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

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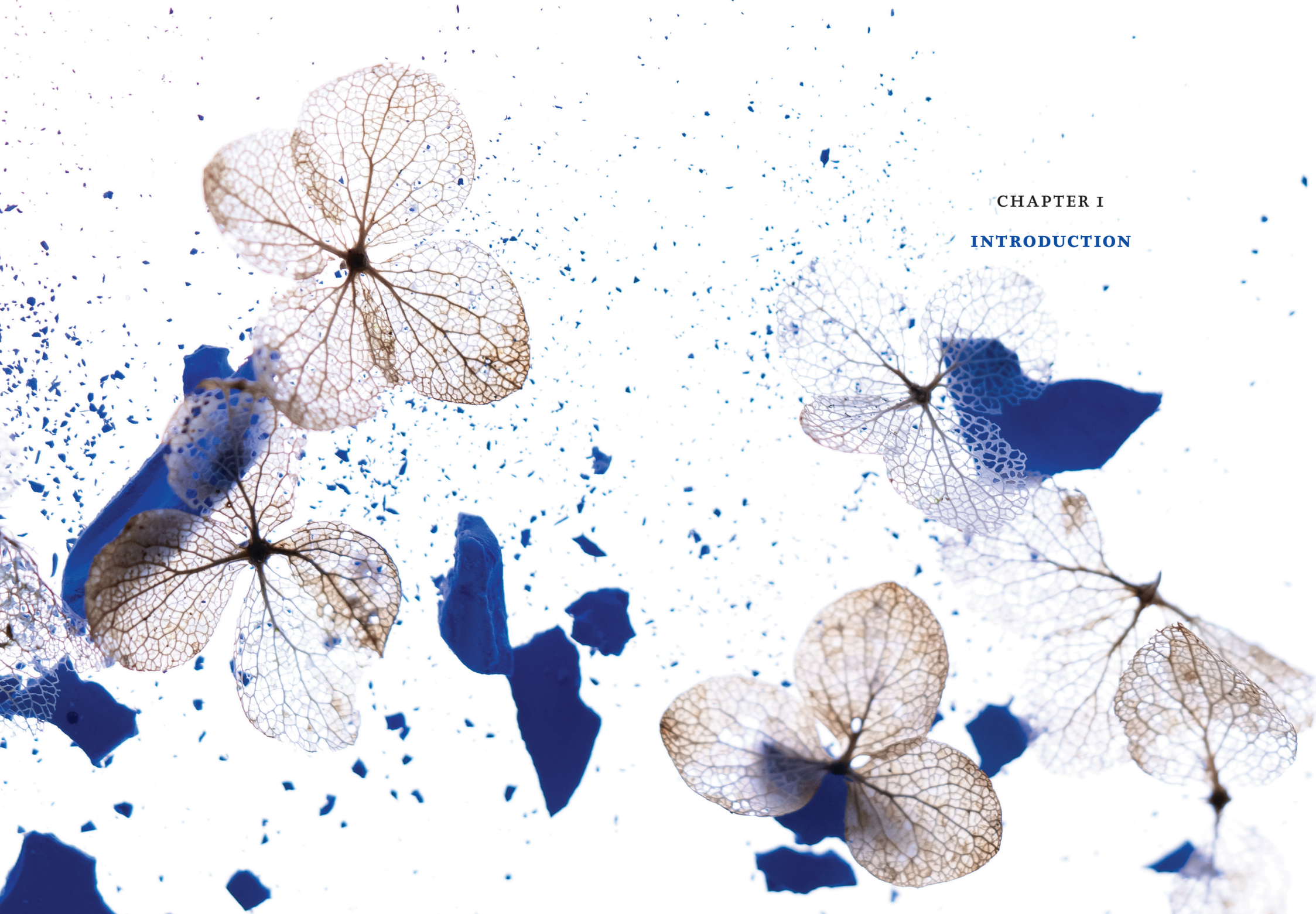


CHAPTER 1

INTRODUCTION

EXCITABILITY OF THE CORTEX, PERIPHERAL NERVES, AND SKELETAL MUSCLES

The human cortex, peripheral nerves and skeletal muscles are excitable tissues. Excitability is defined as the characteristic of certain cells to react to stimuli through fluctuations in membrane potential,[39] thereby allowing our cell membranes to carry electrical signals throughout the body.[1] These electrical signals facilitate the transmission of impulses and are therefore critical to the function of neuronal and muscular tissues. Membrane potential changes are caused by fluctuations in permeability to sodium-, potassium-, calcium- and chloride-ions.[1] Ion channels are responsible for those changes in membrane permeability and the function of electrically excitable cells therefore largely relies on those channels. Voltage-gated ion channels open and close due to changes in membrane potential, neurotransmitter-gated ion channels function in response to neurotransmitters.[2] A wide range of neurological, (neuro-)muscular, and psychiatric diseases is related to abnormalities in excitability. For example, abnormalities in cortical excitability are found in epilepsy;[3] abnormalities in cortical[4–8] and nerve excitability[9–13] in amyotrophic lateral sclerosis (ALS); and abnormalities in skeletal muscle excitability in myotonia congenita.[14] Potential treatments for disorders related to excitability of neurons and muscle cells lie in the modulation of these voltage-gated and neurotransmitter-gated ion channels, which makes these proteins highly interesting as pharmacological targets.[2]

For development of novel drugs targeting excitability, it is critical to have biomarkers for pharmacodynamic effects in the early phase of drug development. Conventional clinical drug development relies on four different phases, starting with testing of safety and tolerability in healthy human subjects. For novel compounds with pioneering biological or therapeutic mechanisms, this linear approach may be unsuitable. Although assessment of safety is crucial, a pharmacological approach to early phase drug development can greatly improve the developmental process.[15] A drug can be confirmed safe and tolerable in a small group of healthy subjects, but the administered dose range may not be pharmacologically active, leading to negative therapeutic findings in patient studies. Alternatively, when a drug is dosed above the therapeutic window, the early studies may show serious safety concerns, leading to discontinuation of further development, although the pharmacological mechanism may have been of great therapeutic value. Solely testing safety and tolerability in early phase studies, without evaluation of pharmacological action of the drug, may therefore lead to risks for study participants and increased developmental costs.[16] Valid biomarkers of pharmacodynamic effects could help determine the likely pharmacologically active dose range. Firstly, such a measure could help translation from preclinical studies to the first administration in humans. Pharmacokinetic-pharmacodynamic modelling then could assist in the prediction of (minimally) pharmacologically active dose between species. Secondly, a pharmacodynamic biomarker could show target engagement in healthy subjects, and thereby provide proof-of-pharmacology, in studies with novel biological mechanisms. Early detection of pharmacological effects could reduce uncertainty and could thereby improve the safety of study participants and add financial value. If such a biomarker responds in a dose-dependent manner, it could also guide dose-escalation studies in healthy subjects alongside safety measures. Lastly, a biomarker for pharmacodynamic effects may assist adequate dose-finding in the translation to patient studies.

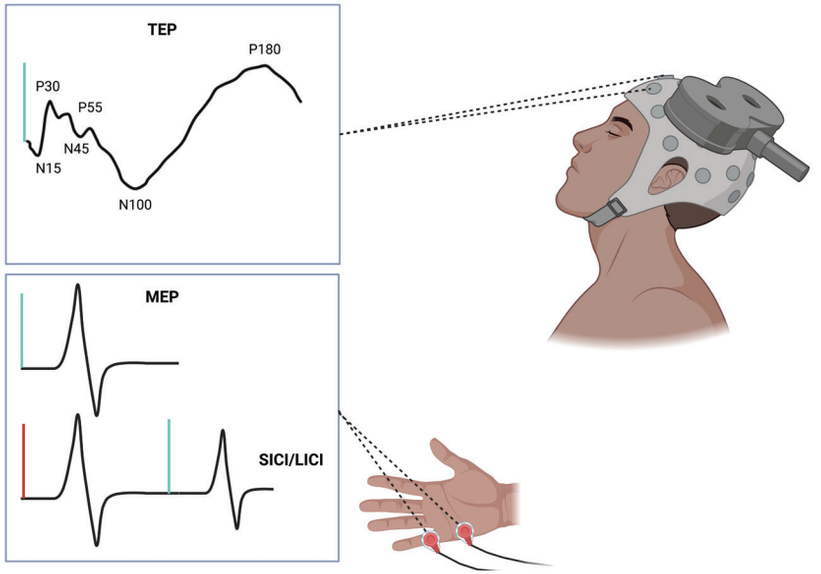
Clinical drug research focused on the field of neuronal and skeletal muscle excitability currently lacks reliable (translational) biomarkers for pharmacological target engagement and would therefore benefit greatly from development of these tools. This thesis describes the validation and implementation of three existing clinical measurements of excitability for use in clinical drug development: transcranial magnetic stimulation (TMS) combined with electromyography (EMG) and electroencephalography (EEG) for the evaluation of cortical excitability; nerve excitability threshold tracking (NETT) to determine peripheral nerve excitability; and muscle velocity recovery cycles (MVRC) to explore skeletal muscle membrane excitability.

CORTICAL EXCITABILITY

TMS is a non-invasive brain stimulation technique (*Figure 1*). Strong electrical currents in the TMS coil generate a magnetic pulse, which can generate a cortical action potential by activation of voltage-gated sodium channels.[17] When directed at the motor cortex, this action potential can lead to muscle activation in a target muscle. To quantify this response, TMS-EMG can be used to measure a motor-evoked potential (MEP), as a measure of cortico-spinal excitability.[18] The MEP is quantified

by measuring the peak-to-peak amplitude of the muscle action potential. Moreover, long- and short intracortical inhibition (LICI and SICI) can be evaluated by measuring the MEP amplitude after paired TMS pulses at different interstimulus intervals (ISI). Alternatively, TMS-EEG can be used to assess the direct brain response as a TMS-evoked potential (TEP).[19] These responses are quantified using amplitudes of positive (P) and negative (N) deflections in the TEP, at set timepoints after stimulation. N15, P30, N45, P55, N100 and P180 therefore reflect positive and negative amplitudes 15, 30, 45, 55 100 and 180 ms after the test pulse.

FIGURE 1 Transcranial magnetic stimulation (TMS) combined with electro-encephalography to explore the cortical response using a TMS-evoked potential (TEP) (upper graph). TMS combined with electromyography to evaluate the motor-evoked potential (MEP) and short- and long intracortical inhibition (SICI/LICI) at the abductor digiti minimi (lower graph). Created with Biorender.com.



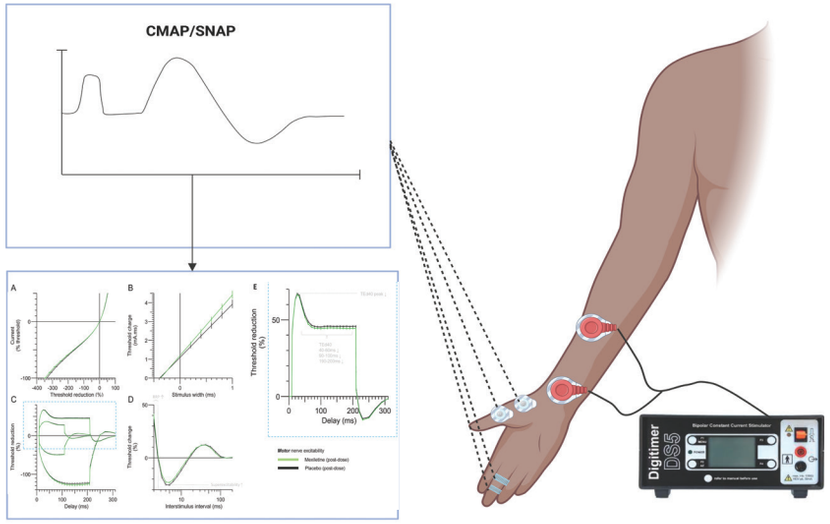
A multitude of studies has been performed evaluating pharmacological effects on cortico-spinal excitability using TMS-EMG.[17] Drug targets investigated using TMS-EMG include (but are not limited to) sodium channel blockers, potassium channel modulators, and γ-aminobutyric acid-A ($GABA_A$) and $GABA_B$ agonists.[20] These studies show that TMS-EMG is sensitive to pharmacological effects of drug targeting cortical excitability. Studies to investigate pharmacodynamic effects on TMS-EEG are more limited, and the knowledge of the neurophysiology behind the TEP is yet largely unclear. Pharmacological challenges using registered drugs, such as benzodiazepines and levetiracetam/brivaracetam, help to identify the meaning of the different TEP components.[21] To our knowledge, studies using TMS-EMG/EEG as biomarker in early phase drug development with novel compounds are scarce. However, effects of a novel potassium channel opener [22] and a α5-$GABA_A$ receptor antagonist [23] were investigated in the early development phase using TMS-EMG/EEG, and these studies support the use of this biomarker for this purpose.

The first study that we performed using TMS, contributes to the growing body of evidence by repeating results from previous TMS-EMG/EEG studies on pharmacodynamic effects of levetiracetam and lorazepam, and adds to existing literature as the first study to evaluate effects of valproic acid on TMS-EEG. Moreover, we evaluated the variability of the measure and feasibility for use in studies to investigate novel drug molecules. The second study applies the technique in early-phase drug development, by exploring effects of a novel α-amino3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptor positive allosteric modulator on TMS-EEG. The latter study also included TMS-EMG, of which the results are published separately.[24] This study demonstrates that TMS-EMG may also be used as translational biomarker of pharmacological effects in animals to humans.

PERIPHERAL NERVE EXCITABILITY

NETT provides information on axonal membrane excitability and ion channel properties at the site of stimulation (*Figure 2*).[25] In our study, the median nerve was stimulated using electrical currents at the wrist. For evaluation of motor nerve excitability, compound muscle action potentials (CMAP) were recorded in the abductor pollicis brevis; for sensory nerve excitability, sensory nerve action potentials (SNAP) were measured at digit two or three. The technique uses a 'threshold tracking' stimulation paradigm, which means that the stimulus intensity is adjusted based on a set threshold.[25] Threshold is defined as 40% of the maximum CMAP. Using four different stimulation protocols, the method explores different properties of the axonal membrane potential.[26]

FIGURE 2 Nerve excitability threshold tracking (NETT) uses electrical stimulation of the median nerve (red electrodes) to measure the amplitude of the compound muscle action potentials (CMAP) at the abductor pollicis brevis; and peak-to-peak amplitude of the sensory nerve action potentials (SNAP) at digit three (upper graph). A stimulation paradigm is used to evaluate different excitability properties (lower graph shows NETT recording). Created with Biorender.com.

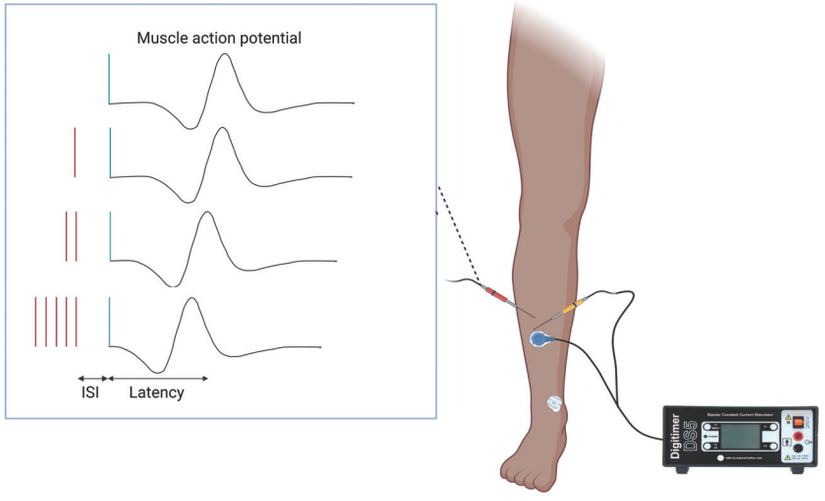


In contrast to the extensive literature describing drug effects on TMS-EMG, only a handful of studies describe pharmacological effects on NETT in humans. Previous studies describe the effects of sodium channel blockers, namely tetrodotoxin due to accidental puffer fish intoxication;[27] effects of a regional nerve block by lidocaine;[28] and effects of mexiletine in patients with chronic pain[29] and Machado-Joseph disease.[30] Moreover, acute pharmacological effects of retigabine and riluzole in patients with ALS have been described.[31,32] In healthy subjects, neuronal excitability during general anaesthesia using propofol and sevoflurane have been studied.[33] Another example where NETT has been used to measure treatment effects, was with nusinersen in patients with spinal muscular atrophy.[34] To our knowledge NETT has not been used as biomarker in early phase drug development. No previous study compared acute, systemic, sodium blocking effects on NETT to placebo in healthy subjects. Therefore, our study evaluates the test-retest reliability, and effects of two registered sodium channel blockers-mexiletine and lacosamide- on both motor- and sensory nerve excitability. Such a study is crucial as proof-of-concept, to explore whether NETT would be valuable as pharmacodynamic biomarker in early phase drug development.

SKELETAL MUSCLE EXCITABILITY

The measurement of MVRC provides a surrogate measure of muscle cell membrane excitability (*Figure 3*).[35] By direct electrical stimulation of the tibial muscle using a needle electrode, the muscle fibres are activated independent of neuromuscular transmission. The method uses the latency from stimulus to muscle action potential as a measure of velocity. A stimulation paradigm is applied with single stimuli, as well as (1, 2 and 5) conditioning pulses, followed by a test pulse at different ISIs. The physiological muscle action potential consists of a refractory period, followed by two periods of depolarization and increased excitability. MVRC can be used to quantify these two periods of supernormality as an increase in velocity due to conditioning pulses.[35]

FIGURE 3 For muscle velocity recovery cycles (MVRC) electrical stimulation of the tibial anterior muscle fibres using a needle electrode (yellow) creates a muscle action potential, which is recorded using a second needle electrode (red). The latency of the muscle action potential is measured after single test pulses (blue), and (1, 2 and 5) conditioning pulses (red) followed by a test pulse (blue) at different interstimulus intervals (ISI). Created with Biorender.com.



MVRC has been used in previous research to discriminate between health and disease, such as myotonic dystrophy [36] and myotonia congenita.[14] No previous studies report the use of MVRC as a pharmacological biomarker. The only article to secondarily describe treatment effects, compares patients with myotonia congenita using sodium channel blocking medication, to patients off treatment, with significant findings.[14] Therefore, this thesis is the first to describe the capabilities of MVRC as pharmacodynamic biomarker and its implementation in an early phase drug study. First, we performed a study to evaluate the variability of MVRC, and to explore whether effects of mexiletine – a sodium channel blocker – can be detected in healthy subjects using MVRC. Mexiletine was chosen as proof-of-concept because it is known to decrease muscle excitability by inhibition of voltage-gated sodium channel subtype 1.4 in muscle fibres.[37,38] After validation of the method, we used MVRC as a pharmacodynamic biomarker in a Phase I trial with a muscle-specific ClC-1 inhibitor – a novel drug developed to enhance muscle excitability in patients with neuromuscular disease. MVRC was implemented in the first-in-human single- and multiple-ascending dose study in healthy subjects to confirm target engagement. After that, effects of ClC-1 inhibition on muscle excitability were evaluated using MVRC in the first-in-patient trial in patients with myasthenia gravis.

AIMS OF THIS THESIS

In conclusion, with the research presented in this thesis we evaluate the potential of TMS-EMG/EEG, NETT, and MVRC, as measures of excitability in early phase clinical drug development. For this purpose, each of the three measurements is first tested in a proof-of-concept study using registered drugs, that are known to influence excitability through ion channel modulation. We assessed whether significant treatment effects could be detected using these techniques. Moreover, we evaluated feasibility and test-retest reliability of the measurements. Secondly, after validation of the methods, and confirmation of their sensitivity to pharmacodynamic effects, we implemented the measurements in early-phase clinical drug studies, which are described in this thesis.

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