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Modulation of neuroplasticity in humans after acute intake of antidepressant, anxiolytic and adaptogenic herbs

Ph.D Thesis

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TABLE OF CONTENTS

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LIST OF ABBREVIATIOS

ACTH	Adrenocorticotropic hormone
AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
AMPARs	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors
BDNF	Brain-derived neurotrophic factor
CAM	Complementary and Alternative Medicine
CNS	Central nervous system
CREB	cAMP response element binding protein
CRH	Corticotropin- releasing hormone
CSP	Cortical silent period
DOPA	Dopamine
EMG	Electromyography
FDA	Food and Drug Administration
GABA	Gamma-aminobutyric acid
GABA T	Gamma-aminobutyric acid transaminases
GAD	Glutamic acid decarboxylase
GABA-ARs	Gamma-aminobutyric acid A receptors
GABA-BR	Gamma-aminobutyric acid B receptor
5-HT	5-hydroxytryptamine
HAMD	Hamilton Rating Scale for Depression
HFS	High frequency stimulation
HPA	Hypothalamic pituitary-adrenal
HYP	Hypericum perforatum
ICF	Intracortical facilitation
ISI	Interstimulus interval
LAI	Long-latency afferent inhibition
LTD	Long-term depression
LTP	Long-term potentiation
M1	Primary motor cortex
MAO	Monoamine oxidase
MEP	Motor Evoked Potential
MDD	Major Depressive Disorder

mGluRs	Metabotropic glutamate receptors
mA	Milliampere
MS	Milliseconds
MT	Motor threshold
NHIS	National Health Interview Survey
NIBS	Non- invasive brain stimulation
NMDA	N-methyl-D- aspartate
NMDARs	N-methyl-D- aspartate receptors
NO	Nitric oxide
NPY	Neuropeptide Y
NRI	Noradrenaline reuptake inhibitor
RRE	Rhodiola rosea extract
SAI	Short-latency afferent inhibition
SAPK	Stress-activated protein kinases
SICI	Short intracortical inhibition
SJW	St. John's Wort
SNRIs	Selective serotonin and noradrenalin reuptake inhibitors
SSRIs	Selective serotonin reuptake inhibitors
TMS	Transcranial magnetic stimulation
TDCS	transcranial Direct Current Stimulation
TRPC 6	Transient receptor potential channels of C type 6
U.K.	United Kingdom
U.S.	United States
VE	Valerian extract
VGCC	Voltage- gated calcium channel

ABSTRACT

Herbal medicine represents one of the most frequently used complementary and alternative approaches for the treatment of psychiatric conditions such as depression, anxiety and sleep disturbance. Among the most used herbal medicines, Hypericum perforatum (HYP) extract, Valeriana officinalis extract (VE) and Rhodiola rosea extract (RRE) are the oldest and most thoroughly researched phytotherapeutic medications. Despite their widespread use, the mechanisms of action and the role of the different compounds of these herbal products are still a matter of debate and warrant the need to develop new approaches to investigate their effects in humans. Noninvasive brain stimulation protocols, such as Transcranial Magnetic Stimulation (TMS) and transcranial Direct Current Stimulation (tDCS) can be used to elucidate the mechanisms of action of psychoactive substances at the cortical level in humans. TMS can be used to test the affect of acute drug intake at the system level in the cerebral cortex in humans. Pharmaco-TMS offers a broad array of measures of cortical excitability and offers the possibility to probe the activity of different forms of inhibitory and excitatory networks. Furthermore, tDCS is a safe noninvasive brain stimulation technique that, combined with TMS, has been shown to induce cortical plastic changes in humans that resemble Long-term potentiation and depression (LTP and LTD)-like plasticity. The studies presented in this thesis explored the neurophysiological effect of the acute intake of herbal products commonly used to treat psychiatric conditions.

Study 1 explored the effect of HYP extract acute dose intake on cortical excitability and plasticity. The results indicated that HYP acute intake affected cortical plasticity induced by cathodal tDCS by modulating LTD-like plasticity in a similar manner of conventional antidepressants.

Study 2 investigated the effects of a single dose intake of VE on cortical excitability. It was found that VE intake modulated intracortical facilitatory circuits explored by TMS.

Study 3 studied the effect of a single oral dose of RRE intake on cortical excitability and plasticity. Results showed that RRE acute intake prevented cathodal tDCS-induced LTD and increased (non-significantly) LTP-like plasticity.

The translational studies described in the thesis add to the understanding of how the herbal products used in psychiatry can affect brain circuitries in humans.

GENERAL INTRODUCTION

Complementary and Alternative Medicine in psychiatry

Complementary and Alternative Medicine (CAM) has been defined by the United States (U.S.) National Center for CAM as a “group of diverse medical and healthcare systems, practices and products that are not considered to be a part of conventional medicine”. These practices include a heterogeneous spectrum of ancient to new-age approaches grouped within five major categories: mind-body medicine, such as meditation, prayer; alternative medical systems, for example traditional Chinese medicine or Ayurveda; biologically based practices, such as herbs, dietary supplements or vitamins; manipulative and body-based practices, such as massage, chiropractic or osteopathy and energy medicine (Coulter & Willis, 2004). The use of CAM interventions, mainly in addition to (complementary) conventional therapies, has increased exponentially in Western industrialized nations over the last few years (Barnes et al., 2008). CAM is mostly used in self-medication and holds widespread appeal, presumably because of its lower cost and higher range of safety when compared to chemically defined pharmaceuticals. In addition, patients who use CAM may favoring a more holistic orientation to health care that might be more compatible with their own value systems and personal beliefs toward their health and life (Frass et al., 2012). CAM has been often used for neck, back, or joint pain, as well as head and chest colds (Barnes et al., 2008). Moreover, the use of such therapies has become common among people with self-defined anxiety and depression (Kessler et al., 2001) (Ravindran et al., 2016). Previous studies showed that CAM users are more likely to be: middle-aged female, well educated and with reported poorer health status than non-users (Harris et al., 2012). CAM prevalence rates and use-related factors are influenced by economic and socio-cultural factors (Welz et al., 2018). Estimate CAM use differs between

countries. Previous surveys in the United Kingdom (U.K.), Germany and Italy showed that between 10% and 70% of the total population use CAM each year (Eardley *et al.*, 2012) (Bodeker & Kronenberg, 2002). In Italy the number of users increased during the last decade, although it still remains beneath the estimates described in many European countries and in the U.S. (Menniti-Ippolito *et al.*, 2002). In the U.S., almost all CAM agents have been defined by the 1994 Dietary Supplement, Health, and Education Act as "dietary supplements", allowing them to avoid the Food and Drug Administration (FDA) regulations (McCabe, 2002). According to the National Health Interview Survey (NHIS), in 2012, 33.2% of U.S. adults used CAM approaches. More specifically, 17.7% of U.S. adults used natural products as they chose complementary health approaches (Clarke et al., 2015). Natural products are a group of complementary health approaches that include a variety of products such as herbs (also known as botanicals or phytomedicine), vitamins, minerals, and probiotics. Research on herbal products has recently flourished with several preclinical in vitro and in vivo studies that validated many herbs as having an effect on the central nervous system (CNS) (Sarris *et al.*, 2011). Numerous studies have demonstrated that the use of herbal products among psychiatric disorders, especially depression and anxiety is a common phenomenon (Liu *et al.*, 2015). Many of these herbal products that are available as over-the-counter psychotropic herbal medicines are fairly safe and have fewer side effects in comparison to conventional drugs such as antidepressants and benzodiazepines (Sarris *et al.*, 2011) (Papakostas, 2008) (Baldwin *et al.*, 2007). The most used herbal products to treat psychiatric conditions are Hypericum Perforatum (HYP), Valeriana Officinalis and Rhodiola Rosea (Sarris *et al.*, 2011).

Antidepressant herb: Hypericum Perforatum Extract

HYP also know as St. John's Wort (SJW) is a perennial flowering herb that grows in open areas throughout much of the world's temperate regions. It is one of oldest and most researched herbal products recommended to treat a wide range of medical conditions. Ancient Greek physicians of the first century suggested HYP as a diuretic, wound-healing herb; as a treatment for menstrual disorders and a remedy for intestinal worms and snakebites. Sixteenth-century scientists recommended it to alleviate pain and to treat depression symptoms. Over the centuries HYP use continued in Europe spreading to other continents. It was commonly made into teas and tinctures for the treatment of anxiety, depression, insomnia, gastritis and inflammation. It has also been used to treat sores, cuts, minor burns and abrasions, especially those involving nerve damage (Castleman, 2001) (Clement et al., 2006).

HYP extract contains different compounds such as naphthodianthrones hypericin and pseudohypericin, a broad range of flavonoids including hyperoside, isoquercitrin and quercetin, and the phloroglucinols hyperforin and pseudohyperforin (Kasper *et al.*, 2010). Compounds that are likely to be responsible for antidepressant activity include hypericin and hyperforin. Flavonoids are also assumed to have an antidepressant effect and they have been shown to improve the pharmaceutical properties of hypericin and other compounds (Butterweck & Schmidt, 2007). The commercially available extracts are usually standardized by either their hypericin or hyperforin contents (Schmidt & Butterweck, 2015). Commercially available extracts are usually extracted from dried aerial parts. Three different types of HYP extracts have been granted as "well-established use" in the treatment of mild to moderate depression: a) a dry extract (3-7:1), manufactured with methanol 80 % (v/v); b) a dry extract (3-6:1), manufactured with 80 % ethanol (v/v); and a

dry extract (2,-8:1), manufactured with 50-68 % ethanol (v/v). For these extract types published clinical data revealed that the evidence of the efficacy in mild to moderate depressive episodes compared to placebo or standard medication was found to be acceptable (Community Herbal Monograph on Hypericum perforatum L.). A large number of HYP extract preparations from different manufacturers are marketed as herbal medicine. Among the different extract preparations, the WS 5570 is one the most studied and the most frequently used as a self-prescribed treatment for depression. It is an 80% methanol extract with a plant-to-extract ratio of between 3:1 and 3:1-7:1. It contains 5-6% hyperforin and 0.12-0.28% hypericin (Szegedi *et al.*, 2005). Clinical trials using various HYP extract preparations allow typical dosages in the range of 300-1800 mg/day (Barnes *et al.*, 2001).

Standardized preparations are most commonly used for the treatment of mild-to-moderate Major Depressive Disorder (MDD). MDD is one of the most prevalent mental illness, it is the second leading cause of disability worldwide and will become the second largest major illness in the world by the year 2020. MDD symptoms include feelings of sadness and loss of interest as well as cognitive dysfunction and sleep disturbances (Ferrari *et al.*, 2013) (Murray & Lopez, 1997). MDD is the psychiatric condition in which the most herbal medicine research has been conducted, with HYP representing the vast majority of that research.

Several controlled clinical trials suggest that HYP extract is significantly more effective than placebo in the treatment of mild-to-moderate MDD, with at least similar efficacy and better tolerability than standard antidepressant drugs (Vorbach *et al.*, 1997) (Kalb *et al.*, 2001) (Lecrubier *et al.*, 2002) (Kasper *et al.*, 2010). A double-blind, randomized, placebo-controlled, multicenter clinical trial has demonstrated the superior antidepressant efficacy of WS 5570 600 mg/day (in one dose) and of WS 5570 1200 mg/day (in two daily doses) compared to placebo in the treatment of patients with a mild or moderate major depressive

episode after 6 weeks of treatment. In the long-term maintenance therapy, this extract showed efficacy in patients with early-onset depression and in those with chronic symptoms. This extract also showed a beneficial effect in preventing relapse after recovery from acute depressive episode (Kasper *et al.*, 2006). Moreover, previous studies confirmed the comparable efficacy of HYP extract to conventional antidepressants such as selective serotonin reuptake inhibitors (SSRIs) for mild to moderate MDD (Ross, 2014) (Rahimi et al., 2009). Regarding, HYP extract WS 5570 was found to be as effective as paroxetine (Szegedi *et al.*, 2005). HYP extract is now recommended as first-line monotherapy for the treatment of mild to moderate MDD and it is recommended as a second-line adjunctive treatment for moderate to severe MDD (Ravindran et al., 2016).

There are many genetic, biological, and psychological factors that contribute to the development of MDD, with considerable variability among individuals. Antidepressant drugs used for MDD treatment increase synaptic availability of monoamines by either blocking serotonin and/or noradrenaline reuptake sites or by inhibiting monoamine oxidase (MAO) enzymes (Duman & Kehne, 2007). The HYP extract antidepressant activity has been observed in both in vitro and in vivo studies and it is attributed to a range of biochemical mechanisms including the inhibition of the synaptic uptake of serotonin, dopamine, and noradrenaline (Muller, 2003). In particular it has been hypothesized that the non-selective inhibition of monoamines occurs in part via hyperforin modulation of the transient receptor potential channels of C type 6 (TRPC6) with consequent sodium influx into the neurons, which finally leads to the release of intracellular calcium and to the decrease of the neurotransmitters reuptake (Schmidt & Butterweck, 2015). Studies on rat brain mitochondria found hypericin to strongly inhibit the enzymes MAO-A and MAO-B (Barnes et al., 2001). However, further studies determined that hypericin's ability to inhibit MAO was lower than was originally estimated (Schmidt & Butterweck, 2015). A modulation of

neuronal excitability via glutamatergic and gamma-aminobutyric acid (GABA) mechanisms was also observed (Schmidt & Butterweck, 2015). Furthermore recent studies showed that HYP is involved in the regulation of genes that control hypothalamic–pituitary–adrenal (HPA) axis function (Kasper et al., 2010). According to the actual state of scientific knowledge, although the single compounds alone have been shown to have antidepressant activity, the total extract appears to be more effective. The presence or absence of the flavonoid compound “rutin” modulates the antidepressant effect of the herb (Wurglics & Schubert-Zsilavecz, 2006). As a result, it seems that the total extract has to be considered as the active substance (Butterweck & Schmidt, 2007). The mechanism of action of HYP extract in human is not yet completely understood and further studies are needed to translate in humans mechanisms underlying the antidepressant activity hypothesized in preclinical studies.

Anxiolytic herb: Valeriana Officinalis extract

Valeriana officinalis (Valerian) is a perennial herb, belonging to the Valerianaceae family. It is native to Europe and Northern Asia and has been used as a mild sedative for more than 2000 years. Ancient Greek and Roman physicians recommended Valerian to treat several disorders including heart palpitations, digestive problems, epilepsy and urinary tract infections. Galeno recommended Valerian as a treatment for insomnia and referred its use in the treatment of epilepsy in children and adults. By the 18 century, Valerian was widely used as a sedative and to treat anxiety, headaches, palpitations, high blood pressure, irritable or spastic bowel and menstrual cramps (Murti *et al.*, 2011). In herbal medicine, the herb's root and rhizomes (underground stems) are chopped and made into a tea or extract to be used primarily as a sedative. Over 150 compounds have been identified in Valerian including alkaloids (actinidine, chatinine, shyanthine, valerianine, valerine); iridoids (valepotriates); sesquiterpenes, contained in the volatile oil (valerenic acid, hydroxyvalerenic acid, acetoxyvalerenic acid) and flavanones (hesperidin, 6-methylapigenin, and linarin). Free amino acids, such as tyrosine, arginine, and glutamine are also present (Hadley & Petry, 2003). The CNS activity is largely related to sesquiterpenoids (valerenic acid) and valepotriates (Navarrete *et al.*, 2006).

In the U.S. Valerian extract (VE) is among the top-selling herbal product and it is marketed as a promoter of restful sleep and to support relaxation (Taibi *et al.*, 2007). Nowadays, VEs are available as over-the-counter dietary supplements, which primarily involve dried root or extracts from the root, formulated into tablets or capsules. VE containing a 70% ethanolic extract standardized to 0,8% valerenic acid is the usual commercially available presentation.

Up to date data about effectiveness of VE as sleep aid are conflicting. Several placebo controlled-studies support its use in case of sleep disturbances. VE showed to promote an improvement in sleep latency and quality in both healthy volunteers and patients with sleep disorder without disrupting normal sleep architecture, with a low rate of side effects (Donath *et al.*, 2000) (Bent *et al.*, 2006) (Salter & Brownie, 2010). In contrast, a recent meta-analysis concluded that VE intake has no significant effect in each sleep parameters considered (sleep onset latency, sleep duration, sleep efficiency) (Leach & Page, 2015). Overall, the evidence, while supporting that Valerian is a safe herb associated with only rare adverse events, does not support its clinical efficacy as a sleep aid for insomnia (Taibi *et al.*, 2007) (Sateia *et al.*, 2017). Although its widespread clinical use to treat anxiety, few studies explored the effectiveness of VE in the treatment of anxiety symptoms. One study demonstrated that VE reduced anxiety among healthy adults in a stress-inducing situation and that these effects happened independently of the beta-blocker propranolol (Kohnen & Oswald, 1988). In a randomized placebo-controlled study with generalized anxiety disorder patients, both VE and diazepam groups were shown to reduce symptoms of anxiety measured by the Hamilton Anxiety Scale, while placebo had no effect (Andreatini *et al.*, 2002). Another randomized placebo-controlled trial involving patients with Obsessive Compulsive Disorder found a positive effect of VE on Young Mania Rating Scale when compared to placebo (Pakseresht *et al.*, 2011).

Current evidence on the pathophysiology of anxiety disorders indicates that serotonergic, noradrenergic, glutamatergic, and GABA-ergic transmissions are involved. This is confirmed by the clinical efficacy of SSRIs, selective serotonin and noradrenalin reuptake inhibitors (SNRIs), and benzodiazepines (Sarris *et al.*, 2011).

VE contains compounds with a range of properties relevant to reduce anxiety symptoms. The sedative effect has been connected to the interaction of some compounds with the

GABA-ergic neurotransmitter receptor system that acts as enhancer of GABA inhibitory signalling in the CNS. It has been proposed that, in animal models, the sedative and antianxiety effects are related to VE modulation of GABA-A receptors (GABA-ARs) (Yuan *et al.*, 2004). The main compound responsible for the observed anxiolytic effect is valerenic acid that allosterically modulates GABA-A Rs. It acts as a subunit-specific (β 3) modulator of GABA-AR. (Benke *et al.*, 2009) (Becker *et al.*, 2014) (Khom *et al.*, 2010). The question of the mechanism of action is still not fully answered and the role and the mechanism of the different compounds is still a matter of debate. Furthermore, the effects of VE intake on cortical inhibitory (GABAergic) circuits in humans still need to be investigated.

Adaptogenic herb: Rhodiola Rosea extract

Rhodiola rosea, known as "golden root" or "roseroot" is a flowering herb belonging to the plant family Crassulaceae that has been traditionally used in Northern Europe, Asia, and Russia for centuries to reduce stress-related symptoms. Rhodiola rosea was used to increase physical endurance, work productivity, resistance to high altitude sickness; for the treatment of fatigue, depression, anemia, impotence, gastrointestinal ailments, infections and nervous system disorders.

In Asia, Rhodiola rosea tea was recommended to treat and prevent cold and flu during the winter. Its traditional use as a general stimulant in Siberian and Russian medicine supported extensive research that identified Rhodiola rosea as an adaptogen, a substance that increases the ability of an organism to adapt to environmental demands (Brown *et al.*, 2002).

Rhodiola rosea contains different compounds including phenylpropanoids (rosavin, rosin, rosarin); phenylethanol derivatives (salidroside or rhodioloside, tyrosol); flavanoids (rodiolin, rodionin, rodiosin); monoterpernes (rosiridol, rosaridin) triterpenes and phenolic acids. The main active compounds thought to be responsible for the CNS effects and adaptogenic action are rosavins and salidroside. The term rosavins is used to include rosavin, rosin, and rosarin. Therefore, Rhodiola rosea extract (RRE) as dried root extract used in human clinical studies is mostly standardized to minimum 3% rosavins and 0.8-1% salidroside (Brown *et al.*, 2002).

RRE has been investigated in several clinical trials that showed its effectiveness in mild depression, anxiety symptoms and in improving fatigue related to mental stress. It is considered as a stimulating adaptogen with antidepressant activity (Sarris, 2018). A randomized, double-blind, placebo controlled clinical study in healthy military cadets in

Russia investigated the influence of RRE (370 and 555 mg doses) on various mental and biological parameters discovering a pronounced antifatigue effect (Shevtsov *et al.*, 2003). Another double-blinded, placebo controlled trial of patients with mild to moderate depression receiving RRE in daily dosages of either 340 mg or 680 mg over 6 weeks showed significantly greater improvement in overall depression, insomnia, emotional instability and somatisation as measured by the Hamilton Rating Scale for Depression (HAMD) scores compared to the placebo group (Darbinyan *et al.*, 2007). RRE has been studied as well for its effect as an adaptogen. A double-blind, placebo-controlled trial of 60 individuals suffering from stress-related fatigue receiving 576 mg of RRE (2 tablets twice a day) for 28 days reported significant effects of the extract versus placebo on a battery of tests measuring fatigue and stress (Olsson *et al.*, 2009). A double-blind, randomized, placebo-controlled trial involving 20 students taking 2 tablets a day of RRE for 20 days during an exam period showed significant improvement in psychomotor function and mental fatigue (Spasov *et al.*, 2000). A multicenter, non-randomized, open-label, single-arm trial of 101 subjects with life stress symptoms receiving 200 mg of RRE twice a day for 4 weeks reported significantly improved scores in a battery of tests used to measure stress and fatigue levels (Edwards *et al.*, 2012). Moreover, RRE has been demonstrated to significantly improve endurance exercise capacity in young healthy volunteers after an acute intake (De Bock *et al.*, 2004). Several studies pointed out the usefulness of RRE as an adaptogen because of its observed ability to increase resistance to various chemical, biological, and physical stressors (Panossian *et al.*, 2010) (Edwards *et al.*, 2012) (Kasper & Dienel, 2017) (Lekomtseva *et al.*, 2017).

The mechanism of action may be related to RRE stimulation of noradrenalin, dopamine, and serotonin receptors in selected brain regions (Amsterdam & Panossian, 2016). MAO inhibition has been proposed to be responsible for the antidepressant activity and rosiridin

has been shown to be a potent inhibitor of MAO A and B in vitro (van Diermen *et al.*, 2009). At the cortical and brainstem level it was demonstrated that sub-chronic treatment (10 days) with RRE increased the concentration of serotonin (Qin *et al.*, 2008). Studies in rats have also shown that the level of serotonin in the brain was increased after oral administration of RRE (Chen *et al.*, 2009). Several preclinical studies conducted in cell lines and animal models showed the presence of biochemical and pharmacological stress-reducing actions of RRE. The administration of salidroside in olfactory bulbectomized rats showed anti-inflammatory effects, regulated the HPA axis activity, induced the transcription of brain-derived neurotrophic factor (BDNF) and improved depressive-like behaviour (Yang *et al.*, 2014). In addition, the extracts and active compounds demonstrated cognitive enhancing effect in rodents (Petkov *et al.*, 1986) (Palmeri *et al.*, 2016). The adaptogenic effects seem to occur via regulation of the HPA axis that modifies the stress response through inhibition of cortisol secretion, mediation of kinase enzymes and modulation of monoamines (Panossian *et al.*, 2010). Moreover anti-oxidative/anti-inflammatory mechanisms have been considered to explain its potential protection against heart and brain disease (Lee *et al.*, 2013) (Anghelescu *et al.*, 2018). Despite numerous preclinical and clinical studies its adaptogenic mechanism of action still needs to be addressed in humans.

Non-invasive brain stimulation to probe cortical excitability and neuroplasticity in humans

Brain stimulation represents a growing area of research with valuable option for clinical application. Non-invasive brain stimulation (NIBS) protocols uses electrical or magnetic stimulations to induce different effects on the neuronal circuits. They offer different ways to target specific brain regions and to identify specific functional role of brain structures. Transcranial direct current stimulation (tDCS) and Transcranial Magnetic Stimulation (TMS) are the most commonly used NIBS methods in humans (He *et al.*, 2018).

Transcranial Magnetic Stimulation

TMS was first introduced in 1985 by Barker et al. to investigate the integrity and function of the human corticospinal system in healthy subjects and in patients with neurological disorders (Barker *et al.*, 1985). This procedure uses magnetic fields to stimulate nerve cells in the brain, based on the principle of electromagnetic induction, as discovered by Michael Faraday in 1838. If a pulse of electrical current passing through a coil placed over a person's head has sufficient strength and short enough duration, rapidly changing magnetic pulses are generated that penetrate the scalp and the skull to reach the brain with little attenuation by extracerebral tissues. These pulses induce an electric field sufficient to cause localized neuronal depolarization and to activate neural networks in the cortex (Paulus *et al.*, 2008). The TMS induced current is oriented parallel to the coil and stimulates neurons that lie in the same plane. The horizontally oriented axons in the cortex are preferentially depolarized when the coil is held tangentially to the head. Therefore, radially oriented neurons are affected by TMS mainly via synaptic input from horizontally oriented elements (Kapogiannis & Wassermann, 2008).

TMS can be applied in single, paired and repetitive pulses which can be delivered through different shaped coils. The most frequently used are round or figure eight coils. The figure eight coil produces a stronger and more focal field than the circular one. TMS of primary motor cortex (M1) is most commonly used to study the human motor system. When single TMS is applied over the motor cortex at appropriate stimulation intensity, a brief, relatively synchronous muscle response, the motor-evoked potential (MEP), can be recorded from a contra-lateral extremity muscle. Single- and paired-pulse TMS paradigms allow for the assessment of cortical excitability: motor threshold (MT), MEP, cortical silent period (CSP), short intracortical inhibition (SICI) and intracortical facilitation (ICF) are the most common parameters used to probe corticospinal excitability (Ziemann, 2004). MT refers to the lowest

TMS intensity necessary to evoke MEPs in the target muscle when single-pulse stimuli are applied to the motor cortex. MT is defined as the lowest intensity required to elicit MEPs of more than 50 μV peak-to-peak amplitude in at least 50% of successive trials in target muscles. It is a global measure of corticospinal excitability (Di Lazzaro *et al.*, 2008) (Paulus *et al.*, 2008). MEP is defined as the overall response of a peripheral muscle, measured by electromyography (EMG), that is induced via TMS delivered over the contralateral motor cortex (Rothwell *et al.*, 1999). MEP amplitude reflects the recruitment and the excitability of the corticospinal tract and it increases with TMS intensity in a sigmoid fashion (Ziemann *et al.*, 2015). The CPS refers to TMS-induced interruption of the EMG activity during a voluntary contraction of a target muscle and depends on inhibitory mechanisms at the level of the motor cortex. The exact mechanism of CPS is still unknown (Caipa *et al.*, 2018). The paired pulse TMS paradigm couples a suprathreshold magnetic stimulus with a preceding (conditioning) subthreshold stimulus and the response to the paired stimuli results in a physiological reduction (inhibition) or increase (facilitation) of MEP amplitude. These effects depend on the interstimulus interval (ISI), measured in milliseconds (ms): at short ISI (1–4 ms) the conditioning stimulus determines an intracortical inhibition with respect to the test stimulus, whereas at longer ISI (>5ms) the effect is an intracortical facilitation (Hallett, 2007). SICI reflects the status of the intracortical GABAergic system mediated mainly by GABA-ARs, as well as dopamine and acetylcholine. On the contrary, ICF reflects a more complex phenomenon and it is an indirect measure of glutamatergic N-methyl-D- aspartate (NMDA) mediated neurotransmission (Paulus *et al.*, 2008).

TMS of the motor cortex can be paired with electric stimulation of a peripheral nerve (median or ulnar nerve) to investigate both short-latency afferent inhibition (SAI) and long-latency afferent inhibition (LAI). If the time interval between TMS and peripheral nerve stimulation is approximately 20–25 ms, MEPs recorded in hand muscles can be

suppressed. This condition is defined as SAI. LAI is the second inhibition period and it is shown for ISIs between 100 and 500 ms (Ziemann, 2004). TMS provides a method to study inhibitory and excitatory circuits in the human brain. TMS has been also used to better understand the physiological effects of drug-induced excitability changes after the administration of CNS active drugs (Ziemann *et al.*, 2015) (Paulus *et al.*, 2008).

Pharmaco-TMS: cortical excitability in humans

Pharmaco-TMS experiments offer an opportunity to explore the neurophysiological effects of psychoactive drugs in vivo in the human brain. Drugs with a known mechanism of action have been used to study TMS measures of motor cortex excitability and to provide a better understanding of several TMS parameters. Moreover, TMS measures have been used to identify the mechanism of action of the studied drug at the system level of the human motor cortex. TMS measures of cortical excitability have been studied at one or several time points after the intake of a single dose compared to a baseline obtained prior to the intake of the drug. The effects on a given TMS measure have been tested in healthy subjects and allowed to identify inference on the physiological mechanisms of these measures (Ziemann, 2013). The best level of scientific evidence for drug effects on TMS measures of motor excitability is provided by randomized placebo-controlled double-blind crossover design studies. Several parameters have been identified as being modulated by drug intake:

-MT is modulated by glutamatergic intracortical synaptic transmission; it increases after voltage-gated sodium channel blockers intake in particular anticonvulsants such as carbamazepine and lamotrigine (Ziemann *et al.*, 1996) (Tergau *et al.*, 2003). On the contrary, it was found that NMDA antagonist ketamine that increases indirectly glutamatergic neurotransmission through the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, decreases MT (Di Lazzaro *et al.*, 2003). GABAergic drugs and other drugs affecting the serotoninergic, dopaminergic and noradrenergic systems do not produce consistent effects on MT (Paulus *et al.*, 2008).

-MEP amplitude: prior human pharmaco-TMS studies showed that this excitatory circuit network is regulated by glutamatergic, GABAergic, noradrenergic and serotonergic neurotransmitters. Positive allosteric modulators of the GABA-AR, such as the benzodiazepines diazepam and lorazepam decrease MEP amplitude. Acute modulation of

other neurotransmission systems (noradrenaline agonists methylphenidate, D-amphetamine, reboxetine and serotonin agonists sertraline and paroxetine) increase it (Ziemann, 2013).

-CSP is modulated by inhibitory mechanisms involving GABA-B receptor (GABA-BR) subtypes. However, this previous hypothesis has been reconsidered because of some drugs, such as baclofen, a specific GABA-BR agonist, have no significant effect on CSP duration. Moreover, the administration of benzodiazepines which act on GABA-AR subtypes showed to lengthen the CSP. It is widely accepted that CSP duration is not affected by drugs that modify facilitatory mechanisms (Caipa *et al.*, 2018).

-SAI is modulated by acetylcholine and GABA as it can be changed with GABA-A agonists.

-LAI probably concerns the basal ganglia or cortical association areas.

-SICI reflects the status of the intracortical GABAergic system mediated mainly by GABA-ARs receptors. Previous studies showed that benzodiazepines and other GABAergic agents that act on GABA-ARs enhance SICI. However, the GABA-AR antagonist flumazenil does not affect SICI and this result was related to the lack of a tonic activity at the benzodiazepine binding site of the GABA-AR in normal human motor cortex (Paulus *et al.*, 2008). Drugs that interfere with membrane excitability and block voltage-gated channels do not have an effect on SICI (Di Lazzaro *et al.*, 2005) (Ziemann *et al.*, 2015). Other pharmaco-TMS studies showed an effect of monoamines on SICI. Previous studies using SSRIs demonstrated that the ingestion of a single oral dose of these drugs induced an increase of SICI and a reduction of ICF. The increase in SICI was linked to the enhanced activity of the GABAergic inhibitory interneurons while the mechanism through which SSRIs exhibited their effects on ICF are not consistent across studies (Ilic *et al.*, 2002) (Eichhammer *et al.*, 2003) (Robol *et al.*, 2004).

- ICF reflects a more complex phenomenon that is modulated by both excitatory and inhibitory mechanisms. It reflects most likely NMDAR-mediated excitatory neurotransmission as the intake of NMDAR antagonists dextromethorphan and memantine leads to its reduction (Ziemann *et al.*, 2015). Although ICF modulation involves facilitation, it does not require any alteration in membrane excitability and therefore remains unaffected by channel-blocking drugs (Boroojerdi, 2002). GABAergic drugs, specifically those with GABA-A activity, have been found to produce a reduction in ICF duration (Ziemann, 2004) (Kapogiannis & Wassermann, 2008). Pharmaco-TMS studies testing ICF agree with the observation that ICF represents an expression of a cortical net facilitation involving facilitation and weaker inhibition (Paulus *et al.*, 2008).

Several factors may contribute to the pharmaco-TMS experiments response, such as genetic polymorphisms of proteins involved in the regulation of neuronal excitability, age and the phase of the menstrual cycle. Moreover, several drugs do not have an exclusive mechanism of action in humans and, consequently, TMS measures often do not allow definite conclusions about the mechanisms involved (Ziemann, 2013).

Transcranial Direct Current Stimulation

TDCS uses low-amplitude currents delivered to the brain area of interest through anodal and cathodal scalp electrodes. The current penetrates the skull, enters the brain, and affects the resting membrane threshold by modulating ion channels or shifting electrical gradients across the neural membrane (Huang *et al.*, 2017).

The current does not induce action potentials, but rather modulates the spontaneous neuronal activity in a polarity-dependent manner. It is considered that tDCS induces persisting excitability changes in the human motor cortex that are selectively controlled by the polarity, duration and current strength of stimulation. During anodal stimulation the current flows from the active electrode to the reference electrode while in the cathodal stimulation the current flows from the reference electrode to the active electrode. Therefore the positively charged electrode is defined as an anode, while the negatively charged electrode is defined as a cathode (He *et al.*, 2018). Anodal tDCS applied to the motor cortex at rest increases the excitability of the underlying cortex (depolarization), while cathodal tDCS applied over the same area decreases it (hyperpolarization). These effects are demonstrated by MEP amplitude changes studied with TMS. After anodal tDCS applied between 5 and 20 min at 1 milliampere (mA), MEP size increases. Cathodal tDCS with these stimulation parameters reduces MEP amplitude (Nitsche *et al.*, 2003) (Nitsche & Paulus, 2000). The after-effects described above are mediated by synaptic plasticity changes and involve the glutamatergic system. Previous research has shown that tDCS may induce neuroplasticity effects similar to long-term potentiation (LTP) and long-term depression (LTD)-like mechanisms (Milev *et al.*, 2016).

LTP-like and LTD-like plasticity in humans

Synaptic plasticity is defined as the continuous remodelling of brain function in response to external stimuli, by way of short and long-term changes of interneuronal connections. It is a CNS adaptive property consisting of the ability of synapses to strengthen or weaken over time, in response to increases or decreases of activity and to optimize the brain network function. Eric Kandel and his group in 1969 found the first evidence for the molecular basis of neuroplasticity by studying the invertebrate sea-slug, Aplysia californica and afterwards with the characterization of LTP in the mammalian hippocampus. In 1973 Bliss and Lomo provided a molecular mechanism for neuroplasticity that follows Hebbian principles (Bliss & Lomo, 1973). The Hebbs's rule states that "when an axon in cell A is near enough to excite cell B and repeatedly and persistently takes part in firing it, some growth or metabolic process takes place in one or both cells such that A's efficacy in firing B, is increased" (Hebb, 1949). The theory has been considered to describe the adaptation of neurons during the learning process and to characterize LTP. To explain the origin and modulation of neural plasticity, several mechanisms have been proposed including LTP and LTD, second messengers pathway activation, gene transcription, neuronal morphological modifications. The two most important forms of long-lasting synaptic plasticity are LTP and LTD, characterized by a long-lasting increase or decrease in synaptic strength, respectively. It is suggested that they are involved in information storage and therefore in learning and memory processes (Collingridge *et al.*, 2010). Regarding LTP, it is induced typically by a 100 Hz high frequency stimulation (HFS) protocol. Its induction is related to the activation of NMDA receptors (NMDA-Rs) during postsynaptic depolarization, with an increase of calcium levels due to calcium influx through the NMDAR channel (He *et al.*, 2018). On the other hand, voltage- gated calcium channels (VGCCs) may be involved in the induction of LTP. These mechanisms may operate cooperatively, for example when calcium influx

through VGCCs leads to the depolarization of the postsynaptic membrane and to the release of the magnesium block from NMDARs, there will be a facilitation of the induction of NMDAR-dependent LTP. The increase in postsynaptic calcium triggers intracellular signaling pathways including the activation of protein and tyrosine kinases, that will result in phosphorylation of AMPA receptors (AMPAR) in the postsynaptic membrane and in new AMPARs (Ziemann *et al.*, 2015).

LTD is characterized by enduring reductions in synaptic efficacy that involves both metabotropic glutamate receptors (mGluRs) and NMDARs. Both forms of LTD result in down-regulation of synaptic transmission and are triggered by lower levels of postsynaptic calcium and are mediated by AMPAR internalization. LTD takes part in many mechanism including the dynamic range in which synapses can operate, the prevention of synapses saturation state, the encoding and the refining of memories (Connor & Wang, 2016). Changes in cortical plasticity can be induced and studied by using NIBS. LTP and LTD have been studied in basic science studies and the knowledge obtained led to the development of several TMS paradigms that allowed to probe neuroplasticity in the awake human cortex. tDCS and TMS can modulate cortical excitability and plasticity. tDCS has been used to induce neuroplasticity in humans and it follows the Hebbian rule of neural plasticity. It is suggested that tDCS induces plasticity in humans by acting on intracellular calcium dynamics since calcium channel blockage abolished LTP-like plasticity induced by anodal tDCS (Nitsche *et al.*, 2003).

Effects of conventional drugs on tDCS-induced LTP- like and LTD-like plasticity in humans

Several studies have shown that tDCS-induced cortical plasticity is modulated by CNS active drugs in humans, providing significant evidence on the effects of these pharmacological interventions on cortical plasticity. The size of the MEP amplitude, evoked by TMS over M1 is used to quantify changes in cortical excitability that are induced by tDCS (Ziemann *et al.*, 2015). tDCS effects on plasticity can be modulated by drugs that affect synaptic dynamics. Previous studies on healthy subjects provided evidence for an NMDA-R-dependence of tDCS induced cortical plasticity. tDCS-induced LTP-like and LTD-like plasticity was blocked by the NMDA-R antagonist dextromethorphan (Liebetanz *et al.*, 2002) while instead the partial NMDA-R agonist D-cycloserine increased tDCS-induced LTP (Nitsche *et al.*, 2004b). Furthermore, the role of serotonin as a plasticity modulator able to affect tDCS-induced plasticity has been supported by previous results in humans. It was shown that a single oral dose of the SSRI citalopram enhanced and prolonged LTP-like M1 plasticity induced by anodal tDCS and converted cathodal tDCS-induced LTD-like plasticity into facilitation in healthy subjects (Nitsche *et al.*, 2009). More complex effects have been shown to appear in other neuromodulators such as dopamine that has a dose-dependent inverted U-shaped like effect on LTP like plasticity (Ziemann *et al.*, 2015). Regarding the noradrenergic system, previous pharmaco-TMS studies in humans showed that amphetamine intake enhanced and prolonged the tDCS anodal effects, while propranolol reduced anodal tDCS-induced LTP like plasticity (Nitsche *et al.*, 2004a). Moreover, the selective noradrenaline inhibitor (NRI) reboxetine reverted cathodal tDCS-induced LTD-like plasticity into facilitation in healthy subjects (Kuo *et al.*, 2017). Anodal and cathodal effects on M1 in humans were abolished by nicotine (Thirugnanasambandam *et al.*, 2011) and

cholinesterase-blockers (Kuo *et al.*, 2007). These previous pharmaco-TMS studies indicate that a large number of CNS active drugs affected these parameters in different ways. The following table by Ziemann et al. 2015, summarizes these results.

Acute effects of CNC active drugs on tDCS induced-like plasticity			
System/Drug	**Mode of action**	**Protocol**	**Effect**
The glutamatergic system			
Cycloserine	Partial NMDA agonist	Anodal tDCS	Increase/facilitation
Dextromethorphan	NMDAR antagonist	Anodal tDCS Cathodal tDCS	Decrease/suppression Decrease/suppression
Voltage-gated ion channels			
Flunarizine	T-type VGCC antagonist	Anodal tDCS Cathodal tDCS	Decrease/suppression No effect
Carbamazepine	VGSC antagonist	Anodal tDCS Cathodal tDCS	Decrease/suppression No effect
The GABAergic system			
Lorazepam	GABAAR agonist	Anodal tDCS Cathodal tDCS	Increase/decrease No effect
The dopaminergic system			
L-Dopa (low/high dose)	DR agonist	Anodal tDCS Cathodal tDCS	Decrease/suppression Decrease/suppression
L-Dopa (medium dose)	DR agonist	Anodal tDCS Cathodal tDCS	Switch to LTD No effect
L-Dopa (low/high dose) + Sulpiride	D1R stimulation	Anodal tDCS Cathodal tDCS	Decrease/suppression Switch to LTP
L-Dopa (medium dose) + Sulpiride	D1R stimulation	Anodal tDCS Cathodal tDCS	No effect Decrease/suppression
Ropinirole (low/high dose)	D2/D3R agonist	Anodal tDCS Cathodal tDCS	Decrease/suppression Decrease/suppression
Ropinirole (medium dose)	D2/D3R agonist	Anodal tDCS	No effect

		Cathodal tDCS	No effect
Sulpiride	D2R antagonist	Anodal tDCS Cathodal tDCS	Decrease/suppression Decrease/suppression
The cholinergic system			
Rivastigmine	Cholinesterase inhibitor m/nAChR stimulation	Anodal tDCS Cathodal tDCS	Decrease/suppression Increase/decrease
Nicotine	Cholinesterase inhibitor m/nAChR stimulation nAChR agonist	Anodal tDCS Cathodal tDCS	Decrease/suppression Decrease/suppression
The serotonergic system			
Citalopram	SSRI	Anodal tDCS Cathodal tDCS	Increase/facilitation Switch to LTP
The adrenergic system			
Amphetamine	Monoamine reuptake inhibitor	Anodal tDCS Cathodal tDCS	Increase/facilitation Decrease/suppression
Propanolol	Beta-adrenergic antagonist	Anodal tDCS	Decrease/suppression

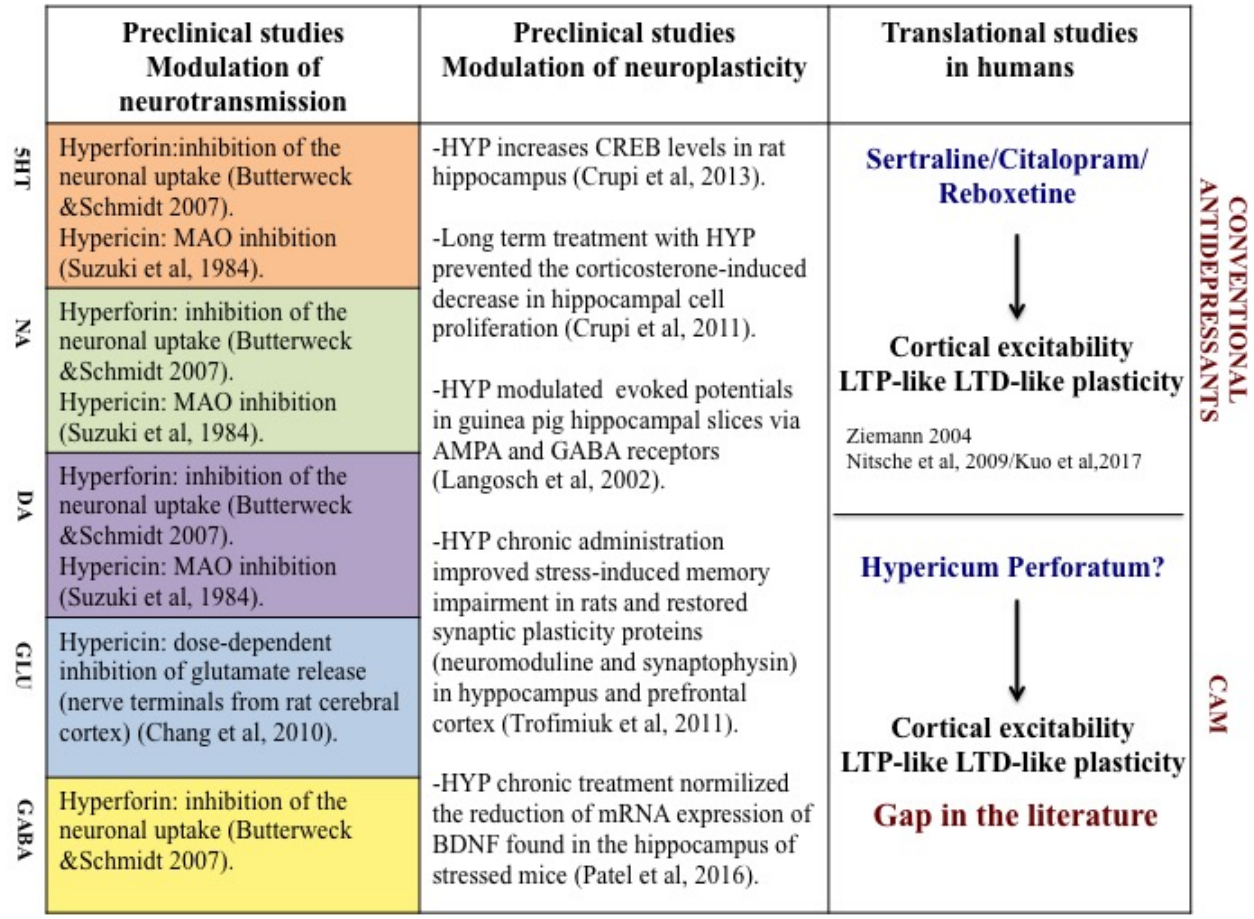
Adapted from Ziemann et al., 2015

STUDY 1

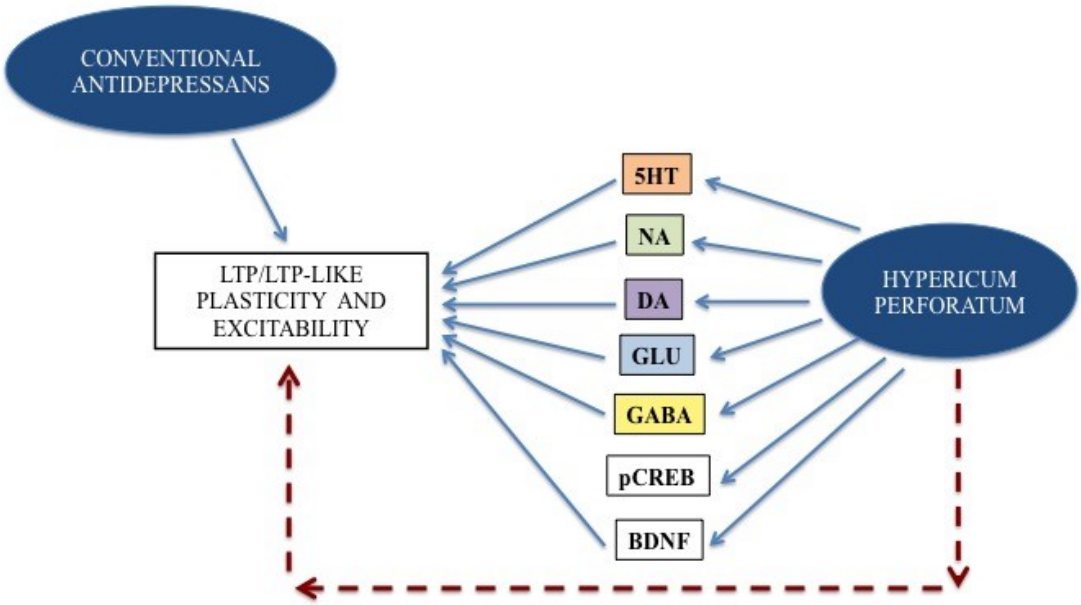
Rational

Preclinical studies support the effect of HYP extract compounds on several neurotransmitters (Butterweck & Schmidt, 2007) (Suzuki *et al.*, 1984) (Chang & Wang, 2010). Modulation of neurotransmitters with conventional drugs has an effect on cortical excitability tested with TMS in humans (Ziemann, 2004). Furthermore preclinical evidence indicates that HYP extract administration induces plastic changes in the brain of rodents (Crupi *et al.*, 2011) (Crupi *et al.*, 2013) (Langosch *et al.*, 2002) (Trofimiuk *et al.*, 2011) (Patel *et al.*, 2016).

Translational studies in humans showed that the administration of conventional antidepressants affected both cortical excitability (reduced ICF, enhanced SICI, changes in MEP amplitude and RMT) and plasticity (Ziemann, 2013). A single dose of the SSRI citalopram enhanced and prolonged LTP-like plasticity induced by anodal tDCS and converted cathodal tDCS-induced LTD-like plasticity into facilitation in healthy subjects (Nitsche *et al.*, 2009). The administration of the NRI reboxetine reverted cathodal tDCS-induced LTD-like plasticity into facilitation in healthy subjects (Kuo *et al.*, 2017). Studies assessing CAM and neuroplasticity in humans are scarce. In this study it was explored whether the administration of HYP extract with well documented antidepressant activity in rodents and humans modulates cortical excitability and neuroplasticity in humans.


Preclinical studies
Modulation of neurotransmission
Preclinical studies
Modulation of neuroplasticity
Translational studies
in humans
5HT
Hyperforin:inhibition of the neuronal uptake (Butterweck &Schmidt 2007).
Hypericin: MAO inhibition (Suzuki et al, 1984).
NA
Hyperforin: inhibition of the neuronal uptake (Butterweck &Schmidt 2007).
Hypericin: MAO inhibition (Suzuki et al, 1984).
DA
Hyperforin: inhibition of the neuronal uptake (Butterweck &Schmidt 2007).
Hypericin: MAO inhibition (Suzuki et al, 1984).
GLU
Hypericin: dose-dependent inhibition of glutamate release (nerve terminals from rat cerebral cortex) (Chang et al, 2010).
GABA
Hyperforin: inhibition of the neuronal uptake (Butterweck &Schmidt 2007).
-HYP increases CREB levels in rat hippocampus (Crupi et al, 2013).
-Long term treatment with HYP prevented the corticosterone-induced decrease in hippocampal cell proliferation (Crupi et al, 2011).
-HYP modulated evoked potentials in guinea pig hippocampal slices via AMPA and GABA receptors (Langosch et al, 2002).
-HYP chronic administration improved stress-induced memory impairment in rats and restored synaptic plasticity proteins (neuromoduline and synaptophysin) in hyppocampus and prefrontal cortex (Trofimiuk et al, 2011).
-HYP chronic treatment normilized the reduction of mRNA expression of BDNF found in the hippocampus of stressed mice (Patel et al, 2016).
Sertraline/Citalopram/ Reboxetine
Cortical excitability
LTP-like LTD-like plasticity
Ziemann 2004
Nitsche et al, 2009/Kuo et al,2017
Hypericum Perforatum?
Cortical excitability
LTP-like LTD-like plasticity
Gap in the literature
CONVENTIONAL ANTIDEPRESSANTS
CAM

Theoretical Framework



Aim

The aim of the study was to investigate the cortical neuromodulatory effects of HYP extract in healthy subjects using a broad array of TMS measures of motor excitability and tDCS-induced LTP-/LTD-like plasticity.

Study design

This is a double-blind, randomized, crossover study in a sample of healthy volunteers.

Independent variable (treatment): two levels HYP extract and placebo.

Multiple dependent variables: TMS parameters of cortico-spinal excitability (RMT, MEP, MEP recruitment, CPS, SICI, ICF, F_{wave}, M_{max}) and the tDCS-induced parameters of cortical plasticity (LTP, LTD).

Participants

Inclusion criteria	Exclusion criteria
Aged between 18 to 50 Being alert, cooperative and both willing and able to participate in the study Being able to provide informed consent	History of epilepsy History of brain trauma History of stroke, brain tumors, brain infections, vascular malformations Exposure to poisons Any bodily metal implants Any device that may be affected by TMS (pacemaker, medication pump, cochlear implant, implanted brain stimulator) Pregnancy and breast-feeding History of long-term alcohol or drug use Sleep deprivation Current use of medications Any current serious medical illness

Research Questions

The research questions being asked are:

RQ1- Does HYP extract intake modulate corticospinal excitability tested with TMS in healthy subjects?

RQ2- Does HYP extract intake modulate LTP-like plasticity induced by anodal tDCS in healthy subjects?

RQ3- Does HYP extract intake modulate LTD-like plasticity induced by cathodal tDCS in healthy subjects?

Research Hypothesis

$H_0$1: There will be no significant difference in corticospinal excitability in healthy subjects after the intake of HYP extract or a placebo.

H_A1: There will be significant difference in corticospinal excitability in healthy subjects after the intake of HYP extract or a placebo.

$H_0$2: There will be no significant difference in LTP-like plasticity induced by anodal tDCS in healthy subjects after intake of HYP extract or a placebo.

H_A2: There will be significant difference in LTP-like plasticity induced by anodal tDCS in healthy subjects after intake of HYP extract or a placebo.

$H_0$3: There will be no significant difference in LTD-like plasticity induced by cathodal tDCS in healthy subjects after intake of HYP extract or a placebo.

H_A3: There will be no significant difference in LTD-like plasticity induced by cathodal tDCS in healthy subjects after intake of HYP extract or a placebo.

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ORIGINAL INVESTIGATION

Hypericum perforatum extract modulates cortical plasticity in humans

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Abstract
Background Hypericum perforatum (HYP) extract is one of the most commonly used complementary alternative medicines (CAMs) for the treatment of mild-to-moderate depression. Non-invasive brain stimulation protocols can be used to investigate the effect of psychoactive substances on the human brain. In this study, we explored the effect of a single dose of HYP extract (WS 5570) intake on corticospinal excitability and plasticity in humans.
Methods Twenty-eight healthy subjects were required to intake 900 mg of either HYP extract or placebo. Cortical excitability was assessed using single and paired transcranial magnetic stimulation (TMS). The electrophysiological parameters of motor threshold, recruitment of motor-evoked potentials (MEPs), cortical silent period (CSP), short interval intracortical inhibition (SICI), and intracortical facilitation (ICF) were tested before and 2 and 5 h after the oral intake. Spinal and neuromuscular excitability and peripheral nerve excitability were measured by *F* response and M-wave. Cortical plasticity was induced using transcranial direct current stimulation (tDCS). Subjects received either HYP extract or placebo before anodal and cathodal tDCS of the primary motor cortex. Plasticity was assessed by MEP amplitudes.
Results HYP extract reversed cathodal tDCS-induced long-term depression (LTD)-like plasticity into facilitation, as compared to placebo. HYP extract did not have a significant effect on anodal tDCS-induced plasticity and TMS measures of motor cortex and spinal/neuromuscular excitability.
Conclusions Our findings suggest that a single oral dose of HYP extract modulates cortical plasticity in healthy subjects and provide new insight into its possible mechanism of action in humans.

Keywords *Hypericum perforatum* extract · TMS · tDCS · Cortical plasticity

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Introduction

Though there are many approved antidepressant treatments for mood and anxiety disorders, inadequate response, delayed onset of clinical improvement, and tolerability issues have led to increasing interest in the potential role of complementary and alternative medicine (CAM) (Kessler et al. 2001). With regard to herbal treatments, *Hypericum perforatum* (HYP) extract is one of the most researched CAM recommended as first-line monotherapy for the treatment of mild to moderate depression and as a second-line adjunctive treatment for moderate to severe depression (Ravindran et al. 2016), (Kasper et al. 2006) (Lecrubier et al. 2002) (Kalb et al. 2001) (Ross 2014). Although the HYP extract mechanism of action still needs to be characterized, several compounds such as phloroglucines (hyperforin, pseudohyperforin), naphthodianthrones (hypericin, pseudohypericin), and flavonoids (quercitrin, isoquercitrin, hyperoside) are considered to play a role in its therapeutic effects

(Butterweck and Schmidt 2007). Furthermore, the commercially available extracts are usually standardized by either their hypericin or hyperforin content, compounds that alone have been shown to have antidepressant activity (Schmidt and Butterweck 2015). Consistent with this evidence, a large number of preclinical studies have suggested that the HYP extract antidepressant activity might be attributed to the inhibition of the synaptic uptake of serotonin, dopamine, and noradrenaline (Muller 2003), to the modulation of glutamatergic and gamma-aminobutyric acid (GABAergic) circuits (Schmidt and Butterweck 2015) and to neuroplasticity (Crupi et al. 2011). Moreover, although the antidepressant activity is confirmed, the mechanisms of action in humans still need to be fully addressed (Mennini and Gobbi 2004). Non-invasive brain stimulation (NIBS) techniques such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) represent a new means for investigating the effect of drugs and potential neuropsychiatric therapeutic interventions on cortical excitability and plasticity. Furthermore, previous pharmaco-TMS studies examined the effects of the administration of a single dose of a neuroactive drug acting on a specific neurotransmitter or neurochemical system on measures of corticospinal excitability. This pharmacological modulation of the TMS parameters provides indirect evidence for neurotransmitters and receptor physiology in the human cortex. In this way, parameters such as motor threshold (MT), motor-evoked potential (MEP) amplitude, cortical silent period (CSP), short interval intracortical inhibition (SICI), and facilitation (ICF) have been characterized and now offer the unique opportunity to translate preclinical pharmacological results in humans. MT is consistently modulated by sodium- and calcium-channel blockers and the N-methyl-D-aspartate receptor (NMDAR) antagonist ketamine. MEP amplitude elicited at a given stimulation intensity reflects transsynaptic activation of corticospinal neurons and is modulated by multiple neurotransmitters (GABA, glutamate, norepinephrine, serotonin). CSP is an interruption of the electromyographic activity induced by TMS delivered during muscle contraction and reflects the activity GABAergic circuits (GABAA and GABAB receptors). SICI reflects the activity of intracortical inhibitory circuits and is modulated by GABA through GABAA receptor, norepinephrine, dopamine, and nicotine while ICF mainly reflects the activity of glutamatergic cortical circuits and has a more complex regulation (GABAergic and Noradrenergic) (Ziemann et al. 2015). In addition, the intake of a single dose of antidepressants i.e., serotonin selective reuptake inhibitors (SSRIs) and norepinephrine reuptake inhibitors (NRIs) affects excitatory and inhibitory cortical circuits tested with TMS (Ziemann et al. 2014) (Ilic et al. 2002) (Eichhammer et al. 2003) (Robol et al. 2004) (Paulus et al. 2008).

TDCS offers the possibility of investigating long-term potentiation (LTP)- and long-term depression (LTD)-like phenomena in the human brain (Nitsche and Paulus 2000) (Nitsche et al. 2008). It is now accepted that psychiatric disorders, such as depression, are accompanied by impaired neuroplasticity (Pittenger and Duman 2008) (Porter et al. 2003) (Malenka and Bear 2004) and that the therapeutic effect of conventional antidepressant treatments are paralleled by functional and morphological plastic changes (Santarelli et al. 2003). Previous studies indicate that depressed patients display a decrease in LTP-like plasticity (Kuhn et al. 2016) (Normann et al. 2007). Importantly, it has been demonstrated that both acute and chronic intake of SSRIs and NRIs modulate LTP-/LTD-like plasticity in healthy subjects (Nitsche et al. 2009) (Batsikadze et al. 2013) (Kuo et al. 2016).

To date, no studies have investigated the effects of HYP extracts intake on cortical excitability and plasticity in humans. Here, in a double-blind, randomized, placebo-controlled crossover design, we investigated the cortical neuromodulatory effects of HYP extracts in healthy subjects using a broad array of TMS measures of motor excitability and tDCS-induced LTP-/LTD-like plasticity. Because of the large number of basic science studies that highlighted the effects of HYP extract on neurotransmitter release and synaptic transmission, we hypothesized that, compared to placebo, HYP extract intake would induce a modulation of intracortical inhibitory and excitatory circuits and would modulate LTP-/LTD-like plasticity in a similar way as conventional antidepressants. Findings will further our understanding of the neuropsychiatric effect of HYP extracts in humans.

Methods

Subjects

A total of 28 right-handed (Oldfield 1971) healthy volunteers participated in the study. Fourteen subjects (eight males, six females, mean age 25.6 ± 4.9 SD) took part in the cortical excitability experiment and 14 subjects (nine males, five females, mean age 27.6 ± 3.7 SD) participated into the tDCS/plasticity experiment.

All subjects were between 18 and 50 years old. They had no history of medical or neuropsychiatric diseases, no family history of epilepsy, no present pregnancy, no medical implants, and no history of any psychopharmacological treatment.

All participants gave their written informed consent. The study was approved by the Institutional Review Board of the New York College of Podiatric Medicine and adhered to the latest version of the Declaration of Helsinki.

Experimental procedure

This was a randomized double-blinded placebo-controlled crossover design. Three coated tablets, each tablet containing 300 mg of a commercially available HYP extract (WS 5570, standardized to 2% Hyperforin) or placebo (equal amount of

vitamin E), were orally administered. The HYP extract dose was selected because it equals typical daily doses in clinical usage (Szegedi et al. 2005) and has already been demonstrated to significantly affect electroencephalography (EEG) measures of cortical brain activity after a single acute dose intake (Schulz et al. 1998). The order was pseudo-randomized and counterbalanced across subjects. Allocation to the HYP extract or placebo was based on a computer-generated list that was prepared by a study-independent researcher. The allocation was concealed in an envelope and revealed after all data analysis was performed. After a washout period of 1 week for the cortical excitability study and 3 weeks for the LTP-/LTD-like plasticity study, subjects were switched to the alternative arm of the study. Researchers were unaware of subjects' allocation.

Cortical excitability

Fourteen subjects were seated on a comfortable armchair. Ag–AgCl surface electrodes were positioned over the muscle belly and the tendon of the right first dorsal interosseous (FDI) muscle. TMS was applied to the contralateral motor cortex through a figure 8 coil (diameter 90 mm) using a Magstim 200 magnetic stimulator (The Magstim Company, Dyfed, UK). The coil was held tangential to the scalp with the handle pointing backwards and 45° away from the midline at the optimal orientation for activation of the corticospinal system (Rossini et al. 2015). The coil was placed at the optimal scalp position (hot spot) to elicit a maximal MEP in the contralateral FDI. The inter-trial interval was 5 s for all measurements. Surface electromyography was monitored on a computer screen to ensure muscle relaxation. The signal was amplified (Digitimer D360, Letchworth Garden, UK), filtered (band pass 20 Hz to 2.5 kHz), digitized at 5 kHz (Power Micro1401, Cambridge Electronics Design, Cambridge, UK), and stored in a laboratory computer.

We tested resting MT (RMT) intensity, defined as the minimum intensity needed to evoke MEPs of at least 50-μV amplitude in at least five out of ten consecutive trials in the relaxed FDI (Rossini et al. 2015). The motor recruitment curve (RC), a measure of pyramidal tract neuron recruitment, was examined by measuring the size of MEPs elicited at stimulation intensities that increased systemically by 10%, from 110% of RMT to 150%. Ten trials were recorded, and the average MEP area was taken as MEP size.

Cortical silent period (CSP) was tested by delivering TMS to the motor cortex during tonic FDI contraction (50% of maximal voluntary contraction, subjects were provided with visual feedback of force displayed on an oscilloscope) at an intensity of 150% of RMT intensity. Ten trials were collected in each subject. The CSP duration was defined as the time from the MEP to the return to the baseline EMG activity. The CSP durations was determined using a custom MATLAB scripts (Mathworks, Natick, MA, USA) according to a previously published method (Daskalakis et al. 2008). Intracortical excitability was studied with paired-pulse TMS. For this purpose, two magnetic stimuli were delivered through the same coil using a "Y" cable. In this TMS paradigm, a subthreshold conditioning stimulus modulates the MEP amplitude produced by a test stimulus, inhibiting it at interstimulus intervals (ISIs) less than 5 ms (SICI) and facilitating it at ISIs above 8 ms (ICF). SICI was induced with a conditioning stimulus set at 80% of RMT and delivered 2 ms before the test stimulus while for the induction of ICF, the ISI was 10 ms (Kujirai et al. 1993). The test stimulus intensity that induced a MEP amplitude of ~ 1 mV was selected. The different conditions (test alone, conditionin/test ISI 2 ms, conditioning/test ISI 10 ms) were delivered in a randomized order and 20 MEPs were recorded for each condition. Spinal and neuromuscular excitability were tested by measuring F-wave amplitude and the maximum M-wave (M-max). Fifteen F-waves from the right FDI muscle were elicited using bipolar supramaximal electrical stimulation of the ulnar nerve at the wrist (constant current square wave pulses of 0.1 ms duration; 1 Hz). The average peak-to-peak amplitude was measured (Manganotti et al. 1997).

TMS parameters were tested according to published guidelines for the use of TMS in clinical neurophysiology (Rothwell et al. 1999) and were administered in a randomized order with a 5-min interval between the different recording. TMS recordings were performed before (T0), 2 h after (T1), and 5 h (T2) after the HYP extract or placebo intake (Fig. 1).

Cortical plasticity

Fourteen participants were seated on a comfortable chair with head and arm rests. TMS was applied over the left motor cortical representational area of the right FDI, and 20 MEPs were recorded using a stimulation intensity that evoked MEPs of ~ 1 mV amplitude. Two hours after HYP extract WS 5570 or placebo intake, a second baseline was determined with the same TMS intensity to control for a possible influence of the herb on MEP amplitude. Cathodal tDCS was applied for 9 min at 1 mA intensity to elicit LTD-like plasticity. To induce LTP-like plasticity, we applied anodal tDCS for 13 min at 1 mA intensity. Twenty MEPs were immediately recorded (T0), 15 (T15), and 30 (T30) min after tDSC cessation and removal of the tDCS electrodes (Fig. 2a). This protocol was used in previous pharmaco-TMS studies to assess the effect of a single dose of antidepressants intake on neuroplasticity in humans (Nitsche et al. 2009) (Kuo et al. 2016) (Kuo et al. 2017).We used a battery-driven constant current stimulator (Activa Tek, Inc. Salt Lake City, UT) with a maximum output of 2 mA. Two saline-soaked surface sponge electrodes (35 cm^2) were applied to deliver the current. One electrode was positioned over the motor cortex representation area

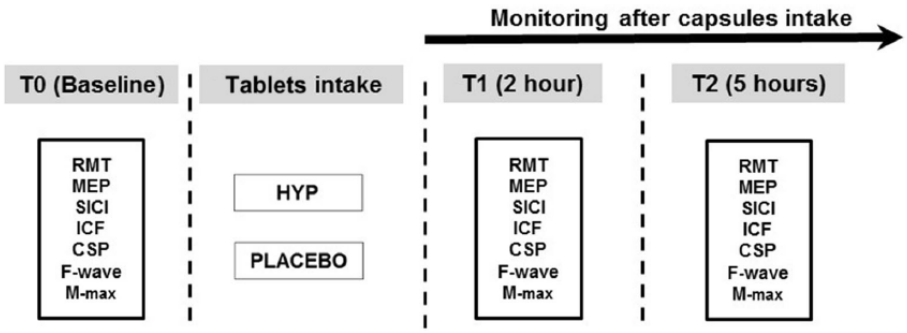

Fig. 1 Experimental design. Fourteen healthy volunteers received three tablets, each tablet containing 300 mg of *Hypericum perforatum* (HYP) extract or placebo in a double-blind crossover design. Transcranial magnetic stimulation (TMS) was applied over the left motor cortical representational area of the first dorsal interosseous (FDI) muscle. Resting motor threshold (RMT), motor-evoked potential (MEP) amplitude recruitment curve, cortical silent period (CSP), short interval intracortical inhibition (SICI), intracortical facilitation (ICF), F-wave amplitude, and maximum M-wave (M-max) were recorded at baseline, (T0), 2 h after (T1), and 5 h (T2) after HYP extract or placebo intake

(hotspot) of the right FDI as identified by TMS, and the other electrode was placed contralaterally above the right orbit. The currents flowed continuously for 9 (cathodal tDCS) and 13 (anodal tDCS) min. These stimulation protocols induce prolonged excitability changes in the human motor cortex: anodal stimulation increases and cathodal stimulation decreases cortical excitability for approximately 1 h after stimulation (Nitsche and Paulus 2001) (Nitsche et al. 2003) (Nitsche and Paulus 2000). All the participants underwent four experimental sessions in a randomized order (placebo-anodal tDCS, placebo-cathodal tDCS, HYP-anodal tDCS, HYP-cathodal tDCS). The experimental sessions were separated by 3 weeks. Cortical excitability changes of the motor cortical representation of the right FDI muscle were monitored by changes to the peak-to-peak MEP amplitudes induced by TMS.

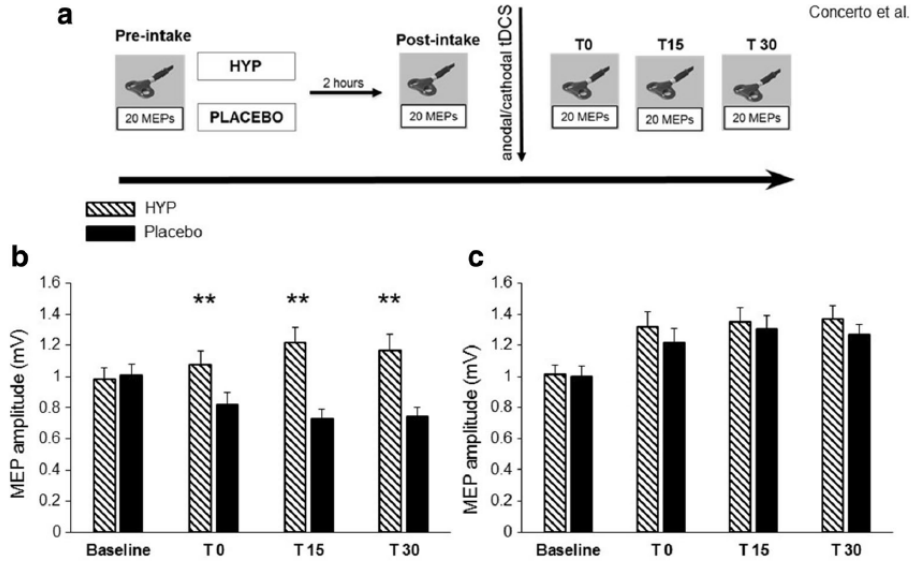

Fig. 2 Effect of a single dose of *Hypericum perforatum* extract on transcranial direct current stimulation (tDCS)-induced motor cortex plasticity. **a** Experimental design: Transcranial magnetic stimulation (TMS) was applied over the left motor cortical representational area of the first dorsal interosseous (FDI) muscle. Motor-evoked potential (MEP) amplitude were measured before (pre-intake) and 2 h after *Hypericum perforatum* (HYP) or placebo intake (post-intake) to control for a possible influence of the herb on MEP amplitude. Transcranial direct current stimulation (tDCS) was applied for 9 (cathodal tDCS) and 13 (anodal tDCS) min with an intensity of 1 mA. Immediately after tDCS, MEPs were recorded to monitor the MEP amplitude changes at three time points covering 30 min after tDCS (T0, T15, T30). **b** Cathodal tDCS. There was no significant difference between the two experimental conditions at baseline. At T0, T15, and T30, the placebo condition showed significant decrease in MEP size (LTD-like plasticity). On the contrary, *Hypericum perforatum* (HYP) extract intake revered the inhibition into facilitation. Error bars represent standard error of the mean. **< 0.01. **c** Anodal tDCS. Anodal tDCS induced an increase in MEP amplitude lasting for 30 min. There was no significant difference in the amount of MEP facilitation (LTP-like plasticity) between different tablets intake at different time points. Error bars represent standard error of the mean

Statistical analysis

The measures of cortical and spinal/neuromuscular excitability were tested using a two-way repeated-measure analysis of variance (ANOVA) with main effect "tablets intake" (two levels: HYP, placebo) and "time" (three levels: T0, T1, and T2). Recruitment curve was analyzed with a three-way repeated-measure ANOVA: main effects "tablets intake," "stimulation intensity" (100, 110, 120, 130, 150% RMT), and "time" (T0, T1, T2). The tDCS-induced plasticity protocol was analyzed using a three-way repeated-measures ANOVA with "tablets intake" and "stimulation" (anodal and cathodal tDCS) as between-subjects factors and "time" (baseline, T0, T15, T30) as within-subjects factor. The repeated-measure analyses were followed by pairwise comparison with Bonferroni correction. The Mauchly's test was used to evaluate the assumption of sphericity, and in the case of significant violations, we applied the Greenhouse-Geisser correction. Data were analyzed using SPSS software (version 22.0; SPSS, Inc., Chicago, IL). Data are presented as means ± SE.

Results

All subjects tolerated tDCS and HYP extract well. None of the participants had any adverse event during the study. RMT was not different ["tablets intake": $F_{(1, 52)} = 0.1, p = 0.74$; "time": $F_{(2, 52)} = 2.3, p = 0.1$; "tablets intake" × "time" interaction: $F_{(2, 52)} = 0.34, p = 0.7$]. A three-way mixed RMANOVA showed that increasing stimulus intensity induced larger MEP without differences between the experimental conditions: "tablets intake" [$F_{(1260)} = 0.14, p = 0.7$]; "intensity" [$F_{(5260)} = 157.23, p < 0.0001$]; "time" [$F_{(2260)} = 4.15, p = 0.2$]; "tablets intake" × "intensity" × "time" interaction [$F_{(10,260)} = 0.98, p = 0.4$].

HYP extract intake did not have significant effect on CSP duration ["tablets intake": $F_{(1, 52)} = 0.55, p = 0.46$; "time": $F_{(2, 52)} = 2.34, p = 0.1$; "tablets intake" × "time" interaction: $F_{(2, 52)} = 1.1, p = 0.33$], SICI ["tablets intake": $F_{(1, 52)} = 0.15, p = 0.69$; "time": $F_{(2, 52)} = 1.1, p = 0.33$; "tablets intake" × "time" interaction: $F_{(2, 52)} = 0.15, p = 0.8$], and ICF ["tablets intake": $F_{(1, 52)} = 0.29, p = 0.5$; "time": $F(2, 52) = 0.46, p = 0.6$; "tablets intake" × "time" interaction: $F_{(2, 52)} = 1, p = 0.3$]. Furthermore, no significant differences were found in spinal cord/neuromuscular excitability [F-wave: "tablets intake": $F_{(1, 52)} = 0.36, p = 0.5$; "time": $F_{(2, 52)} = 0.49, p = 0.6$; "tablets intake" × "time" interaction: $F_{(2, 52)} = 0.44, p = 0.6$; M-max: "tablets intake": $F_{(1,52)} = 0.018, p = 0.8$; "time": $F_{(2,52)} = 0.037\ p = 0.9$; "tablets intake" × "time" interaction: $F_{(2, 52)} = 0.44, p = 0.6$] (Table 1).

In the plasticity experiment, the intake of HYP extract or placebo did not affect MEP size ["tablets intake": $F_{(1,52)} = 0.77, p = 0.38$; "stimulation": $F_{(1, 52)} = 0.06, p = 0.8$; "time": $F_{(1,52)} = 2\ p = 0.16$; "tablets intake" × "stimulation" × "time" interaction: $F_{(1, 52)} = 0.9, p = 0.3$]. Thus, the MEP amplitudes collected before and after the tablets intake were averaged and analyzed as pre-tDCS MEP amplitude. Furthermore, repeated-measure ANOVA showed a main effect "tablets intake": $F_{(1, 52)} = 4.4, p = 0.04$; "stimulation": $F_{(1, 52)} = 10.9, p = 0.001$; and "time": $F_{(3, 52)} = 18.7, p < 0.0001$ with a significant "tablets intake" × "stimulation" × "time" interaction: $F_{(3, 156)} = 19.2, p < 0.0001$. Post-hoc analysis

Table 1 TMS parameters of corticospinal and spinal cord/neuromuscular excitability. All values are means ± SE before (baseline), 2 h after, and 5 h after intake of a single oral dose of *Hypericum perforatum* extract or placebo. There was no significant difference between the two experimental conditions for RMT, MEP amplitude recruitment curve, CSP duration, SICI, ICF, F-wave, and M-max. *RMT* resting motor threshold, *MEP* motor-evoked potential, *CSP* cortical silent period, *SICI* short interval intracortical inhibition, *ICF* intracortical facilitation, *M-max* maximum M-wave

Hypericum perforatum				Placebo		
	Baseline	2 h	5 h	Baseline	2 h	5 h
RMT %	40.5 ± 1.7	41.7 ± 1.6	40.8 ± 1.6	41 ± 1.7	41.7 ± 1.6	41.9 ± 1.5
MEP $_{100\%\ RMT}$(mV)	0.13 ± 0.02	0.18 ± 0.04	0.17 ± 0.03	0.1 ± 0.02	0.18 ± 0.04	0.15 ± 0.04
MEP $_{110\%\ RMT}$(mV)	0.38 ± 0.04	0.40 ± 0.07	0.36 ± 0.04	0.43 ± 0.05	0.44 ± 0.05	0.43 ± 0.02
MEP $_{120\%\ RMT}$(mV)	0.73 ± 0.10	0.89 ± 0.10	0.76 ± 0.11	0.95 ± 0.16	0.91 ± 0.08	0.76 ± 0.1
MEP $_{130\%\ RMT}$(mV)	1.56 ± 0.16	1.70 ± 0.13	1.54 ± 0.18	1.76 ± 0.22	2.05 ± 0.27	1.89 ± 0.23
MEP $_{140\%\ RMT}$(mV)	2.30 ± 0.20	2.63 ± 0.25	2.50 ± 0.27	2.36 ± 0.27	2.83 ± 0.3	2.49 ± 0.29
MEP $_{150\%\ RMT}$(mV)	2.57 ± 0.20	2.99 ± 0.20	2.93 ± 0.25	2.73 ± 0.33	2.97 ± 0.28	2.85 ± 0.38
CSP $_{150\%\ RMT}$ ms	155 ± 7.2	140.7 ± 8.1	150.2 ± 7.1	141.2 ± 8.5	139 ± 8.1	143.1 ± 7.9
SICI	61.6 ± 6.9	65.4 ± 6.4	62.2 ± 6.2	59.5 ± 6.6	62 ± 6.8	57.4 ± 6
ICF	155 ± 7.2	147.5 ± 8	151.2 ± 6.9	146.5 ± 6.9	148.6 ± 6.8	143.5 ± 7.2
M-max (mV)	15.6 ± 0.9	15.2 ± 1	15.7 ± 1	15.6 ± 1.1	15.9 ± 1.2	15.6 ± 1.2
F-wave amplitude (μV)	291.4 ± 20.1	287 ± 18	301 ± 16	289 ± 18.3	273 ± 18	277 ± 21

indicated that, after both HYP extract and placebo intake, anodal tDCS stimulation induced a statistically significant increase in MEP amplitude compared to pre-stimulation (HYP extract: before 1.04 ± 0.07 mV, T0 1.31 ± 0.09 mV $p = 0.0005$, T15 1.35 ± 0.09 mV $p < 0.0001$, T30 1.37 ± 0.08 mV $p < 0.0001$; placebo: before 0.97 ± 0.07 mV, T0 1.21 ± 0.09 mV $p < 0.0001$, T15 1.3 ± 0.08 mV $p < 0.0001$, T30 1.26 ± 0.06 mV $p < 0.0001$). On the other hand, cathodal tDCSt-induced LTD-like effect after placebo (placebo: before 1.09 ± 0.08 mV, T0 0.81 ± 0.07 mV $p < 0.0001$, T15 0.72 ± 0.062 mV $p < 0.0001$, T30 0.74 ± 0.063 mV $p < 0.0001$) while the cathodal tDCS LTD-like effect was reversed into facilitation after HYP extract intake (HYP extract: before 0.97 ± 0.075 mV, T0 1.0.8 ± 0.08 mV $p = 0.008$, T15 1.21 ± 0.1 mV $p = 0.0002$, T 30 1.16 ± 0.1 mV $p = 0.007$). We then performed a paired two-tailed Student's *t* test to investigate differences in MEP amplitudes for each time between the respective tDCS/experimental conditions. Differences were observed at each time point after stimulation in the cathodal/experimental condition (T0 $p = 0.005$, T15 $p = 0.0002$, T30 $p = 0.0.0003$) (Fig. 2b). On the contrary, the results indicate no differences for the anodal/experimental condition (T0: $p = 0.3$; T15: $p = 0.6$; T30: $p = 0.1$) (Fig. 2c).

Discussion

The main finding from this study was that a single dose of HYP extract intake modulated cortical plasticity reversing LTD-like plasticity into facilitation in humans. HYP extract intake did not affect TMS parameters of corticospinal excitability and LTP-like plasticity induced by anodal tDCS.

Many possible processes may explain our findings. Research on antidepressant drug action now focuses on neuroplasticity (Harmer et al. 2017). LTP and LTD are long-lasting increase or decrease in synaptic strength thought to be involved in learning and memory (Collingridge et al. 2010) and to be relevant in understanding the mechanism of action of antidepressants (Santarelli et al. 2003). Several in vivo and in vitro studies indicate that HYP extracts affect synaptic activity. It has been shown that the phloroglucinol constituent hyperforin inhibits the neuronal uptake of serotonin, noradrenaline, and dopamine (Wonnemann et al. 2001) (Schmidt and Butterweck 2015). This effect is due to the activation of the transient receptor potential channel protein 6 (TRPC6) and the consequent decrease of the Na^+ gradient between the neuron and the synaptic cleft. The loss of the gradient decreases the reuptake of the neurotransmitters (Leuner et al. 2007).

Serotonin has mixed effects on neuroplasticity (Kojic et al. 1997) (Ohashi et al. 2002) (Mori et al. 2001) (Ryan et al. 2009) (Bhagya et al. 2011). Data on serotonin and LTD are still controversial and seem to be dependent upon the stimulation of different receptors (Kemp and Manahan-Vaughan 2005) (Normann and Clark 2005) (Jang et al. 2010). Nonetheless, the role of serotonin as a plasticity modulator is further supported by previous results in humans indicating that a single dose of the SSRI citalopram enhances and prolongs LTP-like plasticity induced by anodal tDCS and converts cathodal tDCS-induced LTD-like plasticity into facilitation in healthy subjects. LTP-like plasticity in humans can also be studied by using a paired associative stimulation (PAS) protocol. Citalopram shows a trend towards PAS LTP-like plasticity, whilst abolishing PAS-induced LTD-like plasticity (Batsikadze et al. 2013). Taken together, our results and the published literature suggest that the increased serotonin level in the synaptic cleft induced by HYP extract intake might play a role in the reversal of the LTD-like plasticity we showed in our study.

It is conceivable that our results may have been due to a noradrenergic neuromodulation. In animal slice experiments, adrenergic activation seems to be an enhancer of LTP (Hu et al. 2007) (Tully et al. 2007) (Korol and Gold 2008). Likewise, adrenaline enhances LTD (Marzo et al. 2010), while noradrenaline blocks LTD (Katsuki et al. 1997). In keeping with our results, a previous pharmaco-TMS study in humans showed that the administration of the selective NRI reboxetine reverted cathodal tDCS-induced LTD-like plasticity into facilitation in healthy subjects (Kuo et al. 2017). The specific noradrenaline receptor subtypes that are involved in this mechanism are not clear.

Because HYP extract also affects dopamine release (Schmidt and Butterweck 2015), which has been shown to modulate tDCS-induced plasticity (Monte-Silva et al. 2010) (Fresnoza et al. 2014), the impact of dopamine on cortical plasticity should be taken into account to understand our results. Previous studies suggest a dose-dependent inverted U-shape like effect of dopamine on LTP-like cortical plasticity (Ziemann et al. 2014). With regard to the paradigms we used in our study, it has been demonstrated that the D1-like activation obtained with the intake of sulpiride and L-dopa induces a dose-dependent modification of tDCS plasticity and reversion of cathodal tDCS-induced LTD-like plasticity to facilitation (Fresnoza et al. 2014). These results are consistent with previous data that demonstrate a similar pattern of LTD reversal induced by a D1 agonist in rat hippocampal slices (Mockett et al. 2007). The preparation we used in this study is standardized for hyperforin content. In light of the published literature, hyperforin could have played a pivotal role in the modulation of neuroplasticity. Hyperforin inhibits the uptake of serotonin, norepinephrine, and dopamine (Schulte-Lobbert et al. 2004) (Muller et al. 1997). The modulation of neuroplasticity we described might be due to this non-selective inhibition of the neurotransmitter uptake. Moreover, HYP extracts contain a wide spectrum of different components and we cannot rule out the contribution of further ingredients to the reversal of LTD-like plasticity we demonstrated in the study.

Previous TMS studies in healthy subjects have shown that conventional antidepressants intake affects excitatory and inhibitory cortical circuits tested with TMS, resulting in a pattern of reduced ICF, enhanced SICI, and changes in MEP amplitude and RMT (Ziemann et al. 2014) (Ilic et al. 2002) (Eichhammer et al. 2003) (Robol et al. 2004) (Paulus et al. 2008). The citalopram effect on cortical excitability (SICI) is not consistent across studies and seems to be dependent upon individual genetic factors (Eichhammer et al. 2003) (Robol et al. 2004), while acute administration of the NRI reboxetine modulates ICF(Herwig et al. 2002). Our results suggest that HYP extract only displays a citalopram/reboxetine-like effect on cortical plasticity as both drugs exert a prominent effect on LTD-like plasticity (reversal to LTP) (Nitsche et al. 2009) (Kuo et al. 2017). This indicates that HYP extract induces a complex modulation of cortical activity with both similarities to and differences from conventional antidepressants. Given the widespread nature of brain changes after administration of HYP extract, more studies are needed using different TMS paradigms for a comprehensive understanding of its modulation of cortical circuitries.

This study has strengths and limitations. It is the first study exploring the relationship between herbal products commonly used in neuropsychiatry and brain plasticity in humans. It fills a gap in the literature as commonly used antidepressant drugs have been previously characterized in pharmaco-TMS studies in humans. Although this study contributes significantly to the sparse CAM-synaptic plasticity literature, it does have some limitations. Pharmaco-TMS studies usually employ a drug of known concentration and mechanism of action. On the contrary, herbal extracts comprise several neuroactive substances. Thus, inferences about causality cannot be done and mechanistic interpretation should be taken with caution. This is of pivotal importance especially for HYP extracts which exert modulatory effects on multiple neurotransmitters. We tested only the recommended therapeutic dose and only one commercially available HYP extract. Thus, dose–response relationships need to be investigated to determine the optimal dose necessary to affect neuroplasticity, and there is a need to use different standardized active compounds in order to further characterize the mechanisms underlying the neuromodulatory effect in humans. In addition, there are some methodological limitations: (1) in the paired-pulse paradigms, we used only two ISIs; (2) the CSP duration was tested using only one stimulation intensity; (3) in the plasticity experiment, the after effects were assessed at T1 and T2 (30 min window). Thus, the electrophysiological information we gathered needs to be confirmed with more comprehensive studies. Notwithstanding the important limitations, our results indicate a remarkable cortical modulatory effect of HYP extracts. These results suggest that HYP extracts might be used as an augmentation strategy to conventional plasticity-based interventions (drugs and brain stimulation techniques) in poorly responding depressed patients. This hypothesis should be confirmed in future clinical studies.

Conclusion

In conclusion, the findings of this study show that HYP extract affects cortical plasticity in humans suggesting a modulatory effect that bears some similarities to conventional antidepressants. We should acknowledge that the antidepressant properties of HYP could be unrelated to the observed effect on plasticity. Thus, in order to demonstrate whether the plastic changes might be of relevance in understanding the HYP extract antidepressant effects, it is of pivotal importance to replicate the study in depressed patients. While this study examined the acute effects of an HYP extract, it remains to be determined whether these neuromodulatory effects are sustained over time in patients under chronic treatment and whether they correlate with the clinical outcome.

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Compliance with ethical standards All participants provided their written informed consent. All the procedures were approved by the local ethics committee and complied with the Declaration of Helsinki.

Conflict of interest The authors declare that they have no conflicts of interest.

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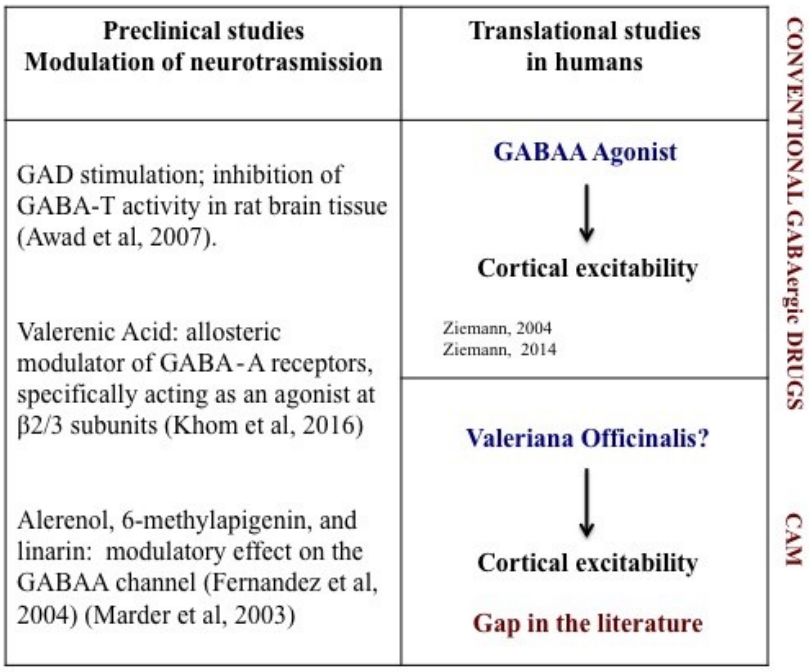
STUDY 2

Rational

Neurobiological preclinical research has begun to show that the herb, especially its active compound valerenic acid, interacts with the GABAAergic system, a mechanism of action similar to benzodiazepines (Marder *et al.*, 2003) (Khom *et al.*, 2007) (Fernandez *et al.*, 2004). For instance, Awad et al. found that VE increased brain GABA level and neurotransmission by stimulating glutamic acid decarboxylase (GAD) and inhibiting GABA transaminases (GABA T) (Awad *et al.*, 2007). Furthermore, valerenic acid stimulates GABergic receptors thereby producing neuronal inhibition (Khom *et al.*, 2016). Paired pulse TMS does not provide direct indexes of GABergic and glutmatergic neurotransmission but does provide indirect neurophysiological measurements closely related to GABA and glutamate receptors mediated function. Translational studies in humans using conventional GABA-ergic agonists modulate cortical excitability (Ziemann, 2004) (Ziemann, 2013). Hence it was planned to evaluate whether VE intake affects TMS parameters.



Theoretical framework

CORTICAL EXCITABILITY

GABA-AR Agonist	MT	MEP	CSP	SICI	ICF	SAI
Diazepam		↓	↓	↑	↓	↑
Lorazepam		↓	↑	↑	↓	↓
Zolpidem		↓	↑	↑	↓	
Midazolam		↓				
Thiopental		↓				
Ethanol			↑	↑	↓	
Vigabatrin			↑		↓	
Tiagabine			↑	↓	↑	
GABA-BR Agonist						
Baclofen			↑	↑↓	↓	
GABA-AR Antagonist						
Flumazenil						

Aim

Although the anxiolytic and sedative effects are mainly attributed to the modulation of GABA-ergic transmission, the mechanism of action has not been fully investigated in humans and no studies have investigated the acute effects of VE administration on human cortical excitability. The aim of the study was to investigate the effects of a single dose of VE on cortical excitability assessed with TMS.

Study design

This is a double-blind, randomized, crossover study in healthy subjects. Independent variable (treatment): two levels VE and placebo. Multiple dependent variables: TMS parameters of cortico-spinal excitability (RMT, MEP, MEP recruitment, CPS, SICI, ICF, SAI, LAI, F_{wave}, M_{max}).

Participants

Inclusion criteria	Exclusion criteria
Aged between 18 to 50 Being alert, cooperative and both willing and able to participate in the study Being able to provide informed consent	History of epilepsy History of brain trauma History of stroke, brain tumors, brain infections, vascular malformations Exposure to poisons Any bodily metal implants Any device that may be affected by TMS (pacemaker, medication pump, cochlear implant, implanted brain stimulator) Pregnancy and breast-feeding History of long-term alcohol or drug use Sleep deprivation Current use of medications Any current serious medical illness

Research Questions

The research questions being asked are:

RQ1- Does VE intake modulate corticospinal excitability tested with TMS in healthy subjects?

Research Hypothesis

$H_0$1: There will be no significant difference in corticospinal excitability in healthy subjects after the intake of VE or a placebo.

H_A1: There will be significant difference in corticospinal excitability in healthy subjects after the intake of VE or a placebo.

Original Paper

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Valeriana officinalis Root Extract Modulates Cortical Excitatory Circuits in Humans

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Keywords
Valerian · Transcranial magnetic stimulation · Short-interval intracortical inhibition · Intracortical facilitation · Cortical excitability

Abstract
Background: *Valeriana officinalis* extract (VE) is a popular herbal medicine used for the treatment of anxiety and sleep disorders. Although the anxiolytic and sedative effects are mainly attributed to the modulation of GABA-ergic transmission, the mechanism of action has not been fully investigated in humans. Noninvasive brain stimulation protocols can be used to elucidate the mechanisms of action of psychoactive substances at the cortical level in humans. In this study, we investigated the effects of a single dose of VE on cortical excitability as assessed with transcranial magnetic stimulation (TMS). ***Methods:*** Fifteen healthy volunteers participated in a double-blind, randomized, cross-over, placebo-controlled study. Subjects were required to take either 900 mg of VE (valerenic acid 0.8%) or placebo (an equal dose of vitamin E). Motor cortex excitability was studied by single and paired TMS before and at 1 h and 6 h after the oral administration. Cortical excitability was assessed using different TMS parameters: resting motor threshold, motor-evoked potential amplitude, cortical silent period, short-interval intracortical inhibition, and intracortical facilitation. Furthermore, we assessed sensorimotor integration by short-latency and long-latency afferent inhibition. ***Results:*** We found a significant reduction in ICF, without any significant changes in other TMS measures of motor cortex excitability. The amount of ICF returned to baseline value 6 h after the intake of the VE. ***Conclusion:*** A single oral dose of VE modulates intracortical facilitatory circuits. Our results in healthy subjects could be predictive markers of treatment response in patients and further support the use of pharmaco-TMS to investigate the neuropsychiatric effects of herbal therapies in humans.

Introduction

Despite the availability of effective pharmacological and psychotherapy strategies, up to 50% of cases of depression, anxiety, and insomnia are nonresponders and

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show relapses [1]. Indeed, there is well-documented evidence that individuals who have found little or no improvement through standard interventions often use herbal remedies for the treatment of these conditions. It has been reported that 50% of patients suffering from depression, anxiety, and insomnia use complementary and alternative medicine [2].

Although herbal therapies have been used for centuries as remedies for psychiatric conditions, research focusing on assessing the effectiveness of botanical psychoactive plants used in psychiatry and their psychopharmacological properties in humans is still inconclusive for many compounds [3]. Among herbal medications for insomnia and anxiety, *Valeriana officinalis* root extract (VE) is one of the most popular [4]. A previous survey study reported that 1.1% of the adult population in the USA (approx. 2 million adults) had used valerian in the past week [5]. Other studies indicate that VE has antioxidant and neuroprotective effects [6–9]. Furthermore, VE modulates brain neurotransmitters [10–14], and shows antianxiety, antidepressant, and antiepileptic activity in animal models [15, 16].

In spite of this preclinical evidence and the large empirical use of VE, there is an ongoing debate in the scientific literature regarding the magnitude of its effects and, to date, no studies have investigated the acute effect of VE administration on cortical excitability in humans. Transcranial magnetic stimulation (TMS), a noninvasive technique widely used to investigate cortical physiology in humans, has been a valid tool to probe the acute pharmacological effects of central nervous system active drugs [17]. Indeed, pharmaco-TMS experiments offer the opportunity of investigating the mechanism of action of psychoactive molecules by analyzing their effects on well-characterized single-and paired-pulse TMS parameters such as resting motor threshold (RMT), motor-evoked potential (MEP) amplitude, cortical silent period (CSP), short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), and short-latency and long-latency afferent inhibition (SAI and LAI) [18]. Indeed, the modulation of the physiological mechanisms underlying these parameters after drug intake offers the possibility to translate the preclinical results to human use, and to probe, noninvasively, the activity on specific cortical functions like membrane excitability (RMT), corticospinal excitability (MEP size), $GABA_B$-dependent inhibition (CSP), intracortical inhibitory and excitatory circuits (SICI and ICF), and sensorimotor integration (SAI and LAI) [18].

Hence, to elucidate the mechanism of action and the neuroactive properties of VE, we sought to investigate the acute effect of a recommended dose in a randomized, double-blind, cross-over study employing a broad array of TMS measures of human motor cortex excitability. In view of the extensive preclinical literature, we hypothesized that compared to placebo VE would induce a modulation of intracortical inhibitory and excitatory circuits.

Methods

Subjects

Fifteen healthy, right-handed college students [19] (9 males and 6 females; mean age 30.2 ± 5.8 years) participated in this study. We excluded subjects who had a history of neurological or psychiatric diseases, metal implants, brain trauma, psychoactive medication use, drug addiction, a family history of epilepsy, or were pregnant. The study conformed to the Declaration of Helsinki and was approved by the Institutional Review Board at the New York College of Podiatric Medicine. All subjects signed a written consent form. None of the subjects took herbal extracts before this study.

Study Design

This was a randomized, double-blind, cross-over study; subjects were required to take 3 capsules (900 mg in total) of VE (the active arm) or placebo. Commercial VE capsules (300 mg each) contained a standardized amount of valerenic acid (0.8%). The "dummy" capsules contained an equal amount of vitamin E; these were prepared by a pharmacist and put into an empty bottle of the commercial product. In this way, the placebo preparation assimilated the typical valerian root odor. The order of drug conditions was pseudorandomized and balanced between subjects. All subjects participated in 2 drug conditions, separated by 3 weeks. In accordance with a previous pharmacokinetic study [20], the subjects were assessed at T0 (before the intake), and at 1 h (T1) and 6 h (T2) after the intake of the capsules.

Cortical Excitability

TMS experiments were performed during the morning hours. Ag-AgCl surface electrodes were positioned over the muscle belly and the tendon of the right abductor pollicis brevis (APB) muscle. Signals were amplified, band-pass-filtered, and sampled using a micro 1401 AD converter (Cambridge Electronic Design, Cambridge, UK) controlled by Signal software (Cambridge Electronic Design v3) and stored on a PC for off-line analysis. TMS was delivered through a focal figure of eight-shaped magnetic coil (diameter of external loop: 90 mm) connected to 2 Magstim 200 magnetic stimulators via a "Y" cable (The Magstim Co., Dyfed, UK). Several parameters of corticospinal excitability were investigated. RMT, a parameter that depends upon neuronal membrane excitability as it is modulated by voltage-gated sodium or calcium-channel blockers, was determined as the minimum stimulator intensity to the nearest 1% to produce an MEP of 50 μV in 5 of 10 trials. We then assessed mean peak-to-peak MEP amplitudes, a parameter that reflects changes in the excitability of the corticospinal tract, using a stimulus intensity of 120% of the RMT (an average of 20 MEPs). CSP was tested by delivering TMS of the motor

Table 1. Comparison of cortical excitability, sensorimotor integration, and neuromuscular and notorneuronal excitability before (T0) and 1 h (T1) and 6 h (T2) after VE and placebo intake

	VE			Placebo			Group × Time interaction
	T0	T1	T2	T0	T1	T2	
RMT, %	43.2±1.9	43±1.4	43.2±1.9	43.4±1.8	43.3±2	43.5±1.8	$F_{2, 56} = 0.01$, $p = 0.9$
MEP, mV	0.67±0.08	0.69±0.07	0.73±0.08	0.75±0.07	0.76±0.1	0.71±0.08	$F_{2, 56} = 0.8$, $p = 0.4$
CSP, ms	139.6±3.6	139.5±3.7	140.2±3.7	143.3±3.7	144.7±2.9	142.8±2.9	$F_{2, 56} = 1.1$, $p = 0.3$
SAI, %	85.5±4.1	82.7±4	84.4±4.1	82.3±4.4	81.7±4.7	82.2±3.6	$F_{2, 56} = 0.03$, $p = 0.9$
LAI, %	73.4±5	74.3±4.6	75±5.1	88.7±5.5	81.5±3.6	82.5±4.8	$F_{2, 56} = 0.03$, $p = 0.9$
M_{max}, mV	16±1.1	16.4±1	16.4±1	15.6±1.2	15.4±1.1	15.7±1.3	$F_{2, 56} = 0.7$, $p = 0.9$
F-wave amplitude, μV	311.1±19.7	310.4±13.1	304.9±12.9	280.8±17.9	284.6±17.9	296.8±19.2	$F_{2, 56} = 0.47$, $p = 0.69$

Error bars indicate standard errors. RMT, resting motor threshold; MEP, motor-evoked potential; CSP, cortical silent period; SAI, short-latency afferent inhibition; LAI, long-latency afferent inhibition; M_{max}, maximum M wave; VE, *Valeriana officinalis* extract.

cortex during tonic APB contraction (50% of the maximal voluntary contraction, assessed and monitored with a visual electromyographic [EMG] feedback). The duration of 15 CSPs was measured from the end of the MEP until the restart of constant EMG activity. EMG traces were rectified but not averaged. CSP is modulated by GABA-ergic and dopaminergic drugs. If a subthreshold (conditioning) stimulus precedes a suprathreshold (test) stimulus at short and long interstimulus intervals (ISI), the MEP generated by the test stimulus is either inhibited (by SICI) or facilitated (by ICF). SICI and ICF were studied with a paired-stimulation paradigm [21]. ISIs of 2 ms (for SICI) and 10 ms (for ICF) were used. Each study consisted of 20 trials for each ISI, and the test stimuli alone were delivered in random order controlled by a laboratory computer. These parameters assess the excitability of intracortical inhibitory and excitatory circuits modulated by GABA-ergic and glutamatergic drugs.

To probe afferent inhibition, the medial nerve was stimulated at the wrist using a Digitimer D-160 stimulator (Digitimer Ltd., Welwyn Garden City, UK) using electrodes with the cathode positioned proximally. Stimulus intensity was adjusted to produce a slight thumb twitch. SAI and LAI were tested at ISIs of 25 and 200 ms. Forty stimuli were delivered at each ISI, and randomly intermingled with 20 trials in which MEPs were elicited by the test stimulus alone. SAI and LAI are modulated by cholinergic and GABA-ergic drugs. For a comprehensive review of the pharmacological modulation of TMS parameters, see Ziemann et al. 2015 [18]. For SICI, ICF, SAI, and LAI, the mean amplitude of the conditioned MEP was expressed as a percentage of the unconditioned (test) MEP mean amplitude.

F-wave amplitude and M_{max} (supramaximal electrical stimulation of the median nerve at the wrist) were tested to investigate changes in spinal motorneuron and neuromuscular excitability. TMS parameters were tested according to the published guidelines for the use of TMS in clinical neurophysiology [22].

Statistical Analysis

Data were analyzed using SPSS software v22.0 (SPSS Inc., Chicago, IL, USA). All the tested parameter of cortical excitability, spinal excitability, and sensorimotor integration (RMT, MEP, CSP, SICI, ICF, SAI, LAI, F-wave, and M_{max}) were analyzed using a 2-way repeated-measures ANOVA with the main effects "Group" (VE or placebo) and "Time" (T0, T1, and T2). We used the Mauchly test to assess the sphericity and applied the Greenhouse-Geisser correction when appropriate. The repeated-measure analyses were followed by pair-wise comparison with the Bonferroni correction. Data are means ± SE. An α value of <0.05 was considered significant.

Results

The study was well-tolerated without adverse events. RMT, MEP amplitude, CSP duration, sensorimotor integration assessed with SAI and LAI, and neuromuscular and motorneurons and excitability assessed with M_{max} and F-wave did not differ between groups (Table 1). We then tested intracortical excitability with the paired-pulse paradigm. SICI was not affected by valerian intake (VE: T0 38.54 ± 4.2%, T1 34.1 ± 4.9%, and T2 39.68 ± 3.7%; placebo: T0 43.17 ± 4.3%, T1 41.1 ± 4.3%, and T2 42.04 ± 4.5% [Group: $F_{1, 56} = 0.71$, $p = 0.4$; Time: $F_{2, 56} = 1.3$, $p = 0.2$; Group × Time interaction: $F_{2, 56} = 0.52$, $p = 0.5$]). However, VE intake decreased the amount of ICF (VE: T0 150.9 ± 7%, T1 114.8 ± 6.9%, and T2 155.7 ± 7.6%; placebo: T0 153.4 ± 6.1%, T1 155.5 ± 5.7%, and T2 159.4 ± 6.3% [Group: $F_{1, 56} = 3.5$, $p = 0.06$; Time: $F_{2, 56} = 17.4$, $p \leq 0.0001$; Group × Time interaction: $F_{2, 56} = 15$, $p \leq 0.0001$]). Post hoc analysis indicated that there was a statistically significant difference between T0 and T1 ($p \leq 0.0001$) but no difference between T0 and T2 ($p = 0.1$). The pair-wise comparison also indicated that in the VE

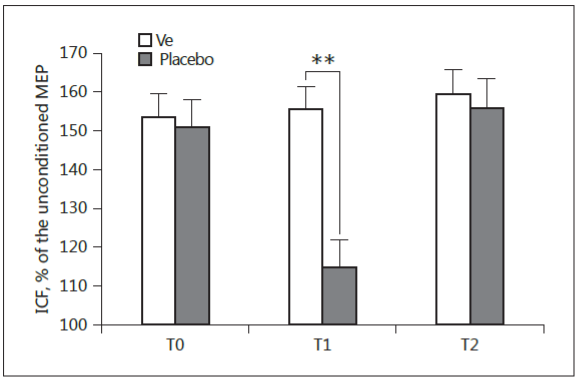

Fig. 1. VE intake induced a reversible decrease in the amount of ICF. Error bars represent standard error of the mean. ** $p < 0.01$. VE, *Valeriana officinalis* extract; ICF, intracortical facilitation; MEP, motor-evoked potential. T0, before the intake; T1, 1 h after the intake; T2, 6 h after the intake.

group, there was a difference between T0 and T1 ($p = 0.001$) but not between T0 and T2 ($p = 0.5$). The amount of ICF did not change in the placebo group (T0 vs. T1: $p = 0.2$; T0 vs. T2: $p = 0.7$) (Fig. 1).

Discussion

The results of this study provide evidence that acute administration of VE in healthy humans affects motor cortex excitability with a specific effect on the ICF. The decrease in the amount of ICF is reversible.

Mechanistically, the impact of acute VE intake on cortical excitability can be explained by taking the physiology of ICF into consideration. In cortical brain slices, a single electrical stimulus to the deep cortical layers evokes a sequence of postsynaptic potentials (PSPs) in the resting neuron: first, a brief excitation, then a short-latency fast inhibition, and then long-latency, more prolonged inhibition [23]. It has been suggested that ICF relates to slow excitatory PSPs induced by activation of the N-methyl-D-aspartate (NMDA) receptor [24], and indexes $GABA_A$ receptor activity (the fast-inhibition PSP) [18]. Previous pharmaco-TMS studies demonstrated that ICF is decreased by the NMDAR antagonists, dextromethorphan [25], memantine [26], and riluzole [27]. In addition, the contribution of $GABA_A$ inhibition to ICF is supported by the decrease in ICF induced by a single dose of lorazepam [28], zolpidem, and diazepam [29], indicating that $GABA_A$ agonists contribute to the net facilitation represented by ICF. In contrast, NE system modulators enhance ICF [30]. These mechanisms are remarkably consistent with the premise that VE, and, particularly valerenic acid, the main component of VE, allosterically modulate $GABA_A$ receptors and, in this way, are thought to induce anxiolytic activity [31–34]. A similar modulatory effect on the $GABA_A$ channel was demonstrated for other components of the VE such as alerenol, 6-methylapigenin, and linarin [35, 36]. To this extent, our findings provide support for a similar modulation in humans. The lack of effect on SICI might be explained by the fact that valerenic acid is a subunit-specific (β_3) allosteric modulator of $GABA_A$ receptors [34, 37]. Furthermore, a point mutation in the β_3 $GABA_A$ receptor subunit prevents the ability of valerenic acid to display anxiolytic-like activity in vivo while the administration of diazepam still maintains the anxiolytic-like activity, as tested with the elevated plus maze and the light/dark choice tests [32]. These data indicate that VE targets neuronal circuits expressing β_3-containing $GABA_A$ receptors, while the anxiolytic activity of benzodiazepines has been shown to be mediated via α_2 $GABA_A$ receptors [38]. This specific inhibitory effect might explain the lack of activity on SICI and the net effect on ICF.

In addition, there is in vitro and in vivo evidence that VE modulates glutamatergic neurotransmission. For instance, valerian and valerenic acid have anxiolytic properties as tested with the dark/light preference task with zebrafish. This anxiolytic effect of valerian and valerenic acid is abolished after the administration of LAP3 (an mGluR I antagonist) and EGLU (an mGluR II antagonist) [39]. Furthermore, VE has a modest inhibitory effect on ^{3H}dizocilpine (MK-801) binding, an indicator of NMDA-valerian interactions [40]. In light of the modest effect on the NMDA receptor, it is likely that the modulation of glutamatergic neurotransmission does not play a pivotal role in inducing the decrease in ICF that we observed in our study.

There is evidence that VE can reduce the turnover of 5-hydroxytryptamine and norepinephrine (NE) in the hippocampus and amygdala, reducing, in this way, the negative effect of stress in mice [14]. In addition, VE administration in rats decreased NE, dopamine, and 5-hydroxytryptamine concentrations in the frontal cortex [41]. A similar effect at the cortical level in humans could be capable of influencing the amount of ICF [18]. We should acknowledge that calcium-channel agonists consistently increase ICF [30]; nonetheless, the assumption of a top-down regulation of ICF induced by the NE brain

Neuropsychobiology 2017;75:46–51
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concentration is speculative at the moment. Furthermore, previous TMS studies carried out in a clinical context highlighted the electrophysiological role of ICF changes as potential markers of a glutamate-mediated adaptive response or compensatory neuroplastic phenomena [42–47]. Thus, we cannot rule out an indirect (adaptive) modulatory effect on ICF.

This study has limitations. First we tested only the recommended therapeutic dose. Future studies should address dose-dependent effects on cortical excitability. In addition, we tested only 1 commercially available, standardized VE that contained a high concentration of valerenic acid. Different VE formulations should be investigated to assess the contribution of different active molecules. In our study, an acute dose of α-tocoferol did not affect cortical excitability in humans, but we cannot exclude a possible modulation of TMS parameters not investigated in the study. Lastly, the results need to be replicated in larger studies measuring the overall significance of the explanatory variables and the way they are combined, not just the individual variables by themselves.

In conclusion, these findings provide the first evidence that VE affects excitatory intracortical circuits in humans. We expect that these results will encourage pharmaco-TMS research aimed at advancing our understanding of the mechanism of action of complementary and alternative medicine currently used as a treatment for a variety of neurological and psychiatric disorders. It remains to be determined whether the VE neuromodulatory effects are present in depressed patients and correlate with disease severity and clinical outcome.

Disclosure Statement

The authors declare no conflict of interest.

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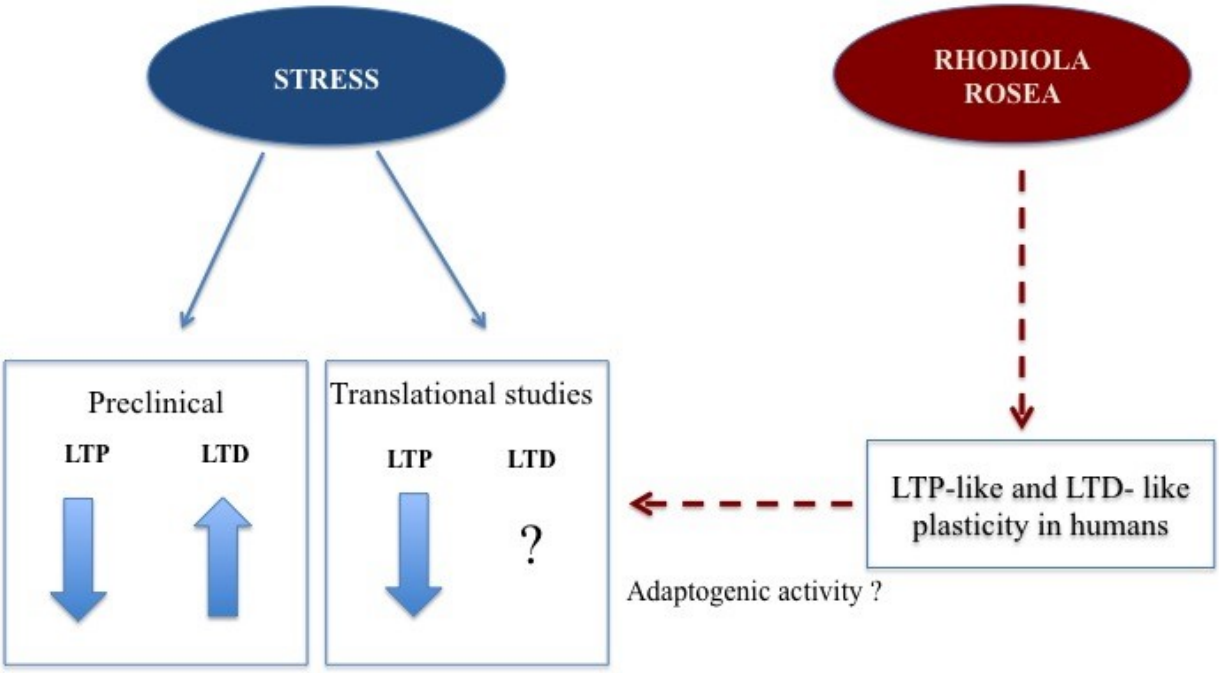
STUDY 3

Rational

RRE acts to normalize cortisol synthesis potentially through inhibition of the Stress-activated protein kinases (SAPK) pathway involved in the pathogenesis of stress symptoms. At the same time through inhibition of the SAPK/JNK pathway, RRE prevents the formation of nitric oxide (NO) and the associated decline in the ATP synthesis (Panossian *et al.*, 2007) (Panossian *et al.*, 2012) (Amsterdam & Panossian, 2016). During stress, high glucocorticoid levels interfere with the physiological occurrence of LTP-like and LTP-like plasticity. It appears that during stress the high glucocorticoid levels and the subsequent increase of glutamate release and the reduced BDNF synthesis alter the homeostasis of brain circuitries with an increase in promoting LTD and the disruption of LTP (Wong *et al.*, 2007). Similar results have been demonstrated in humans using NIBS (Sale *et al.*, 2008) (Concerto *et al.*, 2017). Preclinical studies indicate that RRE increases hippocampal LTP and BDNF expression in the hippocampus of rats and mice (Yang *et al.*, 2014) (Dimpfel *et al.*, 2018). Hence in this study it was planned to investigate whether the administration of RRE would modulate LTD-like and LTP-like plasticity in a manner that counteracts the effect of stress and high cortisol level on neuroplasticity.

Preclinical studies	Stress and plasticity	Translational studies in humans
MAO inhibition (van Diermen et al, 2009). Increase in rat brainstem concentrations of NA, DA and 5HT (Amsterdam &Panossian, 2016).	Stress facilitates the induction of hippocampal LTD-like plasticity and decreases LTP-like plasticity (Wong et al, 2007).	**Stress** ↓ **Neuroplasticity in humans** Sale et al, 2008 Concerto et al, 2017
Increase of BDNF expression in hippocampus in rats and mice (Yang et al, 2014). LTP increase in hippocampus slices (Dimpfel et al., 2018).		
CRH reduction in hoppicampus in rats. Decrease of cortisol level in human saliva, blood serum, rabbits (Yang et al, 2014).		**Adoaptogenic Rhodiola Rosea?** ↓ **Neuroplasticity in humans** **Gap in the literature**
Suppression of elevated phosphorylated kinase (JNK), and cortisol in stress (Panossian et al 2007).		
Stimulation of the expression and release of NPY in neuroglial cells (Panossian et al, 2012).		

Theoretical framework



Aim

The aim of this study was to examine the effect of RRE on human cortical excitability and plasticity and to evaluate its ability to modulate synaptic plasticity in a manner that could potentially reverse or counteract the negative effects of stress.

Study design

This is a double-blind, randomized, crossover-design study in a sample of healthy volunteers. Independent variable (treatment): two levels RRE and placebo. Multiple dependent variables: TMS parameters of cortico-spinal excitability (RMT, MEP, MEP recruitment, CPS, SICI, ICF, F_{wave}, M_{max}) and the tDCS-induced parameters of cortical plasticity (LTP, LTD).

Participants

Inclusion criteria	**Exclusion criteria**
Aged between 18 to 50 Being alert, cooperative and both willing and able to participate in the study Being able to provide informed consent	History of epilepsy History of brain trauma History of stroke, brain tumors, brain infections, vascular malformations Exposure to poisons Any bodily metal implants Any device that may be affected by TMS (pacemaker, medication pump, cochlear implant, implanted brain stimulator) Pregnancy and breast-feeding History of long-term alcohol or drug use Sleep deprivation Current use of medications Any current serious medical illness

Research Questions

The research questions being asked are:

RQ1- Does RRE intake modulate corticospinal excitability tested with TMS in healthy subjects?

RQ2- Does RRE intake modulate LTP-like plasticity induced by anodal tDCS in healthy subjects?

RQ3- Does RRE intake modulate LTD-like plasticity induced by cathodal tDCS in healthy subjects?

Research Hypothesis

$H_0$1: There will be no significant difference in corticospinal excitability in healthy subjects after the intake of RRE or a placebo.

H_A1: There will be significant difference in corticospinal excitability in healthy subjects after the intake of RRE or a placebo.

$H_0$2: There will be no significant difference in LTP-like plasticity induced by anodal tDCS in healthy subjects after the intake of RRE or a placebo.

H_A2: There will be significant difference in LTP-like plasticity induced by anodal tDCS in healthy subjects after the intake of RRE or a placebo.

$H_0$3: There will be no significant difference in LTD-like plasticity induced by cathodal tDCS in healthy subjects after the intake of RRE or a placebo.

H_A3: There will be no significant difference in LTD-like plasticity induced by cathodal tDCS in healthy subjects after the intake of RRE or a placebo.

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Exploring the effect of adaptogenic Rhodiola Rosea extract on neuroplasticity in humans

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ABSTRACT

Objectives: Rhodiola rosea extract is one of the most common herbal treatment for stress. Its mechanism of action in humans still need to be determined. We investigated the effect of a single dose intake of Rhodiola rosea extract on the plastic after-effects induced by anodal and cathodal motor cortex transcranial Direct Current Stimulation in humans.

Methods: Twenty-eight healthy volunteers were required to intake 500 mg of either RRE or placebo. Transcranial Magnetic Stimulation was used to investigate cortical excitability. Motor threshold, recruitment of motor-evoked potentials, cortical silent period, short-interval intracortical inhibition and intracortical facilitation were assessed at different time points. Spinal excitability and peripheral nerve conduction were measured by F-response and M-wave. Furthermore, we assessed the modulation of cortical plasticity using transcranial direct current stimulation after-effects on Motor Evoked Potentials amplitudes.

Results: Rhodiola rosea extract acute intake prevented cathodal transcranial Direct Current Stimulation-induced Long-term depression-like plasticity. The extract intake did not affect cortical excitability.

Conclusions: Our findings suggest that a single oral dose of Rhodiola rosea extract intake modulates cortical plasticity in humans preventing the activity-dependent reduction in the efficacy of neuronal synapses. These results suggest that the adaptogenic and antidepressant effects of Rhodiola rosea extract might be based on its modulation of brain plasticity.

1. Introduction

Herbal medicine has been used for centuries to adapt to stress and counteract its negative cognitive effects, fatigue, depressive symptoms, and insomnia. Adaptogens are “stress-response modifiers that increase an organism's nonspecific resistance to stress by increasing its ability to adapt and survive”.[1] Among the adaptogenic herbs Sedum roseum (L.) Scop, commonly known as Rhodiola Rosea (RR), a plant from the family of Crassulaceae known as Golden/ Artic Root, is one of the most widely used and researched Complementary and Alternative Medicine (CAM) treatment used[2–4] Despite numerous basic science studies and clinical trials,[5] its adaptogenic mechanism of action still needs to be addressed in humans. Several active compounds found in RR extracts (RRE) (salidroside, rosavin, rosarian, triandrin, tyrosol) stimulate the brain and increase the concentration level of neurotransmitters such as norepinephrine (NE), serotonin (5 H T) acetylcholine (Ach), and Dopamine (DA),[6,7] At the cortical and brainstem level it was demonstrated that sub-chronic treatment (10 days) with RRE increased the concentration of serotonin.[7] Furthermore, RRE showed behavioural antidepressant-like effect comparable to imipramine[8] and the administration of salidroside in olfactory bulbectomized rats showed anti-inflammatory effects, regulated the hypothalamic-pituitary-adrenal axis (HPA) activity, induced the transcription of BDNF and improved depressive-like behaviour.[9] In addition, the extracts and active compounds demonstrated

Abbreviations: RRE, Rhodiola rosea extract; TMS, Transcranial Magnetic Stimulation; tDCS, transcranial Direct Current Stimulation; RMT, Resting Motor Threshold; MEP, Motor Evoked Potential; CSP, Cortical Silent Period; SICI, short-interval intracortical inhibition; ICF, intracortical facilitation; Mmax, maximum M-wave; LTP, long-term potentiation; LTD, long-term depression

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cognitive enhancing effect in rodents[10–12] prevented the stress-induced changes in beta-endorphin and stress hormones[13] and modulated hippocampal neurogenesis.[14] These preclinical results have warranted few clinical trials that demonstrated clinically meaningful stimulant, antidepressant, cognitive enhancing, anti-fatigue and anti-stress effects in humans.[15–19] Despite these published data, RRE mechanism of action still needs to be characterized. It is well-known that a period of stress produces hormonal and immunological changes that are associated with a dysregulation of the HPA.[20] At a circuit level, neural plasticity, a fundamental mechanism of neuronal adaptation, is altered by stress and these pathologic plastic changes are thought to play a pivotal role in the pathogenesis of depression and the mechanism of action of antidepressants.[21] Furthermore, in the hippocampus, stress impairs long-term potentiation (LTP) and increases long-term depression (LTD). Because the effects of stress on LTP and LTD parallel the changes in synaptic plasticity that occur following the establishment of LTP with tetanic stimulation (*i.e.*, occluding LTP and enhancing LTD induction),[22] it is conceivable that an adaptogenic compound like RRE might support plastic changes that counteract abnormal LTD induction.[23] A previous study[24] indicated that rosavin, salidroside and commercially available RRE increased LTP in an *in vitro*, slice physiology experiment. Rosavin was more active at higher concentrations than salidroside; while, salidroside was more effective at lower concentration. This data further support the potential for RRE of acting as a plasticity-modulator in mammals.

It is now possible to test in humans the effect of a pharmacologic intervention on cortical excitability and plasticity using Transcranial Magnetic Stimulation (TMS) and transcranial Direct Current Stimulation (tDCS). For instance, several pharmaco-TMS studies investigated the effect of the acute intake of a drug on membrane excitability, cortical inhibitory and excitatory circuits using a well-characterized array of electrophysiological TMS parameters.[25] In addition, it has been demonstrated that tDCS activates glutamate N-methyl-D-aspartate receptors (NMDAR). The subsequent larger Ca^{++} entry in the postsynaptic terminal leads to cortical plasticity that in motor cortex can be quantified with changes in motor evoked potentials (MEP) amplitude.[26] These plastic changes are often described as LTP- and LTD-like phenomena as they share similarities with LTP and LTD tested in brain slices.[27]

The purpose of this study was to investigate the effect of a single oral dose intake of RRE on cortical excitability and plasticity in humans. We hypothesized that RRE intake would induce changes in LTP and LTD-like plasticity consistent with its putative adaptogenic effects.

2. Methods

2.1. Subjects

Twenty-eight right-handed healthy subjects[28] participated in the study. Fourteen volunteers (9 males, 5 females, mean age: 27.6, SD: 3 ± 7) were enrolled into the cortical excitability experiment and fourteen subjects (8 males, 6 females, mean age 29.7 ± 4.7 SD) participated into the cortical plasticity experiment. All the participants were between 18 and 50 years old. They had no history of medical or neuropsychiatric diseases, no metal implants, no brain trauma, no history of any pharmacological treatment, no family history of epilepsy and no present pregnancy.

The study was approved by our Institutional Review Board (2016-04) and it was conducted in accordance with the latest version of the Declaration of Helsinki Principles. All participants gave their written informed consent before participation.

2.2. Experimental procedure

The study was conducted in a double-blinded, placebo controlled crossover design. In both excitability and plasticity protocols subjects were required to intake two capsules, each capsule containing 250 mg of a commercially available RRE (standardized to 3% rosavins and 1% salidroside) or placebo (equal amount of flour). According to the manufacturer (Nature's Way, Green Bay, Wi, USA), the authenticity of the herbal product is certified through TRU-ID certification and that the RRE extract is standardized (each pill contains 7.5 mg of rosavins and 2.5 mh of salidroside). No information are provided regarding the extraction method, quality control and analytical techniques used to the active constituents. The acute dose intake was selected because it equals the daily dose that was used in clinical trials,[19,1516] and has been demonstrated to significantly improve endurance exercise capacity in young healthy volunteers after an acute intake.[29] The order of the experimental conditions (RRE/placebo) was pseudorandomized and balanced between subjects. In the cortical excitability study, the experimental conditions were separated by 1-week washout period while in the cortical plasticity study there was a 3 weeks washout period.

2.3. Cortical excitability

Fourteen participants were comfortably seated in an armchair. Electromyography (EMG) activity was recorded from the right first dorsal interosseous (FDI) muscle using surface electrodes placed in a tendon-belly arrangement. TMS was delivered through a figure-of-eight coil (9 cm) connected to a Magstim 200 magnetic stimulator (The Magstim Company, Dyfed, UK).The coil was placed tangentially to the scalp with the handle pointing backward, 45° away from the midline at a scalp position that induced the largest MEP recorded from the target muscle. Surface electromyography was monitored on a computer screen to ensure muscle relaxation. The signal was amplified, filtered (band-pass 2 Hz to 5 kHz), digitized at 5 kHz (Micro1401, Cambridge Electronics Design, Cambridge, UK).

Several parameters of corticospinal excitability were investigated. We tested resting motor threshold (RMT) defined as the minimum intensity needed to produce MEPs of at least 50-μV amplitude in 5 out of 10 trials.[30] We assessed the motor recruitment curve (RC), a measure of pyramidal tract neuron recruitment, by eliciting MEPs at stimulation intensities that increased systemically by 10%, from 100% of RMT to 150%. Mean peak-to-peak MEP amplitudes (20 trials at each stimulation intensity) were recorded. The cortical silent period (CSP) was induced with a stimulus delivered at 150% of RMT intensity FDI contraction (50% of maximal voluntary contraction). Ten trials were collected in each subject. SICI and ICF were tested by using paired-pulse TMS. In this TMS paradigm, a subthreshold conditional stimulus (80% of the RMT) is delivered 2 ms (SICI) and 10 ms (ICF) before a test stimulus.[31] The different inter-stimulus interval (ISI) were randomly selected and twenty MEPs were recorded for each condition. F-wave amplitude and M-max (supra-maximal electrical stimulation of the median nerve at the wrist) were tested to investigate changes in spinal motor neuron and neuromuscular excitability.[32]

TMS parameters were tested according to published guidelines for the use of TMS in clinical neurophysiology.[33] TMS recordings were performed before (T0), 1 h after (T1), and 24 h (T2) after the RRE or placebo intake (Fig. 1).

2.4. Cortical plasticity

Fourteen volunteers were seated on a comfortable armchair. Twenty MEPs were recorded from the right FDI muscle delivering a stimulation intensity that induced ~ 1 mV amplitude MEPs.

Immediately after the baseline measurement, volunteers received either RRE or placebo. One hour after the intake, 20 MEPs were collected with the using the same TMS intensity. To induce LTD-like plasticity cathodal-tDCS was applied for 9 min at 1 mA intensity. Anodal tDCS was applied for 13 min at 1 mA intensity to elicit LTP-like plasticity. 20 MEPs were recorded immediately (T0), 15 (T15), and 30 (T30) minutes after the end of the stimulation (Fig. 2A).

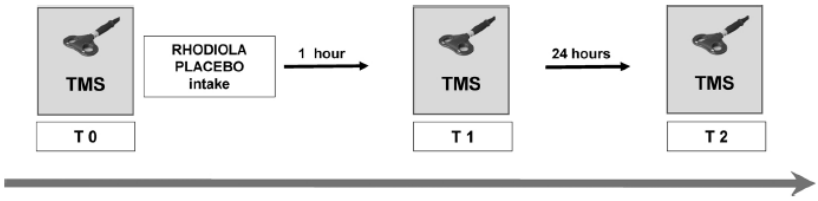

Fig. 1. Experimental design. Neuro physiological parameters of cortical and spinal/ peripheral nerve excitability were tested at baseline, (T0), 1 h after (T1) and 24 h (T2) after Rhodiola Rosea extract or placebo intake. TMS: transcranial magnetic stimulation.

Direct current was transferred by a pair of saline soaked surface sponge electrodes (35 cm^2) and delivered by a battery-driven constant current stimulator (ActivaTek, Inc. Salt Lake City, UT). The stimulating electrode was placed over the motor cortex representation area (hot-spot) of the right FDI as determined by TMS. The return electrode was placed contralaterally above the right orbit. Cathodal tDCS (13 min) or anodal tDCS (9 min) were applied at an intensity of 1 mA.

Previous TMS-studies showed that cathodal stimulation decreases cortical excitability while anodal stimulation increases it for approximately 1 h after stimulation,[3427] This stimulation protocol was used in previous pharmaco-TMS studies that assessed the effect of a single dose of drug intake on cortical plasticity in healthy subjects,[3536]

3. Statistical analysis

Data were analyzed using SPSS software (version 23.0; SPSS, Inc., Chicago, IL) and are presented as means ± SE. All the cortical and spinal excitability parameters were analyzed using a two-way repeated-measure analysis of variance (ANOVA), with main effect "Capsules intake" (RRE, Placebo) and "Time" (T0, T1 and T2). We used a three-way repeated-measure ANOVA to analyze the recruitment curve: main effects "Capsules intake", "Stimulation intensity" (100% RMT, 110% RMT, 120% RMT, 130% RMT, 140% RMT, 150% RMT) and "Time" (T0, T1, T2). The cortical plasticity protocol was analyzed using a three-way repeated-measures ANOVA with "Capsules intake" and "Stimulation" (anodal and cathodal tDCS) as between-subjects factors and "Time" (Baseline, T0, T15, T30) as within-subjects factor followed by posthoc analysis with Bonferroni correction. We tested the assumption of sphericity and used the Greenhouse-Geisser correction.

4. Results

RMT ["Capsules intake": $F_{(1,\ 52)} = 0.04$, p = 0.84; "Time": $F_{(2,\ 52)} = 0.39$, p = 0.67; "Capsules intake" X "Time" interaction: $F_{(2,\ 52)} = 0.13$, p = 0.87], MEP recruitment ["Capsules intake": $F_{(1,260)} = 0.19$, p = 0.8]; "Intensity" $F_{(5,260)} = 103.4$, p < 0.0001; "Time" [$F_{(2,260)} = 3.4$, p = 0.2]; "Capsules intake" X "Intensity" X "Time" Interaction [$F_{(10,260)} = 0.011$, p = 0.4], CSP ["Capsules intake": $F_{(1,\ 52)} = 0.05$, p = 0.82; "Time": $F_{(2,\ 52)} = 0.37$, p = 0.69; "Capsules intake" X "Time" interaction: $F_{(2,\ 52)} = 0.13$, p = 0.87], SICI ["Capsules intake": $F_{(1,\ 52)} = 0.97$, p = 0.33; "Time": $F_{(2,\ 52)} = 0.002$, p = 0.99; "Capsules intake" X "Time" interaction: $F_{(2,\ 52)} = 1.47$, p = 0.23], and ICF ["Capsules intake": $F_{(1,\ 52)} = 0.52$, p = 0.47; "Time": $F_{(2,\ 52)} = 0.14$, p = 0.86; "Capsules intake" X "Time" interaction: $F_{(2,\ 52)} = 2$, p = 0.14] were not different between the experimental conditions. Furthermore, RRE intake did not modulate spinal cord excitability [F- wave: "Capsules intake": $F_{(1,\ 52)} = 0.002$, p = 0.96; "Time": $F_{(2,\ 52)} = 0.09$, p = 0.91; "Capsules intake" X "Time" interaction: $F_{(2,\ 52)} = 0.35$, p = 0.7; M-max: "Capsules intake": $F_{(1,52)} = 0.010$, p = 0.92; "Time": $F_{(2,52)} = 0.24$ p = 0.78; "Capsules intake" X "Time" interaction: $F_{(2,\ 52)} = 0.014$, p = 0.98] (Table 1).

The intake of RRE or placebo did not modulate baseline MEP amplitude ["Capsules intake": $F_{(1,52)} = 0.16$, p = 0.68; "Stimulation": $F_{(1,\ 52)} = 1.91$, p = 0.17; "Time": $F_{(1,52)} = 0.15$, p = 0.69; "Capsules intake" X "Stimulation" X "Time" interaction: $F_{(1,\ 52)} = 0.059$, p = 0.8]. Furthermore, repeated-measure ANOVA showed a main effect "Capsules intake": $F_{(1,\ 52)} = 4.7$, p = 0.03; "Stimulation": $F_{(1,\ 52)} = 51$, p = < 0.001 and "Time": $F_{(3,\ 52)} = 13.6$, p < 0.0001 with a not significant "Capsules intake" X "Stimulation" X "Time" interaction: $F_{(3,\ 156)} = 0.87$, p = 0.4.

Post-hoc analysis indicated that cathodal stimulation induced a statistically significant decrease in MEP amplitude after the placebo intake while RRE intake inhibited LTD-like plasticity (RRE: before 0.82 ± 0.06 mV, T0 0.85 ± 0.07 mV p = 0.53, T15 0.91 ± 0.09 mV p = 0.14, T 30 0.9 ± 0.09 mV p = 0.23; placebo: before 0.89 ± 0.07 mV, T0 0.71 ± 0.08 mV p = 0.0012, T15 0.6 ± 0.08 mV p < 0.0001, T30 0.61 ± 0.047 mV p < 0.0001) (Fig. 2B).

After both RRE and placebo intake, anodal tDCS statistically increased MEP size (RRE: before 0.95 ± 0.049 mV, T0 1.47 ± 0.08 mV p < 0.0001, T15 1.53 ± 0.1 mV p < 0.0001, T30 1.51 ± 0.1 mV

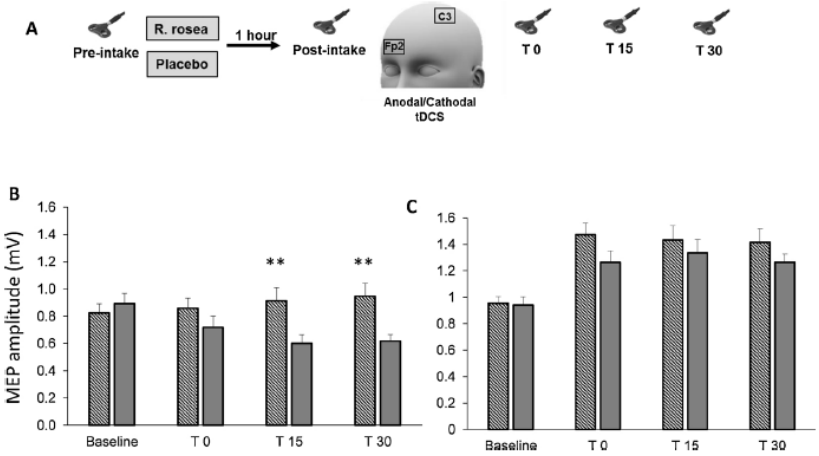

Fig. 2. Rhodiola Rosea extract and neuroplasticity.
A. Motor evoked potential (MEP) were recorded before (pre-intake) and 1 h after Rhodiola Rosea extract (RRE) or placebo intake (post-intake). Cathodal transcranial Direct Current Stimulation (tDCS) (9 min) and anodal tDCS (13 min) were applied with an intensity of 1 mA. MEPs were recorded to monitor MEP amplitude after tDCS (T0: immediately after; T15: 15 min after; T30:30 min after).
B. Cathodal tDCS. MEPs at baseline were not different between the two experimental conditions. Furthermore, Rhodiola Rosea extract (RRE) intake inhibited LTD-like plasticity at each time point after tDCS. Mean ± SE; **: p < 0.01.
C. Anodal tDCS. There was no significant difference in the amount of LTP-like plasticity between the two experimental conditions. Mean ± SE.

Table 1
TMS parameters of corticospinal excitability. All values are means ± SE before (baseline), 1 h after, and 24 h after intake of a single oral dose of Rhodiola Rosea extract (RRE) or placebo. RRE intake did not modulate RMT, MEP amplitude recruitment curve, CSP duration, SICI, ICF, F- wave and Mmax. RMT: Resting Motor Threshold; MEP: Motor Evoked Potential; CSP: Cortical Silent Period; SICI: short-interval intracortical inhibition; ICF: intracortical facilitation; Mmax : maximum M-wave.

Rhodiola Rosea				Placebo		
Baseline		**1 h**	**24 h**	**Baseline**	**1 h**	**24 h**
RMT %	42.9 ± 1.9	42.8 ± 2	42.9 ± 1.8	43.5 ± 1.9	43.2 ± 1.8	43.5 ± 1.8
MEP $_{100\%\ RMT}$(mV)	0.13 ± 0.02	0.18 ± 0.04	0.17 ± 0.03	0.1 ± 0.02	0.18 ± 0.04	0.15 ± 0.04
MEP $_{110\%\ RMT}$(mV)	0.38 ± 0.04	0.40 ± 0.07	0.36 ± 0.04	0.43 ± 0.05	0.44 ± 0.05	0.43 ± 0.02
MEP $_{120\%\ RMT}$(mV)	0.73 ± 0.10	0.89 ± 0.10	0.76 ± 0.11	0.95 ± 0.16	0.91 ± 0.08	0.76 ± 0.1
MEP $_{130\%\ RMT}$(mV)	1.56 ± 0.16	1.70 ± 0.13	1.54 ± 0.18	1.76 ± 0.22	2.05 ± 0.27	1.89 ± 0.23
MEP $_{140\%\ RMT}$(mV)	2.30 ± 0.20	2.63 ± 0.25	2.50 ± 0.27	2.36 ± 0.27	2.83 ± 0.3	2.49 ± 0.29
MEP $_{150\%\ RMT}$(mV)	2.57 ± 0.20	2.99 ± 0.20	2.93 ± 0.25	2.73 ± 0.33	2.97 ± 0.28	2.85 ± 0.38
CSP $_{150\%\ RMT}$ ms	138.3 ± 10.9	136.6 ± 10.9	140.1 ± 12.4	144.5 ± 9	138.5 ± 10.4	141.8 ± 11.2
SICI	57.3 ± 6.98	52.5 ± 5.4	53.2 ± 4.7	45.1 ± 4.9	49.8 ± 5	48.9 ± 3.8
ICF	146.8 ± 6.8	138.9 ± 6.6	141.1 ± 5.4	145 ± 5.8	150.8 ± 5.2	147 ± 4.9
M_{max} (mV)	15.87 ± 1	16.3 ± 1.1	16.1 ± 1.1	15.8 ± 1.1	16.1 ± 1.4	15.9 ± 1.4
***F*-wave amplitude (μV)**	293.7 ± 22.4	293.8 ± 15	306.9 ± 19.3	303.6 ± 19.3	294.8 ± 15.7	293.2 ± 20

$p < 0.0001$; placebo: before 0.99 ± 0.07 mV, T0 1.2 ± 0.08 mV $p = 0.0041$, T15 1.33 ± 0.01 mV $p = 0.0021$, T30 1.31 ± 0.06 mV $p = 0.0033$) (Fig. 2C). Student's *t*-test indicated that in the cathodal/ group there were statistical significant differences at T15 and T30 after stimulation (T0 $p = 0.09$, T15 $p = 0.0013$, T30 $p = 0.0016$). On the contrary, no differences were observed for the anodal/group condition (T0: $p = 0.1$; T15: $p = 0.2$; T30: $p = 0.08$).

5. Discussion

The results of this study provide evidence that the acute administration of RRE in healthy subjects modulated cortical plasticity averting LTD-like plasticity induced by cathodal tDCS. In addition, RRE intake caused a non-significant increase of LTP-like plasticity and did not affect TMS parameters of corticospinal excitability.

In the field of herbal medicine, adaptogens are characterized by their broad pharmacological activity and are recommended for their antistress and nootropic properties.[37] In keeping with this definition, we hypothesized that a putative adaptogenic herb, like RRE, would affect plasticity and we found that the acute intake of RRE decreases LTD-like plasticity. Neuroplasticity is the process by which neuronal circuits adapt to external stimulation.[38] LTD and LTP are a form of activity-dependent synaptic plasticity leading to long-lasting modifications in the efficacy and strength (potentiation/depression) of synaptic transmission.[39] Several reports indicate that stress has a detrimental impact on synaptic plasticity, learning, and memory.[40] Our finding indicates that RRE could counteract the effect of stress on cortical plasticity. Emerging evidence indicate that stress impairs LTP and enhances LTD inducing a non-physiological shift at a network level that favours LTD over LTP induction [41]. For instance, prenatal stress reduce LTP and enhances LTD in the offspring[42] and a similar electrophysiological effect is induced in adult animals exposed to behavioural stress.[43] These effects are thought to be due to the inhibition of the glutamate uptake with a consequent activation of the extra synaptic GluN2B-containing N-methyl-D-aspartate (NMDA) receptors which would results in favouring LTD induction.[44] In line with this report and consistently with our primary hypothesis, RRE intake prevented LTD-like plasticity and increased (non-significantly) LTP-like plasticity. Insofar as the current results indicate plastic changes that might counteract the detrimental effect of stress, these finding have a broader implication for better understanding the adaptogenic properties of pharmacological interventions.

It was proposed that the stress-induced release of glucocorticoids plays a pivotal role in inducing NMDAR and AMPA toxicity,[4546] and LTD enhancement.[47] This is in keeping with a previous report that showed strong enhancement of LTD induction and LTP suppression by high levels of circulating corticosterone or administration of glucocorticoid receptor agonists.[48] Our results might be linked to the fact that RRE counteracts and prevents the negative effect of stress on the HPA.[49,50] For instance, RRE treatment significantly decreased corticotropin-releasing hormone (CRH) mRNA expression levels and the levels of serum corticosterone in the rats exposed to water-floating and exhaustion experiments balancing, in this way the HPA axis.[51] These effects have been reported even after the administration of both a single dose of RRE and its active compound salidroside.[52] Interestingly, the decrease in cortisol production after RRE treatment is paralleled by the decline of proinflammatory cytokines induced by physiological and psychological stresses.[53] Thus, it is conceivable that changes in the HPA homeostasis might, in part, explain our findings. This hypothesis is further supported by previous studies in healthy volunteers that showed that changes in circulating levels of cortisol and high-stress level disrupt cortical plasticity, tested using non- invasive brain stimulation.[54 55]

Indeed, there is evidence indicating that RRE regulates brain neurotransmitters by inhibiting the activity of the enzymes responsible for monoamine degradation, monoamine oxidase A and B and catechol-O-methyltransferase[56] and by facilitating their transport within the brain.[49] The changes in 5-HT and NE concentration in the synaptic cleft[57] might be of importance in explaining the effect of RRE on LTD-like plasticity. For instance, preclinical data in brain slice preparations indicates that 5-HT receptor activation blocks LTD or even converts it into LTP[58–60] In addition, previous works in humans demonstrated that both conventional antidepressants (citalopram and reboxetine) and the antidepressant herb Hypericum perforatum modulate LTD-like plasticity (reduction and/or reversal to LTP) in humans.,[35 6136] Thus, our data might be consistent with a serotoninergic and noradrenergic enhancement induced by RRE intake. Future studies are needed to prove this causal link. Dopamine modulating effects on synaptic plasticity are heterogeneous.[62] Although RRE increases DA brain concentration in animal models, it is speculative to suggest a role for these neurotransmitter on the reduction of LTD-like plasticity we found.

This study has limitations. We tested only the most commonly dosage used in therapeutic trials and we addressed post-tDCS after-effects up to thirty minutes. Future studies should investigate dose-response effects and long-lasting after-effects. Like for the other pharmaco-TMS studies, our results are descriptive. Probing other commercial RREs could further our understanding of the possible mechanisms underlying the adaptogenic and neuromodulatory effect of this herbal treatment. A major limitation of the study is the use of an industrial product. Important information regarding the extraction method and standardization process (that are often proprietary) are missed. Thus, our data

need to be confirmed in future studies. In addition, we have no information about harvest time. This is of relevance because the composition of the aromatic compounds (salidroside and rosavine) of the extract is harvest-time dependent with a significant decrease in concentration in rhizomes harvested at the end of the summer.[63]

6. Conclusion

In conclusion, a wealth of information is emerging about the impact of plasticity in neuropsychiatric diseases. Herb-plasticity interaction studies using non-invasive brain stimulation have an opportunity to provide a new perspective on the therapeutic assessment of herbal medicine and mechanism of action of herbal therapeutics in humans.

Conflict of interest

The authors declared no potential conflicts of interest.

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DISCUSSION

The main aim of this project was to investigate whether the CAM herbal products commonly used to treat psychiatric conditions would modulate cortical circuitries in a similar manner as conventional antidepressant and anxiolytic drugs. In addition, it was investigated, for the first time, the effect of an adaptogen on cortical excitability and plasticity in humans.

In Study 1 it was demonstrated that HYP extract WS5570 modulates LTP-like and LTD-like plasticity in humans in a similar way to conventional antidepressants (citalopram/ reboxetine). The effects of conventional antidepressants on plasticity are supported by a large number of preclinical studies. LTP and LTD, as induced by repetitive electrical stimulation in animal preparations, are modulated by serotonin in a prominent but heterogeneous way. Serotonin enhancement or serotonin receptor activation have been shown to reduce or abolish LTP (Edagawa *et al.*, 1998) (Kojima *et al.*, 2003) (Mnie-Filali *et al.*, 2006) but, serotonin antagonists are also able to abolish LTP (Sanberg *et al.*, 2006) (Huang *et al.*, 2012). Other studies have demonstrated that serotonin activation can enhance LTP (Kojic *et al.*, 1997) (Mori *et al.*, 2001). The impact of serotonin on LTP seems to be affected by age, cortical area, the types of serotonergic subtypes of receptors, and duration of serotonin receptor activation (Kojic *et al.*, 1997) (Ohashi *et al.*, 2002) (Mori *et al.*, 2001) (Ryan *et al.*, 2009) (Bhagya *et al.*, 2011). Moreover, data on serotonin and LTD is still controversial and seem to be dependent upon the stimulation of different receptors. It was shown that 5-hydroxytryptamine (5-HT) 4-receptor activation converts LTD into LTP in hippocampal slice preparations (Kemp & Manahan-Vaughan, 2005). A similar pattern of modulation has been demonstrated in humans using tDCS. Translational studies in humans showed that the administration of conventional antidepressants affected both cortical excitability (reduced ICF, enhanced SICI, changes in MEP amplitude and RMT) and plasticity (Ziemann, 2013). A single dose of the SSRI citalopram enhanced and prolonged

LTP-like plasticity induced by anodal tDCS and converted cathodal tDCS-induced LTD-like plasticity into facilitation in healthy subjects (Nitsche *et al.*, 2009). The administration of the NRI reboxetine reverted cathodal tDCS-induced LTD-like plasticity into facilitation in healthy subjects (Kuo *et al.*, 2017). In this study, the administration of a single oral dose of HYP extract WS5570 reversed cathodal tDCS-induced LTD-like plasticity into facilitation. The increased serotonin level in the synaptic cleft induced by HYP extract intake might have played a role in the reversal of the LTD-like plasticity through a possible activation of the 5-HT4 receptor (Kemp & Manahan-Vaughan, 2005). It cannot be ruled out that noradrenergic activity contributed to the results. Preclinical studies showed an enhancement effect of adrenaline on LTP and LTD, while noradrenaline blocked LTD (Ziemann *et al.*, 2015). It can only be speculated regarding the possible mechanisms underlying these findings. For instance, several in vivo and in vitro studies indicated that HYP extract antidepressant activity is mainly related to its compound hyperforin that inhibits the neuronal uptake of serotonin, noradrenaline and dopamine from synaptic cleft (Schmidt & Butterweck, 2015). In addition, HYP extract increases cAMP response element binding protein (CREB) levels in rat hippocampus (Crupi *et al.*, 2013), prevented the corticosterone-induced decrease in hippocampal cell proliferation (Crupi *et al.*, 2011) and normalized the reduction of mRNA expression of BDNF found in the hippocampus of stressed mice (Patel *et al.*, 2016). Taken together these findings indicated a SSRI-like activity of the HYP extract and a complex modulation of neuroplasticity.

In study 2 it was planned to investigate whether a commonly used anxiolytic herb (VE) would modulate GABA-ergic neurotransmission in humans assessed by TMS neurophysiologic parameters. A large number of studies on herbal extracts with anxiolytic properties, including VE, demonstrated an effect on GABA neurotransmission acting mainly on GABA-AR binding and on the elevation of GABA-AR expression and GABA release in

vitro. For example, both the Noni fruit and the ginseng root display competitive binding to the GABA-AR in vitro. In vitro studies have shown that Kava, German chamomile, hops (Humulus lupulus) and Centella asiatica increase GABA levels in rat brains (Weeks, 2009). The main aim of the study was to demonstrate in humans the putative GABAergic activity of VE by using TMS measures of cortical excitability. GABA-ARs are generally pentameric postsynaptic receptors consisting of different subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π, ρ1– 3) surrounding a central chloride ion-selective channel gated by GABA. GABA-mediated chloride influx results in hyperpolarization of the postsynaptic neuron. Most GABA-ARs contain α, β, and γ subunits, including the most abundant α1β2γ2 receptor which makes up about 60% of all GABA-ARs in the brain. The subunit, particularly the α one, determines the receptor pharmacological characteristics. Thus, receptors that include α1, α2, α3, or α5 are modulated by benzodiazepines (Engin *et al.*, 2018).

GABA-ARs containing the β3 subunit have been identified as the major target which mediates the anxiolytic action of valerenic acid, that is considered the main active compound in VE. For instance, [3H]valerenic acid binding to brain membranes demonstrated that valerenic acid acts as an agonist. Moreover, judging by the ligand selectivity, the GABA-AR binding site of valerenic acid seemed to be different from the known conventional drug (benzodiazepines) modulatory sites of these receptors. Valerenic acid is a subunit-specific (β 3) allosteric modulator of GABA-Rs. Point mutations in the β 2 and β 3 GABA-A receptor subunits inhibited the GABA potentiating effect of valerenic acid on recombinant receptors showing that these subunits have a role in the activity of valerenic acid (Benke *et al.*, 2009).

In this study VE intake affected motor cortex excitability inducing a reduction in the amount of ICF and a lack of activity on SICI. Previous pharmaco-TMS studies demonstrated that these two parameters are modulated by benzodiazepines. For example, positive

modulators of GABA-ARs containing α1-, α2-, α3- or α5-subunits increased SICI meanwhile zolpidem, a benzodiazepine-like hypnotic with largely specific positive modulation of the α1-GABA-AR had no effect on SICI. The GABA re-uptake inhibitor tiagabine and the GABA-BR agonist baclofen decreased SICI, supporting the notion that SICI is controlled by presynaptic GABA-BR mediated autoinhibition of inhibitory interneurons (Ziemann, 2004). In this study the lack of effect on SICI might be explained by the fact that VE targets neuronal circuits expressing GABA-ARs containing β3 subunit, while SICI represents inhibition manly mediated by α2- or α3-GABA-ARs.

The main finding of the study was that a single dose of VE extract intake modulated cortical excitability decreasing ICF. Previous pharmaco-TMS studies demonstrated that NMDAR antagonists dextromethorphan (Ziemann *et al.*, 1998), and riluzole (Schwenkreis *et al.*, 2000) reduced ICF. Furthermore, the contribution of GABA-A inhibition to ICF is supported by the decrease in ICF induced by a single dose of lorazepam, zolpidem, and diazepam indicating that GABA-A agonists contribute to the net facilitation represented by ICF (Ziemann, 2004) (Ziemann *et al.*, 2015). It can only be speculated regarding the mechanism underling the modulation of ICF after VE intake. It is likely that VE intake might have changed the interplay between inhibitory GABAergic interneurons and excitatory glutamatergic interneurons with a net effect on excitation. Future studies are needed to address this issue.

In study 3, it was explored the modulatory effect on brain circuitries of RRE, an herb used for centuries as an adaptogen i.e. stress-response modifiers that increase an organism's nonspecific resistance to stress by increasing its ability to adapt and survive. Preclinical studies indicate that stress induces release of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH,) adrenal hormones and neuropeptide Y (NPY). Glucocorticoids receptors are inhibited by SAPK/JNK and consequently the feedback

downregulation is blocked and cortisol content in blood remains high during stressful conditions. It has been demonstrated that RRE suppresses elevated levels of cortisol and other extracellular and intracellular mediators of stress response, such as elevated NO, SAPK and heat shock proteins Hsp70, which are known to inhibit SAPK. In cellular adaptation to stress, in the increasing of survival, in the enhancing of longevity and in the improvement of cognitive function both NPY and Hsp70 are directly involved (Amsterdam & Panossian, 2016).

Basic science studies demonstrated that stress induces detrimental effects on synaptic plasticity increasing LTD and decreasing LTP. Acute stress facilitates the induction of LTD in the hippocampal CA1 region of the adult rodent brain. The induction of hippocampal CA1 homosynaptic LTD depends on NMDARs, which are heteromeric complexes of NR1 subunits and at least one type of NR2 subunit (NR2A–D). The corticosterone release induced by stress causes the increase of glutamate concentrations in the synaptic cleft by increasing glutamate release and/or decreasing glutamate transport in the hippocampus. The glutamate increase enables the induction of LTD through spillover activation of NR2B-containing NMDARs that are extrasynaptically localized.The expression of LTD is facilitated by the endocytosis of postsynaptic AMPA-Rs. Recent evidence suggests that stress-enabled LTD may result from hippocampal glucocorticoid receptor activation (Wong *et al.*, 2007). Studies in healthy volunteers showed that changes in circulating levels of cortisol and high-stress level disrupt cortical plasticity, tested using NIBS (Sale *et al.*, 2008) (Concerto *et al.*, 2017). In line with this literature and consistently with our primary hypothesis, RRE intake prevented LTD-like plasticity and increased (non- significantly) LTP-like plasticity. Although the inhibition of cortisol might play a role, it is likely that our results might be due to an effect of RRE on neurotransmitters. For instance, preclinical evidence indicates that RRE inhibits MAO activity and the change in serotonin and

noradrenaline in the synaptic cleft might be of importance in explaining the effect of the herb on LTD-like plasticity. Thus, this data might be consistent with a serotoninergic and noradrenergic enhancement induced by RRE intake. It should be acknowledged that pharmaco-TMS studies are descriptive in nature. Thus, it is difficult to make inferences about causality when using a herbal extract that contains numerous active compounds.

CONCLUSION

Herbal medicine represents one of the most frequently used CAM approaches for the treatment of psychiatric conditions. The complexity of herbal products and the concerns regarding their effectiveness warranted the need to develop new approaches to investigate their pharmacological activity in humans. These studies explored the relationship between herbal products commonly used in psychiatry and brain plasticity and excitability in humans. The results fill a gap in the literature as those are the first translational studies on this topic. Furthermore, CAM has been gradually accepted as a useful addition to conventional medicine and these results point towards a powerful modulation of brain circuitries that might be relevant to the understanding of their mechanism of action and their effectiveness.

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