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A Methodological Study Comparing Two Motor Cortex Plasticity Paradigms in Healthy Adults.

Degree Project in Medicine

Kenan Hodzic

Programme in Medicine

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Supervisor: Caroline E. Wass

Division of Pharmacology, Institute of Neuroscience and Physiology, University of Gothenburg

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Abstract

Background:

Neural plasticity is an important function of the brain allowing for change in synaptic transmissions. Modulation in plasticity arises through activity-dependent strengthening; long-term potentiation (LTP), or weakening; long-term depression (LTD), of synaptic transmissions. Such plasticity may be induced by repeated pairing of transcranial magnetic stimulation (TMS) to the human motor cortex, with peripheral median nerve stimulation (PNS), a method called paired associative stimulation (PAS). PAS induces LTP-like (*i.e.* PAS_{LTP}) or LTD-like (*i.e.* PAS_{LTD}) cortical changes in excitability, measured as motor evoked potentials (MEPs) using electromyography (EMG) of the targeted hand muscle.

Aims:

In order to utilize the PAS-method to investigate aberrant neuroplasticity in pathologies, we compared two well-established PAS-paradigms regarding their capacity to induce either PAS_{LTP} or PAS_{LTD}, the impact of time efficiency and frequency of reported adverse events.

Methods:

In the present double-blinded, crossover study we compared two different PAS-paradigms in 14 healthy subjects: 180 paired TMS + PNS stimulations (PAS-180) at 0.1Hz and 225 paired TMS + PNS stimulations (PAS-225) at 0.25Hz. Each paradigm consisted of two protocols: PAS_{LTP} utilizing an interstimulus interval (ISI) between pairings of 25ms inducing increased excitability, and PAS_{LTD} utilizing an ISI of 10ms inducing decreased excitability.

Results:

Responders were defined as having a grand mean of MEPs larger than the averaged baseline for PAS_{LTP}, and a lower grand mean of MEPs for PAS_{LTD}. Both paradigms successfully induced PAS_{LTP} in responders (N=9), however no PAS_{LTD} effects were found in either paradigm. PAS-225 had a lower frequency of reported adverse events and was more time efficient.

Conclusions:

Both paradigms induced equivalent PAS_{LTP} effects in subjects. Due to PAS-225 being more time efficient and associated with less reported adverse events, it is seen as preferential and will be used in future studies examining neural plasticity.

Key words:

Transcranial magnetic stimulation, paired associative stimulation, neural plasticity

1. BACKGROUND

1.1 Synaptic plasticity

Synaptic connections between neurons are the basic building blocks of the brain's circuitry. In 1949 psychologist Donald Hebb refined the notion that learning and memory formation relies on changes in synaptic connections. He hypothesized that a metabolic- or growth response in the synapse between neurons accompanied synaptic activity. Reiterations of activity reinforced synaptic connections, making them more stable and readily traversed[1]. This is now commonly referred to as Hebbian plasticity, and often summarized as "Neurons that fire together, wire together". Hebbian plasticity is defined as strengthening or weakening of synaptic transmission due to activity-dependent modifications in a synapse between neurons. The most investigated examples of Hebbian plasticity are long-term potentiation (LTP), being an increase in synaptic transmission between two neurons, and its inverse counterpart long-term depression (LTD), being a decrease in synaptic transmission [2]. Long-term synaptic plasticity has been thoroughly investigated in the mammalian hippocampus. Bliss et al[3] discovered that a few seconds of high-frequency electrical stimulation of cortical fibers in the hippocampus in rabbits enhanced synaptic transmission for days and even weeks. Subsequent studies further established that LTP required concomitant temporal coupling of postsynaptic depolarization with presynaptic activity [4, 5], *i.e.* presynaptic input preceding postsynaptic depolarization. If the order is reversed, a weakening of the synapse is induced, LTD. This classical model is called spike-timing-dependent plasticity (STDP). This coordinated activity as a mechanism behind synaptic plasticity correlates with Hebb's postulation, and has been demonstrated in a variety of models from hippocampal slices[6] to intact animals[7].

1.2 Basic molecular mechanism underlying long-term potentiation and long-term depression

In our molecular understanding of LTP and LTD, the roles of the *N*-methyl-*D*-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors are crucial [2, 8], although exceptions in *i.a.* hippocampal mossy fibers occurs [9]. The NMDA-receptor had long remained an enigma for neuroscientist. However, Collingridge et al (1983)[10] discovered that NMDA-antagonists induced lower excitatory postsynaptic potentials (EPSP) studying *in vivo* hippocampal slices, and hypothesized that the receptor could be responsible for mediating LTP. Ascher and Nowak (1988) furthered the research by elucidating the NMDA-receptor biomolecular mechanisms of action [11]. Glutamate is the driving force of AMPA- and NMDA-receptors, binding to them as a ligand and inducing neural transmission. However, when the cell is at its resting membrane potential, NMDA is blocked by Mg^{2+} [11, 12]. This blockage is voltage-gated, and the AMPA receptors are responsible for the initial depolarization of the cell [13]. Activation of AMPA receptors by means of glutamate leads to influx of Na^{+}, depolarizing the postsynaptic cell. Depolarization allows for the removal of the Mg^{2+} blocking the NMDA receptor, causing an influx of positively charged ions to the postsynaptic cell, where Ca^{2+} plays a major role. Depending on the nature of the influx of Ca^{2+}, either LTP or LTD triggers. A fast and large increase of intracellular Ca^{2+} leads to LTP, and a slow and small rise leads to LTD [14]. The increased concentration of Ca^{2+} triggers the appropriate response in the cell by causing complicated intracellular cascades that changes the expressions of genes, modifying the synaptic structure and molecular activity[15].

1.3 History of transcranial magnetic stimulation

In 1980 Merton and Morton demonstrated that it was possible to electrically and non-invasively stimulate the cerebral cortex using scalp electrodes in an intact human subject and

evoke motor responses. They found that using brief, but high voltage shocks could elicit movements of the subjects contralateral hand when stimulating the motor cortex[16]. In 1985 the first reliable transcranial magnetic stimulator (TMS) was introduced by Anthony Barker and colleagues [17]. It allowed researchers to electrically stimulate and study the human motor cortex without direct contact with the scalp, in a non-invasive way. The principle is built upon Michael Faraday's law of induction. By discharging current from a large capacitor into a coiled wire, pulsed magnetic fields of 1-4 Tesla in strength are produced. These magnetic fields can be directed to an area of the brain to induce a perpendicular electrical current in the subjects' brain via electro-magnetic induction. The shape of the coil and orientation and location of neurons in the target area affects the quality of the stimulation, and findings show that electrical fields perpendicular to cortical layers are stimulated the most[18]. The coil shape dictates the focality of the induced current. The most commonly used coil is a figure-eight shaped coil, producing a maximal current at the intersection point of the shape. To be able to reproduce stimuli reliably, computer assisted navigation is used allowing infrared-based cameras to track both the coil and the patients head with the use of reflective spheres which are fixed on the coil itself and a head tracker. TMS circumvents the discomforts of direct electrical stimulation through the intact scalp, and also the electrical resistance of the skin, making it possible to induce relatively large currents in the brain. These currents can in turn depolarize neurons and modulate cortical excitability, either increasing or decreasing it[19].

1.4 Transcranial magnetic stimulation and clinical uses

Today the major application of TMS internationally is as a therapy for depression, and Socialstyrelsen recently approved it as treatment of moderate to severe depression in Sweden[20]. For therapeutic purposes, TMS is applied repetitively (rTMS) to the dorsolateral

prefrontal cortex. Several clinical trials have been conducted and recent meta-analysis demonstrate that rTMS is in fact both effective and safe for treating depression [21, 22]. It's also been shown to relieve conditions such as muscular dystonia by normalization of cortical inhibition [23]. Another important application is functional mapping of the brain is made possible using TMS by exciting or inhibiting regions in the brain. Inducing transient functional lesions it could be used for mapping out the laterality of language processing in patients with epilepsy being evaluated for a temporal lobectomy[24]. The technology is still young and many studies are being conducted researching different therapeutic targets.

1.5 Transcranial magnetic stimulation, plasticity and paired associative stimulation

TMS can be used to study plasticity in the brain by pairing a peripheral stimulation of the median nerve with low frequency TMS to the contralateral side of the primary motor cortex, so called paired associative stimulation (PAS). In the motor cortex, the location representing abductor pollicis brevis (APB) is located with single pulses of TMS. Stimulation to this area elicits motor responses in the APB muscle, so called motor evoked potential (MEP), which can be quantified by means of electromyography (EMG). As discussed, induction of cortical excitability or inhibition relies on the temporal pattern and coupling of inputs, *i.e.* STDP. In PAS, varying the interval between peripheral median nerve stimulation (PNS) and TMS pulse, so called the interstimulus interval (ISI), allows for induction of either an increase or decrease in the cortical excitability. A PNS preceding every TMS pulse by 10ms induces a depression in excitability, and an ISI of 25ms induces an increase in excitability [25]. Other intervals (*i.e.* 100, 525 and 1000 milliseconds) do not result in changes in excitability[26].

The proposed model is that the peripheral stimulation of the median nerve, relayed through somatosensory afferents arrive synchronously with the postsynaptic activation via TMS[26]. There is no direct anatomical connection between the primary motor cortex and the primary

somatosensory cortex, and information is thought to be relayed through the postcentral gyrus before reaching the motor cortex[27]. There is also a possibility of isolated afferent inputs directly relayed through thalamic projections to the primary motor cortex [28, 29]. Furthermore, *in vitro* studies have shown that stimulation of local intracortical fibers in the motor cortex paired with stimulation of afferents (cortico-cortical or thalamo-cortical) converging to the same postsynaptic target can induce LTP [30, 31]. Hess et al[31] demonstrated that LTP is facilitated by reducing intracortical inhibition through stimulation of afferent pathways, suggesting that this mechanism increases the excitability of the postsynaptic neuron in addition to possibly providing a synchronous signal. Stefan et al (2002) also concluded that electrical stimulation of the median nerve transiently disinhibits the motor cortex [32], facilitating changes in cortical plasticity.

PAS may reflect the canonical STDP-model as it exhibits the same necessity of being timing-dependent [33], but whether or not this is the actual underlying mechanism remains unknown. Another aspect regarding STDP is the involvement of NMDA receptors. Administration of Dextromethorphan, a non-competitive antagonistic drug of the NMDA-receptor, blocks PAS-induced cortical excitability, *i.e.* PAS-$_{LTP}$, suggesting the involvement of NMDA receptors in PAS-induced plasticity [32].

Classen et al's[26] research gives evidence that the plasticity being induced is on a cortical level. By studying F waves and electrical brainstem stimulation, modalities sensitive to elucidating changes in spinal excitability, investigations have been made to whether the changes in excitability actually occurred within the cortex. Following PAS intervention, no increase in brainstem stimulation induced MEPs could be detected, while TMS induced MEPs resulted in increased excitability. F-waves prior to and after PAS intervention showed unchanged amplitudes, suggesting unaffected excitability in the α-motor neurons of the

median nerve. These results indicate that the occurring changes in plasticity do occur within the cortex, and not on subcortical or spinal levels.

It remains unknown whether the observed changes in plasticity induced by PAS are the same as LTP/LTD, but they do seem to share similar traits such as being timing-dependent, spatially specific regarding stimulated region and effect, and rapidly induced in response to stimulation. Rajji et al[34] also demonstrated that the PAS facilitated potentiation of the motor cortex lasted up to a week, further linking the effects of PAS to physiological changes in cortical plasticity through evolution over time. Because of these associations, the terminology LTP- and LTD-like plasticity, or PAS_{LTP} and PAS_{LTD} is used when referring to the respective protocols.

1.6 Different paired associative stimulation paradigms

PAS was first described by Classen et al (2000). In the original protocol, 90 paired stimulations were delivered at 0.05 Hz[26]. In subsequent studies, various protocols have been used varying the amount of paired pulses and frequency between pairings. Generally, studies using lower frequency (*e.g.* 0.1 Hz) deliver lesser number of stimulations (*e.g.* 90-180 pairings), and higher frequency studies (*i.g.* 0.25 Hz) deliver a greater amount *(e.g.* 180 – 270 pairings)[35]. The goal of the present study was to compare two well established paradigms in our laboratory, namely 225 pulses at 0.25Hz and 180 pulses at 0.1 Hz, using an ISI of 10ms for PAS_{LTD} and 25ms for PAS_{LTP}. Both paradigms have been used in various studies, utilizing induction of changes in plasticity to elucidate different mechanisms in somatic pathologies *i.a.* focal dystonia [36, 37], development of levodopa-induced dyskinesia in Parkinson's disease [38] and possibilities of enhancing motor learning in certain groups of neurological patients[39]. The paradigms are reliable in their induction of PAS_{LTP} and PAS_{LTD}, allowing the use of PAS to investigate the important aspect of aberrant neuroplastic changes in pathologies and the

effects it has in these diseases. What remains unclear is which one of these two paradigms is the most efficacious with regards to magnitude of changes in cortical excitability.

1.7 PAS in future research

In summary, PAS is one of the first electrophysiological methods that may be used to assess changes in synaptic neuroplasticity in the intact human brain. It is thought to reflect the STDP model with synchronous presynaptic and postsynaptic interplay being crucial to the induction of changes in neuroplasticity. Our goal is to establish this method in order to investigate the progressive neuroadaptations in addiction and the mechanism through which our gut hormones drives development of addictive disorders. Previous studies investigating the effects of alcohol on human plasticity has found that acute administration of per-oral ethanol significantly impairs motor cortex LTP in healthy humans [40]. What remains a knowledge-gap is the crosstalk between appetite regulation and addiction. Previous findings have indicated that reward induced by food- and alcohol intake share common mechanisms [41], and receptors for these peptides have been found throughout the reward system of the brain [42, 43]. To investigate the role of gut hormones on the effects ethanol induced changes in human neuroplasticity, we conducted this methodological study to establish PAS in our laboratory by comparing two of the most commonly used PAS paradigms and compare their efficacy and efficiency.

2. AIMS

To establish the PAS_{LTP} and PAS_{LTD} protocols in our laboratory and compare the effects of two commonly used PAS-paradigms which have been proven effective in previous studies, and investigate whether one has advantages over the other in the induction of plasticity (*i.e.* comparing the magnitudes of changes in cortical excitability) in the human motor cortex, time

efficiency and possible adverse events. When established, we aim to use PAS as a method to investigate neuroplastic changes and its effects in certain pathologies.

3. ETHICAL CONSIDERATIONS

The study was conducted in healthy subjects and a cost versus benefit analysis was made as there is no treatment of an illness involved, merely the establishment of a new method. Seizure is a feared adverse effect of TMS, and have been observed in few studies utilizing high frequency rTMS, often in subjects prescribed epileptogenic medications (*i.a.* Fluoxetine)[44]. In these studies, stimuli are delivered with frequencies up to 20Hz. The crude risk of inducing a seizure using rTMS is estimated at 0.1% [45]. Other reported side effects include transient head ache, local pain at the stimulation site and paresthesia [46]. These side effects are more common using higher frequencies, and less probable with lower frequencies. The single-pulse PAS protocol, where stimuli are delivered at frequencies ranging between 0.1 – 0.25 Hz, should result in a markedly lower probability of inducing adverse effects in subjects. To ensure the documentation of possible adverse events, participants were interviewed before and after each session using an adverse effect questionnaire (see Appendix). The benefit of establishing a method which enables the study of plasticity of the human brain is potentially great given that plasticity is a central mechanism in the healthy brain and dysfunctional plasticity is thought to contribute to the pathophysiology of some of the most disabling brain disorders society faces. The study was approved by the ethical committee of Gothenburg (Dnr: 615-14). Each subject received verbal and written information about the trial and procedures involved, and both verbal and written consent was obtained from subjects prior to participation.

4. METHODS

4.1 Subjects

Subjects were recruited through print media advertisements in a local newspaper. Subjects were firstly orally screened by the study personal over the phone and secondly on their first visit to the lab in interviews based on inclusion and exclusion criteria (*Table 1*). The inclusion criteria stated that the subjects were to be psychiatrically healthy (*ergo* no major psychiatric disorder according to the DSM-IV, axis 1, with the exception of having had a depressive episode, now in remission, more than 6 months ago), as well as physically and neurologically healthy. Furthermore, subjects using nicotine, high alcohol consumption (assessed with Alcohol Use Disorders Identification Test (AUDIT) [47, 48]) or narcotic drugs were excluded. Female participants were required to use contraceptives during the study and underwent pregnancy tests prior to testing. All participants were right handed as assessed by the Edinburg Handedness Inventory[49] to keep variability as constrained as possible.

Table 1:
Exclusion and inclusion criteria used in the study.

Exclusion criteria
Use of tobacco within the past 12 months
Alcohol consumption (AUDIT † score ≥ 5 for females and ≥ 6 for males)
Use of narcotic drugs within the past 6 months
Previous treatment of alcohol and/or drug dependence
Breast feeding
Neurological disorder and/or epilepsy/seizures
Psychiatric disorders
Use of neuroleptics
Serious disease (current cardiac, neurological, respiratory or abdominal disease)
Metallic implants in head region and/or cochlear implants
Pacemaker
BMI † > 27

Inclusion criteria
Age 18-45
Willing to sign written consent
Negative pregnancy urine test
Willing to use contraceptives during study (females)
Adequate vision and hearing
Right handed

† AUDIT, Alcohol Use Disorders Identification Test; BMI, Body Mass Index.

4.2 Study design

The study design was a double-blinded, crossover study examining two different PAS-paradigms. Each paradigm consisted of two PAS protocols, *i.e.* PAS_{LTP} and PAS_{LTD}. Each participant therefore underwent a total of four sessions, one for each PAS-protocol (Figure 1). Each subject was assigned a random number and order for which the protocols would be tested. The randomization was handled by software written in C++ Visual Studio 2010. The same software later controlled the delivery and timing of the TMS and PNS pulses based on the randomized subject number, keeping the sessions blinded. The sessions were at least one week apart from each other, and each paradigm was tested a year apart due to limited staff resources during the University semesters.

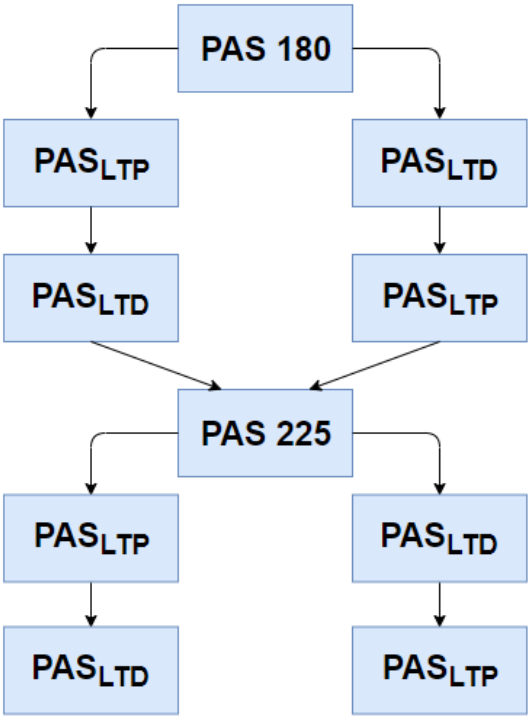

Figure 1: Schematic of the study design. The PAS-180 paradigm was tested first, consisting of two protocols, i.e. PAS_{LTP} and PAS_{LTD}. The order of the protocols were randomized using computer software. Each protocol was tested atleast one week apart. The PAS-225 paradigm was tested one year after the PAS-180 paradigm, utilizing the same randomization process.

4.3 Equipment

TMS stimulation delivery was performed using a figure-of-eight coil connected to eXimia TMS stimulator (Nexstim Ltd., Helsinki, Finland). The coil (outer diameter of each wing, 90mm) was placed with a 45° angle away from the midline of the head, flat against the skull of the subject. Navigated Brain Stimulation software (NBS) (software version 3.2.1, Nexstim Ltd., Helsinki, Finland) was used for targeting and delivering stimulations at desired locations. Subjects were fitted with tracking goggles allowing a stereotactic camera connected to the system to motion track the subjects' head in real time using retroflective materials fitted onto the googles as well as the coil. The software rendered a 3-D model approximating a cortical map of the subjects' brain. When a stimulation was delivered, NBS generated a marker on the virtual cortex corresponding to the relative positional coordinates of the coil to

the subjects head calculated from the information gathered by the stereotactic camera. This spatial accuracy allowed for precise iterations of stimuli to be delivered in a specified region. To identify the cortical region corresponding APB, repeated single pulse TMS stimulations were delivered to the motor cortex. Each pulse elicited a MEP that was recorded with electromyography (EMG) using Ag–AgCl surface electrodes (Amdu Neuroline 720). The active electrode was placed over the muscle belly and the reference electrode over the distal interphalangeal joint of the thumb. The ground electrode was placed on the dorsal side of the hand. The recorded EMG was band pass filtered and amplified, then displayed in the NBS software and visually inspected during each stimuli delivery.

4.4 The paired associative stimulation protocol

The sessions were initiated by finding the cortical region in the motor cortex corresponding to APB in the subject using NBS software. The region eliciting the highest MEPs when stimulated was chosen as a desired region, and the specific cortical location was marked within the software and used as a positioning target. The resting motor threshold (RMT) was defined as the stimulator intensity needed to produce an approximately ≥ 50 μV EMG response in the APB muscle in 5/10 TMS stimuli. Once the location and RMT were determined, the stimulator intensity was set to 120% of RMT and adjusted accordingly to produce a mean of ~0.7 mV in 20 stimuli. A baseline data collection of 20 stimulations at 0.1 Hz was then performed. Electrical nerve stimulation of the median nerve of the hand was performed using a standard stimulation block PNS. The PNS was placed over the ventral right forearm at the level of the wrist, corresponding to the median nerve, assessed by giving repeated PNS stimulations and observing muscle activity in APB. The perceptual threshold for PNS intensity was then examined, being the lowest stimulation intensity the subject could perceive. During PAS, the PNS stimulation intensity was set to 300% of the perceptual threshold to ensure adequate stimulation of the median nerve. The PAS_{LTD} or PAS_{LTP}

protocols were then executed in a random order, determined by the software containing the randomization list, with 180 paired pulses at 0.1Hz in the first part of the trail, and then with 225 paired pulses at 0.25Hz one year later (Figure 1). During the session the subjects were asked to attend to the stimulated hand by looking at it and count the number of stimulations received in order to keep the subjects focused. They were asked to recite the number of stimulations received throughout the session as a way of gauging their focus. Changes in PAS induced MEP amplitudes were then measured with 20 stimulations at 0.1 Hz as a change from baseline at 0, 15, 30, 45 and 60 minutes post-PAS.

4.5 Statistical methods

To estimate the sample size required, an approximation in accordance with previous successful PAS studies where sample sizes varied from 9[40] to 12[34] healthy subjects, was made. As we were uncertain about the effect size actually being of the desired intermediate to strong effect, we initially recruited 21 subjects to make sure we would have enough power to detect a weaker effect. Conducting an *A priori* power analysis after the fact, sample size was calculated using G*Power 3.1 for a repeated measure one-way ANOVA (rmANOVA) within subjects factors, using the following parameters; effect size f =0.3 (equivalent of a Cohen's $d=0.6$), power ($1-\beta$) = 0.8, α = 0.05. This resulted in a sample size of 14.

To quantify the effect size of PAS, each subjects MEP amplitudes were averaged at each time point (T_{Post0}, T_{Post15}, T_{Post30}, T_{Post45}, and T_{Post60}) and normalized to the subject's specific averaged baseline measurement:

$$V(Post0 \dots Post60) = \left(\frac{1}{n}\sum_{i=0}^{n-1} Di\right) / \left(\frac{1}{y}\sum_{j=0}^{y-1} Bj\right)$$

Where V is the subjects normalized variable per time point based on the subject's baseline, n and y equals to the amount of measurements per time point, and D_i and B_j equals the

measured amplitude of a single MEP amplitude in a time point data set {B | $T_{Baseline}$} and {D | T_{Post0}… T_{Post60}}. We then utilized general linear model to execute an rmANOVA with the within-subjects factor time ($T_{Baseline}$ … T_{Post60}) for each of the protocols to analyze whether the protocols in themselves generated a significant change in measured MEP amplitudes over time. PAS may have inter-individual variability based on i.a. attention[50], age[51], psychological well-being[52], time of day when the experiment is conducted[53]. Based on this we defined responders and non-responders as follows:

$$X = \frac{1}{5}\left(\sum_{i=0}^{4} Vi\right)$$

$$responder \rightarrow x > 1 \text{ For } PAS_{LTP}$$

$$responder \rightarrow x < 1 \text{ For } PAS_{LTD}$$

Where X equals the grand mean of a subjects normalized variables, and V_i each normalized variable at the time point of i {V | $T_{Normalized\_mean\_post0}$… $T_{Normalized\_mean\_post60}$}. If the average grand mean was larger than the normalized baseline in the PAS_{LTP} protocol, the subject was considered a responder. In PAS_{LTD}, the average grand mean had to be lower than the normalized baseline to be considered a responder. A subsequent rmANOVA was conducted using only responders to investigate the effects of the different PAS protocols in these subjects. If significance was detected in any test, a post hoc Bonferroni corrected analysis was performed for a pairwise comparison between different time points and baseline. The protocols were compared to each other using a two-way ANOVA with the within-subjects factor as time (T_{Post0} … T_{Post60}) and between-subjects factor as method (PAS_{225}, PAS_{180}). We also compared the RMT between sessions using rmANOVA to ensure minimal variability between sessions. Alpha level was set to 0.05 in all tests and sphericity was tested with Mauchly's test in each analysis.

To gauge subjects' attention during intervention, they were inquired, at 6 predetermined time points, during each PAS intervention to recite the amount of stimulations received. The difference between amounts of stimulation delivered and recited amount received was calculated for each participant at each time point, and a total mean of recited errors between sessions for each participant was calculated for each paradigm. The means were then compared between paradigms using Mann-Whitney U test, due to data having a skewed distribution.

All data are expressed as normalized means ± standard error of the mean (SEM), unless stated otherwise.

5. RESULTS

5.1 Subjects

5.1.1 Demographics

Table 2: Demographical data	
Included women, n(%)	6 (42.9)
Included men, n(%)	8 (57.1)
Age (Mean ± SD, years)	32.28 ± 7.72
Total interviewed, n	39
Total eligible†, n	21
Total completed, n	14
Total drop off, n	7

† Based on inclusion and exclusion criteria, see Table 1.

In total 39 subjects were interviewed, 21 subjects were considered eligible and were asked to enroll in the study. Each paradigm was tested a year apart, and in total 14 subjects were

eligible in regards to inclusion and exclusion criteria throughout the study, and were willing to complete it.

5.1.2 Responders

Table 3: **Number of responders in each protocol**	
Paradigm and protocol	Responders (n)
PAS-180 paradigm	
PAS_{LTP}	9
PAS_{LTD}	3
PAS-225 paradigm	
PAS_{LTP}	9
PAS_{LTD}	3

5.1.2 Reported adverse events

Table 4 **Reported adverse events**		
Adverse event	**PAS-225**	**PAS-180**
Tiredsness, (n)	2	7
Headache, (n)		3
Tingle in extremities, (n)		1
Discomfort, (n)		1

In total there were 12 reported adverse events (*Table 4*) in the PAS-180 paradigm, and 2 in the PAS-225 paradigm. All adverse events were valued as very mild in accordance with the scale used in the adverse event questionnaire (see Appendix).

5.1.3 Resting motor threshold was stable across test sessions

Table 5 Mean resting motor threshold (RMT) for each paradigm	
Paradigm	**RMT(mean ± SEM)**
PAS-180	39.93 ± 5.83
PAS-225	40.32 ± 6.76

Examination of the within subjects test-retest validity between sessions, repeated measures analysis of RMT was analyzed and no significant difference was found $F(3;39) = 1{,}380$, $p = 0.253$.

5.1.4 There was no significant difference in attention between paradigms

Table 6 Total mean of recited errors in each paradigm	
Paradigm	**Mean recited errors ± SD**
PAS-180	5.9 ± 5.52
PAS-225	4 ± 3.81

A Mann-Whitney U test was conducted comparing the means of calculated discrepancy between recited and delivered stimuli during intervention in each paradigm. No statistical significant difference was found $U = 82.5$, $p = 0.475$.

5.2 Paired Associative Stimulation utilizing the 180 paired pulses at 0.1Hz paradigm

5.2.1 PAS_{LTD} did not result in any significant change

In the whole study group (N=14) rmANOVA did not detect any significant PAS_{LTD} effects; F(2.667,34.673)=2.446, *p=0.087*, Greenhouse-Geisser corrected (*figure 2*). The same analysis was conducted in responders only, with no significant within-subjects effects.

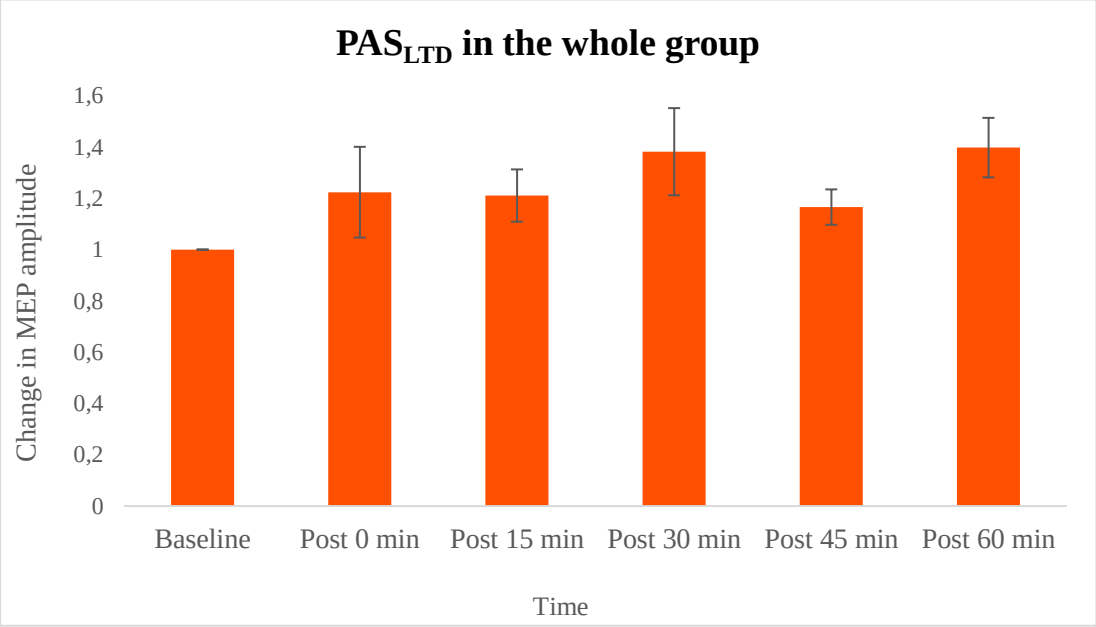

Figure 2: Results shown display normalized mean change of MEPs compared to baseline in the whole group (N = 14) in PAS_{LTD} utilizing the 180 paired pulses at 0.1Hz protocol. Error bars displaying ± 1 SEM. An rmANOVA was performed and no overall significant change was detected F(2.667,34.673) =2.446, p=0.087*, Greenhouse-Geisser corrected.*

5.2.2 PAS_{LTP} resulted in significant enhanced MEP amplitudes in responders but not in the whole study group

rmANOVA detected no significant change in within-subjects effects of PAS_{LTP} in the whole study group; F(3.03;39.38)=2.57, *p=0.067*. When excluding non-responders, a significant within-subjects effect was found; F(5;40)=4.094, *p = 0.004* (*figure 3*). A post-hoc Bonferroni corrected pairwise comparison did not detect a significant difference between different time points.

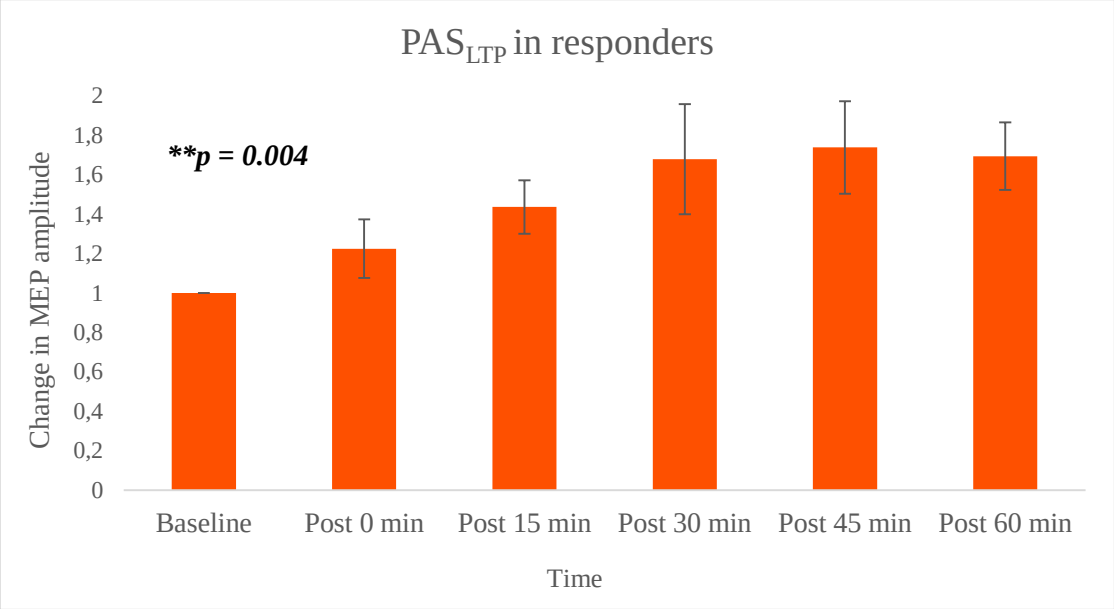

Figure 3: Normalized mean change of MEPs compared to baseline in responders (N = 9) in PAS_{LTP} utilizing the 180 paired pulses at 0.1Hz protocol. Error bars displaying ± 1 SEM. rmANOVA detected an overall significant within-subject effect; F(5;40)=4.094, ***p = 0.004*

5.3 Paired Associative Stimulation utilizing the 225 paired pulses at 0.25Hz paradigm

5.3.1 PAS_{LTD} resulted in a significant enhanced response post PAS MEP in the whole study group

In the whole study group, rmANOVA identified a significant within-subjects effects F(5;65)=2.41, *$*p=0.046$* (*figure 4*). Post-hoc Bonferroni corrected pairwise comparisons did not establish any significant differences between time points. Analyzing responders only, no significant within-subjects effect was found.

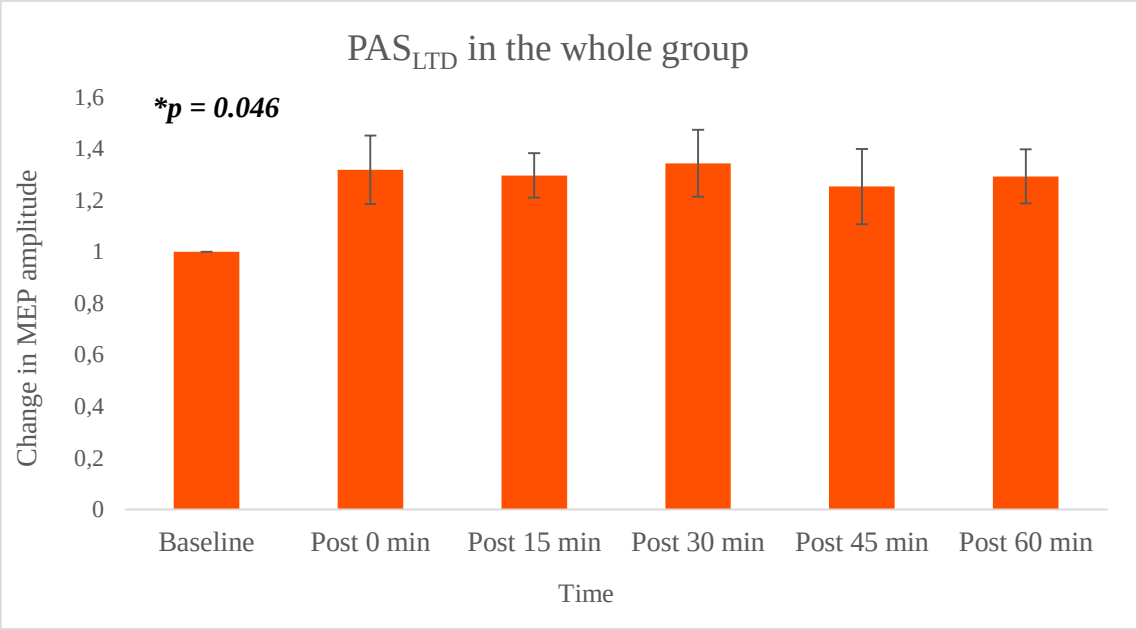

*Figure 4: Results shown display normalized mean change of MEPs compared to baseline in the whole group (N = 14) in PAS_{LTD} utilizing the 225 paired pulses at 0.25Hz protocol. Error bars displaying ± 1 SEM. rmANOVA resulted in an overall significant within-subjects effect F(5;65)=2.41, *p=0.046.*

5.3.2 PAS_{LTP} resulted in significant enhanced MEP amplitudes in the whole group and in responders

In the whole group, PAS-25 resulted in, a significant overall within subjects effect was found; F(5;65)=3.12, *$p=0.014$ (*figure 5A*). Post hoc Bonferroni corrected pairwise comparisons found no significant differences between time points. rmANOVA of responders only identified a significant within subjects effects of PAS_{LTP} with 225 pairings; F(5;40)=6.82, ***$p<0.001$ (*figure 5B*). A post hoc Bonferroni corrected pairwise comparisons in the responder group between baseline and measured time points showed significant change post 45 min: **$p=0.007$ and post 60: *$p=0.015$.

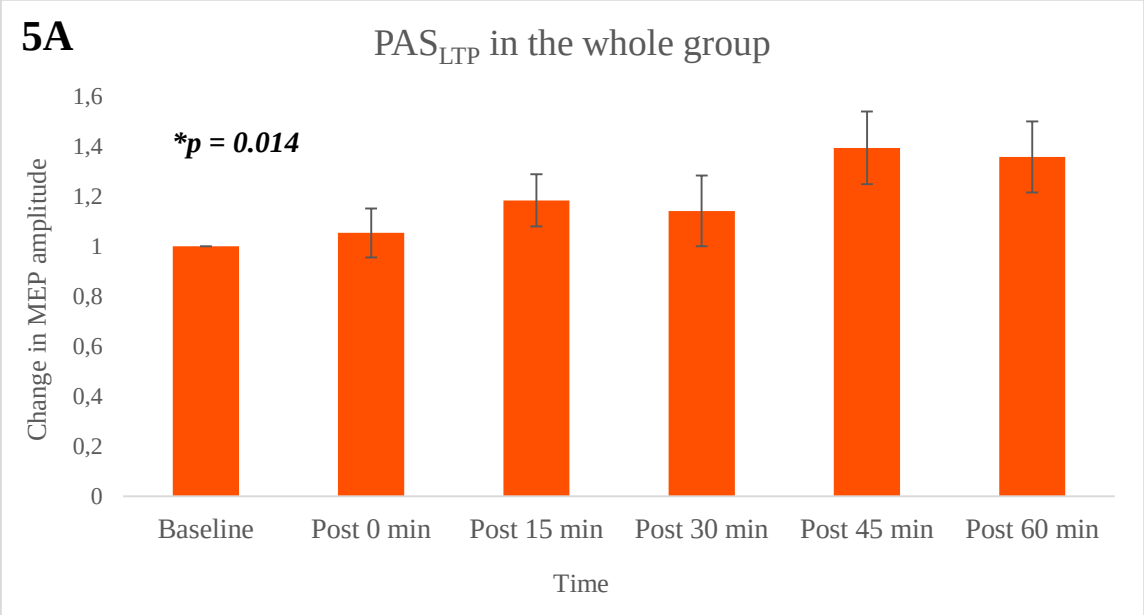

Figure 5A: Results shown display normalized mean change of MEPs compared to baseline in the whole group (N = 14) in PAS_{LTP} utilizing the 225 paired pulses at 0.25Hz protocol. Error bars displaying ± 1 SEM. rmANOVA resulted in an overall significant within-subjects effect F(5;65)=3.12, *$p=0.014$

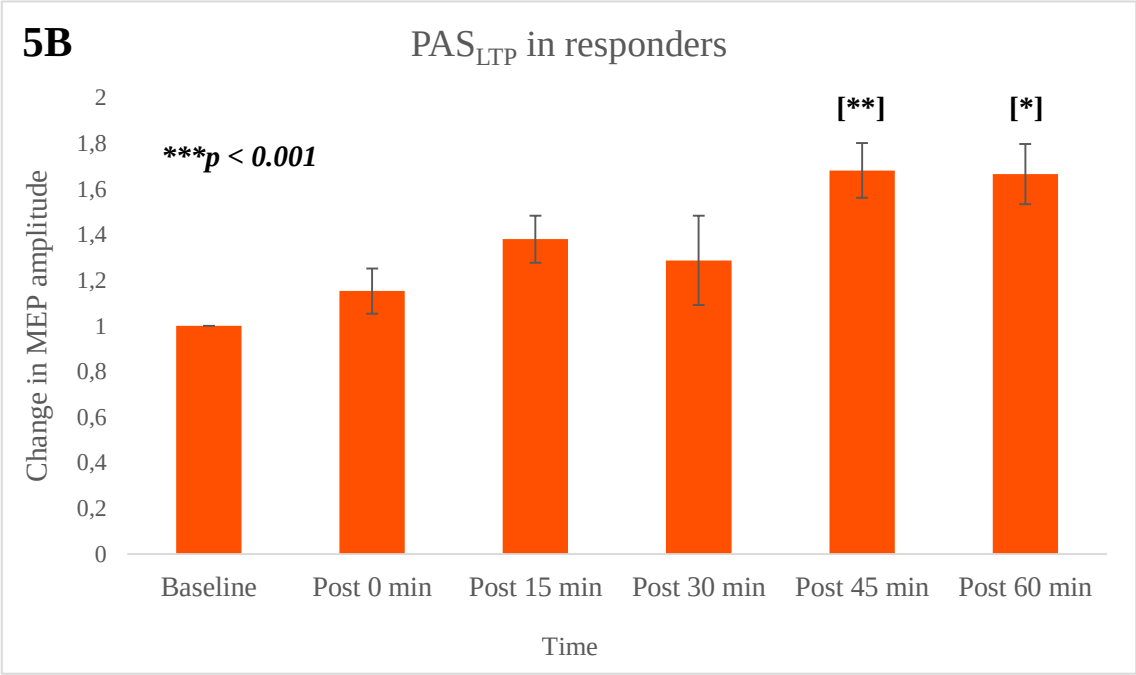

Figure 5B: Results shown display normalized mean change of MEPs compared to baseline in responders (N = 9) in PAS_{LTP} utilizing the 225 paired pulses at 0.25Hz protocol. Error bars displaying ± 1 SEM. rmANOVA resulted in a significant within-subjects effect F(5;40)=6.82, ****p<0.001*. Post-hoc Bonferroni corrected pairwise comparison showed significant difference between baseline and time points post 45 min: **p=0.007 and post 60: **p=0.015*.

5.4 Comparison of PAS_{LTP} between the two paradigms detected no significant difference in the whole study group or in responders

A two-way ANOVA resulted in no significant between-subjects effect F(1;26) = 0.005, *p = 0.946* (*Figure 6*). The same analysis was conducted in responders only, with no significant between-subjects effect F(1;16) = 0.489, *p = 0.494*.

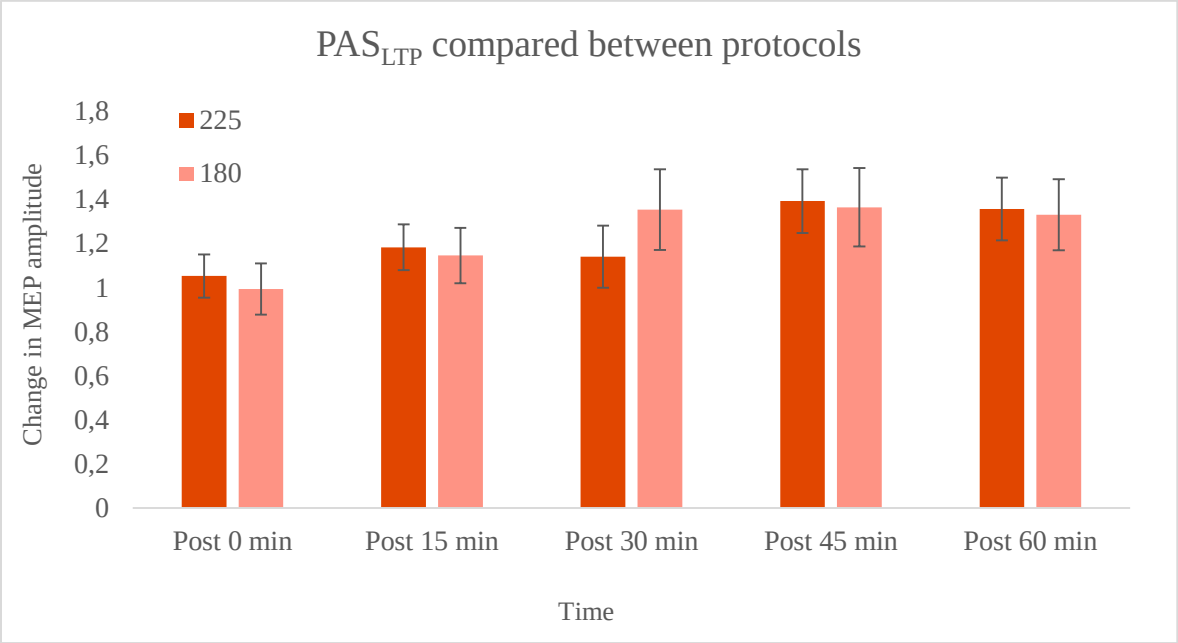

Figure 6: Results display normalized mean change of MEPs. An rmANOVA analysis in the whole group showed no significant change between the two protocols F(1;26) = 0.005, *p = 0.946.*

6. DISCUSSION

The results demonstrate a successful induction of enhanced cortical plasticity, *i.e.* PAS-LTP, in responders for both investigated paradigms, *i.e.* with 180 and 225 paired pulses. The PAS-225 paradigm was also successful in inducing a significant PAS_{LTP} effect in the whole group. On the contrary, PAS_{LTD} could not be induced in any of the protocols. Overall, there was no statistical significant difference between the paradigms in their ability to induce PAS_{LTP}. Despite the absence of difference between paradigms, the PAS-225 paradigm resulted in significant changes from baseline to post PAS 45 and 60 min in responders, while pairwise comparisons in PAS-180 failed to detect such changes, indicating less heterogeneity in PAS-225 data. Although, to the best of our knowledge, the comparison between PAS with 180 and 225 paired pulses has not been examined in one study before, our results coincide with a previous study by Sale et al (2007)[53], comparing a short and long paradigm, *i.e.* 132 paired pulses at 0.2Hz and 90 paired stimuli at 0.05Hz, utilizing the PAS_{LTP} protocol. The results showed a greater facilitation of MEPs in the shorter protocol over three repeated sessions. The exact mechanisms behind these findings remain unclear.

The results do not demonstrate any depression on cortical plasticity utilizing the PAS_{LTD} protocol, and the PAS-225 paradigm instead resulted in significant enhanced cortical plasticity. In a meta-analysis, Wischnewski and Schutter (2016)[35] evaluated the magnitude of PAS effects in 89 studies and found that the effects on cortical excitability are larger for PAS_{LTP} compared to the effects of PAS_{LTD}, but concluded that both PAS_{LTP} and PAS_{LTD} protocols across different parameters are reliable in modulating cortical excitability. When comparing PAS to other methods modulating neural plasticity, *i.a.* theta-burst stimulation, a similar pattern can be seen with lesser inhibitory effects on cortical excitability compared to potentiating effects [54], suggesting underlying mechanisms or interindividual differences favoring facilitation of increased excitability over depression. Surprisingly, the overall effect

of PAS_{LTD} in the PAS-225 paradigm resulted in a significant increase in excitability. Previous studies utilizing the PAS-225 paradigm with the PAS_{LTD} protocol have demonstrated successful induction of depressed excitability in APB after intervention [55, 56]. These studies utilized an interstimulus based on the N20-latency. This technique individually tailors the ISI parameter based on individual differences in conduction time of sensory afferent input to the cortex [57]. The technique is executed through electrical stimulation of the median nerve and measurement of the contralateral N20 component over the somatosensory cortex using electroencephalography (EEG). To this latency, an addition of +2 ms or subtraction of -5ms can be used to generate a more accurate individual ISI for PAS_{LTP} and PAS_{LTD} respectively [56]. Limitations in the study may also have contributed to the discrepancy in the observed effects in the PAS_{LTD} protocols compared to previously observed results. Considering the small sample size, analysis of responder groups was hard to interpret in the PAS_{LTD} protocol. Replication in a larger sample is needed for further evaluation.

Another aspect to consider when comparing the paradigms is the differences in duration and the importance of attention. Stefan et al (2004) [50] demonstrated that attention strongly modulates PAS induced plasticity in the human motor cortex. The exact mechanisms underlying the role of attention in modulating cortical plasticity remain unclear, however basal forebrain cholinergic systems have been implicated to be essential in motor skill learning [58]. In practice, this requires subjects to focus and attend to their hand during a session. Even though the results showed no significant difference in gauged attention, a common complaint among subjects, due to the duration of the PAS-180 paradigm being 30 minutes, was this task feeling arduous. The PAS-180 paradigm had a higher frequency of reported tiredness compared to the PAS-225 paradigm (*Table 3*), which could indicate that the reciting of received stimuli didn't fully represent the subjects' tiredness and attention during the intervention. The PAS-225 paradigm, however, utilizing a slightly higher frequency, was

completed within 15 minutes, leading to subjects perceiving it much less laborious, while generating equivalent results. Fluctuations in attention or arousal may have generated the increased heterogeneous data seen in PAS-180, based on subjects' perceived perception of the intervention. Overall, the PAS-225 paradigm is seen as preferential over the PAS-180 paradigm due to its efficiency.

7. CONCLUSION

The purpose of this study was to establish the PAS method in our laboratory, and compare two PAS-paradigms shown to be effective in previous research. The results demonstrate that the paradigms were unsuccessful in inducing inhibitory effects on MEP amplitudes using the PAS_{LTD} protocols. Replication in a larger sample size and optimization of the protocol is needed for further evaluation. Both paradigms successfully induced equally strong enhancement of MEP amplitudes using the PAS_{LTP} protocols in responders. The paradigm utilizing 225 paired pulses was also successful in inducing enhancement of MEPs in the whole group, and further demonstrated significant changes 45 and 60 minutes post PAS. As the PAS-225 paradigm is more time efficient and also resulted in fewer reported adverse events, it is seen as preferential for future utilization in measuring and inducing LTP-like plasticity in upcoming research.

8. POPULÄRVETENSKAPLIG SAMMANFATTNING

Hjärnans förmåga att förstärka eller försvaga kopplingar mellan nervceller, så kallat hjärnplasticitet, tros vara centralt för att bland annat kunna bilda minnen och är dessutom en viktig egenskap för hjärnans utveckling. Tidigare forskning har även påvisat att vissa sjukdomar som berör hjärnan, exempelvis schizofreni, Parkinsons sjukdom och depression, kan medföra en dysfunktionell hjärnplasticitet.

I den här studien har vi undersökt en ny metod som kan åstadkomma samt även mäta hjärnplasticitet. Detta görs med så kallad transkraniell magnetisk stimulering (TMS). TMS använder sig av elektromagnetiska fält för att åstadkomma en aktivering av nervceller i hjärnan, utan att behöva direkt åtkomst till nervceller via exempelvis kirurgi. Detta tillåter undersökning av hjärnplasticitet på vakna individer, och är förknippat med ytterst få biverkningar.

Teorin bakom hjärnplasticitet bygger på samspelet mellan två nervceller, och timingen av deras aktivering. För att åstadkomma ett förstärkt samspel mellan nervceller, så kallat long-term potentiation (LTP), krävs det att nervcellerna aktiveras nästan samtidigt. Annars så åstadkommer man istället en försvagning i kommunikationen mellan nervcellerna, så kallat long-term depression (LTD).

I denna studie använder vi oss av två protokoll där man stimulerar en nerv i handleden (PNS), som då i sin tur skickar signaler upp till hjärnan. Strax därefter ges en TMS stimulering, och denna stimulering ges i en region i hjärnan som styr tummens muskulatur. Denna metod kallas för paired associative stimulation (PAS). Beroende på timingen mellan PNS och TMS kan man åstadkomma en förstärkning av muskelaktiviteten i tummen (så kallat PAS_{LTP}) alternativt en försvagning av muskelaktiviteten (PAS_{LTD}). Teorin bygger på det ovan nämnda samspelet i aktivering av nervcellerna. Det finns lite olika parametrar som används i PAS, och en specifik uppsättning av parametrar brukar kallas för paradigm i mitt forskningsfält. Vi har undersökt två vanligt förekommande paradigm som nyttjas av forskare världen över. Dessa kallas för PAS-180 och PAS-225, baserat på att man antingen ger 180 parade stimuleringar eller 225 parade stimuleringar.

Målet med studien var att etablera PAS metoden i vårt labb, och att jämföra de två paradigmen PAS-180 och PAS-225 med varandra för att se om något av dem var mer effektivt än det andra.

Våra resultat visar att paradigmen inte kunde åstadkomma en försvagning av muskelaktiviteten i tummen (PAS_{LTD}). De var dock lika bra på att åstadkomma en förstärkning av muskelaktiviteten (PAS_{LTP}). Ett av paradigmen, PAS-225, var dock mycket mer tidseffektivt, där en intervention enbart tog 15 minuter, jämfört med 30 minuter i det andra paradigment PAS-180. Vidare så rapporterade försökspersonerna mycket färre biverkningar i PAS-225 paradigmet. Vid närmre analys av datan så såg man även att paradigmet PAS-225 verkade mer stabilt och påvisade större förändringar mellan de olika mätningar vi gjort.

Sammantaget så tolkar vi resultaten till PAS-225 fördel, och kommer nu att använda oss av denna metoden för att kunna studera hjärnplasticitet vidare i kommande studier.

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11. APPENDIX

11.1 Adverse event questionnaire

Definition på vad som menas med biverkningar

Alla biverkningar som uppstår i samband med behandlingen och har ett tidsmässigt samband med stimuleringen eller det närmaste dygnet efter behandling ska registreras.

Utförande

Fyll i blanketten tillsammans med patienten. I kolumnen ”Före beh.” noteras de symtom som eventuellt förekommer hos patienten innan behandlingen. Om ett symtom föreligger ber man patienten gradera det på en skala från 1 till 4 utifrån riktlinjerna nedan. Följande dagar graderas biverkningarna utifrån förändringar från det första angivna värdet.

1. Inga symtom

2. Lätta symtom

3. Måttliga symtom

4. Allvarliga symtom

Exempel*: en patient anger före behandlingen att hen har lätt huvudvärk motsvarande 2. Dagen efter är huvudvärken värre, motsvarande 3. Påföljande dag är huvudvärken helt borta, d.v.s. 1, och dagen därefter motsvarande 4. Graderingen blir då 2, 3, 1, 4.*

Förklaringar till vissa symtom

Vanföreställningar**:** fråga patienten, men notera också eventuella misstankar på vanföreställningar i det patienten pratar om eller beteende.

Desorganisation**:** värdera utifrån patientens beteende.

Humörsvängning: en akut och drastisk förändring i patientens humör, motsvarande en mani eller en svår depressiv episod. Ange om det rör sig om en förändring ”uppåt” eller ”nedåt”. Här avses inte lättare humörförändringar som skulle kunna vara en eventuell behandlingseffekt.

Svimning**:** gradera ej utan fyll endast i *”ja”* eller *”nej”.*

Kramp**:** gradera ej utan fyll endast i *”ja”* eller *”nej”.*

Ange också sjukdomar som uppträder under behandlingstiden, även om sjukdomen inte är uppkommen till följd av behandlingen.

Biverkningar i samband med behandling

År:_____	**Datum för behandling:**										
	Behandling nr.										
Symtom:	**Före beh.**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Obehag/värmekänsla											
Huvudvärk											
Annan värk:_____											
Öronsusningar											
Trötthet											
Muskelryckningar											
Stickningar											
Domningar											
Synförändringar											
Vanföreställningar											
Desorganisation											
Humörsvängning											
Minnesstörningar											
Koncentrationsproblem											
Yrsel											
Svimning (ja/nej)											
Kramp (ja/nej)											
Annat?											
Ange typ:											
Nytillkommen sjukdom?											
Ange typ:											