropharmacogram changes was found for *Rhodiola rosea* extract. According to discriminant analysis the projection of the effect of Rhodiola shows a rather similar colour in addition to its spacial relationship to the middle dosage of Zembrin®. *Rhodiola rosea* extract is marketed in Germany and has been successfully clinically tested for its activating and stimulant effects (Dimpfel, 2014). Despite having similar electropharmacograms, the frequency pattern of citicoline in the discriminant analysis projects citicoline some distance from Zembrin®. Presumably, the lack of effect of citicoline in the striatum may be the reason for the more distant projection in the discriminant analysis.

The combined results from the electropharmacograms and comparative discriminatory analyses suggest that Zembrin® has dose dependent activity, with potential applications as a cognitive function enhancer, as an antidepressant, and as an analgesic.

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