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Opposite Neurophysiological Findings Induced by *Sideritis scardica* and *Sideritis euboa* Extract in the Rat

Wilfried Dimpfel[1], Björn Feistel[2], Leonie Schombert[3]

[1]Justus-Liebig-University Giessen c/o NeuroCode AG, Wetzlar, Germany
[2]Finzelberg GmbH & Co. KG, Andernach, Germany
[3]NeuroCode AG, Wetzlar, Germany
Email: dimpfel1945@web.de

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Abstract

Psychophysiological effects of Sideritis herba extracts depend on biologically active ingredients, which might be different for several botanical types of this plant. The present investigation aimed at the characterization of extracts from *Sideritis scardica* and *Sideritis euboa in vivo* and *in vitro*. Construction of electropharmacograms on the base of recording of electrical field potentials from four different brain regions was used to compare the possible pharmacological effects to a database of reference drugs with known clinical indications. Whereas *Sideritis scardica* produced decreases of spectral power in line with stimulatory frequency patterns as observed in the presence of *Ginkgo biloba* extract, administration of *Sideritis euboa* produced opposite effects. Electrical stimulation of the Schaffer Collaterals was used to elicit a pyramidal cell response called population spike *in vitro*. The amplitude of this spike was determined in the presence of single as well as theta burst stimuli. Direct exposure of brain matter to *Sideritis scardica* extract led to concentration dependent increases of the population spike amplitude under both stimulation patterns in the range from 12.5 to 100 mg/L. On the opposite, extract from *Sideritis euboa* did not change the electric response up to 50 mg/L. Higher concentrations of this extract attenuated the signal amplitude. A 1:1 blend of both gave intermediate results. The in vitro results are in line with the in vivo EEG recordings, where both extracts induced opposite changes of the electric power with respect to electric frequency patterns. The results from both models suggest a stimulatory and/or memory-enhancing action for the extract from *Sideritis scardica* but not for *Sideritis euboa* extract, where a more tranquillizing effect like that observed in the presence of Humulus cone extract may be expected.

Keywords

Neurophysiology, Rat, Hippocampus Slice, *Sideritis scardica*, *Sideritis euboa*, Greak Mountain Tea, Electropharmacogram

1. Introduction

Preclinical pharmacological characterization of plant derived extracts must be seen as a special challenge since many classic animal models are simply not suited or at the best not sensitive enough like many behavioural models. Plant extracts are multi component mixtures, which have also multifunctional activities based of more than one mode of action. Models based on specific receptor pharmacology have the disadvantage that plant extracts might contain even more than one active ingredient, which complicates interpretation of results. In addition, most models are biased. For screening of plant-derived preparations one needs a non-biased model, which describes drug-induced changes on an intermediate level between the molecular and the behavioural level. Recording the electric activity in several brain regions provides a very good solution to solve this problem since field potentials reflect the activity of neurotransmitter action [1]-[3]. Electric activity reflects the net effect of all neurotransmission in form of a frequency pattern. The information content of these field potentials is therefore very high but unbiased. Since electric changes not only code for motor activity but also for cognitive and emotional features in humans, changes of the frequency content of these signals allow extrapolation of the results from rat to humans. Based on more than 20.000 hours of recording under identical experimental conditions it was recognized that synthetic drugs with similar clinical indications produced similar changes of the frequency pattern [4]. Such a pattern of drug induced frequency changes in several brain areas has been called an electropharmacogram. Feeding these data into a linear discriminant analysis resulted in a matrix of drug actions, which grouped automatically according to clinical indications. Therefore, this matrix can be used to characterize unknown drug actions and even multi-ingredient preparations. The methodology used was identical to that reported earlier [5] [6].

Herba Sideritis ssp. (so called Greak mountain tea) is known concerning their traditional uses for the relief of mild gastrointestinal discomfort and against common colds (HMPC monograph, draft 2015 [7]). Since 2010 new applications for Sideritis species resp. their extracts in the field of CNS activities are described continuously [8]-[11]. In the patent literature (EP2515922B1 [8]) the use for treatment of Mild Cognitive Impairment (MCI) in humans is reported for a Sideritis extract blend based on a *Sideritis scardica* extract and a *Sideritis euboa* extract (ratio 1:1). All references are describing in total the advantage of hydro-ethanolic Sideritis extracts vs. aqueous extracts.

The present experimental series was undertaken in order to compare extracts from two different species of Herba Sideritis by an *in vivo* and an *in vitro* method: firstly, by field potential analysis in freely moving rats to obtain a so-called electropharmacogram

and secondly, by recording the population spike from hippocampal pyramidal cells in vitro after electric stimulation of the Schaffer Collaterals. Both methods have been used extensively in the past during drug discovery.

2. Material and Methods

2.1. Construction of the Electropharmacograms from the Tele-Stereo-EEG *in Vivo*

Eight adult male Fisher 344 rats (8 months of age and day-night converted, weight about 400 g, obtained from Charles River Laboratories, Sulzfeld, Germany) were implanted with 4 bipolar concentric steel electrodes within a stereotactic surgical procedure. 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.

For our investigations we used herbal extracts of Herba *Sideritis scardica* or *euboa* produced by Finzelberg GmbH & Co KG, Andernach/Germany under GMP conditions. Raw material was sourced from cultivations in Bulgaria for *Sideritis scardica* and from Greece for *Sideritis euboa*. Extraction was done by exhaustive percolation with Ethanol 20% V/V at 40˚C (during 12 hours). The Drug-to-Extract-Ratio (DER) native was 6:1 for each. The extracts was gently dried on a vacuum belt with 30% drying excipient (Maltodextrin) and finally milled to a homogene powder (95% under 50% mesh).

Reference extract from Humuli cones was supported by Martin Bauer Group GmbH & Co. KG, Vestenbergsgreuth, Germany (extraction solvent Methanol 40% V/V). Tebonin® tablets (containing standardized Ginkgo biloba extract acc. Ph. Eur.) and Memantine as single compound was sourced from local pharmacies.

2.2. Hippocampal Slice Preparation *in Vitro*

Hippocampus slices were obtained from 25 adult male Sprague-Dawley rats (Charles River Wiga, Sulzbach, Germany). The methodology used was identical to that reported earlier [12]. 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 [13] [14]. All slices were pre-incubated for at least 1 h in Carbogen saturated ACSF (pH 7.4) in a pre-chamber before use [15]. Immersion chamber (List Electronics, Darmstadt, Germany) according to Haas [16] at 35˚C [17]. Recording of extracellular field potentials from the pyramidal cell layer of CA1 [15] was performed according to conventional electrophysiological methods [18].

2.3. Statistics

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 Fischer for positioning of the preparation-induced electric pattern within a matrix of

reference drugs with known clinical indication.

3. Results

3.1. Electropharmacogram of *Sideritis euboa* and *Sideritis scardica* Extract

Effects of vehicle (0.9% NaCl)

The electropharmacogram was recorded continuously for 5 h after administration of the test item, in this case saline. Changes of spectral power within the four brain regions following administration could not be detected. The pattern remained stable for 5 hours as documented in Figure 1.

Effects of *Sideritis euboa* Extract UB2010-57

Spectral frequency changes in the presence of 100 mg/kg of *Sideritis euboa* Extract were dominantly seen in the frontal cortex (a lower dosage of 50 mg/kg did not reveal changes of spectral power). In difference to the results of *Sideritis scardica* increases of power within all frequencies are seen which diminish with time (Figure 2).

Effects of *Sideritis scardica* Extract UB2010-55

Spectral frequency decreases in the presence of 50 mg/kg of *Sideritis scardica* UB2010-55 were dominantly seen during the first hour after administration (Figure 3). All brain areas are involved. Within the hippocampus decreases of spectral beta power are seen throughout the whole recording period.

When comparing the pattern of frequency changes of both Sideritis extracts to changes

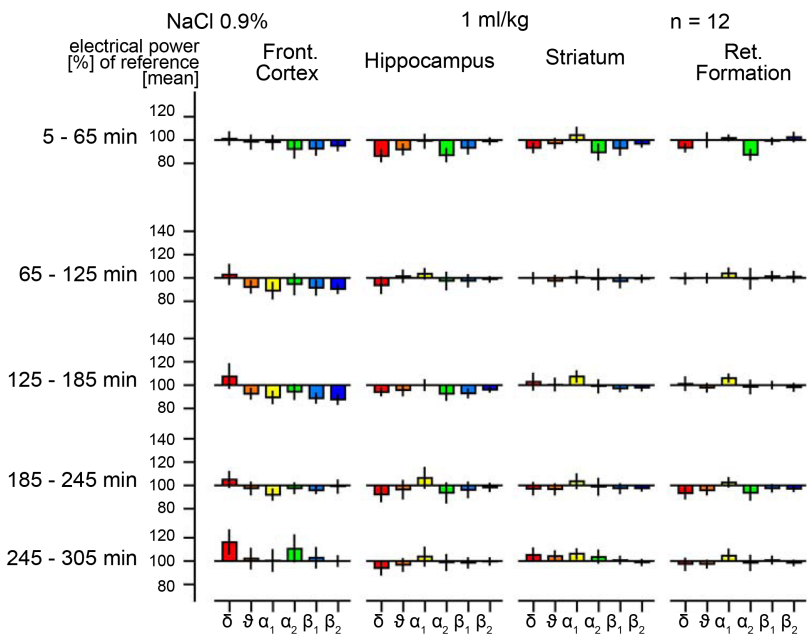

Figure 1. Time dependence of changes of spectral power (ordinate) in % of the 45 min lasting pre-drug baseline recording in four brain regions of the freely moving rat in the presence of control (1 ml/kg saline—0.9% NaCl). Frequency ranges are depicted as coloured bar charts. On the abscissa delta, theta, alpha 1, alpha 2, beta 1 and beta 2 spectral power is depicted from left to right within the four brain areas as mentioned on top of the figure.

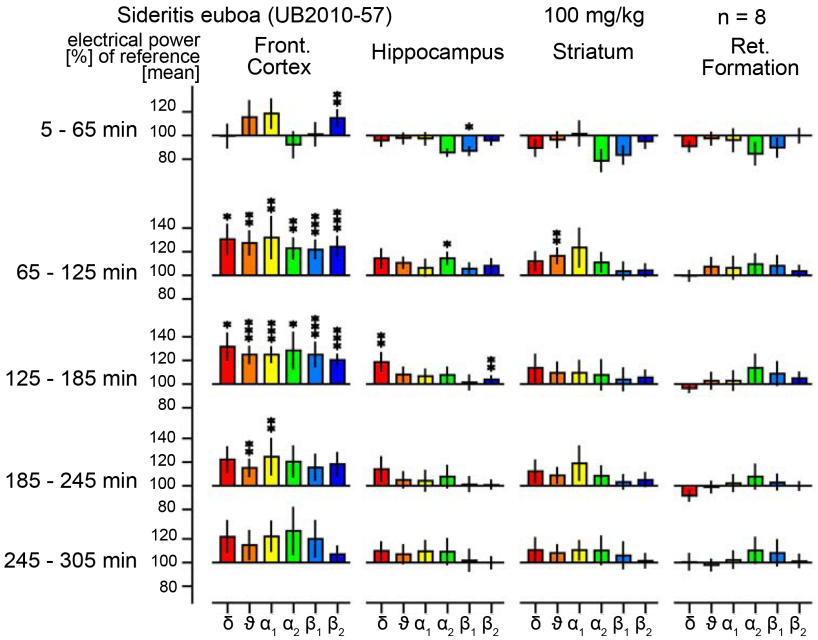

Figure 2. Effects of *Sideritis euboa* (UB2010-57) 100 mg/kg. For other details see legend to **Figure 1**. Statistical significance in comparison to control (saline – 0.9% NaCl) is documented by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

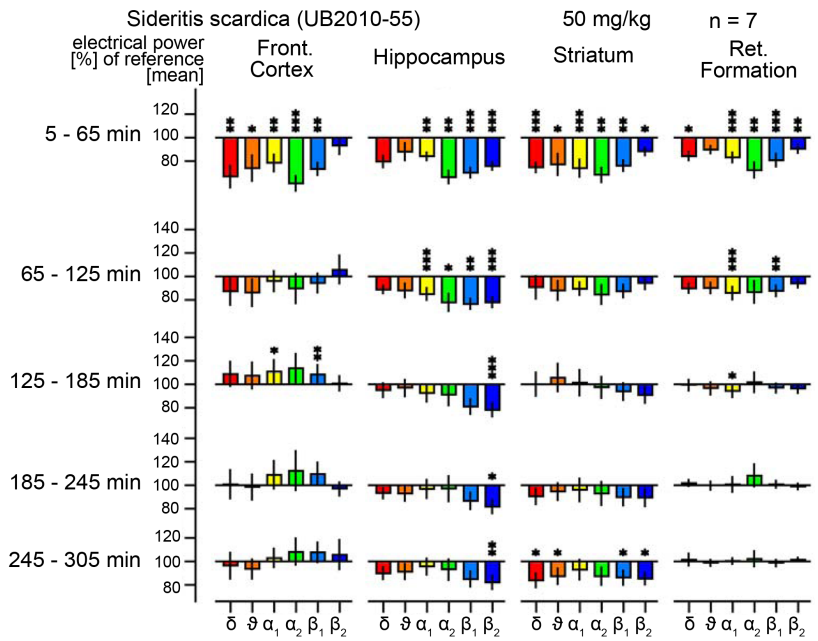

Figure 3. Effects of *Sideritis scardica* (UB2010-55) 50 mg/kg. For other details see legend to **Figure 1**. Statistical significance in comparison to control (saline—0.9% NaCl) is documented by stars: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$.

induced by other preparations a close similarity of *Sideritis scardica* extract to those of Gingko biloba extract (Tebonin®) and Memantine emerges, whereas the change induced by *Sideritis euboa* extract approached that observed in the presence of Humulus

cone extract (Figure 4).

No significant difference was detected with respect to motion. The extracts of *Sideritis scardica* and euboa showed a tendency of increased motion within one hour after administration, followed by a decrease on output level. However, due to high variation of values no safe conclusion can be drawn. Data are documented in Table 1.

3.2. Discriminant Analysis of Electropharmacograms

In order to compare the complicated pattern of frequency changes of the preparations with each other, the procedure of linear discriminant analysis (LDA) was used. Projection of the result of this calculation into space and colour (six-dimensional) led to an obvious separation of the action of some reference compounds with known

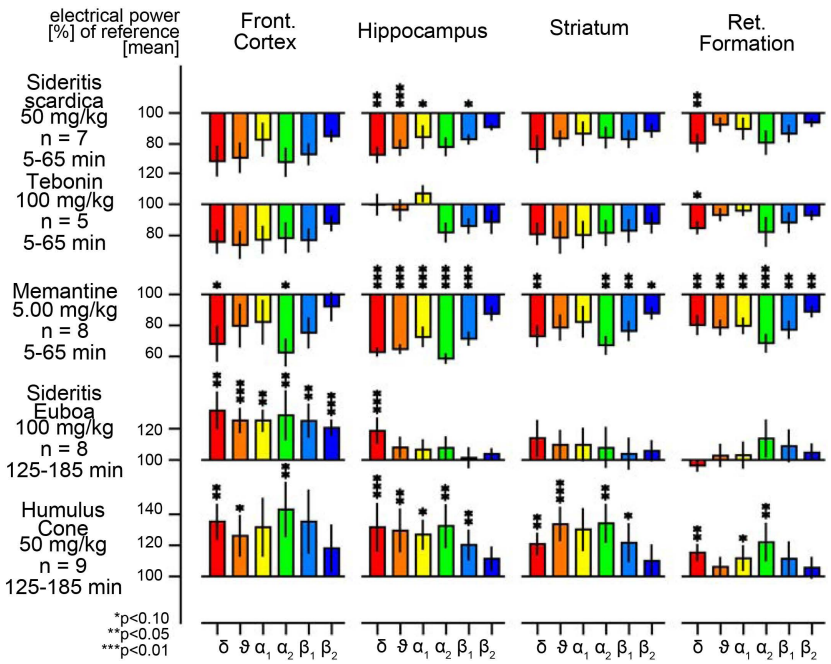

Figure 4. Comparison of the electropharmacograms of Sideritis extracts with those obtained during identical experimental setups of reference extracts or compounds in our database.

Table 1. Changes of motion in the presence of *Sideritis scardica* or *Sideritis euboa* extract. Data are given as means ± SEM. Statistically significant differences were not observed (data for vehicle were averaged from 8 studies because of large variation of values).

	Motion [cm/h]								
Time [min]	Vehicle 1 ml/kg (n = 98)			*Sideritis scardica* 50 mg/kg (n = 7)			*Sideritis euboa* 100 mg/kg (n = 8)		
−45 - 0	905.29	±	56	646.7	±	131	908.08	±	186
5 - 65	866.38	±	43	1041.1	±	180	1065.3	±	220
65 - 125	808.64	±	42	647.40	±	143	808.56	±	193
125 - 185	775.19	±	52	531.37	±	104	903.63	±	213
185 - 245	823.93	±	55	576.36	±	78	856.26	±	245
245 - 305	706.51	±	36	553.01	±	94	827.28	±	276

clinical indication. Using the three spatial coordinates for the first three discriminant axes and green, red and blue colour for the depiction of the result of the second three discriminant functions, indication specific activity changes can be visualized in a more interpretable manner by comparison to the reference compounds. Figure 5 documents the result for the extracts from 2 different Sideritis species. Firstly, three-dimensional space gives evidence of similarity or dissimilarity of drug action. Additionally, equal or similar colours suggest equal or similar mechanism of action. Reference drugs were mostly injected i.p. and were analysed for their action during the first half hour after administration. The orally administered extract from *Sideritis scardica* is analysed for the time period first sixty minutes after administration because of slower uptake by oral administration. Result from *Sideritis scardica* extract groups in the neighbourhood of Ginkgo extract (Ph. Eur. quality) and Memantine. The projection of the *Sideritis euboa* data reveals a different projection into space and colour and thus indicates a different mode of action. But remark that *Sideritis euboa* develops its main action not before the second hour after administration. Its position is found at a completely different position in the discriminant projection not far away from Humulus cone extract.

3.3. Effects of *Sideritis euboa* and *Sideritis scardica* Extract in the Hippocampus Slice Preparation *in-Vitro*

Electric activation of the pyramidal cells can be achieved by single stimuli or a by using a burst pattern of impulses for only one second. The result of this activation is recorded as a so-called population spike. Its amplitude reflects the number of pyramidal cells

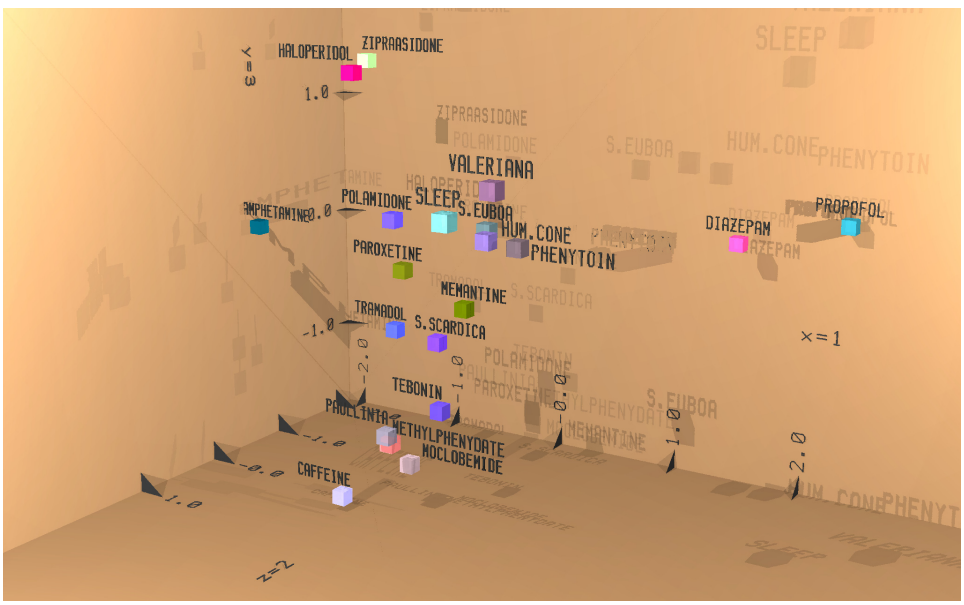

Figure 5. Discriminant analysis of Sideritis extracts in comparison to synthetic reference compounds and plant-derived reference extracts. Results from the first three discriminant functions are projected into space (x, y and z coordinates). Results from the next three functions into colours according to RGB mode. *S. scardica* = *Sideritis scardica* extract; *S. euboa* = *Sideritis euboa* extract; Hum. cone = Humulus cone.

recruited. Responses following single stimuli are stable for hours. Examples for responses to single stimuli or theta burst stimulation are documented in Figure 6 for a single

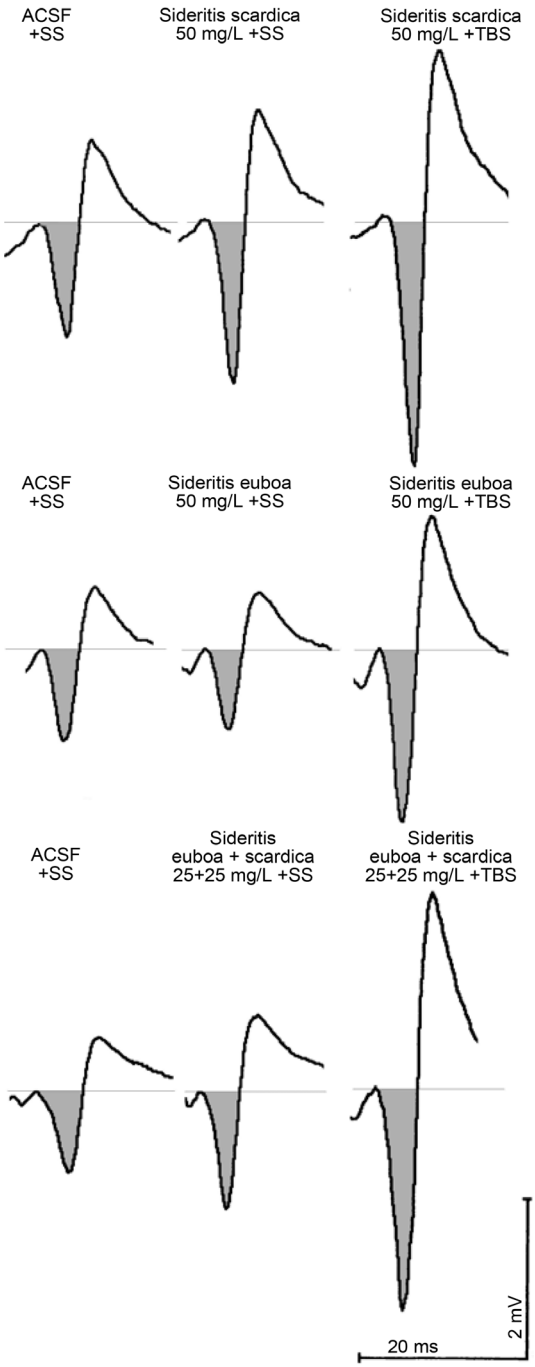

Figure 6. Documentation of original signals showing the effects of using single stimuli (SS, left and middle drawing) or theta burst stimulation (TBS, right drawing) in the presence of artificial cerebro-spinal fluid (ASCF) as control condition and two different Sideritis extracts (upper two panels as well as a 1:1 blend of both (lower panel). The amplitude is calculated from baseline to the lowest point of down reflection of the signal (shadowed). Scales: Time is given in milliseconds (ms), amplitude in millivolts (mV).

slice. Under basic conditions the amplitude of the population spike following a constant current impulse amounts to about 1 mV. As can be seen from Figure 6 there is a profound difference with respect to the activation by the two extracts which could be confirmed in all other slices.

The presence of the two basic Sideritis extracts led to a different concentration dependent effect on the amplitude of the population spike during single shock stimulation in the range of 0 to 100 mg/L. Whereas *Sideritis scardica* induced an increase of the spike amplitude, extract from *Sideritis euboa* produced either no response or at higher concentrations an attenuation of the spike. Maximum increase by *Sideritis scardica* was achieved by 50 mg/L. Decreases induced by *Sideritis euboa* started with 50 mg/L and continuously seemed to induce further decrease of population spike amplitude after 100 mg/L. Effects of the blend point into the direction of an increase of the potential but clearly less at a concentration of up to 50 mg/L in comparison to the effect of *Sideritis scardica* alone. Data for average values (n = 4) of the two extracts and a blend there of are documented in Figure 7 for the recording condition "single shock stimulation, SS".

The presence of the two different Sideritis extracts led to a different concentration dependent effect on the population spike amplitudes also during theta burst stimulation in the range of 0 to 100 mg/L. As already seen after single shock stimulation *Sideritis scardica* produced a clear concentration dependent increase of the amplitude of the population spike, whereas *Sideritis euboa* led to effects in the opposite direction at least at higher concentrations. Again no effect was seen in the case of *Sideritis euboa* up to

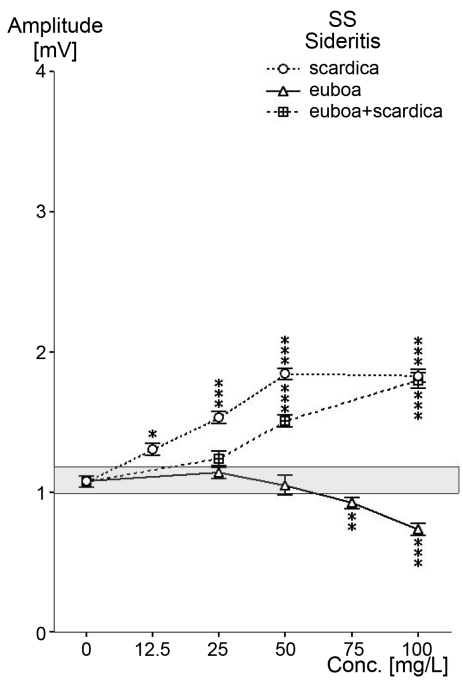

Figure 7. Concentration dependent effects of both Sideritis extracts and their blend (*Sideritis scardica*, *Sideritis euboa* and Sideritis-blend (1:1)) on pyramidal cell activity in terms of changes of population spike amplitudes (as voltage on the y-axis). Results as obtained after using single stimuli (SS). Data are given as mean ± S.E.M. of n = 4 slices.

50 mg/L. After this, a concentration dependent decrease could be documented. A similar picture as seen after single shock stimulation also emerged in the presence of the 1:1 blend. Up to 50 mg/kg effects—as seen after *Sideritis scardica*—were attenuated. Data for mean values (n = 4) of the two extracts and the 1:1 blend thereof are documented in Figure 8 for the recording condition "theta burst stimulation, TBS".

Finally, results were compared to two preparations prescribed for improvement of cognitive performance as tested earlier in this model, namely *Ginkgo biloba* extract (Ph. Eur. Quality) and Memantine (Figure 9). *Sideritis scardica* extract produced a stronger enhancement of the population spike than Ginkgo biloba extract (Ph. Eur. Quality) under same stimulation conditions.

4. Discussion

Testing of herbal preparations for physiological effects is still a major challenge because they may contain more than one active ingredient. This content of active "principles" may also vary within one plant. Therefore it is necessary to examine different species in separate. In the present investigation two species from Sideritis, namely scardica and euboa were tested in an *in vitro* and an *in vivo* model using very sensitive neurophysiological techniques.

The results show a profound difference with respect to effects of *Sideritis scardica* and *Sideritis euboa* extract on the electrically induced population spike amplitude of the pyramidal cells in the rat hippocampus slice *in vitro*. Whereas *Sideritis scardica* obviously

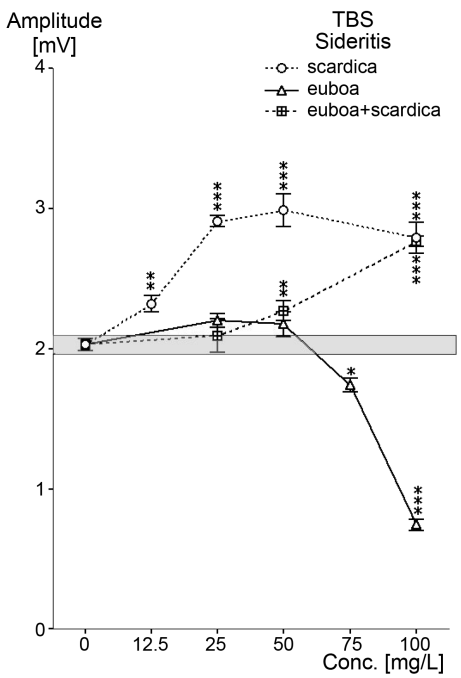

Figure 8. Concentration dependent effects of both Sideritis extracts and their blend (*Sideritis scardica*, *Sideritis euboa* and Sideritis-combination (1:1)) on pyramidal cell activity in terms of changes of population spike amplitudes (as voltage on the y-axis). Results as obtained after using theta burst stimuli (TBS). Data are given as mean ± S.E.M. of n = 4 slices.

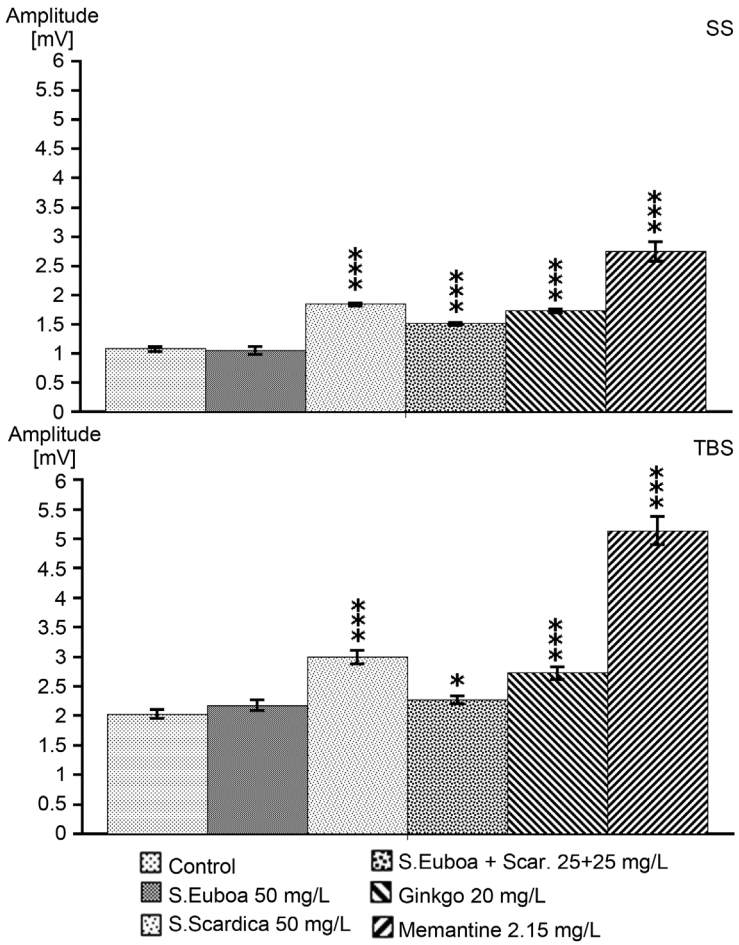

Figure 9. Comparison of results obtained with Sideritis extracts to results from our database. Data are documented for single stimulus (SS) and theta burst stimulation (TBS).

induced a concentration dependent increase of the amplitude, *Sideritis euboa* had no effects up to a concentration of 50 mg/L. Maximum effect was seen for *Sideritis scardica* already with 25 mg/L. According to this a 1:1 blend of both was not more effective than the extract of *Sideritis scardica* alone (please compare the effects of 50 mg/L of *Sideritis scardica* alone and 100 mg/L of the blend (containing 50 mg/L of *Sideritis scardica*). Thus, one can conclude, that—within this concentration range—addition of the extract of *Sideritis euboa* to the extract of *Sideritis scardica* just dilutes the effectiveness in a 1:1 manner.

But we have also to consider the possibility that higher dosages of *Sideritis euboa* would be able to antagonize certain effects of *Sideritis scardica* since higher concentrations—as applied in the hippocampus slice—showed a trend to decrease the population spike amplitude. As we do not know the concentrations reached within the brain in vivo after administration of Sideritis extracts due to possible interferences during absorption from the gut, it is preferable to use only the *Sideritis scardica* extract alone for further studies.

Additional evidence for this proposal comes from *in vivo* characterization of these

two extracts in the freely moving rat. In this model both Sideritis extracts also induce opposite changes. Whereas extract from *Sideritis scardica* induced a decrease in electric power of nearly all frequencies in comparison to baseline recording, extract from *Sideritis euboa* produced increases of power. The decrease is in line with a stimulatory action of the preparation as seen with stimulatory drugs according to our reference database. Interestingly, *Sideritis euboa* induced a trend of increase of power during the first hours after dosing but developed a general statistically significant increase of power during the next hours. This must be interpreted as a calming action of *S. euboa*, which is also confirmed by discriminant analysis, where its effect on spectral signatures was projected into the neighborhood of Humulus cone. This is also in line with the effects observed after high concentrations of the extract from *Sideritis euboa* in the hippocampus leading to attenuation of the pyramidal cell response, which is characteristic for sedative effects. Finally, the effect of *Sideritis scardica* leading to an increase of population spike amplitude recorded from pyramidal cells must be interpreted not only as a stimulatory action but also suggests improvement of certain aspects of memory. Increases of the population spike amplitude have been described in the literature for Memantine [19] or seen in the presence of Ginkgo biloba extract [20] and are interpreted in the sense of better time and spatial memory. In relation of the tested dosages for Ginkgo extract vs. *S. scardica* extract and the therefore needed grade of extract enrichment—DER native rd. 50:1 for Ginkgo extract vs. DER native rd. 6:1 for *S. scardica* extract—the plant *Sideritis scardica* shows a promising potential with respect to CNS activation. The extract from Herba *Sideritis scardica* can therefore be regarded as an appropriate candidate to improve mental fitness and/or memory also in humans.

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Conflict of Interest

There was no conflict of interest. B. Feistel is coworker at Finzelberg and initiated the study.

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