Ten Years of Theta Burst Stimulation in Humans: Established Knowledge, Unknowns and Prospects

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Number of Words (Title): 13

Number of Words (Manuscript): 9581

Number of Figures: 4

Number of References: 157

ABBREVIATIONS

AMT: active motor threshold; BA: Brodmann area; BCM: Bienenstock-Cooper-Munroe; BDNF: Brain Derived Neurotrophic Factor; CAR: cortisol awakening response; CB: calbindin; Cer: cerebellum; CD: cervical dystonia; COMT: catechol-O-methyltransferase; CBS: Corticobasal syndrome. DA; dark agouti; DLPFC: dorsolateral prefrontal cortex; ECT: electroconvulsive therapy; EEG: electroencephalography; GABA: γ-aminobutyric acid; GAD: glutamate decarboxylase; GTS: Gilles de la Tourette syndrome; HD: Huntington's disease; HFO: high frequency oscillation; $InsP_3Rs$: Inositol 1,4,5-trisphoshate receptors; LE: long evans; LIDs: L-Dopa-induced dyskinesias; LTP: long-term potentiation; LTD: long-term depression; M1: primary motor cortex; MEP: motor evoked potential; MO: maximal machine output; MSA: Multiple System Atrophy; NMDA: N-Methyl-D-aspartate; NMDAR: N-Methyl-D-aspartate receptor; PAS: paired associative stimulation; PD: Parkinson Disease; PMd: dorsal premotor cortex; PSP: Progressive Supranuclear Palsy; PV: parvalbumin; RMT: resting motor threshold; rTMS: repetitive TMS; SD: sprague dawley; SEP: somatosensory evoked potential; SMA; supplementary motor area; SNP: single nucleotide polymorphism; STP: short-term potentiation; TBS: theta burst stimulation; cTBS: continuous theta burst stimulation; iTBS intermittent theta burst stimulation; TDCS: transcranial direct current stimulation; TMS: transcranial magnetic stimulation; TRP: transient receptor potential.

ABSTRACT

Background/Objectives: Over the last ten years, an increasing number of authors have used the theta burst stimulation (TBS) protocol to investigate long-term potentiation (LTP) and long-term depression (LTD)-like plasticity non-invasively in the primary motor cortex (M1) in healthy humans and in patients with various types of movement disorders. We here provide a comprehensive review of the LTP/LTD-like plasticity induced by TBS in the human M1.

Methods: A workgroup of researchers expert in this research field review and discuss critically ten years of experimental evidence from TBS studies in humans and in animal models. The review also includes the discussion of studies assessing responses to TBS in patients with movement disorders.

Main findings/Discussion: We discuss experimental studies applying TBS over the M1 or in other cortical regions functionally connected to M1 in healthy subjects and in patients with various types of movement disorders. We also review experimental evidence coming from TBS studies in animals. Finally, we clarify the status of TBS as a possible new non-invasive therapy aimed at improving symptoms in various neurological disorders.

Keywords: Theta burst stimulation; primary motor cortex; plasticity; animal model.

INTRODUCTION

Until the late 1980's transcranial magnetic stimulation (TMS) machines could only deliver 1 stimulus every 4s or so. However a repetitive stimulator was eventually produced that allowed repeated stimulation of the brain at high frequencies. Initially, repetitive TMS (rTMS) was used in "lesion" mode, to interrupt the function of language areas and thereby determine language dominance, or in "activation" mode to locate epileptic foci [Pascual-Leone et al., 1991; Dhuna et al., 1991; 1,2]. However, it was not long before groups began to investigate its potential for inducing after-effects that outlasted the period of stimulation, and which appeared to involve plastic changes in the excitability of cortical synapses. Theta burst stimulation (TBS) is one of many forms of rTMS that were developed after this pioneering work when more advanced stimulators were available [Huang et al., 2005; 3]. Although it was first thought that TBS produced more powerful and reproducible effects than other rTMS methods, a claim that unfortunately has not stood the test of time, its main attraction is the speed of application. It takes 2-3 min or less to apply TBS protocols, making them more acceptable to participants than longer lasting protocols such as 1 Hz rTMS which can take 20-30 min; the same advantage means that it can even be used in unanaesthetised animals. This has led to a large body of literature, which we have tried to survey below. The review mainly focuses on experimental studies performed on the primary motor cortex (M1) or in other cortical regions known to be functionally connected to M1 in healthy subjects and in patients with various type of movement disorders. We also discuss the experimental evidence coming from TBS studies in animals. Finally, we evaluate the status of TBS as a possible new non-invasive therapy aimed at improving symptoms in various types of neurological disorders.

TBS IN HUMAN STUDIES

Neurophysiology of TBS

The original concept of TBS comes from the burst discharge at 4-7 Hz (the theta range in electroencephalography - EEG terminology) recorded from the hippocampus of rats during exploratory behavior [Diamond et al., 1988; 4]. Theta burst patterns of stimulation are commonly used to induce plasticity in animal brain slices [Capocchi et al., 1992; Larson and Lynch, 1986, 1989; 5-7], and it seemed reasonable to adapt these to the human brain using TMS. The parameters were adjusted to match the capabilities of rTMS machines available at the time. Each burst had three pulses at 50 Hz, instead of the four pulses at 100 Hz typically used for stimulating brain slices. Bursts were given at 5 Hz, which is identical to that used in the animal preparation.

The first TBS protocol applied to human subjects was continuous TBS (cTBS) in which TBS was given continuously for 20 seconds [Huang et al., 2005; 3]. It was initially surprising that cTBS reduced the amplitude of the motor evoked potentials (MEPs) for some 20 min since TBS in animal preparations typically enhanced synaptic efficacy resulting in long-term potentiation (LTP) rather than long-term depression (LTD). However, it has been noted that a longer train of stimulation may eventually lead to LTD if the stimulation period is long enough [Heusler et al., 2000; Larson et al., 1986; Takita et al., 1999; 8-10]. The TBS protocol was then adjusted to deliver repeated short trains mimicking what those commonly used for LTP induction in the animal studies. Such intermittent TBS (iTBS) successfully facilitated MEPs [Huang et al., 2005; 3]. The most commonly used varieties of cTBS and iTBS are illustrated in Figure 1A. iTBS enhances cortical excitability for 20 minutes or so whereas cTBS with either 300 or 600 total pulses (20s or 40s duration) leads to inhibition for 20 or 60 min respectively (Figure 1B).

A single TMS pulse to the motor cortex evokes activity in corticospinal fibres that can be recorded directly in conscious humans through electrodes implanted into the epidural space at the high cervical level for the relief of pain [Di Lazzaro and Rothwell, 2014; 11]. Such

recordings have shown that TMS evokes a series of descending waves of corticospinal activity [Di Lazzaro and Rothwell, 2014; 11]. The earliest wave is termed the D-wave because it is caused by direct activation of the axon of corticospinal neurons in the subcortical white matter. The later waves are called I-waves because they are due to synaptic activation of the same corticospinal neurons and they are numbered in order of appearance (I1, I2 etc). ~~These depend on the stimulus intensity, waveform and orientation of the induced current in M1. A conventional monophasic pulse with a posterior-anterior current in the brain, evokes three main components: 1) at low (close to motor threshold) intensities a single descending wave is recorded. This wave is believed to result from monosynaptic activation of corticospinal cells and, in analogy with experimental studies in animals, it has been termed the I1 wave [Amassian et al., 1987; 12] (Figure 2); 2) at higher stimulus intensities later volleys appear, these are termed late I-waves and it has been proposed that they originate from the recruitment of highly synchronized clusters of excitatory and inhibitory neurons producing a high frequency (~600 Hz) repetitive discharge of corticospinal cells (Figure 2); 3) a further increase of TMS intensity leads to direct excitation of the corticospinal axons in the subcortical white matter resulting in a short latency wave termed D-wave (Figure 2). Epidural recordings in a single patient have shown that benzodiazepine administration suppresses late I-waves with no change in the I1 wave [Di Lazzaro et al., 2000; 13], suggesting that I1 and late I-waves are due to activation of different sources of inputs to corticospinal neurons and that only the latter are under the control of γ-aminobutyric acid (GABA)-ergic inhibitory inputs.~~

Epidural recordings before and after TBS show that cTBS and iTBS have differential effects on the I-wave components of the corticospinal volley. The cTBS protocol suppresses the I1 wave, whilst later I waves and the D-wave are much less affected (Figure 2) [Di Lazzaro et al., 2005; Di Lazzaro and Rothwell, 2014; 11,14]. Interestingly, the after effects of cTBS differ from those observed with other stimulation paradigms that suppress MEPs such

as low-frequency (1Hz) repetitive magnetic stimulation and paired associative stimulation with an interstimulus interval of 10 ms (PAS_{10}). These selectively suppress late I waves with no change in the amplitude of the I1 wave [Di Lazzaro and Rothwell, 2014; 11].

In contrast to cTBS, the iTBS protocol enhances late I-waves with no change in the amplitude of the I1 wave [Di Lazzaro et al., 2008; 15]. This suggests that iTBS affects a different population of neurons whose inputs to the corticospinal cells produce the late I-waves (Figure 2). The effect of iTBS might be due enhancement of synaptic transmission in the late I-wave circuit and/or to increased synchronization in the bursting inputs to corticospinal cells. This second effect is supported by the findings obtained in a single patient with chronic stroke who had epidural electrodes implanted in the epidural space of the upper spinal cord for treatment of pain. The I-waves recorded after iTBS of lower limb M1 were not only enhanced in amplitude but also much more synchronised [Di Lazzaro et al., 2006; 16] (Figure 2). The reasons for the differential effects of cTBS vs. iTBS predominantly on the I1-wave vs. late I-waves are currently unknown.

The effects of iTBS and cTBS are blocked by N-Methyl-D-aspartate receptor (NMDAR) antagonists (memantine and dextromethorphan) [Huang et al., 2007; Wankerl et al., 2010; 17,18], while the LTP-like effect of iTBS reverse to an LTD-like effect after D-cycloserine, a partial NMDAR agonist [Teo et al., 2007; 19]. ~~Similar NMDAR dependency has also been noticed in conventional rTMS protocols at a regular frequency, in studies with the original PAS or with modified PAS protocols (i.e. Stefan et al., 2002; Suppa et al., 2013; 20,21) and transcranial direct current stimulation (TDCS) (Nitsche et al. , 2003, Vlachos et al. , 2012, Ciampi de Andrade et al. , 2014; 22,23,24), but not in studies with transcranial random noise stimulation (Chaieb et al. , 2015; 25).~~ In addition, nimodipine, an L-type voltage-gated Ca^{2+} channel blocker, produces a dose-dependent decrease in the effect of cTBS [Wankerl et al., 2010; 18]. ~~There are also few lines of indirect evidence suggesting that the effect of conventional rTMS requires the activation of Ca^{2+} channels (Tan et al. , 2013,~~

~~Lenz et al. , 2015; 26,27). On the other hand, dextromethorphane, a Ca^{2+} channel blocker, prevents the after-effect of anodal, but not cathodal TDCS (Nitsche et al. , 2003; 22).~~ Both the NMDAR and the Ca^{2+} channel are the well-known key receptor/channels at the post-synaptic membrane for induction of synaptic plasticity. These results are consistent with the idea that the after-effects of TBS involve LTP- and LTD-like phenomena.

Theoretical mechanisms of TBS

Based on a simplified post-synaptic mechanism of plasticity, a three-stage theoretical model was devised to explain why changing the pattern of stimulation from cTBS to iTBS reverses its effect [Huang et al., 2011; 28]. Assuming that LTP and LTD are triggered by Ca^{2+} influx to the postsynaptic neuron, the basic assumption of the model is that TBS produces a mixture of excitatory and inhibitory effects that can summate to yield the observed effects on corticospinal excitability. A short burst at 50 Hz leads to a short-latency facilitation together with a longer-latency and weaker inhibition [Huang et al., 2005; Huang and Rothwell, 2004; 3,29]. Hence, iTBS which gives short TBS trains intermittently, keeps the excitatory effect dominant and produces an LTP-like effect. In contrast, cTBS is applied continuously for long enough to allow the inhibitory effect to overcome the facilitatory effect and produces an LTD-like effect. In the first stage of the model, TBS activates the trigger factor, i.e. Ca^{2+} influx to the postsynaptic neuron. The property, including the amount and the rate of the increase, of the trigger factor determines the amount of the build up of inhibiton and facilitation processes that modify the synaptic strength in the second stage. Then, the sum of the amount of inhibiton and facilitation at the end of second stage determine the direction and the amount of after-effects. The assumption of the model is equivalent to say that the trigger factor will concurrently promote LTP and LTD and that the final outcome will be determined by which is dominant. This is supported by a study showing that dysfunction of Inositol 1,4,5-trisphoshate receptors ($InsP_3Rs$) that is required for LTP results in a conversion of LTD to

LTP, while partial blockade of NMDARs to reduce the rate of Ca^{2+} influx results in a conversion of LTP to LTD (Nishiyama et al., 2000; 30). Moreover, in brain slices, the potentiation effect produced by TBS was smaller when 20 bursts were used compared to 10 bursts (Larson and Lynch, 1986; 31), and increasing the number of TBS trains may reduce the LTP effect (Abraham et al., 1997; 32). Beierlein et al. (2003; 33) also showed an initial facilitation followed by depression during a train of stimulation. These results support that a long train of stimualtion favours the inhibitory effect, while a short train of stimulation is likely to produce the facilitatory effect.

Variability in TBS studies

Like all current methods of non-invasive brain stimulation (NIBS), the response to TBS protocols is highly variable from one person to another. This has been highlighted by a number of recent papers that have compared the response of large numbers of individuals [Hamada et al., 2013; Lopez-Alonso et al., 2014; Hinder et al., 2014; 34, 35, 36]. Estimates of the variance differ between studies, but as a rough guide, to detect reliably a difference in response magnitude of about 20% between two groups of individuals requires about 30 people in each group. This is much larger than in most of the studies reviewed below. Several studies have tried to identify factors that might be able to predict an individual's response to a TBS protocol ~~including genes~~.

Cheeran et al. [Cheeran et al., 2008; 37] suggested that some of the difference between people could be due to genetic factors (Figure 3). However, the studies reported thus far have been largely underpowered candidate single nucleotide polymorphisms (SNP) studies, and should perhaps be regarded as preliminary. ~~A common SNP - BDNF Val66Met (rs6265) in the Brain Derived Neurotrophic Factor (BDNF) gene was the first (and subsequently most extensively) evaluated for a role in influencing the response to TBS and other rTMS paradigms, perhaps for these very reasons. Located in the 5-prime 'pro' region of BDNF,~~

~~BDNF Val66Met is critical for activity dependent secretion, and known to influence human episodic memory [Egan et al., 2003; 38]. Chronic rTMS (like chronic electroconvulsive therapy -ECT) was reported to increase serum BDNF levels in patients with depression, and animal studies of chronic rTMS showed an up-regulation of BDNF mRNA [Müller, 2000; Zanardini et al., 2006; Bocchio-Chiavetto et al., 2006; 39,40,41].~~ Cheeran et al. studied the effects of the BDNF Val66Met SNP ~~on the response of healthy subjects to three different plasticity-inducing protocols over M1 [Cheeran et al., 2008; 37]. The BDNF Val66Met SNP significantly influenced TBS in particular,~~ in 18 people. There was ~~with~~ a significant decrease in MEPs after cTBS ~~(n=18)~~ and a significant increase in MEPs after iTBS ~~(n=18)~~ in the Val/Val homozygote individuals (n=9) but not in those with one or more copies of the Met allele (n=9). Two studies have subsequently re-examined its influence on the effects of iTBS with conflicting results. Antal et al. [Antal et al., 2010; 42] reported that LTP-like plasticity could only be induced in 10 Val66Val allele carriers but not in 5 Val66Met allele carriers with iTBS, but Li Voti et al. [Li Voti et al., 2011; 43] found no difference between 7 Val66Met and 14 Val66Val allele carriers in their response to iTBS. Mastroeni et al. [Mastroeni et al., 2012; 44] re-visited the impact of the Val66Met BDNF genotype on the individual response to cTBS ~~,~~and iTBS ~~as well as on homeostatic metaplasticity~~ and did not find a significant effect of BDNF genotype. ~~A (P = 0.081) trend for the polymorphism*time interaction was seen when monophasic MEPs (rather than biphasic MEPs) were assessed, but only a single block of 30 monophasic MEPs at minute 10 post TBS was recorded in this study. A key methodological difference in this study was the use of inverted direction of the induced tissue current with biphasic stimulation in this study (compared to the original Huang et al. study) [Huang et al., 2005; 3], which influences AMT assessment (and consequently stimulation intensity), as well as the population of interneurons stimulated. Hwang et al. [Hwang et al., 2015; 45] demonstrated that stimulation intensity has a significant effect on the influence of BDNF genotype (Val66Met polymorphism) on 10Hz rTMS-induced changes in cortical~~

~~excitability in healthy humans, in keeping with the known biological effects of BDNF on the 'modification threshold' [Hwang et al., 2015; Suppa and Cheeran, 2014; 45,46]. Together, these studies may serve to demonstrate that the effects of this common genetic variation on TBS (and other non invasive brain stimulating protocols) may be more complex and nuanced that originally reported by Cheeran et al. [Cheeran et al., 2008; 37]. Subtle variations in protocol between experiments, or stimulation techniques between labs may have a disproportionate influence on results, over or understating the importance of this SNP. In addition, the role of gender on the effects of the BDNF Val66Met SNP has yet to be examined systematically.~~

Mori et al. [Mori et al., 2011; 47] studied 77 (31 males; mean age, 38.3 +/- 10.2 years) healthy subjects carrying specific allelic variants of NMDAR subunits, specifically NR1 subunit gene (GRIN1 rs4880213 and rs6293) or of the NR2B subunit gene (GRIN2B rs7301328, rs3764028, and rs1805247). Their results showed that individuals carrying the G allele in the rs1805247 GRIN2B SNP show greater long-term potentiation-like cortical plasticity after iTBS. A second paper investigating non-synonymous SNPs in TRPV1 (a member of the transient receptor potential - TRP family receptors), showed no significant effects on TBS. This result is unsurprising, given the fact that TRPV1 functions as a molecular integrator for multiple types of sensory input (activated by capsaicin, endocannabinoids and eicosanoids for example), but is useful as it gives further details of this cohort [Mori et al., 2012; 48]. A cohort of 550 individuals was genotyped, with 77 (31 males; mean age, 38.3 +/- 10.2 years) consenting to TMS studies including cTBS and iTBS. It is unclear why the authors did not report the results for cTBS for GRIN2B or acknowledge the lack of correction for multiple SNP testing in the same neurophysiological dataset. These results have not been replicated to date.

Lee et al. [Lee et al., 2014; 49] studied the effect of the COMT Val158Val (rs4680) polymorphism in 18 elderly subjects (73.78±5.04 years). ~~The COMT gene codes for~~

~~Catechol-O-methyltransferase, which catalyzes the transfer of a methyl group from S-adenosylmethionine to catecholamines This include neurotransmitters like dopamine, epinephrine, and norepinephrine, as well exogenously administered drugs for Parkinson's Disease and hypertension. This functional polymorphism is believed to affect dopamine levels; subjects carrying the Val alleles have increased COMT activity and lower prefrontal extracellular dopamine compared with those with the Met substitution [Stein et al., 2006; 50].~~ Nine participants in this study had the Val/Val allele, while 5 participants were Val/Met carriers, and 4 participants were Met/Met allele carriers. Met allele carriers showed greater cTBS induced suppression of MEP amplitude in healthy elderly subjects. Val/Val subjects appear to show no effect of cTBS in the first 30 min after stimulation, but this was not analyzed in the paper. These results have not been replicated to date.

Factors other than genes contribute to the variability observed in TBS studies. A number of authors have reported several factors leading to between-subject and within-subject variability [Hamada et al., 2013; López-Alonso et al., 2014; 34,35]. Hamada et al. [Hamada et al., 2013; 34] examined 56 people and found that approximately 50% of the variation in TBS response could be attributed to differences in the intracortical network activated by the TMS pulse. Using different coil orientations to activate various intracortical circuits evidence was provided that subjects in whom late I-wave circuits were likely activated by TMS were more likely to respond in the expected direction with both iTBS (LTP-like plasticity) and cTBS (LTD-like plasticity). There is also some evidence that the functional connectivity in cortical networks targeted by stimulation might influence the response to TBS. Nettekoven and colleagues [Nettekoven et al., 2015; 51] demonstrated that "non responders" to an iTBS protocol had greater resting state functional connectivity between M1 and premotor cortex when compared to "responders". Additionally, "responders" demonstrated, in addition to increased MEP amplitudes, increased levels of resting state functional motor network connectivity after iTBS [Nettekoven et al., 2014, 2015; 51,52]. In contrast to the after-effects

of PAS and 6 Hz rTMS that have reported an age-related decline in M1 plasticity [Müller-Dahlhaus et al., 2008, Tecchio et al., 2008; 53,54], Dickins et al. [Dickins et al., 2015; 55] found no age dependent effects in the response to iTBS. Early life events can modify the response to TBS in later life. Pitcher and colleagues [Pitcher et al., 2012; 56] reported that preterm birth was associated with a reduced LTD-like response to cTBS when studied in a group of adolescents. Whether the effects of preterm birth are seen in adulthood is not clear at this stage. Interestingly, in this study the cTBS response of the term born adolescent participants was strong and possibly greater than that seen in adults. This suggests that there might be age dependent effects on the cTBS response but this requires further study. Finally, there are state-dependent and genetic influences on the response to TBS that are outlined elsewhere in this review (Figure 3).

Although there is considerable variation in TBS response between individuals, there is much less variability within an individual from day to day [Hinder et al., 2014; 36]. Taking the results from 30 individuals studied with cTBS on 2 different occasions, a significantly lower proportion of the total variance was accounted for by intra-individual (12.6%) compared with inter-individual effects (41.4%). Similar effects were described by Vallence et al. [Vallence et al., 2015; 57] after cTBS. Many factors may contribute to intra-individual variance such as the state of circulating hormones, time of day, previous levels of activity [see Ridding and Ziemann, 2012; 58]. Clow and colleagues [Clow et al., 2014; 59] recently reported that the magnitude of the initial burst of cortisol seen on awakening (the cortisol awakening response - CAR) was associated with the magnitude of the neuroplastic response to cTBS. When assessed on 4 occasions on different days larger than average CARs were associated with greater cTBS responses. This finding provides evidence that circadian related changes in cortisol secretion within individuals are an important influence on neuroplasticity (Figure 3).

State dependent effects on TBS

TBS is usually delivered at an intensity of 80% active motor threshold (AMT). Estimation of active threshold necessarily involves tonic contraction of the target muscle prior to applying TBS. Under these conditions, cTBS with either 300 or 600 total pulses (20s or 40s total) suppresses MEPs. However, if participants are completely relaxed for >10min prior to TBS, then cTBS with 300 pulses yields a mild facilitatory effect [Gentner et al., 2008; 60]; cTBS with 600 pulses still produces inhibition. The same reversal of effects was seen after phasic muscle contraction [Iezzi et al., 2008; 61] and after administration of the L-type Ca^{2+} blocking drug nimodipine [Wankerl et al. 2010; 18]. It was suggested that after a period of rest, cTBS induces a large Ca^{2+} influx into postsynaptic neurones via NMDA receptors as well as L-type Ca^{2+} channels causing the LTP-like effects. However, nimodipine blocks some of the influx and a smaller amount of Ca^{2+} entry (via the NMDA channels) leads to LTD-like effects. It could be therefore, that prior contraction causes an activity dependent change in L-type Ca^{2+} entry, again resulting in MEP suppression as described originally. Mild (10% of the maximum) voluntary contraction during TBS abolishes the after-effect of TBS [Huang et al., 2008; 62]. One possible reason is that contraction increases the membrane conductance of postsynaptic neurons, so that synaptic current produces less voltage change across the membrane, and consequently less Ca^{2+} entry into the neuron. Interestingly, the same amount of contraction immediately after TBS reversed the inhibitory effect of 20-second cTBS into facilitation and enhanced the facilitatory effect of iTBS [Huang et al., 2008; 62]. This was not seen when the contraction was performed 10 min after 20-s cTBS or immediately after 40-s cTBS [Huang et al., 2008; 62]. The explanation for this is unclear. Immediate contraction could well disrupt the early stages of plasticity induction. Given some minutes to consolidate, contraction at a later time might then have no effect. However, this does not account for the increase in effectiveness of iTBS unless we also propose that contraction only interferes with the LTD-like effects of TBS. If iTBS produces a mixture of inhibitory and facilitatory after-

effects, then removal of the inhibitory component would enhance its facilitation. Mild voluntary contraction of an antagonist muscle during cTBS enhances the depressive effect of cTBS. The authors proposed that reciprocal inhibition of the target muscle reduced the excitatory component of the cTBS effect, increasing the overall amount of suppression. Interestingly forceful (60% of maximum) antagonist contraction blocks all effects of cTBS, perhaps because it is usually accompanied by low levels of activity in the agonist (target) muscle [Fang et al., 2014; 63].

TBS and metaplasticity

Metaplasticity is defined as modification of the direction, magnitude and/or duration of plasticity by previous activity in the same postsynaptic neuron or neural network [Abraham, 2008, Hulme et al., 2013; 64,65]. It is often described in terms of the Bienenstock-Cooper-Munroe (BCM) theory, which implies that plasticity at any given synapse is bidirectional i.e. LTP or LTD can be induced, and that the likelihood for LTP/LTD-induction is not stable over time but depends homeostatically on the activity history of the postsynaptic neuron [Bienenstock et al., 1982; 66]. Work from animal experiments demonstrates that metaplasticity plays significant roles in the regulation of network function and behavior.

~~Beside~~ In addition to a single study showing a non-homeostatic metaplasticity interaction between a suprathreshold 5-Hz rTMS protocol able to elicit short-term potentiation (STP) and iTBS/cTBS-induced LTP/LTD-like plasticity [Iezzi et al., 2013; 67], a number of studies have examined metaplasticity processes tested by subsequent TBS protocols applied to M1. Todd et al. [Todd et al, 2009; 68] initially found that giving iTBS 10min before cTBS converted the expected inhibition (from cTBS alone) into facilitation. A similar pattern of homeostatic interaction was observed by Murakami et al. [Murakami et al., 2012;69] who examined all possible pairs of cTBS and iTBS, separated by an interval of 15min. Application of identical protocols (iTBS→iTBS and cTBS→cTBS) suppressed the

non-primed TBS effects, while pairs of different protocols (cTBS→iTBS, iTBS→cTBS) enhanced the non-primed TBS effects in a homeostatic manner. Murakami et al. also investigated the effects on SICI, and again concluded that plasticity in inhibitory circuits of M1 is also regulated by homeostatic metaplasticity, and could contribute to the homeostatic regulation of excitatory circuits [Murakami et al., 2012; 69]. These results were confirmed by Gamboa et al. [Gamboa et al., 2011; 70] who tested protocols separated by 2, 5 or 20min. In most cases, the interactions were homeostatic.

However, more recent cTBS→cTBS experiments using a 10min interval demonstrate a non-homeostatic interaction with significant lengthening of the LTD-like MEP decrease >120min [Goldsworthy et al., 2012a,b; 71,72], that was resistant to de-depression by voluntary contraction or short-duration iTBS [Goldsworthy et al., 2015; 73]. The mechanisms of these non-homeostatic interactions are currently unclear. It should be noted that this particular combination of paired cTBS with a 10min interval had never been tested in the previous studies that emphasized homeostatic interactions. As reported in other plasticity protocols such as TDCS [Monte-Silva et al., 2010 or 2011?; 74] the interval between TBS blocks may be critical for the after-effects. However, studies with much larger numbers of participants are required to resolve this problem satisfactorily.

TBS and functional brain connectivity

A number of authors have investigated the effect of TBS applied over distant motor and non-motor brain regions in order to produce lasting changes in the excitability of ipsilateral or contralateral M1. In all of the studies reviewed here, TBS effects have been monitored indirectly by measuring changes in MEP amplitudes evoked single-pulse TMS over M1. The most likely explanation for the effects is that TBS changes the excitability of the distant area and modulates the amount of ongoing activity in its connections with M1. M1 excitability is affected because of this changes the balance of inhibitory and excitatory inputs that it

receives. A second possible explanation is that TBS directly stimulates connections from the region of interest onto M1. These then directly change M1 excitability. However, this seems less plausible given that TBS at 80% AMT is unlikely to cause any direct discharge in efferent pathways from the stimulated cortex.

cTBS of M1 in the opposite hemisphere increases, while iTBS decreases MEP amplitudes elicited by single TMS pulses delivered over the target M1 [Ishikawa et al., 2007; Mochizuki et al., 2007; Suppa et al., 2008; Stefan et al., 2008; Di Lazzaro et al., 2008; 15,75,76,77,78]. The hypothesis is that cTBS/iTBS reduces/enhances the amount of tonic activity in long-range (perhaps transcallosal) cortical projections to M1. Given the current view that the interactions between the two hemispheres are largely inhibitory [Ishikawa et al., 2007; Mochizuki et al., 2007; Suppa et al., 2008; Stefan et al., 2008; Di Lazzaro et al., 2008; 15,75,76,77,78], this means that when cTBS reduces the ongoing activity in that connection, it removes inhibition from M1 and increases its excitability. The opposite is true of iTBS. TBS of dorsal premotor cortex (PMd) and supplementary motor area (SMA) also changes excitability of M1 [Mochizuki et al., 2005, Koch et al., 2007; Stefan et al., 2008; Wilkinson et al., 2009; Huang et al., 2009, 2010; 78,79,80,81,82] and can disclose abnormalities in patients with movement disorders such as dystonia [Huang et al., 2010; 83].

CTBS of the lateral cerebellum (Cer) decreases the amplitude of MEPs elicited from contralateral M1 while iTBS of Cer increases MEP amplitudes [Koch et al., 2008; Li Voti et al., 2011; 84, 85]. As with the M1-M1 interaction above, it is thought that cTBS reduces the activity of Purkinje neurons that tonically inhibit the (excitatory) cerebello-thalamo-cortical pathway. This removes excitation from M1 resulting in smaller MEPs. CTBS of Cer also enhances subsequent induction of LTP-like plasticity by PAS at 25 ms interstimulus interval (PAS_{25}) of M1, while iTBS of Cer occludes this form of LTP-like plasticity [Popa et al., 2013; 86], in line with homeostatic metaplasticity. One possible explanation for this would be a homeostatic interaction between the Cer inhibitory priming of M1 and the subsequent

PAS_{25}. However this seems unlikely since the same Cer priming protocols do not interfere with LTP-like plasticity induced by iTBS of M1 [Popa et al., 2013; 86]. It has been suggested that afferent input responsible for the PAS_{25} effect might travel via a cerebellar pathway. If so then the effect of Cer TBS on PAS_{25} might be due to an interaction with the sensory afferent volley rather than a direct effect on M1.

TBS over the primary sensory area (S1) modulates the amplitude of ipsilateral and contralateral somatosensory evoked potential (SEP)' high frequency oscillations (HFOs) but has inconsistent effects on MEP amplitude [Ishikawa et al., 2007; Katayama and Rothwell, 2007, Katayama et al., 2010; Jacobs et al., 2012, 2014; 75,87,88,89,90]. In contrast, several studies applying TBS over higher-order somatosensory areas including Brodmann area 5 (BA5) found increased MEPs after cTBS suggesting that compared to S1, BA5 may have a stronger influence on excitability of ipsilateral and contralateral M1 [Premji et al., 2011; Jacobs et al., 2014; 90,91].

TBS and motor learning

The term motor learning mainly refers to practice-related changes in motor performance induced by repeating a voluntary motor task. Motor learning evolves through an early and a late phase. The early phase of motor learning consists of a practice-related improvement in motor performance that is retained over a relatively short time (motor retention) and then consolidated after several hours (motor consolidation) (Agostino et al., 2008; Iezzi et al., 2010; Teo et al., 2011; 92, 93, 94). Conversely, the late phase of motor learning consists of further incremental performance triggered by additional sessions of motor practice (Agostino et al., 2008; Iezzi et al., 2010; Teo et al., 2011; 92, 93, 94). Plasticity processes in M1 are known to contribute to the early phase of motor learning (Agostino et al., 2008; Iezzi et al., 2010; Teo et al., 2011; 92, 93, 94). There have been few studies of the effects of TBS of M1 on motor learning. In all cases the effects were "non-homeostatic", in that excitatory iTBS

given 10min before the task enhanced learning of ballistic movements [Agostino et al., 2008; Teo et al., 2011; 92, 94)], whereas cTBS impaired learning and retention [Iezzi et al., 2010; 93]. Gating mechanisms may explain why these interactions were non-homeostatic [Ziemann and Siebner, 2008; 95]. Priming the lateral cerebellum with cTBS had no effect on practice-induced changes in peak acceleration of simple movements although it disrupted their retention when tested at a later time [Li Voti et al., 2014; 85]. In contrast, it impaired the skill acquisition of more demanding reaching-to-point movements [Li Voti et al., 2014; 85]. These findings suggest that the lateral cerebellum is involved in long-term memory of these motor skills, and in learning of high-skilled goal-directed voluntary movements.

TBS studies in patients with hypokinetic and hyperkinetic movement disorders

Over the recent years an increasing number of studies have investigated the response to TBS in patients with various types of hypokinetic and hyperkinetic movement disorders. From what is now known about the variance in response to TBS protocols, most of these studies might be considered individually underpowered. Thus results that have not been replicated in more than one centre should be regarded as preliminary.

In patients with Parkinson's disease (PD), the majority of authors have found reduced response to iTBS and cTBS. Whether this is due to changes in intrinsic levels of dopamine in M1 [Wang and O'Donnell, 2001; Molina-Luna et al., 2009; Hosp and Luft, 2013; Hsieh et al., 2014; 96,97,98,99], or to changes in inputs to M1 from basal ganglia and other areas is unknown [Suppa et al., 2011; Bologna et al., 2015; 100,101]. In addition, there is still no agreement on whether abnormal TBS-induced plasticity is normalized by acute or chronic treatment with L-DOPA. Eggers et al. [Eggers et al., 2010; 102] and Suppa et al. [Suppa et al., 2011; 100] first demonstrated reduced responses to cTBS and iTBS, respectively. Although these observations have been confirmed in "de novo" PD patients in the more and the less clinically affected arm [Kishore et al., 2012a; 103] a further study failed to find altered

responses to TBS in parkinsonian patients [Zamir et al., 2012; 104]. The reason for this inconsistency in PD studies may arise from difference in patients' clinical features including disease duration and total daily doses of L-Dopa and other anti-parkinsonian drugs. The effects of "acute" and "chronic" L-Dopa therapy are unclear. Suppa et al. [Suppa et al., 2011; 100] found similar iTBS abnormalities in chronically treated PD patients, on and off therapy, and with or without L-Dopa-induced dyskinesias (LIDs), suggesting no beneficial effect of L-Dopa on TBS-induced plasticity. Kishore et al. [Kishore et al., 2012a; 103] confirmed no beneficial effect of acute L-Dopa challenge in "de novo" patients. In chronically treated PD patients, without LIDs and taking half their normal L-Dopa dose, Huang et al. [Huang et al., 2011;105] found no response to iTBS, while the response to iTBS and the amount of depotentiation elicited by a specifically designed "repeated" TBS protocol were both restored when patients took their full L-Dopa dose. However, in that study Huang et al. [Huang et al., 2011; 105] did not apply the conventional iTBS protocol which was expected to elicit no LTP-like plasticity in PD patients but a modified facilitatory type of TBS (cTBS followed by immediate muscle contraction for 1 min) [Huang et al., 2011; 105]. In addition, in chronically treated PD patients with LIDs, Huang et al. [Huang et al., 2011; 105] found a normal response to the modified facilitatory TBS protocol only when patients received half dose of L-Dopa (not eliciting LIDs), but patients failed to show depotentiation. Further information came from the study of Kishore et al. [Kishore et al. 2012b; 106] in chronically treated PD patients. Kishore et al. [Kishore et al., 2012b; 106] found different types of responses to TBS in patients off and on therapy, according to specific patients' clinical features (stable responders to L-Dopa, fluctuating non-dyskinetics and fluctuating dyskinetics). In chronically treated patients off therapy, TBS elicited normal responses in "stable responders", whereas "fluctuating non-dyskinetics" manifested normal responses to iTBS but not to cTBS. Finally, chronically treated "fluctuating dyskinetics" had reduced responses to both iTBS and cTBS. When tested on therapy, an acute L-Dopa challenge deteriorated responses to cTBS in all

patient subgroups with a paradoxical potentiation instead of depression of MEPs in "fluctuating dyskinetic" patients. The acute L-Dopa challenge also deteriorated responses to iTBS in "fluctuating non-dyskinetics", whereas in "fluctuating dyskinetics", it left responses to iTBS and cTBS globally unchanged. In conclusion, ~~overall~~ these studies in patients with PD point to ~~the~~ a ~~relevant~~ role of specific clinical (i.e. stage of the disease) and pharmacological factors (i.e. total L-Dopa daily dose) in modulating the response to the TBS protocols [Bologna et al., 2015; 101].

Relatively small cohorts of patients with atypical parkinsonisms have also been studied with TBS. In Progressive Supranuclear Palsy (PSP), responses to iTBS were enhanced responses whereas cTBS-induced after-effects paradoxically turned from LTD-like to LTP-like plasticity [Conte et al., 2012; 107]. In contrast, patients with Multiple System Atrophy (MSA) had reduced response to both iTBS and cTBS [Suppa et al., 2014; 108], and the effect was similar in patients with predominant parkinsonian (MSA-P) and cerebellar (MSA-C) features [Suppa et al., 2014;108]. More recently, a study in a small cohort of patients with Corticobasal syndrome (CBS), a rare neurodegenerative disorder characterized by parkinsonism combined with other asymmetric and heterogeneous motor (dystonia and myoclonus) and non-motor symptoms (apraxia, cortical sensory deficit, and alien limb phenomena), showed a more complex scenario [Suppa et al., 2016; 109]. When TBS was applied over the M1 contralateral to the less affected limb (manifesting only parkinsonism), iTBS and cTBS both elicited reduced responses. By contrast, when assessing the M1 contralateral to the more affected limb manifesting parkinsonism plus other motor and non-motor symptoms, TMS elicited heterogeneous responses. A first subgroup of CBS patients disclosed exceptionally decreased M1 excitability possibly due to cortico-spinal neuronal loss, a finding that prevented the examination of M1 LTP/LTD-like plasticity. A second subgroup of patients predominantly manifesting parkinsonism plus other motor symptoms showed reduced responses to TBS, whereas a third subgroup of patients predominantly

manifesting non-motor symptoms was characterized by increased responses to iTBS and cTBS [Suppa et al., 2014; 109]. Overall these findings suggest that TBS may help to understand the pathophysiological bases of the clinical and neurophysiological heterogeneity of patients with atypical parkinsonisms.

Several authors have investigated TBS-induced changes in MEP amplitudes in patients with hyperkinetic movement disorders. The two published studies with TBS in dystonia have apparently conflicting results. Edwards et al. [Edwards et al., 2006; 110] found a prolonged response to cTBS in patients with DYT1 generalized dystonia and cervical dystonia (CD), whereas DYT1 gene carriers without dystonia had reduced responses. They speculated that the prolonged response to cTBS observed in patients, like the increased response to the PAS_{25} [Quartarone et al., 2005; 111] was linked to the pathophysiology of dystonic symptoms, whereas the reduced response to cTBS observed in non-manifesting DYT1 carriers reflects a compensatory mechanisms to protect susceptible individuals from appearance of dystonia [Edwards et al., 2006; 110]. In contrast, Belvisi et al. [Belvisi et al., 2013; 112] found a reduced response to iTBS in patients with focal hand dystonia. The difference between studies could relate to the different versions of TBS, or to the different body part affected by dystonia, but more data is needed to address that question. There is only one study of cTBS in patients in the early phase of Huntington's disease (HD) and in asymptomatic HD carriers [Orth et al., 2010; 113]. Responses to cTBS were reduced in both groups suggesting that altered plasticity may play an important role in the pathophysiology of HD. Responses to iTBS and cTBS have also been reported to be reduced in patients with Gilles de la Tourette Syndrome (GTS) [Suppa et al., 2011, 2014; 114,115]. The effect was comparable in patients with pure motor symptoms and in those manifesting psychiatric comorbidity and unaffected by chronic medication [Suppa et al., 2014; 115]. These findings suggest abnormal LTP/LTD-like plasticity in M1 as a possible factor contributing to the pathophysiology of hyperkinetic movement disorders including GTS [Suppa et al., 2011,

2014; 114,115]. Overall these studies have reported a number of abnormalities in patients with different types of hyperkinetic movement disorders. However, whether and through which physiological mechanisms the above mentioned abnormalities contribute to the pathophysiology of hyperkinetic symptoms remains largely unclear.

TBS IN ANIMAL STUDIES

Animal models supplement human TMS studies by opening the possibility to apply invasive in vivo electrophysiology, post-stimulation in vitro electrophysiology and histology, in addition to behavioral testing. Fortunately, TBS protocols are very suitable for experiments on animals because the short duration allows stimulation of fully awake animals in a stress-free manner after adequate familiarization to the experimental situation including manual restrain [Hoppenrath and Funke, 2013; Mix et al., 2010, 2015; Papazachariadis et al., 2014; Castillo-Padilla and Funke, 2015; 116,117,118,119,120].

A rat model of TMS

The study of TMS in small animals like rats is confronted with a scaling problem. The human brain is about 700x larger than the rat brain, making a focal stimulation of distinct rat brain areas difficult if not impossible. Even recent developments of small rodent coils (Cool-40, MagVenture) do not solve this problem completely. The main limitations are achieving sufficient current flow in a small coil without overheating, a problem that is magnified when applying high-frequency repetitive stimulation. To achieve certain degree of focal stimulation, the peak of the magnetic field is either centered above the cortical area to be stimulated, e.g. for evoking motor responses as in humans [Hsieh et al., 2015; 99], or somewhat eccentric to limit stimulation to one hemisphere [Keck et al., 2001; Rotenberg et al., 2010; 121,122].

Alternatively, the coil is centered on the midline over the corpus callosum with mediolateral orientation of the induced electric field [Benali et al., 2011; Ghiglieri et al., 2012; 123,124]. The former method needs a higher magnetic field strength of about 50-80% of maximal machine output (MO), while the latter requires only 20-30% MO to achieve cellular effects. It is postulated that midline TMS will initiate action potentials in callosal axons and induce primarily supragranular cortical activity in both hemispheres, via synaptic connections with pyramidal cells and interneurons within layer 2/3 and also via action potentials back-propagating to the cells of origin of the callosal projections and to all synapses of local axon collaterals (see Figure 4A_1 and A_2). The lower stimulation intensity needed to activate callosal axons reduces the risk of stimulating deeper parts of the brain directly. In animal models, the principal neuronal effects of patterned stimulation of the human brain using TMS can also be modelled by applying the same stimulation patterns via conductive electrodes, thus enhancing focality and bypassing the necessity of using TMS coils for stimulation [Barry et al., 2014; 125].

Neuronal activity and plasticity markers

The first TBS studies on neuronal activity and plasticity markers in anaesthetized rats demonstrated increased c-Fos and zif268 early gene expression but also decreased amounts of proteins expressed in inhibitory interneurons, like the GABA-synthesizing enzyme GAD67 (67kD isoform of glutamate decarboxylase) and the calcium-binding proteins parvalbumin (PV, Figure 4B) and calbindin (CB) [Aydin-Abidin et al. 2008; Trippe et al., 2009; Benali et al., 2011; 123,126,127]. Studying the changes in protein expression at different times post-iTBS (600 pulses, awake rat) [Hoppenrath and Funke, 2013; 116] revealed that c-Fos, zif268 and GAD65 (65 kD GAD isoform expressed in GABA-ergic terminals) were strongly increased as early as 10 minutes post-iTBS and recovered within 20 minutes, while a reduction in PV, CB and GAD67 expression appeared earliest after 20-40 minutes (see Figure

4C). The former may reflect the acute effects of neuronal stimulation, including activation of GABAergic synapses as indicated by the increase in GAD65. The latter are a sign of neuronal plasticity, probably induced by the degree and temporal pattern of changes in intracellular calcium concentration [Grehl et al., 2015; 128]. The reduction of GAD67 and CB could last for hours, and even days in case of PV [Benali et al., 2011; 123] without further intervention (see below). The effects of stimulation increase in a dose-dependent fashion with each TBS block applied [Volz et al., 2013; Thimm and Funke, 2015; 129,130] and require activation of NMDARs [Labedi et al., 2014; 131]. Interestingly, in dark agouti rats iTBS primarily reduced the expression of PV [Benali et al., 2011; 123], a protein specifically expressed in fast-spiking interneurons mediating perisomatic inhibition and thereby controlling rate and temporal pattern of pyramidal cell output activity [Markram et al., 2004; 132]. In contrast, cTBS had little effect on PV but reduced the expression of CB, expressed in non-fast-spiking interneurons controlling primarily dendritic input to pyramidal cells. It thus appeared that different TBS protocols may be able to affect different subsets of the cortical network, a finding closely related to the different effects of iTBS and cTBS on human cortical I-waves (see above).

Cortical electric activity

A recent study addressing the effects of TBS on rat motor cortex replicated the opposing effects of the two TBS protocols as usually found in human studies, with iTBS increasing and cTBS decreasing the amplitude of MEPs for more than 30 minutes [Hsieh et al., 2015; 99] (see Figure 4D). Thimm and Funke [Thimm and Funke, 2015; 130] analyzed evoked sensory responses in the barrel cortex of anaesthetized rats before, between and after five blocks of either iTBS or cTBS. iTBS disinhibited sensory responses in the layer 3/4 border region by increasing late components of evoked responses (see Figure 4E_1) and by reducing paired pulse suppression at short intervals (20 ms). The effect increased with each of the five blocks.

In contrast, the first cTBS block caused a slight suppression of sensory responses but a weak disinhibitory effect evolved with further repetitions (see Figure 4E$_2$), indicating that the cTBS effect may reverse with repeated or prolonged stimulation [Gamboa et al., 2010, 2011; 70,133]. Another TBS study on rat somatosensory cortex [Benali et al., 2011; 123] showed that iTBS, but not cTBS, increased spontaneous neuronal activity in the gamma frequency range.

Learning and memory

In 2010, Mix et al. [Mix et al., 2010; 117] demonstrated that iTBS, but not cTBS, improved the ability of rats to learn a tactile discrimination task in darkness (Figure 4F$_1$). Analysis of cortical activity marker expression one day after the last session revealed that iTBS, but less cTBS, reduced the expression of PV, CB and GAD67. Since magnetic stimulation was not focused to a particular cortical area, these changes were evident in multiple cortical areas of all animals whether they performed the task or not. However, cortical areas involved in the learning process (frontal and barrel cortex) had significantly less reduction of PV and CB expression than the visual cortex which was not involved in the task (see Figure 4F$_2$). It thus appears that better learning in iTBS-treated rats relates to initial cortical disinhibition, which promotes functional network plasticity. Inhibition normalizes and almost recovers to pre-stimulation conditions during the course of learning related plasticity.

Factors of variability

iTBS and cTBS produce different outcomes in different strains of rat [Mix et al., 2014; 134]. The clear difference between both protocols seen in Dark Agouti (DA) rats, a strong reduction in PV with iTBS but little effect on CB, and vice versa with cTBS, was almost absent in Sprague Dawley (SD) rats. A study on these and a third strain, Long Evans (LE), revealed that the iTBS effects are quite consistent with about 40% reduction in the number of PV+

cells and 20% reduction in the number of CB+ cells in all strains. However, the cTBS effects differed between strains, with opposite effects in SD and DA rats, and the LE in between (see Figure 4G). One factor possibly contributing to the inter-strain variability was seen in the different basal number of interneurons of a certain class, pointing to variations in cortical circuits and a likely genetic factor (see above). A recent rat study further revealed that iTBS-induced reduction in cortical PV expression is age-dependent [Mix et al., 2015; 118]. It cannot be induced before maturation of the perineuronal nets surrounding the cell bodies and proximal dendrites of PV+ interneurons, accompanied by maturation of cortical synaptic inputs. This finding indicates that TBS effects may depend on the developmental changes of cortical areas, which are still in progress during adolescence. Application of iTBS to rats visually deprived from birth to the end of the early cortical critical period prevents the detrimental effect of dark rearing on visual performance of rats which is also associated with iTBS-induced reduction in PV expression but also an increase in cortical BDNF level [Castillo-Padilla and Funke, 2015; 120]. Interestingly, a tactile enriched environment during dark rearing has a similar effect on visual performance and cortical BDNF level but is not associated with the reduction in PV expression observed with iTBS (see Figure 4B).

Disease models

To date, animal disease models using TBS applied via TMS are limited to experimental parkinsonism in rats. The iTBS protocol was found to increase striatal excitability and to rescue long-term depression at cortico-striatal synapses, which had been almost eliminated by 6-hydroxydopamine treatment [Ghiglieri et al., 2012; 124]. Using a similar rat model of experimental Parkinsonism, Hsieh et al. [Hsieh et al., 2015; 99] recently demonstrated that the potential of iTBS to induce M1 plasticity declines with depletion of dopaminergic neurons within the substantia nigra, and with severity of motor deficits. A rat cortical lesion model mimicked TMS-induced cortical activity by applying the iTBS pattern via implanted

electrodes [Barry et al., 2014; 125]. The authors demonstrated that this procedure weakens inter-hemispheric inhibition and improves recovery of motor functions if applied to M1 contralateral to the lesioned hemisphere.

HARNESSING TBS FOR THERAPY

How to harness metaplasticity in brain disease with disordered network activity is currently most extensively studied after cerebral stroke in order to improve functional outcome. Several studies have been ~~B~~based on ~~the certainly oversimplified~~a simple concept of a dysbalanced inter-hemispheric equilibrium with (1) decreased excitability in the ipsilesional hemisphere, (2) increased excitability in the contralesional hemisphere, and (3) exaggerated inhibitory control from the contra- to ipsilesional hemisphere [Ward and Cohen, 2004; 135]. ~~, several proof-of-principal studies~~They have demonstrated that increasing excitability of the ipsilesional M1 with ~~enhancing~~ iTBS ~~of the ipsilesional M1~~ or ~~excitability~~ depressing the excitability of the contralesional cortex with cTBS ~~of the contralesional M1 concurrent with motor practice~~ can improve motor skill and motor learning when applied concurrent with motor practice [Butler et al., 2013, Hsu et al., 2012; 136,137]. ~~Along this line~~Following the same reasoning it was suggested further to increase excitability of the ipsilesional M1 by priming stimulation to enable non-homeostatic gating of subsequent practice-dependent motor recovery [Bolognini et al., 2009; 138]. Accordingly, training of paretic-hand grip-lift kinetics improved after priming (15 min earlier) with iTBS of ipsilesional M1 or cTBS of contralesional M1, but deteriorated after sham TBS in subcortical chronic stroke patients [Ackerley et al., 2010, 2014; 139,140]. Applying the principle of homeostatic metaplasticity to enhance stroke recovery appears somewhat counterintuitive in this context of a prevailing concept of interhemispheric rivalry and reduced excitability of the ipsilesional hemisphere

[Cassidy et al., 2014; 141]. Consequently, only one small-scale clinical trial so far has tested the effects of homeostatic metaplasticity in chronic stroke patients [Di Lazzaro et al., 2013; 142]. Priming of the ipsilesional M1 with excitability decreasing cTBS followed by motor training of the paretic hand/arm resulted in improvement of hand function as tested with the Jebsen Taylor Test in the real cTBS group but not in the sham group [Di Lazzaro et al., 2013; 142]. This provides first preliminary evidence that the concept of homeostatic metaplasticity may be utilized to improve functional outcome after cerebral stroke.

To date, TBS has not been used as extensively as other rTMS protocols in clinical studies [Cramer et al., 2011; 143]. Several studies have examined the potential of cTBS applied to the temporal/temporoparietal cortex for reducing symptoms of tinnitus. The findings are mixed with some studies reporting significant improvements [Forogh et al., 2014; 144] but others no significant effects [Plewnia et al., 2012; 145]. There is some evidence that iTBS applied over the leg region of M1 can reduce lower limb spasticity in multiple sclerosis when applied daily for 2 weeks [Mori et al., 2010; 146] . This effect may be enhanced when iTBS is applied in conjunction with exercise therapy [Mori et al., 2011; 147]. A small number of studies have examined the therapeutic potential of TBS in major depression. For example, a recent study [Li et al., 2014; 148] compared the effects of two weeks of cTBS (right dorsolateral prefrontal cortex - DLPFC), iTBS (left DLPFC), combined cTBS (right DLPFC) and iTBS (left DLPFC), or sham TBS in patients with treatment refractory major depressive disorder. Of note, the TBS trains were extended and involved 1800 pulses. Patients improved in all stimulation conditions but iTBS, and combined cTBS/iTBS, were significantly more effective (with the combined approach being best). Bakker and colleagues [Bakker et al., 2015; 149] compared the safety and effectiveness of 10Hz rTMS and iTBS applied to the dorsomedial prefrontal cortex in medication resistant major depression and concluded that both approaches were equally effective and safe. It should be noted that in this study the

intensity of iTBS was 120% resting motor threshold (RMT), which is significantly higher than that used conventionally.

TBS has also been trialed in several other psychiatric conditions. For example, several case studies have reported that both unilateral [Poulet et al., 2009; 150] or bilateral [Eberle et al., 2010; 151] cTBS applied over the temporo-parietal, or iTBS applied over the left dorsolateral prefrontal [Sidhoumi et al., 2010; 152] cortical areas reduce the medication resistant symptom of auditory verbal hallucinations seen in schizophrenia. However, in a more recent study, real TBS was shown to be no more effective than sham stimulation [Dougall et al., 2015; 153]. In a single case study, cTBS applied in multiple sessions over the right dorso-lateral prefrontal cortex reduced medication resistant symptoms of obsessive-compulsive disorder [Wu et al., 2010; 154]. It is clear that the therapeutic potential of TBS in these and other psychiatric conditions needs to be examined in larger well-controlled studies.

In terms of functional response, perhaps the most impressive studies are those examining the potential of TBS reducing stroke related symptoms of neglect. The design of these studies is based on the interhemispheric imbalance approach described above and were aimed at reducing the excitability of the parietal cortex in the non-stroke affected hemisphere to produce beneficial changes in excitability in the stroke affected parietal cortex. In a randomized, double blind and sham controlled study Koch and colleagues [Koch et al., 2012; 155] demonstrated that 10 sessions of cTBS applied to the posterior parietal cortex (non-stroke left hemisphere) over two weeks resulted in a significant improvement in hemispatial neglect (assessed using the Behavioural Inattention Task) in subacute stroke patients that lasted for at least 2 weeks. Using a slightly different approach, that involved the application of 8 trains of cTBS to the posterior parietal cortex of the non-stroke left hemisphere over 2 consecutive days, large improvements (assessed with the Catherine Bergego Scale) were seen in a group of stroke patients with subacute spatial neglect [Cazzoli et al., 2012; 156].

CONCLUSIONS

In the ten years since its introduction, TBS methods have proved to be a popular and useful addition to the growing number of methods now available to interact with presumed synaptic plasticity in the human brain. The advantages of TBS are its short duration and use of low intensity stimulus pulses, making it more acceptable to participants than some other non invasive brain stimulating protocols. Data from animal studies suggest that the effects observed in the human brain can be replicated in the rat brain and have given some insight into the basic physiological mechanisms involved in TBS effects. Findings indicate that iTBS and cTBS have different effects on inhibitory cortical networks. In particular, iTBS may increase excitability by reducing perisomatic inhibition of pyramidal cells by PV+ fast-spiking interneurons. Experimentally, this increases the amplitude of late sensory evoked responses consistent with modulation of intracortical connections rather than thalamocortical inputs and may correspond to modulation of late I-waves by iTBS in human M1 [Di Lazzaro et al., 2008; 15].

Yet there are still many unknowns. For example there have been no systematic parametric studies. The choice of frequency and intensity of pulses was initially limited by technical factors and safety concerns. But excellent effects have been reported with 30 Hz (rather than 50 Hz) bursts repeated at 10 Hz (rather than 5 Hz) with an intensity of 80% RMT (rather than 80% AMT) [Nyffeler et al, 2006; 157]. More worryingly, the initial parameters of TBS appear to produce highly variable results, at least on M1, and these may well account for some of the discrepancies between studies in the literature. Effectively, many studies have been underpowered with the consequence that reported findings may prove to be less robust and reliable than once believed. Some of the variability may be reduced by careful control of the baseline state of the brain prior to testing, such as by avoiding active muscle contraction

before using TBS over M1, or by testing at a particular time of day. Initial data also suggest that much of the variation is caused by differences between individuals, whereas within an individual, the response may be more repeatable. If so, this means that repeated measures investigations within an individual may prove more reliable than group comparisons between different individuals. But more work needs to be done to investigate the daily variation of TBS effects within individuals so that we have reliable data for power calculations in future studies.

A different approach to the problems of variability has been to search for better ways to administer TBS. A persuasive argument has been that animal experiments have shown that although a single plasticity intervention may induce LTP/LTD-like effects lasting a few hours, repeating the intervention after a gap of several minutes can lead to changes lasting many hours or days. Recent work in humans using TBS also suggests that repeated sessions of TBS may produce a more powerful, long lasting and robust effect than a single session. Confusingly however, there are also reports that two sessions of TBS, rather than reinforcing each other actually oppose each other (i.e. they show a homeostatic interaction rather than a non-homeostatic effect). This might be due to subtle differences in methods and timing between the TBS applications, but no systematic studies have yet been performed to find the optimal combination of inter-session interval or of the number of sessions to apply. These will be of critical importance if this type of approach is to become useful in therapeutic settings and if we are to understand the rules that govern homeostatic versus non-homeostatic interactions. At present, such terms are little more than descriptions of results that have already occurred rather than a priori predictors of response. Thus TBS is, like many other NIBS methods, still in its infancy. The next 10 years will be interesting times.

ACKNOWLEDGEMENTS

Nothing to declare.

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FIGURE LEGENDS

Figure 1: The patterns and effects of TBS. **(A)** The basic element of TBS is a 3-pulse burst at 50 Hz given every 200 ms (i.e. 5 Hz). Two major patterns, including iTBS and cTBS, are commonly used. A short train of 10 bursts lasting for 2 seconds is given every 10 seconds for 20 cycles in iTBS, while 100 or 200 continuous bursts are given continuously for 20 or 40 seconds, respectively, in cTBS. **(B)** iTBS produces a potentiation effect for around 20 min. In contrast, after AMT measurement cTBS for 20 and 40 seconds produces a depressive effect for 20 min and 60 min, respectively.

Figure 2: **Upper part:** schematic representation of motor cortex circuits and possible preferential site of activation using transcranial magnetic stimulation at different stimulus intensities. White circles indicate excitatory neurons, while black filled circle indicate inhibitory neuron. This model includes an inhibitory circuit that have connections with an excitatory bursting interneuron (white circle in a dotted circle) projecting upon the distal

apical dendrites of layer 5 corticospinal cells. It is proposed that low intensity stimulation activates monosynaptic connections to corticospinal cells evoking the I1-wave, at higher intensities late I-waves are evoked through the activation of a complex circuit composed of bursting interneurons and inhibitory neurons that in turn activate the corticospinal cells. At high intensities magnetic stimulation also activates directly the corticospinal axons of corticospinal cells evoking the short latency wave termed D-wave.

Lower part: effects of theta burst stimulation on corticospinal activity.

Left: Epidural volleys recorded in baseline conditions (black trace) and after continuous theta burst stimulation (green trace). Each trace is the average of the responses to 10-25 cortical magnetic stimuli. After cTBS, the amplitude of the I1 wave is suppressed whereas late I-waves and D wave are substantially unchanged.

Middle: Epidural volleys recorded in baseline conditions (black trace) and after intermittent theta burst stimulation (red trace). After iTBS, a selective facilitation of late I-waves is observed with no change in I1 wave.

Right: Epidural volleys recorded in baseline conditions (upper trace) and intermittent theta burst stimulation (lower) in a chronic stroke patient after stimulation of the affected hemisphere. After iTBS, the size and also the number of corticospinal volleys is increased; moreover, after iTBS the corticospinal volleys appear much more synchronised.

Figure 3: Factors possibly contributing to inter-subject and intra-subject variability in the amount of response to theta burst stimulation (TBS) in healthy humans.

Figure 4: Major findings of theta-burst stimulation (TBS) in rats. (**A_1,A_2**) Activation of supragranular cortical layers via stimulation of callosal axons in the rat. Pyramidal cells (Pyr, green) and inhibitory interneurons (PV, CB, red) will be stimulated transsynaptically while Pyr will also be activated antidromically. PV – parvalbumin, CB – calbindin. **(B)**

Diaminobenzidin (DAB) staining of PV+ interneurons in rat visual cortex. Compared to controls (con), iTBS strongly reduces PV expression which is prevented if rats are raised in an enriched environment (EE). Dark rearing (DR) has little effect on PV expression (according to Castillo and Funke, 2015). **(C)** One block of iTBS (600 pulses) causes an early increase in cortical c-Fos and GAD65 expression, reflecting the direct activation of neurons and GABAergic terminals, respectively. Late effects of iTBS are a lasting reduction in PV expression (modified according to Hoppenrath and Funke 2013). **(D)** Increase in motor evoked responses (MEP) after iTBS applied to rat motor cortex and decrease of MEPs after cTBS (modified from Hsieh et al. 2015). **(E_1)** One block of iTBS, but not cTBS, increased somatosensory responses in rat barrel cortex. **(E_2)** Stronger effect after five iTBS blocks and a weak facilitative effect after five cTBS blocks (modified from Thimm and Funke 2015). **(F_1)** Rats treated with iTBS prior to a tactile discrimination task reached the criterion of 75% correct responses significantly earlier (less trials needed) than sham-controls and rats treated with cTBS. **(F_2)** The iTBS-induced reduction in cortical PV expression (red compared to sham controls, yellow; both groups non-learner controls) was diminished after learning (hatched bars, red - iTBS-treated learners, orange – sham-treated learners) in cortical areas involved in the task (frontal and barrel cortex) but not in the visual cortex being not involved (modified from Mix et al. 2010). **(G)** Variability of TBS effects in rats of different strains (SD – Sprague Dawley, LE – Long Evans, DA – Dark Agouti). The iTBS was similar in all strains, reducing PV expression much more than that of CB. The cTBS was variable, causing strong reduction in CB but not PV expression in DA, opposite effects in SD and almost equal but lower reduction of both in LE (modified from Mix et al. 2014).