Inside the Plastic Brain

Chapter 7

Summary and Conclusion

The empirical research presented in this thesis was conducted to gain a better understanding about the application of transcranial brain stimulation for the study of neuroplasticity mechanisms in the human brain. Methodological emphasis was set on combining transcranial magnetic stimulation (TMS) and transcranial alternating current stimulation (tACS) with electroencephalography (EEG) and electromyography (EMG). Together, these modalities of non-invasive brain stimulation and electrophysiological imaging were employed to test the potential of TMS to assess and modulate neuronal excitability for the quantification of neuroplasticity mechanisms. In particular, TMS-induced neuroplasticity effects were compared between healthy individuals and patients with type II diabetes. Furthermore, these effects were tested for their consistency across sessions and their individual reliability in healthy people. At last, potentially influential effects of oscillatory neuronal activity parameters on TMS measures of corticospinal excitability were explored.

The thesis is divided into three parts. In part I (**Chapter 2**), TMS was applied to measure and modulate corticospinal excitability for the investigation of aberrant neuroplasticity mechanisms in a clinical cohort of patients with type II diabetes mellitus. In part II (**Chapters 3 & 4**), the reliability of the neuroplasticity measures obtained from the methodological setup that was used in part I was probed in a different cohort of healthy individuals across separate assessments. Furthermore, neuroplasticity measures based on an alternative methodological design in which TMS was applied to measure and modulate direct cortical activity were tested for their reliability and compared to the corticospinal measures of TMS-induced neuroplasticity. In part III (**Chapters 5 & 6**), the relationship between neuronal oscillations and TMS-induced measures of corticospinal excitability was explored for a better understanding of the mechanisms involved in cortical excitability and their influences on TMS effects. In addition, individual frequency- and phase-dependent transcranial alternating current stimulation (tACS) modulation effects on MEPs were tested to examine how corticospinal excitability is regulated by neuronal oscillation patterns.

Overview of the reported findings

In **Chapter 2**, we combined TMS with EMG and EEG to investigate neuroplasticity mechanisms and the behavioral relevance of aberrant measures in patients with diabetes type II. Single pulse TMS (spTMS) was applied over the primary motor cortex (M1) to induce motor evoked potentials (MEPs) at a single muscle of the contralateral hand (first dorsal interosseous) in a cohort of healthy individuals and in a cohort of diabetic patients. MEP amplitude served as measure of the level of corticospinal excitability. A successful modulation of this measure following the application of a particular TMS protocol (iTBS) that has previously been shown to evoke long term potentiation-like mechanisms in targeted cortical areas (Huang et al., 2005) was expected to be an indication of the presence of functionally operating neuroplasticity mechanisms. By comparing the level of successfully modulated corticospinal excitability between healthy individuals and age matched patients with diabetes type II we aimed to explore the extent to which diabetes type II is related to aberrant

neuroplasticity and whether these alterations are accompanied by behavioral implications. Furthermore, we were interested in testing the combined application of TMS with EMG as a potential measuring tool for neurodegenerative processes in a defined patient group.

Our results revealed that unlike in healthy individuals, neuroplasticity mechanisms are reduced in patients with diabetes type II. The modulatory effect of iTBS on MEP amplitude was lower in the patient group, which suggests that neuroplasticity is impaired in diabetes type II. Furthermore, the measures of reduced neuroplasticity at a relatively early time point after iTBS were related to a decrease in the performance on a cognitive test of verbal learning. This relationship demonstrates the behavioral relevance of aberrant neuroplasticity mechanisms in these patients. In addition, it may represent global alterations in the efficacy of N-Methyl-D-Aspartat (NMDA) receptors in diabetes type II, as both iTBS effects and cognitive impairments are NMDA-receptor dependent (Huang, Chen, Rothwell & Wen, 2007; Di Lazzaro et al., 2008; Rowland, et al., 2005; Newcomer, Farber & Olney, 2000). Our findings are of particular interest, as they may lay the foundation for several application options of the applied method in clinical settings. An objective measure of aberrant neuroplasticity mechanisms in a certain population may allow for new developments of detection strategies that serve as biomarkers for neurodegenerative processes and for adapted interventions at much earlier stages of the pathological progression. Moreover, this measure could serve as a monitoring tool for progressive neurodegeneration and for the level of efficacy of therapeutic interventions or medical treatments of morphologies related to a decline in neuroplasticity or cognition. In summary, the combination of TMS application together with EMG recordings for the modulation of synaptic plasticity with iTBS and for the induction and measurement of MEPs with spTMS in selectively targeted muscles appears to be a promising tool for the investigation and characterization of neuroplasticity in healthy individuals and in patient groups. However, our results are based on one-time assessments and the reliability of these measures using the described stimulation protocol has not been thoroughly established. Thus, in part II of this thesis we report two empirical studies in which we set out to explore the reliability of the aforesaid method and we introduce explorative findings about potential alternative TMS-induced measures of neuroplasticity.

In **Chapter 3**, we tested the reliability of the method we used to assess neuroplasticity in the study reported in part I. For that, we repeated the application of combined TMS and EMG to modulate and measure corticospinal excitability in two identical measurement sessions and in an additional control session in which we replaced the real iTBS application with sham-iTBS. We tested young and healthy adults, because we expected to observe the strongest and most reliable effects in this cohort, as the brain is less prone to already be affected by neurodegeneration at a young age. Our expectations were to find robust modulatory effects of iTBS on MEP measures of corticospinal excitability across the real iTBS sessions, and no modulatory effects in the sham-iTBS session. We were interested in both group level robustness and within participant reliability. Our aim was to demonstrate strong effects of the applied method that prove to be reliable in a healthy cohort. This would allow us to warrant this method's application in other settings, for example in a clinical

population as described in part I, to characterize population specific levels of neuroplasticity and to detect abnormalities of those processes at an early stage of development.

In line with our hypothesis, we found that iTBS successfully modulated corticospinal excitability during a first assessment session. However, we also observed that this positive modulation was reduced during the second assessment session. Furthermore, the modulatory effects of iTBS within a single assessment session were highly variability between individuals and the directions of the effects were not reliable within participants across the two assessment sessions. Taken together, our results support the argument for a facilitatory effect of iTBS on corticospinal excitability. However, it needs to be stressed that this effect was observed on the group level and for an averaged assessment period of sixty minutes following stimulation during a first assessment session and to a weaker extent during a second assessment session. On the individual level, the variability of this effect was high within a single assessment session and the reliability of this effect between sessions was low. These findings are serious reasons for concern about the applicability of this method for the investigation and characterization of neuroplasticity in both research and particularly in clinical settings with the emphasis on individual assessments. This seems to at least hold for the application of this method with the exact parameters that were described in the presented studies. Nevertheless, the reliable measures of neuroplasticity demonstrated in previous studies using cTBS (Vernet et al., 2014) or slightly different protocols for TMS measures of corticospinal excitability following iTBS (Hinder et al., 2014) and also the facilitatory group effects of our studies outlined in chapters 2 and 3 should not be disregarded completely. These findings demonstrate that applying non-invasive brain stimulation for the modulation of the excitability of specific neuronal ensemble has great potential to provide a valuable method for the assessment of neuroplasticity mechanisms. One possible explanation for high variability of iTBS effects between and within individuals could be a generally high level of variability stemming from MEPs, which served as the dependent outcome measure for the interpretation of the effects. High trial-to-trial variability of MEPs has been reported in the literature (Goetz et al. 2014; Wassermann, 2002; Burke, Hicks, Stephen, Woodforth & Crawford, 1995). Thus, one solution to overcome the issue of low reliability of TMS induced neuroplasticity measures could be to replace MEPs with alternative outcome measures for interpretation. Within this framework, we set out to explore the effects of iTBS on TMS-evoked potentials (TEPs) and on neuronal power recorded with EEG directly at the cortical level and to examine a possible relationship between these measures and simultaneously recorded MEPs.

In **Chapter 4**, our objective was to explore potential alternative measures to MEPs for the investigation of TMS-induced neuroplasticity. EEG allows for the assessment of TMS effects on neuronal activity directly at the cortical level, which may provide more specific measures of neuroplasticity. In addition, direct cortical measures have the advantage over MEPs that they can also provide information about neuronal activity from cortical regions outside of M1. In the presented study, we investigated the reliability of TEPs and neuronal power across separate sessions to test whether these measures have the dependability to serve

as potential candidates for the assessment of TMS measures of neuroplasticity. First, we tested the stability of these measures by comparing the amplitudes of TEP components and neuronal power of defined frequency bands across three separate experimental sessions. We found that most of the examined TEP components and neuronal power amplitudes were reliable across sessions. Therefore, these measures seem to qualify as robust ratings for the investigation of neuronal activity. As a next step, we tested the modulatory effect of iTBS on these measures in two identical sessions and in one control session in which real iTBS was replaced with sham-iTBS. We found that the proportional change to baseline after (sham-)iTBS of one particular TEP component (P30-N15), namely the amplitude between the early negative component (N15) around 15ms and the early positive component (P30) around 30ms after spTMS, differed between the real iTBS sessions and the sham-iTBS session. In fact, during the two iTBS sessions, we did not observe significant modulation of P30-N15, but we found a significant reduction in amplitude change in the sham session. The measured post iTBS changes to baseline of the P30-N15 TEP components were not reliable within individuals across both sessions. However, this was not surprising as we did not observe a significant iTBS effect in either of the two sessions and individual measures may thus fluctuate around zero in both sessions, but change slightly in relation to other measures causing nonsignificant intraindividual reliability effects. On the group level, the maintenance of the P30-N15 amplitude following iTBS was consistent across both assessment sessions. The significant change to baseline of the P30-N15 TEP component occurring in the sham session indicates a decrease in amplitude over time. To test for individual reliability of this effect, two identical sham sessions need to be investigated, which was not part of the original design of this study. Our results show that iTBS over M1 prevents the decrease in amplitude of P30-N15, which we observe following sham-iTBS. Therefore, P30-N15 may qualify as a valuable measure for iTBS-based neuroplasticity induction. In contrast to TEP components, measures of neuronal power were not modulated by iTBS.

For the comparison of EEG measures of neuronal activity and EMG measures of corticospinal excitability we tested the relationship of TEP components and neuronal power with MEPs. We found the amplitude of the earliest TEP component around 15ms after spTMS (N15) to be negatively correlated with MEP amplitude. This finding is in agreement with previous studies demonstrating that early TEP components until 30ms are related to measures of corticospinal excitability (Mäki & Ilmoniemi, 2010; Ferreri et al,. 2011) and may thus be of particular interest for the investigation of cortical excitability modulation with iTBS for the study of neuroplasticity. However, we did not find a relationship between the particular P30-N15 TEP component and MEP amplitudes. For the change to baseline values following iTBS we did not observe any relationship between TEP component and MEP amplitudes. For neuronal power, we found a significant interaction of Visit with the relationship between the amplitudes of Low Gamma and MEPs. However, we did not find a significant correlation between both measures. There was no relationship of the amplitude change to baseline post-iTBS between any frequency band and MEPs.

In summary, our findings demonstrate that both TEP components and neuronal power may qualify as reliable measures of neuronal activity. Moreover, TEP components can serve as measures for TMS-induced cortical excitability. Especially the early trough-to-peak amplitude between N15 and P30 appears to be a strong candidate for the investigation of TMS-induced modulations of cortical excitability. This TEP component could become a valuable rating for the study of neuroplasticity at any cortical region accessible to TMS. Future research concerning this matter is required to facilitate the necessary expansion of TMS-based measures of neuroplasticity from corticospinal excitability, which is exclusive to M1, to other cortical regions. At the same time, it remains indispensable to conduct fundamental research on the neurophysiological mechanisms involved in TMS-induced neuronal activity. It is crucial to gain a better understanding of how TMS is processed in targeted neuronal networks and how ongoing neuronal activity affects TMS effects. In part III of this thesis, we investigate the relationship between neurophysiological properties and TMS measures of corticospinal excitability.

In **Chapter 5**, we were interested in possible causes for the observed high variability of trial-to-trial measures of TMS-induced MEPs. Therefore, we investigated whether concurrent neuronal activity at the time and site of TMS application modulates MEPs by testing the relationship between MEP amplitude and neuronal oscillation power and phase. We hypothesized that both power and phase of underlying neuronal oscillations are related to the level of cortical excitability. Hence, it may be speculated that the spontaneous (intrinsic) momentary state of these oscillatory parameters at the moment of the TMS pulse application could influence the TMS-induced MEP amplitude. For that, we recorded both EMG and EEG during TMS application and assessed the effect of preceding power and concurrent phase of neuronal oscillations within the 2Hz – 35Hz frequency range on TMS-induced MEP amplitude.

We found that the phase of neuronal oscillations at intrinsic alpha frequencies is coherently related to TMS-induced MEP amplitude across participants. This finding highlights the importance of apparent fluctuating neuronal excitability for the magnitude of MEP induction with TMS. It appears that TMS is most effective when applied at a particular phase of the ongoing dominant alpha frequency. In our results, this phase was related to the rising slope of the oscillating activity. It may be that neurons are most susceptible for TMS input during this state following rhythmic inhibition of activity after an action potential (Khademi, Royter & Gharabaghi, 2018). Our results could explain why random TMS pulse application without considering the intrinsic neuronal oscillation parameters is accompanied by high variability of MEP amplitude. In respect of these findings, closed-loop application of TMS based on particular neuronal activity parameters from online EEG recordings could prove beneficial for reliable measures of cortical excitability and could provide a better assessment tool of TMS measures of neuroplasticity. In the next chapter, we extended the investigation on the influence of intrinsic neuronal oscillation patterns on TMS-induced measures of corticospinal excitability by testing whether phase locking spTMS to online tACS at individual dominant frequencies has an effect on MEP amplitude.

In **Chapter 6**, we simultaneously applied TMS with tACS at individually calibrated dominant alpha- and beta-band oscillation frequencies to test whether tACS can modulate TMS measures of corticospinal excitability in a phase-dependent manner. We locked spTMS to eight equidistant phases of the online tACS signal over M1 and recorded TMS-induced MEP amplitude.

We found that the modulation of MEP amplitude was phase-dependent for tACS at individual beta-frequency. Moreover, this effect appeared to be specific to low beta-frequency. This indicates that MEP amplitude is dependent on both phase and frequency of the ongoing tACS signal applied at the dominant intrinsic frequency within the low beta band at the time of TMS application. These findings have several important implications. First, our results support the argument for a crucial role of neuronal oscillations in regulating corticospinal excitability. Thereby, we provide further evidence for a potentially causal relationship between neuronal oscillation parameters and fluctuating corticospinal excitability levels reflected in high trial-to-trial variability of TMS-induced MEP amplitude. Second, our results demonstrate the high potential of combining TMS with tACS for the development of controlled and individualized closed-loop applications of TMS, which may lead to less variable MEPs and as a result to reliable TMS measures of neuroplasticity. In addition, combining TMS with other neurostimulation and neuroimaging techniques, such as tACS and EEG, to incorporate individual measures of functional, physiological or anatomical properties of targeted neuronal networks into controlled closed-loop TMS application protocols, may lead to more specified TMS effects (Romei et al., 2016; Romei, Thut & Silvanto, 2016). Ultimately, this could allow for the development of improved or new application protocols for research purposes or clinical applications.