# ECTOPIC MOTOR UNIT ACTIVITY IN MOTOR NEURON DISEASE

Clinical application of surface EMG methods

**Boudewijn Sleutjes**

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# ECTOPIC MOTOR UNIT ACTIVITY IN MOTOR NEURON DISEASE

## Clinical application of surface EMG methods

Ectopische motor unit activiteit bij motor neuron aandoeningen
klinische toepassing van oppervlakte EMG methoden

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# Chapter 1

## General introduction

I Motor neuron disease
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IV Aims and outline of thesis

## I Motor neuron disease

Motor neuron disease (MND) is characterized by the progressive loss of motor neurons that control voluntary muscles. Due to its progressive nature, the muscles gradually lose their function leading to paralysis and, ultimately, death. The most common variant of MND is amyotrophic lateral sclerosis (ALS). Of all the people diagnosed with ALS, 50% die within approximately two to three years after their first symptoms arise and only about 20% live longer than 5 years [1]. Onset is typically around 50 - 70 years of age, but in some patients the onset may be much earlier, around the age of 20 - 30 years. Incidence is higher among men than women, estimated at 2:1 [2-4]. The first symptoms usually occur in the limbs, but muscle weakness may also begin in the bulbar region. Progressive weakness of the respiratory muscles leading to respiratory failure is the most common cause of death. Before the first clinical signs of muscle weakness become apparent, more than 50% of the motor neurons innervating a muscle may already be lost [5]. In the Netherlands, approximately 1,700 people (prevalence 10.3 per 100,000) suffer from ALS [2]. Every year, approximately 500 people (incidence 2.8 per 100,000) in the Netherlands are diagnosed with ALS [2], and about the same number of persons dies every year. In comparison, approximately 570 people in the Netherlands died in traffic accidents in 2013 [6]. The term ALS was first described in 1874 by Jean-Martin Charcot [7]. Despite the tremendous technological progress that has been made in the last 140 years and despite numerous studies that have been conducted to unravel the mechanisms that may cause this deadly disease, relatively little is known about the mechanisms that cause ALS and the progressive degeneration of motor neurons is often unpredictable. The great majority of patients is classified as having sporadic ALS, and only 5 - 10% of the patients have a familial history of this disease. A complex interaction between genetic and environmental factors is believed to contribute to the development of the disease. Several genes have been identified and their discovery gave new insights into the underlying pathophysiological mechanisms. At present, no cure is available, and the only approved and widely used medication (Riluzole) can only marginally slow down the progression of the disease by approximately 3 months [8].

In this section, first the concept of a motor unit as a crucial component being affected by MND will be introduced, together with some basics on how motor units are affected in this condition. Next, one of the most obvious clinical signs, fasciculations, will be discussed, followed by the varying clinical phenotypes. Subsequently, the difficulties in the diagnostic process and the prognosis will be described. Currently, both can be very difficult, especially in the early stages of the disease, even with a thorough clinical and electrodiagnostic examination.

## The motor unit

In MND, the motor neurons that control voluntary muscle movement are particularly affected. The most direct non-invasive measure at the level of single motor neurons is the functioning of motor units (MUs) in a nerve-muscle combination with electrophysiological techniques. Therefore, the structure and functioning of MUs have a central role in the electrodiagnostic examination. An MU is the smallest functional element in the human body that controls skeletal muscles. An MU consists of three basic components, an α- motor neuron (lower motor neuron or LMN) in the brainstem or

spinal cord, an axon and all the muscle fibers it innervates (Fig. 1). The motor axons from the spinal cord innervating the foot muscles can reach lengths of up to 1 meter [9]. Motor axons are bundled in fascicles, and a motor nerve is composed of several of these fascicles. The axon of an MU is enclosed by a myelin sheath, which covers > 99% of the entire axon [10]. Due to the myelin sheath, the axon can transfer action potentials much faster than unmyelinated fibers (a nerve conduction velocity of 50 - 55 m/sec in the median nerve [11]). At the distal end of the MU, the axon branches into several sprouts innervating individual muscle fibers through the neuromuscular junction. Depending on the specific function of the muscle, the number of MUs varies widely. In a healthy individual, MU sizes and the MU size distribution varies depending on the muscle function: from a few muscle fibers per motor unit in muscles exhibiting fine motor control (e.g. extraocular, less than 10 muscle fibers per motor unit) to up to a few thousand muscle fibers in leg muscles (e.g. gastrocnemius, 1,000 - 2,000 muscle fibers per motor unit) [12].

The number of MUs within a muscle can change due to various factors such as age, injury or disease. The major pathophysiological change in MND is the progressive loss of MUs, so methods that estimate the number of MUs can be used as potential tools to monitor disease progression. The loss of MUs leads to the denervation of muscle fibers. These denervated muscle fibers may connect to other motor axons of still functioning MUs. This compensatory process is known as reinnervation, resulting in clustering of muscle fiber types in a muscle (fiber type grouping). As a result, remaining MUs increase in size (more muscle fibers per motor unit), and muscle force can remain more or less unaffected for some time, at least in the early phase.

To control muscles during voluntary movements, electrical impulses that arise in the upper motor neurons (UMN) in the motor cortex are transferred via central motor pathways to the LMN. From there, the electrical impulses continue to travel via the axons and the neuromuscular junction, leading to controlled activation of MUs, resulting in movement of skeletal muscles. The MUs in a muscle are recruited in an orderly manner during voluntary contraction (Henneman's size principle). However, MUs can sometimes show uncontrolled, spontaneous activity, which is known as fasciculations.

## Fasciculations

Fasciculations are regarded as the most obvious clinical characteristic in ALS. Fasciculations can be visible as small, spontaneous, random movements of a muscle when they occur just beneath the surface of the skin. The electrophysiological equivalent of a fasciculation is a fasciculation potential (FP) that occurs due to spontaneous, isolated, sporadic discharges of an individual MU [14]. The identification of this type of ectopic MU activity is an essential part of the electrodiagnostic examination as FPs have been included in the latest diagnostic criteria for ALS [15]. They are often seen at an early stage of the disease in muscles without weakness [16, 17]. FPs may originate at the distal end of the motor axon, along the entire axon, in the cell body or even more central regions [17-21]. However, FPs are not disease specific and can be observed in various peripheral nervous system disorders, and even in healthy subjects [22, 23]. Therefore, FPs as the only abnormality in a muscle is insufficient for the diagnosis of ALS and they should be considered in the context of other clinical and/or electromyographic (EMG) findings [24, 25]. However, the complete absence of FPs raises

clinical doubt in diagnosing patients with ALS [15, 26]. Regarding their prognostic significance, several studies have shown mixed results in disease progression and survival [27-29]. From this data, it can be said that the importance of fasciculations in ALS is recognized, however there is a lack of fundamental understanding of their underlying pathological and non-pathological origins [30].

## Clinical phenotypes

Clinical features of ALS include the combined involvement of UMN loss in the motor cortex and/or degradation of the corticospinal tract, and LMN loss in the brain stem and spinal cord. Clinical symptoms of LMN dysfunction include muscle atrophy, muscle weakness and fasciculations. Typical clinical symptoms of UMN dysfunction are exaggerated deep tendon reflexes (often in weakened muscles), pathological reflexes (Babinski signs) or positive brainstem reflexes, spasticity, and increased uncontrolled crying or laughing, as a sign of loss of inhibition. At clinical presentation, patients may show large variation in the extent of UMN and LMN involvement. Main clinical presentations are a) limb onset, showing combined UMN and LMN signs in the hands and/or legs, or b) bulbar onset, presenting as difficulty with speech, eating and swallowing. A minority of patients with ALS may also show mild behavioral or cognitive impairment overlapping with frontotemporal dementia [31, 32].

MND consists of other somewhat rarer conditions, in addition to the most common variant ALS, including progressive muscular atrophy (PMA), a clinically pure LMN syndrome [33], primary lateral sclerosis (PLS), characterized by pure UMN involvement [34] and progressive bulbar palsy, which solely include LMN dysfunction in the bulbar region [35]. PLS and PMA are related to ALS, often regarded as positioning at either end of the UMN-LMN spectrum [34, 36, 37]. However, PLS is a much rarer variant. In contrary, it is not uncommon that patients initially diagnosed with PMA develop UMN signs in a later stage and need to be re-classified as having ALS [38]. PMA is thought to have similar pathophysiological effects on the peripheral nervous system as ALS [36, 37].

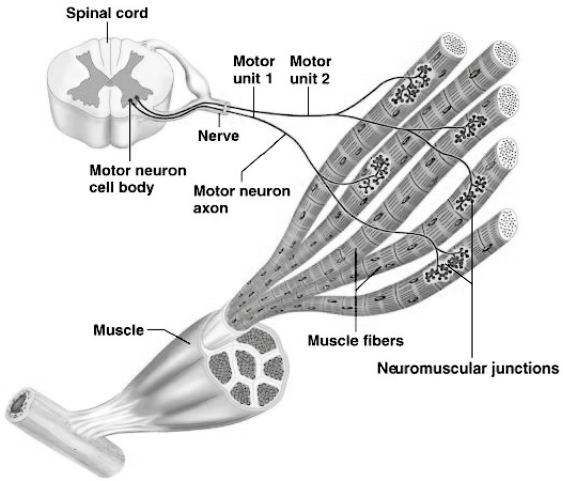


**Figure 1.** *Schematic view of two MUs: The cell body in the spinal cord, the axon in the nerve and the innervation of the muscle fibers, via the neuromuscular junction. (Adapted from [13])*

## Diagnostic and prognostic challenges

Presently, ALS cannot be diagnosed by a specific screening test or biomarker, which makes the diagnostic process a challenging task. Diagnosis of ALS may be difficult as there exist various neurological disorders that may resemble the clinical signs of ALS. For instance, in an early stage when a patient presents with LMN onset with only focal symptoms, which is comparable to segmental spinal muscular atrophy (SMA), often a less severe disorder restricted to a single limb or body region. Some of the most common ALS-mimics include, benign fasciculation syndrome (BFS, abundant fasciculations, no muscle weakness), multifocal motor neuropathy (MMN, fasciculations, muscle weakness, motor nerve conduction block), inclusion body myositis (IBM, weakness predominantly of the flexor muscles of the arms, hands and proximal legs, and swallowing difficulties), cervical myeloradiculopathy (secondary to degenerative spine disease). It has been shown that approximately 10% of patients initially diagnosed as having ALS later turned out to have another disorder [39]. The heterogeneous clinical presentation of ALS with symptoms that may also have an overlap with other neuromuscular disorders contribute to a diagnostic delay of approximately 12 months [40]. The progressive nature of the clinical symptoms and signs in ALS is of importance and 'time' is often seen as the most valuable diagnostic tool by neurologists as the diagnosis becomes more evident with passage of time [41].

### *Diagnostic classification*

An important part in the diagnostic process for ALS is investigating the peripheral nervous system using the electrodiagnostic examination, which can be seen as an extension of the clinical examination. Depending on the clinical situation, several other additional investigations may be performed as well, such as blood tests, muscle biopsy, and neuroimaging (MRI). Electrodiagnostic examination can confirm the underlying pathological process in clinically affected muscles but more importantly, it may also detect changes in clinically still unaffected areas. Furthermore, it can help to rule out other disorders that have a large clinical overlap with ALS [42, 43].

For diagnosis, clinicians mostly rely on identifying the widespread presence of LMN and UMN abnormalities with progressing of clinical symptoms and exclusion of other disorders that may explain the clinical signs. There has been an ongoing debate on the optimal diagnostic criteria for ALS [43], and this has led to several changes in the past decades (El Escorial criteria (EEC), revised EEC, and Awaji [15, 42, 44]). In these criteria, the body is divided into four regions: cranial, cervical, thoracic and lumbosacral based on the origin of nerve roots in the spinal cord or brainstem of the investigated muscles. Diagnostic certainty is based on the number of regions involved. In the latest diagnostic criteria (Awaji criteria, 2008 [15]) equal weight has been given to detecting LMN abnormalities in a muscle using either clinical signs or EMG findings emphasizing the need for clinical neurophysiological examination in the diagnostic process of ALS (Fig. 2).

Detection of UMN signs is limited to clinical signs only. Furthermore, FPs have been added to the diagnostic criteria as an EMG finding of LMN involvement (for detailed EMG criteria, see next section). However, their diagnostic status has been criticised as their presence is not specific, but widespread fasciculations are characteristic of ALS.

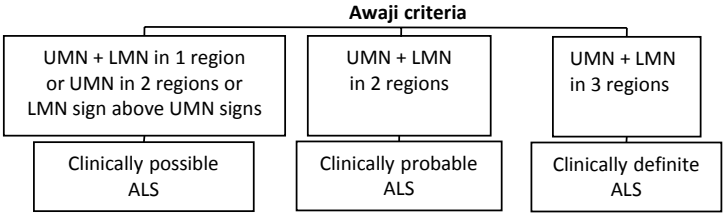


**Figure 2** *(A) Schematic view of the Awaji criteria. Detecting UMN abnormalities is limited to clinical signs only. LMN abnormalities can be found with either clinical signs (muscle weakness, atrophy, and/or fasciculations) or EMG signs (reinnervation/chronic denervation and active denervation, see next section). (Adapted from [45])*

### *Follow-up*

Even after making the diagnosis of ALS, the disease progression varies largely per patient, making it difficult for the physician to predict how the disease will progress. To optimally inform patients and to improve the clinical process, mitigating these uncertainties is crucial. At present, accurate assessment of disease progression is difficult. Therefore, objective measures to assess disease onset and progression are necessary not only for an early diagnosis and improved prognostication, but also to help with patient selection for therapeutic trials. This will be all the more relevant once effective therapies are developed for this fatal disease.

## Scope of this thesis

In the electrodiagnostic studies for MND, several EMG techniques are applied, measuring the electrical activity arising in a muscle or nerve. The EMG signal of an MU is the summation of all muscle fibers that make up the MU, the MU action potential (MUAP). Evaluating the structure and functioning of MUs has a central role in these electrodiagnostic studies. Although current EMG techniques, including invasive needle EMG and single channel surface EMG, have proven their clinical utility, they also have their shortcomings. However, these can partly be resolved using more advanced surface EMG techniques by the manner in which MUs are activated and by the use of multiple surface EMG channels. These techniques may eventually further assist in improving the diagnostic process and lead to a more objective insight into the disease progression. The clinical and pathophysiological similarities between ALS and PMA necessitate a similar clinical approach. Therefore, the broad scope of this thesis is the non-invasive study of MUs in patients with ALS and PMA using advanced surface EMG techniques, further improving their methodology, and assessing their clinical value. This will require an understanding of routine EMG techniques, including the way they detect LMN abnormalities, as well as insight into the application of more advanced surface EMG techniques. These will be discussed in the next section, followed by the specific aims and outline of this thesis.

## II Basics of EMG techniques in clinical practice

Although Galvani already noted the relation between electrical stimulation of the nerve and the contraction of frog limb muscles in 1791, the history of clinical application of EMG is relatively short. A lot of progress has been made in the mid-twentieth century. The first International Congress of Electromyography was held at the University of Pavia in 1961 where speakers such as Frits Buchthald, Eric Kugelberg and Howard Edward Lambert gave famous lectures [46]. Lambert proposed the first electrodiagnostic criteria for ALS in that period [47].

## Electrodiagnostic studies

Electrodiagnostic studies consist of two routinely used methods: nerve conduction studies (NCS) using non-invasive surface EMG, and invasive needle EMG examination. Abnormalities detected on needle EMG have a crucial role in the electrodiagnostic criteria for diagnosing ALS. NCS also have a role in ruling out disorders that may resemble ALS.

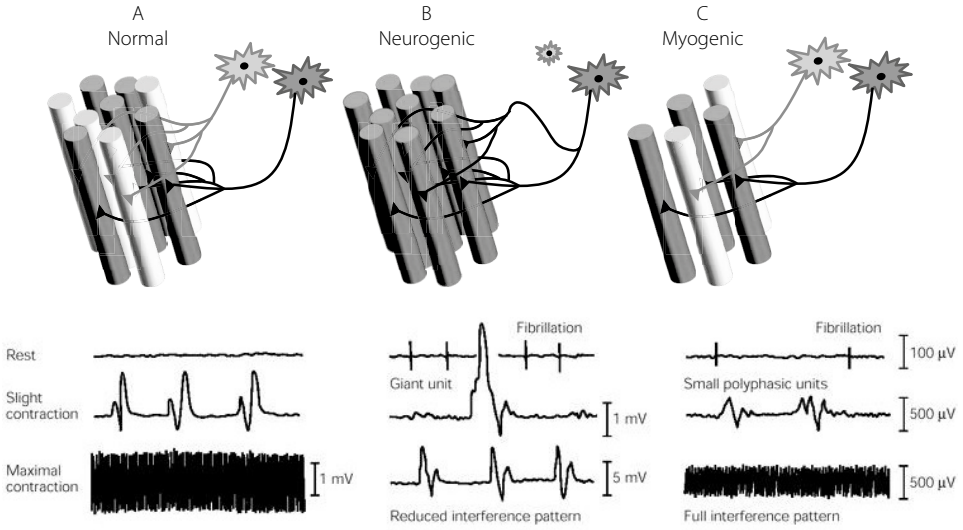


**Figure 3** *Schematic view of two MUs in upper graph: (A) in normal condition, (B) in neurogenic condition (MU loss and increased MU size) and (C) in myogenic condition (muscle fiber loss and reduced MU size). The signals that might be recorded on needle EMG are depicted in the lower graphs: at rest, during slight contraction and maximal contraction. No muscle activity at rest in normal conditions (A), some fibrillations, PSWs, or FPs in neurogenic conditions (B) and some fibrillations or positive sharp waves in myogenic conditions (C). A few MUAPs and a full interference pattern at slight and maximal contraction in normal conditions (A), some giant MUAPs and a reduced interference pattern in neurogenic disorders (B), and smaller MUAPs and a full interference pattern with reduced amplitude in myogenic disorders (C). (Adapted from Kandel, E., et al. (2013), Principles of Neural Science [48] reproduced with permission of McGraw-Hill Education)*

### *Needle EMG*

Needle EMG is useful in the diagnostic process of neuromuscular disorders to find out which part of the MU is affected (Fig. 3). For example, when a patient has muscle weakness, this test can help differentiate whether the nerve fibers or the motor neurons are affected (neurogenic weakness) or whether the muscle fibers are affected (myogenic weakness). However, needle EMG is an invasive method, requiring the insertion of a needle electrode into the muscle under investigation, making it also less suitable for repeated examination.

### *EMG findings of LMN involvement in a muscle*

In the diagnostic criteria, EMG findings of LMN involvement in a muscle is supported by the presence of active denervation and reinnervation (chronic denervation). Active denervation is defined as the presence of either positive sharp waves (PSW), fibrillations or FPs (added to the Awaji criteria). Fibrillations and PSWs are the spontaneous activity of single muscle fibers due to e.g. denervation, where there is no longer a connection between motor axon and muscle fiber. Reinnervation is confirmed by enlarged, frequently polyphasic, unstable, long-duration MUAPs, and a reduced recruitment pattern. In the lumbosacral and cervical regions, two or more muscles innervated by a different spinal root need to be affected to define a body region as being affected. In the thoracic and cranial regions, one or more muscles need to be affected.

The needle EMG signal is most sensitive to the electrical activity of only a few muscle fibers close to the needle tip. Therefore, the needle EMG signal is only an indirect measure of MUAP size. Furthermore, even slight movements have an enormous effect on the needle EMG signal, which makes quantification of MUAP size difficult. The needle tip is surrounded by muscle fibers belonging to only a few MUs. Therefore, the standard procedure is to insert the needle 3 – 5 times at different depths and in several different directions to get an accurate impression of how MUs function in a muscle. As one can expect, this procedure is uncomfortable and painful. Furthermore, needle EMG can be challenging as patients are asked to perform several muscle contractions, which requires a patient's cooperation.

### *Nerve conduction studies*

Nerve conduction studies (NCS) can be divided into motor NCS and sensory NCS. In motor NCS, transcutaneous electrical stimuli are applied to activate the motor nerve and a single channel EMG signal (derived from a single electrode pair above the muscle) records the electrical activity generated in the muscle supplied. In peripheral nervous disorders, several parts of the MU can be affected, such as the myelin sheath surrounding the axon, reflected by the motor nerve conduction velocity (NCV), or the axon itself, reflected by the amplitude of the compound muscle action potential (CMAP). In MND, the myelin sheath generally remains intact and therefore the motor NCV is normal. A significantly decreased NCV could suggest an alternative diagnosis such as demyelinating (inflammatory) neuropathy. In addition, the presence of focal conduction block, detected by a drop

in amplitude between distal and proximal sites of stimulation, would increase the clinical suspicion of multifocal motor neuropathy (MMN). In an advanced stage of MND, axonal loss could result in a reduced maximum CMAP amplitude, however a large number of axons may be lost before this becomes visible as a reduced maximum CMAP amplitude. In sensory NCS, sensory NCV and amplitude are evaluated and these are typically normal in patients with MND.

NCS are non-invasive, so they cause little discomfort for patients. However, it is not possible to measure individual muscle fibers, and the identification of single MUs is less accurate than in needle EMG. Conventional NCS are therefore not well-suited for assessing single MUs.

Although needle EMG and NCS have proven clinical utility, they have shortcomings such as the ones described above. Surface EMG methods that vary in the electrical stimuli applied to activate MUs and that use multiple surface EMG channels can partly resolve these issues and have the potential to complement and enhance the two clinically applied methods at the level of single MUs. This will be discussed in more detail in the next section.

## III Advances in surface EMG methods for clinical application

Due to the recent technical advances, significant interest has grown in the use of advanced surface EMG techniques for the diagnosis and follow-up of neuromuscular disorders. These techniques may reveal electrophysiological characteristics at the level of MUs that cannot be obtained with routine EMG techniques and therefore have the potential to be of clinical value. Furthermore, they may be able to partly replace or limit the use of painfully invasive and extensive needle EMG examination. In devastating diseases such as ALS and PMA, any improvement of diagnostic and prognostic yield as well as patient-friendliness of test methods is more than welcome. Research in the department of Neurology and Clinical Neurophysiology at the Erasmus MC University Medical Center in Rotterdam is at the forefront of these developments. Our focus has been on the development, integration, and application of these novel techniques, both in a research setting and in clinical diagnostic practice. These sophisticated surface EMG methods include the CMAP scan, motor nerve excitability testing, and high-density surface EMG (HDsEMG) recordings. A picture of the complete measurement setup is shown in Figure 4.

Recent progress in the application of these techniques in clinical practice will be described. Due to this progress, it is now possible to identify areas for improvement in the development of these techniques as a clinical tool. This thesis attempts to close the most significant of these gaps.

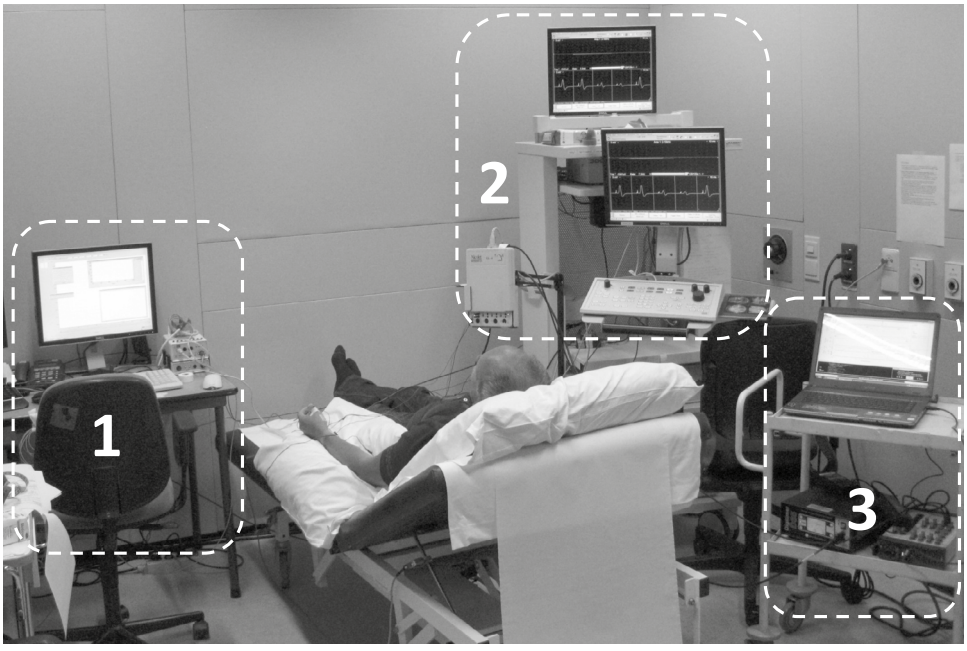


***Figure 4.*** *Illustration of the complete measurement setup with the subject positioned on a clinical examination bed. It shows the equipment for (1), the HDsEMG recording (PC with sEMG software, BioSemi acquisition system), for (2), the CMAP scan recording (Viking Select EMG system), and for (3), the motor nerve excitability testing (laptop with Qtrac software, data acquisition system, DS5 Stimulator)*

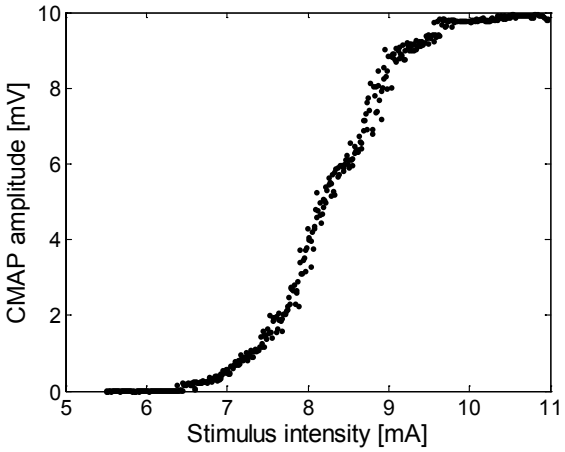


**Figure 5.** *The CMAP scan (stimulus-response curve) of a healthy subject showing a gradual increase in CMAP amplitude.*

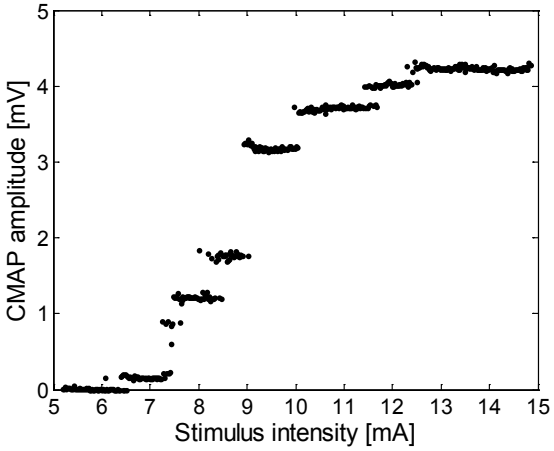


**Figure 6.** *The CMAP scan (stimulus-response curve) of an ALS patient showing gaps or discontinuities due to the presence of enlarged MUs.*

## The CMAP scan

The compound muscle action potential (CMAP) scan is a single channel surface EMG method and can be regarded as an extension of standard motor NCS [49]. The CMAP scan uses the underlying physiological principle that every MU has a specific threshold for activation. When the CMAP amplitude is recorded in response to gradually increasing stimulus intensity (SI), all MUs are successively activated. By plotting the CMAP amplitudes versus the SI, the characteristic pattern of the CMAP scan becomes visible (Fig. 5). In addition, the width of the CMAP scan (lowest to highest MU threshold) gives an indication of excitability properties of all activated axons [50].

The CMAP scan provides clinical information regarding MU and axonal excitability variables that cannot be obtained during routine NCS [49-53]. The CMAP scan has the potential to be a valuable tool to monitor disease progression in patients with ALS and PMA [54]. MU loss and enlarged MUs due to reinnervation is typical in patients with MND and can result in the addition of relatively large MUAPs to the CMAP [51]. This contribution is often visible as gaps in the CMAP scan (Fig. 6). Hence, the presence of multiple gaps is indicative of MU loss and reinnervation. Currently, a manual procedure is required to identify these gaps or so-called steps. In Chapter 2 of this thesis, an automated approach is described to extract the visual characteristics from the CMAP scan, which has the potential to overcome operator-related variability and to reduce time required for the analysis.

## Motor nerve excitability

Motor nerve excitability testing is also a single channel surface EMG technique, however the protocol significantly differs from routine motor NCS by the types of transcutaneous electrical stimuli applied. Motor nerve excitability studies enable the estimation of resting membrane potential and the dynamic changes of the membrane potential after de- and hyperpolarization. The dynamic changes in membrane potential are caused by the activation of ion channels, located at the nodes of Ranvier and inter-nodal segments. Motor nerve excitability testing has already been applied in various neuromuscular disorders. It reveals axonal excitability properties, which cannot be obtained with routine NCS [10]. In ALS, several excitability studies have shown abnormalities in axonal excitability, which have been associated with the occurrence of fasciculations [55-57]. A plausible explanation for the increased excitability was thought to be the combined effect of increased sodium and decreased potassium conductance [55-58]. These abnormalities were more pronounced at the distal motor axon [57, 59]. Motor nerve excitability testing may help to gain insight into the pathophysiological changes in patients with ALS and PMA. In Chapter 5 of this thesis, this technique is combined with HDsEMG to investigate the pathophysiological excitability changes of a type of ectopic MU activity, known as electrically evoked multiplet discharges (MDs), which will be introduced hereafter.

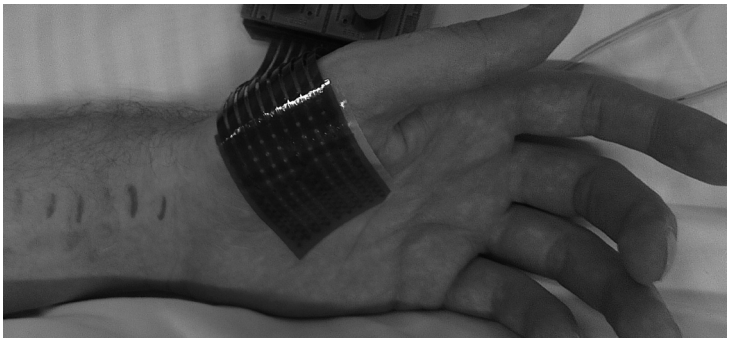

**Figure 7.** *Example of a high-density flexible grid (interelectrode distance of 4 mm) with 9x14 electrodes positioned over the thenar muscles.*

## High-density surface EMG

High-density surface EMG (HDsEMG) makes use of a grid of multiple densely spaced electrodes placed over a muscle (Fig. 7). In The Netherlands, developments and applications in this field were initiated in the department of Clinical Neurophysiology at the Radboud University Medical Centre in Nijmegen [60-68]. In parallel, several other international researchers have been working in this field [69-78]. Multichannel surface EMG signals allow the recording of not only temporal information, but also spatial information of muscle activity (Fig. 8 and 9). The spatial information gives an anatomical position to the MU in a muscle. The MUAP is presented by its spatiotemporal profile, or fingerprint. The most important advantage is that by the presence of topographical information, the recording of individual MUs (either voluntarily recruited, spontaneous or electrically activated) and their changes due to pathology become more reliable and non-invasively accessible. Several clinical applications using HDsEMG based on the unique information on individual MU level are discussed hereafter.

### *Motor unit number estimation*

MND is characterized by the loss of functioning motor neurons. Therefore the number of motor neurons and the rate at which they are lost is likely to be a suitable marker for the severity of the disease and its progression. Because a MU consists of a single motor neuron together with all the muscle fibers it innervates, the number of MUs equals the number of functioning motor neurons. Several studies in patients have shown that serial motor unit number estimation (MUNE) can be used to estimate the rate of progression of ALS [79-82]. By using HDsEMG to estimate the number of motor units, individual MUAP can more reliably be detected and therefore a larger sample of individual MUAPs can be collected compared to conventional single channel surface EMG techniques. Therefore, the motor nerve is electrical stimulated along several sites. At every site, the stimulus intensity is increased until an all-or-none response of an individual MUAP appear. Stimulus intensity is further increased up to a CMAP amplitude where a few individual MUAPs are active. These MUAPs also contribute to the CMAP scan at low stimulus intensity level. The number of MUs is then estimated by dividing the maximum

CMAP amplitude (all active MUAPs; 100%) by the mean amplitude of the collected MUAPs [60, 63]. MUNE using HDsEMG has been applied in Chapters 2, 5 and 7 of this thesis. The benefit of MUNE using HDsEMG is that it may serve as a gold standard for the number of functioning MUs and can also be applied as an objective measure to relate the total number of MUs to MUs expressing ectopic MU activity.

### *Motor unit tracking*

Current methods for estimating the number of MUs and evaluating their function rely on a comparison of samples of MUs. Comparing samples of MUs during the course of the disease, may yield only indirect information of pathophysiological changes in individual MUs. To obtain more direct evidence of possible pathophysiological changes of individual MUs at various stages of the disease, the same MUs need to be tracked longitudinally [83, 84]. However, this is extremely difficult, because properties of individual MUs which allow their repeated identification are also properties that may change with age or in neurogenic disease. Recently, a more powerful motor unit tracking technique, based on HDsEMG recordings, has been developed at our department and it showed that individual MUs could be detected and followed in subsequent sessions in healthy subjects [85]. Following individual MUs over time may yield information on the life cycle of a single MU and on the pathophysiological changes involved in patients with ALS and PMA [86]. To ensure that the observed changes can reliably be ascribed to pathology, other effects need to be taken into account as well. Further development to improve the reliability of this technique to detect pathophysiological changes will be discussed in Chapter 3 of this thesis.

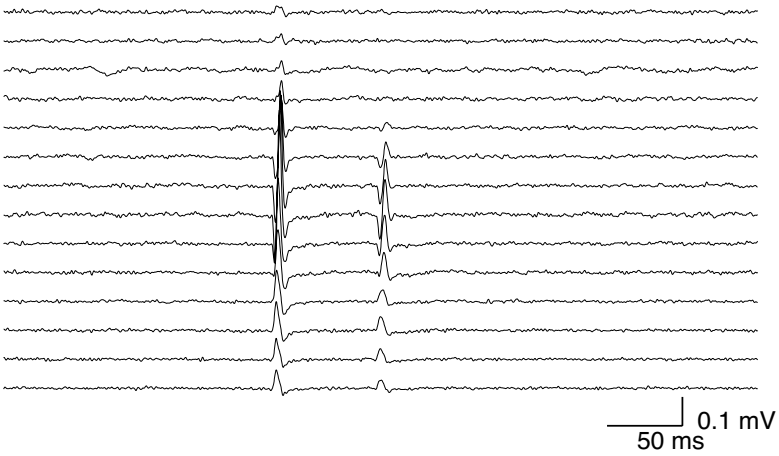


**Figure 8.** *Example of a 0.5 sec HDsEMG recording in the thenar muscles of an ALS patient. Fourteen electrode channels were selected from the HDsEMG grid. Two MUAPs are visible having different spatiotemporal profiles.*

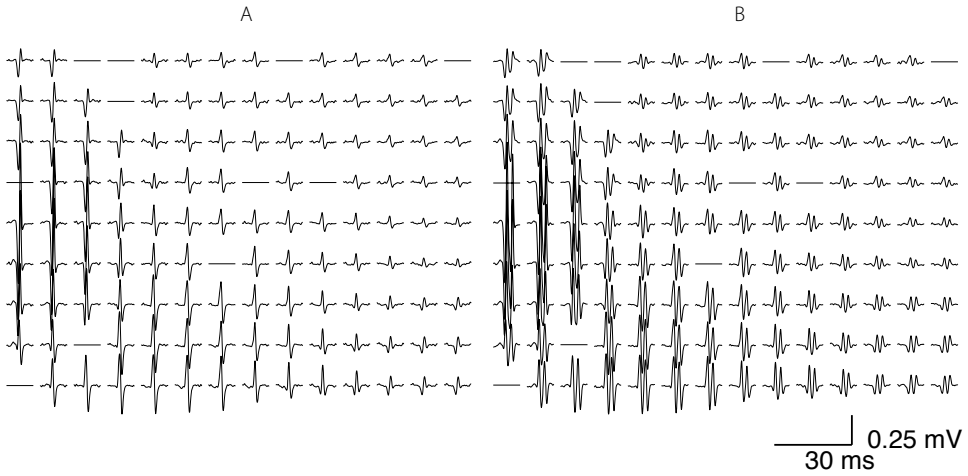


**Figure 9.** *(A) A MUAP fingerprint recorded with the 9 x14 electrode grid in the thenar muscles of an ALS patient after a single stimulus at the threshold intensity of the depicted MU. The MUAP signal is located at the mid-lower left corner of the electrode grid. (B) An MD (doublet) in the same MU as in (A) in a consecutive single stimulus. Each signal in the profile corresponds to the position in the electrode array of the electrode with which the signal was recorded. Electrodes with poor skin contact are visible as flat lines.*

### *Ectopic motor unit activity as fasciculation potentials*

By using HDsEMG, the spontaneous activity of individual MUs, hence also the recording of FPs [21, 75, 87, 88], becomes accessible (Fig. 8). The abundant nature of FPs raises the clinical suspicion of MND in a symptomatic person. However, no study has yet attempted to quantify the abundance of FPs on an individual MU level occurring in a specified time interval to determine their clinical importance. This could probably be due to the fact that needle EMG methods are only sensitive to activity of a few MUs surrounding the needle tip. HDsEMG enables the recording of a much larger sample of MUs, giving a better impression of the functioning of MUs in a muscle. Furthermore, HDsEMG is non-invasive, which makes it possible to perform longer and more stable recordings than needle EMG. By registering the ectopic MU activity in the form of FPs in a standardized setting within a predefined interval may therefore be of clinical relevance. This novel and promising approach will be investigated in Chapter 4 of this thesis.

### *Ectopic motor unit activity as multiplet discharges after electrical stimulation*

Recently, multiplet discharges (MDs) occurring after electrical stimulation have been observed and have been proposed as an approach to study excitability changes in the distal part of the motor neuron [89]. MDs are the re-occurrence of the same MUAPs within 20ms after the first MUAP. In Figure 9 an example is shown of the same MUAP without (Fig. 9A) and with an MD after a single stimulus (Fig. 9B). Currently, very little published information is available of these electrically evoked MDs [90, 91]. This is probably related to the fact that they have a small probability to occur after a single stimulus Additionally, MDs may not be recognized during conventional NCS using single channel surface EMG due to superimposition of multiple MUAPs and the relative small number of stimuli applied. Due to the availability of additional spatial information during HDsEMG the recognition of individual MUAPs, and, hence, the detection of MDs is facilitated. The occurrence of MDs may probably be related to axonal excitability changes at the distal motor end [30]. However, for electrically evoked MDs, evidence for such an association is lacking. This thesis aims to study MDs together with motor axonal excitability testing to improve the understanding of MDs and to assess their clinical significance. Till now, electrically evoked MDs have only been systematically studied in patients with ALS and PMA and in healthy controls [89]. Studying this phenomenon in patients having MND in their differential diagnosis will give more insight into the diagnostic significance of MDs. In Chapters 5, 6 and 7 of this thesis, the pathophysiological and clinical significance of electrically evoked MDs as a neurophysiological feature in ALS and PMA will be further investigated.

## IV Aims and outline of thesis

The ectopic MU activity in the form of FPs and electrically evoked MDs seems to give electrophysiological signs of affected MUs associated with MND. Therefore, they may have diagnostic and prognostic relevance. In this thesis, the study of ectopic discharges of individual MUs in patients with ALS and PMA plays a central role by making use of previous described surface EMG methods. More generally, the objectives of this thesis are the following:

1) To evaluate and further improve the available research methods for the non-invasive study of individual motor units.
2) To develop neurophysiological markers of clinical relevance in individual patients with ALS and PMA, which can be obtained with these surface EMG methods.
3) To clarify the pathophysiological and clinical significance of FPs and electrically evoked MDs.

### *Outline*

In **Chapter 2**, a novel automated method is described that quantifies MU variables from the CMAP scan, overcoming previous issues of operator-related variability and a time consuming manual analysis. How this automated method, when applied to the CMAP scan, is able to give an impression of the pathophysiological changes of MU loss and reinnervation is subsequently illustrated. Afterwards, in **Chapter 3** a concept of an algorithm is proposed to correct for electrode displacement errors to improve the reliability of ascribing MUAP changes to true pathophysiological changes in motor unit tracking studies using HDsEMG. In **Chapter 4**, novel MU discharge properties are identified with the aim to optimally differentiate patients with MND from healthy controls using HDsEMG recordings of MU activity at rest. To provide an understanding of the pathophysiological significance of electrically evoked MDs, in **Chapter 5** the presence of electrically evoked MDs is compared with excitability properties detected with motor nerve excitability testing. In **Chapter 6**, the presence of electrically evoked MDs is examined in patients with suspected MND to clarify the diagnostic accuracy of electrically evoked MDs. Therefore, they are compared with the presence of FPs using needle EMG. It is examined in **Chapter 7** whether electrically evoked MDs are related to disease progression in patients with ALS and PMA. Finally, in the last part, the methodological and pathophysiological aspects together with the clinical implications and future perspectives are discussed in **Chapter 8**. A summarizing overview of this thesis is given in **Chapter 9**.

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# Chapter 2

## CMAP scan discontinuities: Automated detection and relation to motor unit loss

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based on
Clinical Neurophysiology 2014; 125 (2): 388 - 395

## Abstract

*Objective:*
To evaluate an automated method that extracts motor unit (MU) information from the CMAP scan, a high-detail stimulus-response curve recorded with surface EMG. Discontinuities in the CMAP scan are hypothesized to result from MU loss and reinnervation.

*Methods:*
We introduce a new parameter D50 to quantify CMAP scan discontinuities. D50 was compared with a previously developed manual score in 253 CMAP scans and with a simultaneously obtained motor unit number estimate (MUNE) in 173 CMAP scans. The effect of MU loss on D50 was determined with a simulation model.

*Results:*
We found a high agreement (sensitivity = 86.8%, specificity = 96.6%) between D50 and the manual score. D50 and MUNE were significantly correlated below 80 MUs ($r = 0.65$, $n = 68$, $p < 0.001$), but not when MUNE was larger than 120 MUs ($r = 0.23$, $n = 59$, $p = 0.08$).

*Conclusions:*
Discontinuities in the CMAP scan as expressed by a decreased D50 are related to significant MU loss. The determination of D50 is objective, quantitative, and less time-consuming than both manual scoring and many existing MUNE methods.

*Significance:*
D50 is potentially useful to monitor neurogenic disorders and moderate to severe MU loss.

## Introduction

The compound muscle action potential (CMAP) scan is a surface EMG method which gives an overview of all functioning motor units (MUs) in the investigated muscle. The recording set-up used for the CMAP scan is identical to that of standard motor nerve conduction studies. The transcutaneous axonal stimulation protocol of the nerve differs, however, since the CMAP scan makes use of the underlying physiological principle that every MU has a specific threshold for activation. Hence, recording of the CMAP amplitude in response to electrical activation of the motor nerve with gradually increasing stimulus intensity (SI) yields a stimulus-response curve, which reflects the successive activation of MUs (Fig. 1). This CMAP scan provides clinical information regarding MU size, MU number, and axonal excitability which cannot be obtained with conventional supra-threshold CMAP stimulation [1-6].

The CMAP scan can be analyzed automatically by means of a stochastic approach [7, 8]. This computationally intensive method focuses on the estimation of the number of functional MUs in the investigated muscle [9] and is able to monitor the rate of MU loss in amyotrophic lateral sclerosis (ALS) patients [10]. Another approach to CMAP scan analysis is the extraction of several CMAP scan features [2]. Of these features, excessive steps (both in size and number) are probably the most defining characteristics of CMAP scans in patients with marked MU loss (Fig. 1B) [1-4]. Steps are differences between consecutive CMAP amplitudes that are disproportionally large compared to the corresponding increase in intensity of the applied stimulus. Differences between consecutive CMAP amplitudes in the CMAP scans of healthy subjects tend to be small, resulting in a sigmoidal, smooth curve (Fig. 1A). In patients with loss of MUs and/or enlarged MUs due to reinnervation, activation of such an enlarged MU results in the addition of a relatively large MU action potential (MUAP) to the CMAP [1]. This contribution is often visible as an abrupt jump in the CMAP scan. Hence, the presence of multiple steps is indicative of MU loss and reinnervation.

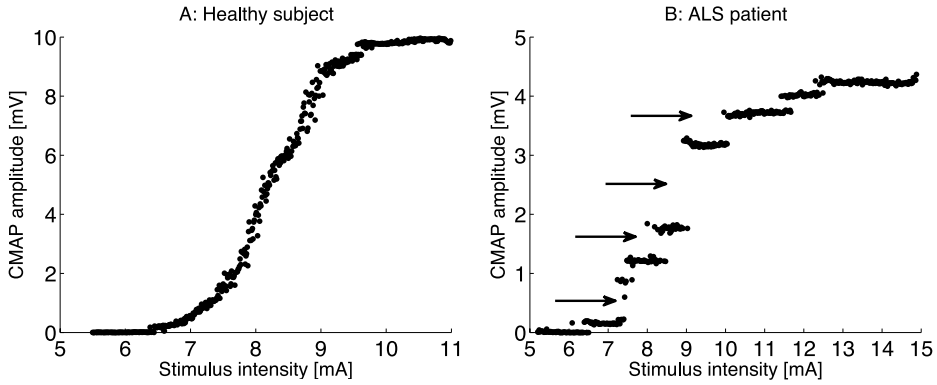


***Figure 1.*** *The CMAP scan, or stimulus-response curve, of a healthy subject (A) and of an ALS patient (B), both obtained from the thenar muscles in response to electrical stimulation of the median nerve. Note the decreased maximum CMAP amplitude and the presence of several discontinuities in the CMAP scan of the ALS patient (arrows).*

***Table 1.*** *Characteristics of the subjects whose CMAP scans were used in the evaluation of the performance of D50 versus the manual step analysis (A) and in the comparison of D50 vs MUNE (B) EDB = extensor digitorum brevis, GBS = Guillain-Barré syndrome, CTS = carpal tunnel syndrome, DM = diabetes mellitus type 1, ALS = Amyotrophic lateral sclerosis, PMA = Progressive muscular atrophy*

| Condition | No. of subjects | Total No. of CMAP scans | Muscle |
|---|---|---|---|
| A) Automated D50 vs Manual step analysis | | | |
| Healthy | 16 | 96 | Thenar |
| Healthy | 31 | 31 | EDB |
| GBS | 25 | 25 | Thenar |
| CTS | 16 | 16 | Thenar |
| DM | 31 | 31 | EDB |
| ALS / PMA | 6 | 54 | Thenar |
| Total | 125 | 253 | |
| B) Automated D50 vs MUNE | | | |
| ALS / PMA | 12 | 92 | Thenar |
| GBS | 51 | 81 | Thenar |
| Total | 63 | 173 | |

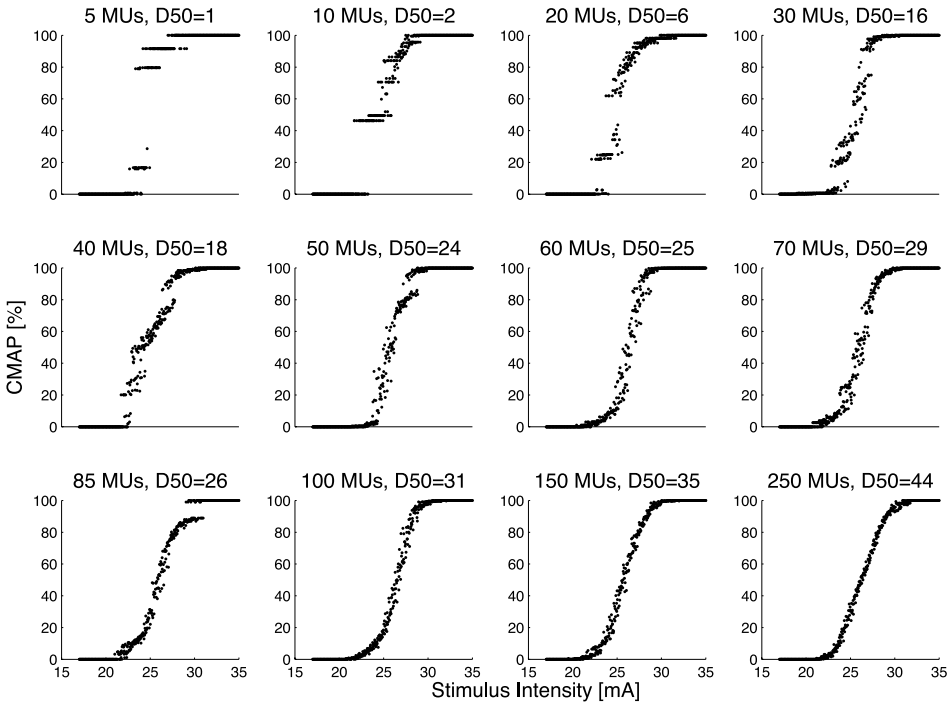


***Figure 2.*** *Examples of CMAP scans generated by a simulation model [2] (further explained in section* Simulation model*) in which the number of MUs was changed between 5 and 250 MUs. The CMAP scans show a gradual transition from a discrete pattern to a smoother sigmoidal curve. The corresponding values for D50 are provided in the labels above each plot.*

Previous work by Maathuis et al. [6] has shown that the quantification of steps can be valuable for monitoring disease progression in patients with motor neuron disease. However, in their procedure steps were identified manually and off-line. The manual nature of the step analysis made the results vulnerable to intra- and inter-operator variability, and the procedure is time-consuming.

Automation could solve these drawbacks and improve the monitoring of CMAP scan changes in motor neuron disease. Furthermore, it would allow more direct feedback to the clinician and potentially facilitate use of the tool in clinical practice.

In this study we present an automated method for step analysis that is based on the detection and size-ordering of all consecutive differences in the CMAP scan. We hypothesize that this method provides similar information to that provided by manual step analysis and that it is useful for monitoring disease progression. To evaluate this hypothesis, we analyzed CMAP scans recorded in patients with a variety of clinical disorders, some with and others without pronounced MU loss. In addition, to validate the method's ability to detect MU loss, we compared its performance with that of a simulation model [2] and with motor unit number estimation (MUNE).

## Methods

### *CMAP scan and MUNE dataset*

The CMAP scan and MUNE datasets used in this study were collected as part of previously performed and ongoing studies carried out at our hospital [3, 5, 6], according to the standardized protocol described below. In all of these studies the experimental protocol had been approved by the institutional Medical Ethics Committee in Rotterdam and all subjects had given informed consent.

To collect a set of CMAP scans that covered the full range of possible patterns, CMAP scans were obtained from healthy subjects and from patients with a variety of neuromuscular disorders. This resulted in the inclusion of a total of 253 CMAP scans from 125 subjects, which had previously been evaluated manually by expert assessors (see below): 127 CMAP scans of 47 healthy subjects (multiple muscles), 25 CMAP scans of 25 Guillain-Barré syndrome (GBS) patients, 16 CMAP scans of 16 suspected carpal tunnel syndrome (CTS) patients, 31 CMAP scans of 31 diabetes mellitus (DM) patients, and 54 CMAP scans of 6 ALS and progressive muscular atrophy (PMA) patients (serial recordings: see Table 1A). From other studies, we used datasets of CMAP scans and MUNEs that were recorded on the same day and in the same muscle. This resulted in 173 pairs of both recordings obtained from the thenar muscles of 51 GBS patients and 12 ALS and PMA patients (Table 1B).

### *CMAP scan pattern analysis and D50*

Figure 2 illustrates the effect of a reduction of the numbers of MUs on the CMAP scan pattern and, specifically, the visual change from a smooth sigmoidal CMAP scan to a more discrete pattern when the number of MUs declines. In general, amplitude differences between consecutive CMAPs may result from the recruitment of new MUs, from alternation and/or from noise. Alternation refers to the variability in the CMAP amplitude resulting from the fact that, when the recruitment ranges of MUs

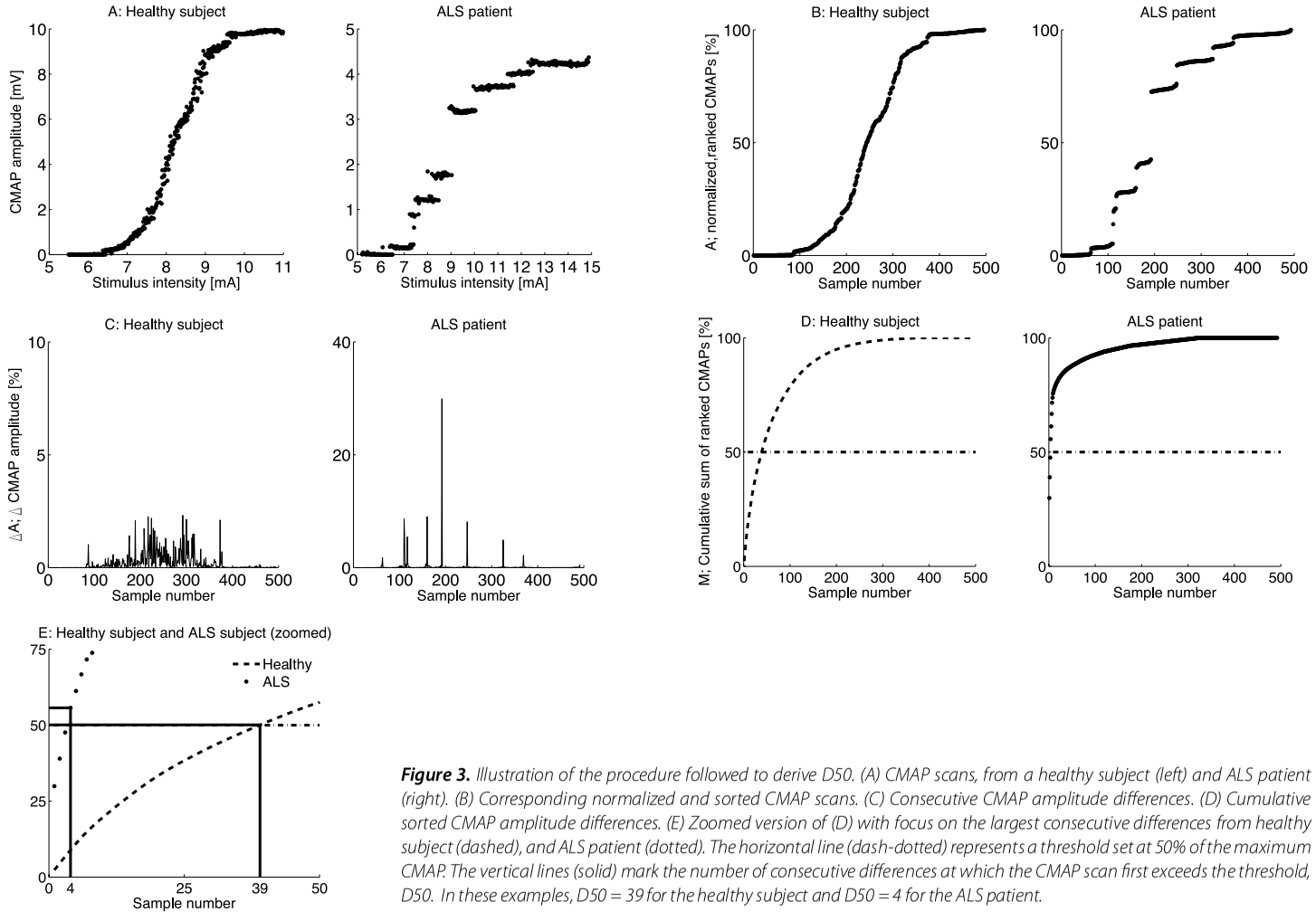


***Figure 3.*** *Illustration of the procedure followed to derive D50. (A) CMAP scans, from a healthy subject (left) and ALS patient (right). (B) Corresponding normalized and sorted CMAP scans. (C) Consecutive CMAP amplitude differences. (D) Cumulative sorted CMAP amplitude differences. (E) Zoomed version of (D) with focus on the largest consecutive differences from healthy subject (dashed), and ALS patient (dotted). The horizontal line (dash-dotted) represents a threshold set at 50% of the maximum CMAP. The vertical lines (solid) mark the number of consecutive differences at which the CMAP scan first exceeds the threshold, D50. In these examples, D50 = 39 for the healthy subject and D50 = 4 for the ALS patient.*

overlap, any combination of these MUs can be activated. The larger a consecutive CMAP amplitude difference, the more likely it is that it results from the activation of an additional single MU. The smallest consecutive differences are mostly the effect of alternation and noise. This implies that, for diagnostic and monitoring purposes, we need to focus on the largest consecutive differences. Therefore, we decided to determine the number of largest consecutive differences that are needed to build-up 50% of the maximum CMAP: D50.

To determine D50, the CMAP scan data was post-processed in Matlab (R2012a: The MathWorks, Natick, MA). First, the N recorded CMAP amplitudes (Fig. 3A) were expressed as percentage of the maximum CMAP and ranked by size:

$$\mathbf{A} = [A_1, A_2, A_n, \ldots, A_N] \quad \text{where} \quad A_1 \leq A_2 \leq A_n, \ldots, \leq A_N \tag{1}$$

with N = 500 in the majority of the recordings (Fig. 3B) and n the sample number. Then, between these ranked CMAP amplitudes, the consecutive differences (Fig. 3C) were calculated:

$$\Delta\mathbf{A} = [\Delta A_1, \Delta A_2, \Delta A_n, \ldots, \Delta A_{N-1}] \quad \text{where} \quad \Delta A_n = A_{n+1} - A_n \tag{2}$$

Next, these consecutive differences were again ranked, this time from largest to smallest, and their cumulative sum was calculated:

$$\mathbf{M} = [M_1, M_2, M_n, .., M_{N-1}] \quad \text{where} \quad M_1 \leq M_2 \leq M_n, \ldots, \leq M_{N-1} \tag{3}$$

with **M** the array of the cumulatively summed ranked consecutive differences (Fig. 3D) and n the sample number. Hence, $M_1$ equals the size of the largest consecutive difference present in the CMAP scan (expressed as percentage of the maximum CMAP), $M_2$ the sum of the largest and second largest difference, and $M_{N-1} = 100$. Because we were primarily interested in the largest consecutive differences (i.e., the elements of **M** with low sample number n), a threshold was set at 50% of the maximum CMAP (this choice is further discussed in section Evaluation). Finally, D50 was determined as the smallest value of n at which **M** exceeded this threshold. In the presence of reinnervation, enlarged MUs will contribute more large MU potentials to the CMAP scan, resulting in a few disproportionally large consecutive differences. As a consequence, D50 will decline and when **M** is plotted versus n, the curve will be steeper for low n. Figure 3E shows this effect for a healthy subject (D50 = 39) and an ALS patient (D50 = 4).

### *Registration*

*CMAP scan*

The CMAP scans were recorded using the CMAP scan application on a Viking Select EMG system (V12, Nicolet Biomedical, Madison, WI) as described in detail elsewhere [2, 4-6]. Recordings were obtained from the thenar muscles of the nondominant hand or the extensor digitorum brevis (EDB) muscle of the left foot by electrically stimulating the median nerve or deep peroneal nerve, respectively (Table 1). First, the SI required to elicit the lowest-threshold MUAP ($S_0$) and the SI required to elicit the maximum CMAP ($S_{100}$) were determined. Then, 500 stimuli (2 Hz, 0.1 ms duration) were applied in a downward direction from $S_{100}$ to $S_0$. Afterwards, the CMAP scan was checked and occasionally additional stimuli were applied in segments of the CMAP scan with relatively few data points.

The CMAP scan data (SI's and corresponding CMAP amplitudes) were imported in Excel (Microsoft, Redmond, WA) and subsequently processed in Matlab. For each of the CMAP scans, D50 was calculated using the algorithm described above. Values for Step% (summed step size as percentage of the maximum CMAP) as derived with the existing manual step analysis procedure were collected from previous and ongoing studies [3, 5, 6].

*MUNE*

MUNE was performed using high-density surface EMG (HDsEMG) [11-13] with an array of 126 electrodes attached to the skin over the thenar muscles. The maximum CMAP amplitude was recorded and a sample of 20 – 30 single MUAPs was collected by positioning the stimulator over several sites along the median nerve. The MUNE was derived by dividing the maximum CMAP amplitude by the mean of the collected MUAPs [12, 13].

*Simulation model*

Use of a computer model to generate simulated CMAP scans has as an advantage that the D50 derived from such a scan can be compared against a known input (number and sizes of the contributing MUs). The simulation model used for this purpose has been described previously by Blok et al. [2]. Figure 2 shows the effect on the CMAP scan pattern when the input of the model changed from 250 MUs down to 5 MUs. In parallel with the changes in MU number, the MU size distribution was changed from a normal distribution for healthy subjects [14] to a distribution with increased MU sizes for a small number of MUs, such as in ALS.

In clinical practice, it is often difficult to have $S_0$ and $S_{100}$ match exactly the onset and offset of the curve. Usually, the CMAP scan has "tails" at both its low and its high end. The responses in the tails carry no information and may be considered lost for the CMAP scan. If excessive, the resulting undersampling of the curve may lead to additional discontinuities and hence spuriously low D50. To incorporate this factor, simulations were performed for three conditions: lower and upper tails of 1 mA (7%), 2mA (14%), and 3 mA (21%), each on top of the "tight" SI range of 14 mA.

In all simulations, the number of stimuli was kept constant at 500, because variation in this number also affects D50. The input number of MUs was altered from 5 MUs up to 400 MUs and to each of the

MUs in such a pool a MUAP size was assigned. Subsequently, for all three SI ranges sets of 50,000 CMAP scans were simulated per pool by randomly assigning activation thresholds to the MUs in each pool. These activation thresholds were normally distributed over the SI range. Finally, for each simulated CMAP scan D50 was calculated.



### *Evaluation*

*Sensitivity and specificity of CMAP scan classification by D50*

As 95% of the CMAP scans from the healthy subjects had a Step% ≤ 18%, this latter value was used as threshold to classify all 253 recorded CMAP scans as normal or abnormal. Subsequently, the performance of D50 against this "gold standard" classification was assessed by means of a receiver operating characteristic (ROC) curve. The ROC curve is a powerful and simple tool for comparing the performance of two methods in binary classification [15, 16]. The performance for D50 in discriminating normal from abnormal CMAP scans was determined by changing its cut-off value from 1 to 70 in discrete steps of 1 and calculating the corresponding sensitivity and specificity. From these data, the ROC curve was constructed and the area under the ROC curve (AUC) was calculated. Subsequently, the maximum of sensitivity multiplied by specificity was taken to mark the optimal cut-off value for D50.

Although the median and peroneal nerves may have somewhat different properties and sensitivity to disease, the threshold in Step% that distinguished normal from abnormal CMAP scans was almost the same for thenar and EDB muscles (18% vs 19%). Therefore, for the purpose of this study, these sets of recordings could be merged.

All statistical analyses were performed using Matlab (R2012a: The MathWorks, Natick, MA). The Lilliefors method was used to test normality. A *p*-value of $< 0.05$ was considered statistically different. Data from Step% and D50 were not normally distributed ($p < 0.05$); therefore, the results will be presented as median and percentiles.

*Choice of threshold at 50% of the maximum CMAP*

Another set of ROC curves was generated to evaluate the choice of D50's threshold at 50% of the maximum CMAP. This threshold represents a balance between two aspects: 1) focus on only those consecutive differences that with the highest probability represent pathologically enlarged MUs, which is optimized by applying a low threshold, and 2) including information of as many MUs as possible, which can be achieved by using a high threshold. This balance was investigated by changing the threshold from 10% of the maximum CMAP up to 90% of the maximum CMAP in discrete steps of 10% (D10, D20, up to D90). As above, the classification obtained with the manual step analysis (Step% ≤ 18%) was used as reference and the AUC was determined for D10 up to D90 separately. Optimal cut-off values for D10 up to D90 were determined as described above. The 95% confidence intervals (CIs) were calculated and the resulting AUCs were compared [15, 17].

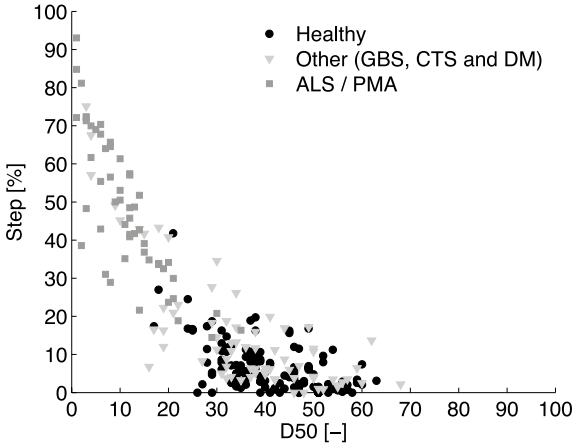


***Figure 4.*** *Step% determined by a human observer versus the automatically determined variable D50 for healthy subjects, other subjects (GBS, CTS and DM) and ALS / PMA patients.*

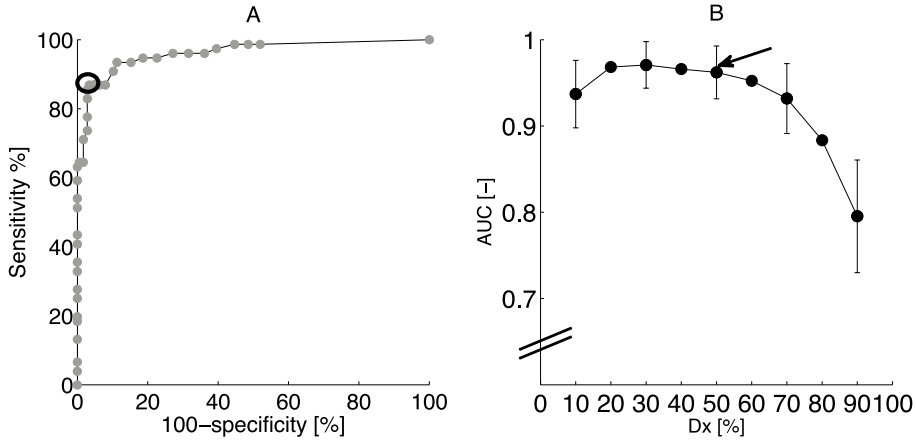


***Figure 5.*** *(A) Receiver Operating Characteristic curve for evaluation of the performance of D50 with respect to identifying normal and abnormal CMAP scans. Values of D50 varied between 1 and 70. Scans were classified as normal or abnormal using the manual step analysis detection method with Step% = 18 as threshold (Step% > 18 is abnormal). The location of the circle indicates the optimal cut-off value at D50 = 25, which corresponds with specificity = 96.6% and sensitivity = 86.8%. The area under the ROC curve is 0.96. (B) The areas under the ROC curve (AUCs) for varying threshold Dx (the number of sorted differences for which their sum exceeds x % of the maximum CMAP). The arrow indicates the overall performance shown in Figure 5A (D50, AUC = 0.96). Error bars represent 95% confidence intervals.*

*D50 vs number of MUs*

Two approaches were used to assess the relation between D50 and the number of MUs. First, the CMAP scan simulation model was used to generate CMAP scans from which D50 was determined; this D50 was then compared to the number of MUs used as model input. Second, from the measured CMAP scans D50 was calculated and correlated with the HDsEMG MUNE data. Because D50 and MUNE were not normally distributed, Spearman's correlation was used, taking into account that for some patients data were obtained from repeated measurements [18].

As a third strategy, we used the MUNE corresponding to a measured CMAP scan as input for the simulation model and subsequently compared the D50 derived from the simulated CMAP scan with the D50 derived from the measured CMAP scan. Because the differences between these two values of D50 were normally distributed (Lilliefors test, $p > 0.05$), the Bland and Altman approach [19] could be used to correct for the multiple measurements per subject.

## Results

To illustrate the relation between D50 and the visual pattern of the CMAP scans, in Figure 2 each subplot is labeled with the corresponding D50. This reveals a clear, inverse relationbetween the extent of discontinuities present and the value of D50.

Figure 4 shows D50 against Step% for all 253 CMAP scans, subdivided for healthy subjects, ALS/PMA patients, and other subjects (GBS, CTS, and DM patients). Median Step% and median D50 are shown in Table 2. The ROC curve for D50 in Figure 5A shows how accurately D50 is able to classify the CMAP scans as normal or abnormal compared to the manual method with threshold at Step% = 18%. The AUC of D50 was 0.96 (95% CI 0.93 – 0.99), indicating that D50 and Step% perform very similarly. The optimal cut-off value was at D50 = 25, with corresponding sensitivity = 86.8% and specificity = 96.6%.

The AUCs for D10 up to D90 as well as the 95% CIs of the AUCs for D10, D30, D50, D70 and D90 are provided in Figure 5B. Overall, the AUCs indicate high accuracy compared to Step%. The AUC level remained constant up to about D50 – D60 and then decreased. There was no significant difference between the highest AUC (for D30) and the AUC for D50 ($p = 0.34$). The optimal cut-off values distinguishing normal from abnormal CMAP scans were at D10 = 3, D30 =12, D50 = 25, D70 = 57, and D90 = 122.

***Table 2.*** *Median values for manual step analysis by Step% and for D50 with $25^{th}$ – $75^{th}$ percentile per subject category*

| Condition (Number of scans) | Manual step analysis Step%, Median ($25^{th}$ – $75^{th}$ percentile) | Automated method D50, Median ($25^{th}$ – $75^{th}$ percentile) |
|---|---|---|
| Healthy subjects (127) | 5.0 (1.9 – 9.6) | 39.0 (34.0 – 57.0) |
| GBS (25) | 6.1 (3.1 – 15.2) | 35.0 (31.5 – 46.3) |
| CTS (16) | 6.3 (3.9 – 10.1) | 37.0 (31.5 – 46.0) |
| DM (31) | 16.3 (7.8 – 39.2) | 38.0 (18.3 – 61.9) |
| ALS / PMA (54) | 47.0 (33.9 – 64.0) | 11.0 (6.0 – 27.6) |

Figure 6 visualizes the results from the two validation approaches, with the curve representing the result of the simulations (median over all simulations, with error bars at the 5$^{th}$ and 95$^{th}$ percentile). The symbols represent the results from the pairs of recordings. For both, the known number of MUs (model input for the simulations and HDsEMG MUNE for the recordings) is shown along the x-axis and the D50 calculated from the corresponding CMAP scan is plotted on the y-axis. In total 34 measured CMAP scans (and MUNEs) were excluded because of abnormal number of stimuli (< 300 or > 700), long tails (< 20% left tail and > 20% right tail), or wide sampling interval (> 0.05 mA between successive stimuli).

Simulations and measurements show a similar trend. There is a linear increase (number of MUs ≈ 2 x D50) in D50 up to 80 MUs; beyond that value D50 levels off. In the presence of 150 or more MUs, D50 stabilizes around approximately 38 consecutive differences (5$^{th}$ – 95$^{th}$ percentile = 32 – 45 at 400 MUs) for the simulated CMAP scans. D50 from measured CMAP scans and MUNE are significantly correlated for ≤ 80 MUs ($r = 0.65$, $n = 68$, $p < 0.001$), weakly correlated for > 80 MUs ($r = 0.47$, $n = 71$, $p < 0.001$), and not correlated ($r = 0.23$, $n = 59$, $p = 0.08$) for > 120 MUs.

Finally, the mean difference Δ D50 between the D50 derived from a simulated CMAP scan with a model input equal to a recorded MUNE and the D50 obtained from the corresponding recorded CMAP scan was 0, with limits of agreement -14 to + 14. This demonstrated that our simulation model generates realistic CMAP scans over a wide range of model inputs.

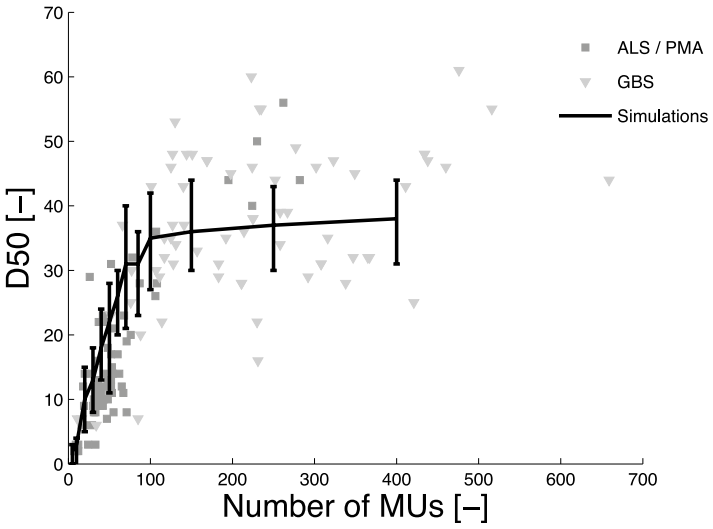


***Figure 6.*** *Validation of D50 by means of a comparison with the number of motor units present. Symbols: D50 of measured CMAP scans versus HDsEMG MUNE from ALS / PMA subjects (square) and GBS subjects (triangle) (n = 139). Curve: simulation results comparing the median D50 of simulated CMAP scans with the model input. The error bars indicate the 5$^{th}$ and 95$^{th}$ percentile of the solid (realistic tail) curve.*

## Discussion

In this study, we have presented an automated approach to obtain information about the number and size of functioning MUs from the CMAP scan. Our method focuses on the discontinuities in the CMAP scan and we have shown that these discontinuities reflect the process of MU loss and reinnervation that occurs in many neuromuscular disorders. Furthermore, we have introduced a variable, D50, that can capture the most pronounced visual characteristics of abnormal CMAP scans in a single number.

Practically, the CMAP scan can be seen as a useful visual tool, supporting and extending conventional electrodiagnostic tests. Little training is required to recognize CMAP scans in the presence of a strongly reduced number of MUs as abnormal (Fig. 2). The pattern changes are quite obvious to the human eye. However, for applications such as follow-up studies and a more objective delineation between normal and abnormal, quantification of these changes is essential. D50 appears to meet this need. The numbers in the labels of Figure 2 adequately reflect the visually perceived smoothness of each curve, with lower D50 representing more discontinuities.

In addition to capturing the characteristic visual aspects of the CMAP scan, D50 performs very similarly as manual step analysis. Because the latter can be used as a follow-up tool to monitor disease progression in ALS [6], the same may be assumed to apply to D50. Probably, the use of D50 will even improve the reliability of the CMAP scan as a follow-up tool, because it eliminates intra- and inter-operator variability. Furthermore, the high correlation between D50 and the number of MUs present (Fig. 6) demonstrates that the characteristic discontinuities in the CMAP scans of ALS patients are directly related to the underlying pathological process. Hence, we conclude that the easily applicable CMAP scan, with its familiar recording set-up, non-invasive nature and quick procedure that is well-tolerated by patients [5] is a promising tool for clinical and scientific use in neurogenic conditions.

A limitation to such use of the CMAP scan is that it can be related to MU number only in conditions of relatively severe MU loss. When there are more than approximately 80 MUs, the linear relation between MU number and D50 levels off (Fig. 6). In these conditions, the CMAP scan becomes smoother (Fig. 2), primarily because the number of possible combinations of activated MUs in response to stimuli of similar intensity (alternation) far exceeds the number of stimuli applied. This has an averaging – and hence, smoothing – effect on the curve. Any model that aims to describe the CMAP scan pattern encounters this issue [7, 9]. Applying more stimuli would only slightly mitigate the effect of such undersampling, at the cost of significant increases in recording duration and patient discomfort.

When there are fewer than 80 MUs present, however, D50 appears to be useful as a rough indicator of this number, with number of MUs $\approx 2 \times$ D50 (Fig. 6). This relation is visible both in the simulation studies and in the comparison with MUNE measurements. It must be noted, however, that the simulated curve represents an average of many simulated CMAP scans. In individual CMAP scans, D50 values can deviate due to alternation, lower and upper tail size, and different MU size distributions between subjects, which is reflected in the wide limits of agreement. Similarly, the wide range in measurement results indicates that the above equation should be applied with caution in individual cases.

In our study, the D50 value that optimally distinguished normal from abnormal CMAP scans was 25, a value that corresponds to approximately 50 MUs according to the above relation. That is, a CMAP scan becomes consistently abnormal when there are fewer than about 50 MUs, a condition that is usually met when ALS patients undergo their first-time (diagnostic) EMG [20]. This cut-off value of 25 was calculated using the findings of the manual step analysis as reference standard. To discriminate between normal and abnormal CMAP scans in this analysis procedure, a Step% threshold was set at 18%. If this threshold was shifted, the AUC curve remained similar to that in Figure 5B (not shown). This is relevant because the CMAP scans were performed in two different muscles (Table 1). In the present small scale study, there was only a minor difference in the upper limit of normal for Step% between thenar and EDB muscles (18% vs 19%) in healthy subjects, but we need to take into account the possibility that the two muscles have somewhat different cut-off values. However, considering the marginal impact on the ROC curve, the use of muscle-specific thresholds is expected to have only a negligible effect on the results of this study.

The fact that there was no significant difference in D50 derived from modeled and measured CMAP scans with the same number of MUs demonstrates that our relatively simple simulation model generates realistic CMAP scans. In turn, this implies that – at least for neurogenic conditions – most of the clinically relevant variation between CMAP scans can be explained by the limited number of parameters incorporated in the model: the number of MUs, their sizes and the activation thresholds. This view is supported by our previous experience with the CMAP scan in various patient populations, which showed that processes of MU loss and reinnervation are consistently reflected in CMAP scan discontinuities, irrespective of differences in the underlying pathology. This is somewhat similar to the characteristic changes observed in needle EMG with neurogenic disorders (e.g., enlarged potentials), which are not specific for a particular condition. As changes in D50 apparently relate to a loss of MUs and reinnervation rather than to a specific pathology, the nature and extent of this pathology seemed not directly relevant for the purpose of the present study. For that reason, we have chosen not to describe our patient groups in great detail in the current paper (of course, details may be found in the publications on the original studies). In addition, this explains why we considered it justified to merge the previously obtained data in such entirely different clinical disorders as ALS and CTS in a single dataset.

Setting a threshold at 50% of the maximum CMAP and counting only the largest differences that contribute to reaching this threshold appears to use the information from discontinuities that is available in the CMAP scan optimally. A threshold at 50% provides a balance between including information of as many MUs as possible (high threshold) and focusing on only those consecutive differences that with the highest probability represent pathology (low threshold). In particular, a threshold above approximately 60% (> D60) of the maximum CMAP negatively affects the procedure's performance, due to the increased influence of alternation (between MU variability), MU instability (within MU variability), and noise (Fig. 5B).

In general, the effect of alternation is likely to be larger than that of noise or within-MU variability, except in conditions of very few remaining MUs (and low D50). For very low numbers of MUs, the

presence of noise or unstable MUs may increase D50: they reduce the consecutive differences and hence, more of these would be required to constitute 50% of the maximum CMAP. In addition, our experience shows that it is important to clearly instruct the patient to take a comfortable position and to relax their muscles as much as possible to minimize movement artefacts. These artefacts may impact the CMAP scan's visual appearance; fortunately, their influence on D50 is usually relatively small because of the CMAP size sorting step in the algorithm.

Other important factors related to the recording are the number of applied stimuli and the length of the tails of the CMAP scan. Previous work has shown that applying 500 stimuli provides an adequate balance between sufficient sampling of the CMAP scan and the recording duration [5]. As the number of applied stimuli influences D50, standardization of this parameter during recording (or normalization during analysis) is necessary. The effect of the tails might be mitigated by modification of the stimulation sequence so that more stimuli are applied in the intermediate range. This would require an adaptive protocol, e.g., one that actively searches for relatively large CMAP amplitude differences. How such an optimization protocol would affect D50 and other parameters needs to be explored, but it has as a potential benefit that the detected large consecutive CMAP amplitude differences can more reliably ascribed to enlarged MUs.

We conclude that D50 yields similar MU information as manual step analysis but can be derived automatically and, hence, objectively. In addition, we have shown that D50 provides an impression of the number of MUs in a range relevant to motor neuron disease. Our method is easy to implement in an EMG system and the computational effort is small, which makes it possible to present D50 directly to the clinician. Therefore, we believe it has significant potential as a monitoring tool in neurogenic conditions.

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# Chapter 3

## Motor unit tracking using high-density surface electromyography (HDsEMG): Automated correction of electrode displacement errors

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## Abstract

*Objectives:*
This study discusses a technique to automatically correct for effects of electrode grid displacement across serial surface EMG measurements with high-density electrode arrays (HDsEMG). The goal is to match motor unit signatures from subsequent measurements and by this, achieve automated motor unit tracking.

*Methods:*
Test recordings of voluntary muscle contractions using HDsEMG were performed on 3 healthy individuals. Electrode grid displacements were mimicked in repeated recordings while measuring the exact position of the grid. A concept of accounting for translational and rotational displacements by making the projection of the recorded motor unit action potentials is first introduced. Then, this concept was tested for the performed measurements attempting the automated correlation of the similar motor unit action potentials across different trials.

*Results:*
The ability to perform automated correction (projection) of the isolated motor unit action potentials was first shown using large angular displacements. Then, for accidental (small) displacements of the recording grid, the ability to automatically track motor units across different measurement trials was shown. It was possible to track 10-15% of identified motor units.

*Conclusions:*
This proof of concept study demonstrates an automated correction allowing the identification of an increased number of same motor unit action potentials across different measurements. By this, great potential is demonstrated for assisting motor unit tracking studies, indicating that otherwise electrode displacements cannot always be precisely described.

## Introduction

Motor units (MUs) are the smallest functional elements of the peripheral motor nerve system. Neuromuscular diseases affect MUs, either directly or indirectly. The follow-up study of MUs during a disease process may, therefore, improve our understanding of neuromuscular diseases. Recently, MU tracking using high-density surface electromyography (HDsEMG) has been introduced as a neurophysiological technique that enables noninvasive follow-up of single MUs [1]. In this technique MU action potentials (MUAPs) are recorded with an array of densely spaced electrodes after electrical stimulation of the afferent nerve. In these HDsEMG recordings, each MUAP is presented as a spatio-temporal profile or fingerprint of the corresponding MU. Use of the characteristic information in the fingerprints facilitates detection of the MUAPs in consecutive recording sessions and, hence, allows for MU tracking.

MU tracking may provide insight into the relationship between MUAP properties and how these are affected during disease progression. However, before changes in a MUAP fingerprint between sessions can be ascribed to an underlying pathophysiological process, other factors that affect it have to be taken into account. It is known that MUAP properties depend on geometrical and anatomical factors such as muscle fiber length, signal stability (electrode-skin contact), electrode location, and electrode orientation [2]. In particular, even small shifts in electrode position can affect the MUAP parameters significantly.

In clinical practice, the recording grid can be rotated, translated and bended in a different way compared to how it was attached in the previous recording session. In the study of Holobar [3], a method was proposed to correct for translational errors for the study of the external sphincter muscle. In this study we extend this correction procedure and show how the effects of rotation between sessions can be automatically compensated for. We illustrate the approach on a low-force voluntary contraction measurement of thenar muscles, following well-defined displacements.

## Methods

### *Recordings*

Test recordings of voluntary contractions were performed on three healthy subjects using a HDsEMG grid with 126 electrodes. The diameter of the electrodes was 1.5 mm and they were spaced equidistantly in 9 rows and 14 columns with inter-electrode distance of 4 mm, as described in detail elsewhere [1]. The grid was placed over the thenar muscles. Initially, four configurations were introduced to illustrate the concept using recordings from a single subject, indicated in Figure 1. Additionally, recording grid in configuration 1 was used as a reference configuration for 5 additional 2-minute recordings in each of 3 subjects: with the grid positioned in reference configuration, and positions rotated for -5, +5, -10 and +10 degrees around it, mimicking accidental grid displacement. The approximate reference position (configuration 1) is identified as the orthogonal to the direction connecting carpometacarpal (CMC) and metacarpophalangeal (MCP) joints of the thumb. This guaranteed the total thenar muscle coverage.

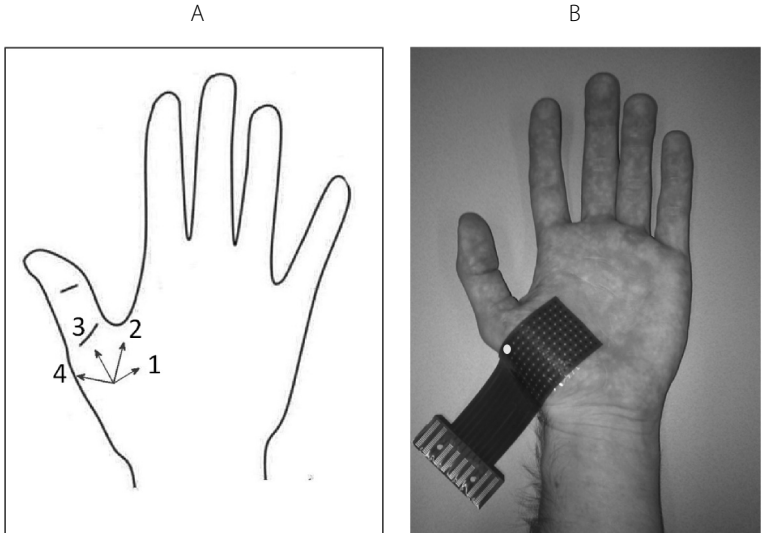


***Figure 1***. *(A) Four test recording configurations; arrows indicate the direction from the side of printed circuit board (PCB) connector towards the horizontal end of the electrode grid; (B) recording grid in configuration 1. White dot indicates a central point for grid rotation.*

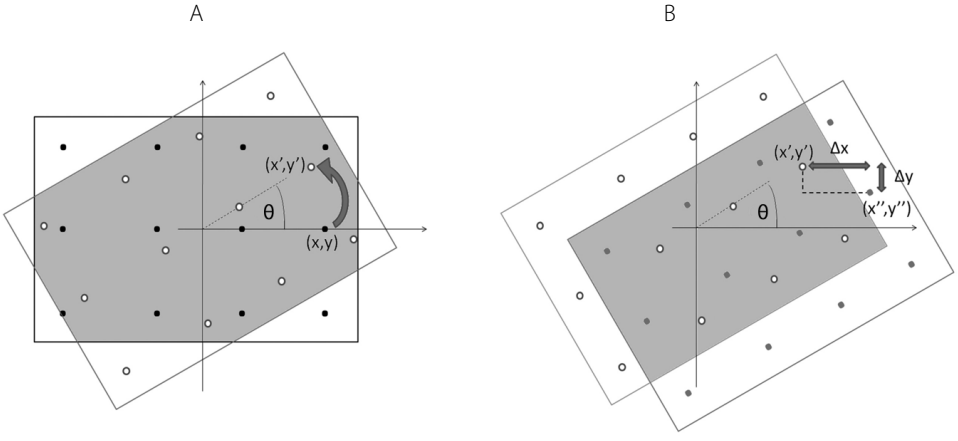


***Figure 2.*** *(A) Calculating the positions of electrodes of a rotated grid; (B) additional translation for finding the position of the best matching.*

In the experimental setup, it was important to ensure that the position of the electrode grid was precise, especially in cases of small (intentional) displacements. For this precise positioning, a double-sided adhesive tape containing small and large gaps was used, with the detailed procedure described in Lapatki et al. [4].

Recordings were performed on a single day for each subject, with half-hour break intervals. Visual feedback in the form of bipolarly filtered signals was used to assist the subject in establishing and maintaining a stable, low-force contraction level, estimated at 1-5% maximum voluntary contraction (MVC).



All signals were first decomposed automatically, separating each into contributions from individual MUs using the algorithm described and verified in Gligorijević et al. [5, 6]. First part of this algorithm [6], responsible for MUAP fingerprint extraction using optimal clustering was utilized. This yielded sets of MUAP fingerprints for each session.

Fingerprints that were observed at least 100 times without being superimposed to other MU fingerprints (uncorrupted) during the two-minute recording were considered reliable, averaged and kept for further analysis.

### *Artificial recording grid displacement*

To assess the effect of rotation of a grid around its center, we first assign coordinates (x,y) to the electrode locations in the original grid placement. After rotation over angle $\theta$, new coordinates (x',y'), representing the position of these electrodes are then obtained by multiplication with a rotation matrix:

$$\begin{bmatrix} x' \\ y' \end{bmatrix} = \begin{bmatrix} \cos\theta & -\sin\theta \\ \sin\theta & \cos\theta \end{bmatrix} \begin{bmatrix} x \\ y \end{bmatrix} \quad (1)$$

To assess the value of a fingerprint in every set of rotated coordinates (x',y'), we use the linear interpolation based on originally recorded values. This was done for each temporal sample to obtain a projection of a fingerprint on a rotated grid. The rotation procedure is depicted in Figure 2A. The interpolation is reliable only in the intersection area (gray) that represents the same physical surface covered by the grid in both recordings, while the rest of the values cannot be reliably assessed and were put to zero.

Apart from rotation, the in-plane translation of the recording grid is practically inevitable and thus has to be taken into account [3]. This translation describes a final transformation of grid coordinates:

$$\begin{bmatrix} x'' \\ y'' \end{bmatrix} = \begin{bmatrix} x' \\ y' \end{bmatrix} + \begin{bmatrix} \Delta x \\ \Delta y \end{bmatrix} \quad (2)$$

Comparing translated observations can be done only in the intersection area (gray in Fig. 2B). Since we can compare only part of each fingerprint, it is necessary to choose a "subgrid" – 6x6 subset of electrodes around the place of the highest spike which is subsequently used for comparison. The subgrid "subfingerprint" is compared with each possible counterpart of the investigated fingerprint with which we attempt to match. The best possible position reveals the translation. This example is shown in Figure 2B.

The minimal translational step in both *x* and *y* directions naturally equals to one inter-electrode distance. This brings a limitation due to the fact that a shifted electrode can measure potentials anywhere between 2 neighboring electrodes. We exploit the property that the potentials between close electrodes can be assessed correctly due to the dense electrode placement of our recording grid. Therefore, we upsample the signal 4 times using bicubic interpolation [7] to increase the translation resolution.

Each fingerprint is upsampled and rotated for a chosen angle, followed by the estimation of optimal translation for the comparison with its "non-rotated" counterpart. We proceed with downsampling the signal to its original dimensions, and then compare rotated and "fixed" fingerprints using 2 parameters: Pearson correlation coefficient and normalized root mean square error (NRMSE). NRMSE is calculated using the following equation:

$$NRMSE = \sqrt{\frac{\sum(\hat{z} - z)^2}{\sum z^2}} \tag{3}$$

where $\hat{z}$ represents the estimated shape obtained by clustering, and z the template (exact) form. Summation is performed over all samples and channels. The peak of the ratio between these values (high correlation and small residue when we subtract fingerprints) indicates MUs that match. Each MUAP fingerprint obtained by a "displaced" grid is compared with its best match from the "fixed" grid measurement. The complete procedure goes as follows: the rotation angle is varied and the optimal translation is calculated until the best overall result is achieved. Fine tuning is then applied around the indicated angle to pinpoint a correct value. The output provides the angle of rotation and the displacement in x and y directions.

## Results

For the study with 4 configurations (Fig. 1A) in total, 25 reliable MU fingerprints were extracted with the decomposition method. Out of these, 3 MUs could be tracked across measurements: 1 MU appeared in 3 configurations (1, 2, 4), and the remaining two were matched across configurations 1 and 2. An example for the automatic correction of the specified 45 degrees displacement is portrayed in Figure 3, showing the correlation and the ratio between correlation and NRMSE. The angle was estimated at 42 degrees, translation at -2 and 3 mm displacement in x and y directions respectively. Figure 4A and 4B indicate the originally recorded fingerprints from positions 2 and 1 respectively. Seemingly different MUs (Fig. 4C) match almost perfectly once the rotational and translational corrections have been applied (Fig. 4D). Part of the procedure used to identify translational displacement following the rotation can be seen in Figure 4E. The subgrid used for comparison is also indicated.

The second part of the study involved 3 subjects and aimed at MU tracking with simulated accidental displacements of the recording grid for configuration 1 (Fig. 1A). On average, 5 fingerprints per subject could be tracked across one or more measurement configurations.

The number of MUs that were found and tracked per subject together with their average correlation before and after electrode placement corrections are summarized in Table 1. It was found that the indicated grid displacements did not always match the ones extracted by our procedure. The largest observed difference was the one for measurement involving subject 1. While the indicated angle was 5 degrees, the calculated (and in fact, correct) one was -2 degrees with respect to the position 1 ("error" of 7 degrees). This may be the result of relative muscle-skin displacement (due to e.g. thumb movement) rather than angle measurement error. On average, estimated displacement was within ±3 degrees from the indicated value.



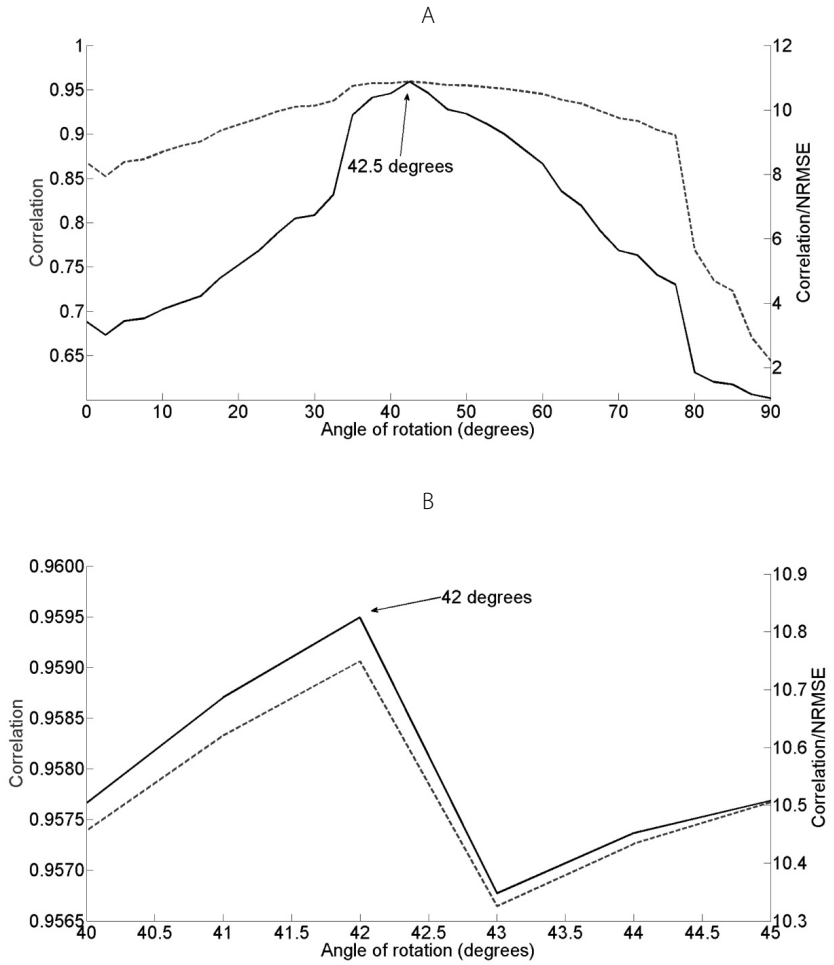


***Figure 3.*** *Correlations (dashed gray) and the ratio correlation/NRMSE (black) between the original and fingerprint obtained by adjusting the displaced electrode grid (A) roughly identified angle of rotation (42.5) as a peak of the ratio correlation/NRMSE and (B) pinpointed precise value of 42.*

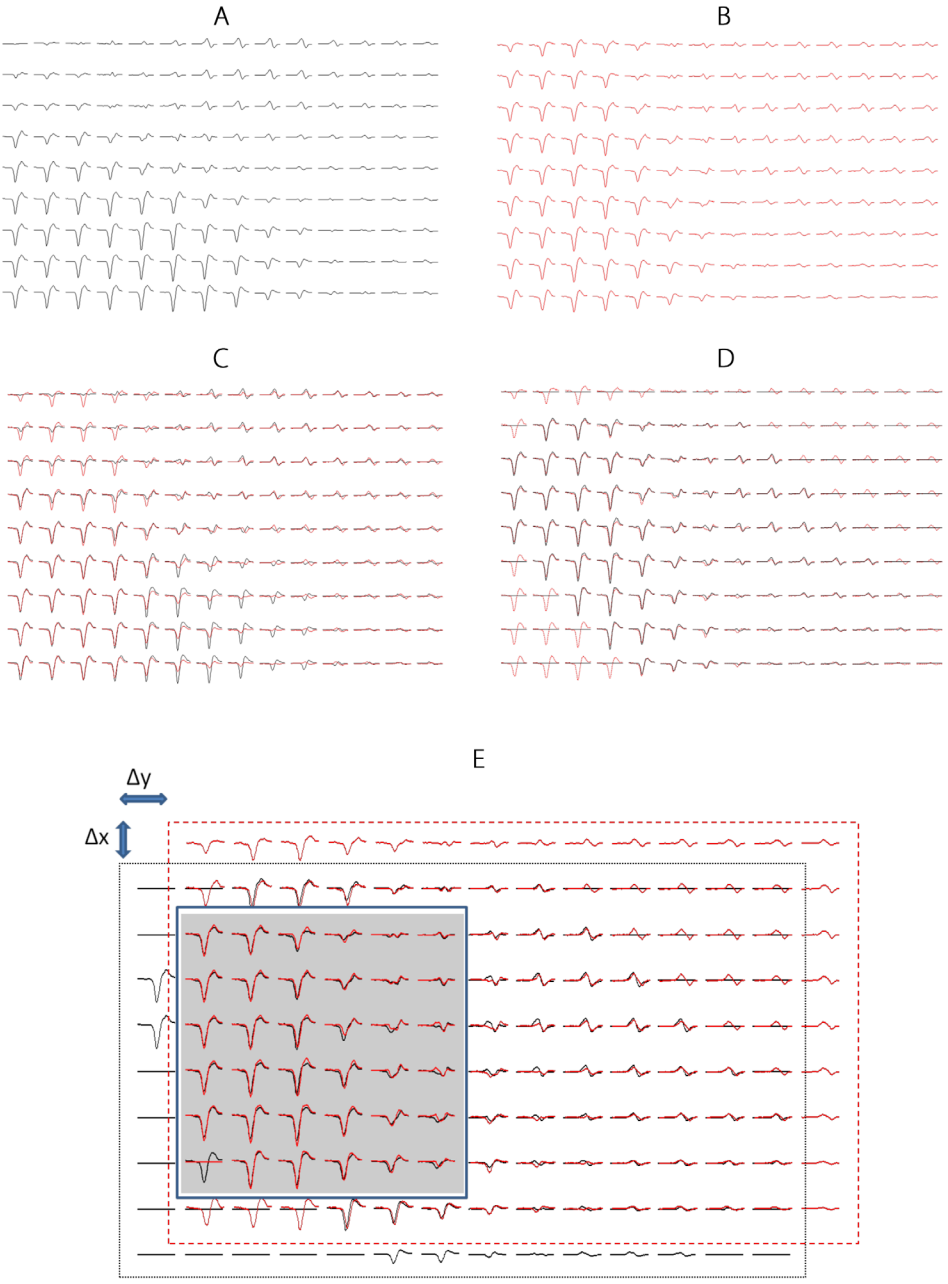


***Figure 4.*** *Matching the MUs from displaced grid recordings; (A) MUAP fingerprint obtained using grid rotated over 42° with respect to signature from (B); (C) overlaid signatures from (A) and (B) without applying correction spatial transforms; (D) overlaid signatures from (B, red) and (C, black) after transform; (E) matching corrected projection of (A) using the operators described in the text to identify matching indicated in (D); Gray area indicates the subgrid used to pinpoint the translation displacement.*

Angular precision could be tested on subject 2, where the same 2 fingerprints were found in two electrode displacement configurations. Searching for the joint peak of agreement revealed the angular ambiguity of 1.6 degrees. Table 2 provides ranges for angular and translational displacements. One should note that translational difference values (Table 2) could be provided up to the resolution of the adjustment step: 1/4 of the inter-electrode distance (IED) in both directions (1mm). Angular displacements should be observed with caution due to the previously indicated (but single case) angular ambiguity.



***Table 1.*** *Comparison of the fingerprints from measurements with displaced (rotated and translated) electrode grid, before and after applying the in-plane projection adjustment*

| Subject | Fingerprints | | Average correlation | |
|---|---|---|---|---|
| | Extracted | Trackable | Before correction % | After correction % |
| 1 | 51 | 8 | 86.3 | 92.4 |
| 2 | 39 | 6 | 84.0 | 93.4 |
| 3 | 30 | 3 | 82.2 | 90.0 |

***Table 2.*** *Ranges for measured displacements and comparison with the indicated values for all subjects*

| Subject | Average angular displacement difference* (°) | Translational difference** (mm) | |
|---|---|---|---|
| | | X direction | Y direction |
| 1 | (-1.1)-7 | 1-4 | 1-3 |
| 2 | (-2)- (-3) | 2-8 | 1-4 |
| 3 | 0.7-4 | 1-6 | 2-6 |

** provided as a range of values of differences between an indicated and calculated angles*
*** provided as a range of absolute values measured in different displacements*

## Discussion

Even when electrode re-placement between sessions is done with great care, repositioning errors may remain. Mitigating the effect of such errors may be expected to improve the reliability of ascribing changes in the MUAP to true (patho) physiological changes. Extrapolating the results of our pilot study with known displacements indicates how this may be achieved when the replacement error is not known.

Thus far, MU tracking using HDsEMG has been performed without corrections for recording grid displacement [1] or accounting for translational displacements only [3].This implied that correlations between identical but displaced MUAPs could be relatively low; indeed, the threshold for considering MUAPs to be similar was set to a correlation value of 80% [1, 3]. Being able to automatically adjust for re-placement errors might allow for a higher threshold, increasing the specificity. It may also simplify greatly the otherwise strict and time consuming procedure for placing the HDsEMG recording grid. Furthermore, in our study, correlations of > 80% did not necessarily mean fingerprints could be considered the same (data not shown) as was observed with visual inspections.

Therefore, we opted to add NRMSE to measure (dis)similarity as well. While correlation reflects similarity in shape, NRMSE addresses the relative residue when 2 shapes are subtracted from one another. This ratio therefore optimizes on matching criteria between MUAPs to minimize the detected similarity between objectively different shapes.

After adjusting the projection using rotational and translational operators, significant improvement in agreement was observable both visually and numerically via the correlation and NRMSE coefficients. Moreover, it was found that this method allows fine-tuning in the order of two degrees in angular direction in rotation and a quarter of the inter-electrode distance accuracy in translation. The correction procedure maximizes correlations between MUs in different sessions. Hard thresholding on these correlation parameters did not enable fully automatic matching in a sense that the visual confirmation on indicated "similar" MUs was still necessary. Further insight into methods for rotation and translation invariant pattern classification might provide more reliable measures for this purpose (e.g. [8]). To define the necessary agreement between fingerprints in order to consider them as originating from the same MU, the minimal disagreement between different MUAPs has to be described. Also, it is important to investigate and describe the changes that could be ascribed to the remaining modeling imperfections such as bending of the electrode grid, and changes in skin-electrode contact. These are likely to result with muscle-specific correction functions that take into account both the physiology as well as the overall geometry of the recording grid. An obvious starting point would be to include proven MUAP propagation models [9-11]. These would enable to differentiate between the physiological changes of the muscle, increase the method performance and define its exact limitations when comparing healthy MUs. This rule would help to maximize the applicability of MU tracking studies.

We addressed the group of complex hand thenar muscles. It is probable that MU tracking would prove much easier and applicable on other muscles and thus, enable qualitative increase in these tracking efforts.

Finally, this proof of concept study was demonstrated on the case of voluntary contractions, granting relatively small number of traceable MUs. This can be explained by several reasons: 1) low force voluntary contractions do not strictly guarantee activations of same MUs; 2) a number of MUs had to be omitted due to larger interference (overlap) with other MUs; and 3) shapes that could arguably belong to the same MUs did not sufficiently match, possibly due to model imperfections that disregarded bending of the grid for these very complex muscles. However, in real MU tracking practice, electrically stimulated recordings would likely take place instead of voluntary contractions. These would enable more precise tracking (accessing limited number of MUs at the same time) and eliminate the need for decomposition avoiding obstacles related to it.

We presented a method to track MUs across measurements more reliably when a recording grid is potentially not perfectly repositioned. The initial results show that the method reliably aligns MUs across sessions. This will allow MU tracking with higher precision than currently possible.

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# Chapter 4

## Identifying fasciculation potentials in motor neuron disease: a matter of probability

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submitted

## Abstract

*Introduction:*
Fasciculations, the spontaneous activity of single motor units (MUs) are a characteristic but nonspecific feature of motor neuron disease (MND). We aimed to identify MU discharge properties to optimally differentiate MND patients from healthy controls.

*Methods:*
High-density surface electromyography recordings were performed in the thenar muscles during 10 minutes of rest. MU discharges were detected and classified based on the interspike intervals as "isolated", "continual" or "other" for each active MU.

*Results:*
In patients ($n = 30$) compared to controls ($n = 14$), more MUs were active (9 vs 3, $p < 0.001$), generating relatively many isolated discharges (35% vs 10%, $p = 0.01$). Two or more MUs with isolated discharges occurred more frequently in patients compared to controls (24% vs < 1% of 10-second windows, $p < 0.001$).

*Conclusions:*
More frequent occurrence of different MUs showing isolated discharges (≥ 2 MUs per 10-sec window) allows improved identification of patients with MND.

## Introduction

Fasciculations are small, random movements of a muscle that can be present in various disorders of the peripheral nervous system as well as in healthy subjects [1-3]. However, widespread fasciculations are a prominent clinical feature in motor neuron disease (MND) and they are considered highly consistent with MND. The clinical relevance of fasciculations is underlined by their incorporation in the electrodiagnostic criteria of amyotrophic lateral sclerosis (ALS) [4]. As the electrophysiological equivalent of a fasciculation is a fasciculation potential (FP), the identification of FPs forms an essential part of the routine clinical EMG examination in MND. This procedure is complicated by the fact that FPs, as spontaneous activity of single motor units (MUs), can only be distinguished from other, non-pathological MU discharges by their firing pattern.



Currently, the identification of FPs relies on implicit knowledge (pattern recognition) of the electromyographer, acquired during his or her training as a medical specialist and in the practice of seeing many patients under expert supervision. Explicit, quantitative criteria that define an FP are lacking. FPs are often described as the spontaneous, irregular discharges of an individual MU [5-7], but then "irregular" is rarely specified. Previous studies have shown a wide range of discharge rates from only a few FPs per minute [8] up to discharge rates of 10 Hz, occasionally containing multiplet discharges [9, 10]. In addition, FP firing properties may closely resemble those of MUs that are voluntarily activated at low thresholds [11, 12]. Consequently, in clinical practice differentiation between normal and pathological (FP) discharges generally is difficult [13]. Yet, misclassification may impact diagnosis as well as the understanding of underlying pathophysiological mechanisms [9, 14].

In this study, we set out to determine which firing characteristics best set FPs apart from normal MU activity. Our first aim was to quantify the firing characteristics and abundance of all MU activity at rest for both patients with MND and healthy controls. This MU activity would include both FPs and some low-threshold voluntary activity due to incomplete relaxation. For this purpose, we used high-density surface EMG (HDsEMG) recordings because they are more comfortable for subjects and allow for longer and more stable recordings than needle EMG. Furthermore, this technique has a much larger pick-up area, which enables a more complete inventory of activity in a muscle. Second, we addressed the question how the observed firing properties can be used to optimally differentiate between MND patients and healthy subjects.

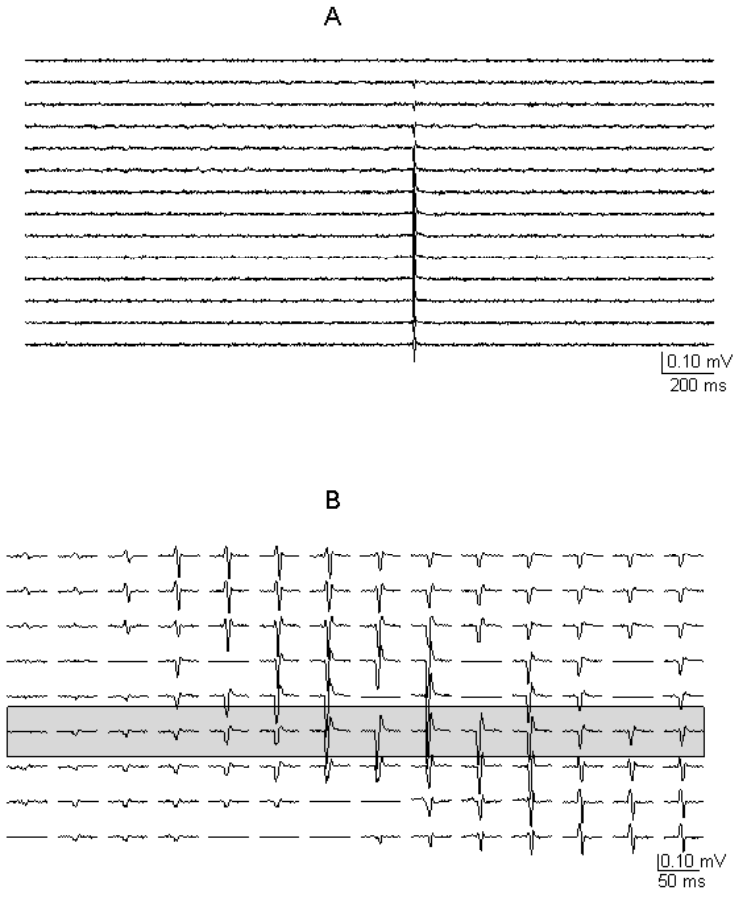


***Figure 1***. *(A) Example of an isolated discharge of an MU in a healthy subject. The signals correspond to a row selected from the 9 x 14 electrode grid. (B) Fingerprint of the same isolated discharge. The position of each signal in the profile corresponds to the position in the electrode array of the electrode with which the signal was recorded. Electrodes with poor skin contact are represented by flat lines. The gray rectangle illustrates the row of electrodes from which the signals in Figure 1A originate.*

## Materials and Methods

### *Subjects*

In total 30 patients (20 men, 10 women; median age 64 years, range 32 - 78 years; median disease duration since symptom onset, 17.1 months), clinically diagnosed with either probable or definite ALS (n = 20) or PMA (n = 10), participated in this study. Exclusion criteria were clinical symptoms and/or electrodiagnostic evidence of carpal tunnel syndrome. Our age-matched control group consisted of 14 healthy subjects (4 men, 10 women; mean age 65 years, range 41 - 74 years) without any clinical history of neurological disorders. The study protocol was in accordance with the principles of the Declaration of Helsinki and approved by the medical ethical committee of the Erasmus MC University Medical Center, Rotterdam, The Netherlands. All patients gave written informed consent.



### *MU discharge registration with HDsEMG recordings*

HDsEMG recordings were made using a 126-electrode high-density array attached to the skin over the thenar muscles of the non-dominant hand, in combination with an HDsEMG amplifier system (ActiveTwo, Biosemi, Amsterdam, The Netherlands) [15]. The reference electrode was attached to the dorsal side of the metacarpophalangeal joint of the second finger, and the ground electrode to the dorsum of the hand. The HDsEMG signals were sampled at 4096 Hz per channel, band-pass filtered (2 Hz - 500 Hz), and stored for further processing. The acquisition software allowed visual feedback of the EMG activity during the recordings (Fig. 1A).

Subjects were asked to take a comfortable position on a clinical examination bed with a pillow under their non-dominant hand and to relax their muscles. In case of excess EMG activity during the recording, patients were additionally instructed and/or helped to relax their muscles. Each recording lasted for 10 minutes (n = 41), except in three patients with recordings of 5 minutes (n = 2) and 8 minutes (n = 1). Recording for multiple minutes (instead of the 5-10 seconds that are common in clinical practice) increased the probability that infrequently firing MUs were captured.

### *Decomposition into discharges from single MUs*

The recorded HDsEMG signals were post-processed in Matlab (R2014a: The MathWorks, Natick, MA). The detection and classification of discharges from single MUs (MU action potentials, MUAPs) was performed using a decomposition algorithm previously described in detail [16]. This algorithm automatically assesses the number of clusters (present MUs) and assigns MUAPs to these clusters on the basis of shape similarity within a cluster and differences between clusters. Per MU that was active, the output of the algorithm provides a list of discharge times of this unit and a spatiotemporal profile ("fingerprint") of the MUAP over the electrode array (Fig. 1B). Next, extracted clusters were visually inspected and, if necessary, adjusted interactively. Clusters were merged when similarities in their fingerprints and a matching discharge pattern indicated they originated from the same MU. Clusters were subjected to re-classification if they contained misclassified MUAPs (MUAPs with fingerprints that were already assigned to another cluster). To ascertain that only true MUAPs (rather than artefacts)

were included, only MUs with more than 15 discharges in the 10-minute recording were accepted. Finally, all discharges from intervals that included the simultaneous activity of > 8 MUs were excluded, as these may have resulted from suboptimal muscle relaxation.

### *Quantification of MUAP firing pattern and discharge rate*

According to the definition of FPs by the American Association of Neuromuscular & Electrodiagnostic Medicine (AANEM), FPs most commonly occur sporadically as single FPs [17], implying that there is a relatively long interval before and after the MU discharge without a discharge of the same MU. Therefore, as a first approach, we calculated the interspike interval (ISI) before and after each discharge. Physiologically, as a lower boundary, voluntarily recruited MUs have an onset frequency of approximately 4 Hz [7] or, as its reciprocal, an ISI of 250 ms. We therefore considered discharges for which both the preceding and following ISI were equal to or less than 250 ms part of a spike train and indistinguishable from voluntary activity. Henceforth, these discharges will be referred to as *continual*. If both ISIs preceding and following a discharge exceeded 250 ms, this discharge was considered to meet the AANEM criteria of a single, sporadic discharge and will be referred to as *isolated*. Finally, if one ISI was larger than 250 ms and the other was smaller, a discharge was classified as *other*.

Single MU activity can now be characterized by a combination of these three discharge types. Sporadically firing FPs will likely be mainly dominated by isolated discharges. In contrast, voluntarily activated MUs are expected to be dominated by continual discharges. However, irrespective of the discharge types within a single MU, the absolute number of discharges per MU may vary extensively from only a few per minute to a large amount of discharges with firing rates in the range of voluntarily activated MUs. Therefore, the numbers of continual, isolated, and other discharges were expressed as percentage of the total number of discharges of an MU. This means that the sum of isolated, continual, and other MU discharges equals 100%. The joint ISI plot in Figure 2A illustrates these concepts, where the two gray areas correspond to the continual discharges (lower left) and the isolated discharges (upper right), and the two unmarked areas correspond to the other discharges (upper left and lower right).

As a second approach, we attempted to quantify the amount of MU activity at rest. The clinically observed abundant random muscle twitching in MND patients may originate from only a single MU with many discharges, but can also be due to discharges originating from multiple MUs. Rather than merely counting the total number of discharges per patient, we therefore defined the discharge load as the number of discharges of a single MU per minute. In addition, the emphasis is commonly placed on the sporadic nature of FPs and their supposedly abundant nature. Therefore, we quantified their ectopic behaviour by determining how many different MUs showed only isolated discharges. Since this number of MUs strongly depends on the duration of the recording, this analysis was performed by splitting the full 10-minute recording into 200, 60, and 20 consecutive recording windows with a duration of 3, 10, and 30 seconds respectively. Afterwards, in each recording window the number of MUs with only isolated discharges was determined.

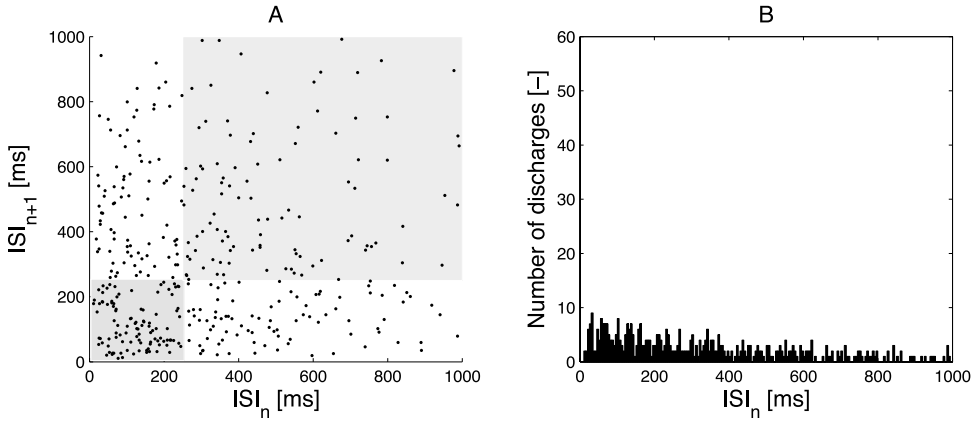


***Figure 2.*** *(A) Joint ISI plot, showing the preceding ISI ($ISI_n$) and following ISI ($ISI_{n+1}$) of the discharges from a single MU in an ALS patient, where the two gray areas mark the continual (lower left) and isolated (upper right) discharges, the two white areas mark the other (upper left and lower right) discharges, and (B) its corresponding ISI histogram. Note that isolated and other discharges are also present for $ISI_n$ and $ISI_{n+1}$ beyond 1000 ms.*

### *Statistical analysis*

All statistical analyses were performed using Matlab (R2014a: The MathWorks, Natick, MA). A p-value of $< 0.05$ was considered statistically significant. The Lilliefors method was used to assess normality. Data regarding discharge type were not normally distributed ($p < 0.05$). Therefore, those results are presented as median and percentiles. Differences between groups were tested by the Mann-Whitney U-test.

***Table 1.*** *Summary of motor unit discharge characteristics.*

| | MND patients median (25%-75%) | Healthy controls median (25%-75%) | p-value |
|---|---|---|---|
| Number of subjects | 30 | 14 | - |
| Total number of active MUs | 242 | 44 | - |
| Number of MUs (# per subject) | 9 (4 – 10) | 3 (1 – 4) | < 0.001 |
| Discharge load (# discharges / min) | 14 (7 – 53) | 25 (7 – 72) | 0.07 |
| MU action potential size (μV) | 28 (17 – 52) | 25 (16 – 35) | 0.16 |
| Firing rate (Hz) | 2.5 (0.6 – 7.4) | 6.0 (2.2 – 10.1) | 0.001 |

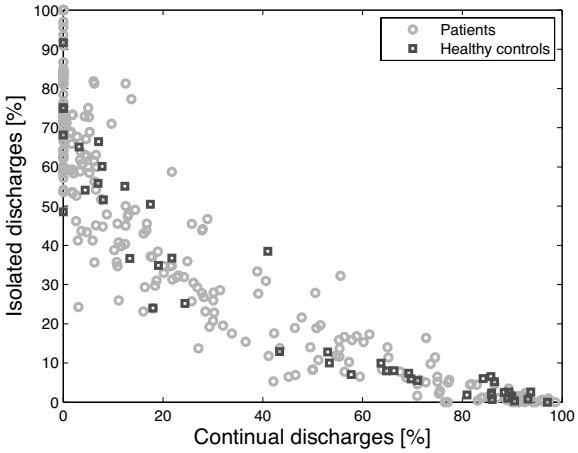


***Figure 3.*** *The percentage of isolated discharges versus the percentage of continual discharges per MU for all MUs with discharges in patients (circles) and healthy controls (squares). All combinations of continual and isolated discharges can occur in both patients and controls, including MUs with predominantly isolated discharges.*

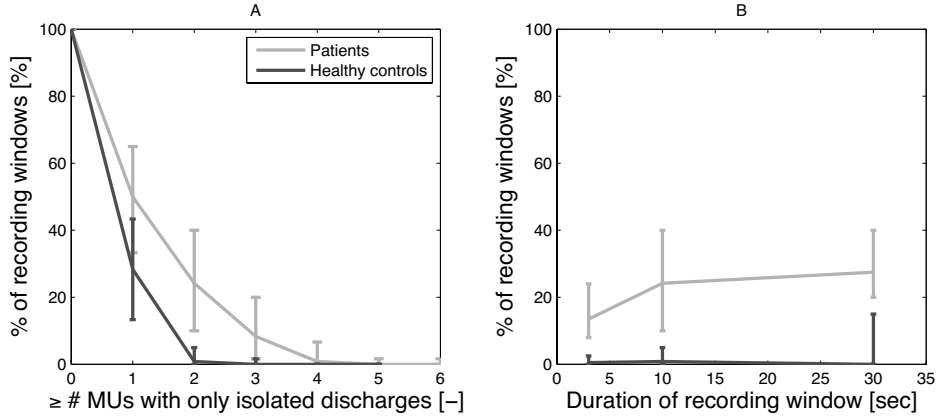


***Figure 4.*** *(A) Percentage of 10-sec recording windows versus the number of simultaneously active MUs in such a window for patients and healthy controls. Curves indicate median and error bars the interquartile range (25% - 75%). (B) The percentage of recording windows with a duration of 3, 10, and 30 seconds (simulated by dividing the full 10-minute recording in small segments) in which there are ≥ 2 MUs having only isolated discharges (light grey curve, patients; dark grey curve, healthy controls).*

## Results

Table 1 summarizes the characteristics of the observed MUs and their discharges in the 30 patients with MND and 14 healthy controls. The mean number of MUs that were active during the ten minutes of the recording was higher in patients with MND compared to healthy controls. The firing rate of these active MUs was lower in patients than in healthy controls, while the mean discharge load tended to be lower in patients than in healthy controls (Table 1). The same number of active MUs was found in ALS (n = 20) and PMA (n = 10) patients (9 vs 9 MUs, p = 0.71) and the discharge load was also similar (16 vs 14 discharges/minute, p = 0.27). To visualize the different firing patterns, Figure 3 shows the percentage of continual discharges versus the percentage of isolated discharges for each detected MU. This reveals a clear inverse relation, with firing patterns dominated by isolated discharges in the top left corner and those with predominantly continual discharges in the bottom right. Figure 3 also reveals that there are many MUs with a mix of both discharge types. More importantly, in controls the same inverse relation was observed as in patients, with several healthy MUs showing predominantly isolated discharges.

In MND patients compared to controls, MU discharges were classified more often as isolated (35% vs 10%, p = 0.01) or other (33% vs 27%, p = 0.04) and less frequently as continual (18% vs 54%, p = 0.003). Again, no significant difference was found between ALS and PMA patients.

Finally, Figure 4A shows the results for the analysis of the number of simultaneously active MUs with only isolated discharges, evaluated using a recording windows with a duration of 10 seconds. Two or more simultaneously active MUs with only isolated discharges were observed in 28 out of 30 patients with MND and in 24% (IQR: 10% - 40%) of the 10-sec recording windows. By contrast, this phenomenon was observed only sporadically in healthy controls (in 7 out of 14 subjects and in < 1% (IQR: 0% - 5%) of the recording windows). Figure 4B illustrates how the presence of ≥ 2 simultaneously active MUs with only isolated discharges expressed as percentage of the recording windows depend on the duration of the recording windows. The percentage of recording windows that showed ≥ 2 MUs with only isolated discharges was elevated in patients with MND compared to healthy controls for all recording window durations (3, 10, and 30 seconds), The probability that ≥ 2 MUs with only isolated discharges are detected first increases and then stabilizes for the duration of recording windows longer than 10 seconds. When using a 30-sec recording window, ≥ 2 simultaneously active MUs with only isolated discharges were present significantly more frequently in our patient group than in our healthy control group (MND: 28% of the 30-sec recording windows, IQR: 20% - 40%, vs healthy controls: 0%, IQR: 0% - 15%, p < 0.001).

## Discussion

In this study we set out to define criteria for identifying FPs, starting from the assumption that these criteria should be based on firing characteristics. Existing definitions suggested that FPs can be recognized by their sporadic nature [6, 17] or, alternatively, by specific interspike interval histograms [9]. In addition, a lower firing rate has also been suggested [18], which was observed in the current

study in patients with MND compared to healthy controls. What we further found is that firing patterns of active MUs in MND patients are more frequently dominated by isolated discharges and less frequently by continual discharges compared to controls. Furthermore, in patients more MUs are simultaneously active (Fig. 4). By combining these findings, our quantitative approach objectifies that isolated discharges are much more likely to occur in a patient than in a healthy subject. It implies that if a specific firing is to be called a FP (pathological discharge), such a judgement is a probabilistic one that requires taking into account the context of other MUs that show (or do not show) isolated discharges. Of course, other clinical and/or electrophysiological features (fibrillations, giant potentials) may also provide information which increases the a priori likelihood that isolated discharges are FPs, but difficulties may arise in the absence of such features [19].

These difficulties were encountered when examining only the discharge patterns of single MUs in this study. We found that it is not possible to identify firing patterns that are specific for pathology: various combinations of isolated, continual, and other discharges can be observed in relaxed muscles of both healthy subjects and MND patients (Fig. 3). More specifically and contrary to our expectations, in 7 out of 14 healthy subjects MUs were found with predominantly isolated discharges. Whereas in the patient group the discharges of the MUs in the relaxed muscle may have been both normal (incomplete relaxation) or pathological (FP) in nature, we consider it highly unlikely that the isolated discharges in the controls would have resulted from some unknown, underlying pathology in as many as seven healthy subjects. Hence, we have to conclude that fasciculating MUs in MND patients cannot be distinguished from normal MUs solely by their discharge characteristics. Furthermore, because the MU firing pattern, once correctly detected, is independent of recording technique, this conclusion appears equally valid for HDsEMG and needle EMG.

Subsequently, we tried to develop criteria that with a high probability could be ascribed to pathology and that make use of the fact that isolated discharges are much more common in patients. Figure 4 suggests that a criterion based on the number of simultaneously active MUs with only isolated discharges may meet this aim. Alternatively, the number of isolated discharges themselves (optionally expressed as percentage of the total number of discharges) might be used. Assessing whether or not there is sporadic activity of ≥ 2 different MUs appears very well feasible both with HDsEMG and with needle EMG. Such an approach finds further support in the work of Shiga et al. (2000), who demonstrated that two consecutive FPs originating from different MUs are pathognomonic for ALS [20]. The objective parameters for discharge types and number of MUs with only isolated discharges may be implemented relatively easily into an EMG system having an MU analysis program [21, 22].

Of course, the sensitivity, specificity, and optimally discriminating value for these new parameters depend on the muscle investigated, recording technique used, and recording duration. Spontaneous MU activity differs between muscles in patients as well as in healthy controls [23, 24], and for needle EMG, with its more limited uptake area than HDsEMG, the optimal value for the objective parameters are expected to be different. Therefore, further studies in other muscles and with needle EMG are required to determine appropriate normal values for the number of different MUs showing only isolated discharges to assess its diagnostic accuracy. With respect to recording duration, in general terms this duration should be as short as possible to make a clinically useful test, but sufficiently long

to allow detection of isolated discharges from multiple MUs. A previous HDsEMG study found that approximately 70 - 120 seconds are required to detect 1 - 5 FPs [25]. We evaluated the duration of the recording windows (3, 10, and 30 seconds) and noted that for windows longer than 10 seconds the percentage of recording windows for ≥ 2 MUs showing only isolated discharges stabilizes. In these longer windows, some MUs did not exclusively show isolated discharges but also some continual discharges and, therefore, were excluded. Our results thus indicate that a recording duration of 10 seconds usually suffices to detect ≥ 2 MUs with only isolated discharges in patients with MND. However, it should be noted that ≥ 2 MUs with isolated discharges was observed in only 24% of 10-sec recording windows. Therefore, a longer duration may be required when the occurrence of isolated MU discharges is low. Finally, in this study only healthy subjects were used for comparison. Therefore for further investigation it is very important to define how specific the criterion for the number of different MU with only isolated discharges is for patients with MND in relation to other disorders in which spontaneous MU discharges are frequently present.



To discriminate between isolated and continual discharges, in this study a threshold was set at 250 ms, based on the physiological lower limit of voluntarily recruited MUs. Shifting this threshold to slightly higher or lower values did not result in marked changes in the overall curve in Figure 3B (not shown). However, a crucial factor that does need to be taken into account in the interpretation of our data is to what extent the subjects were able to relax their muscles. The operator needed to ensure that the specific muscle under recording was relaxed as completely as possible. We attained this situation by positioning the subject comfortably on the examination bed and putting a pillow below the hand. Nevertheless, we cannot preclude that some MUs became spontaneously active due to non-pathological factors, such as incomplete muscle relaxation or unintentional contraction of the muscle [11]. Specifically, isolated discharges may have been induced by unintended subtle ballistic-type movements, which have already been reported to cause double discharges [26].

A final factor that must be taking into account is that some of the discharges in our ALS patients may have originated from low-threshold motor neurons recruited by the hyperexcitable corticospinal system and/or from abnormalities in the cortical inhibitory system (neuronal fasciculations) [9, 27-29]. The firing patterns of such neuronal fasciculations are basically indistinguishable from those of low-threshold voluntarily activated MUs. Hence, we believe it is generally not possible to determine the nature of continual discharges. With respect to isolated discharges, however, we conclude that it appears to be feasible to distinguish healthy from diseased muscles, but this requires that the identification of FPs be considered a probabilistic rather than a deterministic process by taking into account the activity of multiple MUs.

In conclusion, we present a potential clinically relevant method for objective quantification of MU discharges. Our results imply that in addition to solely assessing the presence of FPs and their abundance during routine clinical EMG, supplemental relevant information can be obtained by assessing the number of different MUs from which they originate. This especially can be helpful in the early stage of MND, or in clinically unaffected muscles, where FPs might be the only feature present. This information can potentially be used to aid in differentiating MND from other disorders.

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# Chapter 5

## Increased supernormality in patients with multiplet discharges: Evidence for a common pathophysiological mechanism behind multiplets and fasciculations

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## Abstract

*Objective:*
To determine whether there is a relation between electrically evoked multiplet discharges (MDs) and motor axonal excitability properties. We hypothesized that electrically evoked MDs share their underlying pathophysiological mechanism with fasciculations.

*Methods:*
High-density surface EMG and motor nerve excitability recordings of the thenar muscles were performed in 22 patients with motor neuron disease (MND) in their differential diagnosis and who were referred for EMG examination.

*Results:*
Supernormality (hyperexcitable phase following the refractory period) was significantly increased in patients with MDs (n = 10) compared to patients without MDs (n = 12) (25.5% vs 17.0%; p = 0.02). Depolarizing threshold electrotonus differed significantly between both groups as well (TEdpeak, 76.6% vs 66.6%, p < 0.01; TEd90-100ms, 51.7% vs 44.3%, p < 0.01).

*Conclusions:*
Our findings imply that the same pathophysiological excitability changes are involved in generating MDs and fasciculations. Yet, MDs may be quantified more easily, and may be more specific for abnormal distal excitability than fasciculations, because fasciculations may originate along the motor axon as well as in the neuron cell body.

*Significance:*
MDs are potentially useful as objective measure of increased distal axonal excitability at individual motor unit level and might complement clinical studies in MND.

## Introduction

Altered motor axonal excitability is commonly suggested to provide a mechanism for the generation of fasciculations [1-3]. In patients with motor neuron disease (MND), where fasciculations are an important clinical feature, several studies have observed changes in axonal excitability as evidenced by an increased supernormality, elevated depolarizing threshold electrotonus, and an increase in the strength-duration time constant (SDTC) [2, 4, 5]. These changes are believed to originate from increased sodium and reduced potassium channel conductance in the distal segments of the motor axon [2-4, 6].

Altered excitability properties may also explain the occurrence of electrically elicited multiplet discharges (MDs), which were observed in MND patients in a previous high-density surface EMG (HDsEMG) study [7]. Detailed analysis of these distally evoked MDs showed that their spike intervals were restricted to the supernormality period. This suggests that MDs result from altered axonal excitability in much the same way as fasciculations of distal axonal origin [8].

If electrically elicited MDs and fasciculations do indeed arise from the same membrane instability, this may imply a similar diagnostic significance in MND patients [9, 10]. However, whereas fasciculations appear at random and may originate at various anatomical sites including the cell body [8, 11-15], MDs have the benefit of being highly localized (in the most distal part of the axon) and under control of the investigator. As yet no relation has been established between MDs and the above mentioned motor nerve excitability changes associated with the generation of fasciculations. Assessing this relation was the purpose of the present study.

## Materials and Methods

### *Patients*

Twenty-two patients (17 men, 5 women; mean age 62 years, range 32 - 78 years; median disease duration from symptom onset to recording 15.0 months) participated in this study. All patients had MND in their differential diagnosis and after progression of symptoms, were classified according to the revised El Escorial criteria as probable or definite amyotrophic lateral sclerosis (ALS) (n = 15) or progressive muscular atrophy (PMA) (n = 6). One patient was initially diagnosed with PMA, but due to atypical progression of symptoms, some indication of conduction block, and the absence of a clinical response to repeated intravenous immunoglobulin (IVIg), a differential diagnosis of multifocal motor neuropathy (MMN) could not be excluded. Therefore, this patient will be further referred to as "undetermined".

All patients were recruited through our neuromuscular outpatient clinic at the time they were referred for an EMG examination. Both MD registration and motor nerve excitability testing were restricted to the thenar muscles (cervical region). Hence, the scoring (presence or absence) of fasciculation potentials (FPs) recorded by needle EMG during the EMG examination from muscles only in this region was considered as relevant for this study. Patients with clinical symptoms and/or electrodiagnostic evidence of carpal tunnel syndrome were excluded. The median time interval

between HDsEMG and motor nerve excitability recordings in the 22 patients was 4.5 weeks. We used control data from 29 normal subjects (21 men, 8 women; mean age 39 years, range 23 - 58 years), which was available through the Qtrac software [16]. The experimental protocol was approved by the medical ethical committee of the Erasmus MC. All patients gave written informed consent.

### *Motor nerve excitability testing*

Nerve excitability was assessed using the QTRAC-S software package (TROND-F, version 20/01/2010, Institute of Neurology, London, UK). The cathode of the stimulator (Red dot electrode; 3M Health care) was fixed at the level of the wrist over the median nerve; the anode was placed approximately 15 cm more proximally on the ulnar side of the forearm. The active recording electrode was attached to the skin over the abductor pollicis brevis muscle (APB) and the reference electrode was placed over the interphalangeal joint of the thumb. A ground electrode was placed at the base of digits 3 and 4. Skin temperature was kept > 30 °C and measured near the stimulation site during the study.

The protocol consisted of five tests. In short, in the initial test, a stimulus-response curve was derived and used to set a target response (40% of maximum CMAP amplitude) for the other four excitability tests (strength-duration, recovery cycle, threshold electrotonus, and current-threshold). In the strength-duration test, the change in stimulus intensity required to reach the target response was obtained for different stimulus durations. To assess the recovery cycle, after every supramaximal conditioning stimulus a test stimulus was applied at varying time intervals to determine the changes in axonal excitability. In the threshold electrotonus and current-threshold (I/V) relationship tests, a conditioning stimulus was applied to depolarize or hyperpolarize the axons. In general, a depolarizing current increases the excitability of an axon, decreasing the strength of the subsequently applied test stimulus that is necessary to elicit the target response. A hyperpolarizing current has the opposite effect. The strength of the test stimulus required to elicit the target response is automatically adjusted. By varying the intensity and duration of the conditioning stimulus and by applying test stimuli at varying intervals during or just after the conditioning stimulus, axonal membrane properties can be characterized [17, 18].

### *MD registration with HDsEMG recordings*

To detect MDs we used a 126-electrode high-density array attached to the skin over the APB in combination with an HDsEMG amplifier system (ActiveTwo, Biosemi, Amsterdam, The Netherlands) [19]. The reference electrode was attached to the dorsal side of the metacarpophalangeal joint of the second finger, the ground electrode to the dorsum of the hand. The stimulator (circular felt-pad electrodes, diameter of 5 mm) was positioned over several sites along the median nerve, all in vicinity of the site of the cathode in the motor nerve excitability tests. At each site, stimulus intensity was gradually increased until a few (usually 4 to 6) MUs were activated, according to the visual feedback of the response on HDsEMG. Since most MUs that generate MDs are known to do so in response to only a small percentage of applied triggers, many stimuli may need to be administered to elicit and detect an MD. Therefore, subsequently 500 stimuli (2 Hz, 0.1 ms) were applied at each site, and

responses were recorded. In this way, up to approximately 20 different motor unit action potential (MUAP) profiles were collected per patient. Finally, the maximum CMAP amplitude was recorded and divided by the mean of the collected MUAPs to derive a motor unit number estimate (MUNE), as described in more detail elsewhere [20].

MD analysis was performed by post-processing the HDsEMG recordings in Matlab (R2013a: The MathWorks, Natick, MA) using previously described decomposition software [20, 21] that was slightly adapted to facilitate MD analysis. In contrast to single-channel surface or needle EMG, HDsEMG presents a MUAP as a spatiotemporal profile. The extra spatial MU information provided by the array of electrodes aids the recognition of single MUAPs during decomposition. The result of this analysis is an overview, listing per stimulation site, per MUAP profile found, and for each of the 500 applied triggers whether or not the MU corresponding to a MUAP profile was activated by the trigger. Not all MUs were activated all 500 times, due to the probabilistic nature of MU activation near the activation threshold.



Subsequently, the decomposed recordings were checked visually for the presence of MDs according to the following three criteria [7]. First, any extra discharge needed to have an identical spatiotemporal profile as the MUAP M-wave (direct response). Second, the M-wave with the extra discharge needed to be identical to the regular M-wave without extra discharge. Last, the extra discharge should occur within a time interval of 20 ms following the M-wave (Fig. 1). For every MU that generated at least one MD, the number and type of MDs (doublet, triplet, etc) was established. Other variables that were determined included the total number of MUs showing MDs, the absolute number of MDs, and the relative number of MDs as percentage of the total number of M-waves.

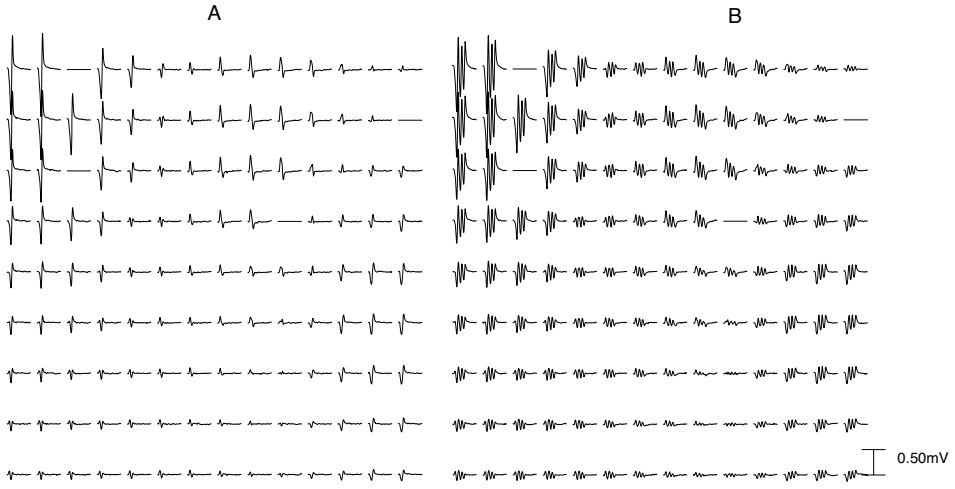


***Figure 1.*** *Example of a spatiotemporal MUAP profile recorded with the 9x14 high-density electrode array and evoked after a single stimulus in an ALS patient (A). The same MUAP showed a triplet evoked after a single stimulus during the same recording (B). The position of each signal in the profile corresponds to the position in the electrode array of the electrode with which the signal was recorded.*

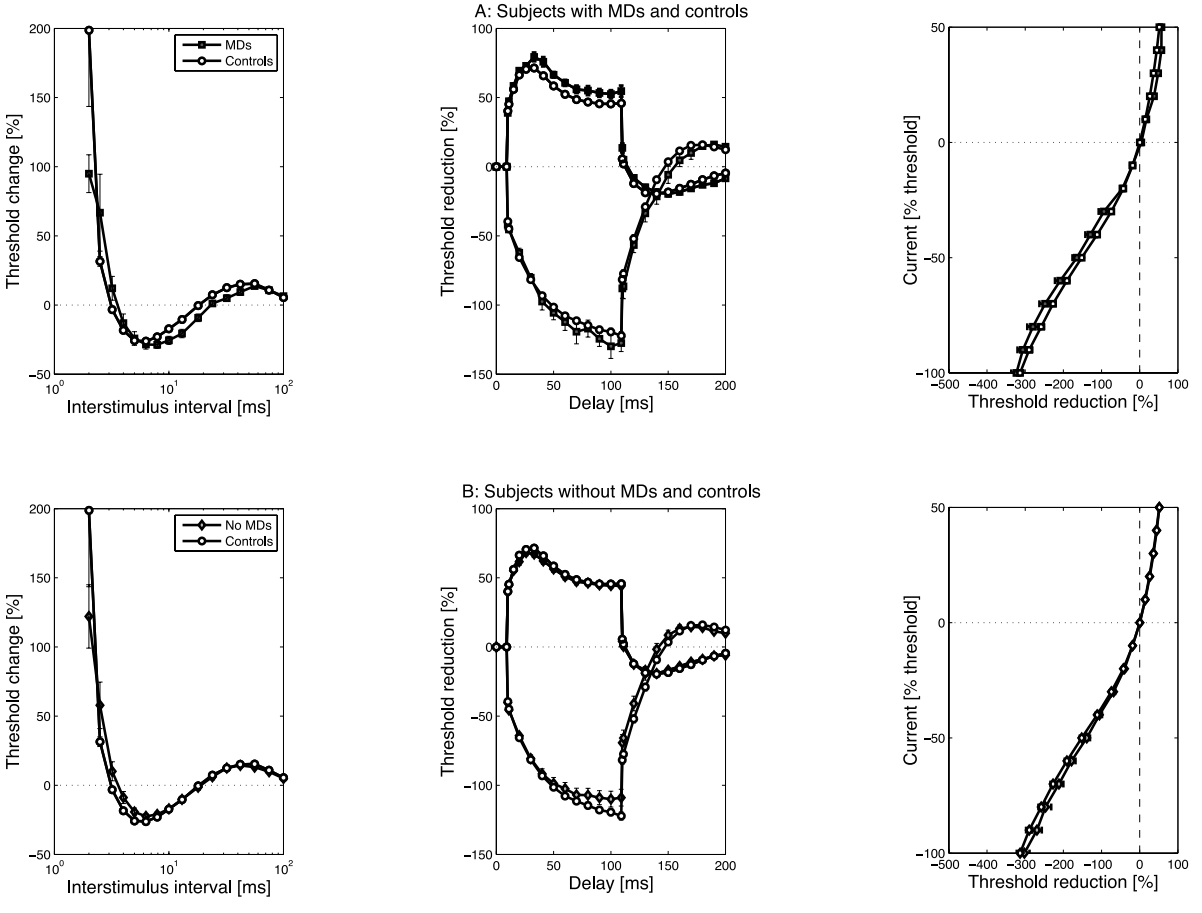


***Figure 2.*** *Illustration of the recovery cycle (left), threshold electrotonus (middle) and current-threshold relationship (right) for (A) patients showing multiplet discharges (MDs; black square, n = 10), and (B) patients not showing MDs (gray diamond, n = 12), both with healthy controls (open circle, n = 29). Curves are shown as mean ± SEM. Note that for visualization curves were not adjusted for age and temperature.*

In some of our patients, the disease had progressed further than in others. In more advanced MND, fewer MUs will have survived, which might lead to a reduction in the number of MDs per recording. Yet, disease progression may also lead to more pronounced excitability changes and, hence, to more MDs. To investigate these effects, we determined the number of MDs per surviving MU, by dividing the number of MDs by the MUNE, and correlated this number with supernormality.

### *Statistical analysis*

Statistical analyses were performed for the strength duration time constant (SDTC), supernormality at 10 ms, and for the depolarizing threshold electrotonus variables TEdpeak and TEd90-100ms. Data are shown as mean ± standard error of the mean (SEM). Because excitability is influenced by age and temperature, the data of the control subjects were age- and temperature-adjusted according to previously established relations [16, 22] before statistical analysis to be able to compare them with our measurements. The Shapiro-Wilk test showed that the data was normally distributed. One-way ANOVA was used to determine differences between group means and Bonferroni post-hoc testing was applied when comparing differences between multiple groups. The relationship between supernormality and MDs per surviving MU was analyzed using a Spearman's rank correlation test. Statistical tests were performed using Matlab (R2013a: The MathWorks, Natick, MA) or SPSS (Version 21.0, Inc., Chicago, IL). A p-value of $< 0.05$ was considered statistically significant.



## Results

MDs were found in 10 patients (7 ALS, 2 PMA, 1 undetermined). The number of motor units (MUs) showing MDs varied from 1 to 5 per patient, with a study total of 21 MUs showing 135 MDs. The MDs were observed following 1.9% of the stimuli that triggered M-waves of these 21 MUs. Most of the MDs were doublets (133 of 135). In one ALS patient triplets were found (2 of 135). The occurrence of MDs in the thenar muscles was not related to the disease duration (symptom onset to recording) of the patients. To what degree the thenar muscles were involved by the disease was expressed by the number of functional MUs estimated by HDsEMG MUNE, which varied from 26 up to 311 with a median of 159. MD registration was restricted to the thenar muscles (cervical region). In this region, FPs were present in 18 patients (13 ALS, 4 PMA, 1 undetermined). One ALS patient showed MDs in the absence of FPs.

Figure 2 illustrates the results of the recovery cycle, threshold electrotonus, and current-threshold relationship tests for patients with electrically evoked MDs, patients without electrically evoked MDs, and healthy controls. Further statistical analysis showed no significant difference in SDTC between patients that showed MDs and patients without MDs (0.46 ± 0.02 ms vs 0.48 ± 0.03 ms, $p = 1.00$). In the recovery cycle, the supernormality at 10 ms was significantly increased in patients with MDs (25.5 ± 2.9% vs 17.0 ± 2.1%; $p = 0.02$, Fig 2A) when compared to patients without MDs. The depolarizing threshold electrotonus (TEd) variables also differed significantly between these two subgroups (TEdpeak, 76.6 ± 2.6% vs 66.6 ± 1.7%, $p = 0.001$; TEd90-100ms, 51.7 ± 2.0% vs 44.3 ± 1.5%, $p = 0.003$;

Fig 2B). The undetermined patient showed large supernormality at 10 ms (39.9%) and fanning out in the threshold electrotonus test (TEd90-100ms = 59.0% and TEh100 = -173.0%). Furthermore, axonal degeneration was also present in this patient as the number of MUs was markedly reduced (MUNE = 27).

As ALS and PMA are often regarded to be part of the same clinical spectrum [23, 24] and since we did not find any excitability differences between these subgroups, we merged them for the comparisons with the healthy controls. When the 21 ALS and PMA patients were thus compared to the healthy controls (n = 29), a significant difference was found for the SDTC (0.47 ± 0.02 ms vs 0.40 ± 0.02 ms; p = 0.01), the supernormality at 10 ms (20.0 ± 1.8% vs 13.0 ± 1.1%, p = 0.001), and for the depolarizing threshold electrotonus (TEd) variable TEdpeak (70.2 ± 1.7% vs 66.1 ± 0.9%, p = 0.02). TEd90-100ms (47.1 ± 1.4% vs 44.3 ± 0.8%, p = 0.06) was not significantly different. Finally, when the number of MDs was normalized for the number of functional MUs present (to compensate for axonal degeneration), a significant correlation was found between supernormality at 10 ms and the number of MDs (as percentage of the number of stimuli applied) per MU (r = 0.47; p = 0.03).

## Discussion

In this study, we used two electrophysiological methods, one measuring excitability properties directly and the other addressing ectopic axonal activity, to clarify the pathophysiological mechanisms in patients showing electrically evoked MDs. Our results demonstrate that axonal excitability is increased in patients in whom MDs can be found but not in patients without MDs.

The differences in excitability properties between healthy controls and the 21 MND patients that we observed are consistent with previous findings [2, 4-6]. The prolonged SDTC reflects increased persistent Na+ channel conductance. An increased supernormality can result from either membrane hyperpolarization or a reduction in K+ conduction [2, 4]. Of these two possibilities, the latter seems to be the most likely explanation, as membrane hyperpolarization could only partly account for the observed excitability changes. Furthermore, it has been shown in previous studies that a reduced $K^+$ conductance is associated with the occurrence of fasciculations [2, 4-6]. Due to the reduced $K^+$ conductance, an imbalance may arise between the inward sodium and outward potassium currents. This imbalance can result in axonal hyperexcitability, increasing the probability that an axon generates a spontaneous discharge (fasciculation).

Our present findings demonstrate that the altered excitability properties which have been suggested to result in fasciculations are also related to the presence of electrically evoked MDs. MDs in MND have a distal origin and they probably originate at the terminal branches, which is also thought to be the trigger site of distal fasciculations [3, 7, 8]. In line with this view, after blocking of presynaptic $K^+$ channels, a single electrical trigger has been shown to be able to induce repetitive discharges [25, 26]. Hence, this implies that a single mechanism (a reduced K+ conductance associated with axonal hyperexcitability that increases the probability of spontaneous activity) may explain both fasciculations and MDs.

In this context, it is interesting to look in some more detail at the single included patient showing electrically elicited MDs with the as yet undetermined diagnosis as our aim was to establish the relation between altered motor axonal excitability and electrically elicited MDs. This patient who met the inclusion criteria had a high supernormality, fanning out, and a small number of functional axons. Previously, such excitability changes in MMN patients have been ascribed to membrane hyperpolarization just distal from the conduction block [27]. It was suggested that at the level of the lesion, the membrane becomes depolarized due to an increase in extracellular $K^+$ concentration. At the same time, surrounding segments become hyperpolarized, as the result of increased, compensatory electrogenic pump activity. This effect of local membrane de- and hyperpolarization was associated with fasciculations [28, 29]. Since our undetermined patient also showed MDs, again we may tentatively assume a common mechanism here. This implies that these excitability changes could also be evaluated on a single motor axonal level by the detection of electrically evoked MDs in a controlled and objective manner.

Fasciculations are well established in the diagnosis of MND in current practice recommendations [9, 30]. They are thought to arise both from central regions, at the motor neuron cell body, and at the motor axon [8, 11-15]. Spontaneous (not electrically activated) MDs, just as fasciculations, are known to occur in MND patients but are also relatively common in healthy subjects [31-34]. With respect to MDs in healthy subjects, several studies have suggested a proximal (motor neuronal cell body) rather than axonal origin [31, 35, 36]. Furthermore, we have previously studied electrically evoked MDs and did not observe any in 10 healthy subjects and a total of 300 MUs [7].

Based on this collective evidence, we tentatively assume that spontaneous activity (whether fasciculations or MDs) originating proximally can be normal, whereas distally generated ectopic discharges are usually abnormal. If so, an important clinical implication would be that diagnostic approaches that can selectively address ectopic discharges that originate in the distal axon might have a higher specificity for abnormal motor axonal function. In general, registration of fasciculations does not allow discrimination as to their origin unless highly specialized techniques to determine the firing pattern are used [8]. By contrast, with electrically elicited MDs we are able to study distal excitability changes in isolation.

Registration of MDs as applied in the current study has as another advantage that MDs may be quantified more easily: the number of applied stimuli is known and under the control of the operator. This makes our approach a potentially valuable, objective addition to current electrodiagnostic tests. Furthermore, MD registration focuses on single, possibly affected MUs, whereas excitability tests study groups of axons amounting to 40% of the functional axons present. In earlier phases of the disease, abnormalities in excitability testing can be obscured due to a relative low number of affected MUs included in the 40% CMAP target response. Therefore, MDs are potentially more sensitive to observe pathophysiological changes in the early phase of MND, when only a few MUs are affected. Still, the probability that an electrical stimulus elicits an MD remains small. This is probably why not all ALS and PMA patients showed MDs. Considering the previously established relation between excitability properties on the one hand and disease progression and prognosis in ALS on the other [5, 37], further investigation seems warranted.

A limitation of the present study is that the excitability recordings were made at the level of the wrist whereas MDs in MND originate more distally, at the level of the nerve terminal branches [7]. Hence, the two tests address different sections of the axon and as the biophysical properties at the nerve terminal branches differs, this difference influences the excitability measures [3, 38]. Excitability studies focused on a more distal part of the motor axon could provide further insight into the genesis and relation between electrically evoked MDs and FPs. However, as excitability tends to be most abnormal at the most distal site, our study design probably results in under- rather than overestimation of the relation between MDs and axonal excitability.

Second, we recognize that our use of the Qtrac data as normal control set may appear to be a limitation of the study. However, we did collect a control data set prior to and with test conditions as in our current study, which showed no significant deviation from the Qtrac data. We chose to use the Qtrac data as control set because it is widely available in the research community and hence, its use in a possible replication study would eliminate one source of potential differences. We also believe that setting our patient data against a "gold standard" facilitates future comparisons of these data with other findings.

Another factor that may have influenced our results is the prescription of Riluzole (100 mg/day) to all ALS and PMA patients at the time of the excitability tests. Recent findings suggest that administration of Riluzole results in a decreased supernormality and a non-significant reduction in depolarizing threshold electrotonus [39]. If Riluzole normalizes excitability, this factor probably does not affect our main conclusion, because our findings would likely have been more explicit when we had examined the patients prior to using this drug.

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# Chapter 6

## Diagnostic accuracy of electrically elicited multiplet discharges in patients with motor neuron disease

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## Abstract

*Objective:*
To determine and compare the diagnostic accuracy of electrically elicited multiplet discharges (MDs) and fasciculation potentials (FPs) in motor neuron disease (MND).

*Methods:*
Patients were eligible when they had MND in their differential diagnosis and were referred for electromyogram (EMG). Stimulated high-density surface EMG of the thenar muscles was performed on the same day as standard EMG examination. High-density recordings were analysed for presence of MDs and needle EMG of any muscle investigated in the cervical region for presence of FPs.

*Results:*
Of the 61 patients enrolled in this diagnostic study, 24 patients were clinically diagnosed with amyotrophic lateral sclerosis (ALS) and 11 patients with progressive muscular atrophy (PMA). Another diagnosis was made in 26 patients. Sixteen patients in whom MDs were detected were diagnosed with either ALS (n = 11) or PMA (n = 5) (sensitivity = 47.1%, PPV = 94.1%). MDs were detected in only one patient initially diagnosed with PMA, but in whom later on multifocal motor neuropathy could not be excluded (specificity = 96.2%). Electrically elicited MDs had a higher specificity than FPs (96.2% vs 53.9%, $p < 0.001$, n = 26) and lower sensitivity (47.1% vs 85.3%, $p = 0.002$, n = 34). When considering presence of MDs in MND as neurogenic EMG abnormality, lower motor neuron involvement of ≥ 1 EMG region increased from 50.0% to 73.5% ($p = 0.008$, n = 34).

*Conclusions:*
Electrically evoked MDs are highly specific for ALS and PMA and are an early sign of lower motor neuron dysfunction.

## Introduction

Establishing the diagnosis of amyotrophic lateral sclerosis (ALS) or progressive muscular atrophy (PMA) tends to be a challenging task due to a clinically heterogeneous presentation and the absence of a reliable biomarker. Owing to the disastrous course of these forms of motor neuron disease (MND), accurate and early diagnosis is of importance. Yet, currently the diagnostic delay is about 12 months [1]. According to the latest electrodiagnostic criteria [2], neurophysiological evidence of lower motor neuron (LMN) dysfunction should be considered equivalent to clinical evidence, which underlines the importance of the electrodiagnostic examination. Any electrophysiological measure that enhances the detection of LMN dysfunction early in the diagnostic phase can, therefore, potentially improve the diagnostic process in MND.

Electrically elicited multiplet discharges (MDs) may be such a promising measure. The registration of MDs has been introduced recently as an approach to study excitability changes in the distal part of the axon [3]. MDs are thought to result from the same pathophysiological mechanism as fasciculations of distal origin and may, therefore, have similar diagnostic significance [4]. Fasciculations may also arise at the soma, however, and spontaneous activity of the soma, whether in the form of fasciculations or MDs, is relatively common even in normal conditions [5,6]. By contrast, distal spontaneous activity in healthy subjects appears to be rare [3]. Hence, we hypothesized that the specificity of distally evoked MDs for ALS and PMA might be higher than that of fasciculations and set out to assess the diagnostic accuracy of electrically elicited MDs and fasciculations following the STARD guidelines [7].



## Materials and methods

### *Patients*

Patients were eligible for this study when they had MND in their differential diagnosis and were referred by the neuromuscular outpatient clinic of the Erasmus MC Rotterdam to its clinical neurophysiology department for an EMG examination. MND did not have to be the most likely diagnosis, as any suspicion on clinical grounds sufficed for inclusion. The study was approved by the medical ethical committee and all participating patients gave written informed consent. Patients were recruited as part of a larger high-density surface EMG (HDsEMG) study [4], where exclusion criteria were clinical symptoms and/or electrodiagnostic evidence of carpal tunnel syndrome.

### *MD registration with HDsEMG recordings*

HDsEMG recordings were performed to obtain electrically evoked MDs as previously described [3]. HDsEMG obtains temporal and spatial information from an array of 9x14 electrodes spaced densely over the muscle [8-10]. As a result, it allows collection of a larger sample of different motor unit action potentials (MUAPs) than single-channel EMG techniques, and aids the recognition of single MUAPs and corresponding MDs if present.

The HDsEMG recordings were made with the array attached to the skin over the thenar muscles of the non-dominant hand. The reference electrode was attached to the metacarpophalangeal joint of the second finger on the dorsal side and the ground electrode to the dorsum of the hand. A sample of up to approximately 20 different single MUAPs was collected by positioning a stimulator over several sites along the median nerve. At each site, the stimulus intensity was gradually increased until a few (usually 4 to 6) motor units (MUs) were activated, according to the visual feedback of the responses on HDsEMG. Since, most MUs that generate MDs are known to do so in response to only a small percentage of applied triggers, many stimuli may need to be administered to elicit and detect an MD. Therefore, subsequently 500 stimuli (2 Hz, 0.1 ms) were applied at each site, and responses were recorded.

An MD analysis was performed by post-processing the HDsEMG recordings in Matlab (R2012a: The MathWorks, Natick, Massachusetts, USA) using previously described decomposition software [9,10], that was slightly adapted to facilitate MD analysis. The result of this analysis showed per MUAP profile found, whether or not this MUAP was activated by each of the 500 applied triggers (due to the probabilistic nature of MU activation near the activation threshold, not all MUs were activated all 500 times). In addition, for every MUAP that generated at least one MD, the number and type of MDs (doublet, triplet, etc) were established by criteria described elsewhere [3] and registered.

### *Study design*

To detect electrically elicited MDs, high-density surface EMG recordings were performed on the same day as the standard EMG examination, which included detection and scoring of fasciculation potentials (FPs). An expert assessor conducted the HDsEMG recordings blinded to the results of the EMG examination. A resident neurologist performed and evaluated nerve conduction studies and needle EMG under direct supervision of an experienced clinical neurophysiologist. The EMG examination protocol was based on the differential diagnosis and adapted to EMG findings during the examination, and hence, differed between patients. For the same reason, the number of investigated muscles also varied between patients. Since MD registration was restricted to the thenar muscles (cervical region), only the presence or absence of FPs from muscles in the cervical region was considered relevant for the current comparison with electrically evoked MDs. The shape and complexity of FPs were not evaluated for the present study.

The clinical diagnosis of ALS or PMA was made by an consultant neurologist specialized in neuromuscular disorders, based on the symptoms and progression of the disease, neurological examination and the results from routine tests, including EMG, MRI, and blood tests that were predominantly performed to exclude other disorders that could explain the clinical symptoms. The revised El Escorial criteria were applied to categorize the patients with MND as to diagnostic probability [11]. The consultant neurologist was blinded to the HDsEMG results. Information regarding the neurological examination and patient characteristics was obtained from each patient's history. The vital capacity (VC) and ALS functional score (ALSFRS-R) were measured on the same day as the EMG examination. The ALSFRS-R (ALS functional rating scale – revised) is a questionnaire yielding

a maximal score of 48 (representing normal functioning) that assesses various aspects of activities of daily living over four subcategories (subscores): bulbar, fine motor, gross motor, and respiratory [12]. Finally, the clinical diagnosis was reviewed and, if necessary, revised after several months of progression of symptoms. Using this clinical diagnosis as reference standard, we determined and subsequently compared the diagnostic accuracy of electrically evoked MDs and fasciculations.

### *Statistical analysis*

Statistical analysis was conducted using Matlab (R2012a: The MathWorks, Natick, MA). Differences between groups were tested by the Mann-Whitney U-test or Student t-test. The Kolmogorov-Smirnov method was used to assess normality. Data is presented as either mean and standard deviation (SD) or median with interquartile range (IQR). The diagnostic accuracy of MDs and fasciculations was investigated using 2x2 contingency tables. To compare the proportions of patients in whom the number of affected EMG regions increased when MDs were used as additional electrophysiological parameter, the McNemar exact test was applied. A p-value of $< 0.05$ was considered statistically significant.

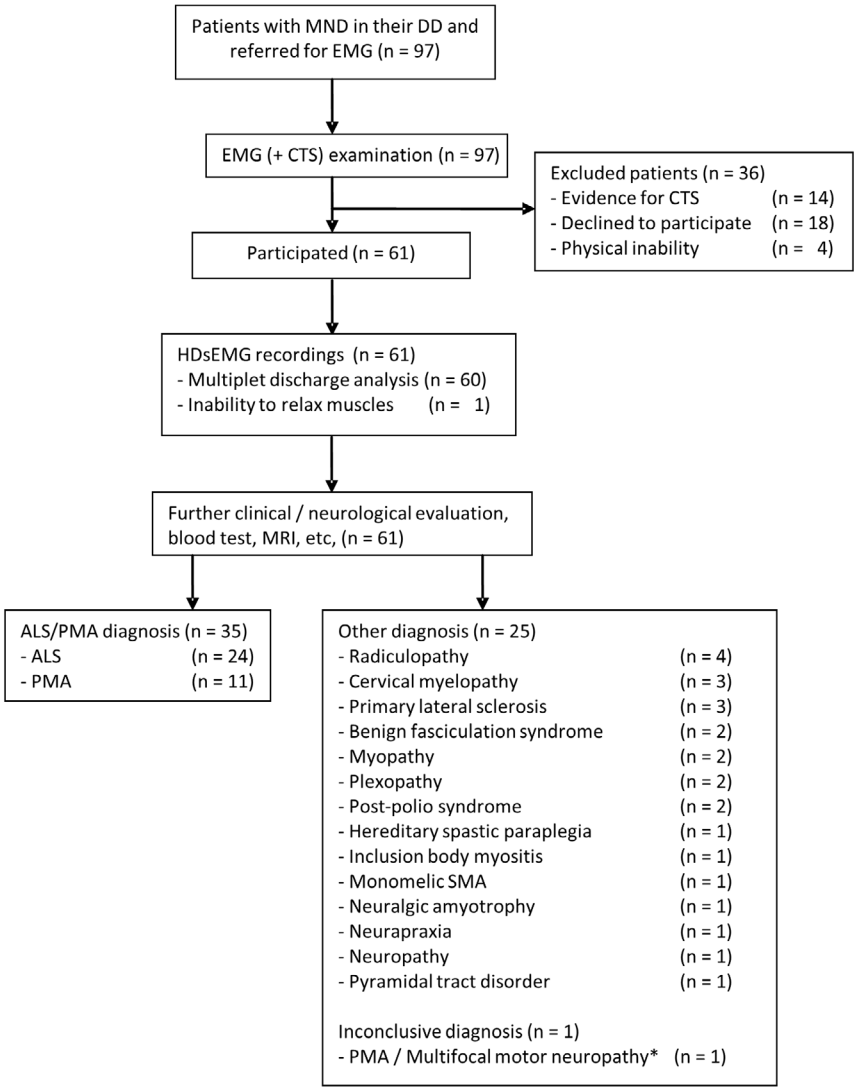


***Figure 1.*** *Schematic flow diagram of patient inclusion and diagnoses according to the STARD criteria.*
**Initial diagnosis of PMA; due to atypical progression, MMN could not be excluded*

***Table 1.*** *Patient characteristics*

| | All participating patients* (n = 61) | Patients with ALS / PMA* (n = 35) | Patients with other diagnosis* (n = 26) |
|---|---|---|---|
| Age, yrs | 58 (47 – 69) | 62 (50 – 71) | 52 (45 – 61) |
| Gender, men / women | 40 / 21 | 23 / 12 | 17 / 9 |
| ALSFRS-R** | 43.0 (40.0 – 46.0) | 42.0 (37.5 – 43.8) | 45.0 (42.0 – 47.5) |
| VC***, % of predicted | 89.4 (74.8 – 95.8) | 83.8 (69.0 – 94.3) | 92.3 (80.3 – 108.6) |
| Disease duration****, mnths | 18.3 (9.5 – 33.3) | 12.2 (9.6 – 22.6) | 34.4 (9.5 – 63.6) |

** Values in median ($25^{th}$ - $75^{th}$ percentile), or otherwise when indicated*
*** In 8 patients with ALS / PMA and in 3 other patients the ALSFRS-R score was not obtained*
**** In 1 patient with PMA and 1 patient with another diagnosis VC measurement was not possible*
***** Symptom onset to the time of EMG examination*

## Results

Inclusion started in August 2010 and ended in June 2013. During this period, 61 patients enrolled in this study (Fig. 1). Their demographic and clinical characteristics are shown in Table 1.

A subset of these patients was also part of a previous study carried out at our hospital [4]. Of the 61 patients, 24 patients were eventually clinically diagnosed with ALS, 11 patients with PMA, and 26 patients got another diagnosis (Fig. 1). Based on the revised El Escorial criteria [11], the 24 patients with ALS could be categorized after progression of symptoms as having 'possible' ALS (n = 1), 'probable' (n = 1), or 'definite' (n = 22) ALS. During follow-up, four patients with PMA progressed to 'probable' (n = 1) and 'definite' (n = 3) ALS. One patient, initially diagnosed with PMA, showed atypical progression of symptoms. In addition, in a repeated EMG examination, a conduction block was observed, and although there was no clinical response to repeated intravenous immunoglobulin (IVIg), a differential diagnosis of multifocal motor neuropathy (MMN) could not be excluded. This patient will further be referred to as 'inconclusive'. For the diagnostic accuracy assessment, this patient will be analysed as having another diagnosis. No familial cases in the patients with ALS and PMA were present. Ten patients with ALS had a bulbar onset of symptoms. By April 2014, 13 (37.1%) of the 35 patients with ALS and PMA had died.

All 61 patients tolerated the HDsEMG recordings well. VC measurements were not obtained in two patients, due to equipment error (n = 1) and inability of the patient to conduct the measurement (n = 1). For logistic reasons the ALSFRS-R questionnaire was not scored in the first 11 patients. In one patient, clinically diagnosed with PMA, no reliable single MUAP profiles could be obtained due to inability to sufficiently relax the thenar muscles. On account of this, statistics were based on 60 patients or 34 patients with ALS and PMA when involving MDs.

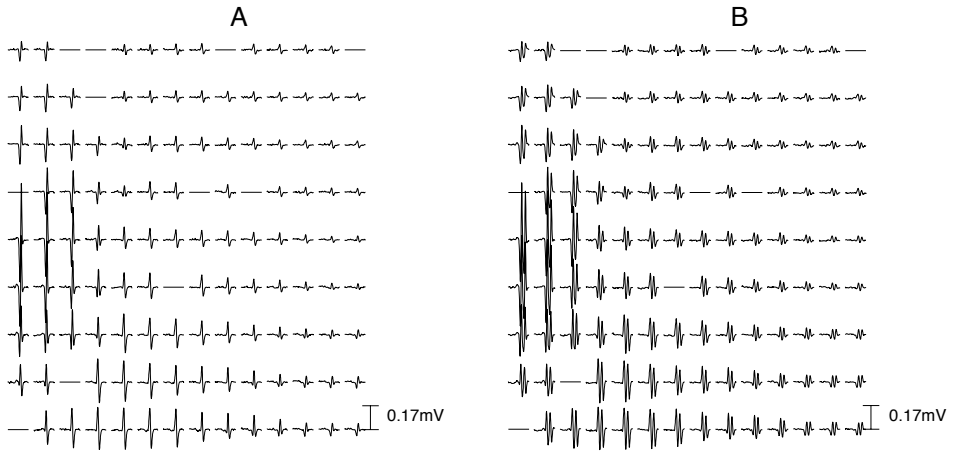


***Figure 2.*** *(A) Example of an M-wave from a MU after a single trigger, recorded with the 9 x 14 electrode grid in an ALS patient. (B) An electrically evoked doublet of the same MU as in (a) in the consecutive trigger. The position of each signal in the profile corresponds to the position in the electrode array of the electrode with which the signal was recorded. Electrodes with poor skin contact are visible as flat lines.*

***Table 2.*** *Contingency tables for (A) presence/absence of electrically elicited MDs in the thenar muscles of 60 patients, (B) presence/absence of FPs in the cervical region in 61 patients.*

| (A) Outcome of MD analysis using HDsEMG recordings | ALS / PMA | No ALS / PMA |
|---|---|---|
| MDs present | 16 | 1 |
| MDs absent | 18 | 25 |
| **(B) Outcome of FP evaluation using needle EMG** | | |
| FPs present | 30 | 12 |
| FPs absent | 5 | 14 |

***Table 3.*** *Number of body regions involved in the electrodiagnostic evaluation without electrically elicited MDs and with MDs (considered as neurogenic EMG abnormality) in the 34 patients diagnosed with ALS / PMA*

| Number of positive EMG regions | LMN dysfunction without electrically elicited MDs - , (%) | LMN dysfunction with electrically elicited MDs -, (%) | p-value |
|---|---|---|---|
| ≥ 1 | 17 (50.0) | 25 (73.5) | p = 0.008 |
| ≥ 2 | 9 (26.5) | 11 (32.3) | NS |
| ≥ 3 | 2 (5.9) | 3 (8.8) | NS |

Electrically elicited MDs (Fig. 2B) were found in 11 patients with ALS and in 5 patients with PMA and in the 'inconclusive' patient. In those 17 patients with MDs, in total 31 MUs were found that generated MDs, with 1 – 5 MUs showing MDs per individual patient. From these 31 MUs, in total 333 MDs were recorded in response to 3.3% of all stimulations. Since not every trigger elicits an MD from a MU (Fig. 2A and B), the number of MDs per MU was also expressed as a percentage of the number of elicited M-waves. This percentage, a measure of MD persistence, varies between the MUs with MDs (Fig. 3). The highest persistence was found in an ALS patient in whom 93 (71%) out of the 131 triggers that elicited an M-wave showed an MD. Most of the MDs were doublets (331 of 333). In one ALS patient triplets were found (2 of 333). There was no significant difference in the number of MUs that showed MDs between patients with ALS and PMA (21 vs 9, respectively) nor in MD persistence. In the 'inconclusive' patient a single MU showed MDs with a persistence of 0.3%. Finally, we found no relation between age and the number of MUs showing MDs in patients ($r = 0.10$, $p = 0.43$, $n = 60$).

The diagnostic accuracy of electrically elicited MDs was determined by means of Table 2A, which shows that the presence of MDs is significantly related to the clinical diagnostic outcome (Fisher exact test, $p < 0.001$, $n = 60$). Sixteen patients in whom MDs were detected were later diagnosed with either ALS ($n = 11$) or PMA ($n = 5$) (PPV = 94.1%; sensitivity = 47.1%, CI: 32% - 62%). MDs were detected only in the 'inconclusive' patient (specificity = 96.2%, CI: 91% - 100%).

The accuracy assessment of fasciculations shows that the presence of FPs in the cervical region is also significantly related to the clinical diagnostic outcome (Fisher exact test, $p = 0.002$, $n = 61$, Table 2B). In 30 of the 35 patients with ALS and PMA, FPs were observed (sensitivity = 85.7 %, CI: 76% - 95%) as well as in 12 patients with another diagnosis (PPV = 71.4%; specificity = 53.9%, CI: 39% - 69%): inclusion body myositis, post-polio syndrome, 'inconclusive', monomelic spinal muscular atrophy, primary lateral sclerosis, neuropathy, plexopathy, hereditary spastic paraplegia, radiculopathy (2x), benign fasciculation syndrome, and cervical myelopathy. Overall, compared to MDs, FPs were more commonly observed both in patients with ALS and PMA and in patients with another diagnosis. This resulted in a significantly higher sensitivity for FPs (85.3% vs 47.1%, $p = 0.002$, $n = 34$). By contrast, the specificity was significantly higher for electrically evoked MDs (53.9% vs 96.2%, $p < 0.001$, $n = 26$).

Another perspective on these findings can be obtained by considering the electrodiagnostic criteria used to establish the diagnosis of ALS or PMA from the standard EMG examination [11]. Of the 34 patients clinically diagnosed with ALS or PMA, 17 patients had ≥1 EMG region with electrophysiological signs of LMN dysfunction, 9 patients had ≥2 EMG regions involved, and 2 patients had ≥3 EMG regions with LMN dysfunction. Would the presence of MDs have been added as electrodiagnostic criterium (and considered as neurogenic EMG abnormality), then the percentage of patients with LMN involvement of ≥ 1 EMG region would have increased significantly (from 50.0% to 73.5%, $p = 0.008$, $n = 34$; Table 3).

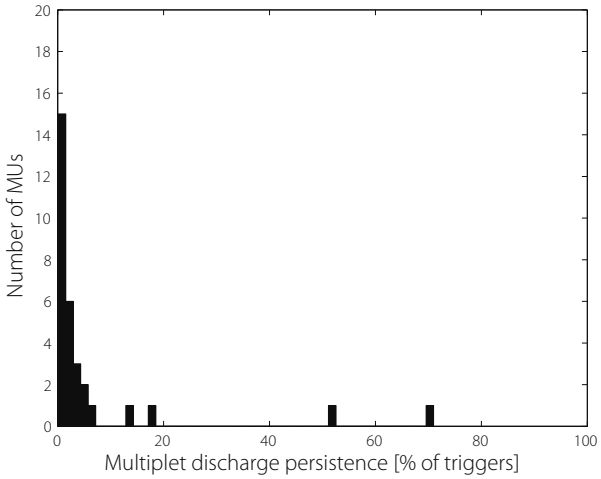


***Figure 3.*** *Histogram of MD persistence (percentage of applied triggers of a MU that showed an MD) for all 31 MUs.*

## Discussion

In this study, we have shown that electrically elicited multiplet discharges appear to be a highly specific electrophysiological marker of LMN dysfunction. This finding has important diagnostic implications. Of the patients with MND in their differential diagnosis who showed MDs, 94% eventually had ALS or PMA. In addition, despite being a well-known clinical hallmark in patients with MND, fasciculations are less specific for MND, as they were also commonly observed in other neurogenic disorders.

Fasciculations can probably arise in various ways along the motor neuron [13]. Some of these ways may be normal (i.e., occur in healthy subjects as well), other are pathological. Several motor nerve excitability studies in patients with neurogenic disorders have shown abnormalities in sodium and potassium channel conductance, which are most pronounced in the distal part of the motor neuron in patients with ALS [14] or at the site of the lesion in MMN patients [15]. These abnormalities are thought to lead to an imbalance between inward sodium and outward potassium currents and, resulting in axonal hyperexcitability. This hyperexcitability increases the probability that an axon generates a spontaneous discharge (fasciculation) and also provides a plausible explanation for electrically evoked MDs. Such an explanation is supported by a recent study in which we showed that the supernormality (hyperexcitability following the refractory period of the recovery cycle) of the distal axon is increased in patients that show MDs but not in others [4]. Although MDs were detected in the clinically inconclusive patient with possible conduction block that does not affect our main findings of higher specificity for MDs in MND compared to fasciculations.

Currently, information on electrically evoked MDs is scarce. These have been observed in two patients with ALS [16] and in some patients with unspecified neurogenic disorders [17], but their diagnostic value has never been investigated. Probably, the fact that MDs are so rarely mentioned in the literature is explained by the small probability that a trigger elicits an MD. Only 3.3% of the applied

triggers in this study resulted in an MD, meaning that MDs will not usually occur in neurophysiological recording procedures – unless long trains of stimuli are applied. Another factor is the use of single electrode surface EMG in conventional (surface) EMG examination, which makes it difficult to discriminate superimposed MUAPs and recognize MDs. HDsEMG adds spatial information, which aids in the recognition of individual MUAPs, and, hence, the detection of MDs.

In the present study half of the patients with ALS and PMA showed electrically elicited MDs whereas in a previous study all patients did [3]. This is probably due to our study design, which comprised only a single HDsEMG recording. In the previous study multiple sessions during disease progression were performed. The latter approach may be expected to increase the number of recorded MDs, because of the increased number of triggers applied [3]. However, this previous study on MDs only included healthy subjects besides patients with ALS and PMA, where in none of the healthy controls electrically elicited MDs were observed [3]. To get better insight into the specificity of distally evoked MDs, in the current study we extended the clinical spectrum by including patients having MND in their differential diagnosis.

In contrast to electrically evoked MDs, voluntarily activated MDs have been observed frequently in healthy subjects and also in neuromuscular disorders [18-20]. These MDs are thought to have a proximal (at the motor neuron cell body) rather than distal axonal origin [18-20]. In patients with MND there may, therefore, be both proximally and distally generated MDs – as there are proximally and distally generated fasciculations. Indeed, the frequency of voluntarily activated MDs is higher in patients with ALS than in healthy subjects and this has been considered an early marker of MN dysfunction [20]. Furthermore, spontaneous doublets have been suggested to have diagnostic value in routine EMG examination [21], and doublet fasciculations were more prominent in ALS when the disease progressed [22].

Unfortunately, the design of our study does not allow us to draw any conclusion regarding the nature and specificity of proximal, voluntarily activated MDs in patients with MND. Yet, the evidence collected thus far, by ourselves and others, suggests that spontaneous activity (whether fasciculations or MDs) originating proximally can be normal whereas distally generated discharges are usually abnormal. There is presently no easy way to distinguish proximally from distally arising fasciculations, leading to a mixture of both normal and abnormal spontaneous activity on needle EMG. The two types of MDs however can be easily separated eliciting the MDs electrically. This likely explains the higher specificity of electrically elicited MDs compared to fasciculations in our study.

Finally, our study comprises only a relatively small number of patients. To determine the diagnostic accuracy of MDs more reliably, a larger set of patients is required. We are of the opinion that such an effort is warranted by the present findings, which show that MDs are a potentially valuable measure in the diagnostic phase of ALS and PMA. Currently, the HDsEMG recordings needed to detect electrically evoked MDs are time-consuming and require specialized equipment. However, this method may be helpful in making the diagnosis ALS or PMA earlier in patients that are otherwise difficult to diagnose. A reliable and early diagnosis will be all the more relevant when novel effective therapies become available.

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# Chapter 7

## Electrically evoked multiplet discharges are associated with more severe clinical deterioration in motor neuron disease

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submitted

## Abstract

*Objectives:*
To evaluate whether electrically evoked multiplet discharges (MDs) are related to severity of clinical deterioration in motor neuron disease (MND).

*Methods:*
Stimulated high-density surface EMG (HDsEMG) recordings were performed in the thenar muscles. Data was collected from 31 MND patients. MDs from the HDsEMG recordings were determined at baseline. ALSFRS-R scores were obtained at baseline and at a maximum of sixteen weeks follow-up.

*Results:*
The presence of MUs showing MDs was associated to progressive clinical deterioration for ALSFRS-R ($p = 0.02$) and fine motor function (FMF) ($p < 0.001$). Patients showing higher number of MUs with MDs ($r = 0.61$, $p < 0.001$) as well as patients showing higher number of MDs (as percentage of applied stimuli) ($r = 0.59$, $p = 0.001$) corresponded with a more severe decline in their FMF.

*Conclusions:*
Electrically evoked MDs are associated with more severe clinical deterioration in patients with MND.

## Introduction

Amyotrophic lateral sclerosis (ALS) and progressive muscular atrophy (PMA) are neurodegenerative motor neuron diseases (MND). The progressive loss of motor neurons leads to muscle weakness and eventually death. The pathophysiological mechanisms are still poorly understood, which makes it even more difficult to develop effective treatment strategies. There are indications that hyperexcitability of the motor system is a pathophysiological mechanism that almost always occurs during the course of MND [1, 2]. Ectopic motor unit activity in the form of fasciculations is suggested to be caused by excitability changes [3-5]. These hyperexcitability properties have been observed in the distal segments of the motor axon [4, 6, 7], but functional changes in the anterior horn cell body or even more central hyperexcitable motor pathways may also play a role [8-11]. It has been suggested that ectopic distal axonal activity in the form of multiplet discharges (MDs) after electrical stimulation are caused by distal pathophysiological excitability changes [12, 13]. Electrically evoked MDs can be reliably detected using high-density surface EMG (HDsEMG) recordings as this method aids in the recognition of individual motor unit action potentials (MUAPs) by adding spatial information [14, 15] (Fig. 1). Because the number of applied stimuli are under full control of the examiner, the procedure can be standardized, which enhances the quantification of MD registration. Several studies have shown that there is a relation between excitability properties and disease progression and prognosis in ALS [16, 17]. If electrically elicited MDs indeed are an expression of hyperexcitability they may have an important clinical relevance. Therefore, our aim was to investigate whether ectopic MU activity in the form of electrically elicited MDs is related to disease progression in ALS and PMA patients.



## Materials and Methods

### *Patients*

Data was collected from 31 consecutive patients with MND obtained from previously performed and ongoing studies carried out at our hospital [12, 13]. These patients were classified according to the revised El Escorial criteria as probable or definite ALS (n = 22) or PMA (n = 9). The study protocol was approved by the medical ethical committee of the Erasmus MC. All patients gave written informed consent.

In these 31 patients, we performed HDsEMG recordings and determined the ALS Functional Rating Scale-revised (ALSFRS-R) scores. HDsEMG recordings were performed at baseline and ALSFRS-R scores were taken both at baseline and a maximum of sixteen weeks follow-up (mean (SD): 10.9±3.2). The ALSFRS-R was used to quantify disability in patients with ALS [18]. The ALSFRS-R score ranges from 0 (maximum disability) to 48 (normal) and can be divided into four subscores; bulbar, fine motor, gross motor and respiratory function. Because our HDsEMG recordings were performed in the thenar muscles which would more closely reflect hand muscle function, we also used the fine motor function (FMF) subscore for comparison (handwriting, cutting food and handling utensils, dressing and turning in bed and adjusting bed clothes; 4 points per item, maximal score: 16) [18]. The rate of decline in ALSFRS-R has been shown to be an important predictor of survival in ALS [19, 20].

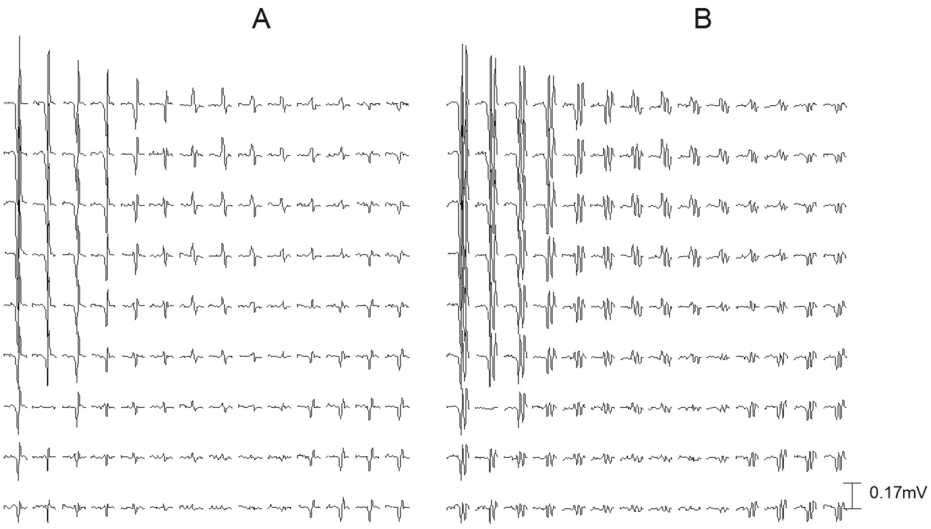
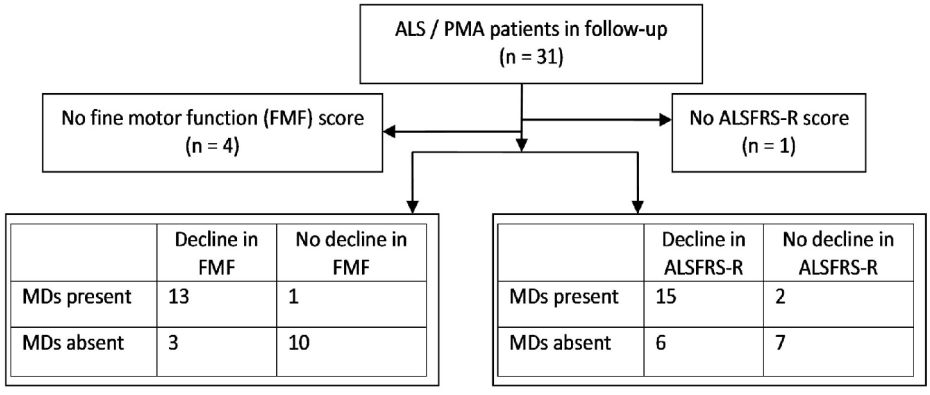



***Figure 1.*** *(A) A motor unit action potential (MUAP) profile recorded with the 9 x 14 electrode grid in an ALS patient over the thenar muscles. (B) An electrically evoked doublet of the same MU as in (A) in the consecutive stimulus. The position of each signal in the profile corresponds to the position in the electrode array of the electrode with which the signal was recorded.*




| | Decline in FMF | No decline in FMF |
|---|---|---|
| MDs present | 13 | 1 |
| MDs absent | 3 | 10 |

| | Decline in ALSFRS-R | No decline in ALSFRS-R |
|---|---|---|
| MDs present | 15 | 2 |
| MDs absent | 6 | 7 |

***Figure 2.*** *Flow diagram and contingency tables of the decline in ALSFRS-R score and fine motor function between baseline and follow-up session measured in the 31 ALS and PMA patients.*

***Table 1.*** *Patient characteristics*

| | ALS/PMA patients* |
|---|---|
| Diagnosis, ALS/PMA | 22 / 9 |
| Gender, M/F | 21 / 10 |
| Age, yrs | 65 (32 – 78) |
| Onset to diagnosis, mnths | 12.2 (7.0 – 24.4) |
| Survival time**, mnths | 35.8 (21.4 – 50.7) |
| Died***, yes/no | 20 / 11 |
| ALSFRS-R, (max 48) | 42.0 (40.0 – 44.0) |
| Fine motor function (FMF), (max 16) | 14.0 (11.0 – 16.0) |
| Patient with MU showing MDs, yes/no | 18 / 13 |
| MUNE | 107 (45 – 214) |

** Values in median ($25^{th}$ - $75^{th}$ percentile), or otherwise when indicated*
*** Symptom onset till death (n = 20);*
**** 15 ALS and 5 PMA patients had died*

The decline in ALSFRS-R and FMF was defined as the absolute change in the scores between both sessions. To correlate the rate of disease progression with MD variables, the absolute change in score was divided by the time interval to correct for the variation in time interval between sessions.

### *High-density EMG recordings and multiplet discharge registration*

Detection of MDs after electrical stimulation was performed using HDEMG recordings with an 9 x 14 electrode array attached to the skin over the thenar muscles [12]. Since the probability of eliciting an MD for most MUs is low, 500 stimuli (2 Hz, 0.1 ms) were applied at several sites along the median nerve (4 to 6 sites), and the responses were recorded. Stimulus intensity level was adjusted to activate the first few low-threshold MUs. This resulted in a sample of up to 20 single MUAPs per patient. For every subject the number of single MUAPs that elicited an MD was registered. In parallel, the number of MUs was estimated by dividing the maximum CMAP amplitude by the mean amplitude of the collected single MUAPs [14, 15]. In addition, for single MUAPs that elicited an MD, the number of stimuli when the MU was active and the number of stimuli that elicited an MD were registered to determine their proportional occurrence (Fig. 1).

### *Statistical analysis*

Statistical analyses were performed using Matlab (R2014a: The MathWorks, Natick, MA). Normality was tested by the Lilliefors method. The association between the presence of electrically evoked MDs and any change in ALSFRS-R scores were investigated using 2x2 contingency tables. When normally distributed, data are presented as mean and standard deviation, otherwise as median and percentiles. Differences between groups were compared by t-tests or Mann-Whitney U-tests as applicable. Spearman's correlation was used to assess the relation between MD variables and the change in ALSFRS-R scores. Differences with a p-value of $< 0.05$ were considered statistically significant.

## Results

The characteristics of the 31 ALS/PMA patients at baseline session are shown in Table 1. Electrically elicited MDs were detected in 18 of the 31 patients with a total of 56 MUs (range 1 – 9 MUs showing MDs per patient). The median electrically evoked MD occurrence (as percentage of the number of applied stimuli) was 1.5% (IQR: 0.4% - 3.0%). There was no significant difference in number of MUs showing MDs between ALS and PMA patients (median: 1 vs 1, $p = 0.91$). Complete ALSFRS-R scores were available in 30 patients. The FMF scores could be obtained for both sessions in 27 patients (Fig. 2). We did not find any difference in ALSFRS-R score at baseline and decline of ALSFRS-R score between ALS and PMA patients. No relation was found between patients' age and the number of MUs showing MDs. Furthermore, the time interval between sessions did not significantly differ in patients with and without MDs (10.9±2.9 vs 10.9±3.7 weeks, $p = 0.96$). At baseline MUNE was not related to ALSFRS-R ($r = 0.21$, $p = 0.27$). During the follow-up period, the ALS/PMA patients significantly deteriorated, as reflected by the absolute decline in ALSFRS-R score (median (IQR): 2 (0 – 4), $p < 0.001$, $n = 30$).

The presence of electrically evoked MDs at baseline was associated with progressive clinical deterioration by ALSFRS-R (Fisher exact test, $p = 0.02$, $n = 30$, Fig. 2). Fifteen (15/17) patients in whom electrically evoked MDs were detected showed a decline in ALSFRS-R in the follow-up session. The number of MUs showing MDs at baseline showed a trend towards decline in ALSFRS-R at follow-up ($r = 0.33$, $p = 0.08$, $n = 30$). Patients who eventually showed a relatively more severe decline in ALSFRS-R at follow-up had a higher occurrence of MDs (as percentage of the number of applied stimuli) at baseline ($r = 0.38$, $p = 0.04$, $n = 30$).

Similarly, baseline presence of electrically evoked MDs was associated with progressive deterioration of (FMF) (Fisher exact test, $p < 0.001$, Fig. 2). Significant majority (13/14) of the patients that showed MDs had a decline in FMF between both sessions. The majority of the patients (10/13) without MDs showed no progressive deterioration in the functional scores. In patients with both a higher number of MUs showing MDs ($r = 0.61$, $p < 0.001$, $n = 27$) and higher occurrence of MDs (as percentage of number of applied stimuli, $r = 0.59$, $p = 0.001$, $n = 27$) at baseline, significant correlations with severe decline in FMF at follow-up were seen.

## Discussion

We used HDsEMG recordings to assess whether ectopic motor unit activity is related to disease progression in ALS and PMA patients and showed that electrically evoked MDs measured at baseline are associated with severity of disability progression reflected by the decline in ALSFRS-R and FMF at follow-up.

In a previous small scale study it was indicated that electrically evoked MDs were likely to be related to clinical deterioration in MND patients averaged over several sessions in follow-up [12]. In this extended study we showed that already a single baseline measurement of MD variables is predictive of a more severe decline in disability scores in a larger patient group.

Distal evoked MDs were hypothesized to be related to pathophysiological excitability changes [12]. A recent motor nerve excitability study demonstrated further evidence confirming this hypothesis where the presence of electrically evoked MDs was related to altered excitability properties [13]. This study showed that the same pathophysiological excitability changes are probably involved in generating MDs and fasciculations of distal origin. Furthermore, these distally evoked MDs were shown to be highly specific for MND as an early sign of lower motor neuron dysfunction [21]. These findings together with the association between MD variables and disease progression found in this study further suggest that the mechanisms leading to the generation of electrically evoked MDs is related to the pathophysiological changes in MND.

Our findings showed a significant association between the occurrence of electrically evoked MDs and overall disability progression (decline in ALSFRS-R). Furthermore, a trend could be observed between the number of MUs showing MDs and the progressive decline in ALSFRS-R, although it was not significant. A plausible explanation may be that the number of patients in our study could have been too small to show such a relation. Furthermore, the detection of electrically evoked MDs was restricted to the thenar muscles by the study methodology. Hence the measurements are likely to be more representative of fine motor skills rather than global functional skills. In addition, the thenar muscles are involved in the clinically often observed split hand syndrome, where they are relatively more affected than other muscles. This may also partly explain the lack of association with changes in global functional skills, although this phenomenon was not specifically assessed in this study. The split hand sign has been suggested to be an early feature in patients with ALS [22, 23] and motor axonal excitability studies have shown that the motor axons innervating these hand muscles are differently affected [24]. Importantly, the recording of electrically evoked MDs in these muscles may therefore give further insight into the underlying pathophysiological mechanism of this phenomenon at a single motor axonal level and further insight into their relation with disease progression. More generally, other factors may also play a role in overall disease progression such as variation in site of onset and pattern of spread over body regions [25, 26]. Furthermore, excitability properties were suggested to change over time and therefore are different depending on the disease stage [4, 17]. Hence, in individual cases the results should be interpreted with caution and further research is required to determine if this neurophysiological finding can be used as a marker for the rate of disease progression in MND. Repeated MD measurements after a predefined time interval of for instance 1 month as well as their detection at multiple measurement sites may improve their ability to predict overall disease progression.

A limitation of the present study is that the time intervals between both sessions varied. Ideally, the patients should have had similar time intervals between assessments of functional scores. Some patients showed no change in ALSFRS-R score, probably due to a relatively slow progression, or may be due to a relatively short follow-up. However, the follow-up period seems to be adequate to be able to detect changes, because of the significant decline in function reflected by the ALSFRS-R for the whole patient group. There was no significant difference in the follow-up time between patients with and without MDs, and hence this factor would have a negligible effect on our findings. To relate

MD variables to disease progression, the results were corrected for the time interval, assuming a linear disease progression as reflected by the ALSFRS-R score. Although it is difficult to determine how this assumption is met, various studies consider a linear disease progression in ALS to be an adequate estimate [27-30].

In conclusion, the presence of electrically evoked MDs at baseline measurement is associated with a more severe clinical deterioration reflected by the disability scores at follow-up. The detection of electrically evoked MDs makes it possible to non-invasively examine and selectively investigate excitability properties at the distal part of the motor axon. A larger set of patients should further evaluate the prognostic value of electrically evoked MDs to predict clinical deterioration and their usefulness in clinical practice. This study of MDs seems to be a suitable tool for further research and larger clinically oriented studies.

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# Chapter 8

## General discussion

In the previous chapters, the application of several advanced surface EMG techniques in a research setting and in clinical practice in patients with motor neuron disease (MND) has been explored. The possibilities offered by these techniques could eventually support the diagnostic process and give a more objective insight into the course of the disease, and could potentially facilitate the evaluation of novel therapies. For this purpose, this thesis predominantly focused on fasciculation potentials (FPs), a clinical hallmark in MND, and multiplet discharges (MDs) after electrical stimulation. These two types of ectopic activity of individual motor units (MUs) are thought to be an expression of affected MUs. Therefore, they may have diagnostic and prognostic relevance. The main goals of this thesis were the following:

1) To evaluate and further improve the available research methods for the non-invasive study of individual motor units.
2) To develop neurophysiological markers of clinical relevance in individual patients with ALS and PMA, which can be obtained with these surface EMG methods.
3) To clarify the pathophysiological and clinical significance of FPs and electrically evoked MDs.

The most important findings described in this thesis and the formulated goals will be discussed here, based on their methodological and pathophysiological aspects. Subsequently, the clinical implications will be evaluated, followed by recommendations for future studies.

## Methodological aspects

The first goal of this thesis was covered through the development of an automated method to quantify the CMAP scan pattern in a single marker, D50 (**Chapter 2**). The automated method mitigates susceptibility to operator-related variability in the current manual procedure and can therefore potentially improve the utility of the CMAP scan as a follow-up tool [1]. This marker can be easily determined, so the results can become directly available to the clinician during electrodiagnostic examination.

By relating D50 to MU loss, the second goal of this thesis was realized. D50 was found to be sensitive to MU loss and reinnervation, which is already commonly present in the diagnostic phase in patients with MND. The sensitivity of D50 to MU loss and reinnervation decreases when there are more than approximately 80 MUs innervating a muscle, because then a smooth, gradually increasing CMAP scan pattern becomes visible. This smoothing effect is caused by the large number of possibilities in terms of active MUs at a particular stimulus intensity. This effect is also encountered by more computationally intensive methods [2-4]. Still, the CMAP scan can be applied in other conditions such as Guillain-Barré, because it contains useful clinical information on axonal excitability [5, 6].

Practical factors remain important for the quality of the CMAP scan (and thereby the accurate assessment of D50), such as avoiding redundant applied triggers, instructing patients to relax their muscles, and minimizing movement artefacts. Alternative stimuli protocols are interesting options to explore for the further improvement of the CMAP scan as a diagnostic instrument and follow-up tool. In this way, the CMAP scan could be made more sensitive to MU loss, reinnervation, and possibly also for measuring higher number of MUs. The successfully implemented CMAP scan simulation

model and motor unit number estimation (MUNE) using high-density surface EMG (HDsEMG) can provide useful insights into the above approaches. The effect of alternative protocols on D50 and other parameters need further exploration.

A longitudinal study to track changes in individual MUs, referred to as motor unit tracking, may provide novel pathophysiological information on disease progression in patients with MND [7]. In an identified MU action potential (MUAP) profile, changes can occur between sessions. These can result from either suboptimal reproducibility, such as replacement error of the high-density electrode grid, or from pathophysiological changes. Hence, the further repositioning errors can be reduced, the finer the changes that can be reliably ascribed to disease progression.

The first goal of this thesis was achieved by illustrating the feasibility of correcting HDsEMG electrode grid displacement errors (**Chapter 3**). Yet, other factors need to be taken into account as well (e.g. adopting the same hand position), which requires standardization of the set-up. Even though, motor unit tracking is still laborious and currently unsuitable for general clinical practice, it remains a promising research tool. The automated adjustment for correcting electrode grid replacement errors, as described in **Chapter 3**, may help to improve this research tool for follow-up studies of neuromuscular disorders.

The clinical relevance of fasciculation potentials (FPs) is underlined by the fact that they are incorporated in the latest electrodiagnostic criteria for ALS [8]. FPs are the spontaneous activity of single MUs, and they are a characteristic, but nonspecific feature of MND. During EMG examination, FPs are most commonly observed as isolated discharges [9]. However, in routine electrodiagnostics, there are no quantitative criteria that define an MU discharge as a pathological discharge (FP). Therefore, the identification of FPs relies on operator's experience, which implies that an element of subjectivity remains present.

The findings in **Chapter 4** covered the first goal of this thesis through the development of a potential clinically relevant method for objective quantification of MU discharges. The results revealed that a pathological discharge cannot be identified based on information from only a single MU. However, we obtained an improved identification of patients with MND compared to healthy controls when using a new marker that incorporates the activity of multiple MUs showing isolated discharges in a specific time interval. This implies that the presence of a few sporadic FPs (originating from a single MU) itself should not be considered an abnormality. Only when the FPs originate from multiple MUs a link can be made to MND as underlying pathology. These findings correspond with the clinically often observed abundant nature of fasciculations in patients with MND. Considering the impact on the diagnosis of this finding, it must be noted that for the diagnosis of MND other signs (muscle weakness / atrophy, fibrillations and giant MUAPs) are very important. However, in the early phase of the disease, FPs might be the only feature present, which makes it more difficult to deduce their pathological significance [10]. This difficulty can then potentially be mitigated using a marker that objectifies the quantity of FPs (discharge pattern of multiple MUs).

As a step towards the second goal of this thesis, this marker may therefore be a useful feature in the diagnostic phase of MND, or in clinically still unaffected muscles by assessing the number of different MUs from which the spontaneous activity originate. Future clinically oriented studies should determine its optimal discriminative value, because this will depend on the muscle under investigation [11, 12], the applied recording technique and recording duration.

## Pathophysiological aspects

The pathophysiological mechanisms in MND are still not well understood. Altered excitability properties of motor axons have been suggested to be the underlying pathophysiological mechanism in MND that would lead to the occurrence of fasciculations [13-15]. Recently, electrically evoked multiplet discharges (MDs) by means of HDsEMG were introduced as an approach to examine the distal part of single motor neurons in patients with MND [16]. Currently, little information on electrically evoked MDs is available [17, 18]. They were hypothesized to be related to pathophysiological excitability changes. However such a relation was not established. A better understanding of the etiology of MDs is required to bring them closer to clinical use.

Regarding the third goal of this thesis, the findings in **Chapter 5** confirm that patients showing MDs had altered axonal excitability properties that were similar to changes observed in other excitability studies addressing fasciculations [14, 15, 19-21]. Electrically evoked MDs were thought to originate at the terminal branches of the axon [16], just like distal fasciculations [22, 23]. Our findings suggest there is a common mechanism involved behind the generation of distal fasciculations and electrically evoked MDs in MND. The study described in **Chapter 5** demonstrates that excitability properties in single MUs can be evaluated by detecting electrically evoked MDs by means of HDsEMG in a controlled and objective manner.



### *Diagnostic relevance of electrically evoked multiplet discharges*

To clarify the diagnostic significance of electrically evoked MDs patients, we examined patients with a suspected diagnosis of MND (**Chapter 6**). The third goal of this thesis was achieved through this study by establishing the diagnostic relevance of electrically evoked MDs for MND. The findings indicated that they were more specific than FPs.

The location where MDs arise appears to be an important factor in explaining their higher specificity for abnormal motor axonal function. FPs can arise from various locations [15, 22, 24-27], where some of them are normal, because they also occur in healthy subjects ([11, 28, 29], **Chapter 4**), while others are pathological. Voluntarily activated MDs (not electrically evoked) have been commonly observed in healthy subjects and also in various other neuromuscular disorders [30-32]. These voluntarily activated MDs are thought to originate proximally at the motor neuron cell body. Proximal MDs can also occur due to subtle ballistic contractions [31, 33, 34]. Hence, in MND there could be a mixture of both distally and proximally generated MDs, just as there are proximally and distally generated fasciculations. By evoking MDs electrically, the distal and proximal types can be separated. Our findings, and findings by others, suggest that MU discharges (FPs or MDs) that originate proximally are usually normal

(physiological), whereas distally generated discharges are usually abnormal [35, 36]. This may explain the higher specificity of electrically elicited MDs compared to FPs in our study.

It must be noted that the occurrence of FPs varies between muscles [11], which may also be the case for electrically evoked MDs. Therefore, the specificity of electrically evoked MDs in other muscles is currently unclear. Theoretically, there may be other physiological mechanisms and disease processes with similar changes in the membrane potential that could cause this type of discharge, indicating that they may also represent different phenomena [37-39]. Nonetheless, it can be argued that in the given clinical context, electrically evoked MDs are of diagnostic relevance for patients with ALS and PMA as we observed in our study (**Chapter 6**).

### *Ectopic motor unit activity: an early expression of the beginning of the end?*

It has been shown that FPs have prognostic relevance [40, 41]. FPs have been suggested to be an early manifestation of progressive lower motor neuron dysfunction [35]. With regard to the third goal of this thesis, the findings in **Chapter 4** suggest that pathophysiological information can be deduced from spontaneous MU discharges when they originate from multiple MUs showing such discharges. Sporadic MU discharges may have a normal physiological origin [42], but their exaggerated presence (originating from multiple MUs) may be more likely due to an underlying pathology, which could be induced by pathophysiological excitability changes [14, 15]. How the abundance of spontaneous MU discharges evolves during disease progression remains to be defined. Ectopic MU activity in the form of electrically evoked MDs has been associated with MU loss and clinical deterioration in patients with MND [16]. The findings in **Chapter 7** showed that the number of MDs at a baseline session in patients with ALS/PMA was related to the rate of functional decline. This further suggests that distally evoked MDs provide an indication of pathophysiological excitability changes. Whether the excitability changes are a result or a cause of axonal degeneration currently remains unclear.

Altered excitability properties are likely the mechanism behind FPs and electrically evoked MDs. More generally, in patients with MND, there are several indications that hyperexcitability is a pathophysiological mechanism that almost always occurs during the course of the disease [43, 44]. The interspike intervals of distally evoked MDs correspond with the supernormality period and the presence of electrically evoked MDs is related to increased supernormality (**Chapter 5**). Despite the fact that ion channel expression may differ at the terminal branches, potassium conductance impairment seems to be a likely explanation for the increased supernormality [14, 15, 19, 21]. In animal studies, blocking of presynaptic potassium channels located at the nerve terminal branches has shown to lead to repetitive discharges after a single electrical trigger [45, 46]. When this observation can be extrapolated to humans, this may further point towards reduced potassium conductance. Interestingly, in disorders characterized by potassium channel dysfunction, the presence of many (spontaneous) MDs is noteworthy [39, 47]. Preliminary findings in a motor unit tracking study in MND showed increasing occurrence of MDs in individual MUs over time (unpublished data). This may indicate ongoing potassium conductance impairment related to motor neuron degeneration.

Despite the probable relation described above, the mechanisms of motor neuron death in MND are still far from fully understood and there may be various other degenerative pathways. Our results demonstrate that by studying ectopic MU activity in the form of FPs and electrically evoked MDs, a contribution can be made towards further unravelling the pathophysiological mechanisms in MND (**Chapter 4, 5, 6, 7**).

## Clinical implications

### *Diagnostic phase*

Because of the absence of a specific biomarker for MND, any measure that could facilitate the diagnosis of MND would fill a large clinical gap. The CMAP scan may be a useful tool in the diagnostic phase, because the novel marker, D50, can detect underlying neurogenic abnormalities. Therefore, it can be used as a sensitive and objective assessment in addition to the clinical symptoms (muscle weakness, muscle atrophy). In this way, the CMAP scan provides information that complements routine NCS and needle EMG findings. Needle EMG will still be required as a diagnostic test because it can detect neurogenic changes and spontaneous muscle fiber activity not accessible with surface EMG. However, needle EMG may not always be necessary in some clinical situations, and in those cases, the CMAP scan can potentially be a useful alternative. The CMAP scan has the advantage of activating all MUs, while needle EMG relies on a limited sample of MUs in a muscle.

Future research is required to determine the sensitivity of the CMAP scan to detect neurogenic abnormalities in comparison with needle EMG. Owing to its non-invasive nature and the relatively easy procedure, the CMAP scan can be more readily applied than needle EMG, e.g, in children, and in longitudinal studies [48].



In the Awaji diagnostic criteria of ALS, equal weight is given to clinical and electrophysiological findings in defining the presence of lower motor neuron dysfunction [8]. In these criteria, the presence of FPs is considered equivalent to fibrillations and positive sharp waves [8]. The findings in **Chapter 4** add to this criterion and imply that in addition to solely assessing presence of FPs, supplemental relevant information can be obtained by assessing the number of different MUs from which they originate. This especially can be helpful in the early stage of MND, or in clinically unaffected muscles, where FPs might be the only feature present. This information can potentially be used to aid in differentiating MND from other disorders.

Initial implementation of our quantitative method requires incorporation into routine needle EMG software that includes an MU analysis program [49, 50]. Then, further clinical investigations are needed to establish adequate norms for pathology when our method is used with needle EMG. Finally, a next step could be to relate the quantity of FPs (originating from multiple MUs) in a specified time interval to the diagnosis.

In addition, electrically evoked MDs were demonstrated to be of diagnostic relevance for patients with MND. The findings indicated that they were more specific than FPs (**Chapter 6**). Hence, electrically evoked MDs may fit into the existing diagnostic criteria as an additional electrophysiological sign. Even though, electrically evoked MDs are obtained from a single nerve, they can provide useful clinical information in the clinical context of patients with a suspected diagnosis of MND.

Currently, detecting electrically evoked MDs requires relatively laborious HDsEMG recordings and analysis, which makes it not directly applicable in clinical practice. Future studies should concentrate on modifying the recordings and analysis to mitigate this laborious procedure. An earlier diagnosis may improve patients' perspectives, especially when novel effective therapies finally become available, and because it may help to reduce stress and uncertainty for patients and their families.

### *Disease progression and prognosis*

For evaluating the effectiveness of novel therapies, an accurate, reproducible marker is required that can monitor disease progression. Motor unit number estimation (MUNE) has been shown to be a more sensitive marker of disease progression than muscle force measurements, or ALS questionnaires [51-53]. The CMAP scan can be performed quickly, is well tolerated by patients, and has shown promising results in monitoring disease progression in MND [1, 54-56]. The automated method described in **Chapter 2** may therefore be preferable to existing MUNE techniques, because it can be performed quickly and easily. Additionally, the automated method can be implemented in routine EMG software and the computational effort is small.

Various studies have shown that the widespread presence of fasciculations in multiple body regions is of prognostic significance [40, 41]. Applying the quantitative method of identifying FPs, as described in **Chapter 4**, allows FPs to be systematically evaluated at an individual MU level and related to disease progression and prognosis. In addition, a longitudinal study that combines ectopic MU activity, motor nerve excitability testing and MUNE has been performed, and the measurements are ready to be analyzed. This study can potentially give important information on how these various features relate to each other during the course of the disease. For future longitudinal studies, it seems to be adequate to perform four follow-up sessions, considering the impact of such studies on patients. Follow-up sessions should be combined as much as possible with the regular visits to the hospital.

The findings described in **Chapter 7** showed the relation between electrically evoked MDs and more severe decline in fine motor skills. Owing to the variability between patients, these results should be interpreted with caution in individual patients. Therefore, a larger study should evaluate whether electrically evoked MDs could be of help in order to better inform patients about their increased risk of decline in fine motor functions, such as hand skills. Further down the line, they might also be used in combination with clinical signs, such as muscle weakness and atrophy, in order to guide clinicians regarding further treatment.

## Concluding remarks

For electrodiagnostic examination of MND, a proper evaluation of individual MUs is required. This thesis has provided novel pathophysiological and clinical insights into MND at the level of individual MUs. The applied surface EMG techniques and enhanced methodologies yielded novel and complementary diagnostic and prognostic information that could not reliably be obtained with current routine techniques. Furthermore, the HDsEMG system can be applied as a useful reference tool to existing EMG techniques and new EMG techniques. The findings in this thesis warrant further exploration of these techniques, but their demonstrated utility outweighs the challenges of data analysis and data acquisition for their eventual integration. In the field of electrodiagnostic examination, there is an increasing interest in the use of surface EMG, because it is more comfortable for patients than routine needle EMG [57]. This thesis forms a crucial basis towards clinical application of surface EMG in the field of clinical neurophysiology. This could help to improve the clinical process and the development of novel therapies for patients with ALS and PMA.

## Future perspectives

In this thesis, several surface EMG methods have been applied and the results showed promising avenues for future studies. This presents opportunities for improving the diagnosis and clinical management of MND. Future steps can be made in the following directions:

Before the CMAP scan can be widely implemented in clinical practice, normative data need to be collected (D50, and potentially other future variables) in multiple muscles.

The split-hand syndrome has been shown to be a useful diagnostic sign in patients with ALS [58, 59]. The diagnosis of ALS can potentially be further facilitated by applying the CMAP scan to the split-hand muscles.

A longitudinal study in multiple muscles (e.g. split-hand) can determine the usefulness of the CMAP scan in monitoring the rate of disease progression in MND for phase II clinical trials. A comparison with other measures (neurophysiological index [60], ALSFRS-R) is recommended.

Although implementation of HDsEMG on a wide scale is currently still limited due to the specific equipment and software required, the following steps may help to make it a more accepted technique in basic research and clinically oriented studies.

First, the high specificity of electrically evoked MDs in patients with MND described in **Chapter 6** warrants ongoing research in this direction. This may be in the form of a study to further investigate the diagnostic value and applicability of this approach in current clinical practice. A close cooperation with specialized ALS centers is recommended to collect a sufficiently large group of patients and controls. Such a study should also be able to clarify the prognostic value of electrically evoked MDs.

Another way forward would be to undertake the technical challenges involved in the HDsEMG technique [61, 62]. Data analysis software of the procedures for MUNE, motor unit tracking, and spontaneous/voluntary MU activity can be further refined to reduce time for data analysis, and to enhance the detection of clinically relevant variables, e.g. the widespread presence of fasciculations. Although it is not without difficulties, the approach described in **Chapter 4** can potentially support the clinical examination, because it is a more sensitive and objective screening method in measuring spontaneous activity in muscles.

A small-scale motor unit tracking study, in conjunction with motor nerve excitability testing (applied on single MUs [42, 63]), could be applied in patients with MND versus healthy controls, or in patients with MND in multiple follow-up sessions. This way, the changes of MU properties may reveal novel pathophysiological insights into the disease process in individual MUs. If so, this may provide a better understanding of the processes related to MU degeneration.

The promising findings of this thesis provide a basis for, and will hopefully stimulate, future methodologically, pathophysiologically and clinically oriented studies of surface EMG in electrodiagnostics. The combined effort into these three areas is required to bring surface EMG closer to clinical application. This thesis offers tools along this path, which may help us to reach the ultimate goal: to improve quality of life and life expectancy in patients with MND.

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# Chapter 9

## Summary
## Samenvatting

## Summary

Motor neuron disease (MND) is a severe and incurable disease, characterized by the progressive loss of motor neurons that control voluntary contraction of the muscles. The most common variants of MND are amyotrophic lateral sclerosis (ALS) and progressive muscular atrophy (PMA). Owing to the relentless course of the disease, an early and accurate diagnosis is important especially when new and effective drug therapies become available. Evaluating the effectiveness of novel therapies (to ultimately improve quality of life and life expectancy) requires an accurate and reproducible marker that can monitor disease progression. For this purpose, the smallest functional element of the neuromuscular system for voluntary muscle contraction is studied, known as the motor unit (MU). In MND, these functional components are affected, which is why evaluating the functioning of MUs is an important aspect in the electrodiagnostic examination. As described in **Chapter 1,** current standard electromyographic (EMG) techniques have their limitations, especially when measuring at the level of individual MUs. These limitations can partly be resolved by more advanced surface EMG techniques. The possibilities offered by the advanced surface EMG techniques may eventually support the diagnostic process and give a more objective insight into the course of the disease. This thesis predominantly focused on fasciculation potentials (FPs), a clinical hallmark in MND, and multiplet discharges (MDs) after electrical stimulation. These two types of ectopic activity of individual MUs are thought to be an expression of a diseased state of MUs due to MND. Therefore, they may have diagnostic and prognostic relevance. The research described in this thesis has the following aims: (1) to evaluate and further improve the available research methods for the non-invasive study of individual MUs, (2) to develop neurophysiological markers of clinical relevance in individual patients with ALS and PMA, which can be obtained with these surface EMG methods, and (3) to clarify the pathophysiological and clinical significance of FPs and electrically evoked MDs.

One of the surface EMG methods that needed to be further developed was the compound muscle action potential (CMAP) scan, which reflects the successive activation of all MUs in a muscle. The CMAP scan provides clinical information on MU size, MU number and axonal excitability that complements the electrodiagnostic findings obtained with routine motor nerve conduction studies and needle EMG. Currently, a manual procedure is applied to extract the clinical information from the CMAP scan, which is time-consuming and susceptible to operator-related variability. In order to overcome these issues, an automated method is described in **Chapter 2** that has been developed to objectively quantify the largest discontinuities (visual as gaps) in the CMAP scan. This has resulted in a novel neurophysiological marker, D50, which was applied to CMAP scans originating from healthy subjects and patients with a variety of neuromuscular disorders. This marker was shown to be sensitive to MU loss and reinnervation, which are processes highly consistent with ALS and PMA. Therefore, it is potentially useful to monitor disease progression in patients with MND. Due to the small computational effort in determining D50, the results can become directly available to the clinician during the electrodiagnostic examination.

Another research method, motor unit tracking using high-density surface EMG (HDsEMG), has been introduced as a novel approach for monitoring individual MUs over time. This may yield new information on pathophysiological changes of the disease process on individual MU level. At present, motor unit tracking is performed without correcting for displacement of the HDsEMG electrode array between recording sessions. To achieve this, we developed an algorithm to correct for HDsEMG electrode displacement errors in motor unit tracking (**Chapter 3**). This proof-of-concept study in healthy subjects shows that automated correction is feasible when identifying MU action potentials between the measurement sessions. When incorporated in the software, the automated correction may improve the certainty with which changes in MU action potentials can be ascribed to pathophysiological changes. This will allow motor unit tracking for follow-up studies with a higher precision than currently possible.

FPs are incorporated in the latest electrodiagnostic criteria for ALS, but they can also be observed in healthy subjects. They are commonly detected as isolated single MU discharges. However, in routine electrodiagnostics, the identification of FPs relies on subjective assessment. Therefore, in **Chapter 4** a novel objective approach was introduced that characterizes MU discharges in a muscle at rest. In the automated approach applied to HDsEMG recordings in patients with ALS and PMA and healthy controls, MU discharges were quantified based on the interspike interval before and after each discharge. This approach revealed that, based on the discharge pattern of single MUs, it was actually not possible to identify pathological discharge patterns. Considering the common clinically observed abundance of fasciculations, a new marker was determined that takes into account the activity of multiple MUs showing isolated discharges in a specific time interval. By using this marker, we obtained an improved identification of patients with MND compared to healthy controls. This can be especially helpful in clinically unaffected muscles, where fasciculations might be the only feature present. Furthermore, when this marker is implemented in routine needle EMG software, it may serve as a more objective approach to determine the level of spontaneous MU activity during electrodiagnostic examination.

Electrically evoked MDs using HDsEMG have been recently applied to study the distal involvement of the motor neuron in patients with ALS and PMA. These MDs were thought to be related to pathophysiological excitability changes. The findings described in **Chapter 5** confirm this relation between the presence of electrically evoked MDs and altered motor axonal excitability properties in patients with suspected MND. Moreover, the excitability changes were similar to changes observed in previous studies addressing fasciculations in patients with ALS. This implies a common pathophysiological mechanism for electrically evoked MDs and distal fasciculations. These MDs may therefore have similar diagnostic significance as FPs in patients with MND. To investigate this, a diagnostic study was performed in patients with suspected MND (**Chapter 6**). The results demonstrated that electrically evoked MDs are a potentially valuable measure in the diagnostic phase of patients with ALS and PMA. Furthermore, the findings indicated that they are an even more

specific electrophysiological sign of lower motor neuron dysfunction than FPs. A subsequent study was performed to assess whether electrically evoked MDs were related to the disease progression in patients with ALS and PMA (**Chapter 7**). The findings showed that electrically evoked MDs in patients with ALS and PMA are associated with more severe clinical deterioration. This suggests that the mechanism that promotes the occurrence of electrically evoked MDs is related to pathophysiological changes. Finally, in **Chapter 8** the main findings of this thesis are discussed, as well as directions for future work. This thesis has led to clinically relevant insights for patients with MND, which cannot be obtained with routine EMG techniques. Several interesting approaches and challenges remain present to further improve the applied surface EMG methods as a research tool and to bring them closer to a hopefully wider clinical application.

## Samenvatting

"Motor neuron aandoeningen" zijn een categorie van zenuwaandoeningen die in het Engels beter bekend staat onder de term "motor neuron disease" (afgekort MND). Deze ernstige en ongeneeslijke aandoeningen worden gekenmerkt door het progressieve verlies van motorische neuronen die zorgen voor de vrijwillige aanspanning van de spieren. De meest voorkomende varianten van MND zijn amyotrofische lateraal sclerose (ALS) en progressieve musculaire atrofie (PMA). Vanwege het meedogenloze ziekteverloop is het belangrijk om in een vroeg stadium een nauwkeurige diagnose te stellen. Dit is vooral van belang wanneer er effectieve medicijnen beschikbaar komen. Voor het evalueren van de effectiviteit van nieuwe therapieën (om uiteindelijk kwaliteit van leven en de levensverwachting te verbeteren) is er een nauwkeurige, reproduceerbare marker nodig om het ziekteverloop te meten. Hiervoor is de kleinste functionele eenheid in het neuromusculaire systeem voor de vrijwillige aanspanning van een spier bestudeerd, die ook wel de motorische eenheid ("motor unit" in het Engels, afgekort MU) wordt genoemd. Individuele MUs zijn belangrijke functionele componenten die aangedaan zijn door MND. Vandaar ook dat de evaluatie van het functioneren van MUs een belangrijk onderdeel is bij het elektrodiagnostisch onderzoek. Zoals beschreven in **Hoofdstuk 1**, hebben de huidige elektromyografische (EMG) technieken die gebruikt worden bij het elektrodiagnostisch onderzoek enkele nadelen, vooral bij het meten op het niveau van individuele MUs. Deze nadelen kunnen door meer geavanceerde oppervlakte-EMG-technieken ("surface EMG" in het Engels, afgekort sEMG) gedeeltelijk verholpen worden. Deze sEMG-technieken hebben als mogelijkheid om uiteindelijk te helpen bij het stellen van een diagnose en het ziekteverloop in kaart te brengen. Dit proefschrift is grotendeels gericht op het meten van fasciculatiepotentialen ("fasciculation potentials" in het Engels, afgekort FPs), een belangrijk klinisch kenmerk in MND, en repetitieve ontladingen ("multiplet discharges" in het Engels, afgekort MDs) na elektrische stimulatie. Deze twee typen van ectopische activiteit van individuele MUs worden gezien als een uiting van zieke, aangedane MUs als gevolg van MND. Daarom kunnen ze mogelijk ook van diagnostische en prognostische waarde zijn. Het onderzoek beschreven in dit proefschrift heeft de volgende doelen: (1) het evalueren en verder verbeteren van de beschikbare onderzoeksmethoden om op niet-invasieve wijze individuele MUs te kunnen bestuderen, (2) het ontwikkelen van klinisch relevante neurofysiologische markers bij individuele patiënten met ALS en PMA, die kunnen worden verkregen met deze sEMG-methoden, en (3) het verduidelijken van de pathofysiologische en klinische betekenis van FPs en MDs na elektrische stimulatie.

Eén van die sEMG-technieken die verder ontwikkeld moest worden, was de "compound muscle action potential" (CMAP) scan. Hierin worden door elektrische stimulatie van een motorische zenuw achtereenvolgens alle MUs in een spier geactiveerd. De CMAP-scan geeft klinische informatie over de grootte van MUs, het aantal MUs en de prikkelbaarheid van de zenuw. Dit is een waardevolle aanvulling op de bevindingen die worden verkregen tijdens het motorische geleidingsonderzoek en het naald-EMG. Op dit moment wordt er een handmatige methode gebruikt om de klinische

informatie uit de CMAP-scan te halen. Dit proces is tijdrovend en gevoelig voor variabiliteit tussen gebruikers. Om deze tekortkomingen te verhelpen, wordt er in **Hoofdstuk 2** een automatische methode beschreven, waarbij de grootste discontinuïteiten (zichtbaar als stappen) in de CMAP-scan nu objectief gekwantificeerd worden. Dit heeft geresulteerd in een nieuwe neurofysiologische marker, D50, die toegepast is op CMAP-scans van zowel gezonde proefpersonen als patiënten met verschillende neuromusculaire aandoeningen. Daarbij hebben we aangetoond dat deze marker gevoelig is voor MU-verlies en reinnervatie. Deze processen zijn allebei duidelijk aanwezig bij patiënten met ALS en PMA. Daarom is deze methode mogelijk ook te gebruiken bij het meten van het ziekteverloop bij patiënten met MND. Het bepalen van D50 vergt weinig rekenkracht, dus het resultaat kan direct beschikbaar komen voor de arts tijdens het elektrodiagnostisch onderzoek.

Een andere methode, genaamd 'motor unit tracking' met behulp van 'high-density' oppervlakte-EMG ("high-density surface EMG" in het Engels, afgekort HDsEMG) is onlangs geïntroduceerd om individuele MUs in de tijd te volgen. Dit kan mogelijk informatie opleveren over de pathofysiologische veranderingen van het ziekteproces op het niveau van een individuele MU. Op dit moment wordt motor unit tracking uitgevoerd zonder rekening te houden met verplaatsingen van het HDsEMG-elektrodenmatje tussen meetsessies. Om dit te verhelpen, hebben we een algoritme ontwikkeld waardoor verplaatsingsfouten van het HDsEMG-elektrodenmatje bij motor unit tracking worden gecorrigeerd (**Hoofdstuk 3**). Uit dit 'proof-of-concept'-onderzoek bij gezonde proefpersonen blijkt dat automatische correctie mogelijk is bij het identificeren van specifieke MU-actiepotentialen die gevonden zijn tijdens meerdere metingen. Wanneer deze methode wordt opgenomen in de software voor de analyse van motor unit tracking, kunnen hiermee mogelijk de veranderingen in de MU-actiepotentialen met grotere betrouwbaarheid toegeschreven worden aan werkelijke pathofysiologische veranderingen. Hierdoor kan motor unit tracking in vervolgonderzoek mogelijk met een hogere nauwkeurigheid worden uitgevoerd dan momenteel mogelijk is.



FPs zijn opgenomen in de meest recente elektrodiagnostische criteria voor ALS, maar ze worden ook gezien bij gezonde proefpersonen. Vaak worden ze gedetecteerd als enkele, geïsoleerde MU-ontladingen, maar uiteindelijk worden FPs in het elektrodiagnostisch onderzoek op een subjectieve manier geïdentificeerd. Daarom wordt er in **Hoofdstuk 4** een nieuwe objectieve manier geïntroduceerd voor het karakteriseren van MU-ontladingen in een spier in rust. In de automatische analyse, toegepast op HDsEMG-metingen bij patiënten met ALS en PMA en gezonde proefpersonen, worden MU-ontladingen gekwantificeerd op basis van de tijdsintervallen voor en na elke ontlading. Echter, uit de automatische analyse bleek dat het op basis van enkele MUs niet mogelijk was om een pathologisch ontladingspatroon te identificeren. Aangezien er tijdens klinisch onderzoek vaak een overvloed aan fasciculaties wordt gevonden, werd er een nieuwe marker bepaald die rekening houdt met de activiteit van meerdere MUs met geïsoleerde ontladingen in een specifiek tijdsinterval. Met deze marker bleken patiënten met MND beter te kunnen worden geïdentificeerd in vergelijking met gezonde proefpersonen. Dit kan vooral nuttig zijn in klinisch niet aangedane spieren waarin alleen

nog fasciculaties waargenomen worden. Als deze marker tevens in standaard naald-EMG-software wordt geïmplementeerd, zou deze mogelijk een objectievere manier zijn om de mate van spontane MU-activiteit te bepalen tijdens het elektrodiagnostisch onderzoek.

Elektrisch geactiveerde MDs zijn onlangs toegepast om het distale gedeelte van het motorneuron te bestuderen bij patiënten met ALS en PMA. Men vermoedde dat deze MDs gerelateerd waren aan pathofysiologische veranderingen in de prikkelbaarheid van de zenuw. De bevindingen in **Hoofdstuk 5** bevestigen dat bij patiënten met een klinische verdenking op MND de aanwezigheid van elektrisch geactiveerde MDs gerelateerd is aan de veranderde eigenschappen van de prikkelbaarheid van zenuwvezels. Bovendien waren de veranderingen in prikkelbaarheid vergelijkbaar met veranderingen waargenomen bij eerdere onderzoeken naar fasciculaties bij patiënten met ALS. Dit wijst op een gemeenschappelijk pathofysiologisch mechanisme voor elektrisch geactiveerde MDs en distale fasciculaties. Deze MDs hebben daarom ook mogelijk dezelfde diagnostische betekenis als FPs. Om dit te onderzoeken, is er een diagnostische studie uitgevoerd bij patiënten met een klinische verdenking van MND (**Hoofdstuk 6**). De resultaten toonden aan dat elektrisch geactiveerde MDs in de diagnostische fase bij patiënten met ALS en PMA een potentieel waardevolle maat zijn. Uit de bevindingen bleek tevens dat ze een specifieker elektrofysiologisch kenmerk zijn voor aantasting van het perifeer motorisch neuron dan FPs. Een volgende studie was uitgevoerd om na te gaan of deze MDs ook gerelateerd zijn aan het ziekteverloop bij patiënten met ALS en PMA (**Hoofdstuk 7**). Uit de bevindingen blijkt dat elektrisch geactiveerde MDs bij patiënten met ALS en PMA samenhangen met een sterkere klinische achteruitgang. Dit doet vermoeden dat het mechanisme dat zorgt voor het optreden van elektrisch geactiveerde MDs gerelateerd is aan pathofysiologische veranderingen. Tot slot worden in **Hoofdstuk 8** de belangrijkste bevindingen en richtingen voor toekomstig onderzoek besproken. Dit proefschrift heeft geleid tot klinisch relevante inzichten bij patiënten met ALS en PMA, die niet kunnen worden verkregen met de huidige EMG-technieken. Er blijven verschillende interessante opties en uitdagingen over voor de verdere verbetering van de toegepaste sEMG-methoden als onderzoeksinstrument en van de methoden zelf, waardoor deze methoden dichter bij een hopelijk bredere klinische toepassing komen.

## List of abbreviations

| | |
|---|---|
| AANEM | American Association of Neuromuscular & Electrodiagnostic Medicine |
| ALS | amyotrophic lateral sclerosis |
| ALSFRS-R | amyotrophic lateral sclerosis functional rating scale – revised |
| APB | abductor pollicis brevis |
| AUC | area under the curve |
| BFS | benign fasciculation syndrome |
| CI | confidence interval |
| CMAP | compound muscle action potential |
| CMC | carpometacarpal |
| CTS | carpal tunnel syndrome |
| DM | diabetes mellitus |
| EDB | extensor digitorum brevis |
| EEC | El Escorial criteria |
| EMG | electromyography |
| FMF | fine motor function |
| FP | fasciculation potential |
| GBS | Guillain-Barré syndrome |
| HDsEMG | high-density surface electromyography |
| IBM | inclusion body myositis |
| IED | inter-electrode distance |
| IQR | interquartile range |
| ISI | interspike interval |
| IVIg | intravenous immunoglobulin |
| I/V | current-threshold |
| LMN | lower motor neuron |
| MCP | metacarpophalangeal |
| MD | multiplet discharge |
| MMN | multifocal motor neuropathy |
| MND | motor neuron disease |
| MRI | magnetic resonance imaging |
| MU | motor unit |
| MUAP | motor unit action potential |
| MUNE | motor unit number estimation |
| MVC | maximum voluntary contraction |
| NCS | nerve conduction studies |
| NCV | motor nerve conduction velocity |
| NRMSE | normalized root mean square error |

| | |
|---|---|
| PCB | printed circuit board |
| PLS | primary lateral sclerosis |
| PMA | progressive muscular atrophy |
| PPV | positive predictive value |
| PSW | positive sharp waves |
| ROC | receiver operating characteristic |
| SD | standard deviation |
| SDTC | strength duration time constant |
| SEM | standard error of the mean |
| sEMG | surface electromyography |
| SI | stimulus intensity |
| SMA | spinal muscular atrophy |
| TE | threshold electrotonus |
| TEd | depolarizing threshold electrotonus |
| TEh | hyperpolarizing threshold electrotonus |
| UMN | upper motor neuron |
| VC | vital capacity |

# Publications

## *Journals*

- **Sleutjes BTHM**, Montfoort I, van Doorn PA, Visser GH, Blok JH. *Diagnostic accuracy of electrically elicited multiplet discharges in patients with motor neuron disease*, Journal of Neurology, Neurosurgery and Psychiatry, 2014, In press

- **Sleutjes BTHM**, Montfoort I, van Doorn PA, Visser GH, Blok JH. *Increased supernormality in patient with multiplet discharges: evidence for a common pathophysiological mechanism behind multiplets and fasciculations*, Clinical Neurophysiology, 2014, In press

- Gligorijević I, **Sleutjes BTHM**, De Vos M, Blok JH, Montfoort I, Mijović B, Signoretto M, Van Huffel S. *Correcting electrode displacement errors in motor unit tracking using high-density surface electromyography (HDsEMG)*, Methods of Information in Medicine, 2014, In press

- **Sleutjes BTHM**, Montfoort I, Maathuis EM, Drenthen J, van Doorn PA, Visser GH, Blok JH, *CMAP scan discontinuities: Automated detection and relation to motor unit loss* Clinical Neurophysiology, 2014, 125 (4), 388-395

- **Sleutjes BTHM**, Maathuis E, van Doorn PA, Blok JH, Visser GH. *Electrically evoked multiplet discharges are associated with more severe clinical deterioration in motor neuron disease*, submitted

- **Sleutjes BTHM**, Gligorijević I, Montfoort I, van Doorn PA, Visser GH, Blok JH. *Identifying fasciculation potentials in motor neuron disease: a matter of probability*, submitted

- **Sleutjes BTHM**, Kemps HMC, Thijssen EJM, van de Vosse FN, Schep G, Peters CH, Wijn PFF, *The reliability of continuous measurement of mixed venous oxygen saturation during exercise in patients with chronic heart failure*, European Journal of Applied Physiology, 2008, 102(4), 493-496

- Kemps HMC, Thijssen EJM, Schep G, **Sleutjes BTHM**, de Vries WR, Hoogeveen AR, Wijn PFF, Doevendans PA, *Evaluation of two methods for continuous cardiac output assessment during exercise in chronic heart failure patients*, Journal of Applied Physiology, 2008, 105(6), 1822-1829

# Publications

## *Conferences*

- **Sleutjes BTHM**, Montfoort I, van Doorn PA, Visser GH, Blok JH. *Diagnostic accuracy of electrically elicited multiplet discharges in patients with motor neuron disease*, 30th International congress of clinical neurophysiology (ICCN), March 20-23, 2014, Berlin, Germany

- **Sleutjes BTHM**, Montfoort I, van Doorn PA, Visser GH, Blok JH. *Multiplet discharges are related to increased supernormality period in ALS and PMA patients*, 24th International symposium on ALS/MND, December 6 -8 , 2013, Milan, Italy

- **Sleutjes BTHM**, Montfoort I, Blok JH. *Automated CMAP scan pattern analysis detects motor unit loss*, Oral presentation at the 4th Dutch Biomedical Engineering Conference 2013, January 24-25, 2013, Egmond aan Zee, The Netherlands

- **Sleutjes BTHM**, Montfoort I, Blok JH. *CMAP scan pattern analysis detects motor unit loss*, 59th Annual Meeting of the American Association of Neuromuscular & Electrodiagnostic Medicine (AANEM), October 3-6, 2012, Orlando, Florida, USA. **Winner of the President's Research Initiative Award**

- Gligorijević I, **Sleutjes BTHM**, De Vos M, Blok JH, Montfoort I, Mijović B, Signoretto M, Van Huffel S. *Correcting electrode displacement errors in motor unit tracking using high-density surface electromyography (HDsEMG)*, 7th International Workshop on Biosignal Interpretation, July 2-4, 2012, Como, Italy

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# Dankwoord

Nu is het dan zover dat ik aan mijn dankwoord kan beginnen. Dat betekent dat mijn promotie er bijna op zit. In het begin van mijn onderzoek, als er door mijn omgeving gevraagd werd waarnaar ik onderzoek deed en ik daarbij de zenuwaandoening ALS liet vallen, bleef het rinkelen van het spreekwoordelijke belletje vaak achterwege. Daarin is vooral afgelopen jaar veel veranderd door de wereldwijde hype met de Ice Bucket Challenge. In dit gedeelte van mijn proefschrift wil ik graag terugblikken op de afgelopen jaren en vooral stilstaan bij de mensen die direct en indirect betrokken zijn geweest bij mijn promotieonderzoek.

Beste promotor, beste Pieter, vanaf het begin van mijn project heb je me gesteund. Ook na het vertrek van zowel Joleen als Gerhard ben je blijven vertrouwen in een succesvolle afronding. Het laatste jaar heb je zonder problemen min of meer de rol van directe begeleider overgenomen, ondanks je drukke agenda. Dankzij jou heb ik een blik kunnen werpen in het werk van een neuroloog. Bij onze afspraken nam je de tijd om mij te dwingen helder, kort en bondig te verwoorden wat daadwerkelijk belangrijk is in de klinische praktijk door het stellen van de juiste en kritische vragen. Bij jou geldt afspraak is afspraak en dat is erg veel waard. Ik waardeer het zeer dat je me ook bij andere zaken hebt betrokken, zoals de neuro-immunologiebijeenkomsten op de woensdagmiddag en de vele neurologie-activiteiten. Hierin ben je in staat om de juiste balans en sfeer te creëren tussen plezier hebben en hoogwaardig onderzoek doen. Naast dit proefschrift, heeft dit geresulteerd in onze eigen Ice Bucket Challenge. We zitten nog steeds te wachten op de video van de uitgedaagden. Dank voor je begeleiding en de ruimte die ik gekregen heb om mijn onderzoek af te ronden.

Beste copromotoren, beste Joleen en Gerhard, bij mijn sollicitatiegesprek met jullie eind 2009 kwam ik in eerste instantie voor een andere functie, maar ik liep anderhalf uur later naar buiten met een promotieonderzoek. Bij de KNF waren jullie de drijvende kracht achter veel lopende onderzoeksprojecten naast dat van mij. Daarom was het ook een enorme aderlating toen jullie na 1,5 jaar van mijn promotieonderzoek relatief kort na elkaar aangaven te vertrekken bij de KNF. Hierna was het niet alleen voor mij, maar ook voor jullie, zoeken naar de juiste balans om het project tot een goed einde te brengen. Dat was niet altijd even gemakkelijk, maar nu ik het dankwoord kan schrijven, betekent dat dus dat het is gelukt! Joleen, jij bent als geen ander in staat om zowel de meest ingewikkelde details als de grote lijnen binnen enkele minuten helder en overtuigend te verwoorden en tevens duidelijk op papier over te brengen. Na je vertrek is Rotterdam – Delft dan ook bij mijn vaste routes gaan horen. Dank voor de vele waardevolle commentaren op mijn artikelen en je begeleiding tijdens het project. Gerhard, na je vertrek is ook Rotterdam – Heemstede een bekende route voor me geworden. In onze besprekingen kon je altijd duidelijk mijn onderzoek en resultaten in het KNF-perspectief zetten en vanuit dat oogpunt aangeven welke elementen belangrijk waren. Ik wil je dan ook bedanken voor je begeleiding.

Graag wil ik hierbij ook de leden van de kleine commissie, prof.dr. P.A.E. Sillevis Smitt, prof.dr.ir. D.F. Stegeman en dr. H. Franssen, bedanken voor het beoordelen van het manuscript. Prof. Sillevis Smitt, bedankt dat u de tijd wilde nemen om het manuscript te beoordelen tussen uw drukke werkzaamheden door. Prof. Stegeman, dank voor uw waardevolle suggesties om het manuscript te verbeteren. Dr. Franssen, dank voor uw hulp bij de interpretatie van enkele meetresultaten tijdens mijn promotieonderzoek. De overige leden van de commissie, prof.dr. H.J. Stam en prof.dr. H.C.W. Beijerinck wil ik bedanken voor hun bereidheid om zitting te nemen in de promotiecommissie. Prof. Beijerinck, dank dat u op deze manier bij de afsluiting van mijn promotietraject wilt zijn, nadat ik via u ook in Rotterdam terechtgekomen ben.

Beste Inger, uiteindelijk ben je relatief kort bij de KNF geweest, mede door je zwangerschap, maar ook omdat je hart uiteindelijk toch meer ergens anders lag. Ondanks dat, was je bij bijna alle studies van mijn promotieonderzoek betrokken. Dank voor de vele tips voor het schrijven van artikelen en het regelen van de zaken rondom mijn promotie.

Beste Ellen en Judith, bij de start van mijn project waren jullie de andere twee promotieonderzoekers bij de KNF. Dank voor het wegwijs maken in de wereld van de EMG-onderzoekstechnieken. Ellen, jouw onderzoek heb ik voortgezet. Ondertussen ben je moeder geworden en begonnen met de huisartsenopleiding. Judith, jij bent nu neuroloog op de afdeling. Bij jou kan ik altijd terecht voor waardevolle discussies over de mogelijke klinische toepassingen van ons onderzoek. Alle twee veel succes met de afronding van jullie promotie!

Dear Ivan, thank you for our research related discussions in Leuven. Within a few weeks, we finished our "Como" paper. Eventually, this cooperation resulted in a second paper as well. I will never forget our trip to Milan and Como. I had a great time, so I hope we keep in touch!

Beste Joseph, ik waardeer het zeer dat je ruim de tijd nam om te discussiëren over mijn resultaten en mijn artikelen te bekijken in de laatste maanden van mijn promotie. Het was niet ongewoon dat ik na een gesprek met jou naar buiten liep met relevante wetenschappelijke artikelen in mijn Inbox. Daarbij was je altijd oprecht geïnteresseerd in hoe mijn bevindingen van nut konden zijn in de praktijk.

Erg veel praktische zaken tijdens mijn promotieproject zijn een stuk gemakkelijker verlopen dankzij Monique en Jacqueline. Dank voor het werk dat jullie mij uit handen hebben genomen, de tijd die jullie namen voor een praatje en de gezelligheid die jullie meebrachten!

Tijdens de periode waarin de patiënten bij mij langskwamen had ik regelmatig contact met Cisca. Jij bent degene die de patiënten opvangt als ze het vreselijke nieuws hebben gehoord. In dit moeilijke proces ben jij een steun voor hen. Ik vind het bewonderenswaardig met hoeveel toewijding je dit doet. Het was een plezier om met je samen te werken! Hier wil ik dan ook de patiënten bedanken voor hun deelname aan mijn onderzoek. Hun persoonlijke situaties hebben veel indruk op mij gemaakt.

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Anouk, jij was met je keuzeonderzoek bezig bij de KNF toen ik met mijn promotieonderzoek begon. Daarna hebben we contact gehouden en inmiddels ben jij ook bijna klaar met je promotieonderzoek. De afgelopen jaren hebben we regelmatig samen koffiepauzes gehad, waarin we met veel plezier het lief en leed van het doen van promotieonderzoek met elkaar deelden. Succes met de afronding van jouw promotie!

Gedurende mijn project zijn er meer medewerkers bij de KNF en Neurologie, en collega-onderzoekers in meer of mindere mate bij mijn promotie betrokken geweest. Ik kan jullie hier niet allemaal noemen, want dan ga ik zeker namen vergeten. Allemaal bedankt voor de mooie tijd!

Dan zijn er nog veel mensen buiten mijn werk om die voor een onvergetelijke tijd hebben gezorgd tijdens mijn promotie. Familie, vrienden en kennissen waarmee ik de afgelopen jaren opgetrokken heb. Oud-studiegenoten van BMT, mede-"topsporters" van het schaatsen, tennissen en hardlopen uit Rotterdam en de regio Eindhoven. Bernard, je was altijd belangstellend over de vorderingen van mijn promotieonderzoek. Succes met het afronden van je coschappen! Frederieke, dank voor de steun, het doorlezen van de teksten en het letten op de taalkundige formuleringen. Je was echt betrokken bij de laatste loodjes van mijn proefschrift. Myron en Joost, bedankt voor de vele mooie stapavonden in Rotterdam en avontuurlijke vakanties. Verder ook mijn improvisatiegroepje, we vormen een mooi, chaotisch stelletje bij elkaar. Het was heerlijk om na een dag werken plots midden in een ruimtereis te zitten, een giraffe met hoogtevrees te hebben verkocht en panda's voorbij te zien vliegen.

Paul en Ot, ik ben zeer vereerd dat jullie mijn paranimfen willen zijn. Ot, na onze studie BMT in Eindhoven hebben we altijd contact gehouden. Door mijn verhuizing naar Rotterdam, de afronding van jouw promotie en je nieuwe baan, hebben we elkaar de laatste jaren minder vaak gezien. Ondanks dat hielden we elkaar op de hoogte van de belangrijke zaken in het leven. Paul en Samantha, bij jullie staat de deur altijd voor mij open, dank daarvoor. Paul, de beste besprekingen waren misschien wel onze wekelijkse "meetings" op de dinsdagavond in Rotterdam of Den Haag. We kunnen lachen om de meest onzinnige dingen, praten over de meest serieuze zaken in het leven, inhoudelijk discussies voeren over ons werk en werkelijk alles daar tussenin. Het feit dat we daar zo gemakkelijk tussen kunnen switchen is bijzonder. Ik hoop onze "meetings" nog lang voort te kunnen zetten.

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Boudewijn Sleutjes
Rotterdam, maart 2015

## Curriculum vitae

Boudewijn Sleutjes werd geboren op 2 juni 1983 te Boxtel. In 2001 behaalde hij zijn Gymnasium diploma aan het Jacob-Roelandslyceum te Boxtel. Aansluitend studeerde hij Biomedische Technologie aan de Technische Universiteit Eindhoven. Deze studie sloot hij met grote waardering af in 2007. Zijn afstudeerwerk deed hij op de afdeling Klinische Fysica en Sportgeneeskunde van het Máxima Medisch Centrum te Veldhoven. Hierna begon hij met de ontwerpersopleiding "Design and Technology of Instrumentation" van het Stan Ackermans Instituut. Als onderdeel van deze ontwerpersopleiding werkte hij 1 jaar bij Philips Research in Eindhoven en 4 maanden bij Ballard Power Systems in Vancouver, Canada. Deze tweejarige ontwerpersopleiding rondde hij af in december 2009. In maart 2010 startte hij met zijn promotieonderzoek bij de afdeling Klinische Neurofysiologie en Neurologie van het Erasmus MC te Rotterdam, resulterend in dit proefschrift.

# PhD portfolio

| Summary of PhD training and teaching | Year | Workload (ECTS) |
|---|---|---|
| *General courses* | | |
| Structure and organization of the nervous system | 2010 | 3.0 |
| Motor systems | 2011 | 3.0 |
| Neurological disorders | 2011 | 3.0 |
| Biomedical English writing and Communication | 2011 | 3.0 |
| Course for quantitative researchers | 2012 | 3.0 |
| Repeated measurements | 2012 | 1.4 |
| Missing values in clinical research | 2012 | 0.7 |
| | | |
| *International conferences* | | |
| 3rd and 4th Dutch Biomedical Engineering Conference | 2011, 2013 | 2.0 |
| 7th International Workshop on Biosignal Interpretation | 2012 | 0.5 |
| 59th Annual Meeting of the AANEM | 2012 | 1.0 |
| 24th International symposium on ALS/MND | 2013 | 0.5 |
| 30th International congress of clinical neurophysiology (ICCN) | 2014 | 0.5 |
| | | |
| *Seminars and workshops* | | |
| Reanimatie bij volwassenen, basic life support | 2012 | 0.1 |
| Trainingsdag dialoogtraining | 2012 | 0.2 |
| Loopbaanoriëntatie | 2012 | 1.0 |
| PhD day | 2010, 2012 | 0.5 |
| | | |
| *In depth courses* | | |
| MUNE techniques + MUAP quantitation at AANEM | 2012 | 0.5 |
| | | |
| *Other* | | |
| Prinses Beatrix Spierfonds, Spierziektecongres | 2012 | 0.5 |
| ALS congres | 2010 | 0.2 |
| Presentaties bij KNF en Neurologie | 2012 - 2015 | 1.0 |
| Presentaties bij neuro-immunologiebijeenkomsten | 2011 - 2015 | 1.0 |
| Wetenschapsmiddag, Erasmus MC | 2013 | 0.1 |
| KNF dagen | 2014 | 1.0 |
| | | |
| *Teaching activities* | | |
| Supervising medical students | 2010 | 1.5 |
| Teaching of 2nd year master of neuroscience students | 2011 - 2013 | 1.5 |