Measurement of cortical, nerve, and muscle excitability in early phase clinical drug development

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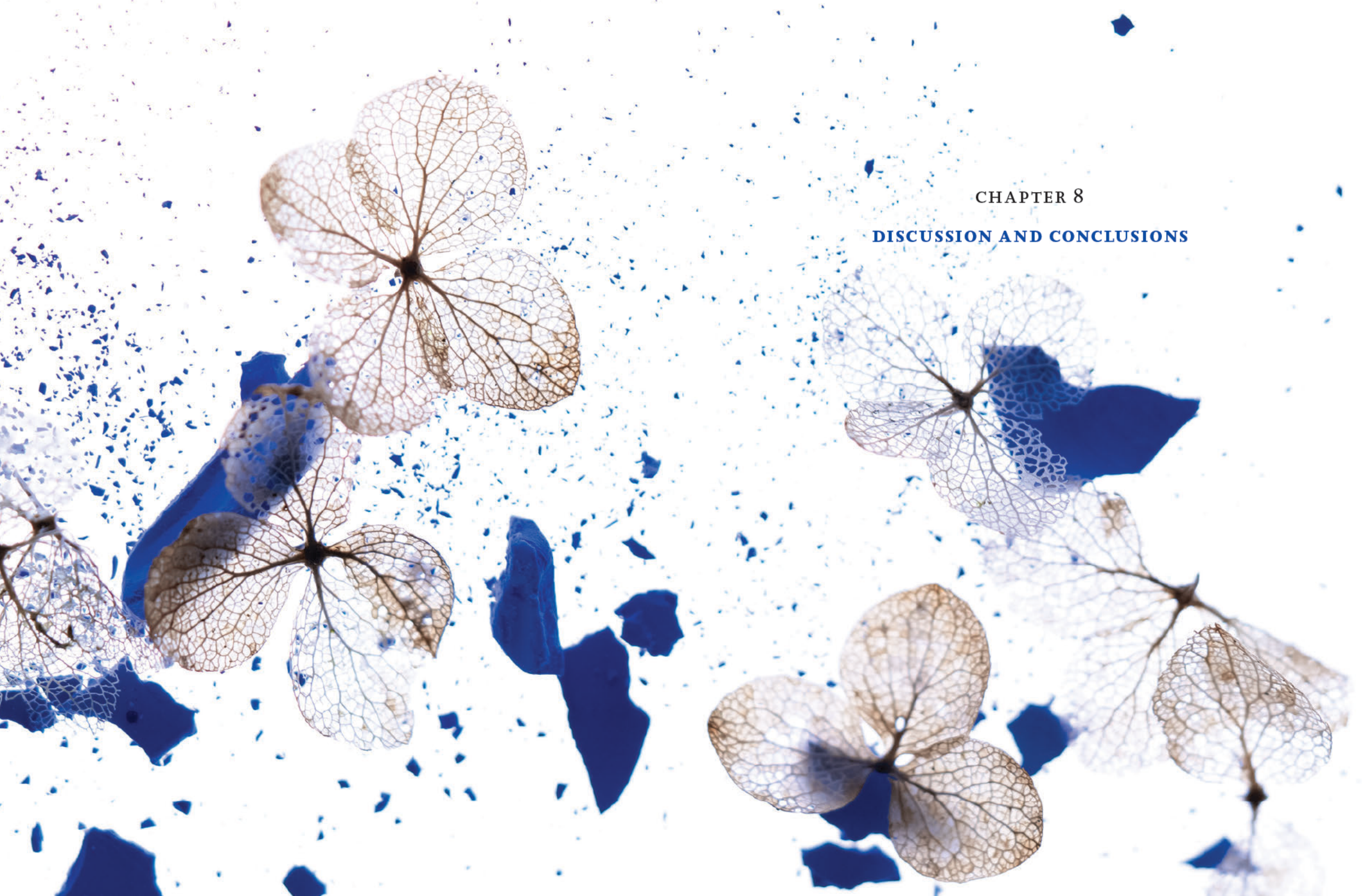

CHAPTER 8

DISCUSSION AND CONCLUSIONS

Abnormalities in cell excitability can be found in multiple neurological and neuromuscular disorders, such as epilepsy;[1] amyotrophic lateral sclerosis (ALS);[2–6] and myotonia congenita.[7] Excitability is driven by voltage- and neurotransmitter-gated ion channels.[8,9] Pharmacological modulation of these ion channels is therefore promising as treatment for excitability-related diseases.[8] In early phase drug development, the use of biomarkers for pharmacodynamic effects in healthy subjects and first-in-patient studies is pivotal.[10] In this thesis I describe the potential of three measures of excitability to detect pharmacodynamic effects of ion channel modulators: transcranial magnetic stimulation (TMS) combined with electromyography (EMG) and electroencephalography (EEG), nerve excitability threshold tracking (NETT), and muscle velocity recovery cycles (MVRC). We used TMS-EMG/EEG to evaluate effects on cortical excitability; NETT to assess peripheral nerve excitability; and MVRC to explore muscle cell excitability. Firstly, we tested these measurements in proof-of-concept studies using registered drugs known to influence excitability. These studies were used to explore if the measurements are sensitive to drug-induced changes in excitability, and the test-retest variability and feasibility to apply them in the context of a clinical study in healthy subjects were evaluated. After validation of the methods, we used the measurements in early phase clinical drug studies with novel drug candidates. In the following discussion the implications of our findings for use of these methods as biomarkers in future drug development programs will be discussed: an evaluation of their general value as pharmacodynamic biomarkers for ion channel modulators; their feasibility for use in early phase drug studies; and finally, ideas for future research.

TRANSCRANIAL MAGNETIC STIMULATION

The development of a non-invasive biomarker for pharmacodynamic effects on cortical excitability is useful for clinical application and for drug development, especially in the context of epilepsy, where cortical hyperexcitability is an important disease factor.[1] The prospect of novel pharmacological treatments aimed to target cortical excitability, led us to implement TMS-EMG/EEG in a clinical study setting to validate it as pharmacodynamic biomarker. Firstly, we focused on the pharmacodynamic effects of registered antiepileptic drugs –levetiracetam and valproic acid- and benzodiazepine lorazepam, on TMS-EMG/EEG in healthy subjects, as

described in **CHAPTER 2**. We evaluated effects on single pulse (sp) and paired pulse (pp) TMS-EMG/EEG in a double-blind, placebo-controlled, four-way crossover study. Levetiracetam, valproic acid, and lorazepam decreased the motor-evoked potential (MEP) amplitude compared to placebo. Moreover, levetiracetam significantly increased TMS-evoked potential (TEP) component N45, and decreased N100.[11] The decrease in MEP amplitude – observed for all three study drugs – corresponds to inhibition of cortico-spinal excitability. We therefore concluded that TMS measures can detect general changes in excitability induced by these antiepileptic drugs, and that TMS biomarkers could be helpful in early phase drug development.

After the proof-of-concept study described above, we implemented TMS in an early phase drug study, to evaluate effects of TAK-653, a α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) -positive allosteric modulator, on TMS-EEG (**CHAPTER 3**). TAK-653 is a drug candidate under development for major depressive disorder. We found that 6 mg TAK-653 affected the TEP amplitude 60-70 ms after the TMS pulse. Although further research is needed to confirm these results, our findings indicate that TMS-EEG is sensitive to AMPAR modulation.

We conclude from these studies that TMS-EMG/EEG is feasible in small studies in the early phase of clinical drug development. The measurement is non-invasive and can be repeated multiple times before and after drug administration. However, the measurement also has limitations. It is challenging to perform reproducible TMS measurements, resulting in a relatively high variability in TMS outcome measures, particularly between individuals. This limitation may make it difficult to detect more subtle drug effects. The measurement is therefore most suitable for use in a cross-over design, rather than parallel (single- or multiple-ascending dose) studies. Furthermore, TMS-EMG endpoints provide an indirect measure of excitability and reflect general changes in cortico-spinal excitation or inhibition.[12] TMS-EMG could therefore be a valuable tool to demonstrate drug effects on general excitability, but this makes TMS-EMG less suitable to distinguish between different pharmacological mechanisms of action, or to highlight certain channel activity. TMS-EEG provides a more direct insight into the cortical response[13] and may therefore be more useful for this purpose, however the physiology of the TEP response is still largely unknown. A third limitation is the lack of consensus within the TMS community when it comes to stimulation

methods (e.g. interstimulus intervals of interest) and analysis methods (e.g. artifact removal methods).[14] This heterogeneity makes it difficult to compare results from different research groups.

In our opinion these limitations do not disqualify the use of TMS-EMG/EEG as pharmacodynamic biomarkers for proof-of-mechanism studies. Although the variability is relatively high, significant treatment effects on cortical excitability were detected at therapeutic dose levels for all tested compounds. Moreover, with further research and further measurement standardization, evaluation of TEPs may even prove to be useful for differentiating specific pharmacological mechanisms of action.

NERVE EXCITABILITY THRESHOLD TRACKING

We had two driving factors for the introduction of NETT as pharmacodynamic biomarker. Firstly, there is a considerable interest from the pharmaceutical industry in the development of non-addictive and safe analgesics, among which (selective subtype-specific) voltage-gated sodium channel (Na_V) blockers.[15] Secondly, potassium channel activators, like retigabine, are of interest to lower increased nerve excitability in amyotrophic lateral sclerosis,[16] and effects of retigabine have been demonstrated using NETT.[17] Therefore, we decided to study whether pharmacological inhibition of Na_V conductance can be detected in healthy volunteers using NETT (**CHAPTER 4**). This was assessed in a randomized, double-blind, three-way crossover study, comparing effects of two registered Na_V channel blockers – mexiletine, and lacosamide – to placebo. Motor and sensory nerve excitability measurements were evaluated at multiple pre- and post-dose time points. We found that mexiletine and lacosamide significantly decrease motor and sensory nerve excitability, corresponding to their mechanism of action.[18]

Our results show that NETT can detect (subtle) drug-induced changes in motor and sensory nerve excitability, with significant dose-effect relationships, even in a small group of healthy subjects. Furthermore, the inter- and intra-subject variability of the NETT endpoints is low- an important characteristic of a valuable pharmacodynamic biomarker. We conclude that NETT can be a useful tool in the clinical development of novel Na_V channel modulators, and potentially other modulators of (peripheral nerve-specific) ion channels. A general limitation of NETT (as well as TMS, and MVRC) is that it requires specialized equipment

and trained staff. However, the low variability in our study indicates that the measurement can be performed repeatably and in standardized manner. Furthermore, a possible limitation of NETT in the context of pain research, is that it measures excitability at the stimulation site [19] (in this case the median nerve- a large, myelinated nerve), not the target site (unmyelinated nociceptive nerves).

Further research, with other modulating drugs, could help to create a channel-specific nerve excitability profile in healthy human subjects. If a distinct fingerprint of affected NETT variables could be linked to a specific channel, this could facilitate confirmation of target engagement in early phase drug studies with novel pharmaceuticals. For example, if nerve excitability profiles of Na_V subtype 1.6, 1.7, and 1.8 blockers are compared, findings may help determine target engagement and/or off-target effects. Moreover, further information may be gained by combining these findings with computational nerve modelling.[20]

MUSCLE VELOCITY RECOVERY CYCLES

The need for a pharmacodynamic biomarker for drugs targeting muscle excitability arose when we were planning to perform a Phase I study with an inhibitor of muscle-specific chloride channel ClC-1. The drug candidate (NMD670) was a first-in-class compound, designed to increase muscle excitability and subsequently improve muscle function in neuromuscular diseases such as myasthenia gravis (MG). MVRC, a measurement that estimates muscle cell excitability, had been shown to be sensitive to abnormalities in ClC-1 function in patients with myotonia congenita,[7] which is caused by a congenital loss-of-function mutation of ClC-1.[21] Therefore, we decided to perform a proof-of-concept study to explore the potential of MVRC as pharmacodynamic biomarker for drugs targeting muscle excitability (**CHAPTER 5**). We compared effects of a registered drug known to influence muscle excitability by inhibition of Na_V channels (mexiletine)[22–24] to placebo in a randomized, double-blind, two-way crossover study in healthy subjects. MVRC recordings were evaluated at baseline and at multiple post-dose time points. We found that MVRC could detect a decrease in muscle membrane excitability by mexiletine.[25] Our results indicate that MVRC is sensitive to pharmacodynamic effects, and we concluded that MVRC could potentially be a useful tool for our planned first-in-human study with ClC-1 inhibitor NMD670.

In **CHAPTER 6** we described the first administration of NMD670 to healthy human subjects. Safety, pharmacokinetics, and pharmacodynamics of NMD670 were assessed in male and female subjects in a randomized, double-blind, placebo-controlled, single- and multiple-ascending dose study. Pharmacodynamic effects were evaluated using MVRC. We found that NMD670 increased muscle excitability, as expected from the pharmacological mechanism of action. This was reflected in increased MVRC parameters of early supernormality, and clinical symptoms of myotonia at the highest dose levels. The effects on MVRC parameters were similar to findings in myotonia congenita,[7] and therefore indicate proof-of-mechanism of ClC-1 inhibitor NMD670. These findings further emphasize that MVRC can detect pharmacological changes in muscle excitability in healthy subjects.

After evaluation of the safety of NMD670 in healthy subjects, we tested pharmacodynamic effects of NMD670 in patients with MG as proof-of-mechanism, with multiple pharmacodynamic measurements, including MVRC.

In **CHAPTER 7** we describe the findings of preclinical studies with NMD670, and the clinical pharmacodynamic effects of NMD670 in patients with MG. In the patient study, pharmacodynamic effects were evaluated by clinical evaluation of myasthenic symptoms using the Quantitative myasthenia gravis (QMG) score, and also neurophysiological tests, among which MVRC. In patients with MG, we found significant improvements on the clinical QMG score after NMD670, indicating that ClC-1 inhibition may indeed have positive effects on muscle function in these patients. The effects of NMD670 on MVRC in MG patients are not described in this thesis and will be published in a separate manuscript on this study focusing on MVRC only. To summarize our findings, MVRC endpoints were affected in the same direction as in the healthy volunteer study, an effect that reached statistical significance for one parameter after NMD670 1200 mg compared to placebo in patients with MG (unpublished data). These findings were dose dependent, in line with the findings in healthy participants, and corresponding to an increase in muscle cell excitability (unpublished data). Therefore, these data confirm the suggested ClC-1 target engagement of NMD670 in patients with myasthenia gravis. Moreover, our findings show that MVRC can detect pharmacodynamic effects of ClC-1 inhibition in both health and disease, further encouraging the use of this biomarker for assessment of pharmacological effects on muscle excitability.

In conclusion, our findings support the use of MVRC as pharmacodynamic biomarker in early phase drug development. The measurement is able to detect effects of different types of drugs on muscle cell excitability, in both healthy subjects and patients with MG. Our findings support pharmacological target engagement of both mexiletine (Na_V blocker) and NMD670 (ClC-1 inhibitor). Furthermore, MVRC variables have a relatively low inter- and intra-subject variability, the measurement can be performed quickly, and it can be repeatedly measured. Although the measurement is more invasive than TMS and NETT, because it uses recording and stimulation needles inserted in the muscle, this measurement is well-tolerated. What might limit MVRC's feasibility is its relative complexity to perform, and to interpret – although we have shown this is not a limitation at our centre.

IDEAS FOR FUTURE STUDIES

The use of TMS-EEG, NETT, and MVRC as pharmacodynamic biomarkers is still in its infancy. In our opinion, the next step in the validation of these biomarkers would be to investigate their sensitivity to a range of different ion channel modulators. Firstly, because this would extend the use of these biomarkers to a larger variety of pharmacological targets and disease indications. Secondly, it would be important to compare the excitability profiles of these different ion channel modulators, to see if the distinct excitability variables can be used to differentiate pharmacological effects on a channel level. In other words, the proposed studies should inform if certain variables provide a high specificity for modulation of corresponding channels, as opposed to a more general indication of increased and decreased excitability. Ideally, this would lead to a channel-specific fingerprint of variables, which can be used for proof of target engagement.

It would be interesting to investigate whether findings on TMS-EMG/EEG, NETT, and MVRC in healthy subjects are translatable to clinical treatment effects in patients. One way towards answering this question, would be to evaluate whether differences in excitability can be detected in the target patient population when compared to normal controls, and whether these variables change in the direction of normal after treatment administration. For example, differences in TMS-EMG/EEG variables can be detected between (drug-naïve) epilepsy patients and

healthy subjects.[1] It would be useful to investigate whether treatment with (novel) anti-epileptic drugs could change this enhanced excitability in patients with epilepsy. We are currently performing such a study in our unit. Additionally, although outside the scope of drug development, it would be extremely helpful if a biomarker, such as TMS-EMG/EEG, could reliably predict seizure control after subscription of anti-epileptic drugs in the clinic. This would require long-term follow-up studies to investigate whether significant (acute) effects on TMS-EMG/EEG correlate with seizure control.

In the case of myasthenia gravis, on the other hand, there is theoretically no clear abnormality in muscle membrane potential, because the pathophysiology of myasthenia gravis is based on the loss of acetylcholine receptors in postsynaptic membrane of the neuromuscular junction, leading to disturbed neuromuscular transmission.[26] Therefore, we may not be able to use pathophysiological changes of myasthenia gravis on MVRC outcomes, to evaluate treatment effects of drugs targeting muscle excitability. It should thus be noted that the use of MVRC in our study with NMD670 was based on the mechanism of action of the drug, not on the pathophysiology of myasthenia gravis. With MVRC, we were able to detect significant effects of NMD670 on muscle excitability in healthy subjects, and in patients with myasthenia gravis. The finding of increased muscle excitability provides us an indication of target engagement and proof-of-mechanism in healthy subjects, but it does not indicate that there was a clinical improvement of muscle function in patients with myasthenia gravis. The combination of MVRC and clinical evaluations (such as QMG score) in patients is therefore recommended. In our study described in **CHAPTER 6** and **8**, the MVRC results for NMD670 strengthen our positive findings on the clinical QMG score, because it indicates that increased muscle excitability may indeed be responsible for an improvement of muscle function in patients with myasthenia gravis.

The same may be considered when investigating novel analgesics using NETT. There are conflicting findings in literature on the presence of abnormalities in NETT variables in chronic pain,[27,28] possibly because NETT does not examine the excitability of nociceptive nerve fibers, but of a large, myelinated nerve.[28] In our study with mexiletine and lacosamide (**CHAPTER 4**), we found significant excitability lowering effects of Na_v channel blockers on NETT in healthy subjects, which shows target engagement at the median nerve. In the same study, we have also performed

evoked pain tests. Mexiletine increased cold pressor pain tolerance and lowered cold pain perception; lacosamide showed no analgesic effects on these tests (unpublished data). Considering the abundant effects of lacosamide on NETT, it can be questioned whether a significant effect on peripheral nerve excitability as measured with NETT, corresponds with analgesic effects. However, it should be noted that investigation of NETT may still be useful to investigate pharmacological target engagement on channels that populate both myelinated axons and unmyelinated nociceptive nerve fibres, because the confirmation of target engagement can be pivotal in the early phase of drug development (in healthy subjects).

Lastly, it may be useful to investigate the potential of TMS-EMG/EEG, NETT, and MVRC as biomarkers in the translational phase from animal models to clinical drug studies. **CHAPTER 3** describes effects of TAK-653 on TEPs, and effects of TAK-653 on MEP amplitude were assessed in the same study and published elsewhere.[29] The study showed that comparable plasma concentrations of TAK-653 increased both MEP amplitude in humans, and motor responses to TMS in rats (registered with mechanomyography). These results indicate that MEP amplitude can be considered a useful translational biomarker for AMPA receptor modulation in future drug development programs.[29] NETT might also be helpful as a tool to navigate between preclinical studies and early phase clinical studies. NETT protocols have been used to investigate pharmacological effects on nerve excitability properties in vitro, for example using Na_v channel blockers (lidocaine, mexiletine, and tetrodotoxin),[30,31] propofol,[32] and amitriptyline.[33] Effects of K_v7 potassium channel activator flupirtine were even evaluated in vitro, as well as *in vivo* (a randomized, placebo-controlled trial in healthy subjects), in the same study.[34] In addition, measurement of peripheral nerve excitability using NETT in animals is extensively performed. Among this research, several studies investigated effects of Na_v,[35] potassium- and HCN[36] channel modulators on NETT in animals. Moreover, effects of a novel drug molecule, namely a compound that selectively inhibits $Na_v1.8$, were successfully tested in a mouse model of Charcot-Marie-Tooth disease.[37] The possibility to perform NETT in animal models, and even in vitro, encourages further investigation of its potential as translational biomarker. MVRC measurements have also been performed in animals. Two studies in pigs used MVRC to evaluate muscle membrane properties in faecal peritonitis.[38,39] Also, a recent publication comparing MVRC in mice and humans, showed differences in muscle

excitability between the species.[40] So, MVRC could also be considered as a translational biomarker for drug effects, although these differences between species should then of course be taken into account. Future studies should indicate whether pig models are better translatable to humans than mice.

CONCLUSION

This thesis describes a set of excitability measurements – TMS-EMG/EEG, NETT, and MVRC- and the applicability of these tools in early phase clinical drug development. We validated the biomarkers in healthy subjects with registered drugs and showed that the measurements are all repeatable and sensitive to pharmacological effects, even in a small number of subjects. Furthermore, we have evaluated effects of a novel AMPA-PAM with TMS-EMG/EEG, and a first-in-class ClC-1 inhibitor with MVRC, and the findings helped us to confirm proof-of-mechanism of these compounds in healthy subjects. In conclusion, these measurements proved to be valuable pharmacodynamic biomarkers in two drug development programs, encouraging their further use in clinical development of other future drug candidates targeting cortical-, neuronal-, and muscle cell excitability. The use of such clinical pharmacodynamic biomarkers could improve the quality and efficiency of the development process of drugs for e.g. amyotrophic lateral sclerosis, chronic pain, depression, treatment-resistant epilepsy, and neuromuscular diseases.

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