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Mechanism of Action of Low Dose Preparations from *Coffea arabica*, *Gelsemium* and *Veratrum* Based on *in Vivo* and *in Vitro* Neurophysiological Findings

Wilfried Dimpfel[1*], Andreas Biller[2]

[1]NeuroCode AG, Wetzlar, Germany
[2]Dr. Loges + Co. GmbH, Winsen/Luhe, Germany
Email: [*]w.dimpfel@neurocode-ag.com

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Abstract

Low dose remedies are widely administered in medicine. We used Tele-Stereo-EEG and the hippocampal slice preparation to measure physiological effects of orally given *Coffea* D6 (40 mg/kg), *Gelsemium* D4 (10 mg/kg) and *Veratrum* D6 (30 mg/kg) in rats. Adult rats were implanted with electrodes positioned stereotactically into four brain regions. Changes in field potentials were transmitted wirelessly. After frequency analysis data from 6 - 8 animals were averaged. For *in vitro* testing, preparations were superfused directly on hippocampal slices. Stimulation of Schaffer Collaterals by single stimuli (SS) or theta burst stimulation (TBS) resulted in stable population spike amplitudes. All three low dose preparations produced decreases of spectral power. Statistically significant changes were observed in delta, theta and alpha2 spectral power. In the hippocampal slice preparation *Coffea* facilitated signal transfer presumably by enhancing glutamate AMPA receptor transmission. *Gelsemium* showed a similar effect, but only after single shock stimulation. Opposite to this, attenuation of the electric pathway was recognized after theta burst stimulation due to AMPA receptor and glutamate metabotropic II receptor mediated transmission. *Veratrum* was able to attenuate glutamatergic due to receptor-mediated signalling sensitive to AMPA and NMDA. The results strongly speak in favour of the existence of biologically active molecules in these low dose preparations.

Keywords

Neurophysiology, Rat, *Gelsemium sempervirens*, *Veratrum album*, *Coffea arabica*,

[*]Corresponding author.

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Electropharmacogram, Hippocampus Slice

1. Introduction

Homeopathic dilutions of plant-derived extracts are widely administered. Nevertheless the debate on measurable effects of such preparations keeps going on. In order to search for answers it seems appropriate to deal initially with some plant-derived preparations in the order of dilutions of D4-D6, where for sure some molecules are left within the preparation after the dilution procedure. A second reason to get engaged into this matter was the availability of two very sensitive physiological methods used for years to characterize successfully classic synthetic drugs as well as plant derived extracts: Tele-Stereo-EEG for *in vivo* characterization [1] and hippocampus slice for *in vitro* characterization [2] [3]. The former methodology provides evidence for *in vivo* characterization of dose and time dependent drug effects including predictions for clinical use; the latter provides information on concentration dependent effects of water-soluble preparations on physiological transmission of brain electric signals including hints on the mechanisms of action. During the course of the present investigation we looked at three preparations: *Coffea* D6, *Gelsemium* D4 and *Veratrum* D6. All three were tested in these two models in the presence of different dosages *in vivo* and several concentrations *in vitro* in order to get data for a possible modulation of physiological parameters.

2. Materials and Methods

2.1. Construction of Electropharmacograms (Tele-Stereo-EEG *in Vivo*)

Eight adult Fisher 344 rats (8 month of age and day-night converted, weight about 400 g) were implanted with 4 bipolar concentric steel electrodes within a stereotactic surgical procedure. All four electrodes were placed 3 mm lateral within the left hemisphere. Anterior coordinates are 12.2, 5.7, 9.7 and 3.7 mm for frontal cortex, hippocampus, striatum and reticular formation (according to the atlas of [4]. A 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 four electrodes were connected to each other to give a short circuit reference. The base plate was carrying a plug to receive later on the transmitter (weight: 5.2 g including battery, 26 × 12 × 6 mm of size).

Animals were given two weeks for recovery from this procedure. After this, the transmitter was plugged in for adaptation and control experiments with saline. 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 Animal Health Guidelines.

EEG signals were recorded from frontal cortex, hippocampus, striatum and midbrain reticular formation 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 of 0.25 Hz resolution [1]. The method earlier described was used exactly [5]. 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: 0.8 - 4.5 Hz; theta: 4.75 - 6.75 Hz; alpha1: 7.00 - 9.50 Hz; alpha2: 9.75 - 12.50 Hz; beta1: 12.75 - 18.50 Hz; beta2: 18.75 - 35.00 Hz). These frequency ranges were recognized to change independently from each other in all earlier trials. Spectra were averaged in steps of 3 minutes each and displayed on-line. In an off-line procedure spectra were averaged to give longer periods for further analysis and data presentation.

Two dosages of each extract were tested by oral administration (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, D98704 Langewiesen, Ger-

many). 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.

A crossover design with at least one week of drug holidays in between the administrations was used. Controls consisted of oral administration of 1 ml/kg of a physiological saline solution. After a pre-dose period of 45 minutes for baseline recording, drug effects were observed continuously for 65 minutes after a lag time of 5 minutes for calming of animals after oral administration. Changes of electricpower density (μV^2) are expressed as % of the 45 min absolute pre-dose spectral power values within each frequency band. Data were averaged from all animals, which gave valuable recordings for the particular experimental day. Data are expressed as mean values averaged from n = 6 - 8 animals. Statistics were calculated according to Wilcoxon, Mann and Whitney. Finally, data were compared to a database containing about 200 preparations tested under identical conditions. Comparison also included use of linear discriminant analysis according to Fisher for positioning of the preparation-induced electric pattern within a matrix of reference drugs with known clinical indication [6].

The remedies were provided by Dr. Loges + Co. GmbH, D 21423 Winsen, Germany, in form of lactose triturations.

2.2. Hippocampal Slice Preparation *in Vitro*

Hippocampus slices (for overview see [7]) were obtained from 25 adult male Sprague-Dawley rats (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 [3] [8]. Animals were exsanguinated under ether anaesthesia, the brain was removed in total and the hippocampal formation was isolated under microstereoscopic sight. 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 [9].

During the experiment the slices were held and treated in a special superfusion chamber (List Electronics, Darmstadt, Germany) according to Haas [10] at 35˚C [11]. Five slices were used from one rat per day under one of the test conditions (control or different concentrations of test compounds). The preparation was superfused with artificial cerebrospinal fluid (ACSF) at 220 ml/h. Electrical stimulation (200 µA constant current pulses of 200 µs pulse width) of the Schaffer Collaterals within the CA2 area and recording of extracellular field potentials from the pyramidal cell layer of CA1 [9] was performed according to conventional electrophysiological methods using the “Labteam” Computer system “NeuroTool” software package (MediSyst GmbH, Linden, Germany). Measurements were performed at 10 min intervals in order to avoid potentiation mechanisms after single stimuli (first recording at 10 min is discarded for stability purposes). Four stimulations—each 20 s apart—were averaged for each time point. After averaging the last three of four responses to single stimuli (SS) to give one value, potentiation was induced by applying a theta burst type pattern (TBS; [12]). The mean amplitude of three signals 20 seconds apart were averaged to give the mean of absolute voltage values (microvolt) ± standard error of the mean for each experimental condition (single stimulus or theta burst stimulation). Electrical stimulation of the Schaffer Collaterals within the C2 area with single stimuli resulted in stable responses of the pyramidal cells in form of population spikes with an amplitude of about 1 mV and about 2 mV after theta burst stimulation (TBS).

For stimulation of glutamate receptors (NMDA, AMPA, Kainate and metabotropic receptor) four agonists were used, respectively: *trans*-1-Aminocyclobutan-1,3-dicarboxylic acid (ACBD [13], (S)-(-)-*α*-Amino-5-fluoro-3,4-dihydro-2,4-dioxo-1(2*H*)-pyrimmidinepropanoic acid (S-Fluorowillardiine [14]-[16], (RS)-2-Amino-3-(3-hydroxy-5-tert-butyliosxazol-4-yl)propanoic acid (ATPA; [17]-[20] and (±)-1-Aminocyclopentane-*trans*-1,3-dicarboxylic acid (t-ACPD; [21]-[23]. All agonists were tested in pilot experiments in order to detect a concentration leading to strong increases of population spike amplitude in the presence of single stimuli (SS) and theta burst stimulation (TBS).

For antagonization of the stimulus-induced physiological effect at NMDA, AMPA, Kainate and metabotropic glutamate receptors the following chemicals were used respectively: CGS 19755 [24], NBQX [25], UB 301 [26], and RS-APICA [27]. All chemicals were from BIOTREND Chemikalien GmbH, Cologne, Germany.

3. Results

3.1. Effects of Low Dose Preparations Seen in the Tele-Stereo-EEG *in Vivo*

Coffea arabica D6 was tested orally in the model "Tele-Stereo-EEG" at 10 mg/kg and 40 mg/kg. For technical reasons some results of experiments are only based on 6 or 7 rats. There is a quantitative difference between the effects on different brain regions. Strongest effects were seen within the frontal cortex (**Figure 1** at the top). The presence of *Coffea arabica* led to a massive statistically significant attenuation of alpha2 waves followed by a still prominent and significant attenuation of delta and theta waves. Finally, also alpha1 and beta1 waves were attenuated but not beta2 waves. Within the hippocampus the effects were similar with regard to the pattern of changes but clearly less pronounced. The same pattern of frequency changes was also seen within the striatum and reticular formation, but here the effects became hardly significant, even at the higher dosage of 40 mg/kg. Data reveal a clearly dose dependent effectiveness of *Coffea arabica*.

Gelsemium sempervirens D4 was tested orally in this model at 10 mg/kg and 20 mg/kg. At the lower dosage strongest effects were seen within the frontal cortex consisting in attenuation of delta (statistically significantly different from control) and alpha2 waves (**Figure 1** at the middle part). Attenuation of theta waves also became statistically significant. Within the hippocampus and striatum a similar picture evolved with significant attenuation of delta, theta, alpha2 and beta1 spectral frequencies. There was hardly an effect visible within the reticular formation. Doubling of the dosage led to smaller effects in all brain regions.

Veratrum album D6 was tested orally at 10 mg/kg and 30 mg/kg. At the lower dosage hardly an effect was documented. But increasing the dosage to 30 mg/kg led to significant attenuation of delta and theta spectral frequencies within the frontal cortex and hippocampus (**Figure 1** at lower part). In the hippocampus also alpha2 and beta1 spectral power was statistically significantly attenuated. Only small effects were seen in the striatum and reticular formation at this higher dosage.

In summary, all three low dose preparations produced decreases of spectral power mainly within the frontal

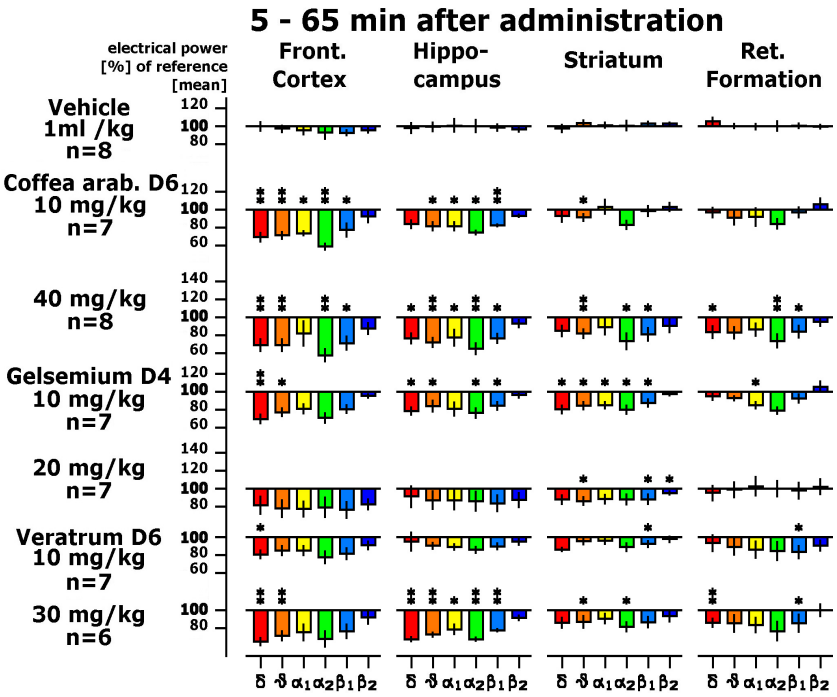

Figure 1. Effect of *Coffea arabica* D6, *Gelsemium* D4 and *Veratrum* D6 on spectral frequencies in four brain areas. Values are given in % of the 45 minutes lasting pre-drug baseline period. Colored bars represent frequencies: red: delta waves, orange: theta waves, yellow: alpha1 waves, green: alpha2 waves, turquoise: beta1 waves, blue: beta2 waves. Statistical significances according to Wilcoxon, Mann and Whitney are indicated by stars: $^{*}p < 005$, $^{**}p < 0.01$.

cortex and hippocampus, clearly less or no change within the striatum and reticular formation. Main changes were observed in delta, theta and alpha2 spectral power. With respect to motion no significant differences were observed in comparison to vehicle administration (**Table 1**).

3.2. Effects of Low Dosepreparations on the Hippocampus Slice Preparation *in Vitro*

In order to test the preparations with direct contact to brain matter, a strong dilution seemed to be necessary, since one can only expect a small amount of molecules to enter the brain. Preparations were tested up to an amount of 50 mg/L for *Gelsemium*, up to 100 mg/L for *Veratrum* and up to 150 mg/L for *Coffea* D6. *Coffea* D6 was able to increase the amplitude of the population spike recorded from pyramidal cells after single stimuli to the Schaffer Collaterals as well as after theta burst stimuli in a concentration-dependent manner up to a concentration of 100 mg/L. However, the highest concentration of 150 mg/L used in this assay was less effective producing a more bell-shaped curve (**Figure 2(A)**). This effect was antagonized by the presence of a very specific glutamate receptor antagonist, namely NBQX, at a concentration of 0.05 µM during single stimuli (**Figure 2(B)**) and during theta burst stimulation (**Figure 2(C)**). No antagonization was shown with three other receptor specific chemicals (**Figure 2(B)**, **Figure 2(C)**). Thus, a very specific mechanism of action was uncovered with respect to glutamatergic hippocampal neuronal transmission.

In the presence of *Gelsemium* D4 also a bell-shaped response was detected. At 12.5 mg/L and 25 mg/L an increase of the amplitude of the population spike was obvious during single shock stimulation, but the effect vanished at 50 mg/L (**Figure 3(A)**). However, in the presence of 25 and 50 mg/L the response to theta burst stimulation led to a linear strong statistically significant attenuation of the amplitude. Under the same experimental condition four glutamate receptor agonists (trans-ACPD, RS-ATPA, fluorowillardine and ACBD) led to an increase of the amplitude of the population spike (**Figure 3(B)**, **Figure 3(C)**). Among these were two, in the presence of which *Gelsemium* D4 was able to antagonize their action in a statistically significant manner: trans-ACPD (at 0.025 µM) and trans-ACBD (at 0.05 µM). This feature was observed during administration of single stimuli as well as after theta burst stimulation.

In the presence of 75 or 100 mg/L of *Veratrum* D6 the amplitudes of the population spikes were attenuated statistically significantly after single as well as after theta burst stimulation (**Figure 4(A)**). The same glutamate receptor agonists as used in the presence of *Gelsemium* were used to possibly detect an antagonistic action of *Veratrum* D6. Only the effects of fluorowillardine and trans-ACBD were influenced by *Veratrum* D6 in a statistically significant manner during administration of single stimuli (**Figure 4(B)**) and during theta burst stimulation (**Figure 4(C)**).

In summary, all three low dosepreparations were able to modulate the intra-hippocampal electric communication in a different way and with different results. *Coffea* D6 facilitated signal transfer presumably by enhancing glutamate AMPA receptor transmission. *Gelsemium* D4 showed a similar effect, but only after single shock stimulation. However, attenuation of signalling within this electric pathway was recognized after theta burst

Table 1. Result of motion analysis for the first hour after administration. Motion is given in cm/hour.

Vehicle 1 ml/kg	747.26 ± 100
Coffea D6 10 mg/kg	628.94 ± 69
Coffea D6 40 mg/kg	931.47 ± 242
Gelsemium D4 10 mg/kg	540.95 ± 89
Gelsemium D4 20 mg/kg	825.15 ± 111
Veratrum D6 10 mg/kg	736.77 ± 129
Veratrum D6 30 mg/kg	870.46 ± 169

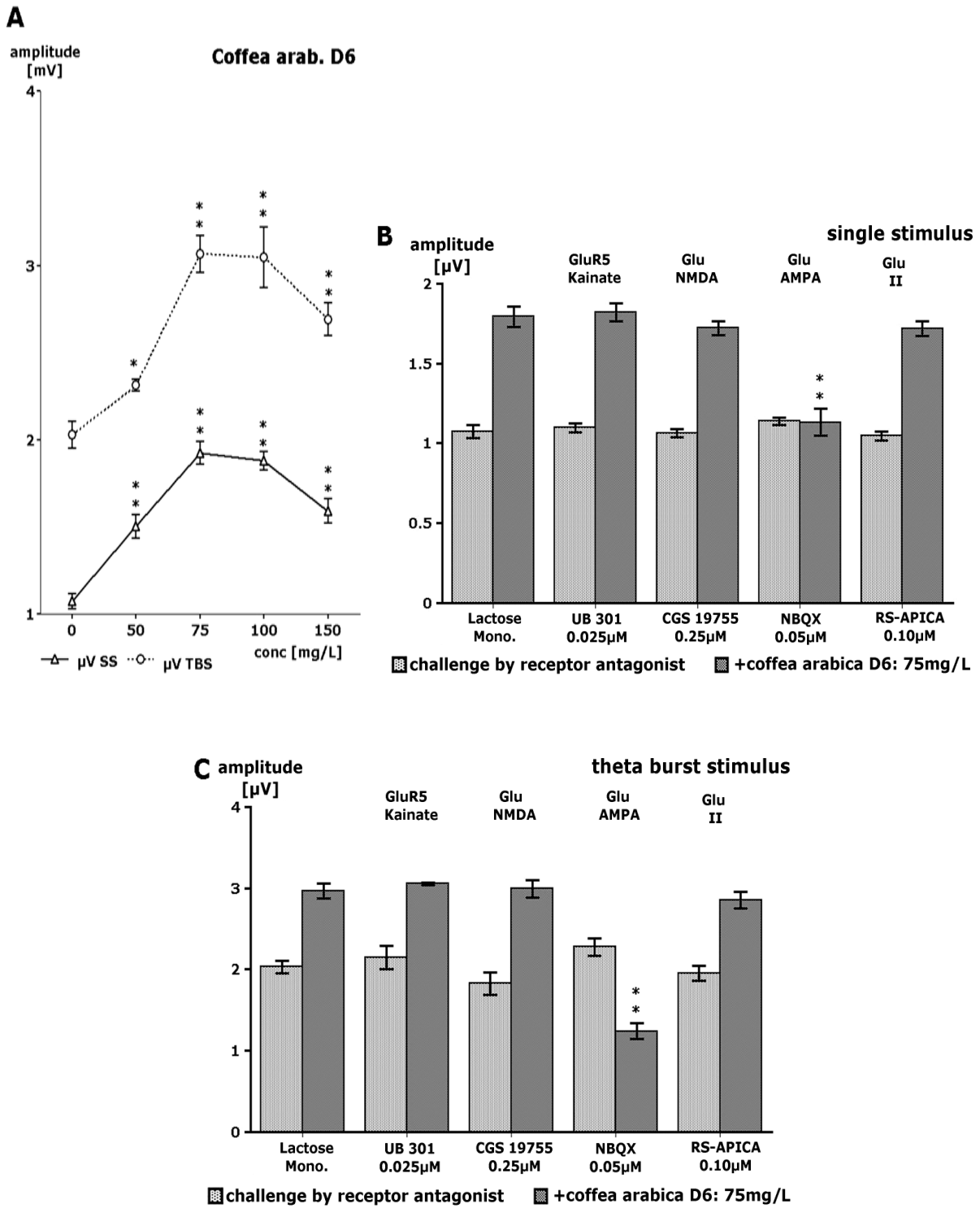

Figure 2. Effect of *Coffea arabica* D6 on population spike amplitude of pyramidal cells after stimulation of Schaffer Collaterals. (A) Lower curve obtained in the presence of single shock stimulation, upper curve after theta burst stimulation; (B) Challenge of the effect by various glutamate receptor antagonists after single shock stimulation and (C) after theta burst stimulation. Abbreviations of antagonists are given on the abscissa. Statistical significance according to Wilcoxon, Mann and Whitneyis indicated by stars: $^{*}p < 0.05$; $^{**}p < 0.01$.

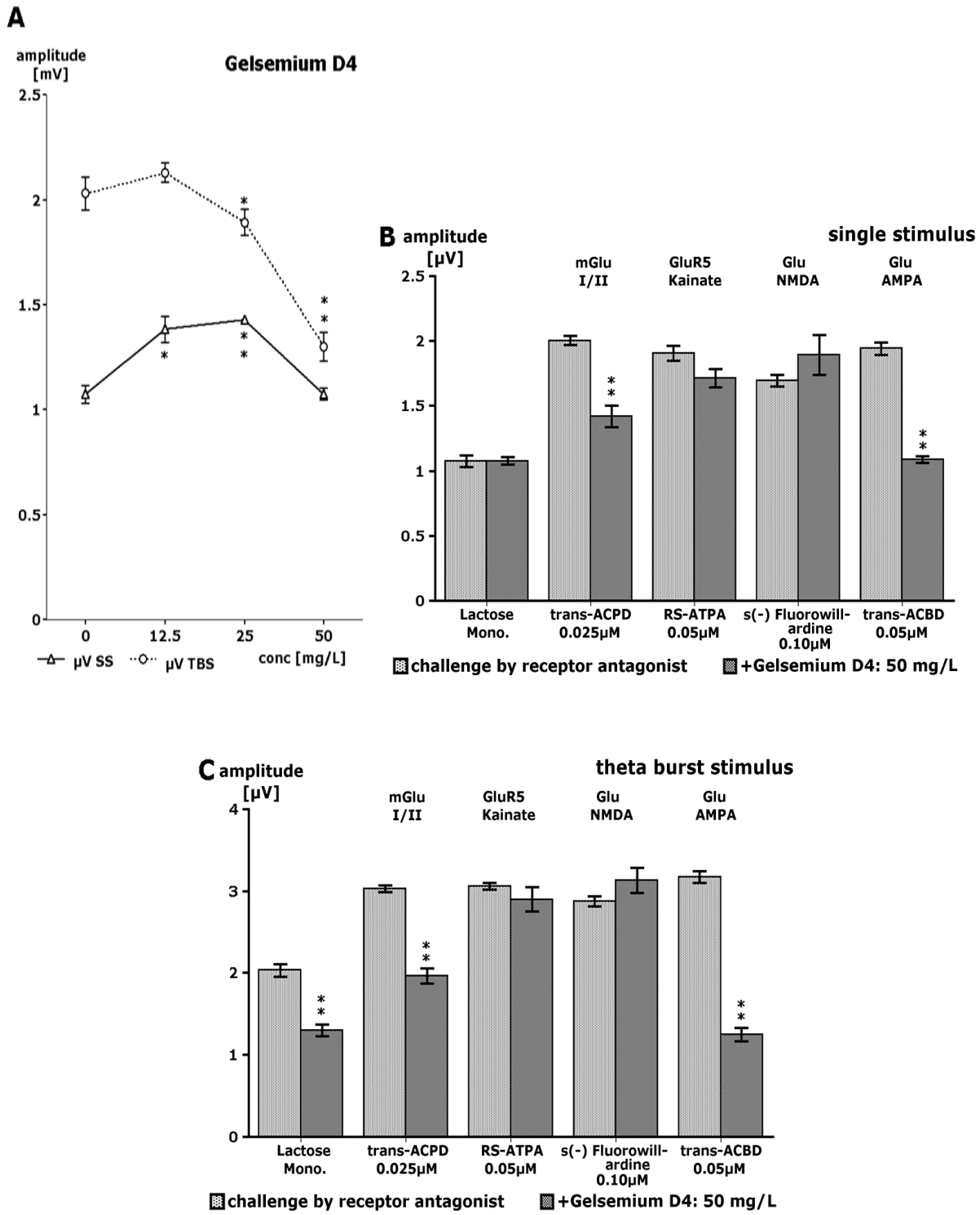

Figure 3. Effect of *Gelsemium* D4 on population spike amplitude of pyramidal cells after stimulation of Schaffer Collaterals. (A) Lower curve obtained in the presence of single shock stimulation, upper curve after theta burst stimulation; (B) Challenge of the effect by various glutamate receptor antagonists after single shock stimulation and (C) after theta burst stimulation. Abbreviations of antagonists are given on the Abscissa. Statistical significance according to Wilcoxon, Mann and Whitney is indicated by stars: $^{*}p < 0.05$; $^{**}p < 0.01$.

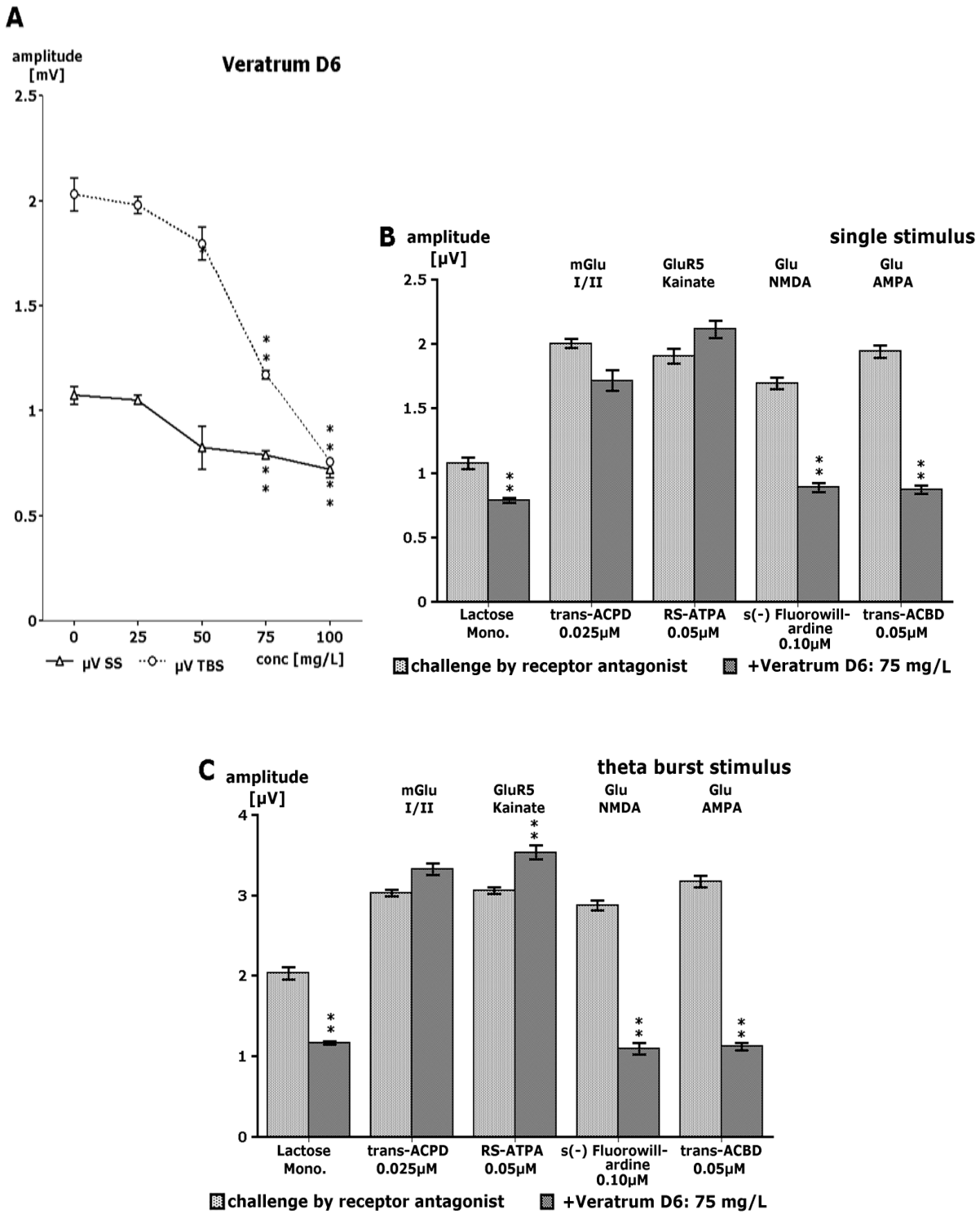

Figure 4. Effect of *Veratrum* D6 on population spike amplitude of pyramidal cells after stimulation of Schaffer Collaterals. (A) Lower curve obtained in the presence of single shock stimulation, upper curve after theta burst stimulation; (B) Challenge of the effect by various glutamate receptor antagonists after single shock stimulation and (C) after theta burst stimulation. Abbreviations of antagonists are given on the Abscissa. Statistical significance according to Wilcoxon, Mann and Whitney is indicated by stars: $^{*}p < 0.05$; $^{**}p < 0.01$.

stimulation. Responsible for this is probably glutamate AMPA receptor and glutamate metabotropic II receptor mediated transmission. *Veratrum* D6 was able to attenuate glutamatergic transmission during both stimulation procedures presumably according to modulation of the two ionic glutamate receptors sensitive to AMPA and NMDA.

4. Discussion

With regard to the electropharmacograms of *Coffea* D6, *Gelsemium* D4 and *Veratrum* D6 similar but not identical patterns of changes were observed *in vivo*. Comparison of the results with preparations tested earlier in this model revealed a close similarity to a synthetic compound acting by inhibition of monoamino-oxidase A, used for the treatment of depression in humans (**Figure 5**). But there were obvious differences to drugs used for other clinical indications like diazepam for treatment of nervousness or sleep disturbances, haloperidol for treatment of schizophrenia, fentanyl as an analgesic drug, valproic acid used for the treatment of epilepsy or amphetamine, which is a strong stimulatory compound (**Figure 5**).

In order to be able to consider the whole frequency pattern with all 24 variables (six frequency range times four brain areas), one has to use more complex mathematical tools. As used and published earlier, the three low dose preparations were fed into a linear discriminant analysis based on a larger number of reference drugs with known clinical indications in humans. The result of this kind of analysis is depicted in **Figure 6**. All three preparations group together in the vicinity of Ginkgo, a plant-derived preparation with cognition enhancing and antidepressive capability. A list of the reference drugs including their abbreviations and dosages administered is given in **Table 2**. From this one could expect a similar action in humans if administered at an appropriate dosage. Further investigation into this direction seems promising.

Information on biological effects of the three plants under investigation is rare. Recently, [28] reported anxiolytic effects of an extract from *Gelsemium sempervirens*. However, they found this effect in mice at a dosage

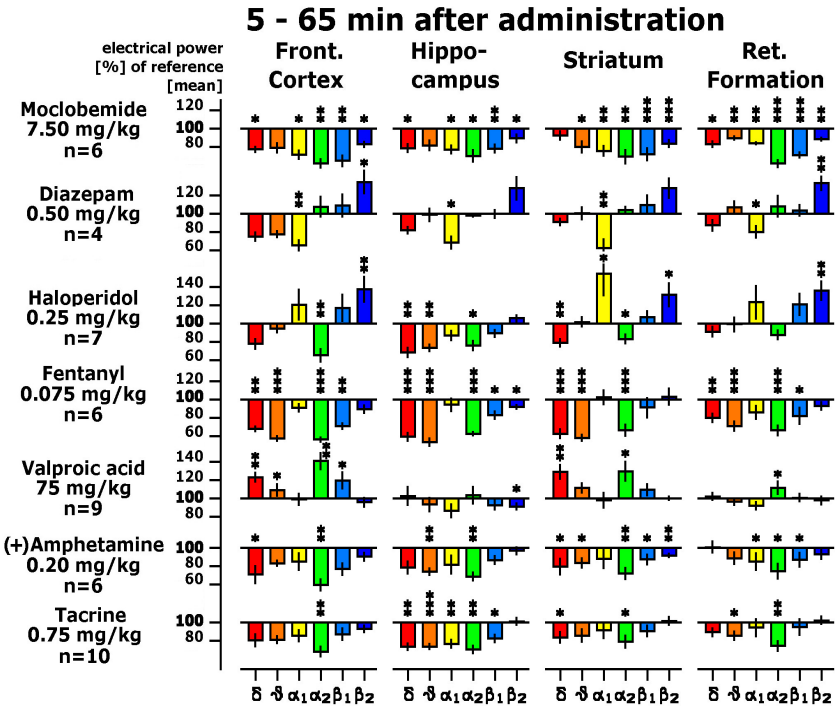

Figure 5. Effect of reference compounds from our database on spectral frequencies in four brain areas. Values are given in % of the 45 minutes lasting pre-drug baseline period. Colored bars represent frequencies: red: delta waves, orange: theta waves, yellow: alpha1 waves, green: alpha2 waves, turquoise: beta1 waves, blue: beta2 waves. Statistical significances according to Wilcoxon, Mann and Whitney are indicated by stars: $^{*}p < 005$, $^{**}p < 0.01$; $^{***}p < 0.001$. Please note different patterns of frequency changes for drugs used in different clinical indications.

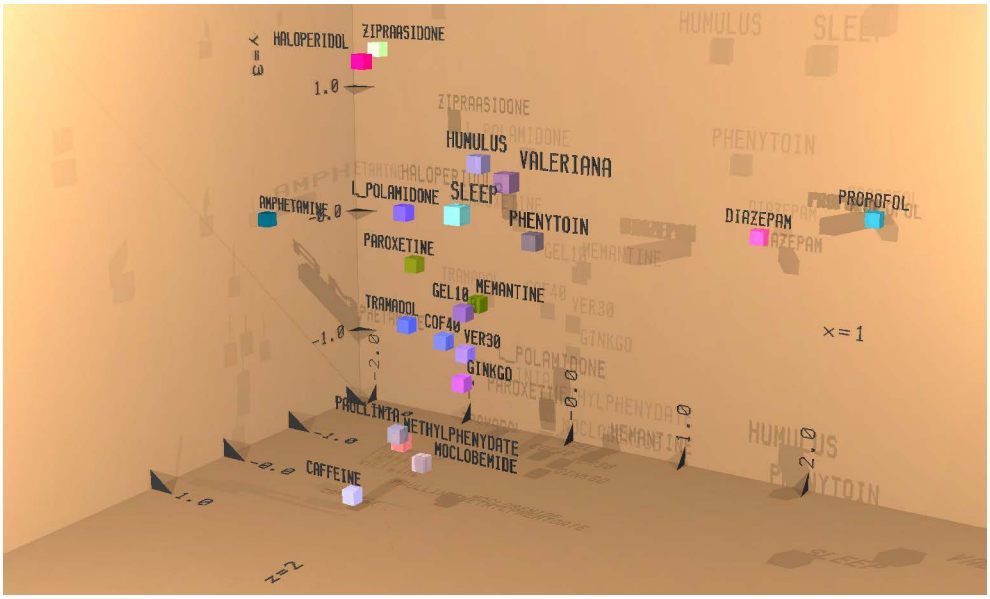

Figure 6. Documentation of the result of discriminant analysis. Extracts are labelled as COF40 for *Coffea* D6, GEL10 for *Gelsemium* D4 and VER30 for *Veratrum* D6. Number refers to the dosage classified. Other reference drugs with their dosage used are listed in **Table 2**. Results from first three discriminant functions are shown by the coordinates x, y and z, respectively. Results from fourth to sixth discriminant function are depicted by the colour (for details see methods).

Table 2. Listing of reference drugs tested earlier under identical conditions. Doses and time of recording are given in separate lines. Drugs were given i.p except for low dosepreparations.

Substance definition	Dose [mg/kg]	Time	Substance analysis	Dose [mg/kg]	Time
Diazepam	0.50	5 - 35 min	Caffeine	2.50	5 - 35 min
Memantine	3.00	5 - 35 min	Tramadol	5.00	5 - 35 min
L-Polamidon	1.00	5 - 35 min	Paullinia	15	5 - 65 min
Ziprasidone	1.00	5 - 35 min	Ginkgo extr.	100	5 - 65 min
Haloperidol	0.50	5 - 35 min	Valeriana	100	125 - 185 min
(+)Amphetamine	0.40	5 - 65 min	Humulus	50	125 - 185 min
Paroxetine	1.00	5 - 35 min			
Propofol	60.0	5 - 65 min			
Methylphenidate	2.50	5 - 35 min			
Moclobemide	5.00	5 - 35 min	GEL10 (*Gelsemium* D4)	10.0	5 - 65 min
Phenytoin	4.00	65 - 125 min	VER30 (*Veratrum* D6)	30.0	5 - 65 min
Sleep		65 - 125 min	COF40 (*Coffea* D6)	40.0	5 - 65 min

of 150 mg/kg of original extract and a fraction of the extract at 10 mg/kg, which is well above the dosage used in the present experiments. On the other side there is one report on modulation of emotional responses of mice to novel environments in the presence of high centesimal dilutions of *Gelsemium sempervirens* [29]. But there is also one report on a clinical study with centesimal dilutions of *Gelsemium sempervirens*, which did not reveal any effects on anticipatory anxiety in a double-blind, randomized, single centre, placebo-controlled study in 180 humans [30].

The results as obtained from the *in vitro* approach indicate very strong concentration dependent effects on intra-hippocampal neuronal communication. The synapse between Schaffer Collaterals and the pyramidal cells uses glutamate as neurotransmitter. But there are different ionotropic and metabotropic receptors involved for control of this pathway. Interestingly, the three plant-derived low dosepreparations exert a different action with respect to receptor-modulated physiological signals. Since the effect of *Coffea* can be attenuated by a chemical, which inhibits AMPA mediated signal transfer (NBQX), it must be assumed that this receptor is involved in the action of *Coffea* D6, whereas no effect was detected in the presence of NMDA- or Kainate-dependent signal transfer. This result speaks in favour of a highly specific intervention of *Coffea* D6 on brain matter. Since the effect was not only observed during single stimuli but also during theta burst stimulation, the resulting increase of long term potentiation as induced by this stimulation pattern leads to the conclusion that time and space dependent memory processes should be improved in the presence of *Coffea*. For discussion of LTP in the literature see [31].

A different picture arises with respect to *Gelsemium* D4. An increase of the response was shown during single stimuli but a decrease after theta burst stimulation at the higher concentrations of 25 and 50 mg/L. Using several glutamate specific receptor agonists to modulate the signal transfer between Schaffer Collaterals and pyramidal cells one can get information with respect to possibly involved ionotropic and/or metabotropic receptors in the action of drugs [1]. In the case of *Gelsemium* the agonistic action of trans-ACPD and trans-ACBD were attenuated. These two chemicals are very specific for intervention with the metabotropic glutamate receptor II and ionic AMPA receptor, respectively. Thus, physiological signal transfer mediated by these two receptors seem to be the target of *Gelsemium* D4 giving first hints on the mechanism of action of this preparation.

There is some similarity of the action of *Veratrum* D6 to that documented for *Gelsemium* D4 with respect to theta burst stimulation in that an attenuation of the amplitude of the population spike is observed. But this time a concentration-dependent attenuation of the amplitude of the population spike was also seen during administration of single shock stimulation. According to the use of several glutamatergic receptor agonists a different pattern in comparison to *Gelsemium* emerges, since two ionic glutamate receptors seem to be involved at the same time: the AMPA and the NMDA receptor. Thus, an ionic glutamate receptor mediated signal transfer obviously can be regarded as mechanism of action for *Veratrum* D6.

5. Conclusion

In summary, all three plant-derived low dose preparations exerted dose dependent physiological effects *in vivo* with respect to quantitative assessment of field potentials and *in vitro* with respect to specific glutamate receptor mediated signalling in the hippocampus slice of the rat. For interpretation of modulation of glutamate dependent therapeutic effect see [32] [33]. The results prove that there is an effect of low dose preparations on neurophysiological parameters, which probably stems from highly specific actions of some molecules still present in low trituration of these plant extracts. The results not only proof a clear dose dependent pharmacological action of these preparations but also provide hints on their mechanism of action. The two neurophysiological models used in this approach seem to be suited to gather more information also with other low dose preparations. For more information and results obtained using this methodology, please refer to [34].

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Conflict of Interest

There was no conflict of interest.

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