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Assessment and modulation of cortical inhibition using transcranial magnetic stimulation

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Abstract: Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique, which is used for diagnostic, therapeutic and scientific purposes in the field of neurology and psychiatry. It is based on the physical principle of electromagnetic induction and allows for the local activation of cortical areas through the intact skull of conscious humans. When applied repeatedly (repetitive TMS; rTMS) sustained changes of cortical excitability can be observed. Hence, TMS resembles a promising approach for assessing and modulating neuronal networks in a non-invasive manner. However, despite its broad clinical application, the cellular and molecular mechanisms of rTMS-based therapies remain not well understood. Established therapeutic concepts assume that pathologically altered cortical excitability is normalised, which may involve 'long-term potentiation' or 'long-term depression' of excitatory synapses. Indeed, animal studies demonstrate that rTMS induces long-term changes of excitatory neurotransmission. However, it is unclear through which mechanisms synaptic changes, which are caused by external electromagnetic activation of the cortex and therefore are not specific for context or behaviour, could have a positive impact on complex brain function. More recent findings suggest that not only excitatory but also inhibitory neuronal networks are modulated by rTMS. It was shown for example that 10 Hz rTMS leads to a calcium-dependent long-term depression of inhibitory GABAergic synapses. Since the reduction of inhibitory neurotransmission (= disinhibition) is considered important for the expression of associative plasticity at excitatory synapses, it is conceivable that rTMS-induced disinhibition may promote context- and behaviour-specific synaptic changes. Hence, the model of *rTMS-induced local disinhibition* represents an attractive explanation for the observation that a seemingly unspecific (exogenous) magnetic stimulation could induce specific (endogenous) structural, functional and molecular changes of cortical synapses. Current research focuses on the effect of rTMS-induced disinhibition on synaptic plasticity in suitable animal models (both *in vivo* and *in vitro*). Thus, apart from its diagnostic and therapeutic potential TMS represents a promising method for conducting clinically-oriented translational plasticity studies.

Keywords: non-invasive brain stimulation, synaptic plasticity, disinhibition, interneurons

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Introduction

The ability of the brain to respond with structural, functional and molecular changes to a specific stimulus is generally called plasticity (Konorski, 1948). Activity-dependent changes at neuronal contact sites, so-called synaptic plasticity, play a crucial role in physiological brain functions, such as learning, memory and orientation in space and time. The cellular and molecular mechanisms of associative synaptic plasticity (Hebb, 1949), i.e., the cellular and molecular correlate of context- and behaviour-specific modifications in the brain, have been thoroughly investigated during the past decades [see for example (Bliss and Collingridge, 1993; Nicoll and Roche, 2013)].

Evidence for plastic changes in the human brain comes from TMS-studies (among others; Ziemann et al., 2008). TMS is a non-invasive brain stimulation method utilising the principle of electromagnetic induction. A very brief (~500 µs) and strong magnetic field (> 1 Tesla) is generated over the skull of a healthy subject or patient using an appropriate stimulation coil (Fig. 1*a*). This magnetic field results in the induction of electric fields in the brain, which in turn activate neurons. Since magnetic fields penetrate skin and the intact skull (= transcranial), this stimulation technique is regarded as *non-invasive*. TMS is considered safe if performed according to established security guidelines and is in general well tolerated (Rossi et al., 2009).

Typically, the effects of single TMS pulses over the primary motor cortex are assessed and quantified by re-

cording of so-called motor evoked potentials (MEP) in the target muscle (Fig. 1*b*). Combined stimulation of the motor cortex and the spinal cord can be used to diagnostically assess the central motor conduction time, which is prolonged in demyelinating diseases like multiple sclerosis. Application of consecutive trains of stimuli with up to several hundreds of TMS pulses (repetitive TMS, rTMS) over the motor cortex can lead to changes of cortical excitability beyond the period of stimulation: high-frequency rTMS (≥ 5 Hz) typically increases cortico-spinal excitability, measured as an increase of MEP amplitude, whereas low-frequency rTMS (1 Hz) reduces it. Based on pharmacological studies and analogies to conventional plasticity experiments, it has been hypothesised that rTMS-induced changes of cortical excitability may represent long-term potentiation or long-term depression of excitatory synapses (Ziemann et al. 2008). Indeed, using organotypic brain slice cultures, it was shown that repetitive magnetic stimulation induces structural, functional and molecular changes of excitatory synapses, corresponding long-term potentiation and thus associative excitatory synaptic plasticity (Vlachos et al., 2012). Consistent with these findings it was shown that rTMS promotes learning in animal experiments (Mix et al., 2010) and motor rehabilitation following ischemic stroke in humans (Brodie et al., 2014; Volz et al., 2016). However, it remains unclear how complex brain function in healthy subjects and in patients is improved by activity-dependent modifications of neuronal networks, caused by several hundred of exogenous magnetic pulses.

Role of inhibitory networks in associative synaptic plasticity

In recent years the role of inhibitory neuronal networks on physiological brain functions has been carefully investigated (Trembley et al., 2016). It is now well accepted that inhibitory interneurons control the activity and excitability of neuronal networks. This observation led to the hypothesis that a decrease of inhibitory neurotransmission, i.e. *disinhibition*, may promote associative synaptic plasticity and thus learning and memory formation. Indeed, evidence has been provided that a reduction of GABAergic inhibition improves learning and memory, whereas increased inhibition impairs plasticity induction (Trembley et al., 2016). Thus, our current understanding is that *local disinhibitory networks* play a central role in regulating learning and memory formation (Letzkus et al., 2015).

Apart from plasticity of GABAergic synapses (Froemke, 2015), local disinhibition can be achieved through different network motifs: for example by 1) decreased glutamatergic activation of local inhibitory interneurons, 2) increased activity of inhibitory projection neurons, which inhibit local inhibitory interneurons of other cortical areas,

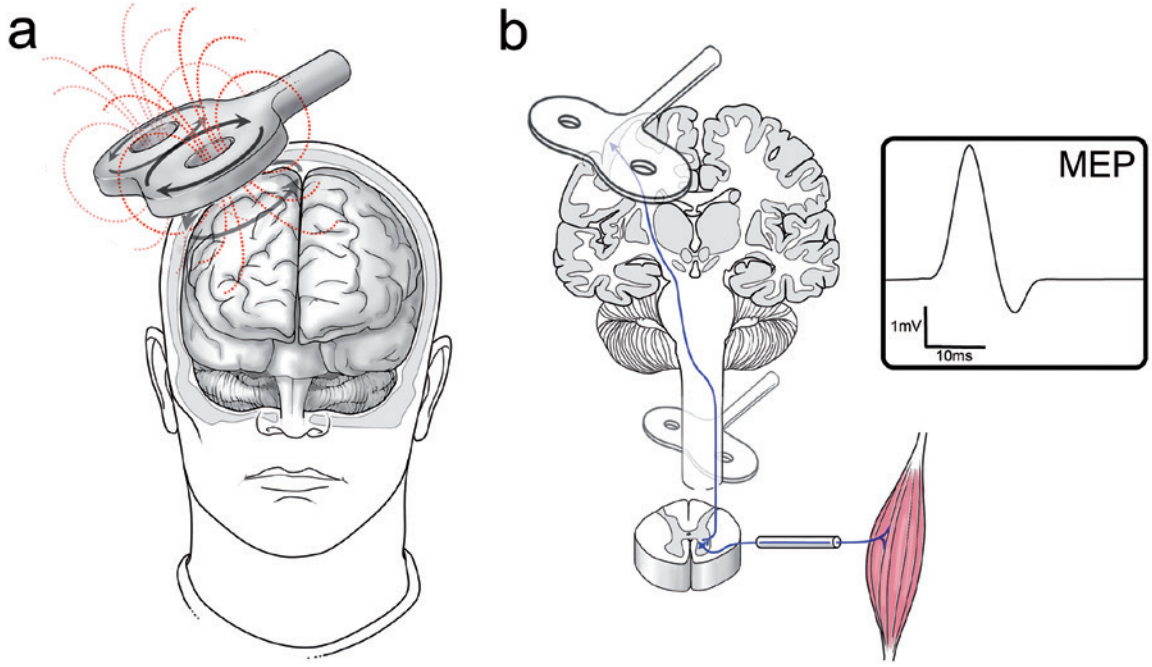

Fig. 1: Transcranial magnetic stimulation (TMS). (a) Visualisation of transcranial magnetic stimulation (b) of the motor cortex and the spinal cord. The effects of TMS are quantified by recording of motor evoked potentials (MEPs).

or 3) by neuromodulatory projection pathways that activate specific local interneurons, which in turn inhibit other local interneurons [Fig. 2; see also (Letzkus et al., 2015)].

The clinical relevance of disinhibition [e.g. (Nelson and Valakh, 2015)] is also reflected by the fact that alterations of excitation/inhibition-balance are considered a major cause for neurological and psychiatric symptoms. Therefore, from a clinical point of view tools/approaches enabling the assessment and modulation of (dis)inhibitory networks seem highly attractive. Appropriate diagnostic and/or interventional measures could detect and moreover influence the ability of the human brain to express endogenous plasticity, making TMS an interesting clinical tool.

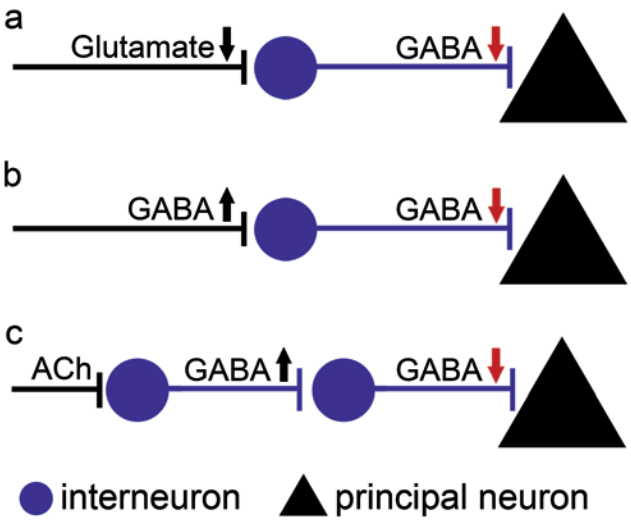

Fig. 2: Network motifs of local disinhibition. Besides a 'long-term depression' of inhibitory synapses on pyramidal cells local disinhibition can be achieved by several network motifs: e.g. by (a) decreased glutamatergic activation of local inhibitory interneurons, (b) increased activity of inhibitory projection neurons, which innervate local interneurons or by (c) neuromodulatory projection pathways (e.g. cholinergic projections; acetylcholine, ACh), which activate specific local interneurons, which in turn mediate inhibition of other local interneurons.

TMS-based assessment of inhibitory cortical networks

Functional experiments of intra- and intercortical inhibition in the motor cortex of the human brain are mainly based on TMS-studies (Di Lazzaro and Ziemann, 2013). In recent years, several specific double-pulse protocols have been established in this context: the so-called 'short-interval intracortical inhibition' (SICI) is based on application of a subthreshold conditioning TMS pulse, which does not induce MEPs itself, followed by a suprathreshold test pulse within a short interval (1–5 ms). In contrast, 'long-interval intracortical inhibition' (LICI) consists of pairing of two suprathreshold TMS pulses with a longer interval (50–200 ms). In both cases, inhibition of the test MEP amplitude can be observed. While the fundamental network mechanisms still remain elusive (Di Lazzaro and Ziemann, 2013), it is assumed that the initial pulse is responsible for a characteristic (direct or indirect) activation of inhibitory interneurons, triggering a quantifiable reduction of the test MEP. Meanwhile, pharmacological studies indicate that SICI reflects the influence of ionotropic $GABA_A$ receptors, whereas LICI is rather attributed to metabotropic $GABA_B$ receptors (Ziemann et al., 2015). Thus, characteristic TMS protocols allow for the non-invasive, functional analysis of local inhibitory networks in the human motor cortex.

However, an important limitation in this context is the fact that quantification of TMS effects require recordings of MEPs. These potentials are not recorded at the point of stimulation, meaning at least two synapses beyond the stimulated motor cortex (Fig. 1*b*). Also, these protocols cannot be translated to other (disease-related) brain regions. Furthermore, because of possible 'filtering' of cortico-spinal synapses and the motor endplate, a direct correlation between MEP amplitude and cortical excitability remains controversial. Accordingly, efforts are currently focusing on a more direct analysis of cortical inhibition, which is independent of the stimulated area of the cortex, using TMS-evoked electroencephalographic (EEG) potentials (Rogasch and Fitzgerald, 2013; Premoli et al., 2014). This attempt is supported by other techniques, e.g. GABA-magnetic resonance spectroscopy (Stagg et al., 2011). However, it remains to be shown, to what extent plasticity inducing rTMS protocols change TMS-evoked EEG signals and what the underlying mechanisms might be.

rTMS-induced modulation of inhibitory networks

While the effects of rTMS on excitatory synapses have been demonstrated in several animal models (both *in vivo* and *in vitro*; Tang et al, 2015), rTMS-induced changes in inhibitory synapses remain not well characterized. However, robust experimental data show that specific rTMS protocols differentially affect calcium-binding molecules, which are regarded as activity- and plasticity-markers of inhibitory interneurons (Caroni, 2015). Thus, for example intermit-

tent theta-burst stimulation (iTBS) causes a reduction of parvalbumin (PV) expression in inhibitory interneurons of the rat cortex, whereas a continuous theta-burst stimulation (cTBS) and low-frequency rTMS (1 Hz) rather reduce calbindin expression in another set of interneurons (Benali et al., 2011). Since PV-expressing interneurons mediate somatic inhibition of principal neurons and calbindin-positive interneurons affect dendritic inhibition, these studies suggest that rTMS can influence specific aspects of network inhibition (e.g. control of efferent and afferent activity on pyramidal cells) based on the particular rTMS protocol employed. Notably, current studies mainly show a decrease of the relevant marker molecules, which is consistent with rTMS-induced disinhibition.

Unpublished data suggest that interneurons with reduced PV expression receive fewer neocortical glutamatergic inputs than those with high PV expression (also see Fig. 2). Similar correlations between PV expression and the balance of excitatory and inhibitory synaptic inputs were earlier described in the hippocampus of mice (Donato et al., 2013). Another electrophysiological study in the primary somatosensory cortex of rats further demonstrates that a decrease of PV expression, induced by iTBS-rTMS, is accompanied by an increase of sensory responses (Thimm and Funke, 2015). This observation supports the notion that rTMS-induced disinhibition could underlie the previously described improvement in learning behaviour (Mix et al., 2010). Albeit, studies of interneuronal activity- and plasticity-markers are not directly demonstrating rTMS-induced functional changes of inhibitory synapses.

rTMS-induced long-term depression of inhibitory synapses

In a recent study the effects of repetitive magnetic stimulation (rMS) on structural, functional and molecular properties of inhibitory synapses were studied (Lenz at al., 2016). Using single cell recordings of CA1 pyramidal neurons in mouse brain slice cultures, we showed that rMS indeed reduces inhibitory neurotransmission. These functional changes were accompanied by structural and molecular changes of inhibitory postsynapses, i.e. the reduction and destabilisation of gephyrin clusters, the major scaffolding protein at inhibitory postsynapses to which $GABA_A$ receptors anchor (Tyagarajan and Fritschy 2014; Kneussel and Hausrat 2016).

The rMS-induced reduction in inhibitory neurotransmission requires the activation of voltage-gated sodium channels, NMDA receptors and L-type voltage-gated calcium channels during stimulation. Furthermore, the effects of rMS on inhibition are not detectable in the presence of cyclosporine A, an inhibitor of calcineurin-dependent phosphatase. Based on these results we conclude that rMS induces calcium-dependent phosphorylation/dephosphorylation reactions, which destabilize gephyrin scaffolds and thereby mediate a long-term depression of GABAergic neurotransmission. In our experimental setting tonic inhibition, mediated by extrasynaptic GABA receptors, was not affected by rMS (Lenz et al., 2016).

Pharmacological inhibition of calpain-dependent proteases did not alter the effects of rMS. Since calpain has been linked to the calcium-dependent degradation of gephyrin under conditions of hyperexcitation (Tyagarajan and Fritschy 2014), these data indicate that the applied 10 Hz rMS protocol does not result in excessive and potentially toxic excitation of neuronal networks, which is usually accompanied by proteolytic degradation of essential synaptic elements.

Since rTMS-induced changes in gephyrin were also observed *in vivo*, using anaesthetized mice, our results indicate that rTMS could be a promising interventional tool to regulate gephyrin-dependent inhibitory synaptic plasticity. This conclusion is of clinical relevance since changes of the human gene locus for gephyrin are linked with an increased risk to develop autism, schizophrenia and epilepsy (Lionel et al., 2013). Together with the studies described above, these results show that rTMS is able to mediate long-term changes of inhibitory neurotransmission consistent with rTMS-induced disinhibition.

Interactions of rTMS-induced inhibitory and excitatory synaptic plasticity

Apparently, rTMS is capable of modulating inhibitory and excitatory neurotransmission. Interestingly our experiments show that both processes are calcium-dependent and require the activation of NMDA receptors and L-type voltage-gated calcium channels (Vlachos et al., 2012; Lenz et al., 2015, 2016). Based on this observation, the question arises how rTMS-induced inhibitory and excitatory synaptic modifications interact with each other (at the temporal, spatial and molecular level).

Based on the proposed concept of *rTMS-induced disinhibition*, it is interesting to hypothesize that rTMS mainly modulates inhibitory synapses whereas changes of excitatory synapses rather reflect the result of a 'disinhibition-in-

duced gating of associative excitatory synaptic plasticity'. Indeed, our *in vitro* experiments reveal that 10 Hz rMS is not immediately followed by a post-tetanic increase of excitatory synaptic strength as seen in classic LTP-experiments using local electric stimulation. Instead, a slow-onset potentiation of excitatory synapses within 1–2 h after stimulation, plateauing after 2–4 h, is observed (Vlachos et al., 2012). In contrast, expression changes of inhibitory marker molecules in the rat cortex can already be detected after 30 min following rTMS (Hoppenrath and Funke, 2013). These findings support the hypothesis that plasticity of excitatory synapses is not directly attributed to rTMS. In light of this hypothesis experiments addressing the exact temporal sequence of rTMS-induced long-term depression of inhibitory synapses (Lenz et al., 2016) and of rTMS-induced modification of excitatory synapses (Vlachos et al., 2012) seem crucial. Moreover, studies in which network activity is systematically modulated before, during and after rTMS (e.g. by applying pharmacological or optogenetic techniques) could help answering the question to what extent the effects of rTMS depend on the activity-state of the network. We are optimistic that this work will also shed a light on the influence of rTMS-induced disinhibition on metaplasticity and homeostatic plasticity. Furthermore, it could contribute to the recent discussion about the significant inter- and intra-individual variability of rTMS-effects in humans (Muller-Dahlhaus et al., 2008; Hamada et al., 2013; Lopez-Alonso et al., 2014). In this context it is interesting to speculate that the cellular and molecular processes, induced by a specific rTMS protocol *per se*, are not that variable, whereas the long-term effects could very well depend on various inter- and intra-individual parameters [(e.g. age, alertness, time of stimulation, etc. (Ridding and Ziemann, 2010)].

Synapse-specific effects of rTMS

rTMS-induced disinhibition represents an interesting explanation for the observation that a seemingly unspecific exogenous stimulation of cortical networks can lead to context- and behaviour-specific changes. For example, it might be possible to apply 10 Hz rTMS or iTBS prior to rehabilitation methods following stroke, in order to support specific training effects by 'disinhibition-induced gating of associative plasticity' at specific excitatory synapses (Volz et al., 2016).

Indeed, evidence has been provided that rMS is able to induce plasticity of specific synapses on principal neurons (Fig. 3): 10 Hz rMS mainly affects dendritic but not somatic inhibition (Lenz et al., 2016). In turn, the same stimulation protocol results in potentiation of excitatory synapses on small dendritic spines close to the soma of pyramidal neurons (Vlachos et al., 2012; Lenz et al., 2015). The exact mechanisms leading to these synapse/input-specific effects of rTMS need to be determined. Nevertheless, from an anatomical point of view it is an interesting concept that specific rTMS parameter may selectively enhance excitatory synapses close to the soma of projection neurons, e.g. thalamo-cortical projections in lamina 4 of the cortex, while dendritic disinhibition could facilitate the ability to express plasticity in dendrites of the superficial associative cortical layers. It remains to be clarified to what extent the synapse-specific effects of rMS, which are detected on CA1 pyramidal cells of organotypic hippocampal cultures, can be transferred to other neuronal cell types and networks of the cortex.

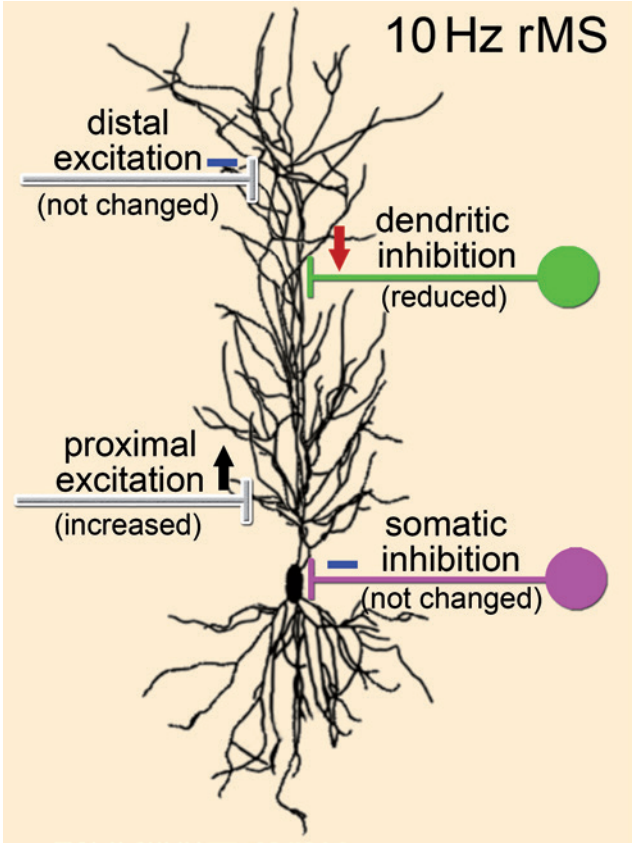

Fig. 3: Synapse-specific effects of repetitive magnetic stimulation (rMS). Despite the relatively large induced electromagnetic field, rMS is able to differentially affect distinct synapses on pyramidal cells. *In vitro* studies show that 10 Hz rMS results in decreased dendritic inhibition, and in an increase of excitatory synaptic neurotransmission on small dendritic spines close to the soma of pyramidal neurons. The exact mechanisms of these input-specific effects of rMS are currently under investigation.

Effects of TMS during stimulation

An import, still unanswered question concerns the neural effects of TMS during stimulation. Most of the assumptions in this context are based on computational approaches (Esser et al., 2005; Rusu et al., 2014). One of the major technical challenges are the strong electromagnetic fields generated during stimulation. These fields impede electrophysiological recordings, i.e. single-cell recording of neural activity, despite a few studies, which were performed with a low field strength and adequate position of the recording electrodes (Moliadze et al., 2005; Mueller et al, 2014; Pashut et al., 2014). Meanwhile 'contact-free' functional microscopy techniques, e.g. via calcium- or voltage-sensitive dyes (Kozyrev et al., 2014; Murphy et al., 2016), indicate that a low stimulation intensity mainly activates inhibitory interneurons, whereas projection neurons will be recruited with higher intensities [see (Pashut et al., 2014)]. Hence, these studies convey a plausible explanation for the already described SICI double-pulse protocol, where the subthreshold conditioning TMS pulse results in a $GABA_A$ receptor-dependent inhibition of the test MEP response.

In order to understand the effects of rTMS in more detail it will be important to simultaneously record the activity pattern of whole neuron populations with a high temporal and spatial resolution. Such studies may also shed light on the role of so-called backward propagating action potentials, which seem to play an important role in rTMS-induced synaptic plasticity (Lenz et al., 2015). Certainly, the currently available information is not enough to reliably predict effects of rTMS using computer-based simulations. Our pharmacological studies have so far only reveal that during stimulation, the activation of voltage-gated sodium channels, NMDA-receptors and L-type voltage-gated calcium channels is required in order to mediate rTMS-induced changes in excitatory and inhibitory neurotransmission *in vitro*. In this context, the potential influence of TMS on glia cells (astrocytes, oligodendrocytes and microglia) as well as on the neurovascular system also needs to be considered.

Open questions and perspectives

30 years after the development of the first TMS device by Anthony Barker and colleagues (Barker et al., 1985) important information about the cellular and molecular mechanisms of TMS have started to emerge. However, current rTMS-based therapies do not result in significant and long-lasting improvement of neurological and psychiatric symptoms (Lefaucheur et al., 2014). The fact that the mechanisms of rTMS are still poorly understood hinders substantial progress in this clinically relevant area. Furthermore, our limited knowledge about the role of synaptic plasticity under pathological conditions restricts a more efficient application of rTMS.

Studies from recent years showed that alterations of synaptic plasticity are not necessarily reflecting neurological or psychiatric diseases (Maggio and Vlachos, 2014). In fact, a decrease in the ability of neurons to express plasticity could also protect networks from 'maladaptive changes'. At the same time potential negative effects on the success of rehabilitation measures need to be considered in this context. Our current understanding is that the threshold for the expression of plasticity changes during the course of a disease. Changes in the excitation/inhibition-balance seem to play a crucial role since, as it is discussed in this article, changes of inhibitory synapses influence plasticity of excitatory synapses. It is clear that a comprehensive understanding of the role of various forms of synaptic plasticity (associative, homeostatic plasticity and metaplasticity) under pathological conditions needs to be obtained in order to successfully employ plasticity modulating therapeutic strategies.

Future diagnostic and therapeutic interventions could be aimed at assessing and modulating the ability of neural networks to express plasticity at different time points in the course of a disease and in identified cortical regions. Apparently, TMS resembles an attractive clinical tool in this respect. In fact, TMS may also overcome or complement deficits of conventional pharmacologic treatment, which lacks the required temporal and spatial specificity. Accordingly, the diagnostic and therapeutic potential of rTMS regarding inhibitory networks needs to be further elaborated and additional cellular and molecular effects of rTMS need to be considered, e.g. influence on gene expression, mRNA transport/stability, local protein biosynthesis, interaction with mitochondria and role of glial and vascular factors. Yet, *rTMS-induced disinhibition* represents a promising working hypothesis for future studies.

In this context it will also be necessary to consider the influence of rTMS on the described network motifs of local (dis)inhibition (Fig. 2); thus, the influence of rTMS on (1) glutamatergic synapses on inhibitory interneurons, (2) on intercortical inhibitory projections and (3) neuromodulatory projection pathways. Moreover, the effects of different TMS stimulation parameters need to be studied systematically, in order to determine for example whether it is also possible to increase inhibition and/or modulate somatic inhibitory neurotransmission (Fig. 3). Current studies are

trying to read activity states of neuronal networks in the brain ('brain states') by EEG in real time and to apply repetitive TMS pulses specifically only at time points of a certain state (Zrenner et al., 2016). This approach is based on the theory that the excitability of neuronal networks is regulated by pulsed inhibition (Jensen and Mazaheri, 2010). We anticipate that brain state-dependent rTMS, in contrast to conventional 'open-loop' stimulation, will significantly improve the effects of rTMS toward long-term plasticity. Thus, the future of therapeutic rTMS protocols may lie in 'closed-loop' approaches, which consequently utilise spontaneous rhythmic oscillations of inhibitory activity.

Perspectively, computational approaches could be helpful in selecting 'ideal' stimulation parameters/protocols for a given individual. However, the required basic information regarding the exact modes of action of TMS during and after the stimulation currently still remains elusive. It is clear that a better understanding of rTMS-induced plasticity will support the optimisation of existing therapeutic concepts, which already focus on modulating excitation/inhibition-balance (e.g. in the context of stroke or epilepsy therapy). We are convinced that the concept of *rTMS-induced local disinhibition* will gain attention in this context. Thus, apart from its diagnostic and therapeutic potential TMS represents a promising methodical tool to perform clinically orientated translational plasticity studies, addressing the relevance of disinhibition and synaptic plasticity under physiological and pathological conditions.

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