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Neurophysiological effects of a special extract of *Cyperus esculentus* L. (Cyperol)

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ABSTRACT

The tuberous rhizomes of *Cyperus esculentus* L. have been used as a food by hunter-gatherer and agricultural societies for millennia. Varieties and selections of the plant are cultivated in southern Europe, north Africa and west Africa. There is popular food and beverage uses, and traditional medicinal uses suggest that the “tubers” may have functional food potential.

The first standardized extract of *Cyperus esculentus*, Cyperol, has been developed and commercialized. In order to characterize the central nervous system activity of this extract a series of neurophysiological studies were undertaken in-vitro, in-vivo and in a pilot clinical study.

The results of the studies indicate that Cyperol induces changes in brain electrical activity (EEG), demonstrating that bioactive compounds from the extract are absorbed, are bioavailable, and that these compounds or their metabolites cross the blood brain barrier. Taken overall, the results of the reported studies indicate calming activity without sedation. This extract does indeed have functional food potential and could be used to maintain a calm state of mind while dealing with cognitively demanding work. Preliminary indications suggest that the extract may have anxiolytic potential which should be explored in future clinical studies.

Key words: *Cyperus esculentus*, Cyperol, stress, calm, anxiolytic, non-sedating

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INTRODUCTION

Cyperus esculentus L. is a perennial monocot sedge with edible tuberous rhizomes belonging to the Division *Magnoliophyta*, Class *Liliopsida*, Order *Cyperales* and Family *Cyperaceae*. *Cyperus esculentus* var. *esculentus* is a variety common as a weed among cultivated crops worldwide, whereas *Cyperus esculentus* var. *sativus* is cultivated for the larger size of the edible 'tubers'. Other varieties include *Cyperus esculentus* var. *hermannii* (Florida), *Cyperus esculentus* var. *leptostachyus* (USA), *Cyperus esculentus* var. *macrostachyus* (USA), *Cyperus esculentus* var. *sativus* (Asia) (Maduka & Ire 2018). Cultivars and selections of *Cyperus esculentus* var. *sativus* are being cultivated, including a selection used to produce a commercialised extract known as Cyperol. Traditional African selections can be found on local markets distinguished by the yellow, brown or black colour of the tuberous rhizomes.

C. esculentus (CE) is indigenous to the Mediterranean of north Africa and southern Europe ¹, and has since spread to all the continents of the world with the exception of Antarctica. The edible tuberous rhizomes are popularly called tubers and the use of the word tubers throughout this text refer to the CE tuberous rhizomes.

CE tubers are called tigernuts, earth almond, chufa (Spanish), almond de terre, souchet comestible (French), erdmandel (German), habb el'-aziz, habb el-zala and felfel es-Sudan (Arabic), indawo (Zulu), ofio (Yoruba), akiausa (Igbo).

The history of consumption of the tubers is very ancient, dating back at least 6000 years ². Tubers of CE are one of the early cultivated crops of Ancient Egypt, and were consumed as a sweet made simply of ground tubers mixed with honey. CE tubers have been found in tombs from the 4th millennium BC to the 5th century AD³, and they have also been depicted on murals in ancient Egyptian tombs, including the mural in Theban tomb No. 100, 18th Dynasty,⁴, stored in large amounts.

CE tubers have been used as a wild root vegetable in southern Africa⁵, and by Native Americans in the United States ^{6,7}. To this day cultivated CE tubers are widely used as food, and are commonly sold in local produce markets in North Africa, and in West Africa, and are a common item in supermarkets in Spain. Minimally processed CE tubers, including peeled, sliced and milled into a flour, are popular in many countries as a whole food and for use in mueslis, granolas, snacks and in baking. In Spain a popular traditional water extract of the tubers is sold in restaurants and supermarkets as a beverage known as *horchata de chufa*, which is typically sweetened and flavoured with cinnamon.

Notwithstanding its common use as a food, traditional medicinal uses of CE tubers have also been reported, including uses as a health tonic, an aphrodisiac, for enhancing fertility, treating colds, abdominal pain, colic, diarrhoea, and for increasing lactation ². CE tubers have been reported to have contradictory uses, either as a sedative or as a stimulant ⁸, which may depend on the selection or cultivar.

Pharmacologically active compounds that are bioavailable exert their action in the organism by interaction with molecular targets, including receptors, enzymes, channels, and transporters. With respect to central nervous system activity, modulation of neurotransmitter activity is a main target of CNS active pharmaceuticals and botanicals. Changes in neurotransmitter activity induce changes in the downstream signalling cascade, which finally ends up with changes in 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 ion channel activity. Thus, drug-induced changes in local field potentials reflect the changes in the underlying neurotransmission of local neural networks.

Frequency analysis of quantitative electroencephalograms (qEEG) in the presence of CNS-active pharmaceuticals, botanicals or foods, can be depicted as the so-called electropharmacogram, which has been widely used to

characterize drug actions on rat⁹ and human brains¹⁰. Interpretation of the changes in activity of spectral frequency bands can be made with respect to the likely major underlying neurotransmitter activity responsible for these changes, and aims at advancing an understanding of possible clinical applications of these substances in humans. For example, the relationship between EEG delta waves and underlying cholinergic neurotransmission was first recognised in 2005¹¹, while theta waves are influenced by agonists acting on the norepinephrine α_2 receptor¹² and dopaminergic activity is associated with changes in α_2 frequencies¹³.

A proprietary spray-dried standardized extract of a special selection of *C. esculentus* var *sativus* tubers, Cyperol, has been developed. In view of the contradictory traditional uses as sedative or stimulant, and anecdotal reports that Cyperol has a calming activity, the present study was undertaken in order to interrogate the CNS activity of this extract using in vitro CNS screening and frequency analysis of qEEG with a view to characterize this activity for applications in functional foods and supplements.

MATERIAL & METHODS

Materials

Cyperol is a commercialized standardized proprietary extract of CE produced by Nektium Pharma, Spain. Previous screening of separate aqueous, ethanolic, ethyl acetate, and hexane fractions from Nektium's cultivated selections of CE tubers, using in-vivo qEEG, had revealed that each fraction displays a distinct pattern on electropharmacograms, including distinct sedative-hypnotic, calming, and stimulant signatures (Study no: NeuroCode AG NCAG 38/15 TEEG of 3rd March 2016). From the interpretation of these results, a special cultivated CE tuber selection was made for the production of extract Cyperol with the expectation that this extract should exhibit the desired calming activity, without unwanted sedation.

In-vitro CNS screening. Radioligand binding assays

The activity of Cyperol at a concentration of 300 µg/ml was tested by means of radioligand binding assays using specific radioligands against a panel of 70 human neuroreceptors, ion channels binding sites and neurotransmitter transporters: adenosine A1, A2A and A3 receptor; α_1 adrenoceptors (non-selective) and α_2 adrenoceptors (non-selective); angiotensin AT1 and AT2 receptors; benzodiazepine (BZD) binding sites (central) and BZD peripheral binding sites; bombesin B1 receptors (non-selective); bradykinin B2 receptors; cannabinoid CB1 and CB2 receptors; cholecystokinin CCKA (CCK1) receptor and CCKB (CCK2) receptors; Corticotropin-hormone releasing receptor Type 1 (CRF1); dopamine D1, D2S, D3 and D4.4 receptors; endothelin ETA and ETB receptors; GABA receptors (non-selective); AMPA receptor; Kainate receptor; histamine H1, H2 and H3 receptor; Imidazoline I2 receptor; CysLT1 leukotriene (LTD4) receptor; melanocortin MC4 receptors; melatonin MT1 receptors; muscarinic cholinergic receptors M; neurokinin NK1, NK2 and NK3 receptors; neuropeptide Y receptor; nicotinic N neuronal $\alpha_4\beta_2$ receptor; orexin OX1 receptors (NOP); NMDA receptors (phencyclidine PCP binding site); PPAR γ receptor; Prostaglandin E2 receptor 2 (EP2); Estrogen receptor (ER); prostacyclin (PGI2) IP receptor; ATP P2X and P2Y receptors; Sigma receptor; progesterone receptor (PR); Androgen receptor (AR); Thyrotropin-Releasing Hormone Receptor Type 1 (TRH1); vasopressin V2 receptor; glucocorticoid receptors (GR); vasoactive intestinal peptide VIP1 receptors (VPAC1); vasopressin V1a receptors; Voltage-gated K⁺ (KV) channel; Ca²⁺ channel (L, verapamil site or L. diltiazem site); ATP-sensitive potassium (KATP) channel; small conductance calcium-activated potassium SKCa channel; Na⁺ channel (site 2); Cl-channel (GABA-gated); norepinephrine transporter; dopamine transporter; GABA transporter; Choline transporter (CHT1); 5-HT transporter; Serotonin 5HT1 receptor.

Experiments were performed in duplicate. The specific ligand binding to the receptors was

defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabeled ligand. The results are expressed as a percent of inhibition of the binding of a radioactively labeled control ligand specific for each target obtained in the presence or absence of the test material Cyperol and were calculated from the formula $[100 - ((\text{measured specific binding} / \text{control specific binding}) \times 100)]$. An inhibition or stimulation higher than 70% is considered as a significant effect of the test compounds.

Quantitative EEG in vivo

To characterize the pharmacodynamic action of Cyperol, quantitative electroencephalography (EEG) was recorded wirelessly from freely moving rats following the method of ¹⁴. A dose of 100 mg/kg was administered by gavage. Eight Fischer rats were implanted with a set containing 4 bipolar concentric steel electrodes and a transmitter that sent local field potentials wirelessly to a computer for frequency analysis. Transmitted data were processed by Fast Fourier Transformation (FFT) and spectral power documented for 6 frequency ranges (delta, theta, alpha1, alpha2, beta1 and beta2) within frontal cortex, hippocampus, striatum and midbrain reticular formation in hourly intervals. Twenty-four variables (4 electrode positions x 6 frequency ranges) were fed into a linear discriminant analysis. Results from linear discriminant analysis was documented by depicting the results from the first three functions into space (x, y and z coordinates), from the second three functions into three colors (RGB mode as used in TV). CNS active pharmaceuticals and botanicals typically show a specific EEG "fingerprint" (the electropharmacogram) and the spectral signature of the test substance can be compared with those of a pre-evaluated drug. A test substance with a similar spectral signature to a pre-tested drug or botanical can be expected to have similar CNS activities. Statistic evaluation in comparison to control is performed by a non-parametric Wilcoxon test.

Long-term Potentiation in the hippocampus slice preparation

The effect of Cyperol on Long-Term Potentiation (LTP) in the hippocampus was investigated. The characterization of the electrical activity in the hippocampus-slice preparation has been reported earlier¹⁵. The treatment doses were 10mg/L, 20mg/L, 40mg/L, 70mg/L and 100 mg/L of Cyperol. Measurements were performed at 10 min intervals to avoid potentiation mechanisms. Four stimulations – each 20s apart – were averaged for each time point. After obtaining three stable responses to single stimuli (SS) long term potentiation was induced by applying a theta burst type pattern (TBS). The mean amplitude of three signals were averaged to give the mean of absolute voltage values (Microvolt) \pm standard error of the mean for four slices representing one of the experimental conditions. Four slices were used from 1 rat per day. Four slices were averaged to give one value. For statistical purposes the non-parametric Wilcoxon, Mann and Whitney U-test was used.

Quantitative EEG. Pilot human clinical trial

Objective

The primary objective was to evaluate the psychophysiological and cognitive effects of Cyperol extract versus placebo by aid of quantitative EEG recordings in combination with psychometric testing after administration of a single dose in healthy adults. The secondary objective was to evaluate tolerability and the response to stressful situations.

Population

Sixteen healthy male and female subjects were invited to participate. All participants were German-speaking, mother tongue German or fluent German as a second language. They were between 18 and 40 years old, right-handed and with an unremarkable medical history. Consent to participate in the study after education in written and oral form (informed consent) was obtained in writing.

The study was performed in accordance with the current version of the declaration of Helsinki

(52nd WMA General Assembly, Edinburgh, Scotland, October 2000). The trial was conducted in agreement with the International Conference on Harmonization (ICH) guidelines on Good Clinical Practice (GCP). The study gained full approval from ethics committee.

Clinical trial

The effect of a single oral dose of 1000 mg of Cyperol extract (5 capsules, each of 200 mg) was compared with a placebo. A randomized, double blind, placebo controlled cross-over design was used. Efficacy evaluation took place on three different levels of evidence: filling out a questionnaire, performance of six psychometric tasks and recording of quantitative EEG during performance of the psychometric tasks. Subjects were sitting alone in a quiet separate room with dimmed light in a comfortable chair. Psychometric tests were performed before and after intake of the trial preparations. A total of 6 psychometric tests were performed in the presence of quantitative EEG recording: concentration d2-test, memory test (ME), calculation performance

test (CPT), reaction time test (RT), number sequence test (NST) and number connection test (NCT).

The quantitative EEG was performed as previously described¹⁶. The EEG was recorded from 17 different brain regions within the 6 defined frequency ranges (delta, theta, alpha1, alpha2, beta1 and beta2). A baseline recording (first recording) was made for 6 minutes under the condition of eyes open (EO) in a relaxed state, followed by the psychometric tests as show in Figure 1. EEG data were recorded twice: before (baseline) and 90 min after the intake of the trial preparation. Between the measurement subjects spent their time in the facility's recreation room. All experiments took place at the same time of the day (starting at 7 o'clock in the morning). Data were analyzed from 1.86 to 35 Hz using the CATEEM® software. Data recorded during the relaxed state were fed into a linear discriminant analysis to be compared to reference preparations.

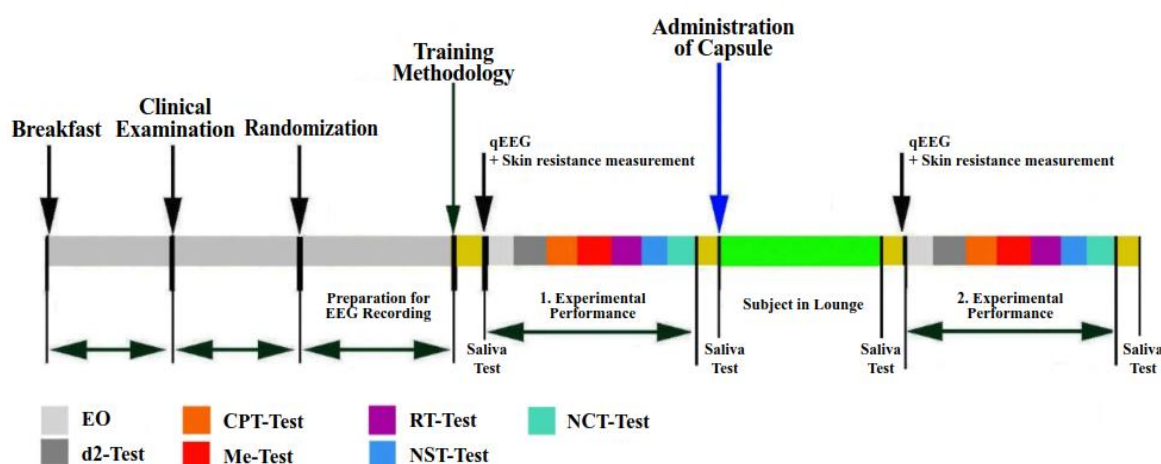


Fig. 1: Time line of the experimental day. Recording conditions: Eyes open (EO) and during different cognitive tests: attention (d2-test), memory test (Me-Test), calculation performance test CPT-Test, reaction time test (RT-Test), number sequence test (NST-Test), number connection test (NCT-Test).

In order to be able to compare the activity of Cyperol extract with placebo, two regions of special interest (ROI) were defined: the frontal cortex (average from the electrode positions F_z, F₇ and F₈) and the association cortex (average from the

electrode positions P₃, P₄, T₅ and T₆). These two brain areas have previously been shown to be responsible for higher mental activity and to have changed their EEG frequency content during cognitive testing in previous experiments. By

setting the absolute spectral power during baseline recording to 100%, preparation induced changes can be documented as % change from this baseline. Thus, in the presence of placebo no major changes should emerge.

The recorded data were fed into a linear discriminant analysis to be compared to reference preparations. This mathematical tool is used to differentiate the CNS activity of various preparations from each other. Thus, if the plotted projections of preparations cluster together in a 3-dimensional space, it is interpreted that they have rather similar effects on the spectral power, in general. In addition, if preparations are projected with similar colour (an additional 3 dimensions), this can indicate that they may have the same or a similar pharmacological mechanism of action¹⁷.

The Profile of Mood States (POMS) questionnaire was filled out before intake of the trial preparation and 90 minutes after intake. It is used to assess transient, distinct mood states. The questionnaire contains 65 words/statements that describe feelings people have. Scores are given for Dejection (S1); Sullenness (S2); Fatigue (S3) and Thirst for action (S4).

Statistics

EEG data from the first recording session before intake of the capsules are calculated as absolute numbers (μV^2). For explorative statistical evaluation the non-parametric Sign test was used. For mathematical differentiation of the different mental loads the linear discriminant analysis according to Fischer was used. Results from the first three discriminant functions were projected into space (X, Y and Z coordinates), whereas results from the fourth to sixth discriminant functions were coded into red, green and blue color, respectively, followed by an additive color mixture (so-called RGB-mode). In order to document statistically the different electric reactions of the brain to various cognitive loads, data from each challenge were documented as absolute spectral power (μV^2). Comparison of Cyperol versus placebo was accomplished by evaluation of the second recording of the day 90 minutes after

intake in comparison to the baseline values. Data from the first recording (baseline) were set to 100% and electrophysiological changes produced by placebo or the extract were depicted as %-changes thereof.

Since this study was an exploratory study with a small number of participants and EEG data are not normally distributed, the non-parametric sign test was chosen for comparison between placebo and Cyperol extract. Exploratory statistics gave p values, which are presented at the appropriate site. A time line of the experimental day is depicted in Figure 1.

Results

In-vitro CNS screening. Radioligand binding assays

Cyperol was evaluated through vitro CNS screening by radioligand binding assays to evaluate whether there was any selective pharmaceutical-like CNS activity. The threshold for a selective "hit" on any target was defined to be more than 70% displacement of radioligand binding. None of the control agonist or antagonist ligands, was displaced more than 50% by the extract at the high concentration of 300 $\mu g/ml$ (Figure 2). The lack of selectivity of Cyperol against any of the tested pharmaceutical CNS targets is consistent with its status as a functional food.

In vitro Long-Term Potentiation (LTP)

To evaluate the effect of Cyperol on neuroplasticity we used the hippocampus slice model in vitro for testing Long-term Potentiation (LTP). The study demonstrated that compared to the saline control Cyperol led to increases of the population spike amplitude during both single shock stimulation (SS) as well as during theta burst stimulation (TBS). (Figure 3). This result can be related to potential improvements in spatial and time dependent memory.

Quantitative evaluation of field potentials (EEG) in vivo

The ability of Cyperol to alter brain electrical activity in-vivo was studied using quantitative evaluation of field potentials. It was found that

Cyperol administered by gavage at a dose of 100 mg/kg resulted in a statistically significant attenuation of delta, theta, alpha1, alpha2, beta1 and beta2 power within the frontal cortex. These changes lasted until the third hour after administration. During the first hour after administration changes in the frontal cortex were more pronounced than in the striatum, hippocampus and reticular formation. During the second and third hour all frequencies were attenuated in a highly

statistically significant manner in the frontal cortex (Figure 4 A). This pattern of frequency changes in the brain is rather similar to that caused by administration of cannabidiol (CBD) a natural non-psychoactive cannabinoid isolated from hemp flowers (*Cannabis sativa*) and is popularly used to decrease the subjective experience of stress and anxiety (Figure 4 B). The electropharmacogram for CBD is from a previous study.

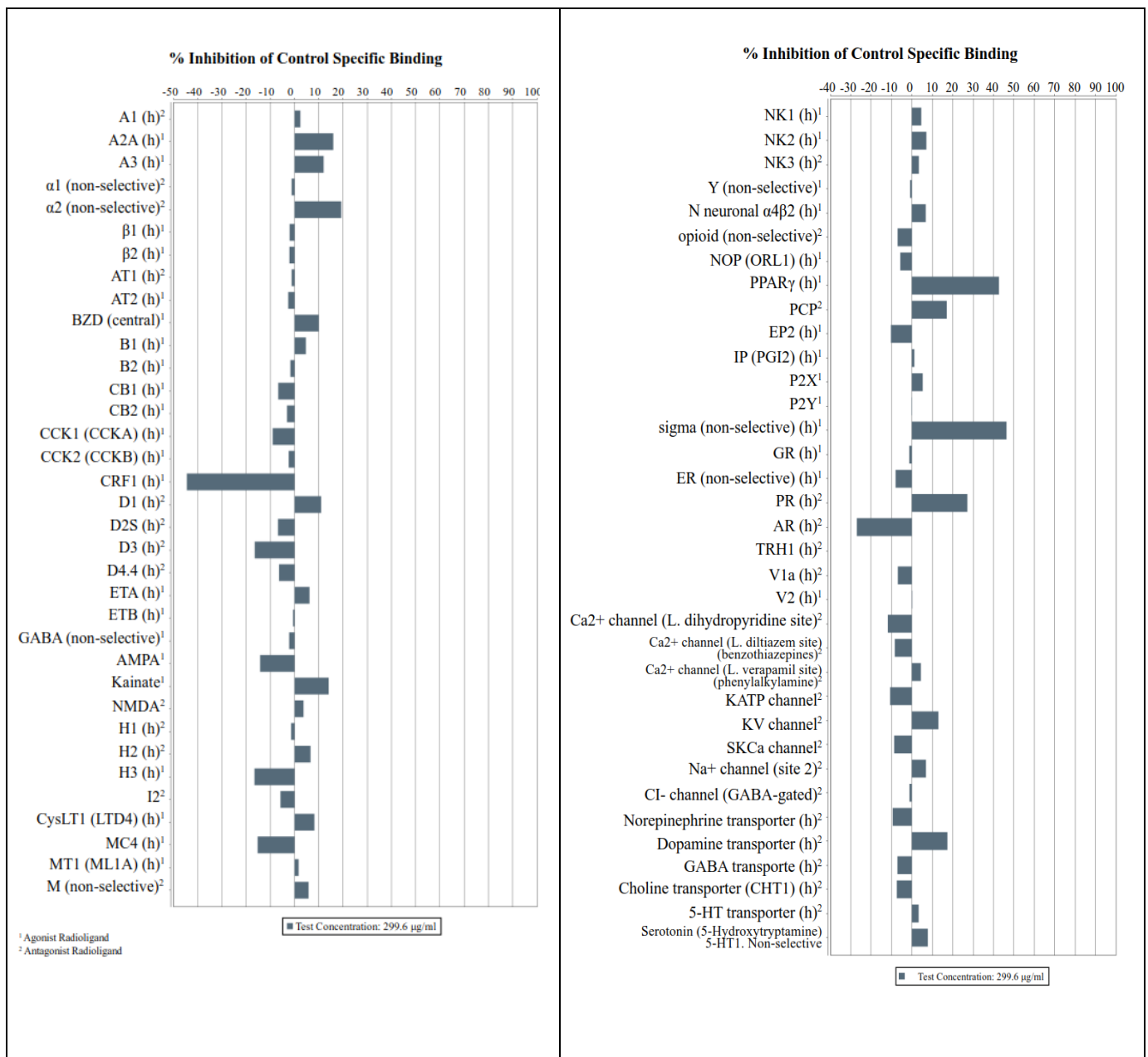


Fig. 2: Results shown as % change in control radioligand binding of Cyperol at a concentration of 300 µg/ml against classical CNS agonist or antagonist ligands.

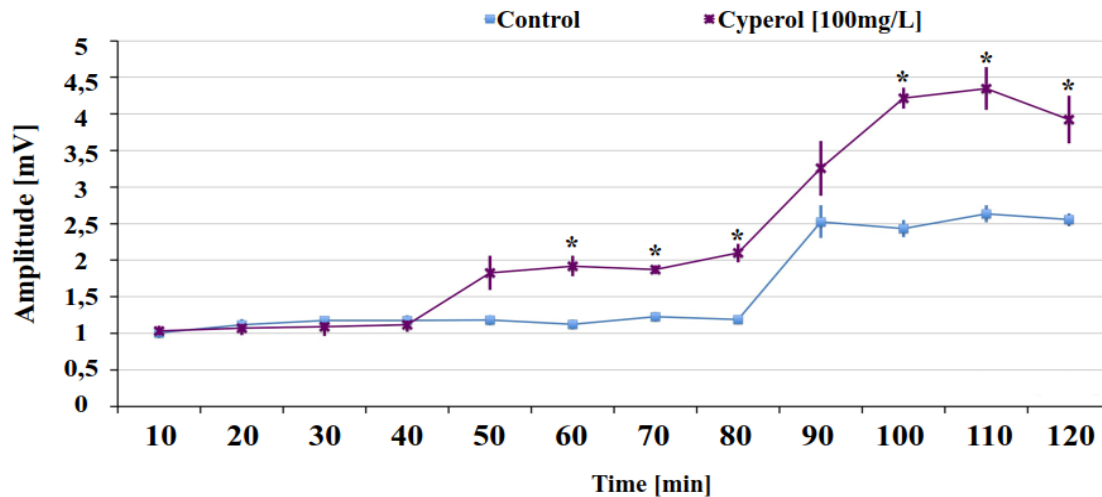


Fig. 3: Cyperol increases Long-Term Potentiation (LTP): The effect of control (saline) and Cyperol (100 mg/L) on hippocampal pyramidal cell activity in terms of changes in population spike amplitudes (represented as voltage on the vertical axis). The results are obtained after performance of a single stimulus (10-80 mins) or after a burst of stimuli (90-120 mins). Data are given as the mean \pm SEM of $n=12$. Statistical analysis was performed using Mann-Whitney non-parametric test against identical time periods from control data. $*=p<0.01$.

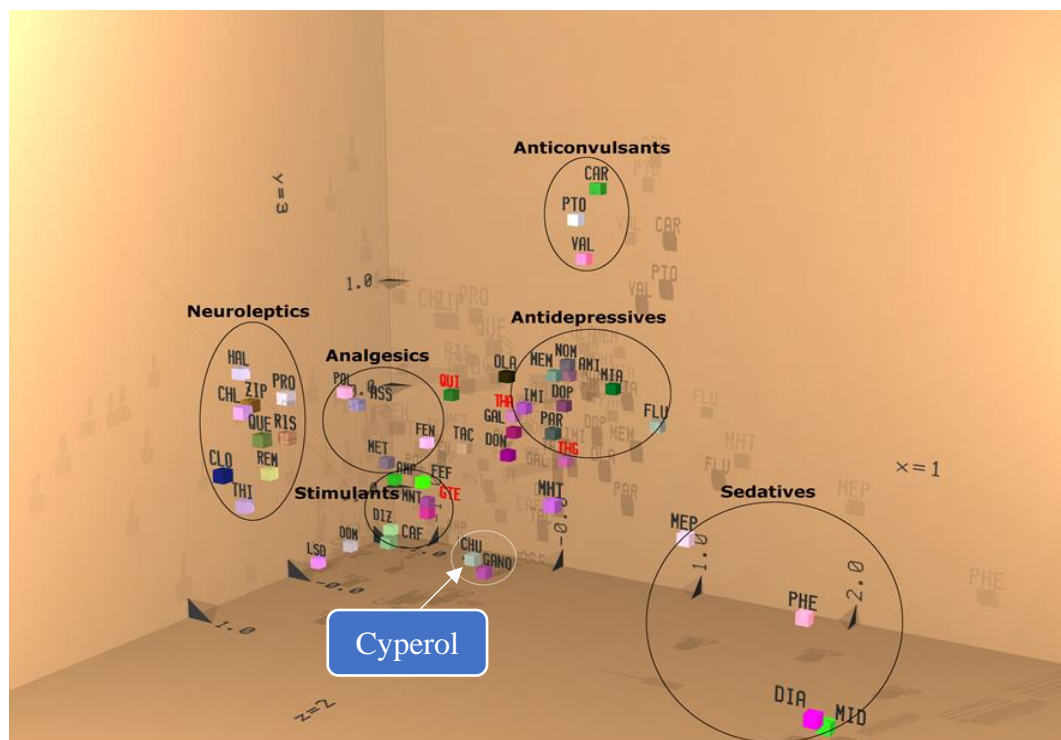


Fig. 5: Discriminant analysis of the electropharmacogram data from reference pharmaceuticals and two botanical extracts administered to freely moving rats. Results from the first three discriminant functions are depicted with the space coordinates x , y and z . Results from the next three discriminant functions are depicted as RGB color mixture like in TV technology. CHU=Cyperol, Gano=Ganoderma. For other abbreviations see Table 1

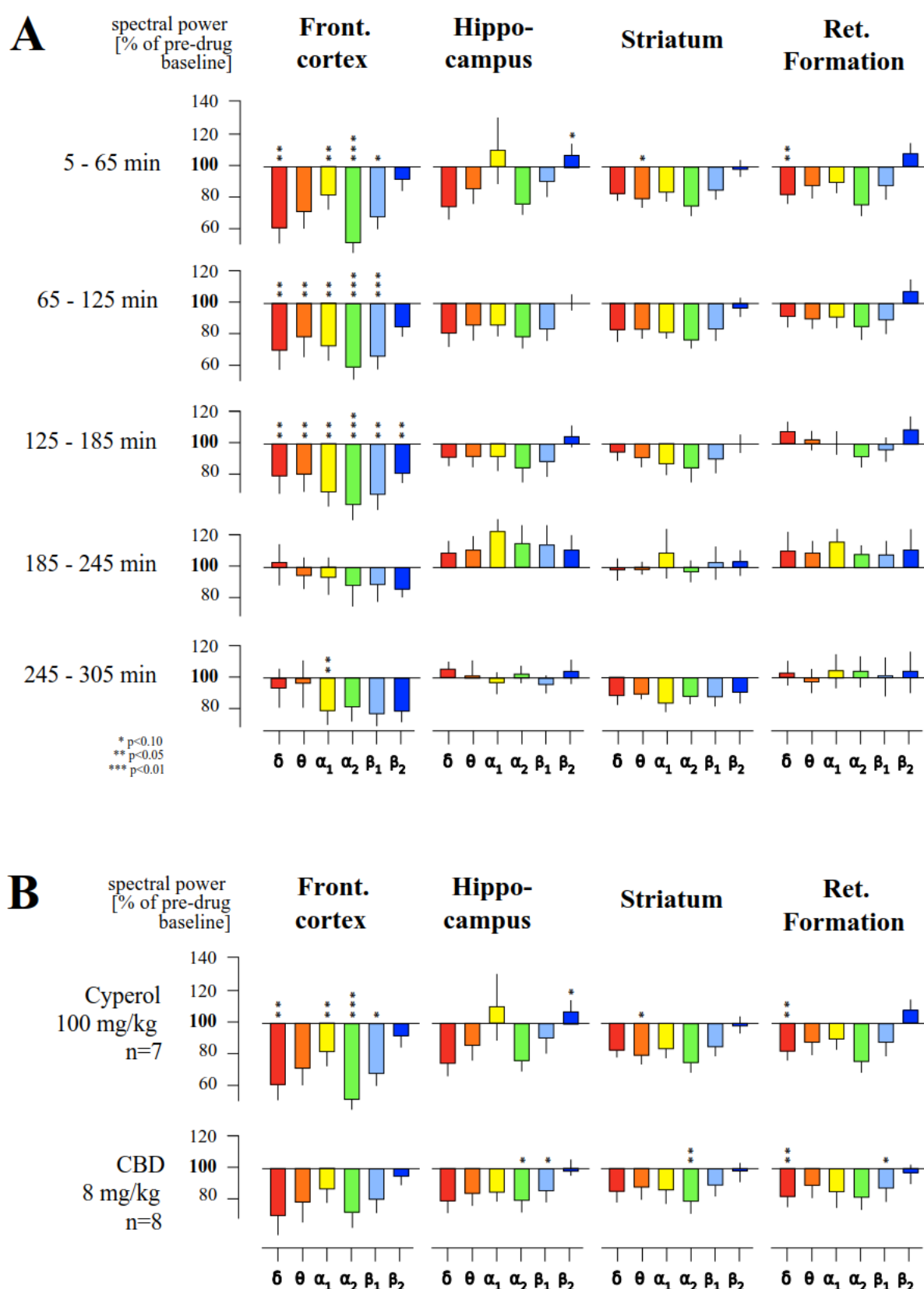


Fig. 4: Time dependent changes of spectral power in 4 brain regions. Electropharmacograms after administration of Cyperol by oral gavage (A). Comparison between the electropharmacograms of Cyperol and CBD (cannabidiol) (B). Data are presented as % of the baseline values recorded for 45 minutes before oral administration by gavage. Frequency ranges are given on the horizontal axis: from left to right: δ (red), θ (orange), α_1 (yellow), α_2 (green), β_1 (turquoise), β_2 (blue). Statistical analysis was performed using non-parametric test against identical time periods from vehicle data. *= $p<0.1$; **= $p<0.05$ and ***= $p<0.01$.

Placebo

Cyperol

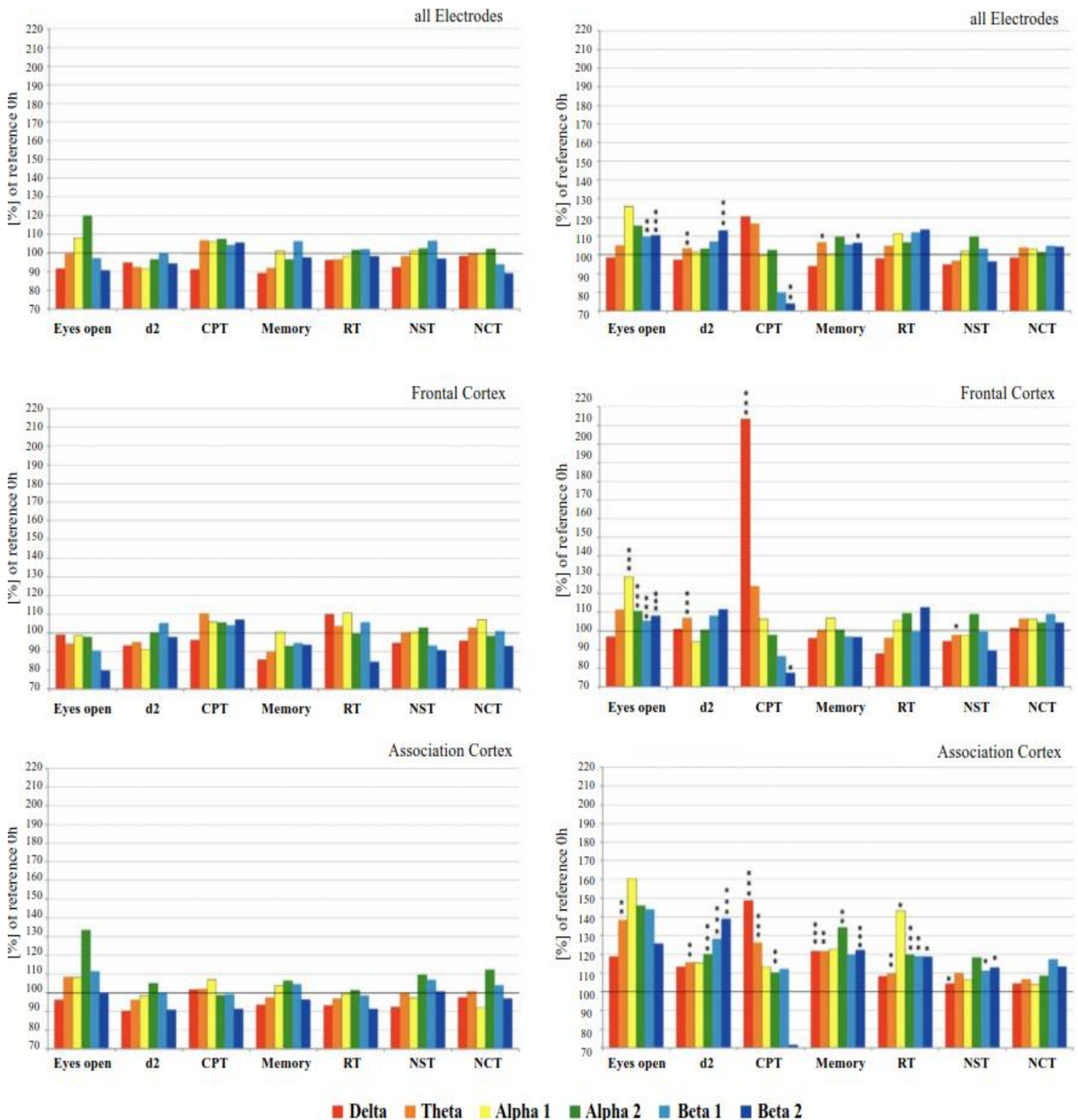


Fig. 6: Spectral frequency changes in % of the baseline after intake of Cyperol in the relaxed state (eyes open) and during performance of six cognitive challenges. Data are documented as median of all electrode positions (upper graph), for frontal cortex (middle graph) and associative cortex (lower graph) both for placebo and for Cyperol. Red color: delta; orange: theta; yellow: alpha1; green: alpha2; turquoise: beta1 and blue: beta2 spectral power concentration. Cognitive tests: Concentration test (d2), memory test (ME), Calculation Performance Test (CPT), reaction time test (RT), number sequence test (NST) and number connection test (NCT). Statistical significance (Sign-Test) between Placebo and Cyperol is indicated by asterisk. *= $p < 0.11$; **= $p < 0.08$ and ***= $p < 0.02$.

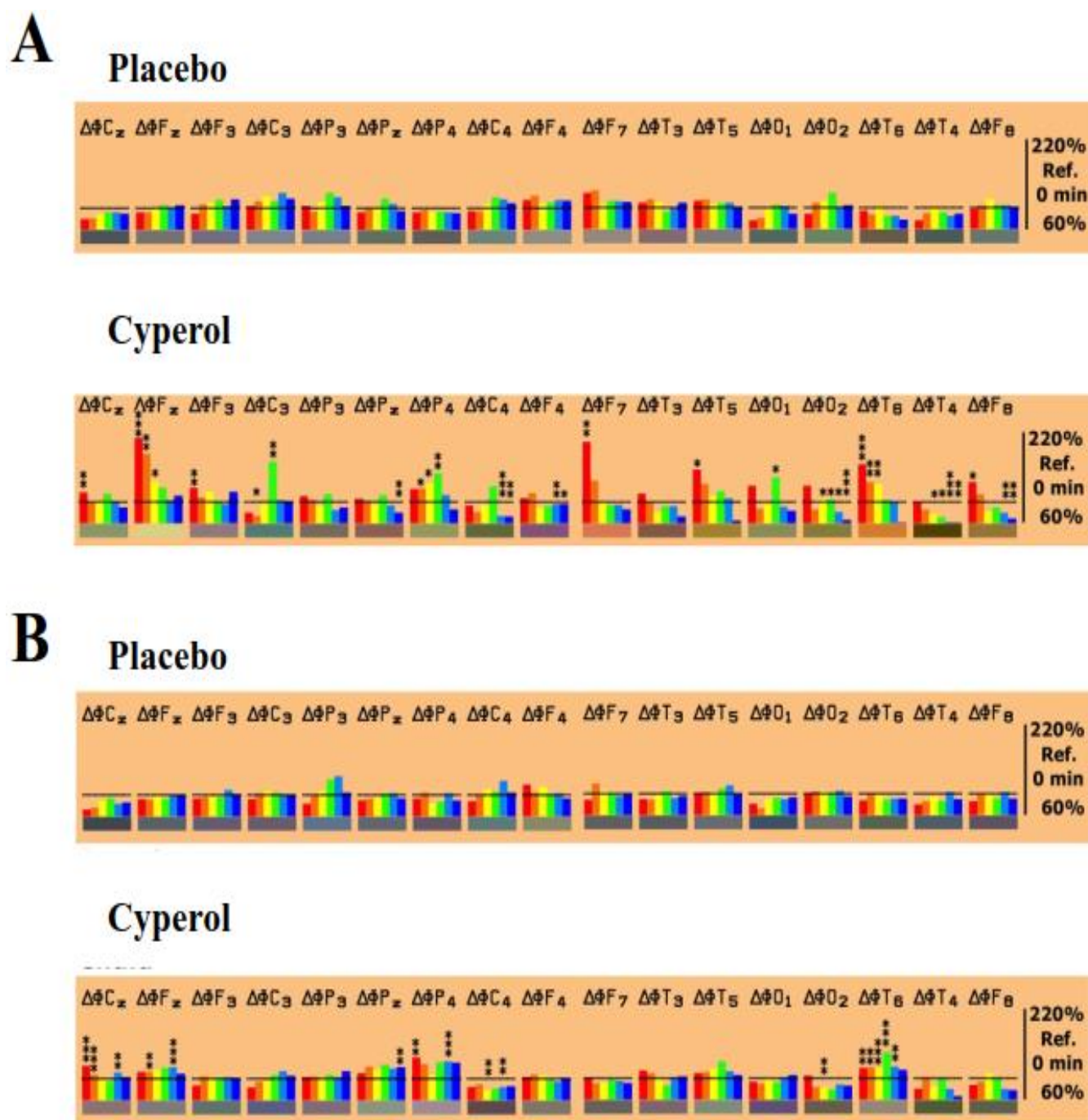


Fig. 7: Overview on changes of the frequency content at all 17 brain regions (electrode positions) for Placebo and Cyperol under the recording condition “CPT (calculation performance test)” (A) and Memory Test (B). Overview on spectral frequency changes in % of the baseline values (set to 100%) in the presence of all trial preparations. C=central, F=frontal, P=parietal, T=temporal, O=occipital. Red color: delta; orange: theta; yellow: alpha1; green: alpha2; turquoise: beta1 and blue: beta2 spectral power. Statistical significance (Sign-Test) between Placebo and Cyperol is indicated by asterisk. *= $p < 0.11$; **= $p < 0.08$ and ***= $p < 0.02$.

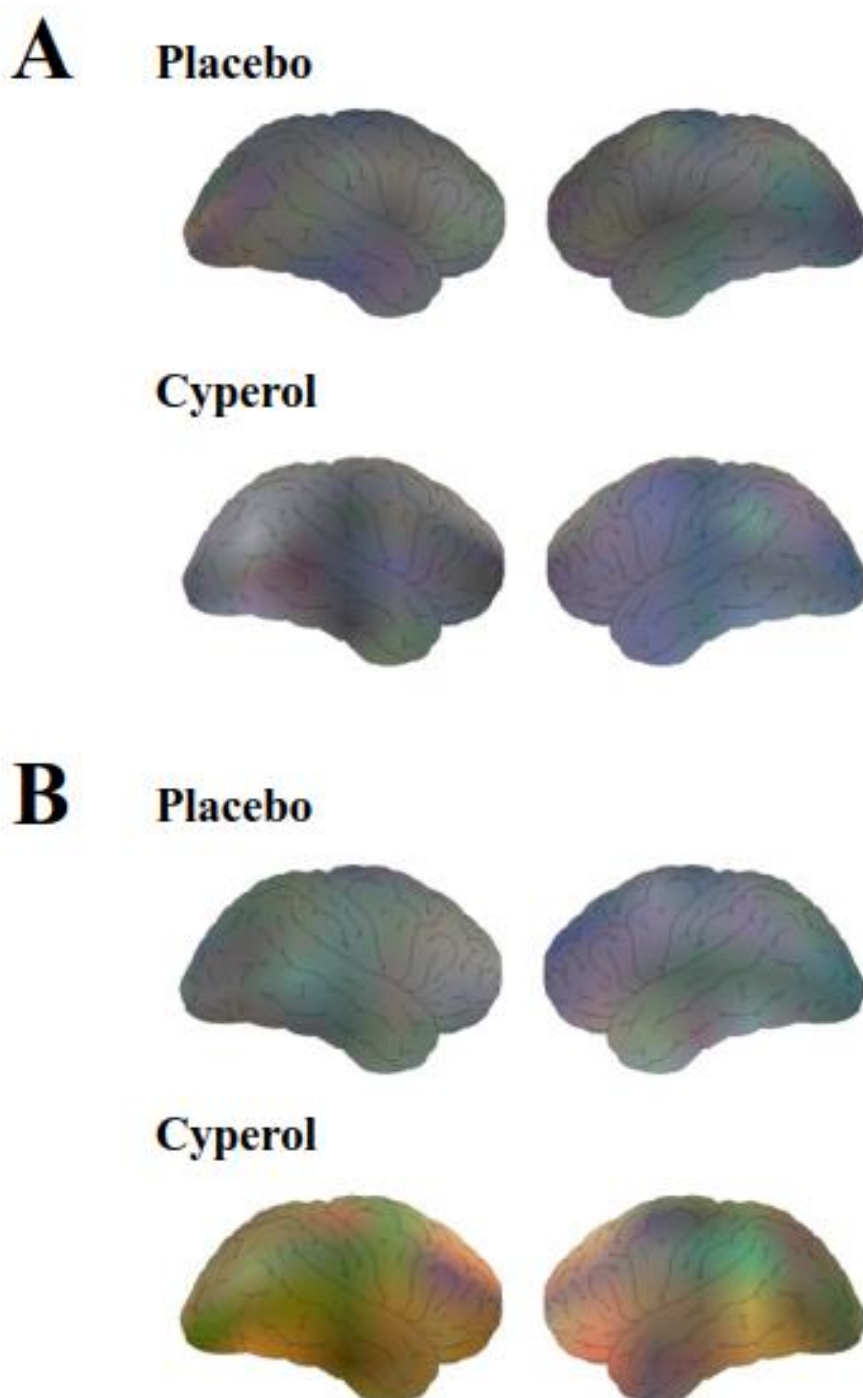


Fig. 8: Overview of spectral frequency changes in % of the baseline values (set to 100%) during d2 (A) or CPT (B) test in the presence of placebo and Cyperol documented as brain maps. Spectral power was translated into spectral colors followed by additive color mixture according to RGB processing as used in TV. Spherical projection of the cortex hemispheres with frontal areas in the middle part of the image. Left hemisphere on the right side. Cognitive tests: Concentration test (d2) and calculation performance test (CPT).

Pilot human randomized controlled trial

Quantitative EEG

The changes in brain electrical activity (quantitative EEG) produced by Cyperol during the

“eyes open” resting state and during psychometric challenges were compared in a small exploratory single dose human clinical trial.

The EEG results for placebo and Cyperol of the “eyes open” resting state and during performance of cognitive challenges are shown graphically in Figure 6.

Analysis of changes in spectral power changes in the presence of Cyperol compared to placebo showed marked increases in alpha1 spectral power in the “eyes open” state for the average of the recordings from all electrodes, for the frontal cortex where the increase in alpha1 spectral power reached statistical significance ($p < 0.02$), and in the association cortex, where the increase in alpha1 did not reach statistical significance. For the “eyes open state there was a trend of increase of beta1 spectral power for the average recorded for all electrodes ($p < 0.08$), statistically significant increase in beta1 power ($p < 0.02$), and an increase in the association cortex not reaching statistical significance. For the “eyes open” state there was a statistically significant increase of beta2 spectral power for the average recorded for all electrodes ($p < 0.02$), and in the frontal cortex there was a statistically significant increase in beta2 spectral power ($p < 0.02$), and an increase in the association cortex not reaching statistical significance. The statistically significant increases of alpha1 and beta2 spectral power in the frontal cortex during the resting eyes open state indicate a calm state of mind.

Psychometric test

The results of the psychometric tests attention d2-test, memory test (ME), calculation performance test (CPT), reaction time test (RT), number sequence test (NST) and number connection test (NCT) are shown in Table 2.

There were no significant differences in comparison to placebo during performance of any psychometric test 90 min after ingestion of Cyperol. However, during performance of several psychometric tests, significant changes in spectral power were seen in the presence of Cyperol. In the most cognitively challenging of the tests, the calculation performance test (CPT), a special pattern of frequency changes emerged in the presence of Cyperol consisting of large

statistically significant increases of delta spectral power in frontal and posterior temporal electrode positions (Figure 7), and in the frontal cortex ($p < 0.02$) and association cortex ($p < 0.02$) (Figure 6).

During the memory test (ME) Cyperol induced the strongest effects in the right parietal and posterior temporal area (Figure 7).

During performance of the memory test and the d2-test a conspicuous increase of theta power and a significant increase of beta2 power emerged indicating mental activation. During performance of the CPT a significant attenuation of beta2 power was seen (Fig. 6). Increases of theta power were statistically significant under all recording conditions except for the number sequence test (NST) and number connection test (NCT). Increases of beta power reached statistical significance during performance of cognitive tests except for the CPT and the NCT test (Fig. 6).

The frequency changes induced in the presence of Cyperol are also reflected in brain maps (Fig. 8). Focal increases of alpha power (green) are seen in the association cortex of both hemispheres. Increases of beta power during performance of the d2-test are represented by dominance of blue color, mainly in the temporal lobe. During performance of the CPT, massive delta and theta increases lead to red-orange color in frontal and temporal brain regions.

Profile of Mood States (POMS)

The Profile of Mood States questionnaire (POMS) was used to evaluate any changes in how participants felt during the study. Under the experimental stress condition represented by the psychometric tests, Cyperol tended to improve ($p = 0.073$) the sub-score “sullenness” of the POMS at 90 minutes after intake in comparison to the baseline values before intake (table 3). The scores for “dejection” and “fatigue” were also lower after intake of Cyperol but did not reach statistical significance. The score “thirst for action” increased, however without reaching statistical significance.

Tab. 1: List of abbreviations of the reference pharmaceuticals and botanicals tested in the same in-vivo EEG model with their respective dosages (mg·kg⁻¹) and times of EEG recording

Abbreviature	Drugs or botanicals	Doses	Time EEG
<i>Neuroleptic</i>			
RIS	Risperidone	0.25	5-35 min
ZIP	Ziprasidone	1.00	5-35 min
QUE	Quetiapine	2.50	5-35 min
HAL	Haloperidol	0.50	5-35 min
REM	Remoxipride	10.00	5-35 min
CHL	Chlorpromazine	0.50	5-35 min
PRE	Prothipendyl	1.00	5-35 min
CLO	Clozapine	3.00	5-35 min
THI	Thioridazine	5.00	5-35 min
OLA	Olanzapine	3.00	5-35 min
<i>Anticonvulsants</i>			
VAL	Valproic acid	75.00	95-125
PTO	Phenytoin	4.00	95-125
CAR	Carbamazepine	15.00	95-125
<i>Hallucinogens</i>			
DIZ	Dizocilpine	0.25	5-35 min
LSD	Lysergic acid diethylamine	0.05	5-35 min
DOM	Dimethoxymethylamphetamine	0.20	5-35 min
<i>Sedatives</i>			
MHT	Methohexital	20.00	35-65 min
MEP	Meprobamate	60.00	35-65 min
DIA	Diazepam	0.50	35-65 min
MIA	Midazolozepan	0.05	35-65 min
PHE	Phenobarbitone	60.0	35-65 min
<i>Analgesics</i>			
ASS	Acetylsalicylic acid	200.0	5-35 min
MET	Metamizol	100.0	5-35 min
POL	L-Polamidone	1.00	5-35 min
FEN	Fentanyl	0.075	5-35 min
<i>Stimulants</i>			
CAF	Caffeine	1.00	5-35 min
FEF	Fenfluramine	1.00	5-35 min
MNT	metanicotine	1.00	5-35 min
AMP	Amphetamine	0.20	5-35 min
<i>Antidementia</i>			
TAC	Tacrine	0.75	5-35 min
GAL	Galantamine	1.00	5-35 min
DON	Donepezil	1.50	5-35 min
DOP	DOPA	2.50	5-35 min
<i>Antidepressants</i>			
PAR	Paroxetine	2.00	5-35 min
MEM	Memantine	3.00	5-35 min
IMI	Imipramine	10.00	5-35 min
MIA	Mianserine	5.00	5-35 min
AMI	Amitriptyline	10.00	5-35 min
FLU	Fluvoxamine	40.00	5-35 min
NOM	Nomifensine	1.00	5-35 min
GTE	Green tea extract	75.00	5-65 min
THG	Theogallin	20.00	5-65 min
THA	L-Theanine	30.00	5-65 min
QUI	Quinic acid	10.00	5-65 min

Tab. 2: Psychometric performance during several tests and measurement of reaction time (RT given in milliseconds) for Cyperol. ME=Memory Test, CPT= Calculation Performance Test, RT= Reaction Time Test, NST= Number Sequence Test, NCT= Number Connection Test.

	Cyperol		Placebo	
Tests	Time 0h	Time 1.5h	Time 0h	Time1.5h
D2-Test	16.26 ± 3.92	17.52 ± 3.66	16.09 ± 3.29	17.24 ± 3.36
CPT	3.82 ± 3.47	4.67 ± 3.12	3.96 ± 3.08	4.41 ± 2.89
Me-Test	8.59 ± 2.31	9.91 ± 1.49	8.87 ± 1.64	9.37 ± 1.69
RT-Test	360.15 ± 45.02	342.79 ± 40.10	361.84 ± 52.51	371.27 ± 41.77
NST	9.93 ± 4.22	16.62 ± 5.12	9.80 ± 4.41	10.46 ± 4.82
NCT	170.67 ± 55.31	184.92 ± 39.67	172.79 ± 48.65	192.31 ± 45.50

Values are expressed as mean ± S.D. (n=60). Differences between Cyperol and placebo were evaluated by one-way analysis of variance (ANOVA).

Tab. 3: Results from the questionnaire “Profile of Mood States” for Cyperol compared to placebo before (baseline) and 1.5h after intake of Cyperol or placebo. The four mood states used for the evaluation of the POMS questionnaire were: S1=Dejection, S2=Sullenness, S3=Fatigue, S4=Thirst for action, SD=Standard deviation

	Cyperol		Placebo	
Questionnaire	Baseline (0h)	1.5h after intake	Baseline (0h)	1.5h after intake
S1	0.63 ± 0.83	0.31 ± 0.35	0.60 ± 1.05	0.29 ± 0.57
S2	0.30 ± 0.39	0.10 ± 0.14*	0.50 ± 0.91	0.20 ± 0.43
S3	1.81 ± 1.16	1.21 ± 1.05	2.05 ± 1.24	1.37 ± 0.99
S4	1.95 ± 1.30	2.41 ± 1.06	2.54 ± 1.46	2.72 ± 1.39

Values are expressed as mean ± S.D. (n=60). Differences were evaluated by one-way analysis of variance (ANOVA).

*=p<0.073 compared with placebo.

Discussion

The current experimental series has revealed entirely new neurophysiological effects for the extract Cyperol, which have not previously been reported for *Cyperus esculentus*.

In-vitro screening of Cyperol against 70 CNS receptor, enzyme and transporter targets did not reveal profound pharmaceutical-like binding activity, consistent with the intended uses of the extract in functional foods, beverages and supplements. In the hippocampal slice preparation,

Cyperol was able to enhance the excitability of hippocampal pyramidal cells under both single-stimulus and theta burst stimulation. This increased LTP activity indicates the extract has potential to enhance spatial and time-dependent memory.

Quantitative EEG recorded wirelessly in-vivo demonstrated that compared to the control, Cyperol administered by gavage resulted in statistically significant attenuation of delta, theta, alpha1, alpha2, beta1 and beta2 power within the

frontal cortex which lasted up to three hours after administration (Figure 4 A). This demonstrates that bioactive compounds from the extract are absorbed, and that these compounds and/or their metabolites are able to cross the blood brain barrier and modulate the electrical activity of the frontal cortex. Referring to the increased LTP in the hippocampal slice model, excitability of hippocampal pyramidal cells is mediated by the neurotransmitter glutamate. Since beta1 waves on EEG are under the control of glutamatergic transmission¹⁸ the significant attenuation of beta1 waves by the extract in-vivo supports the modulation of glutamatergic signaling as an activity of the extract.

Figure 4B shows that the electropharmacogram of Cyperol has a similar pattern of attenuation of delta, theta, alpha1, alpha2, beta1 and beta2 power within the frontal cortex to that of cannabidiol (CBD) a natural cannabinoid extracted from hemp flowers (*Cannabis sativa*). This suggests that while both may not have the same molecular mechanisms of action (there was no significant binding activity on CB1 or CB2 receptors in the in-vitro screen), both Cyperol and CBD have similar net integrated effects on underlying regional neurotransmitter activity, and likely similar mental health and wellbeing applications. Both human observational studies and in vivo studies have demonstrated a broad range of therapeutic effects for CBD including attenuating anxiety and depressive-like behaviors, and CBD may also decrease the intensity of symptoms commonly associated with post-traumatic stress disorder, including chronic anxiety in stressful environments¹⁹. Cyperol can thus be anticipated to be helpful for anxiety and stress, applications which should be investigated in future controlled clinical studies.

In the plot of the discriminant analysis (Figure 5) it can be seen that the two botanical extracts Ganoderma (*Ganoderma lucidum*) and Cyperol plot immediately adjacent to each other in 3 dimensional space, and rather distinct from the plots for pharmaceutical clusters. Since Ganoderma is known to have anxiolytic activity²⁰ and

antidepressant-like activity²¹, Cyperol may have anxiolytic and antidepressant potential too. At the same time, Cyperol and Ganoderma are distinctly different from each other in the RBG colour plots which representing an additional 3 dimensions in the discriminant analysis, suggesting they modulate brain electrical activity by different underlying molecular mechanisms of action.

Cyperol induced clear changes in spectral power in the frontal cortex of the brain during the resting “eyes open” state with statistically significant increases in alpha1 spectral power ($p=0.02$), beta1 spectral power ($p<0.02$), and in beta2 spectral power ($p<0.02$). These statistically significant increases during the resting eyes open state support can be interpreted as indicative of a calm yet attentive state of mind.

During the most stressful psychometric test, the calculation performance test (CPT), Cyperol induced a special pattern of frequency changes consisting of large statistically significant increases of delta spectral power in frontal and posterior temporal electrode positions (Figure 7), in the frontal cortex ($p<0.02$) and in the association cortex ($p<0.02$) (Figure 6). The frequency changes induced by Cyperol are also reflected in computer-generated colour of brain maps (Fig. 8). During performance of the CPT massive delta and theta increases lead to red-orange color in frontal and temporal brain regions indicating mental activation, readily distinguished from the colour changes induced by the placebo. Thus, subjects taking Cyperol are clearly not sedated, and the brain activation during CPT suggests that the can rise to the mental challenge of a cognitively demanding psychometric test.

In this small exploratory human clinical study no significant changes were seen in any of the psychometric tests compared to the placebo.

In the Profile of Mood States (POMS) questionnaire, Cyperol improved ($p=0.073$) the sub-score “sullenness” of the POMS at 90 minutes after intake in comparison to the baseline values before intake. The scores for dejection and fatigue were

also lower after intake of Cyperol but did not reach statistical significance. The score thirst for action increased, however without reaching statistical significance. These results from the POMS questionnaire are very preliminary, from a small sample size of healthy people subjected to the stress of cognitive challenges. It will be instructive to look at the effect of acute and chronic ingestion of Cyperol on stress, anxiety, and mood in a larger study.

In conclusion, the series neurophysiological studies on Cyperol reported here are to the best of our knowledge the first to have explored the CNS activity of an extract of *Cyperus esculentus*. The results indicate that orally ingested Cyperol, a polymolecular functional food, has distinct effects on brain electrical activity in-vivo, lasting up to 3 hours after ingestion, as well as on brain electrical activity of healthy humans, demonstrating that bioactive compounds from the extract are absorbed, are bioavailable, and that these bioavailable compounds, or their metabolites, cross the blood brain barrier. In addition, taking into consideration changes in the patterns of brain electrical activity induced by Cyperol in-vivo, the similarity of the in-vivo electropharmacograms for Cyperol and CBD (cannabidiol), the close positioning of Cyperol with *Ganoderma lucidum* on discriminant analysis of the in-vivo EEG data, and interpreting human EEG studies at rest and under a stressful cognitive challenge (the CPT test), the activity of Cyperol can be characterized as calming without sedation. Thus Cyperol has the potential to support a calm state of mind in stressed people of all ages, including office workers and students, who need to feel calm and attentive without feeling sedated. The anxiolytic potential of the extract should be explored in future clinical studies.

Conflict of interest statement

Drs. Wiebe, López-Ríos, Pérez-Machín, Vega-Morales and Gericke, and Mr. Mateos are employed in Research & Development at Nektium Pharma. Dr. Aydogan declares no conflict of interest.

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