From micro to macro

Chapter 9

General Discussion

The overall aim of this thesis was to investigate the underlying neuroplastic mechanisms induced by transcranial magnetic stimulation (TMS) in humans, through an interdisciplinary series of studies using a variety of techniques. The experimental chapters of this thesis describe six different studies, beginning with three in an *in vitro* human neuronal cell culture model, and moving to three in human participants. In human neurons, the aim was to investigate the plasticity-related mechanisms induced by both excitatory and an inhibitory repetitive TMS (rTMS) protocols, specifically through measuring neural activity (calcium imaging), gene expression, and morphological and structural changes. We found evidence for immediate changes to cell activity following stimulation, but few changes in gene expression and morphology. In humans, the aim was to use indirect measures of assessing plasticity after the same excitatory protocol used in the first three chapters. We found in healthy participants that repeating multiple stimulation sessions in a single day did not promote additive plasticity effects. We also did not find evidence that TMS could be used to assess plasticity in participants with altered neuroplasticity (insulin resistance). Finally, we show using concurrent EEG-TMS and fMRI that excitatory stimulation to left dorsolateral prefrontal cortex (LDLPFC) was able to promote activation in several important cortical and subcortical structures. Overall, from stimulation of living human cell cultures to human participants rTMS has been used in this thesis to induce and investigate neuroplastic changes with a range of microscopic to macroscopic outcome measures.

Summary of findings

This thesis begins with molecular studies *in vitro*, and moves towards TMS stimulation of human participants. For the molecular, *in vitro* studies, the human SH-SY5Y cell line was introduced. SH-SY5Y cells are derived from a human neuroblastoma, and they can be differentiated *in vitro* to a mature, neuronal-like state (1-3). They can be used as a model of human neuronal plasticity, as they express many plasticity-related genes as well as morphological and functional characteristics of mature neurons (3-5).

SH-SY5Y cells are grown in a cell culture medium that is commonly supplemented with 3-10% fetal bovine serum (FBS, serum). To differentiate SH-SY5Y cells to a mature state, retinoic acid (RA) is often added to the cell culture medium, and the concentration of supplemented serum is reduced, for example from 10% to 3% (1, 6, 7). Once fully differentiated, the remaining supplemented serum is commonly removed from the culture medium before experimentation. This is to synchronize the cells to the same phase of the growth cycle (8) as well as to remove all growth factors and proteins which may have confounding effects on the experimental intervention (9, 10). However, the acute effects of such a complete serum removal from

differentiated SH-SY5Y cells had not previously been examined, in particular the effects on plasticity gene expression and structural outcome measures.

In Chapter 2**,** the effects of serum removal on gene expression and morphological markers of plasticity were assessed. We found that serum removal from differentiated SH-SY5Y cells does cause acute changes in plasticity markers, in particular in the gene expression and morphological outcome measures, which we were interested in investigating after iTBS and cTBS. Therefore, in all subsequent chapters we did not deprive differentiated SH-SY5Y cells of serum before stimulation. This chapter also provides important information for future studies using differentiated SH-SY5Y cells as a model for plasticity effects on gene expression and morphology. We show that the removal of serum causes acute changes in the expression of genes related to plasticity and in neuron morphology, which may mask any plasticity effects of the particular intervention. It is therefore important to consider the impact of serum removal before experimentation, and perhaps refrain from or thoroughly verify that such acute removal would not affect specific target genes/markers of interest before applying the intervention.

Chapter 3 describes the first in-vitro study using the theta burst stimulation (TBS) protocols on human neuron-like cells in culture. We were first interested in whether stimulation with intermittent TBS (iTBS), a three-minute protocol assumed to have excitatory effects, and continuous TBS (cTBS), a 40-second protocol assumed to have inhibitory effects (11) have opposite effects on immediate neuronal excitability. To do this, we used differentiated SH-SY5Y cells to investigate functional changes in neuronal activity, as measured through live calcium imaging. Cells were incubated with a fluorescence calcium indicator (Fluo-4AM, Thermo Fisher) which binds calcium at concentrations in the 100nM-1mM range (12). Resting calcium levels in neurons are between 50-100nM (13), therefore there is almost no fluorescence signal detected at baseline. Cells were then stimulated with iTBS, cTBS, or sham stimulation. Immediately after stimulation, there was a slight increase in fluoresce levels, however, when a depolarization was chemically induced with 1M KCl, a large increase in fluorescence levels was recorded. Importantly, cells that had been stimulated with the excitatory protocol (iTBS) showed a stronger increase in fluorescence compared to sham stimulated cells. Cells stimulated with the inhibitory protocol (cTBS) showed a lower fluorescence response to chemical depolarization compared to sham stimulated cells. These results provide support for the expected opposite effects of iTBS/cTBS on neuronal excitability, namely that iTBS can increase and cTBS can decrease neural responsiveness to subsequent depolarization. Further research in more complex human neuron models is needed, however these results provide preliminary support for the generally assumed effects of these two commonly used protocols, as well as provides a potential outcome measure for assessing the responsiveness of neurons to different rTMS protocols.

To investigate the longer-lasting effects of iTBS and cTBS, we chose to measure changes in gene expression and neuron morphology. This is done in Chapter 4, using the same human neuronal model (differentiated SH-SY5Y cells) as in Chapter 3. Gene expression changes specifically related to plasticity were measured, as well as morphological changes in the organization of proteins βIII-Tubulin and MAP2, which have also been related to plasticity (14-17). We found that stimulation did not lead to dramatic morphological or gene expression changes in any of the plasticity markers measured. There was however a slight increase in two genes we measured, *NTRK2* and *MAPK9,* 24 hours after stimulation. iTBS has been shown to increase excitability, as shown in studies in human motor cortex (11), as well as in SHSY5Y cells in Chapter 3 of this thesis. Therefore, an increase in the expression of these genes may indicate plasticity processes induced by iTBS.

Thus, in Chapters 2-4 the human neuronal cell model (differentiated SH-SY5Y cells) was introduced and established, and the effects of rTMS stimulation on immediate neuronal activity, gene expression, and morphology in this model were described. Evidence for increased neuronal excitability was shown, as well as some support for iTBS-induced plasticity effects on gene expression.

There are benefits to using a human *in vitro* neuronal model to measure the plasticity-inducing mechanisms of rTMS, which are further discussed below. However, it is important to complement human *in vitro* studies with *in vivo* ones. There are many large differences between the human brain and human neurons grown in a dish, and to fully understand and optimize rTMS for use in research and clinically, its effects need to be examined across all levels. Chapter 5 offers an example of how cellular and animal studies can be used to inform and design rTMS protocols for use in the clinic. This chapter reviews the concept of metaplasticity, and the importance of fundamental research to inform the necessary timing between subsequent rTMS sessions to maximize stimulation effects. This review is also important in the interpretation of the results of Chapter 6**,** where iTBS sessions are repeated five times in a single day in order to maximize excitatory effects of stimulation.

In the first chapter involving healthy human participants (Chapter 6), we were interested in investigating the effects of 'accelerated iTBS', a protocol consisting of repeated iTBS sessions in a single day, which has shown efficacy in the treatment of depression (18, 19). Despite its success in the clinic, the efficacy of accelerated iTBS over motor cortex using motor evoked potentials (MEPS) as an outcome measure, had yet to be shown. In this chapter, the effects of accelerated iTBS on corticospinal excitability (using MEPs) for up to 90 minutes following the stimulation were assessed. Effects on MEP amplitude were compared to that after a single iTBS session, and to sham. In a fully within-subject design, we found that there was no effect of accelerated iTBS on motor cortex excitability compared to sham. As discussed in

Chapter 5, evidence from animal studies showed that longer breaks (60 minutes) may be required to maximize excitability effects in repeated stimulation protocols. This could explain why, in Chapter 6, we were unable to measure stronger excitability effects, as the breaks between stimulation sessions were maximally 15 minutes and thus may have been not long enough to promote measurable neuroplastic effects.

However, in Chapter 6, the effects of iTBS on neuroplasticity were assessed only through motor evoked potentials (MEPs), which can be influenced by many sources of variability (biological, experimental, etc. (20)). Therefore, in Chapter 7**,** MEPs were combined with TMS-evoked potentials (TEPs) to assess excitability after iTBS. Additionally, this chapter investigated iTBS-induced neuroplasticity in type II diabetes (T2DM) patients, known to have altered neuroplasticity mechanisms. T2DM patients (and high-BMI matched control participants) were classified using blood samples as having insulin resistance (IR) or as being matched controls. The aim of this study was to investigate whether the degree of insulin resistance correlated with TMS-based measures of excitability (MEPs, TEPs). We found no difference in TMS-based neuroplastic responses between the IR and matched control groups, and no correlation between IR and TMS-based measures of excitability. However, we did not find evidence for iTBS-inducing neuroplastic mechanisms in our control group, indicating that future studies using a more effective plasticity inducing protocol, such as accelerated iTBS, are needed to draw any conclusions from this clinical population.

In Chapters 6 and 7, iTBS protocols were applied to either a healthy or clinical population, and TMS-based measures of assessing neuroplasticity (MEP/TEP's) were used. In both these studies, we found no effect of iTBS stimulation on promoting neuroplasticity when stimulating the motor cortex. This adds to recent reports from other groups showing difficulties in replicating the assumed excitatory effects of iTBS (21-23).

In the clinic, iTBS has been shown to be an effective option for the treatment of depression (24), where stimulation is delivered to frontal cortical areas such as the DLPFC (24-26), as opposed to the motor cortex. Therefore, it would be beneficial to assess neuroplastic effects of iTBS directly in the DLPFC rather than the motor cortex.

The study described in Chapter 8 uses a multimodal approach combining TMS, EEG and fMRI, pioneered several years ago at Maastricht University (27, 28), to examine the neuroplastic effects of iTBS in the DLPFC. This chapter presents preliminary results of a within-subject design on eight healthy participants. Offline iTBS stimulation (or sham) was delivered to the left DLPFC, followed by concurrent single TMS-pulses in the 3T MRI, while simultaneously recording the EEG signal. The project is ongoing, but preliminary results suggest that TMS pulses to the DLPFC are able to activate deeper cortico-limbic structures such as the anterior cingulate cortex (ACC) and insula, and

that alpha power can modulate the signal elicited by high intensity TMS pulses at subcortical structures.

Throughout the six experimental chapters in this thesis, the neuroplasticity mechanisms induced by TMS have been investigated using several different techniques and experimental setups. The effects of commonly used rTMS protocols iTBS and cTBS have been assessed in a human neuronal cell culture model (neural activity, gene expression, morphology), a healthy human population (MEPs), a clinical human population (TEPs, MEPs), and finally in a multimodal approach combining TMS-EEG and fMRI. Our human neuronal cell model was used to establish strong functional effects of iTBS and cTBS, as recorded using live calcium imaging (Chapter 3), while effects on plasticity-related gene expression and neuron morphology showed fewer clear differences between iTBS and cTBS over several later time points (Chapter 4). In two of the chapters describing human studies (Chapters 6 and 7), we were unable to replicate the established effects of iTBS on MEP amplitude. This difficulty replicating the expected iTBS effects has been reported by several other studies (21, 22, 29). Limitations of the findings *in vitro* and of the indirect human neuroplasticity outcome measures *in vivo* are described in the section below.

A human neuron-like model for assessing TMS effects

SH-SY5Y human neuroblastoma cells were chosen as a human neuronal model for the studies described in Chapters 2-4 of this thesis, as they are relatively easy to culture, can be fully differentiated to a mature neuronal-like state (1, 2, 7), and express mature neural markers and functional synapses which have been well documented in the literature (3, 30-33). This makes them a good model for exploratory, pilot studies such as those described in this thesis.

Why move to human *in vitro* neural models?

Animal models have been critical in advancing our understanding of the underlying mechanisms of TMS (for reviews; see (34-36)). Animal models have provided evidence for the hypothesized opposing neuroplastic effects of iTBS and cTBS (37-39), and showed that TMS is capable of inducing an immediate release of intracellular calcium following stimulation (40, 41). However, there are several key aspects which limit the use of animal models when modelling the complexity of the human brain (42). Several animal studies have been carried out in cat (43) and non-human primates (44-46), though most studies have used rodents or cell cultures derived from rodents in TMS studies (37-41, 47-52). Despite the obvious difference in size and organizational complexity between the human and rat brain, human neurons also show different gene regulation and expression patterns (42, 53), and different baseline neuronal excitability (54-56). Thus, as TMS is thought to work through altering neuronal excitability (57), it is important to use human neurons to verify findings from animal models. Before the experiments described in this thesis, only two studies had used a

differentiated SH-SY5Y cells to investigate the effects of rTMS (33, 58), and none had investigated the effects of iTBS/cTBS in a human neuron or neuron-like model.

Chapters 3 and 4 are the first to describe the effects of iTBS/cTBS on calcium activity, gene expression, and morphological markers of plasticity in a human neuron-like model. In contrast to animal studies (38, 39, 59), in Chapter 4 we did not show strong, opposite effects of iTBS/cTBS on plasticity markers. However, this may be due to the different stimulation parameters used, and the lack of cortical organization and inhibitory interneurons in our human neuronal setup. We stimulated at 100% maximum stimulator output, in order to ensure that TMS was able to induce activity in our cells. This is verified by our findings from Chapter 3, which convincingly show that iTBS and cTBS are able to immediately induce changes in response to chemical depolarization using calcium imaging. However, animal studies often repeat iTBS/cTBS protocols several (up to five) times (38, 39, 59), while we stimulated our cells with a single iTBS/cTBS protocol. Therefore, the many more stimulation pulses could explain our lack of strong findings in SH-SY5Y cells on the gene expression and morphological levels.

Additionally, our findings in Chapter 4 describing the gene expression and morphology effects after stimulation can also be explained by several limitations of our SH-SY5Y human neuron-like cell model. First, they are derived from human neuroblastoma cells; and while differentiation protocols aim to establish mature, neuron-like phenotypes in the majority of cells (1, 7), there is still dish-to-dish variation between cultures (6). For instance, there are different ratios of mature, neuron-like cells and undifferentiated, epithelial-like cells, which likely respond differently to TMS.

Second, SH-SY5Y cells develop a catecholaminergic-like phenotype, with the potential to synthesize dopamine and noradrenaline (30). They do not represent the mix of excitatory (glutamatergic) and inhibitory (GABAergic) neurons thought to be most involved in rTMS response (34), or the complex spatial organization of cortical columns within the human brain. Animal studies often investigate TMS effects over the whole cortex, or in slice cultures, which contain various neural cell types. Animal studies have found TMS effects to be specific to the cortical region (59), and largely working through inhibitory interneurons (37, 39, 48, 60-62) both of which are not represented in our SH-SY5Y setup.

In humans, many biological and experimental factors contribute to the high degree of variability associated with indirect assessment of TMS-induced neuroplasticity using MEPs (20, 21, 36). By performing experiments in a human neuron-like model, we show the value of systematically assessing the neuroplastic effects induced by iTBS/cTBS from the most basic level up. In Chapter 3 we show that iTBS can increase the excitability of neurons as expected. Future studies can build on this finding, in subsequently more complex human neural models (from iPSC-derived neurons, to

cerebral organoids, to human cortex), to better understand which layers contribute to variability of TMS responsiveness. Through *in vitro* human studies, these factors can be measured and controlled, and can contribute to a better understanding of how TMS is able to induce neuroplasticity in human neurons.

Future Directions of human *in vitro* modelling

Future studies using the SH-SY5Y cells could be informative by applying different TBS protocols (such as accelerated protocols, or repeating sessions up to five times as in some rodent studies (38, 39, 59)), different gene expression or morphological assessment techniques, or following up on protein/phosphorylation changes in these cells. However, based on the limitations of SH-SY5Y cells as a model for human neurons following TMS, it would also be beneficial to consider more complex, advanced human neuronal setups. The studies described in this thesis were important in piloting the setup in SH-SY5Y cells, and serve as a basis for future studies in more complex human *in vitro* neural models to better understand TMS neuroplasticity effects.

For example, it is now possible to take fibroblasts from a skin biopsy and transform them through treatment with a series of reprogramming factors to become induced pluripotent stem cells (iPSCs) capable of differentiating to any cell fate, including neurons (63). Since this ground-breaking discovery in 2006, protocols for direct differentiation from fibroblast to neural progenitor cells have become available (64-66). After differentiation to functional neural progenitors, neurons can then be further differentiated into specific neural phenotypes (glutamatergic, GABAergic, dopaminergic, serotonergic, motor neurons, etc.) (67). iPSCs can also organize into a 3D structure, or cerebral organoid, which can be used to model the complexity of neurodevelopment of the human brain (68, 69).

The development of these advanced, *in vitro* human neural model systems has allowed for progress in disease modelling, but also in personalized medicine. For example, TMS is widely used as a clinical treatment for a range of psychiatric and neuropsychological disorders (70). It is most widely used as a treatment for depression (25, 26, 71), however many patients are nonresponsive to treatment, with about a third of patients completing stimulation treatment in remission (72). iPSC-derived neurons offer the possibility to pre-screen patient-derived neurons for responsiveness to particular stimulation protocols, *before* undergoing TMS treatment. In this way, the parameters of stimulation protocols could first be optimized *in vitro*, which would likely improve the remission rate in the clinic. Building on the results presented in Chapter 3, one relatively quick way to assess whether neurons respond to specific TMS protocols could be to test their responsiveness to rTMS protocols with calcium imaging. This method could potentially verify whether patient-specific neurons respond to a particular stimulation protocol within a relatively limited time

window. This method would furthermore benefit from validation in iPSC-derived neural setups, while forming a proof-of-principle study of how *in vitro* human neural models and interdisciplinary research can be extremely valuable for the future of TMS applications.

Indirectly assessing neuroplasticity in humans

In the first half of the thesis, techniques such as calcium imaging, qPCR and immunocytochemistry were used to directly measure the molecular neuroplasticity changes induced by iTBS and cTBS in SH-SY5Y cells. In the second half, such a direct assessment of molecular changes following stimulation was not possible. The studies done in humans relied on indirect measures to assess neuroplasticity, such as corticospinal (MEP) or cortical (TEP) excitability assessment measures, as well as neuroimaging (fMRI). In Chapters 6 and 7, we report difficulty in replicating the established, excitatory effects of iTBS. In Chapter 6 and 7, we found no difference between MEP amplitude induced by sham, iTBS or accelerated iTBS. Essentially, we were unable to validate the assumption that iTBS increases cortical excitability in humans, as measured by corticospinal assessment (MEP amplitude).

This difficulty in replicating an iTBS-induced increase in corticospinal excitability as assessed through MEP amplitude has also been reported by several other groups (21, 23, 29). Our null findings do not necessarily indicate that iTBS does not work as previously hypothesized, but highlight the limitations of using MEP amplitude as an indirect assessment outcome measure following stimulation.

For example, the use of MEPs can be confounded by substantial variability related to both experimental and biological factors (20). Several uncontrollable neurobiological dynamics such as cortical network activity, developmental factors and neurotransmitter availability are thought to influence the variability of responses (36). Substantial intra-subject variability has also been reported, for example with subjects showing highly variable responses to iTBS stimulation on different experimentation days (22). To control for as many factors as we could, in Chapters 6 and 8 we planned all experiments (if possible) at the same time of day, 1 week apart, and told participants to maintain a normal routine and drink the same amount of caffeine as normal. While we aimed to control for as many confounding factors as possible, MEPs remained a variable outcome measure in these two studies.

Additionally, brain-state has been suggested as a factor contributing to variability of individual responses to rTMS protocols (73). For example, in a setup such as that in Chapter 6, where iTBS sessions were repeated multiple times within a single day, the effects of brain state can strongly influence results. Even the effect of the metal visualization of activity can prime the motor cortex and affect MEP amplitude (74-76). In Chapter 6, we aimed to control for this impact of mental visualization differences

between participants during the breaks between iTBS sessions. We did this by playing the same video clips to all participants, to hopefully maintain a relatively controlled group brain state.

An alternative to MEPs as an assessment of corticospinal excitability is to use simultaneous TMS-EEG, and to record TMS-EEG evoked potentials (TEPs). TEPs are recorded in the ongoing EEG signal, where positive (P) and negative (N) fluctuations at predictable latencies milliseconds after the TMS pulse (N15, P30) can indicate neural excitability (77-79). TEPs are not yet commonly used to assess neuroplasticity, however they have been shown to be highly reproducible (80, 81), in contrast to MEPs (22). In Chapter 7, we found no effect of iTBS on any TEP component measured (N15, P30, N15-P30). As this study was carried out in an elderly population of participants with high BMI or clinical T2DM diagnosis, it is difficult to draw conclusions on the use of TEPs to assess neuroplasticity, as iTBS may have been unable to induce the hypothesized neuroplastic effects in this clinical population. Since we also found no effect of iTBS on MEP amplitude, it is feasible that a single iTBS protocol is not sufficient to induce neuroplastic changes in this participant group. However, the accelerated protocols, such as those used in Chapter 6 may be a promising alternative. The mini-review in Chapter 5 also hypothesizes that accelerated protocols with longer breaks between stimulation sessions may be more effective at promoting stronger neuroplasticity effects in the clinic.

In this thesis, both Chapters 6 and 7 describe a lack of iTBS effects on indirect outcome measures; MEPs and TEPs. Additionally, in Chapter 4, gene expression and morphological markers of plasticity show little to no modulation by iTBS at the cellular level. Therefore, three chapters in this thesis do not provide support for the assumed excitatory-effects of iTBS. Importantly, we do show evidence for an immediate modulation of neural calcium activity induced by iTBS at the cellular level (Chapter 3). Cells that were stimulated with iTBS showed a stronger fluorescence response to chemical depolarization thank sham and cTBS stimulated cells. This fundamental finding in a simple, monolayer human neural setup is important to contrast with the null in vivo and *in vitro* findings described in Chapters 4, 6 and 7.

Homeostatic Plasticity

The underlying neuroplastic effects of rTMS are thought to work through synaptic plasticity, as well as by altering molecular mechanisms, which maintain a dynamic threshold for subsequent plasticity (82-85). Therefore, if the first stimulation primes the neuron for a particular direction of plasticity (for example LTP), homeostatic plasticity would act against this to promote plasticity in the opposite direction (LTD, in this example) following the subsequent stimulation. A full review on metaplasticity and its relation to brain stimulation studies can be found in Chapter 5.

Accelerated iTBS (Chapter 6) is also important to interpret in light of the mini review of metaplasticity in Chapter 5. With accelerated iTBS in particular, homeostatic metaplasticity mechanisms may act against intended stimulation effects. Accelerated protocols have also been shown to be effective when given with 50 minutes between stimulation (86), which aligns with animal research describing that 40-60 minutes between stimulation sessions is required to ensure additive LTP effects (87, 88). Accelerated iTBS protocols offer a promising alternative as an optimized treatment protocol, but it is important to consider homeostatic plasticity effects from *in vitro* studies, to best determine optimal spacing between stimulations.

Interdisciplinary Research

The research in this thesis aims to provide some insight into the neuroplasticity mechanisms induced by iTBS and cTBS. More generally, the interdisciplinary aspect (studies in both *in vitro* and *in vivo,* and using a range of techniques) of this research can highlight the value of these combined approaches. For example, as described in the mini review in Chapter 5, fundamental research on *in vitro* models is critical for determining the optimal parameters for clinical or research stimulation protocols in humans. Additionally, starting from a simple, unorganized neuronal model such as SH-SY5Y cells and building up to more complex *in vitro* models containing relevant functional organization can provide valuable information on processes influencing the large variability of rTMS responses in humans. Combining both fundamental, cellular studies where such stimulation protocols can be directly tested for effectiveness, and clinical studies where these protocols can be tested in patients are important. In the future, this presents the possibility for personalized medicine, by deriving patient or person-specific neural cells (iPSC-derived) and testing the effects of various stimulation protocols, before stimulation of the patient/person in the clinic or lab. This could greatly benefit the effectiveness of brain stimulation protocols.

However, there have been some additional challenges in the dissemination of some of the studies described in this thesis, which may be partially related to their interdisciplinary nature. For example, we have received many critiques of our studies, such as not fitting the scope of the journal, and not having a strong, realistic human neuronal model. While there are certainly limitations to the use of SH-SY5Y cells (discussed in detail in the discussion of Chapters 2,3 and 4 as well as above), the novel and interdisciplinary aspect of the studies was often overlooked. Therefore, in addition to the high-risk nature and variety of techniques in these studies, this difficulty with dissemination is an additional barrier that we have encountered in this interdisciplinary research.

Different perspectives, skill sets, and communication methods are critical to start unravelling the complexities of the human brain. For the studies included in this thesis in particular, if we are better able to understand the tools we use to study the brain,

such as TMS, we can move towards optimizing them, personalizing treatment options, and predicting the most successful outcome measures. The exploratory studies presented in this thesis provide a basis from which future research can build on, to hopefully better understand, develop and optimize rTMS protocols for patient/research use.

Concluding remarks

The main aim of this thesis was to investigate the neuroplasticity mechanisms induced by TMS (iTBS/cTBS) in humans. Starting from the neuronal level and working up to studies in human participants, the experiments described in this thesis begin to unravel the neuroplastic mechanisms induced by TMS, and pave the way for future understanding, optimization and maybe even personalization of TMS protocols.