Central Nervous System Profiling of *Hericium erinaceus* Biomass Powder by an Electropharmacogram Using Spectral Field Power in Conscious Freely Moving Rats

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SUMMARY

Mushroom extracts seem to exert an action on brain function. In order to objectify such an effect changes of the electric activity of the brain have been successfully used in earlier experiments in the presence of diverse food extracts.

Changes of field potentials recorded from implanted electrodes into the depth of the brain of rats served to analyse the action of plant-derived extracts in comparison to food supplements and reference drugs. Frequency analysis of the data and feeding of the results into discriminant analysis allowed indication dependent classification of the effects. The present investigation aimed at the neuro-physiological characterization of the effect of a preparation of *Hericium erinaceus* in this model.

The presently tested mycological preparation "MRL's *Hericium erinaceus* hyphal powder" induced a pattern of frequency changes consisting in a statistically significant attenuation of delta, theta, alpha2 and beta1 spectral power, but not alpha1 power in all brain regions during the first 2 hours after administration. The lack of alpha1 spectral power attenuation in combination with attenuation of delta, theta and alpha2 power is shared by some other preparations tested earlier under identical conditions like Zembrin®, Acetylsalicylic acid, Methylphenidate, Taxifolin and Ginkgo extract. From this, calming, analgesic, antidepressive and cognition enhancing properties might be deduced for the tested mushroom biomass. However, due to the fact, that only one dosage was tested, interpretation of the results is limited. As active ingredients recently discovered erinacines from *Hericium ericaneus* mycelia might be considered, which show up in the brain as early as 30 min after oral administration.

INTRODUCTION

Drugs, food supplements and functional food exert their action within the organism by interaction with targets defined biochemically (e.g. receptors, enzymes, channels transporters, large protein molecules sometimes also sitting at the outer surface of cells). With respect to the central nervous system neurotransmitter receptors represent main targets. 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 externally administered molecules with their targets within the concert of neurotransmission.

Frequency analysis of the field potentials in the presence of drugs leads to the so-called electropharmacogram, which has been widely used in the past to characterize drug actions on rat (Dimpfel, 2007) and human brains (Dimpfel, 2011)(Dimpfel, 2015). Interpretation of the results was performed with respect to neurotransmitter activity as well as aiming at possible clinical indications in humans. A relation between EEG delta waves and cholinergic neurotransmission has been suggested for the first time by Dimpfel (Dimpfel, 2005). Theta waves have been recognized as being influenced by drugs acting at the biochemically defined norepinephrine alpha2 receptor (Dimpfel & 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 (Dimpfel et al., 1989). Dopaminergic activity is reflected by changes in alpha2 frequencies (Dimpfel, 2008). Drug induced changes in the beta1 frequency domain relate to glutamatergic transmission, whereas drugs acting at GABA receptors induce increases in beta2 frequencies.

Hericium erinaceus (Bull.: Fr.) Pers. is an edible and medicinal basidiomycete fungus belonging to the class Agaricomycetes, order Russulales and family Agaricomycetes (Kirk et al. 2008). It is commonly known as *Shishigashira* or *Houtou* (meaning "monkey head") in China and *Yamabushitake* (meaning "mountain priest") in Japan. English names for the fungus include Lion's Mane, Monkey's Mushroom, Bear's Head, Hog's Head Fungus, White Beard, Old Man's Beard and Pom Pom (Thongbai et al., 2015). The fruiting body has historically been prescribed as part of traditional Chinese medicine (TCM) and Kampo medicine in Japan, including for treating neurasthenia and general debility (Ying et al., 1987).

Hericium is found across the northern hemisphere in Asia, Europe and North America (Thongbai et al., 2015). In recent years the fruiting bodies and cultured mycelia of Hericium have become increasingly popular in North America and Europe in the form of nutraceuticals and food supplements for improving health and well-being, including for enhancing cognitive function. Hericium fruiting bodies and mycelium can be grown on industrial scale on diverse substrates, including inexpensive agricultural wastes. Both the fruiting body and the cultured mycelia have been reported to produce several classes of bioactive molecules, including polysaccharides, proteins, lectins, sterols, phenols, and terpenoids (Thongbai et al., 2015).

The following *in vitro, in vivo* and human clinical studies have demonstrated that powders, extracts and fractions of Hericium have activities on the central and peripheral nervous systems:

In vitro studies

Secretion of nerve growth factor (NGF) from astrocytes has been noted to be increased with 150µg/mL of the ethanolic extract. Lion's Mane has been noted to increase mRNA expression of NGF in isolated astrocytes to around 5-fold that of control at 100-150µg/mL

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of ethanolic extract in a concentration dependent manner (Mori et al., 2008). Isolated erinacines (A-C), present in the mycelium, are known to stimulate NGF secretion at 1mM concentrations (Kawagishi et al., 1994). Glutamate neuronal excitability appears to be attenuated in the presence of Hericium extracts *in vitro* (Moldavan et al., 2007).

In vivo studies

An increase in NGF mRNA has been detected in the hippocampus, but not cortex, of mice given 5% of the diet as lion's mane for a period of seven days to around 1.3-fold of control (Mori et al., 2008). Hericium appears to protect rats against cognitive decline caused by β-amyloid pigmentation at 5% of the diet (Mori et al., 2011).

In an *in vivo* study in rats, Hericium aqueous extract of fruiting body was able to promote neuronal regrowth after crushing injury to the gluteal nerve. Rats that had induced gluteal nerve damage were able to walk better after ingestion of the extract (Wong et al., 2011).

Compared with saline-treated mice, dietary administration of Hericium ethanolic fruit body extracts at 60 mg/kg once a day for 4 weeks reduced anxiety and depressive-like behaviour in healthy mice assessed through elevated plus-maze, tail-suspension and forced swimming tests. This was associated with increased proliferation of hippocampal progenitors and enhanced neurogenesis (Ryu et al., 2018).

Antidepressant-like effects of ethanolic extract of Hericium mycelium enriched in erinacine A were studied in depressive mice challenged by repeated restraint stress (RS) . The extract at 100, 200 or 400 mg/kg body weight/day was orally given to mice for 4 weeks. After 2 weeks of Hericium administration, all mice except the control group went through with 14 days of RS protocol. Stressed mice exhibited behavioural alterations, including extended immobility time in the tail suspension test (TST) and forced swimming test (FST), and increasing the number of entries in open arm (POAE) and the time spent in the open arm (PTOA). The levels of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) were decreased in the stressed mice, while the level-s of interleukin (IL)-6 and tumour necrosis factor (TNF)-α were increased. These changes were significantly reversed by the administration of Hericium extract, especially at the dose of 200 or 400 mg/kg body weight/day. Additionally, the extract was shown to activate the BDNF/TrkB/PI3K/Akt/GSK-3β pathways and block the NF-κB signals in mice. Taken together, this erinacine A-enriched Hericium mycelium extract could reverse the depressive-like behaviour caused by induced RS and was accompanied by the modulation of monoamine neurotransmitters as well as pro-inflammatory cytokines, and regulation of BDNF pathways. Thus the erinacine A-enriched Hericium mycelium extract has potential for the treatment of depressive disorders (Chiu et al., 2018).

Hericium extracts with known amounts of erinacine A and hericenones C and D were tested in a frail mouse model of physiological aging. Two-months oral supplementation with Hericium reversed the age-related decline in recognition memory. Proliferating cell nuclear antigen (PCNA) and doublecortin (DCX) immunohistochemistry in the hippocampus and cerebellum in treated mice supported a positive effect of the extract on neurogenesis in frail mice (Ratto et al., 2019).

Clinical studies

A double-blind, parallel-group, placebo-controlled trial performed on 50 to 80 year old Japanese men and women diagnosed with mild cognitive impairment, 30 subjects were randomized into two 15 person groups. The subjects of the active group took four 250 mg tablets containing 96% of Hericium hyphal and dry powder 3 times a day for 16 weeks. After termination of the intake, the subjects were observed for the next 4 weeks. At weeks 8, 12 and 16 of the trial, the Hericium group showed significantly increased scores on the cognitive function scale compared with the placebo group; at week 4 after the termination of the 16 weeks intake, the cognitive function scores decreased significantly. The results indicated that Hericium is well tolerated, and improves mild cognitive impairment (Mori et al., 2009).

A recent study carried out on 77 overweight or obese volunteers reported that a daily, 8-week oral supplementation with Hericium (80% mycelium extract and 20% fruiting body extract), coupled with a low calorie diet regimen improved depression, anxiety, sleep, and binge eating compared with subjects undergoing low calorie diet only. This improvement was correlated with increased circulating pro-BDNF levels and pro-BDNF/BDNF ratio, despite the lack of any significant changes in BDNF circulating levels (Vigna et al., 2019).

The present preparation consisting of mycelium powder from *Hericium erinaceus* biomass (Hericium-MRL) was tested as the first study in the animal model of field potential analysis in order to see whether any ingredients can pass the blood barrier and exert an activity on the electric activity of the central nervous system. The electropharmacogram of this preparation should also provide more insight into the effectiveness with respect to time dependence. Since many publications also deal with so-called EEG gamma activity -representing frequencies above 35 Hz-, this parameter was also measured.

Materials and Methods

The *Hericium erinaceus* used in this study was derived from a specific superior isolate and cultivated and homogenised in the form of a biomass on sterile (autoclaved) substrate in Europe, under ISO 22000:2018 standards. The proprietary technology used in the cultivation process ensures that the resulting standardised biomass is free from contamination by other fungi. The obtained biomass contains mycelium and primordia (young fresh fruiting body) of

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this respective mushroom and was supplied by Mycology Research Laboratories Ltd (Luton, United Kingdom).

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 (Dimpfel et al.,1986) (Dimpfel et al.,1988) (Dimpfel et al., 1989), (Dimpfel, 2003). In short, after automatic artefact rejection electric 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; beta1: 12.50 - 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 off-line procedure spectra were averaged to give longer periods for further analysis and data presentation.

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 previous studies, for example with different dosages of caffeine. Solutions were prepared fresh for each experimental day and administered orally by gavage after 45 minutes of pre-drug Vehicle recording. Vehicle was water.

The study was performed at the preclinical laboratories of NeuroCode AG, Sportparkstr. 9, D-35578 Wetzlar/ Germany. Allowance according German guidelinesfor animal protection was received (Genehmigung gem. §8 Abs.1 des Tierschutzgesetzes, Ref. # 17 0736 540 13 00007), dated 24th of May 2017).

Table 1
Test compounds

Hericium erinaceus Biomass (Hericium-MRL)	LOT 16K118	150 mg/kg	Mycology Research Laboratories The Spires, Suite 8, Adelaide Str., Luton.UK LU1 5BB
VEHICLE 0.9% NaCl		1.0 ml/kg	Braun, Melsungen, Germany

Nine adult Fisher 344 rats (5 months of age and day - night converted, weight about 350 - 400 g, provided by Charles River Laboratories, D-97633, Sulzfeld, Germany) were used in this experimental series. Animals were implanted with electrodes into the brain and were given 2 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.

The animals were allowed to acclimatise for at least 4 weeks before the study started. There was automatic control of light cycle, temperature and humidity. Animals were day-night reversed (12h/12h). Daily monitoring indicated that temperature and humidity remained within the target ranges of 22 degree Celsius and 44, 5% humidity, respectively. Cages, bedding, and water bottles were changed at regular intervals, i.e. every 2-3 days. Standard Diet (Nohrlin H10, Altromin, D-32791 Lage, Germany) was available ad libidum. The animals had access to domestic quality mains water ad libidum.

Rats were implanted with 4 bipolar concentric steel electrodes within a stereotactic surgical procedure during anaesthesia with Ketamine. All 4 electrodes were placed 3 mm lateral within the left hemisphere. Dorso-ventral coordinates were 4, 6, 4.2 and 8 mm and anterior coordinates were 3.7, 9.7, 5.7 and 12.2 mm for frontal cortex, striatum, hippocampus, and reticular formation, respectively (according to the atlas of (Paxinos & Watson, 1982)). A pre-constructed base plate carrying 4 bipolar stainless steel semi-micro electrodes (neurological electrodes "SNF 100" from Rhodes Medical Instruments, Inc., Summerland, CA 93067, USA) and a 5-pin-plug was fixed to the skull by dental cement interacting with 3 steel screws placed on distance into the bone. The distant recording spot of the electrode was the active electrode, whereas the proximal spots of the 4 electrodes were connected to each other to give a common reference. The base plate was carrying a plug to receive later on the transmitter during the experimental session (weight: 5.2 g including battery, 26x12x6 mm of size).

A crossover design with at least 1 week of drug holidays in between the administrations was used. Oral administration of 1.0 ml/kg of vehicle (0.9% NaCl) or *Hericium erinaceus* biomass preparation was performed. After a pre-drug period of 45 min for pre-drug recording, drug effects were observed continuously on the screen (artefact control) for 300 minutes subdivided into 15 min periods after a lag time of 5 minutes for calming of animals after oral administration. Changes of electric power µV2 are expressed as % of the 45 min lasting absolute pre-drug spectral power values within each frequency band. Data were averaged from 8-9 animals. Data are expressed as mean values ± S.E.M. Statistics were calculated by means of the Wilcoxon, Mann, Whitney U-test.

Dosage was chosen by taking in account the human dose

recommendation and a relationship factor of 5–10:1 based on kilogram body weight as recommended in the literature (Reagan-Shaw et al, 2008). Dose level of *Hericium erinaceus* biomass 150 mg/kg was discussed with the Sponsor on the base of his experience. The animals were dosed orally using a solution of a constant volume of 1.0 ml/kg body weight for vehicle and active preparation. The dosage administered to each animal was determined every day by the weight of that animal at the time of administration.

Statistically, the Wilcoxon-Mann-Whitney U-test was used for comparison to results obtained by vehicle administration at the particular timing. Comparison of data to reference compounds tested earlier under identical conditions was performed using linear discriminant analysis according to Fischer. A total of the classic 24 variables (6 frequency ranges times 4 brain areas) was 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 was performed using the 3 spatial coordinates for the results of the first 3 discriminant functions. 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 (frozen) for classification of unknown preparations. Only data from 20 to 65 min after administration were classified. Data are archived as raw data on hard disk and magneto-optic devices for backup.

RESULTS: Oral administration of the vehicle (0.9% NaCl) did only result in very minor changes of spectral gamma power within the striatum and reticular formation from the second hour on. A complete time course is given in Fig. 1. Oral administration of the *Hericium erinaceus* biomass preparation (150 mg/kg) resulted in a statistically significant attenuation of spectral delta and theta power in the hippocampus and reticular formation. Theta and alpha2 spectral power were statistically significantly attenuated in all brain areas. Also, beta1 spectral power was significantly attenuated in all brain regions. Changes were still visible during the second hour after administration but did not reach statistical significance (Fig. 2).

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 (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.

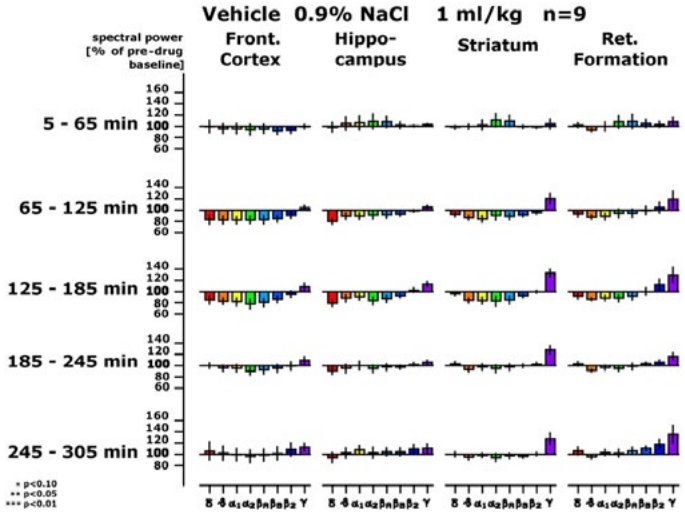

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Fig.2 Effects of *Hericium erinaceus* biomass (150 mg/kg) bodyweight)

For other details see legend to Fig. 1. Data from 1 animal were not evaluated due to technical problems. Statistical significance in comparison to control (vehicle) is documented by stars: *=p<0.10; **=p<0.05; ***=p<0.01.

With respect to motion no statistically relevant differences were observed in comparison to vehicle administration during the first 3 hours. Only during the 4th hour a significant reduction was seen (Tab.3).

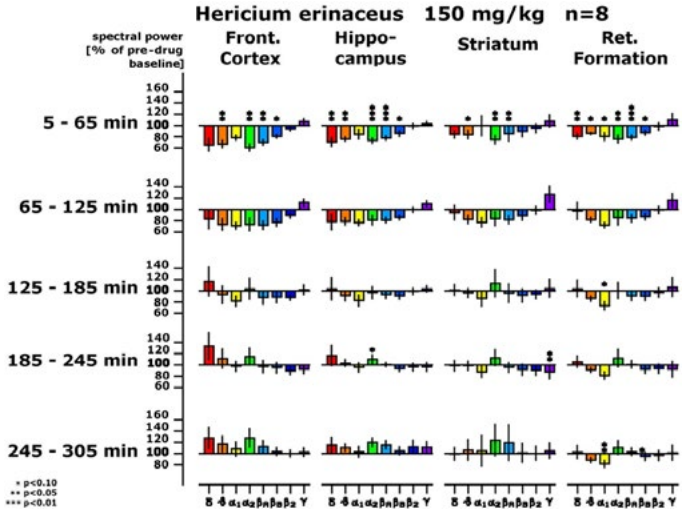


Table 3. Effects of Motion

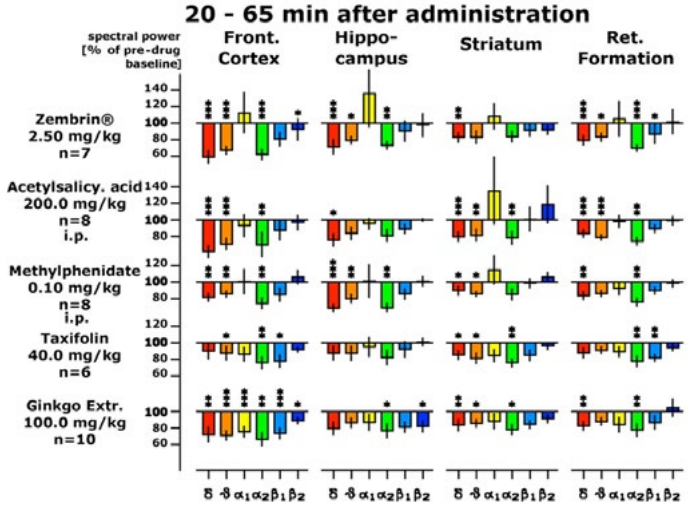
Changes in motion (cm/h) given for the whole time line of 5 hours after drug administration in hourly intervals. Mean average values are given ± S.E.M. Statistical comparison to the results with control (Vehicle) were determined using the Wilcoxon, Mann, Whitney U-test (p values are given on the right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

Motion [cmlh]							
Time (min)	VEHICLE 0.9% NaCl 1.0ml/kg n ±9			Hericium 150mg/kg n=8			
-45-0	626.67	±	120	884.22	±	48	
5-65	676.49	±	117	899.44	±	50	
65-125	794.39	±	97	984.71	±	68	
125-185	1051.10	±	81	975.87	±	67	
185-245	913.30	±	89	540.38	±	46	0.027
245-305	695.38	±	80	811.92	±	70	

Fig.3 Effects of reference preparations

For other details see legend to Fig. 1. Statistical significance in comparison to control (vehicle) is documented by stars: *=p<0.10; **=p<0.05; ***=p<0.01. Please note, that analysis of the data is referring to the time period of 20 to 65 minutes after administration.

Feeding the data into linear discriminant analysis revealed, that classic drugs with well-known clinical indications grouped together according to their prescription in patients (Dimpfel, 2003). Analysis of the presently tested *Hericium erinaceus* biomass (MRL) confirmed the observed similarity to some reference preparations since the *Hericium erinaceus* biomass was projected into the vicinity of Zembrin®, Acetylsalicylic acid, Taxifolin and Ginkgo (Fig. 4). For comparison to reference drugs the first time period of 20 to 65 min. was chosen.



DISCUSSION

The animal model "Tele-Stereo-EEG" (Dimpfel et al., 1986) 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. In general, drugs produced different individual patterns of spectral changes. However, drugs with similar clinical indications induced similar changes among each other. Therefore, unknown preparations can be compared to drugs with well-established use.

The presently tested herbal preparation *Hericium erinaceus* biomass induced a pattern of frequency changes consisting in a significant attenuation of delta, theta, alpha2 and beta1 spectral power, but not alpha1 power (except for the reticular formation). The lack of alpha1 spectral power attenuation in combination with attenuation of delta, theta and alpha2 power is shared by some other preparations like Zembrin®, Acetylsalicylic acid, Methylphenidate, Taxifolin except for Ginkgo extract (Fig. 3). Due to the similarity to these drugs with well-known clinical efficacy calming, analgesic, antidepressive and cognition enhancing properties might be deduced for the *Hericium erinaceus* biomass. However, due to the fact, that only one dosage was tested, interpretation of the results is limited.

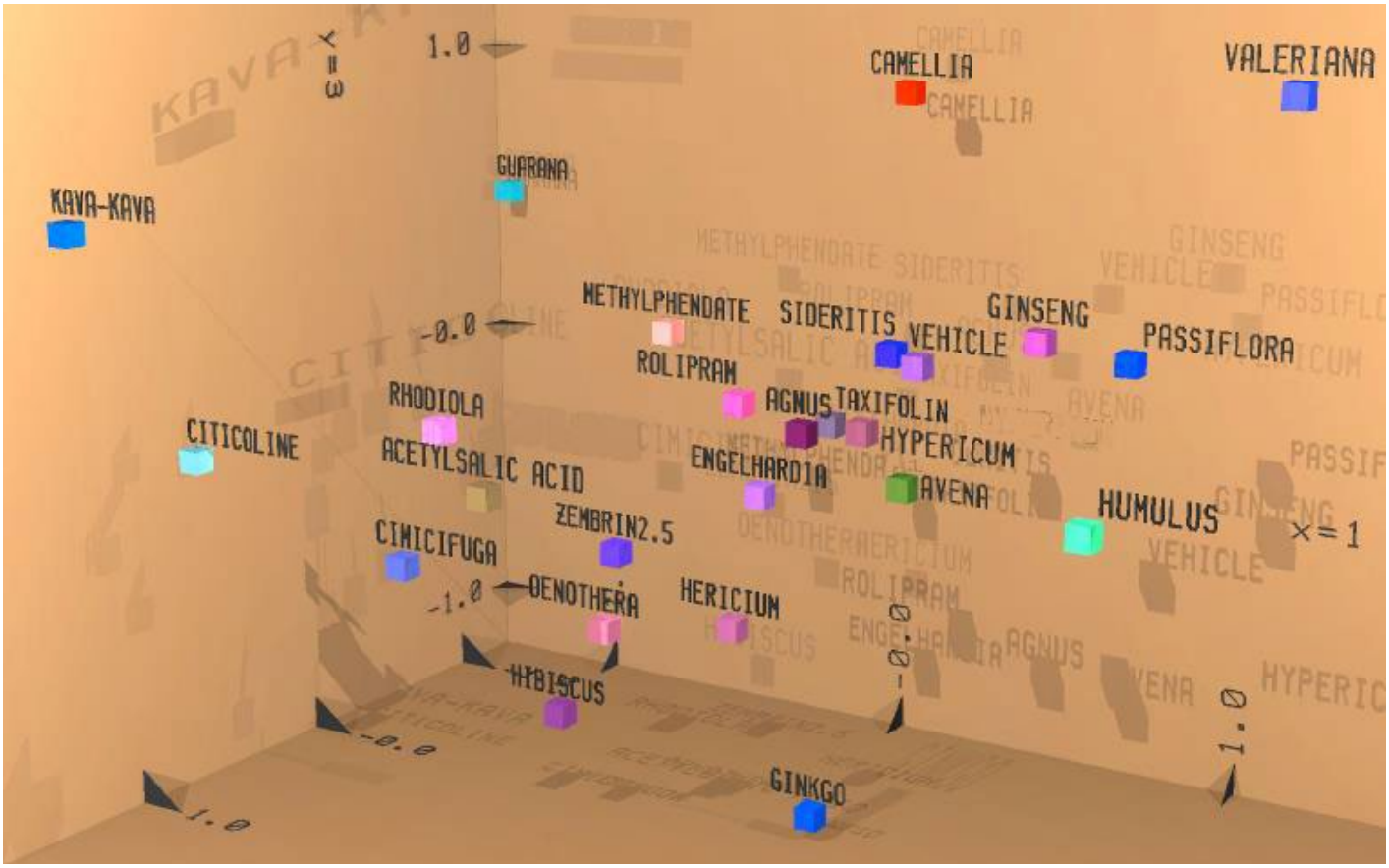


Fig. 4 Discriminant analysis of electropharmacograms

Comparison of the electro-pharmacogram of orally given ***Hericium erinaceus*** biomass MRL (150 mg/kg) with patterns of reference drugs. It provides similar spectral frequency changes according to the results of the first 3 discriminant functions.

A great similarity with respect to space and colour to some reference drugs signalizes similar net effects with respect to clinical indications (Dimpfel, 2003, 2013). Data from the first recording period 20 to 65 min. after administration (s. Tab. 2).

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Table. 2 Listing of reference compounds used for discriminant analysis. Doses and time of recording are given.

Substance Definition	Dose [mg/kg	Application	Time
Kava-Kava	200	orally	20 - 65 min
Guarana	25	orally	20 - 65 min
Humulus	50	orally	125 - 185 min
Valeriana	100	orally	125 - 185 min
Ginkgo	100	orally	20 - 65 min
Agnus-Castus	50	orally	20 - 65 min
Rhodiola	100	orally	20 - 65 min
Hypericum	250	orally	20 - 65 min
Substance Analysis	**Dose [mg/kg]**	**Application**	**Time**
Avena	100	orally	20 - 65 min
Ginseng	100	orally	20 - 65 min
Passiflora	100	orally	20 - 65 min
Oenothera	50	orally	20 - 65 min
Cimicifuga	75	orally	20 - 65 min
Camellia sin.	25	orally	20 - 65 min
Citicoline	48	orally	20 - 65 min
Rolipram	0.1	orally	20 - 65 min
Sideritis	100	orally	20 - 65 min
Taxifolin	40	orally	20 - 65 min
Zembrin	2.5	orally	20 - 65 min
Acetylsalicylic acid	200	i.p	20 - 65 min
Metylphenidate	0.1i.p.	i.p	20 - 65 min
Vehicle	1 ml	orally	20 - 65 min
Engelhardia	75	orally	20 - 65 min
Hericium-MRL150	orally	orally	20 - 65 min
Hibiscus (SUP_EEG_HSA50)75	orally	orally	20 - 65 min

Due to similarity to some reference preparations tested earlier under identical conditions, calming, analgesic, antidepressive and cognition enhancing properties might be deduced. Potential antidepressive effects of *Hericium erinaceus* have been described recently besides those of *Scutellaria baicalensis* and *Rhodiola rosea* within a review (Limanaqi et al., 2020). At some matter of fact a reduction of depression and anxiety in 30 females has been reported by (Nagano et al., 2010) after intake of Hericium cookies for 4 weeks. A review on the therapeutic potential of *Hericium erinaceus* for depressive disorder has been published recently (Chong et al., 2019), stating that Hericium ameliorates depressive like behaviour through the modulation of monoamine transmitters. According to the catecholamine hypothesis of affective disorders (Schildkraut, 1965) (Bunney & Davis, 1965) norepinephrine, serotonin and dopamine activity are disturbed in depressed patients. Main effects of Hericium in the present investigation are seen on theta and alpha2 frequencies, which correspond to norepinephrine (theta) and dopamine (alpha2) neurotransmission. This is in line with our interpretation of a potential positive effect of Hericium on depression.

Available information on Hericium, including its taxonomy, phylogeny, health-promoting benefits, and medicinal properties is reviewed by (Thongbai et al., 2015). Studies on secondary metabolites have resulted in the isolation of an exceptionally large amount of structurally different and potentially bioactive components including erinacines, hericerins, steroids, alkaloids, and lactones (Friedman, 2015). Biologically active ingredients of Hericium have been mainly recognized to be polysaccharides. Over the past decade, it has been demonstrated that Hericium polysaccharides possess various promising bioactivities, including antitumor and immunomodulation, anti-gastric ulcer, neuroprotection and neuroregeneration, anti-oxidation and hepatoprotection, anti-hyperlipidemia, anti-hyperglycemia, anti-fatigue and anti-aging (He et al., 2017).

Lipoxin A4 (LXA4) is an endogenously produced eicosanoid that acts as an endogenous "breaking signal" in the inflammatory process. *Hericium erinaceus* biomass (MRL) supplementation has been shown to significantly up-regulate Lipoxin A4 in the brain of rats within 90 days when compared to a separate control group (Trovato et al., 2016). In the brain of rats receiving Hericium maximum induction of Lipoxin A4 was observed in cortex and hippocampus followed by substantia Nigra, striatum and cerebellum. These brain regions correspond very much to those where effects of Hericium were observed in our acute study (Cortex and hippocampus).

With respect to active compounds of Hericium only erinacine A has confirmed pharmacological actions in the central nervous system in rats and to date only erinacines have been documented to cross the blood brain barrier (BBB). Therefore, current effects of Hericium on brain activity as observed in this study might very well derive from erinacines. However, no direct evidence has yet shown that other compounds of the whole extract could pass through the blood-brain barrier. Erinacines are groups of cyathin diterpenoids that show biological activities as stimulators of NGF synthesis and could be useful as a treatment for neurodegenerative disorders and peripheral neuropathy. To date, 15 erinacines (erinacines A–K and P–S) have been identified and further investigations have demonstrated that 8 of them have various neuroprotective properties, such as enhancing NGF release (erinacines A–I), reducing amyloid-β deposition, increasing insulin-degrading enzyme (IDE) expression (erinacines A and S), or managing neuropathic pain (erinacine E), while others are either being currently discovered or have other pharmacological activities (Li et al., 2018). Erinacine S, so far known to have been produced only in *Hericium erinaceus* mycelia, has just recently

been discovered and is able to reduce amyloid plaque growth and improve neurogenesis in aged brain of rats (Hu et al., 2019). Erinacine S was detected in the brain, as early as half hour after administration (2.069 ± 0.503 µg/g), peaked at 2h after administration (11.294 ± 9.662 µg/g) (Hu et al., 2019). These values correspond quite well with the early effects of Hericium as observed on brain electricity in the present study. Preclinical studies have also shown that there can be improvements in ischemic stroke, Parkinson's disease, Alzheimer's disease, and depression if *Hericium erinaceus* mycelia enriched with erinacines are included in daily meals (Li et al., 2018).

Conclusion:

From this preliminary study in a small number of animals (n=8) it can be concluded that MRL's *Hericium erinaceus* hyphal powder contains compounds that are bioavailable and cross the blood brain barrier resulting in an EEG signature that can be interpreted by discriminant analysis to have potential calming, analgesic, antidepressant and cognitive-enhancing activities. Based upon many years in the evaluation of electropharmacogram studies in both pharmaceutical and natural products, the dose of 150 mg/kg body weight used in the present study may translate to a human dose of 15 mg/kg body weight, or 1 050 mg in a 70 kg adult, within the daily dose range of 1-3g recommended by the Sponsor (Nektium)).

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Conflict of interest

The study was financially supported by Nektium Pharma Julia Wiebe is co-worker at Nektium Pharma. There was no conflict of interest.

Contributions:

Dr Julia Wiebe designed the experimental set up of the study. Prof Wilfried Dimpfel performed the experiments and wrote part of the manuscript. Dr Nigel Gericke wrote part of the manuscript and provided many references.

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