

**NEUROMUSCULAR MODELLING OF SKELETAL
MUSCLE CONTRACTION FROM EXPERIMENTAL
MOTONEURONAL ACTIVITY**

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I declare that, except where reference is explicitly made to the work of others, this dissertation is the result of my own work and has not been submitted for any other degree at Imperial College London or any other institution.

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ABSTRACT

To investigate human voluntary muscle contraction, computational neuromuscular models are commonly modelled with a single Hill-type muscle actuator receiving a unique experimental neural control from electromyography (EMG). Despite countless successful applications, this approach is limited as it cannot describe nor control the recruitment and neuromechanical dynamics of the individual motor units (MUs) constituting the muscle. As detailed in the first systematic review of the field reported in this thesis, current Hill-type modelling approaches are therefore limited to simulate and investigate neuromuscular control during human voluntary muscle contraction.

The aim of this thesis was to develop a novel neuromuscular model described as a collection of Hill-type MU actuators and controlled by adequate experimental neural inputs to allow the forward simulation of the MU pool dynamics during human voluntary muscle contraction.

A large portion of this thesis focused on the experimental and computational methods necessary to obtain reliable neural inputs to the modelled MUs. Motoneuron (MN) spike trains were experimentally identified from the decomposition of high-density EMG (HDEMG) signals. With refinements in the experimental and processing protocols, the number and quality of the identified spike trains were maximized. Despite that, only small portions of the active MU pool were identified, and the HDEMG spike trains did not provide an accurate approximation of the real neural command to muscle. To address this limitation, a novel computational pipeline was developed to infer from the HDEMG spike trains the discharge activity of the complete MN population. The pipeline used mathematical relationships between eight MN electrophysiological and anatomical properties, that were derived for the first time from the processing of an extensive set of mammal studies. The new extrapolated set of discharging MN proved to both accurately replicate the HDEMG data and accurately approximate the neural command to muscle.

The reconstructed MN spike trains were input to a state-of-the-art neuromuscular model to control its collection of Hill-type MUs. In doing so, this MN-driven model intrinsically accounted for the dynamics of MU recruitment and forward simulations of human voluntary muscle contraction were performed. The model was scaled with subject-specific musculoskeletal quantities and the predicted muscle force, obtained with advanced models of the MU neuromechanical properties, was experimentally validated.

By bridging the gap between recently available experimental data at the MN level and Hill-type neuromuscular modelling, this thesis provides a state-of-the-art tool for investigating neuromuscular control during human voluntary contractions, with possible important applications in neurorehabilitation and human-machine interfacing.

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NOMENCLATURE

The key acronyms and notations used in this thesis are reported in the following table.

MN	Motoneuron
MU	Motor Unit
EMG	Electromyogram / Electromyographic signal
HDEMG	High-Density EMG
bEMG	Bipolar EMG
CNS	Central Nervous System
AP	Action Potential
MSK	Musculoskeletal
MRI	Magnetic Resonance Imaging
TA	Tibialis Anterior
MVC	Maximum Voluntary Contraction
PNR	Pule-to-Noise Ratio
CST	Cumulative Spike Train
eND	effective Neural Drive
F^{th}	MN/MU Force recruitment threshold

Chapter 1

INTRODUCTION

1.1. Context of this research

Contrary to cardiac or smooth muscles, skeletal muscles are voluntary muscles, which means that the timing and the amplitude of their contraction process can be voluntarily controlled by the central nervous system (CNS), i.e., by the brain and the spinal cord. Because they are attached to the bones with tendinous insertions, the contracting skeletal muscles transmit a pulling force to the skeletal structure, allowing vital functions for vertebrate animals, including limb motion, maintaining body posture, and securing joints. The voluntary force generated by a skeletal muscle arises from a succession and an interplay of complex phenomena between the CNS and the muscle's contractile machinery. The neural message received from the CNS is first converted by a pool of alpha-motoneurons (MNs) into a neural command to the muscle. This neural command is broken down into sets of neuromuscular controls, each of which is specific to one MN and the specific bundle of muscle fibres it innervates, that altogether constitute a motor unit (MU) of the muscle. The neural controls to the MUs are then transformed in a series of chemical-mechanical mechanisms into molecular forces, that sum across the multiscale architecture of the muscle to build the muscle force. The afferent neural system is not studied in this thesis.

The mechanisms responsible for force generation and voluntary neuromuscular control have been extensively investigated in experimental studies in fishes, amphibians, and mammals for millennia, with the first known experiments on nonhuman skeletal muscles reported in the ancient Greece in 4th century BC (Cohen & Loew, 1984). From this research, important phenomenological macroscopic properties of the skeletal muscles were understood. For example, the force developed by a voluntary contracting muscle depends on its length (Huxley & Hanson, 1954), its elongation speed (Hill, 1938), its contraction history like muscle fatigue (Behm et al., 2021), the discharge frequency of the controls received by the MUs, and the number of MUs that receive this control, i.e. that are recruited (Heckman & Enoka, 2012). When the generated muscle force is gradually increased, the Henneman's size principle (Henneman, 1957; Henneman, 1981; Henneman, 1985) states that, within a MU population, the MUs are sequentially recruited in the increasing order of the force they can generate, the size of their innervating motoneuron, and the number of muscle fibres. Furthermore, past experimental studies in animals advanced our understanding of comprehensive molecular properties and mechanisms involved in the multiscale development of whole muscle contraction. For example, the whole muscle force builds upon billions of molecular forces generated by micrometre-scale structures, called sarcomeres, that undertake cross-bridge cycling dynamics (Huxley & Niedergerke, 1954). Also, the neural message is received by the MUs from neurotransmitters crossing synaptic gaps, and travels along the MUs by means of membrane electric depolarizations elicited by inward-outward flow of

charged ions. Importantly, these macroscopic and molecular properties and mechanisms were found to be highly dependent on the task performed, and on the species, muscle, and subject investigated, as reviewed and discussed in this thesis.

Our understanding of these properties and mechanisms is less advanced for the human living body, for which the invasive *in vivo* practices and *in vitro* approaches commonly taken with animals are reduced to exceptional electrode insertions (Van Cutsem et al., 1997; Muceli et al., 2022) and muscle biopsies (Gollapudi & Lin, 2009; Ferguson & Shur, 2022) for ethical reasons. Our experimental understanding of human neuromuscular control and force generation dominantly relies on non-invasive techniques, including recent advances in surface electromyography (EMG) (Del Vecchio et al., 2020; Waasdorp et al., 2021) and imaging (Pincheira et al., 2022), and whole muscle force protocols. Although they provide a crucial window onto important principles of human neuromuscular control and force generation, the information provided by these non-invasive techniques is limited. For example, measured joint torques do not inform on muscle co-activation, bipolar surface EMG (bEMG) signals cannot distinguish the recruitment dynamics of the MUs from their discharge behaviour, high-density EMG (HDEMG) approaches can only identify the discharge activity of small portions of the MN pool, and HDEMG and most imaging techniques are only possible in isometric or quasi-isometric conditions, while the muscle's excitation-contraction coupling dynamics can only be recorded with invasive techniques. Besides these limitations, most of these non-invasive techniques are challenging to setup and process, which makes it difficult to experimentally observe the activity of several muscles simultaneously. Consequently, although the human muscle's force-generating mechanisms share obvious similarities with animal's, including the well-admitted Force-Length, Force-velocity, and Force-frequency relationships, our experimental knowledge of human motor control and on how most neuromuscular properties scale from animals to humans remains limited.

To support experimental investigations, mathematical neuromuscular models of skeletal muscles have been developed since the late 19th century. They aim to mimic the dynamics of muscle contraction by predicting the time profile of relevant muscle-related quantities that are challenging or impossible to obtain in humans *in vivo*, including neural drive, muscle force and fascicle length and velocity. In the field of muscle modelling, Hill-type models (Figure 1-1A) are globally more popular than other available solutions, such as analytical descriptions of the muscle twitch (Fuglevand et al., 1993; Ding et al., 2000; Frey Law & Shields, 2006), Huxley-type cross-bridge models (Huxley & Hanson, 1954; Huxley & Niedergerke, 1954; Julian, 1969; Zahalak, 1981; Smith et al., 2005; Lemaire et al., 2016), continuum models (Röhrle et al., 2012; Heidlauf et al., 2017; Röhrle, Oliver, Yavuz et al., 2019; Gfrerer & Simeon, 2021), and other comprehensive models (Tamura et al., 2008), for several reasons. With sarcomere-to-muscle multiscale simplifications and phenomenological modelling approaches, Hill-type models

rely on fewer parameters and are computationally cheaper and conceptually simpler (Winters & Stark, 1987; Lemaire et al., 2016; Hamburger, 2017; Schmitt et al., 2019) than Huxley-type or continuum models, while they are complex enough to describe the chain of neuromechanical events responsible for muscle force generation, which makes them more accurate, flexible and prone to further refinements (Schmitt et al., 2019) than analytical models.

To advance our understanding of the muscles' process of force generation, Hill-type models have been used in the past 50 years in countless applications. Some studies modelled and investigated the muscles' contractile mechanics (Till et al., 2008; Bayer et al., 2017) and neuromechanics (Mörl et al., 2012), energetics (Houdijk et al., 2006; Ross et al., 2018; Ross et al., 2020), and variations in properties with various factors such as age or neuromuscular impairments (Thelen, 2003; Shao et al., 2009; Hasson & Caldwell, 2011). Other Hill-type studies for example performed computational investigations of the interplay between muscles and their surrounding structures, by focusing on the muscle-tendon interplay (Zajac, 1989; Caldwell, 1995), the muscles' relative force contribution to joint torques, motion and contact forces (McLean et al., 2003; Shao et al., 2009; Nikooyan et al., 2012; Putra et al., 2015; Bicer et al., 2022), the indeterminacy of the load sharing system (Sarshari et al., 2020; Michaud et al., 2021), or muscle-induced stress development in skeletal bodies (Moody et al., 2009; Phillips et al., 2015; Favier et al., 2021a; Favier et al., 2021b). With if/then scenarios, Hill-type models found practical uses in clinical applications for predicting the outcome of surgical procedures (Delp et al., 1990; Giat et al., 1994; Tigue et al., 2022) or optimizing rehabilitation (Sartori et al., 2012; Hassani et al., 2014; Ai et al., 2016; Peng et al., 2016). Useful in sports, Hill-type models were employed for optimizing performances (Edman et al., 1978; Pandy et al., 1990; Anderson & Pandy, 1999) or predicting the effects of muscle fatigue on performance (Giat et al., 1993; Liu et al., 2002; Callahan et al., 2016; Potvin & Fuglevand, 2017; Penasso & Thaller, 2018; Sharma et al., 2009).

Finally, Hill-type muscle models were used to explore motor control, including muscle reflex (Winters, 1995; Geyer et al., 2003; Geyer & Herr, 2010), and, most importantly for this thesis, voluntary neuromuscular control (Winters & Stark, 1985; Walter et al., 2014; Caillet et al., 2022; Caggiano et al., 2022). In the Hill-type literature, voluntary neuromuscular control can be investigated in inverse dynamics simulations with static optimization methods to infer the level of muscle activation necessary to match the external dynamic joint moments occurring during a recorded force or motion. This approach however typically relies on an assumption of minimal energy expenditure, which is not entirely realistic, often underestimating the dynamics of antagonistic muscle co-contraction (Kian et al., 2019). Otherwise, human voluntary neuromuscular control has been modelled for the past 40 years in forward simulations of muscle contraction with bipolar EMG (bEMG)-driven approaches (Hatze, 1980; Hof & Van den Berg, 1981; Zajac, 1989; Lloyd & Besier, 2003; Pizzolato et al., 2015). In these

bEMG-driven approaches, the EMG signal recorded by bipolar electrodes is processed to provide, as displayed in Figure 1-1A, a unique neural control to a Hill-type model that describes the whole muscle's dynamics. The bEMG-driven approach is well-established and implemented in open-source and automated tools (Pizzolato et al., 2015) and has advanced our understanding of neuromuscular control, with important applications in human-machine interfacing (Sartori et al., 2012; Ao et al., 2017; Caggiano et al., 2022; Cimolato et al., 2022; Sartori et al., 2009).

However, the information provided by the bEMG-driven approach on the interplay between CNS and muscle machinery remains limited. The recorded bEMGs lump into a single signal the dynamics of MU recruitment and discharge frequency, which limits the interpretability of this neural control and constrains the muscle to be modelled with a unique Hill-type actuator, that usually overlooks the continuous distribution of the MN neuromechanical properties across the MU pool. While some studies attempted to address this limitation with a single actuator (Hatze, 1977; Hatze, 1980; Dorgan & O'Malley, 1997; Song et al., 2008; Dick et al., 2017; Hussein et al., 2021), others proposed to describe the muscle as a collection of Hill-type actuators, as displayed in Figure 1-1B, each of which would describe the dynamics of individual MUs (Hatze, 1980; Hamouda et al., 2016; Carriou et al., 2019). This multi-MU structure is ideal for carefully mapping the continuous distribution of the electrophysiological and neuromechanical properties of the MUs across the MU pool, modelling in detail the excitation-contraction coupling properties of the MUs, and avoiding some detrimental multiscale simplifications typically made at the muscle scale. However, because they lacked adequate experimental data, these multi-MU studies used synthetic neural inputs to the individual Hill-type MUs of their model, and could not investigate voluntary neuromuscular control with their refined MU-scale models.

Recent advances in the decomposition of HDEMG signals (Negro, Muceli et al., 2016; Del Vecchio et al., 2020; Hug et al., 2021) allow the non-invasive recording of the discharge activity of individual MUs during human voluntary contraction. The MN spike trains obtained with this experimental approach intrinsically account for the dynamics of MU recruitment and provide the time-history of MN discharges for the identified MNs. Although the MN spike trains provided by HDEMGs have previously been lumped into a single neural control to drive single Hill-type actuators (Sartori et al., 2017; Kapelner et al., 2020), like in bEMG-driven approaches, they can theoretically control the individual Hill-type units of the aforementioned multi-MU muscle descriptions, as suggested in Figure 1-1B. The MN-driven multi-MU model proposed in Figure 1-1B would be the first to reconcile the experimental behaviour of individual MNs with a model of the contractile dynamics of their innervated MUs. This opens avenues for investigating the dynamics of the MU pool in MN-driven forward simulations and refining our understanding on the interplay between the CNS and the neuromuscular machinery for a

better appreciation of neuromuscular control during human voluntary contraction. Later important applications would be conceivable in neurorehabilitation and the design and control of neuroprosthetics (Farina et al., 2017; Farina et al., 2021).

This thesis proposes novel methods on how to best control multi-MU muscle models with experimental HDEMG MN spike trains in MN-driven forward simulations of human voluntary neuromuscular contraction.



Figure 1-1: Description of a whole muscle with Hill-type actuators. Hill-type models typically include two passive spring-like elements to account for the passive properties of the muscle-tendon tissue, and a force generator (FG) that describes the active neuromechanical dynamics of the muscle tissue. (A) A unique Hill-type model is used to describe the muscle's macroscopic properties responsible for force generation. It is driven by a unique neural control, that is obtained from processed bipolar EMG signals in bEMG-driven methods. (B) The whole muscle is described as a collection of in-parallel Hill-type actuators that describe the neuromechanical dynamics of individual MUs. The muscle is driven by multiple neural controls, that are the MN spike trains obtained from processed HDEMG signals, three of which are represented here. Methods for developing such MN-driven multi-MU neuromuscular model are proposed in this thesis.

1.2. Thesis aims and objectives

The aim of this thesis is to propose experimental and computational methods for developing, controlling, and validating the novel MN-driven multi-MU neuromuscular actuator in Figure 1-1B for forward simulations of human voluntary muscle contraction. More specifically, aims can be broken down as follows:

- (1) To develop a state-of-the-art neuromuscular model described as a collection of MU Hill-type actuators
- (2) To determine and obtain the necessary experimental data for controlling, scaling, and validating the neuromuscular model in voluntary contractions
- (3) To determine computational ways for realistically controlling the neuromuscular model with the decomposed HDEMG data

The aims will be met by the following objectives:

- (1) Performing a systematic review of the field of Hill-type modelling for precisely identifying the state-of-the-art and gaps in neuromuscular modelling - Chapter 2
- (2) Obtaining experimental force and imaging data to scale and validate the neuromuscular model - Chapter 3
- (3) Identifying some key factors in the process of HDEMG signal acquisition and decomposition to maximize the number and the quality of the output MN spike trains - Chapter 3
- (4) Determining whether the MN spike trains from (3) are reliable quantities to control a neuromuscular model in voluntary contraction - Chapters 3 and 4
- (5) Developing computational methods to artificially augment the empirical data from (3) to reliably control neuromuscular models in voluntary contraction – Chapter 4 and 5
- (6) Developing the MN-driven multi-MU neuromuscular model in Figure 1-1B, with advanced modelling of the MU excitation-contraction coupling properties and careful assignment of the MU properties across the MU pool - Chapter 6

1.3. Summary of chapters

The thesis is divided into seven chapters including the introduction. Each chapter is motivated by and builds upon the findings of the previous chapters, as displayed in Figure 1-2. In-between-chapter transitions link the key chapter conclusions to the thesis aims and objectives described in Section 1.2. Each chapter starts with a summary of its key methods and conclusions, followed by a background section. This approach was considered the clearest way to step-by-step develop the workflow converging towards the final MN-driven neuromuscular model.

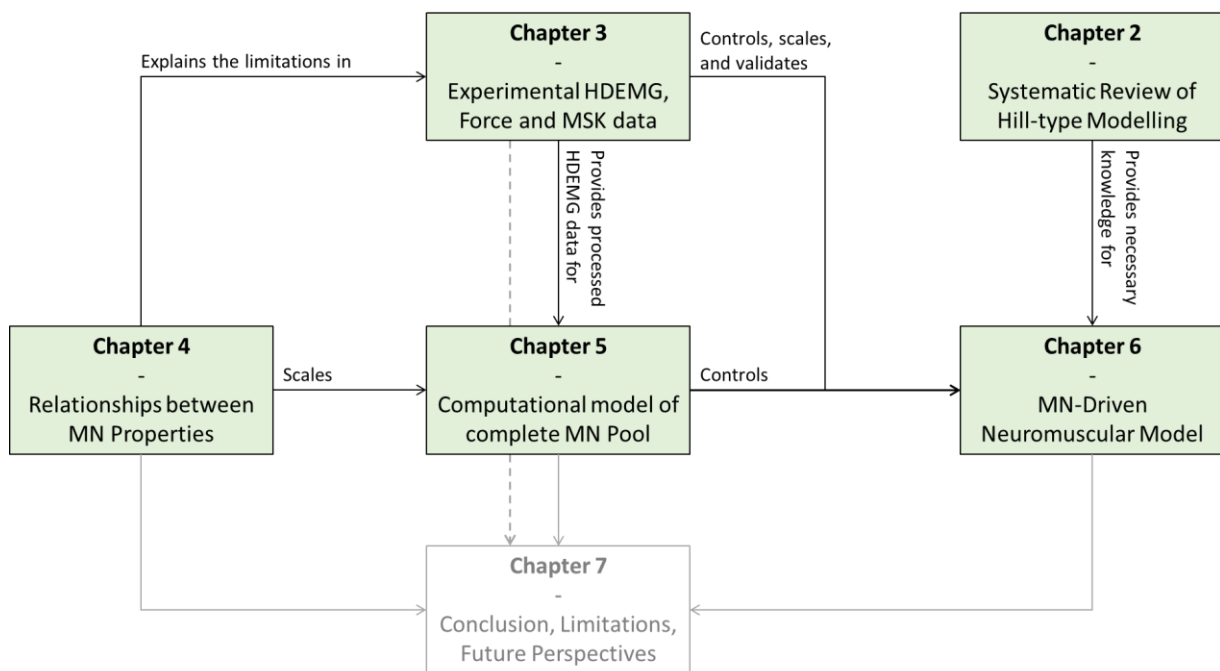


Figure 1-2: Inter-relation between the chapters 2 to 7 of this thesis.

Chapter 2 presents the first systematic review of the field of Hill-type neuromuscular modelling. 55 eligible studies, which include a Hill-type model that contributes to the state-of-the-art in Hill-type modelling, are extracted from a systematic search and analysed with two scoring schemes. The first scoring scheme assesses the number of neuromechanical properties described by the model, while the second scoring scheme evaluates the level of model validation, reusability, and calibration, and the modelling strategies proposed in the study. This review provides the necessary knowledge for understanding the main trends and gold standards in Hill-type modelling and how to challenge them, and precisely identifies the state-of-the-art and gaps in the field.

Chapters 3 provides the experimental data necessary to control, scale, and validate the MN-driven neuromuscular model. First, a state-of-the-art subject-specific musculoskeletal (MSK) model is obtained from segmented magnetic resonance imaging (MRI) scans to scale the neuromuscular model in Figure 1-1B with subject-specific quantities. Second, experimental ankle dorsiflexion torques are

recorded to allow the validation of the whole muscle force predicted by the MN-driven model. Third, sets of experimental MN spike trains are obtained from the decomposition of HDEMG signals recorded during voluntary isometric muscle contractions. A sensitivity analysis shows that more MN spike trains of better quality than typically obtained in the literature can be obtained by increasing the number of electrodes covering the muscle and fine-tuning the decomposition algorithms. Although optimized for number and quality, these experimental spike trains alone are shown not to give a complete description of the real neural command delivered by the CNS to the muscle. Alone, this experimental neural data is not reliable enough to directly control a pool of MUs like in Figure 1-1B.

Chapter 4 derives for the first time a set of mathematical relationships between eight key MN electrophysiological and anatomical properties. To do so, the data reported by an extensive set of experimental studies is processed, merged, fitted, and transformed. The resulting relationships provide a quantification of the Henneman's size principle, support well-admitted methods of MN modelling, provide answers to why the experimental spike trains in Chapter 3 do not entirely explain the real neural drive to muscle, and deliver a key feature of the computational method developed in Chapter 5.

Chapter 5 develops a novel computational method to address the limitations of the experimental neural data identified in Chapter 3. In four steps, the information intrinsic to the experimental MN spike trains is processed and augmented to infer the discharge activity of the MNs that were overlooked from the HDEMG acquisition and decomposition. To do so, cohorts of computational MN models, driven with a synaptic control derived from the spike trains identified in Chapter 3, are realistically scaled with the mathematical relationships derived in Chapter 4. The discharge behaviour of the completely reconstructed MN pool, inferred from the experimental data with this computational method, is shown to better approximate the real neural drive to muscle than the experimental data alone. Therefore, Chapter 5 concludes the three-chapter workflow necessary to obtain reliable neural controls for the MN-driven multi-MU neuromuscular model in Figure 1-1B.

Chapter 6 develops the computational neuromuscular model displayed in Figure 1-1B. This MN-driven muscle model is described as a cohort of in-parallel MUs, the generated forces of which linearly sum to build the whole muscle force. Each MU is modelled with a Hill-type model that describes the excitation-contraction and mechanical properties and dynamics specific to that MU. Taking advantage of this favourable rheological structure, advanced models of the MU activation dynamics are proposed with a phenomenological description of the free calcium and calcium-troponin transients in the sarcoplasm. Using the subject-specific quantities obtained in Chapter 3 and findings from the literature, care is given to the assignment of specific properties to the MUs constituting the MU pool.

All non-subject-specific neuromechanical properties are scaled with available human data, or if necessary, cat or rodent data from the literature. The interplay between the neural and mechanical interfaces of the neuromuscular system is accounted for with activation and length inter-dependencies of their dynamics. With validations of the predicted muscle force against the experimental force recorded in Chapter 3, it is shown that the neuromuscular model behaves better when controlled by the computationally reconstructed inputs from Chapter 5 than with the sole experimental sets of MN spike trains obtained in Chapter 3.

Finally, Chapter 7 reflects on the work carried out by listing the accomplishments made in this study and suggesting future work to further refine the neuromuscular model developed in Chapter 7 and extend the pipeline to other muscles and contraction tasks for future practical applications in neuromuscular control of human voluntary contractions.

Chapter 2

HILL-TYPE COMPUTATIONAL MODELS OF NEUROMUSCULAR ACTUATORS: A SYSTEMATIC REVIEW

NOTE: Aside minimal alterations, this chapter, excluding Section 2.1.1, was uploaded as a preprint manuscript to the bioRxiv server under the terms of the CC-BY 4.0 international license after it was peer-reviewed by the involved co-authors.

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Chapter Summary

This Chapter 2 fulfils the Objective (1) listed in Section 1.2. To develop a novel MN-driven Hill-type model that advances the field of neuromuscular modelling, it is important to first obtain an extensive knowledge of the content of the field and identify its state-of-the-art and current gaps. Lacking recent reviews, the field of Hill-type modelling is dense and hard-to-explore, with detrimental consequences on knowledge transmission, inter-study consistency, and innovation. To help navigating the literature and build the necessary knowledge for this thesis, the first systematic review of the field of Hill-type muscle-tendon modelling is proposed in this Chapter 2. To clarify its content, and identify its state-of-the-art and current caveats, fifty-five criteria-abiding studies were extracted using a systematic search and their Hill-type models analysed with to two scoring schemes. The first scheme assessed the number of neuromechanical properties described by the model, while the second evaluated the level of model validation, reusability, and calibration, and the modelling strategies proposed in the study. It was concluded that most models (1) do not significantly advance the dated gold standards in muscle modelling and do not build upon more recent advances, (2) overlook the importance of parameter identification and tuning, (3) are not strongly validated, and (4) are not reusable in other studies. Most importantly for this thesis, this review confirmed that it has never been proposed in the literature a MN-driven multi-MU neuromuscular model like in Figure 1-1B, that would account for the dynamics of MU recruitment and receive experimental data from individual discharging motoneurons in forward simulations of human voluntary contraction. Such modelling approach would tackle several of the current gaps and limitations in the field reported in this review, including the modelling limitations related to the fibre-to-muscle multiscale simplifications, the absence of existing models describing the dynamics of motor unit recruitment based on experimental motoneuronal data, the limitations of the current phenomenological multiscale descriptions of the muscles' excitation-contraction coupling properties, and the key gap of having never controlled a pool of individual MUs with experimental motoneuronal control in human voluntary muscle contraction.

2.1. Introduction and context of Hill-type muscle modelling

2.1.1. Skeletal muscle physiology and mechanisms responsible for muscle force generation

Before focusing on the mathematical and computational methods for modelling the neuromuscular dynamics with Hill-type models, this introductory section to Chapter 2 provides some background knowledge on the physiology of the human skeletal muscles and on the mechanisms responsible for muscle force generation during voluntary muscle contractions.

2.1.1.1. Skeletal muscle physiology

A skeletal muscle is an organ of the muscular system that is attached to the skeletal structure of the body (the bones) and is controlled by the CNS by means of α -MNs. As displayed in Figure 2-1, a skeletal muscle is a multiscale architecture. The organ, at the macroscopic scale, includes hundreds of thousands of muscle fibres for a typical skeletal muscle of the human lower limbs (Feinstein et al., 1955). The fibres are grouped into approximately a hundred of compartments (Buchthal & Schmalbruch, 1980), named fascicles or fibre bundles, that are separated by the perimysium collagenous matter. The muscle fibres are also grouped into MUs, each of which includes a group of muscle fibres showing similar contractile behaviour and a single MN innervating them. The MNs, which are constituted of dendritic trees, a soma, an axon, and terminal branches, link the spinal cord to the muscle fibres with synaptic interfaces. As discussed in Chapter 3, human skeletal muscles of the lower limbs generally include a few hundred MUs. Three MUs are identified in purple, red and green in Figure 2-1. At the subcellular scale, each fibre includes thousands (Placzek & Boyce, 2016) of myofibrils, each of which is a longitudinal repetition of molecular units called sarcomeres (molecular scale in Figure 2-1). A sarcomere is a hexagonal $1\text{-}4\mu\text{m}$ -long structure, represented in plane in Figure 2-2 (top), that includes five key proteins: myosin, actin, titin, tropomyosin, and troponin, the collaborative dynamics of which produce infinitesimal forces which are transmitted to the tendon after multiscale propagation.

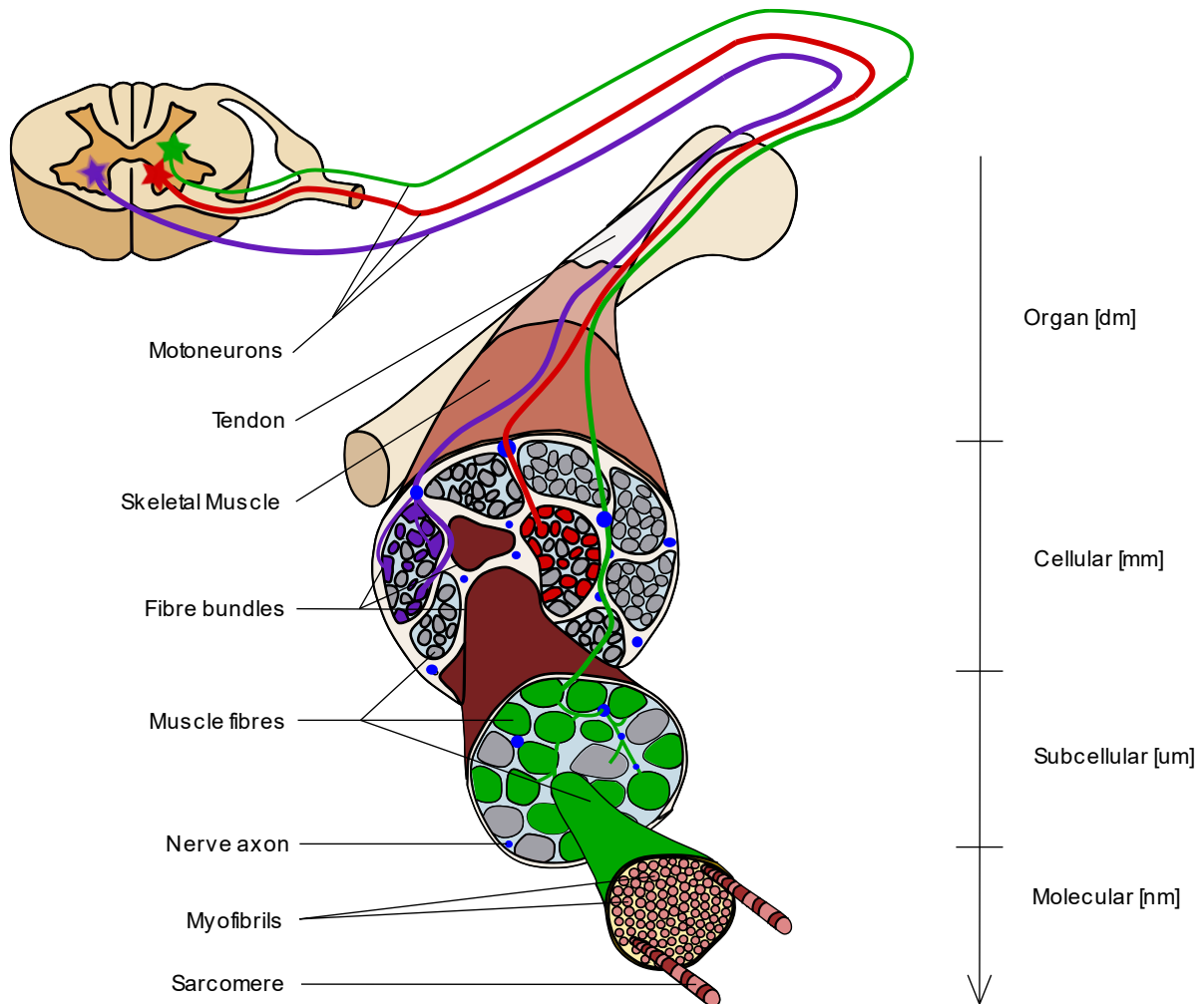


Figure 2-1: multiscale architecture of a skeletal muscle. Three MUs (purple, red, and green) are identified.

2.1.1.2. Mechanisms responsible for muscle force generation

To generate voluntary muscle force, the neuromuscular system transforms the neural message elicited by the CNS into dynamics of muscle contraction. For each active MU, a train of membrane electric depolarizations, called action potentials (APs), is transmitted by the spinal cord to the MN dendritic trees, travels along the MN axon, crosses the MN-fibre synaptic gap called neuromuscular junction, journeys along the membrane of the innervated fibres, and enters the aforementioned fibres through narrow tunnels (t-tubules) that wrap the myofibrils. In more details, this train of APs is triggered by an influx of synaptic neurotransmitters that bind to postsynaptic receptors, which open ligand-gated channels that elicit inward-outward flows of positively and negatively charged ions. When this mechanism displaces the membrane voltage equilibrium beyond a certain threshold, a self-feeding positive feedback of inward positive ions diffusion onsets and produces an AP. After the spike initiation, the MN membrane remains inert at the discharge location during an absolute refractory

period, while the fired AP triggers the opening of voltage-gated ion channels in the next nearby MN locations, allowing the unidirectional journey of the AP train.

In the t-tubules, the train of APs mechanically opens for finite amounts of time a chamber, the sarcoplasmic reticulum, which releases sparks of calcium ions Ca^{2+} into the myofibrils. From there, the Ca^{2+} ions diffuse in the sarcoplasm, reach the sarcomeres (Step 2 in Figure 2-2-bottom) and trigger the cross-bridge cycling mechanism (Figure 2-2-bottom) (Huxley & Niedergerke, 1954). Once a Ca^{2+} ion binds a troponin molecule, the tropomyosin shifts and makes available an actin's binding site (Step 3 in Figure 2-2-bottom). The available actin binding site and a surrounding myosin head form a link, called a cross-bridge, which receives a power stroke from a hydrolysing ATP molecule and mechanically shortens the sarcomere in a concentric 'sliding' motion of its filaments (Step 4 in Figure 2-2-bottom) (Huxley & Hanson, 1954). The cross-bridge then detaches, and the process repeats upon Ca^{2+} ion availability. The unitary sarcomere forces produced by the ATP power strokes during the cross-bridge cycling event and triggered by the trains of APs propagate and sum across the multiscale architecture of the muscle to build the whole muscle force that is transmitted to the skeletal structure by the tendon (Figure 2-1).

2.1.1.3. Factors influencing the force-generating process

In first approximation, the level of force generated by a skeletal muscle at each time instant is linearly correlated to the total number N_s of formed cross-bridges in the force-generating state, that simultaneously deliver sarcomere unitary forces. N_s varies with four main mechanisms.

Firstly, the maximum number of cross-bridges that can be formed in an individual sarcomere depends on the number of myosin heads able to reach the actin sites. This number is given by the amount of overlap between the actin and myosin filaments, which is determined by the sarcomere length. In human skeletal muscles, a maximum number of cross-bridges can form at the optimal sarcomere length $2.6\mu m$ (Burkholder & Lieber, 2001; Gordon et al., 1966), below and above which some myosin heads cannot find a pairing actin site (Force-Length relationship in the next section). In isometric contractions (fixed muscle length), the whole muscle force (or N_s) consequently varies with the muscle length, which determines the average sarcomere lengths across the muscle, assuming homogeneous sarcomere lengths despite contradicting experimental evidence (Gollapudi & Lin, 2009).

Secondly, the quicker the sarcomere filaments concentrically slide, the lower is the likelihood of an actin-myosin link to form or remain attached against the pulling sliding force. Consequently, the force generated by the whole muscle (or N_s) hyperbolically decreases with increasing instantaneous shortening velocity (Force-Velocity relationship in the next section). On the contrary during eccentric

contractions, when the contracting muscle develops force while lengthening, the force generated by the whole muscle increases with increasing lengthening velocity.

Thirdly, the number of MN APs received per second by the innervated fibres of a MU, also called AP discharge rate or firing frequency, determines the level of concentration of the Ca^{2+} ions diffusing in the sarcomeres of the MU. The higher the AP discharge rate, the higher the Ca^{2+} concentration in the sarcoplasm, the higher the number of troponin proteins binding Ca^{2+} ions, and the higher the likelihood of tropomyosin shifting and uncovering actin sites for cross-bridge formation.

Finally, the whole muscle force depends on the number of 'recruited' MUs that receive spike trains from the spinal cord. Following Henneman's size principle (Henneman, 1957; Henneman et al., 1965a; Henneman, 1981; Henneman, 1985) and as discussed in Chapters 3 and 4, the MU population is recruited in a specific order, starting with the 'small' MUs that include a few hundred fibres and generate low maximum tetanic forces and ending with the large high-force MUs of a few thousand fibres.

To conclude, at each time instant, depending on the muscle's instantaneous length and contraction velocity and on other nonlinear mechanisms not described here, the CNS varies both the number of MUs which are recruited and their discharge rate to meet the target generated force. In theory, a muscle is expected to produce its maximum instantaneous possible force at optimal fibre length, during eccentric contractions, with all possible MUs recruited and discharging at maximum firing rates.

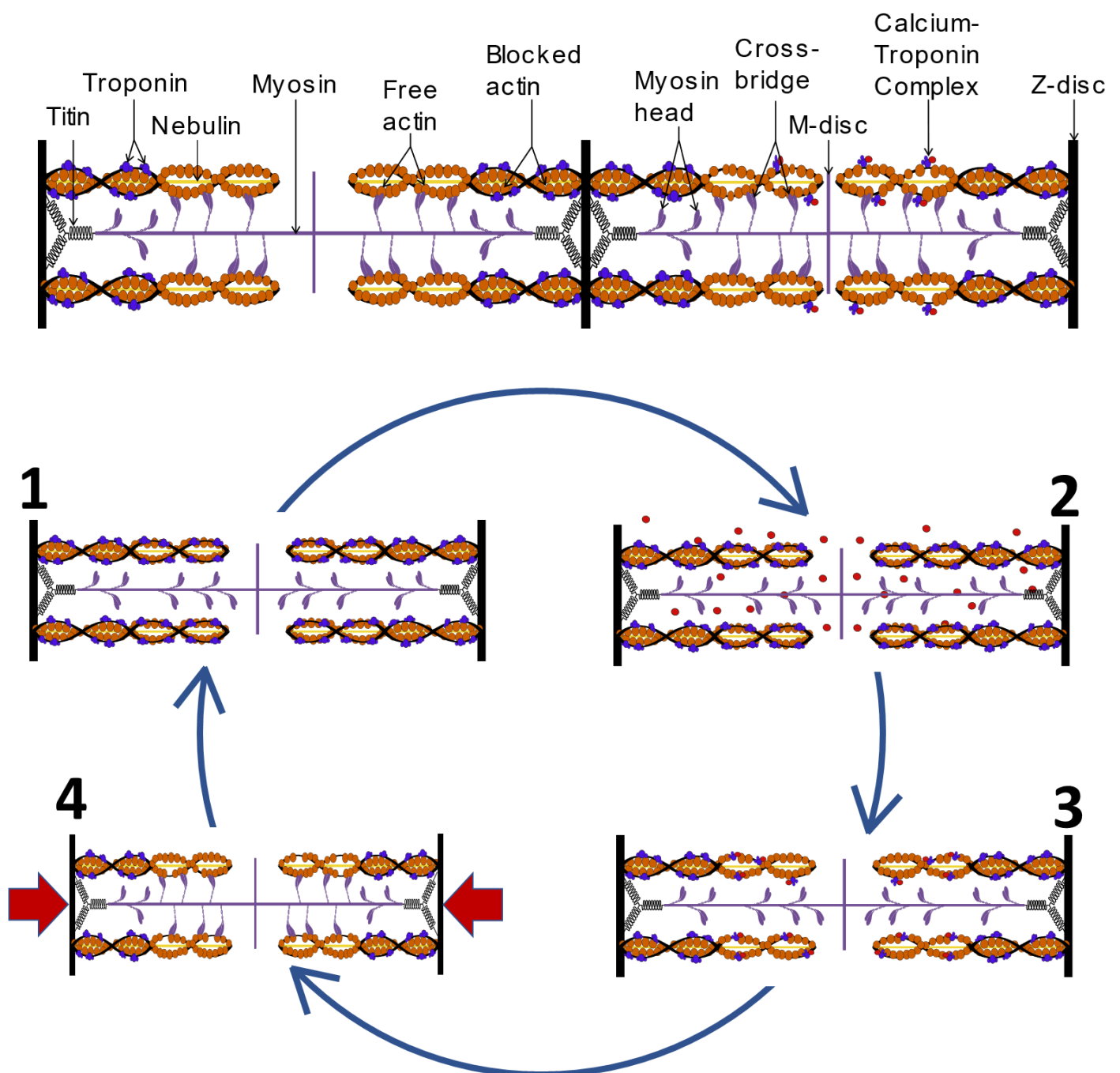


Figure 2-2: (Top) Description of the architecture of a sarcomere. (Bottom) Four-step process (1-to-4) of the cross-bridge cycling mechanisms responsible for whole muscle shortening and force production.

2.1.2. Hill-type muscle modelling

As introduced in Section 1.1, computational Hill-type models are commonly used to mimic the muscle contraction dynamics summarized in Section 2.1.1 and predict the time profile of relevant muscle-related quantities that are challenging or impossible to obtain in humans *in vivo* (Gollapudi & Lin, 2009; Dick et al., 2017; Monte et al., 2020; Zhang et al., 2021), including neural drive, muscle force and fascicle length and velocity. As shown in Section 1.1, Hill-type models were used in a wide range of applications in the past 50 years. This is explained by their high flexibility, their ability to receive experimental neural inputs for EMG- or FES-driven forward simulations of muscle contraction, their availability in user-friendly biomechanical simulation platforms (Delp et al., 2007; Seth et al., 2018; Dembia et al., 2020), and a century of advancing research upon which they build, an informative genesis of which is provided in Appendix A.1. Hill-type models are reliable tools, the fair predictive accuracy of which has been extensively validated for various modelling variants, species, subjects, muscles and tasks, with a suitable compromise between model complexity and accuracy.

To choose the optimal Hill-type variant, one must have a clear understanding of the content of the literature in Hill-type modelling. Yet, the field of Hill-type modelling is currently extremely dense, with over a thousand models proposed in the past 50 years, and hard-to-explore since the last available narrative reviews date back 20 years or more and the more recent studies investigating the existing modelling approaches and assessing the gaps in the literature are not exhaustive. While this situation drastically limits knowledge transmission and complicates for everyone the time-consuming identification of the ‘right’ Hill-type model in the literature, it also slows down the pace of innovation, despite important remaining limitations, as discussed in this study, as it is difficult to build upon an ill-defined state-of-the-art. This situation also promotes detrimental tendencies, such as ill-referencing, inter-study inconsistency in terminologies, definitions and notations, and overlooking most recent contributions. As advised in the 90s (Epstein, 1998), contemporary literature and systematic reviews of the literature must be available to organize and clarify the contents and state-of-the-art of the field, as recently done in skeletal muscle continuum modelling (Dao & Tho, 2018), multiscale neuromuscular modelling (Röhrle, Oliver, Yavuz et al., 2019), cervical spine MSK modelling (Alizadeh et al., 2020), and lower limb force modelling during gait (Trinler et al., 2018).

In this respect, this study provides the first systematic review of the field of Hill-type muscle-tendon modelling. Its contents and state-of-the-art are assessed with two scoring schemes, a completeness assessment, and a modelling evaluation, applied to a set of 55 eligible studies, that were extracted from a systematic search for published Hill-type models. The eligible studies were chosen to be representative of all the modelling approaches taken to describe 23 properties underlying the muscle-

force generation process. The completeness assessment identifies which muscle properties are described in an eligible model, while the modelling assessment evaluates the level of validation and reusability of the model and attention given to its modelling strategy and calibration. The results of the scoring schemes are used to discuss the current trends and gold standards in Hill-type modelling, challenge them, investigate the state-of-the art, and identify the current gaps and lines of improvement. This study is supported by extensive supplementary material and can be used as a convenient time-saving tool to navigate through the literature, as any published model can be retrieved by combining the modelling approaches proposed in the eligible studies. The knowledge built with this systematic review will serve in this thesis to develop the novel MN-driven multi-MU Hill-type-like neuromuscular model displayed in Figure 1-1B.

2.2. Methods

This systematic review was performed following the PRISMA guidelines (Moher et al., 2009), the commented checklist of which is provided in Appendix A.2.

2.2.1. Terminology, definitions, and acronyms

The definition of a Hill-type model has been continuously refined and extended since Hill's preliminary Nobel prize-winning works (Hill, 1922; Hill, 1938; Barclay & Curtin, 2022), as detailed in Appendix A.1. A Hill-type model refers today, consistently with the terminology proposed in milestone reviews in the field (Zajac, 1989; Winters, 1990; Epstein, 1998), to any rheological arrangement of an active force generator (FG), which builds the active muscle force, and in-series and in-parallel MT passive elements, which passively transmit force through the stretch-resisting dynamics of their constitutive material. In the literature, the behaviour of these active and passive rheological elements is governed by mathematical expressions that describe up to 23 phenomenological passive or active MT properties, which are displayed along with their acronyms in Table 2-1 and Figure 2-3.

In this review, the contractile element (CE) and the neuromechanical element (NE) interact to define the FG (Figure 2-3). The CE lumps any of the 10 CE-related contractile properties (Table 2-1) that describe the multiscale mechanical events that occur during the active contraction dynamics of the FG. The NE groups the events involved in the neuromechanical interface between the central nervous system and the muscle machinery; it accounts for the excitation, activation, and motor unit (MU) recruitment dynamics of the neuromuscular system, that define the neural control to the CE.

For future purposes, a 'baseline' Hill-type model (Figure 2-4) refers to any rheological arrangement of one passive element, one NE and one CE, the behaviour of which is respectively described by one passive property, one CE-related property, and one NE-related property. In the following, a Hill-type model is termed 'innovative' if it provided a methodological contribution to the field at the time of its publication, and more precisely a novel modelling approach for at least one of the 23 properties reported in Figure 2-3, that could not be retrieved from a previously published paper identified from the systematic search.

If not stated otherwise, this review only refers to 'generic' models (Zajac, 1989), that are normalized by the subject-muscle-specific slack length of the Series Elastic Element (SEE), the optimal length of the CE and the muscle maximum isometric force. For simplicity and conciseness, muscle pennation is disregarded in this review, despite limitations for highly-pennated muscles (Zajac, 1989; Scott & Winter, 1991; Carbone et al., 2016).

Table 2-1: Hill-type models are described with 9 possible rheological elements and 23 possible phenomenological properties. This table provides for these elements and properties brief descriptions and the acronyms that are used in this review. ADL, ADV, FLA, FVL and FVA refer to a nonlinear dependency of the model activation dynamics (AD) and Force-Length (FL) and Force-Velocity (FV) relationships to the model active (A), length (L) and velocity (V) states.

Rheological element	Properties	Acronym	Description and corresponding physiological events and mechanisms
NE (Neuromechanical Element)	Excitation dynamics	ED	Electro-chemical dynamics of the neural drive from the spinal pre-synaptic input to the depolarization of the nerve axons (neural ED – nerve action potentials (APs)) and of the muscle fibre sarcoplasm and T-tubules (muscle ED – muscle APs)
	Motor unit recruitment dynamics	MURD	Dynamics of the motor unit recruitment
	Activation dynamics	AD	Dynamics of the excitation-contraction coupling properties from the release of calcium ions Ca^{2+} in the sarcoplasm to the transient myosin-actin cross-bridge attachment mechanism
	Calcium-dependent activation dynamics	ADC	Explicit inclusion of a sub-model of the transient dynamics of calcium concentration in the model of AD
	Length-dependent activation dynamics	ADL	Inclusion of the nonlinear dependency of the AD to the length of the CE, that affects calcium transients and enhances the probability of troponin-calcium binding and of cross-bridge formation (Blinks et al., 1978; Rassier et al., 1999; Rockenfeller & Günther, 2018)
	Velocity-dependent activation dynamics	ADV	Inclusion of the nonlinear dependency of the AD upon the velocity of the CE
CE (Contractile Element)	Force-length relationship	FL	Phenomenological instantaneous isometric relationship at maximal muscle activation between the CE length and the CE force, consistently with the sliding filament theory and the average myosin-actin overlap in the sarcomeres

	Activation-dependent force-length relationship	FLA	Inclusion of the nonlinear activation-dependency of the FL relationship accounting for the phenomenologically decreasing optimal CE lengths with higher activation levels
	Concentric Force-velocity relationship	FVC	Instantaneous relationship at maximal activation and optimal CE length between the concentric / eccentric contraction velocity and force of the CE. It relates to the varying probability of cross-bridge attachments between sliding sarcomere filaments and to the titin activation dynamics
	Eccentric Force-velocity relationship	FVE	
	Length-dependent force-velocity relationship	FVL	Nonlinear length-dependency of the FV relationship, involving lower maximum shortening velocity at decreasing lengths below optimal CE length
	Activation dependent force-velocity relationship	FVA	Nonlinear activation-dependency of the FV relationship, involving an increased value of maximum shortening velocity and decreased curvature of the relationship with higher levels of activation
	Force depression	FD	Decrease in the isometric muscle force after active shortening compared to purely isometric conditions, potentially related to titin activation dynamics
	Residual force enhancement	RFE	Increase in the isometric muscle force after active lengthening compared to purely isometric conditions, potentially related to titin activation dynamics
	Other time-history dependent mechanisms	OTHD	Sag: a short-term force decrease specific to fast fibres Yield: a slow force decrease related to an increasing rate of cross-bridge detachment during isotonic contractions reducing the number of cross-bridges (Brown et al., 1999) Short-range stiffness: a short-term force increase/ decrease at the beginning of a lengthening/ shortening task, attributed to deformation of attached muscle cross-bridges

	Fatigue	Fat	A decline in the ability of a muscle to generate sustained force over time
PEE	Parallel passive stress-strain relationship	PEE	Instantaneous force-length relationship of parallel tissue structures passively resisting muscle stretch
PEE2	2 nd parallel passive stress-strain relationship	PEE2	Instantaneous force-length relationship at short lengths accounting for the deformation of the sarcomeres building a repulsive passive force
PDE	Parallel passive force-velocity relationship	PDE	Instantaneous force-velocity relationship accounting for the viscoelastic properties of the parallel passive structures
M	Muscle inertia	M	Muscle mass, impacting the inertial dynamics of the MT complex
SEE	Series passive stress-strain relationship	SEE	Stress-strain relationship accounting for the passive resistance to stretch of in-series tendinous and / or muscular tissues; usually models the behaviour of the tendon, in which case it is often referred as T in the literature
SEE2	2 nd series passive stress-strain relationship	SEE2	Stress-strain relationship accounting for the passive resistance to stretch of in-series muscular and musculotendinous tissues; usually models the stiffness of the cross-bridges, of the titin in active conditions, and of the aponeurosis
SDE	Series passive force-velocity relationship	SDE	Instantaneous force-velocity relationship accounting for the viscoelastic properties of the in-series passive structures

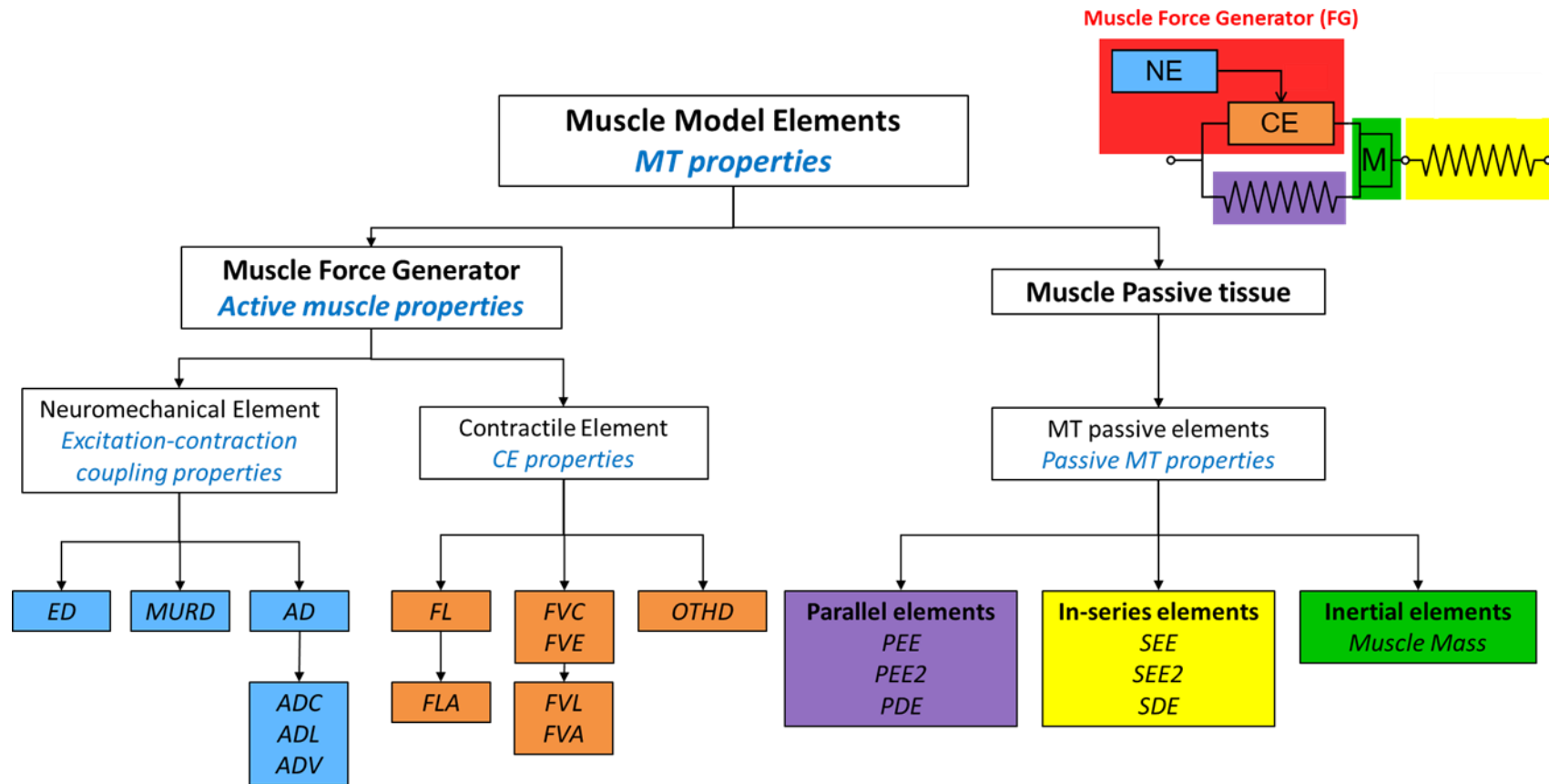


Figure 2-3: Hill-type models typically include passive elements and a muscle Force Generator (FG). In the FG, the Neuromechanical Element describes the muscle's excitation-contraction coupling properties (light-blue boxes) and drives the muscle Contractile Element, described by the contractile properties (orange boxes) and responsible for force generation. Some properties are nonlinearly dependent to the level of activation (FLA, FVA), length (ADL, FVL) and contraction velocity (ADV) of the FG. Besides inertial elements (green box), Hill-type models include passive elements in-parallel (purple box) and in-series (yellow box) with the FG. The acronyms for the 9 rheological elements and the 23 muscle properties are defined in Table 2-1.

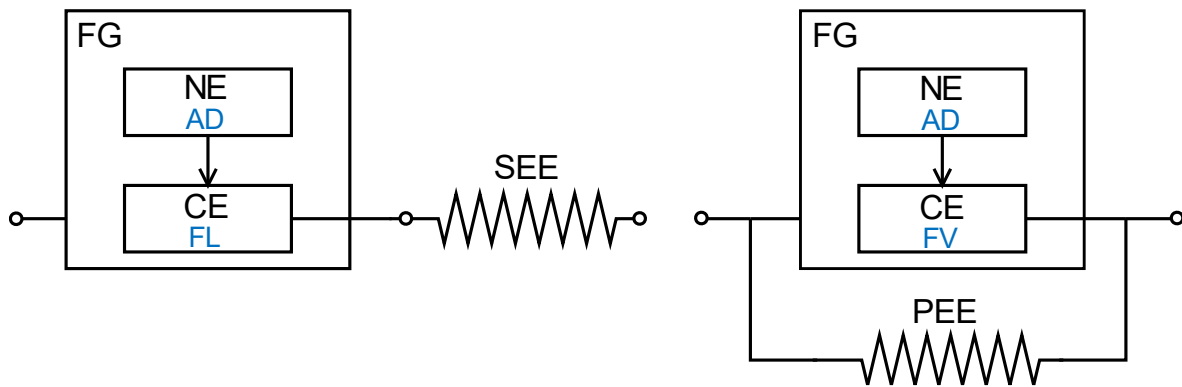


Figure 2-4: Example of two 'baseline' Hill-type models. They include one passive element (Left: SEE and Right: PEE), and an FG that involves a NE, described here by one AD property, and a CE, described here by one FL (Left) or FV (Right) relationship. The acronyms are defined in Table 2-1.

2.2.2. Systematic search for eligible studies

The research studies published between 1938 and 25/09/2022 that involved a Hill-type model were systematically searched for on four computational databases: PubMed, Web of Science, MedLine via Web of Science and EMBase via Ovid. Specific keyterms, reported in Appendix Table A-1, were searched for in the titles and abstracts of the research studies. The keyterms were grouped into four concepts (C1 to C4) listed in Table 2-2 with OR Boolean operators. The four groups of keyterms were then joined into a unique search string of the form 'C1 AND C2 AND C3 NOT C4'. Details on the search strategy are provided in Appendix A.3. The results of the systematic search were stored by year of publication in the reference manager Mendeley.

The final set of 'eligible' studies retained for scoring assessment was identified according to six eligibility criteria (EC1 to EC6) listed in Table 2-2, aiming for milestone studies that provide detailed descriptions of 'complete' Hill-type models representative of the genesis and the state-of-the-art in Hill-type modelling. After removing duplicates, the studies that did not meet EC1 to EC5 were chronologically removed based on title screening, abstract screening, and if necessary, screening of the core text of the paper. The filtering was then repeated with the reference lists of the papers that passed EC1 to EC5, to identify further potentially eligible studies that were missed by the systematic search. The final set of studies that met EC1 to EC5 were chronologically compared for application of EC6. To identify the 'innovative' studies, inheritance diagrams were built for the AD, FL, FV, PEE and SEE properties to map over the past fifty years the chronological chain of references between studies. The inheritance diagrams distinguish the parent studies that proposed novel modelling approaches to the field from the child studies that took inspiration from or replicated the parent ones.

Table 2-2: Definition of the four keyterm concepts C1-C4 for the systematic search. Definition of the eligibility criteria EC1-EC6.

C1	The Hill-type MT model
C2	The muscle, the tendon
C3	The model, the actuator, the simulation
C4	The smooth muscle, the cardiac muscles
EC1	The studies are Journal articles in English
EC2	Only skeletal muscles are considered
EC3	The muscle model is of the Hill-type
EC4	An explicit description of the properties of at least a 'baseline' Hill-type model is provided
EC5	The dynamics of the model rely on length- and force- dependent state variables
EC6	The model is 'innovative'

2.2.3. Scoring assessments

2.2.3.1. Completeness assessment

Each eligible model was assessed for 'completeness' by scoring 1 for each of the 23 properties (Figure 2-3) that was included in the Hill-type model, yielding a 'completeness' score out of 23, with a minimum of 3 according to EC4. According to Table 2-1, the modelling of the NE, CE and passive elements can respectively score 6, 10 and 7, yielding a balanced significance between these three types of elements. The completeness score enables an inter-study assessment of the level of complexity and the relative modelling strengths and weaknesses of the eligible models for mimicking the true muscle neuromechanical interface, mechanical events, and passive dynamics. The average score across eligible studies for each property was also calculated to identify the most common practices and gaps in Hill-type modelling.

2.2.3.2. Modelling assessment

Four methodological practices (I-IV) considered by the reviewers to be crucial for a suitable transmission of incremental knowledge in the field of Hill-type modelling were reviewed in a 'modelling assessment' of the eligible studies. With the 10 questions (Q1 to Q10) listed in Table 2-3, it was evaluated the strategy taken by the eligible studies for (I) model validation, (II) promoting model reusability, (III) detailing the modelling choices, strategy, and limitations, and (IV) model calibration. Q1 to Q10 were scored 0, 1 or 2, which indicate no, incomplete and satisfying methodological practice respectively, and yield a 'modelling score' out of 20. To avoid bias and subjectivity, a detailed scoring grid was built for each question before the studies were assessed and is provided in Appendix Table A-2.

Table 2-3: The 10 questions constituting the modelling assessment. The eligible studies can score 0, 1 or 2 to these questions according to a detailed scoring scheme provided in Appendix Table A-2.

Practices	Question number	Questions
(I) Model Validation	1	Is the accuracy of the predictions obtained with the Hill-type model assessed against ad hoc experimental data?
	2	Is the model validated against reliable muscle-specific experimental quantities?
	3	Is the accuracy of the model evaluated with quantitative mathematical metrics?
(II) Model reusability	4	Is enough information provided in the study to fully reimplement the computational model and reproduce the results published in the study?
	5	Is the model optimized for numerical stability and computational speed?
(III) Modelling choices and strategy	6	Are the taken simplifications in modelling the rheological actuator clearly stated?
	7	Are the modelling choices of the rheological model clearly stated and / or justified with respect to the objectives of the study?
	8	Is there a discussion about the strengths and limitations of the normalized Hill-type model?
(IV) Model calibration	9	Are the mathematical expressions describing the properties of the Hill-type model scaled with experimental data consistent with the scope of the study?
	10	Is the Hill-type model scaled or normalized with species-patient- or muscle-specific scaling parameters?

2.2.3.3. Global score

For each eligible paper, the completeness and modelling scores were normalized by 23 and 20 respectively and averaged to yield a final global score in percentage. The global score measures the global degree of completeness, validation and reusability of an eligible model and the level of attention given to its modelling strategy and calibration. The global score is not an indicator of the overall quality of the eligible studies, considering that some of them were not modelling studies, with focus and contributions beyond the scope of the scoring assessments.

2.3. Results

2.3.1. Systematic search for eligible studies

The systematic search returned 55 eligible studies, listed in Table 2-4, that meet EC1 to EC6 (Table 2-2). As displayed in the flowchart in Figure 2-5 (see Appendix Figure A-1 in Appendix A.5 for additional details), the three databases returned 2492 journal articles in English without duplicates, among which 125 were identified by the reviewers to meet EC2 to EC5. After screening the reference lists, 163 studies were carefully screened in several iterations to apply EC6, using the inheritance diagrams stored in Appendix A.10 as support. Fifty-five eligible models met EC6 for the reasons listed in Appendix Table A-3, while the 98 excluded studies were either replicates or a combination of the 55 eligible models. As displayed in Figure 2-5, 61 non-eligible studies were retained and briefly analysed in Appendix A.7 because 32 of them proposed models, assessed for completeness in Appendix Figure A-5, that were innovative (EC6) but did not meet EC4, while the others brought significant contributions to the field of Hill-type modelling, including narrative reviews, sensitivity analyses, or comparisons between published models. The 55 eligible (Table 2-4) and 32 non-eligible (Appendix Table A-6) models that meet EC6 are considered to be representative of all the modelling approaches proposed to date in the field of Hill-type modelling for the properties listed in Table 2-1.

As displayed in Table 2-4, 32 of the 55 eligible studies aimed to develop the field of Hill-type modelling by proposing innovative muscle models and investigating the sensitivity of model predictions to parameter values, challenging some modelling assumptions, or investigating the strengths and limitations of Hill-type models to predict some physiological mechanisms. Eight eligible studies developed new methods to use Hill-type models in neuromusculoskeletal simulations. Fourteen eligible studies used Hill-type models to investigate physiological mechanisms including some material stress-strain dynamics, joint mechanisms related to muscle activity, posture and motion control in response to perturbations or the muscle redundancy problem. For clarity, the studies are referred to 'First Author Year of publication' in the figures of this review. Also, the two eligible complementary studies (Kim et al., 2015; Kim & Kim, 2018) were grouped together in this review.

Table 2-4: List of the 55 eligible models and the main aim of their related study

Topic of the study	References
Advancing the field of Hill-type modelling	(Bahler, 1968a; Hatze, 1977; Hatze, 1978; Giat et al., 1993; Durfee & Palmer, 1994; Van Zandwijk et al., 1996; Curtin et al., 1998; Shue & Crago, 1998; Nussbaum & Chaffin, 1998a; Brown et al., 1999; Cheng et al., 2000; Ettema & Meijer, 2000; Lloyd & Besier, 2003; Thelen, 2003; Günther et al., 2007; Mavritsaki et al., 2007; Song et al., 2008; Menegaldo & Oliveira, 2009; Tsianos et al., 2011; Maceri et al., 2012; Blümel et al., 2012; Mörl et al., 2012; Callahan et al., 2013; Lee et al., 2013; Millard et al., 2013; Elias et al., 2014; Kim et al., 2015; Hamouda et al., 2016; Dick et al., 2017; Siebert et al., 2018; Ross, Nigam et al., 2018; Kim & Kim, 2018; Rockenfeller, R. et al., 2020; Hussein et al., 2022)
Developing methods for including Hill-type models in neuromusculoskeletal simulations	(Wyss & Pollak, 1984; Pierrynowski & Morrison, 1985; Otten, 1987; Winters, 1995; Dorgan & O'Malley, 1997; Kistemaker et al., 2006; Proctor & Holmes, 2010; Wang, J. et al., 2012)
Investigating physiological mechanisms using Hill-type models	(Bobbert & van Ingen Schenau, Gerrit Jan, 1990; van Soest & Bobbert, 1993; Happee, 1994; Caldwell, 1995; DeWoody et al., 2001; Gallucci & Challis, 2002; McLean et al., 2003; Stelzer, Maximilian & Von Stryk, 2006; Gömmel et al., 2007; Moody et al., 2009; Rengifo et al., 2010; John et al., 2013; Marcucci et al., 2017; Warner et al., 2020)

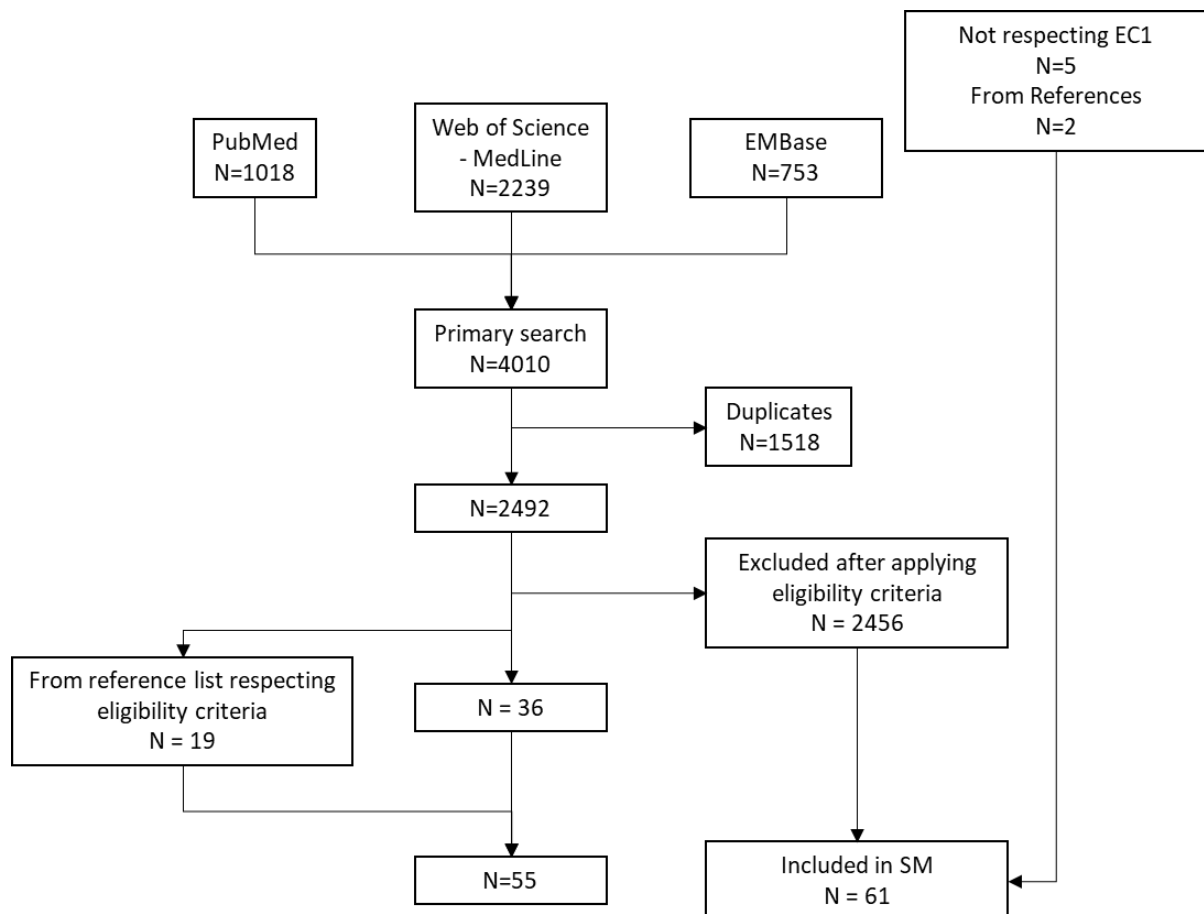


Figure 2-5: Flowchart of study inclusion according to EC1 to EC6. 36 eligible studies were obtained from the systematic search and 18 from the reference lists of the studies respecting EC1 to EC5. 61 non eligible studies were listed in Appendix Table A-6 and investigated in Appendix A.8 for their important contribution to the field of Hill-type modelling. See Appendix A.5 for a more detailed version of this diagram.

2.3.2. Completeness assessment

2.3.2.1. General results

The per-study results of the completeness assessment are displayed in Figure 2-6 (additional details in Appendix Figure A-2). The 55 eligible models scored in the range [3-16] out of 23 possible MT properties (mean: 8, standard deviation: 2.6) and showed no correlation between model completeness and publication year. As shown in Figure 2-7A, most of the models included between seven and nine MT properties, seven models included five or fewer properties, consistently with the model description by Zajac (1989), and five models included at least 12 properties (Winters, 1995; Dorgan & O'Malley, 1997; Cheng et al., 2000; Song et al., 2008; Tsianos et al., 2011), that is more than half of the identified muscle properties reported in Table 2-1. The specific properties included in each of the 55 eligible studies can be identified in the bar graph in Appendix Figure A-2. The rheological arrangements of the eligible models are displayed in Appendix Figure A-7 in Appendix A.9.

2.3.2.2. Modelling trends

The frequency graph in Figure 2-7B shows that the six properties AD, FL, FVC, FVE, PEE and SEE are widely considered key in Hill-type modelling as they respectively appear in 98%, 96%, 98%, 83%, 76% and 85% of the eligible studies. These most popular properties have been described and extended in the past 50 years with various modelling approaches and mathematical descriptions, the time-evolution of which is detailed in Appendix A.10 with inheritance diagrams and a summary of the mathematical equations proposed in the eligible studies.

The dynamics of the NE are most commonly lumped with a phenomenological model of AD consisting of a transient first-order ordinary differential equation (ODE) inspired from (Winters & Stark, 1988; Zajac, 1989; He et al., 1991; Winters, 1995) in 30 of the 55 eligible studies, or with a steady-state nonlinear transfer function (7/55), while several other eligible studies (17/55) describe the calcium dynamics (Hatze, 1977; Hatze, 1978; Otten, 1987) in an ADC approach. A quarter of the eligible models (15/55) drove the muscle AD with an excitatory drive derived from a model of ED, including for example feedback controllers (3/55), first-order phenomenological ODEs (8/55) or comprehensive compartmental Hodgkin-Huxley-like models of the motoneuron discharging mechanisms (Proctor & Holmes, 2010; Kim & Kim, 2018). A quarter of the eligible models (13/55) considered the dynamics of MU recruitment (MURD) with, for example, detailed phenomenological models (Hatze, 1978; Dorgan & O'Malley, 1997; Callahan et al., 2013), simpler models relying on recruitment thresholds (4/55), or different recruitment characteristics for slow and fast representative units identified from EMG wavelet analysis (Lee et al., 2013; Dick et al., 2017).

The dynamics of the CE are dominantly modelled (50/55) with a length-dependent FL relationship, that linearly scaled by the active state, and is typically described with quadratic (11/55) (Woittiez et al., 1984) and gaussian (16/55) (Hatze, 1977) mathematical descriptions according to Appendix A.10.3. One study (Hamouda et al., 2016) tackled the issue of the non-physiological instability of the descending limb of the FL relationship. Most eligible studies (39/55) modelled the FVC relationship with Hill's two-parameter or normalized hyperbola (see Appendix A.10.2.2), the 20% force overestimation of which near static conditions is tackled with a double-hyperbolic shape (Curtin et al., 1998). Around half (21/55) of the eligible studies modelled the FVE relationship with a two-parameter hyperbolic curve (Mashima et al., 1972; FitzHugh, 1977), while less than a quarter (12/55) used an inverted hyperbola respecting a C2 continuity between concentric and eccentric FV branches.

The muscle passive properties are typically modelled with a PEE and/or a SEE, described as nonlinear passive springs, the stress-strain relationships of which are mostly defined with quadratic (Bobbert & van Ingen Schenau, Gerrit Jan, 1990; van Soest & Bobbert, 1993), exponential (Hatze, 1977), and

piecewise linear-exponential (Otten, 1987; Giat et al., 1993) expressions (see Appendices A.10.4 and A.10.5). Approximately half of the eligible studies (25/55) used a linear PDE to model the slight viscoelasticity of the muscle's inner passive structures, or to prevent numerical singularities when inverting the FV relationship.

2.3.2.3. Less commonly modelled properties

As shown in Figure 2-7B, between 10% and 30% of the eligible studies enrich the aforementioned modelling approaches by considering a PEE2 (10/55), describing the short-length repulsive force due to sarcomere deformation, muscle mass (13/55), accounting for muscle-specific inertial characteristics and preventing numerical instabilities (Cheng et al., 2000; Song et al., 2008), or the nonlinear state-dependency of the ADL (12/55), FVL (15/55) and FVA (15/55) relationships. The eight remaining properties listed in Table 2-1 (ADV, FLA, FD, RFE, OTHD, Fat, SEE2 and SDE) are rarely modelled and appear in less than 10% of the eligible studies. As seen in Figure 2-7C, most of the least commonly modelled properties have not been considered in the eligible models in the past ten years and were mostly developed in the 1990s and 2000s.

2.3.2.4. Model parameters

Most eligible studies maintained low model complexity, as half used between 5 and 14 parameters to define their normalized properties, a third used 15 to 24 parameters, and 5 studies used more than 35 parameters up to around 60. As supported by Appendix Figure A-4 in Appendix A.8, on average two parameters were used to define one property, with higher ratios for the studies modelling the MURD (Hatze, 1978) or comprehensive descriptions of the NE (Proctor & Holmes, 2010; Kim & Kim, 2018).

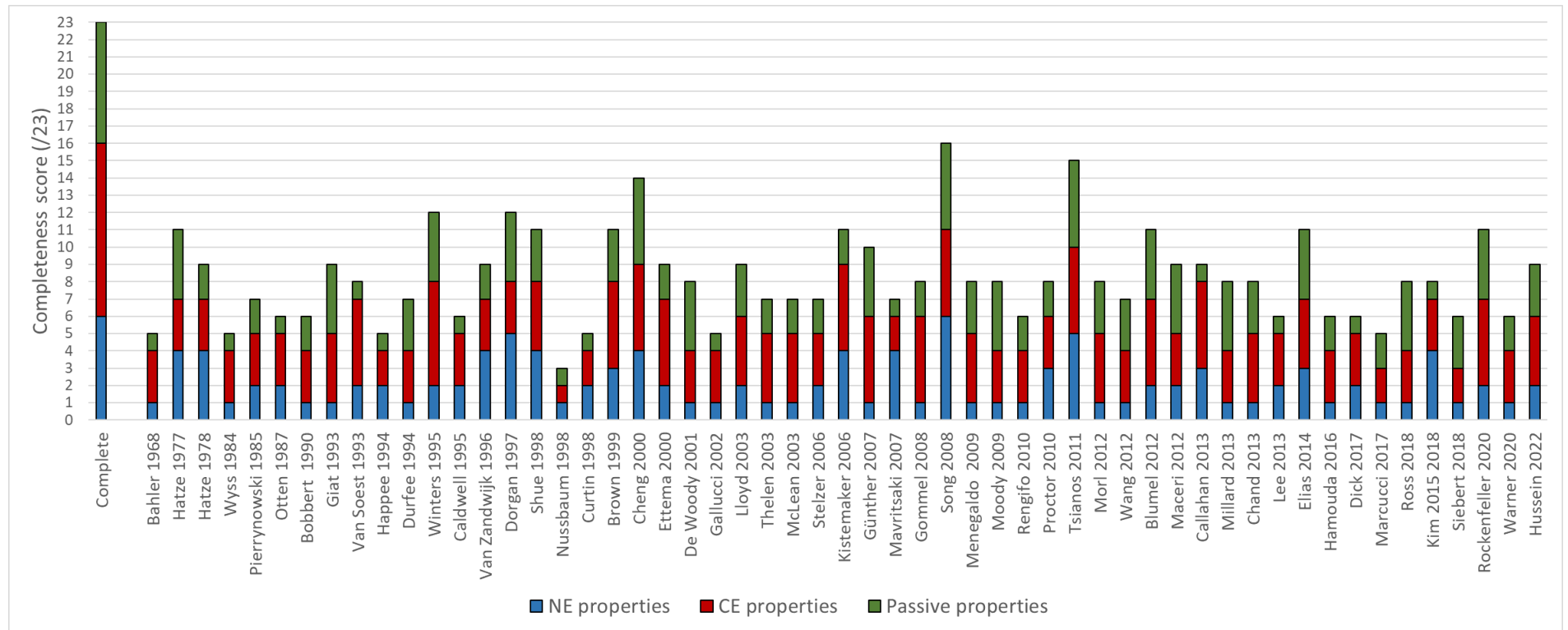


Figure 2-6: Results of the completeness assessment for the 55 eligible studies. A 'complete' bar representative of a theoretical complete model is provided for comparison. Blue: the 6 properties of the NE. Red: the 10 properties of the CE. Green: the 7 properties of the passive elements. The 55 studies are chronologically ordered. A more detailed version of this graph is provided in Appendix A.8.

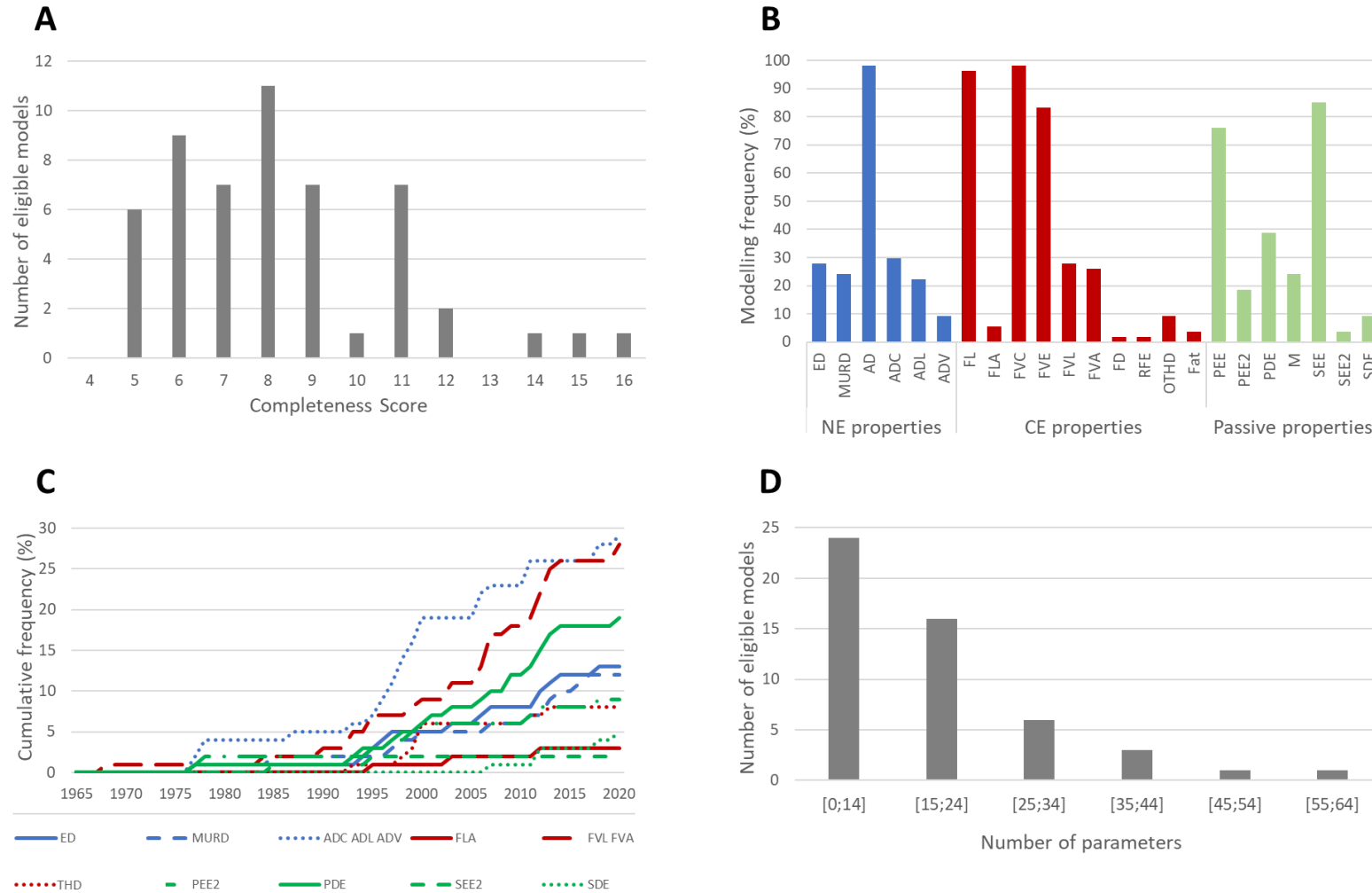


Figure 2-7: Results of the Completeness Assessment of the 55 eligible studies. (A) Frequency distribution of the eligible models according to their completeness score (out of 23). (B) Modelling frequency of each of the 23 properties among the eligible studies, where the modelling occurrence of each property is normalized to a percentage. (C) Time-accumulation of the eligible models that considered the 12 least commonly modelled properties. For clarity, some properties are grouped together: ADC-ADL-ADV, FVL-FVA, and FD-RFE-OTHD-Fat under ‘THD’. (D) Frequency distribution of the eligible models according to their number of parameters

2.3.3. Modelling assessment

2.3.3.1. Global results

Figure 2-8A shows that the eligible studies scored in the range [4-18] out of 20 (mean: 11, standard deviation: 3.5), 22 studies scored less than 10/20, and 9 studies scored above 15/20. The per-study results of the modelling assessment are displayed in Figure 2-9 (additional details in Appendix Figure A-17) and show no correlation between modelling score and publication year. As shown in Figure 2-8B, the four categories (validation, reusability, modelling choices and strategy, and calibration) detailed in Table 2-3 globally received the same level of attention with average scores of 1.0, 1.0, 1.2 and 1.2 respectively, while the eligible studies scored an average in [0.8-1.2] for most questions, except Q3 and Q5 for model optimization and model reusability which scored below 0.75/2. Appendix A.12 reports for Q1 to Q10 the most popular modelling approaches proposed in the eligible studies.

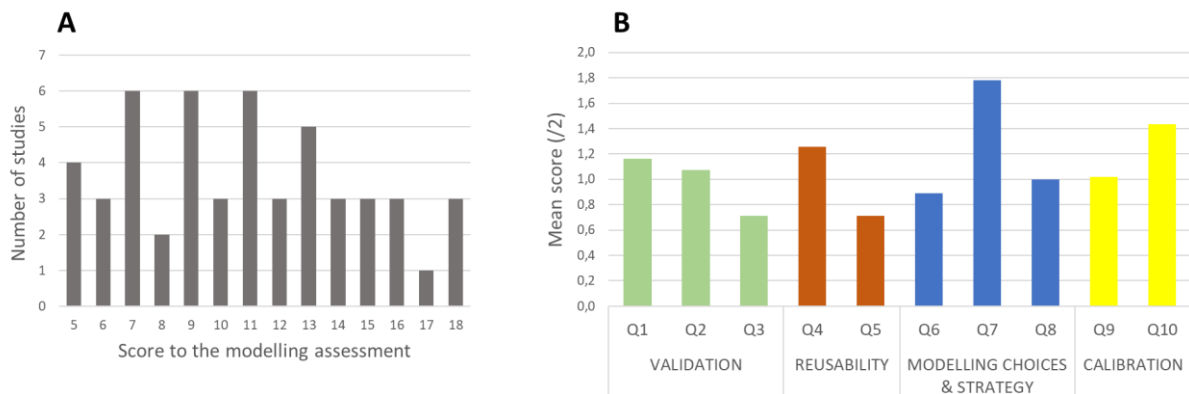


Figure 2-8: (A) Distribution of the 55 eligible studies according to their total score in modelling assessment (out of 20). (B) Mean scores among the 55 eligible studies for the 10 questions of the modelling assessment. The yielded mean scores are out of 2.

2.3.3.2. Model validation (Q1 to Q3)

Less than half of the eligible studies (25/55) validated the model predictions against ad hoc experimental animal data, mostly mammalian muscle force (10/55) or human (15/55) quantities such as joint torque (12/55). A quarter (15/55) of the studies proposed validations against results from available experimental datasets, the protocol of which were reproduced in simulations, and the remaining eligible studies (15/55) either proposed no model validation or qualitative validations against other results from the literature. Less than a third (17/55) of the eligible studies used mathematical or statistical metrics for the analysis and interpretation of the model predictions, while the remaining studies (39/55) only proposed qualitative and visual assessments of their analysis. Consequently, approximately a third of the eligible studies proposed sufficient validation for a complete evaluation of the performance of their model.

2.3.3.3. Model reusability (Q4 and Q5)

Approximately two thirds (36/55) of the eligible studies did not provide enough information to make possible a re-implementation of their Hill-type models and a reproduction of the published results, missing some reference or some description for the chosen rheological arrangement, the mathematical equations describing the modelled properties, and/or the parameter values scaling these properties. Out of the 19 remaining eligible studies, which presented models that could theoretically be re-implemented, only five provided full access to the actual implementation of their computational model (Cheng et al., 2000; Mavritsaki et al., 2007; Song et al., 2008; Millard et al., 2013; Kim & Kim, 2018), while open-source versions for two more (Thelen, 2003; John et al., 2013) were then made available as the result of other studies. Most of the eligible models (37/55) were not optimized for stability and computational speed, while eight imposed C1 or C2 continuity for all modelled properties, and twelve avoided singularities in the inverted FV relationship (see Appendix A.12 for additional details). Consequently, most of the eligible models cannot be readily reused in other studies.

2.3.3.4. Modelling choices and strategy (Q6 to Q8)

More than half of the studies (33/55) commented on some modelling simplifications and assumptions regarding the muscle physiology and geometry, while less than a third (16/55) discussed the multiscale modelling approach. Around three quarters of the eligible studies (43/55) justified their modelling choices with references and in accordance with the aim of the study, although most of them (40/55) did not discuss and compare the strengths and weaknesses of the modelling approach in relation to other studies in the literature. Consequently, most of the eligible studies do not draw conclusions on the future works necessary to improve their model.

2.3.3.5. Model calibration

Around a third (20/55) of the eligible studies made at least one of the model properties subject-muscle-specific by tuning its parameters either with parameter calibration minimizing a cost function between simulated and experimental quantities, or with ad hoc experimental measurements. Half of the eligible studies (28/55) used the aforementioned methods to scale the normalized Hill-type model with subject-specific values for the slack length of the Series Elastic Element (SEE), the optimal length of the CE and the muscle maximum isometric force. Consequently, most of the eligible models are generic.

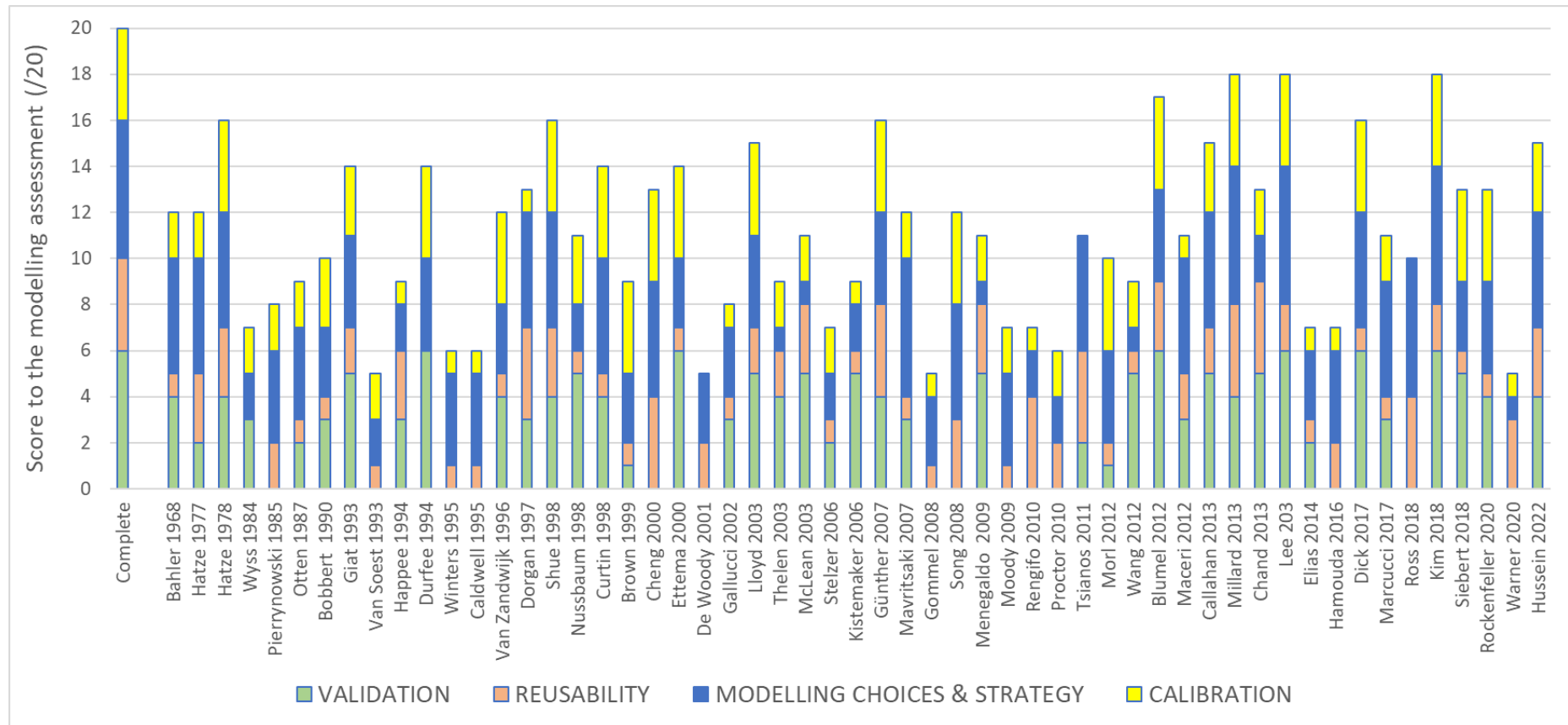


Figure 2-9: Results of the modelling assessment for the 55 eligible studies. A 'complete' bar representative of a model scoring 2 to the 10 questions is provided for comparison. Green: model validation. Red: Model reusability. Blue: Modelling choices and strategy. Yellow: model calibration. A more detailed version of this graph is provided in Appendix A.11.

2.3.4. Global assessment

Figure 2-10 provides the global score for the 55 eligible studies. A high score globally suggests that an eligible model describes numerous muscle properties, is carefully described, calibrated, and validated, and is easily reusable. However, the global score is not an indicator of the overall quality of the eligible studies. The eligible studies scored in the range [26%; 67%] (mean: 46%, standard deviation: 12%). Ten eligible studies scored below 33%, 25 in the range [33%; 50%], and nine above 60% (Hatze, 1978; Shue & Crago, 1998; Cheng et al., 2000; Günther et al., 2007; Song et al., 2008; Tsianos et al., 2011; Blümel et al., 2012; Millard et al., 2013; Kim et al., 2015; Kim & Kim, 2018)

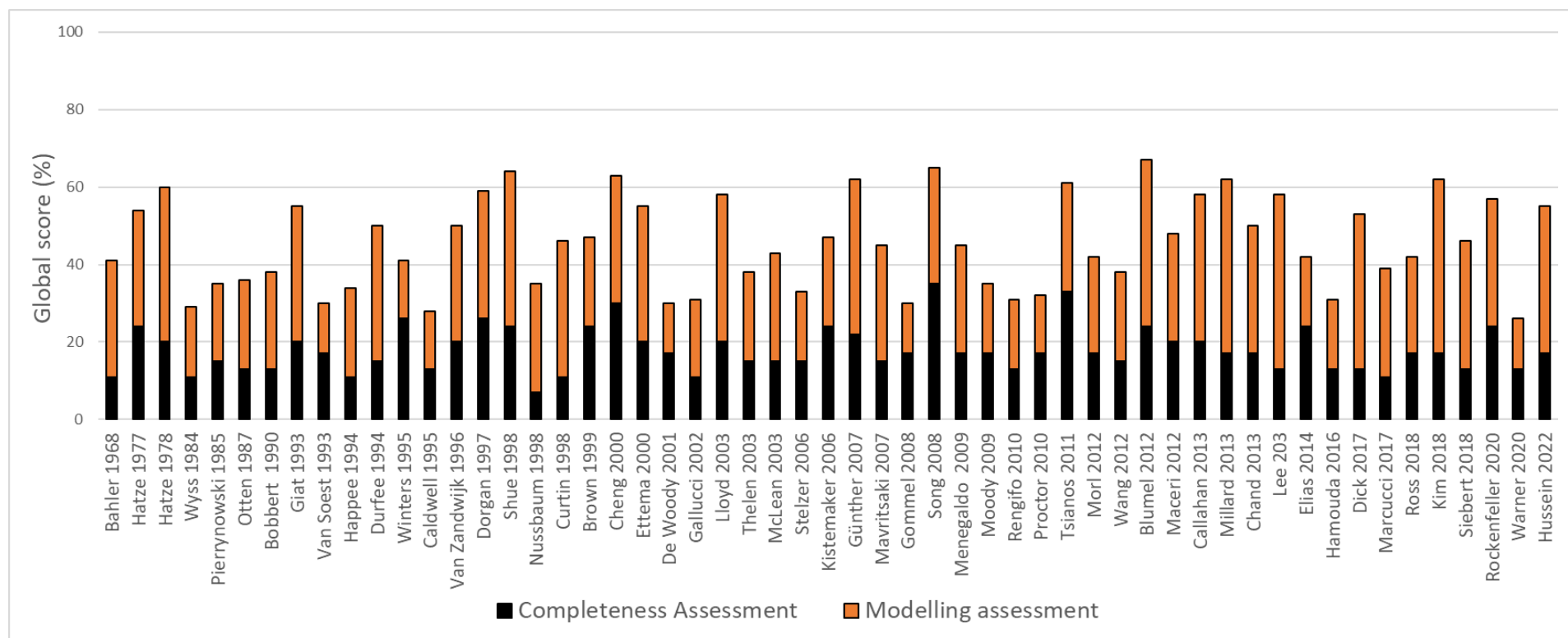


Figure 2-10: Global scores for the 55 eligible studies. Completeness (black) and modelling (orange) assessments were given the same weights to derive the global score.

2.4. Discussion

2.4.1. Summary and main objectives

This systematic review was performed to build sufficient knowledge for confidently developing a novel MN-driven multi-MU Hill-type neuromuscular model that would advance the field by answering some of its current limitations. To clarify the dense and hard-to-explore field of Hill-type modelling, for which recent reviews are lacking, and assess the state-of-the-art and current gaps in property modelling and methodological practices, 55 eligible studies (Table 2-4), which were extracted from the systematic search (Figure 2-5) according to six eligibility criteria (EC1 to EC6, Table 2-2) that impose a minimum level of model complexity (Figure 2-4) and innovation (Appendix A.6), were investigated according to two scoring schemes. In the following, the results of the completeness and modelling assessments (Figure 2-6 to Figure 2-10) are used to (1) define the main trends and gold standards in Hill-type modelling, (2) challenge these gold standards with advanced modelling methods, (3) clarify the state-of-the-art by investigating properties non-commonly modelled in the field, (4) discuss the importance of model validation, and (5) open the discussion on the necessity of more regular reviews and global recommendations in the field of Hill-type modelling, with an emphasis on model reusability for optimal transmission of knowledge.

2.4.2. Main trends and gold standards in Hill-type modelling

According to the conclusions drawn in the Results Section from the completeness and modelling assessments, to Figure 2-7B, Figure 2-8B for Q6-8 and to Appendix A.12, Hill-type models in the literature are most commonly described with a Voigt-Kelvin structure that includes a NE, a CE, a PEE and a SEE (see examples Appendix Figure A-7) to model the ‘classic’ AD, FL, FVC, FVE, PEE, SEE properties in a multiscale approach where a representative muscle fibre represents the whole muscle. Typically, a single phenomenological ODE produces a time-history-dependent active state $a(t)$ that describes the lump activity of the muscle’s neuromuscular interface, and linearly scales the instantaneous length-dependent and velocity-dependent forces $f_{FL}(l^{FG})$ and $f_{FV}(v^{FG})$ determined by the independent CE’s FL and FV properties, to define the total FG force f^{FG} as $f^{FG}(t, l^{FG}, v^{FG}) = a(t) \cdot f_{FL}(l^{FG}) \cdot f_{FV}(v^{FG})$. The FG, in-parallel with the PEE, is in equilibrium with the SEE, which passively transmits the active force as $f^{SEE} = f^{FG} + f^{PEE}$. These equations define a common baseline for most of the Hill-type models in the literature and are typically rearranged to isolate and solve for unknown state variables, such as the FG length l^{FG} or force F^{FG} . The most common mathematical descriptions for the AD, FL, FV, PEE and SEE properties are reported in Appendix A.10.

2.4.3. Challenging the gold standards in Hill-type modelling

The aforementioned gold standards in Hill-type modelling, most of which were proposed between 1938 and 1976 (see Appendix A.1 for details), can be challenged and the main trends in defining and modelling the multiscale approach, the FL and FV properties, the active state, the interplay between the NE and the CE and the in-series passive elements can be extended.

2.4.3.1. Multiscale approach

Hill-type models are flexible enough to describe, with a single actuator and adequate multiscale simplifications, the structure and dynamics of either individual sarcomeres, fibres, MUs or, most commonly, whole muscle-tendon systems. Despite successfully reducing the cost and complexity of Hill-type muscle modelling, multiscale approaches come with important assumptions. When a whole muscle is described as one of its ‘representative’ unidirectional fibre or MU, it implies that all muscle fibres or MUs have homogeneous ‘average’ material properties and dynamics, and are in parallel, coplanar, of the same length spanning across the whole muscle length, making the same pennation angle with the tendon and simultaneously excited with the same neural control and electromechanical delay with an homogeneous repartition of activated fibres across the muscle, and work independently without lateral force transmission. Multiscale sarcomere-to-muscle approaches further imply that the muscle’s sarcomeres all have the same length and neuromechanical characteristics and work independently from their in-parallel neighbours. These multiscale simplifications may contradict experimental data ((Blemker & Delp, 2005; Blemker et al., 2007), for example), preventing modelling of some muscle properties, such as MURD, and affecting the physiological accuracy of the muscle description with limitations in reconciling microscopic and macroscopic scales and mechanisms (Winters et al., 2011; Wisdom et al., 2015; Hou, 2018; Günther et al., 2018), with subsequent potential downgrades in prediction accuracy (Huijing & Slawnych, 2000).

Yet, only a few eligible studies (see Appendix A.11 and Figure 2-8 Q6-7), including the 9 modelling single fibres or MUs with a Hill-type model, explicitly evoke the multiscale approach and its implications on Hill-type modelling. This lack of clarity feeds and is strengthened by a lack of consensus between studies in the notation defining the muscle quantities, with 22 different notations observed in the eligible studies for the length of the contractile unit ($L, L_M, L_{CE}, L_m, L_f, L_{fibers}, L^M, L^m, L^F, l, l_{CE}, l_{CC}, l_m, l_f, l_{ce}, l^M, l^{CE}, X_m, x, x_{eye}, x_{CE}, \lambda$), and often the same notation (l or l_m) for different quantities (muscle and muscle-tendon length). Overlooking the multiscale conditions, the eligible studies often use experimental data to fit the Hill-type properties at a scale inconsistent with the modelled physiology. This is an important limitation, as the FL relationship is for example known to be piecewise linear at the sarcomere scale but to be smoother with larger

plateau (Hatze, 1980) and more slowly decreasing descending limb beyond optimal fibre length at larger scales (Herzog et al., 2015), a multiscale difference sometimes debated (Winters et al., 2011; Moo et al., 2020). Also, the curvature of the FV relationship decreases with higher scales (compare (Ranatunga, 1982; McDonald et al., 1994; Roots et al., 2007) for works on rat skinned fibres, bundles of fibres, and whole muscles), while the Young's modulus in the exponential stress-strain relationship of the PEE nonlinearly increases from the fibre to the muscle scale (Ward et al., 2020), with dominant contributions to the total passive force of the titin structures and of the extracellular matrix at the fibre and muscle scales respectively (Prado et al., 2005; Ward et al., 2020; Wheatley, 2020). Consequently, the multiscale Hill-type modelling must be correctly applied and commented to maintain credible descriptions of the muscle physiology and dynamics.

2.4.3.2. FL, FV and FVL properties

Current gold standards in FL and FV modelling can be advanced. The non-physiological instability in the classic description of the descending limb of the FL relationship can be corrected with a time-history-dependent approach accounting for the initial length at activation onset (Hamouda et al., 2016), or with a two-mode Hill-type model (Akazawa, 2019; Akazawa, 2020). To avoid an overestimation of instantaneous force at low shortening velocities, the FVC relationship should be modelled as double-hyperbolic (Edman et al., 1978; Edman, 1988; Curtin et al., 1998; Edman, 2005; Alcazar et al., 2019), while the standard FVE modelling reaching higher-than- F_0^M forces during eccentric contractions is experimentally challenged (Sutarno & McGill, 1995). As modelled in only a few eligible studies (Figure 2-7B), the FVC property nonlinearly varies with instantaneous muscle length (Askew & Marsh, 1998) as the maximum shortening velocity decreases with shorter sub-optimal muscle lengths (Abbott & Wilkie, 1953; Gordon et al., 1966; Bahler, 1968a), despite a lack of consensus (Edman, 1979; Edman, 1988), and the curvature of the FVC relationship (defined by the a_f parameter in Appendix A.10.2.2) decreases with shorter sub-optimal muscle lengths (Granzier et al., 1989) and increases for longer over-optimal lengths (Edman, 1988), suggesting an increased probability of cross-bridge attachment during filament sliding when the inter-filamentary spacing is reduced, which can be debated to be redundant with the properties described by the FL relationship.

2.4.3.3. Definition of the muscle active state

The active state of a CE is commonly defined as the intracellular concentration of calcium ions (Thelen, 2003) or the proportion of available troponin receptors bound to calcium ions (Ebashi & Endo, 1968). These definitions prevent extending the phenomenological description of the NE behaviour to subsequent important transient events that are typically not reflected in the instantaneous CE properties, such as the calcium-troponin binding dynamics or the probabilistic cycling mechanism of

cross-bridge formation between detached, weak bound and force-generating states (Shorten et al., 2007; Tamura et al., 2008; Zot & Hasbun, 2016), that are typically overlooked in Hill-type modelling. The active state of a CE can be defined as the normalized instantaneous ratio of formed cross-bridges in the force-generating state in a population of myosin heads, irrespective of the degree of filamentary overlap, relative inter-filamentary sliding speed and other external mechanical mechanisms, which should be modelled in the contraction dynamics of the CE. This definition is not paradoxical with any influence fibre length or velocity can have on the mechanisms involved in the overall AD discussed below. To the authors' knowledge, only three analytic (Wexler et al., 1997; Ding et al., 2000; Neidhard-Doll et al., 2004) and five Hill-type models (Otten, 1987; Van Ruijven & Weijs, 1990; Rassier et al., 1999; Wilson et al., 2006; Mavritsaki et al., 2007) have proposed phenomenological time-dependent ODEs to explicitly account for the transient mechanisms occurring between the events of calcium ion release and force generation, which are typically lumped with a steady-state pCa-force sigmoidal relationship (Hatze, 1977; Lloyd & Besier, 2003).

2.4.3.4. Interplay between the NE and the CE – FLA, ADL, FVA, ADV properties

The real muscle mechanisms rely on an interplay between the CE's and NE's activation, length, and velocity states, which is commonly overlooked in Hill-type models as shown in Figure 2-7B and Appendix Figure A-5. Modelling the behaviour of the NE to be independent from the state of the CE and linearly scaling with the active state the FL and FV relationships, which are typically obtained from experimental observations at maximal muscle activity, are important limitations which partly explain the poor performance of Hill-type models in submaximal activation tasks (Perreault et al., 2003). As performed in some of the eligible studies (Figure 2-7B), this interplay is considered with ADL, ADV, FLA and FVA approaches, which are potentially redundant, and where the FG properties are nonlinearly related to the active state a , length l^{FG} and velocity v^{FG} as described in Appendix A.10.

The FL relationship is empirically observed (Rack & Westbury, 1969; Stephenson & Wendt, 1984; Guimaraes et al., 1994; Huijing, 1996; Balnave & Allen, 1996; Rockenfeller & Günther, 2018; Goisard de Monsabert et al., 2020) to distort for submaximal contractions towards peak forces occurring at longer CE lengths. It is explained, at the CE level, by changes in the force transmission pathways between the multiscale layer of the muscle (Huijing, 1999) to minimize the muscle internal work and optimize force production (Holt & Azizi, 2014). The FLA property is phenomenologically captured in three eligible models (Winters, 1995; Lloyd & Besier, 2003; Blümel et al., 2012) with an activation-dependency of the CE optimal length $l_0^{FG}(a)$, which improves the accuracy of the model predictions (Sun et al., 2018). However, it is believed that the FLA approach phenomenologically encapsulates in these studies some ADL properties at the NE level (Rassier et al., 1999), that are responsible for the

length-dependencies of the experimental frequency-force (Rack & Westbury, 1969; Brown et al., 1999), EMG-force (Goislard de Monsabert et al., 2020) and pCA-force (Stephenson & Williams, 1982) relationships. It is proven that the calcium transients are length-dependent (Blinks et al., 1978; Baylor et al., 1983; Close & Lännergren, 1984; Konishi et al., 1991) as the muscle geometry affects the dynamics of calcium release from the sarcoplasmic reticulum and calcium diffusion in the sarcoplasm. The reaction rate for calcium-troponin (CaTn) unbinding decrease with increase sarcomere length (Matsuo et al., 2010). Furthermore, lengths above l_0^{FG} increase the sensitivity of cross-bridge formation to calcium ions (Endo, 1973; Moisesescu & Thieleczek, 1979; Stephenson & Williams, 1982; Stephenson & Wendt, 1984), the probability of CaTn formation by geometrically increasing the sarcomere cross-sectional surface density of troponin at constant volumetric calcium concentration (Hatze, 1977; Rockenfeller & Günther, 2018), and the probability of cross-bridge formation by geometrically reducing the intrafilamentary spacing between myosin and actin filaments (Godt & Maughan, 1981). The latter phenomenon may be redundantly modelled with the FVL approach described previously.

The modelling of a nonlinear α -dependency of the FVC relationship (FVCA) proposed in 14 eligible studies is justified by the important decrease in maximum shortening velocity with decreasing activation observed in human joint torques (Chow & Darling, 1999; Camilleri & Hull, 2005), cat dissected muscles (Petrofsky & Phillips, 1981) and in rabbit skinned fibres (Moss, 1986) and explained by an increase in inactive inertia (Ross & Wakeling, 2016). Yet, this phenomenon could also be modelled as an ADV property in the NE because of both a lower ratio of fast-contracting MUs at low activation, and an increase in the number of cross-bridges bearing tensile force against shortening dynamics, as cross-bridge detachment rates slow with lower levels of sarcomeric calcium concentration. While denied (Camilleri & Hull, 2005) or neglected in a suitable manner (Winters, 1995), three eligible studies (Winters, 1995; Günther et al., 2007; Blümel et al., 2012) consider another FVCA property, where the curvature of the FVC relationship decreases at higher activation (Petrofsky & Phillips, 1981; Bayer et al., 2017), which is potentially explained by the lower ratio of slow versus fast recruited MUs, which exhibit different FV contractile properties. Five eligible studies (Brown et al., 1999; Cheng et al., 2000; Song et al., 2008; Tsianos et al., 2011) proposed an ADV property to model the yielding phenomenon, which relies on the same physiological mechanisms as short-range stiffness (Malamud et al., 1996) during eccentric contractions. Upon stretch deformation, cross-bridges provide rapid tensile stiffness (Campbell & Lakie, 1998; Getz et al., 1998) before forcibly detaching leading to the force drop related to yielding (Sutarno & McGill, 1995), the effects being emphasized in slow-type fibres (Malamud et al., 1996; Brown et al., 1999) in fast eccentric contractions (Nichols, 1984; Malamud et al., 1996), where the rates of cross-bridge detachment (Joyce et al., 1969) and attachment are respectively higher and slower. Therefore, the SRS dynamics should be modelled as a passive property

(De Groote et al., 2017), while yielding is both a passive and a time-history dependent FVA property, for the decrease of weak-bound and force-generating cross-bridges respectively due to mechanical stretches. Although SRS and yielding should not be modelled in the NE according to the definition of AD previously proposed, they influence the time-course of the muscle ED and MURD (Cordo & Rymer, 1982; Sutarno & McGill, 1995).

2.4.3.5. In-series elastic elements

Tendon slack length and stiffness have a key influence on model predictions, according to the sensitivity studies reported in Appendix A.7. The tendon behaviour is commonly modelled with an SEE in-series with the FG (see Appendix A.9 for rheological structures) and simple nonlinear stress-strain relationships (see equations in Appendix A.10). Besides complicated multiscale tendon models (Maceri et al., 2012), advanced phenomenological models of the tendon behaviour that would for example account for its hysteresis (Herzog, 2019) have however not yet been proposed in the literature of Hill-type modelling. Most eligible models describe the additional passive activity of the muscle aponeurosis and other sub-scale passive structures with a SEE2, although they should be considered in-parallel with the FG and included in the PEE in first approximation (Herzog, 2019). In sarcomere-scale Hill-type structures, a SEE2 can however encapsulate the passive titin and cross-bridge elasticities, which are in-series with the force-generating cross-bridges, although they are mechanically not in-series with the macroscopic tendon due to the in-parallel arrangement of laterally-connected myofibrils and fibres, contradicting some previous sarcomere-to-muscle multiscale modelling approaches (Pierrynowski & Morrison, 1985; Zajac, 1989; Pandy et al., 1990). To avoid such inaccurate simplifications in sarcomere-to-muscle multiscale approaches, it is advised to neglect the SEE2 dynamics when the elasticity elastic energy storage is predominantly taken by a long and/or compliant free tendon (Zajac, 1989). The increment in tendon velocity-dependent stiffness arising from the SDE viscoelasticity can be likewise neglected for slow-to-medium contraction speeds (van Ingen Schenau, G J et al., 1988; Meijer et al., 1998; Mörl et al., 2012).

2.4.4. Current gaps in Hill-type modelling and lines of improvement

According to the results of the completeness assessment in Figure 2-6 and Figure 2-7A, the eligible studies globally score relatively low (mean and median of 8/23) and do not extend the baseline approach (Figure 2-4) much beyond the gold standards discussed previously. Individually, they however tackle numerous gaps in Hill-type modelling and open the field to new lines of improvement, some of which were discussed in the previous section.

2.4.4.1. Modelling the neuromechanical interface

Most eligible studies limit the modelling of muscle's excitation-contraction coupling properties to a 'black box' 1st-order ODE and/or a steady-state sigmoid transformation (see Appendix A.10 for examples) to transform the excitation state into the active state of the FG. Such models of AD are hardly relatable to actual physiological mechanisms and are therefore difficult to interpret, calibrate, validate, and advance (Hussein et al., 2022). This is a major limitation when numerous highly nonlinear mechanisms actually define the subject-muscle-task neuromechanical dynamics of the MU pool and the neural drive to muscle, and are proved to have a dramatic influence on force generation (Heckman & Enoka, 2012; Farina & Negro, 2015). To tackle this limitation, more complex modelling approaches must be proposed to describe the discrete NE dynamics, from the spinal synaptic influx to the definition of the active state previously proposed.

In this respect, some eligible studies (17/55) explicitly model the time-course of the calcium transients in the sarcoplasm (see Appendix A.10 for equations). In describing actual physiological mechanisms, this ADC approach enables a confident modelling of the length-dependency of the AD dynamics, discussed previously, and a direct validation against experimental (Hollingworth et al., 1996; Baylor & Hollingworth, 2003) or simulation (Baylor & Hollingworth, 1998; Holash & MacIntosh, 2019) studies evaluating calcium transients and pCa-force relationships. When modelling ADC, it is worth noting the preferable choice of the mag-2-fura indicator in experimental studies, as well as the strong dependency of the calcium transients to animal species and temperature (Hirota et al., 1989; Raju et al., 1989; Konishi et al., 1991; Delbono & Stefani, 1993; Delbono et al., 1995; Zhao et al., 1996; Capote et al., 2005; Hollingworth, Stephen et al., 2008; Baylor, Stephen M. & Hollingworth, 2011; Hollingworth, Stephen et al., 2012). While some comprehensive models of calcium transients (Shorten et al., 2007; Heidlauf & Röhrle, 2014; Pizarro & Olivera, 2020) can also be included in Hill-type models, additional AD dynamics such as the CaTn transients have been proposed in a recent MN-driven Hill-type model (Caillet et al., 2022).

As suggested in Figure 2-7B, because of the limitations related to the fibre-to-muscle multiscale approach, most eligible studies disregard the dynamics of MURD and Henneman's size principle (Henneman, 1957; Henneman, 1981; Henneman, 1985). The MU recruitment, discharge and mechanical properties are inter-related, MU-specific, and continuously distributed across the MU pool from small low- to large high-force and -threshold MUs, as quantitatively reviewed (Caillet et al., 2022), and the relative importance of MURD and discharge frequency in the determination of the neural drive to muscle is task-dependent (Heckman & Enoka, 2012). In most multiscale eligible studies, it is unclear whether the complete MU pool is assumed to be recruited at all times and the excitation state is an indicator of the overall MN discharge frequency, or if the excitation state, typically obtained from

processed EMG signals in this case, is a lump representation of the MU pool recruitment and discharge dynamics. To avoid confusion, some studies proposed a phenomenological model of MURD in a single multiscale NE (Hatze, 1978; Hatze, 1980; Legreneur et al., 1996; Riener et al., 1996; Dorgan & O'Malley, 1997; Riener & Fuhr, 1998; Song et al., 2008; Tsianos et al., 2011; Callahan et al., 2013). Recent studies also proposed an explicit description of the MURD with a complete reconstruction of the MU pool in muscle models built as a collection of MUs described analytically (Raikova et al., 2018), with Hill-Huxley-type structures (Carriou et al., 2019) or with Hill-type actuators (Caillet et al., 2022). The MURD are described with the model from Fuglevand et al. (1993) in the first two studies, while other approaches could be considered (Heckman & Binder, 1991; Liu et al., 2002; Dideriksen et al., 2010; Dideriksen et al., 2011; Dideriksen et al., 2012; Potvin & Fuglevand, 2017; Petersen & Rostalski, 2019). MU pool reconstruction makes it easier to describe the distribution of the MU neuromechanical properties in the muscle, which can also be lumped in two FGs for slow-type and fast-type populations (Wakeling et al., 2012; Lee et al., 2013; Dick et al., 2017).

MU pool reconstruction and detailed models of the MURD motivate an advanced modelling of the ED, i.e. of the electrical-chemical mechanisms underlying the time-course of the MU-specific neural message from the spinal synaptic influx to the depolarization of the t-tubules. In the literature, analytical, phenomenological or Hodgkin-Huxley-like approaches are proposed to model the discharge trains of action potentials that drive MU-scale Hill-type models (Hatze, 1977; Hatze, 1978; Happee, 1994; Legreneur et al., 1996; Van Zandwijk et al., 1996; Mavritsaki et al., 2007; Proctor & Holmes, 2010; Maceri et al., 2012; Kim et al., 2015; Kim & Kim, 2018) and permit realistic reconstruction of the MU force as a summation of individual twitches, as opposed to muscle-scale approaches that receive continuous neural controls, such as EMG-driven models. The modelling of the robust process of neuron-fibre synaptic transmission that always elicits post-synaptic action potentials (Bigland-Ritchie et al., 1982; Wood & Slater, 2001; Kovyazina et al., 2010) can be neglected.

It is noteworthy that both the modelling of the ED and MURD can be by-passed if MN spike trains and their intrinsic discharge and recruitment dynamics are experimentally obtained, e.g. by decomposing HDEMG signals (Del Vecchio et al., 2020) or intramuscular recordings (Muceli et al., 2022) and extended to the recruitment and discharge behaviour of the complete MU pool with physiologically realistic methods (Caillet et al., 2022). This approach is technically challenging but it is preferable over merging these MN-specific quantities into a unique neural control to muscle-scale models (Sartori et al., 2017; Thompson et al., 2018; Kapelner et al., 2020).

2.4.4.2. Titin-induced time-history-dependent mechanisms

The classic steady-state instantaneous CE properties should be extended to describe the muscle time-history dependent transient properties, as performed in a few eligible models (Figure 2-7B), to avoid potential high prediction errors (Winters, 1990) and describe certain behaviours like balance control against perturbations (Seiberl et al., 2013; De Groote et al., 2017). While comprehensive models of muscle fatigue exist (Shorten et al., 2007), the proposed methods for modelling FD and RFE in Hill-type models are phenomenological and not related to the physiological mechanisms responsible for these time-history dependent properties. RD and RFE are explained by the various dynamics of titin (Herzog et al., 2016; Tahir et al., 2020), rather than the nonuniformity of sarcomere lengths (Herzog et al., 2015). Titin can shorten with calcium-mediated binding (Herzog et al., 2010; Herzog et al., 2015) or winding (Hessel et al., 2017) motions and increase its stiffness upon activation (Bagni et al., 2002; Bagni et al., 2004; Powers, Krysta et al., 2014; Rassier et al., 2015), which besides stabilizes the aforementioned descending limb of the instantaneous FL relationship (Schappacher-Tilp, 2018). With an unfolding-folding behaviour at activation (Brynnel et al., 2018), titin can also provide power strokes to the force-generating cross-bridges and accelerate muscle shortening (Rivas-Pardo et al., 2016; Eckels et al., 2019). Consistent with the previously discussed ADL properties, titin promotes cross-bridge-attachment by passively pre-stretching non-active sarcomeres (Fusi et al., 2016; Piazzesi et al., 2018). Already included in Huxley-type (Rode et al., 2009; Schappacher-Tilp et al., 2015) and continuum (Heidlauf et al., 2017) models, the titin activity could be modelled in a hybrid CE-PEE property that alternates between active (Li, Yong et al., 2018) and passive (Brynnel et al., 2018; Ward et al., 2020) states, and is in-series or in-parallel with the cross-bridges upon calcium activation (Herzog, 2019).

2.4.4.3. Inertial effects

Although it typically increases the numerical cost of the model by involving 2nd-order ODEs, considering muscle mass in Hill-type models can increase numerical stability (Cheng et al., 2000) and reduce the muscle maximum shortening velocity and mechanical work (Ross & Wakeling, 2016; Ross et al., 2020). A mass element is necessary to investigate the contraction-related effects of muscle mass flow (Guo et al., 2020), on which depends the MSK centre of mass location, and wobbling masses (Gruber et al., 1998) on the reduction of impact forces (Liu, W. & Nigg, 2000; Gittoes et al., 2006), joint contact forces and torques (Gruber et al., 1998; Pain & Challis, 2006), or bone internal stresses (Moody et al., 2009).

2.4.4.4. Choice of parameters

The behaviour of the NE, CE and passive elements is highly dependent on the parameter values that describe the properties displayed in Figure 2-3 and discussed previously. These parameter values, which are typically obtained from fitting experimental data with parametrized mathematical

relationships, vary with multiscale considerations as discussed, and are mostly also species-, muscle-, temperature-, and subject-specific. For example, the a_f parameter describing the curvature of the FVC relationship increases from fibre- to muscle-scale as discussed, and between slow-type and fast-type fibres (Bottinelli et al., 1996; Bottinelli et al., 1999; Widrick et al., 2002), while the parameter defining the half-time of relaxation of the calcium transients (ADC) drastically decreases with increasing temperatures (Hollingworth et al., 1996), between slow and fast fibres (Baylor, Stephen M. & Hollingworth, 2012) and between mammalian (Baylor & Hollingworth, 2003) and amphibian (Hatze, 1977) species. Potentially because of limitations in knowledge transmission in the field of Hill-type modelling, discussed later in this section, it was observed that most eligible studies overlooked the importance of these numerical values (Figure 2-8B Q9), which were not provided in most publications. Several eligible studies proposed CE and passive properties described with parameters experimentally obtained from different species and typically copied from milestone but dated Hill-type modelling studies (Hatze, 1977; Otten, 1987; Winters, 1995). More recent experimental data are usually not exploited as dated frog data (Gordon et al., 1966) is commonly used over more recent human data (Gollapudi & Lin, 2009) to describe human muscles.

According to sensitivity studies listed in Appendix A.7 (Scovil & Ronsky, 2006; Redl et al., 2007; Xiao & Higginson, 2010; De Groote et al., 2010; Ackland et al., 2012; Carbone et al., 2016; Bujalski et al., 2018), the predictions of muscle forces in MSK simulations are most sensitive to three MT parameters: the tendon slack length, the optimal length of the CE and the muscle isometric force. . It is worth noting that these observations were made with mostly ‘baseline’ models, and additional sensitivity studies on ‘more complete’ models are required to generalize these conclusions. While the parameters describing the muscle properties can remain generic, these three scaling parameters must be tuned to be subject-muscle-specific (Zajac, 1989). As referenced in Appendix A.12, some eligible studies (Hatze, 1978; Bobbert & van Ingen Schenau, Gerrit Jan, 1990; Lloyd & Besier, 2003; John et al., 2013; Kearney et al., 2021) proposed calibration methods, although various configurations of parameter values can explain similar muscle dynamics (Bujalski et al., 2018), depending on the role of the muscle in the MSK task (Carbone et al., 2016; Bujalski et al., 2018; Kian et al., 2021). Other eligible studies experimentally measured these properties in animal (Giat et al., 1993; van Soest & Bobbert, 1993; Günther et al., 2007) or human (Dick et al., 2017) ad hoc experiments, or used available experimental datasets (Brown et al., 1999; Callahan et al., 2013). Some also scaled the MT parameters using subject-specific anthropometric measurements and generic literature data (Cheng et al., 2000; Menegaldo & Oliveira, 2009; Wang, J. et al., 2012; Dick et al., 2017). In this approach, recent datasets obtained from large populations of young adults and recent experimental techniques should be preferred for estimating the FG optimal length (Ward et al., 2009) and the muscle maximum isometric force (Handsfield et al.,

2014), as performed previously (Rajagopal et al., 2016), to smaller dated datasets (Wickiewicz et al., 1983; Delp, 1990) obtained from specimens of advanced age that present important age-related muscle atrophy and smaller PCSAs (Young et al., 1984; Klein et al., 2001; Thelen, 2003; Hasson & Caldwell, 2011). Finally, close to half of the eligible studies used generic data from the literature to determine these parameters.

2.4.5. Principles of model validation

The performance of a muscle Hill-type model can be evaluated by comparing its predictions against experimental quantities obtained in situ or from complete experimental datasets made available in the literature (Perreault et al., 2003; Wakeling et al., 2021). The gold standard quantity for model validation is the time-course of individual fibre or muscle forces, which can be obtained or derived from dissected muscles of anesthetized animals as in some of the eligible studies, from non-invasive ultrasound measurements of fascicle or tendon length in humans (Lichtwark et al., 2007; Dick et al., 2017; Monte et al., 2020; Zhang et al., 2021; Zhang et al., 2022), or from specific protocols that minimize muscle co-activation during joint torque measurements (Hatze, 1981). Since the latter methods are relatively complex, most models are validated by comparing experimental and predicted joint torques, also often used for model calibration for the same activity, joint angles, and neural EMG-like signals, as discussed in the Results Section and detailed in Appendix A.12, despite limitations in validation accuracy as simulated and experimental quantities accumulate errors independent from muscle contraction mechanisms. In all cases, different experimental datasets must be used for model calibration and validation. Finally, the validation of model performance should be quantified with mathematical metrics (see Appendix A.12 for details related to the eligible studies) to prevent qualitative assessments and bias and improve readability. These important considerations were overlooked by many eligible studies (Figure 2-8B, Figure 2-9).

Once validated, a model may be implemented in other studies providing its performance is objectively considered in light of the modelling choices taken and the physiological plausibility of the calibrated parameter values. Most eligible studies overlooked such practices, as reported by the modelling assessment (Figure 2-8B Q3 and 6 to 8). Model reusability is also promoted when published results can be reproduced, which is possible when the model can be fully reimplemented, either with an open-source implementation of the model, or from a detailed description in the text of the modelled properties, rheological arrangement of the elements, parameter numerical values and methods for solving the muscle dynamics, most of which the eligible studies failed to provide (Appendix A.12 and Figure 2-8B Q4 and 5).

2.4.6. Limitations of the review

This systematic review, despite following the PRISMA recommendations (see Appendix A.2), comes with some limitations related to the custom design of the eligibility criteria and scoring schemes.

By constraining the eligible models to the six EC in Table 2-2, milestone studies specialized in research areas interfacing with the modelling of MT properties in Hill-type models were overlooked, including for example advances in parameter estimation (Modenese et al., 2016; Falisse et al., 2016), volumetric muscle definitions (Woittiez et al., 1984; Modenese & Kohout, 2020), the optimization of computational runnability (Van Den Bogert et al., 2011; De Groote et al., 2016), MSK modelling (Delp et al., 2007; Rajagopal et al., 2016) and in-vivo neural inputs for motor control (Sartori et al., 2017; Thompson et al., 2018; Kapelner et al., 2020). Besides disregarding other types of muscle models (EC3), such as Huxley-type (Zahalak, 1981; Lemaire et al., 2016; Carriou et al., 2019) and continuum (Röhrle et al., 2012; Röhrle, Oliver et al., 2019; Gfrerer & Simeon, 2021) approaches, some key ‘innovative’ Hill-type models that met EC6 but did not include a NE (EC4) were also not considered for global scoring in this study; to complement the extensive results presented in this study for the 55 eligible studies, those 32 additional studies are reported in Appendix A.7 and assessed for completeness in Appendix A.8. Besides, although it provides a window on the state-of-the-art in Hill-type modelling, the application of EC6 biases the results towards concluding that the field of Hill-type modelling is populated with rather more complete Hill-type models (Figure 2-6, mean: 8/23) than the majority of models proposed in the literature, which are actually closer to baseline models (Figure 2-4) and would score 5/23 or less.

Besides the 23 properties considered in this study, some important neuromuscular properties, such as the titin dynamics, were not considered for completeness assessment, as they were never described in Hill-type models. Also, it is important to reiterate that the completeness assessment provides information about the complexity of a model and its capacity to describe specific muscle mechanisms but does not provide any indication on the quality of the modelling approach. Likewise, the outcomes of the modelling assessment do not provide any indication on the accuracy of the eligible model predictions. The choice of model complexity and number of parameters, a key topic in Hill-type modelling (Audu & Davy, 1985), could have been considered in this scoring scheme, beyond the results in Figure 2-7D. Although care was taken to achieve a balanced scoring approach, as discussed in the Methods Section for both the scoring schemes, some modelling and methodological approaches may be under-represented, like the choice of rheological structure or the use of the multiscale simplifications that only account for 2.5% of the global score each (Figure 2-10). Finally, if the 55 eligible models are representative enough of the field of Hill-type modelling to describe its state-of-the-art, they may not be representative of the methodological approaches globally taken in the literature

about model validation, reusability, modelling choices and strategy and calibration (Table 2-4). This reduced window may weaken the impact of some conclusions drawn from the Modelling Assessment in this Discussion Section, considering for example that some non-eligible studies would have reached high modelling scores (Siebert et al., 2008; Hasson & Caldwell, 2011).

2.4.7. Required recommendations in the field of Hill-type modelling

As shown in the chronological graph in Figure 2-7C, the state-of-the-art in Hill-type modelling seems to have plateaued since the 80s-90s when substantial modelling innovations occurred. It is notably observed that following the turn of the century modelling advances have focussed on describing discrete muscle properties and never build upon other contemporary innovations, as they keep relying on the 50-year-old gold standards, rather than challenging their important limitations, discussed previously. This tendency is typical of a dense field, for which the most recent narrative reviews (listed in Appendix A.7) date back 20 years or more, and where the transmission of knowledge is therefore limited. To permit innovation and incremental breakthroughs, Epstein (1998) stresses the importance of regular literature reviews to periodically clarify and summarize the field, update on its state-of-the-art and avoid the emergence of detrimental inclinations that further aggravate the situation. One of them is the increasing inconsistency observed between the eligible studies in terminology and notations referring to, for example, the multiscale FG length as optimal length, normalized length, maximum isometric force, tendon slack length, maximum force in eccentric contractions, but also in the definition of isometric contraction as constant muscle or muscle-tendon lengths. Another detrimental tendency is the chain of references between Hill-type studies breaking over time; in such case, inheritance diagrams (Appendix A.10) are necessary to trace the current modelling techniques back to their source studies. This unclear literature also pushes researchers towards reusing and not adapting the milestone studies from the 80s-90s with more recent available experimental data, as discussed previously. In this context, the lack of systematic good practices stressed by the modelling assessment (Figure 2-8B) further hinders the transmission of knowledge and the reusability of published models, as it is difficult to build upon a model that is not carefully validated and described, and for which no open-source implementation is available. In these conditions, researchers seem rather to opt for open-source ready-to-use models (Schutte et al., 1993; Thelen, 2003; Millard et al., 2013; John et al., 2013), as 20% of the studies obtained from the systematic search and respecting EC1 to EC3 since 2006 used the muscle models embedded in the simulation platform OpenSim (Delp et al., 2007; Seth et al., 2018). While these models are shown to make good and fairly accurate predictions for maximal shortening and sub-maximal lengthening contractions respectively, and motivate advances in fields related to muscle modelling, the studies using these models however do not contribute to advancing the Hill-type modelling field itself.

Besides regular reviews that clarify the field and summarize its state-of-the-art, the field of Hill-type modelling would strongly benefit from globally-accepted recommendations, as proposed in other related fields (ESB recommendations). These could propose (1) standard definitions, notations and terminology for the common variables and dynamics of Hill-type models with respect to the chosen multiscale approach and rheological structure. As done for MSK systems (Hicks et al., 2015), those recommendations could provide a checklist and advise on (2) some best methodological practices, including the choice of mathematical metrics and experimental data for model validation, stating the assumptions and limitations related to the multiscale and rheological approaches, choosing the model complexity and completeness with regards to the study aim, taking the best approach for scaling and calibrating the model, and discussing its strengths and weaknesses in light of other findings in the literature. These recommendations should (3) refer to the sensitivity analyses in the field that have identified the most important model parameters, for which datasets compiling published experimental data for different species, muscles and scales should be produced for an optimized scaling of the models. Finally, they should provide (4a) a checklist of the details to include in the main manuscript or appendix for the full re-implementation of the model including the rheological structure, the equations describing the modelled properties, and a table listing the parameter values describing these properties and scaling the model, and (4b) suggest to modellers to release open-source implementations of their models so that the results presented in their study can be reproduced.

2.4.8. How can this review be used for developing a novel neuromuscular model?

By describing the state-of-the-art in Hill-type muscle modelling, this review identified some important limitations in the field, that this thesis aims to address with the development of a MN-driven multi-MU neuromuscular model (see Figure 1-1B). This Chapter and its related appendix provide all the necessary tools to develop this novel neuromuscular model. Figure 2-6, Figure 2-9 and Figure 2-10 (more detailed versions in Appendix A.8 and Appendix A.11) identify the best available published Hill-type models for model completeness, validation, reusability, or calibration, and modelling choices and strategy, from which inspiration was drawn. Also, the modelling strategy was built upon the detailed reviews of the rheological structures proposed in the field, and of the main modelling approaches for properties ADC, AD, FVC, FVE, FVL, FVA, FL, FLA, PEE and SEE, which are proposed respectively in Appendix A.9, and in Appendix A.10, with lists of mathematical equations and inheritance diagrams.

Chapter 3

EXPERIMENTAL DATA FOR CONTROLLING, PERSONALISING, AND VALIDATING THE NEUROMUSCULAR MODEL

The author wishes to thank Dr. Luca Modenese for providing the subject-specific musculoskeletal model presented in Section 3.3.1.3. The author also wishes to thank Dr. Simon Avrillon for the collaborative work in writing Section 3.2 and for his help in processing the HDEMG data presented in that section.

Transition

The systematic review in Chapter 2 confirmed that no MN-driven multi-MU neuromuscular model driven by individual MN discharges exists in the literature. This Chapter 3 describes how a maximal number of experimental MN spike trains were obtained from HDEMG signals, to empirically control in forward simulations the individual Hill-type MUs constituting the model. To validate and scale the muscle-tendon system, experimental joint torques and subject-specific MSK geometries were also obtained in this Chapter 3.

Chapter summary

This Chapter 3 fulfils the Objectives (2) and (3) listed in Section 1.2. In this Chapter 3, experimental MN spike trains and ankle dorsiflexion torques were simultaneously recorded during trapezoidal isometric contractions of the Tibialis Anterior (TA) muscle. The number of MNs identified from the HDEMG decomposition techniques was maximized to obtain highly reliable neural controls to the MN-driven muscle model introduced in Chapter 1. To do so, it was assessed the sensitivity of the output of the HDEMG decomposition to the density and size of the grids of electrodes. With large high-density grids of 256 electrodes, as many as 81 spike trains were recorded in the TA muscle, which is around three times the typical numbers obtained in the literature with classic 64-channel grids. Despite important improvements, the MN spike trains identified in this study cannot however entirely explain the neural drive to muscle, and further necessary refinements will be made in Chapter 5. Because of the importance of scaling and validating muscle models with subject-specific quantities, as underlined in Chapter 2, subject-specific musculoskeletal geometries were obtained from the manual segmentation of magnetic resonance imaging (MRI) data. The chapter starts with a description of the key mechanical properties of the MU population in a typical human TA muscle.

3.1. Introduction to the mechanical properties of the TA muscle

3.1.1. Anatomy

In humans, the TA muscle is one of the ten skeletal muscles that span the ankle joint. It originates along the lateral tibial condyle with a short and stiff tendon and inserts into the medial cuneiform and first metatarsal bones of the foot with a medium-compliant long tendon. Innervated by the deep fibular nerve, it is the dominant of the three muscles, including the extensor hallucis longus (EHL) and extensor digitorum longus (EDL) muscles, responsible for ankle dorsiflexion. Its average volume and maximum isometric force F_0^M are 100 cm^3 (Handsfield et al., 2014) and 1227 N (Rajagopal et al., 2016), respectively. Its two main antagonistic muscles are the tibialis posterior and the peroneus longus. The transmission of TA force to the skeletal system allows important daily activities like walking, for example, as it makes it possible the swing phase of the gait stride and stabilizes the ankle during heel strike. The TA muscle is one of the few muscles in the human body to be surrounded on one side by a bone (the tibia) and not by an adjacent muscle, thus reducing crosstalk issues in EMG measurements. The skin-fat layer that covers the TA muscle is typically thin. For these reasons and others, the TA muscle is one of the most common investigated human muscles in HDEMg protocols and is the muscle investigated in this thesis.

3.1.2. Properties of the TA MU pool according to the literature

In this section, properties specific to the MU pool of the human TA muscle are derived from the literature. These generic properties will scale the pool of individual Hill-type MUs that constitute the MN-driven model introduced in Chapter 1.

3.1.2.1. Size of the MU pool in a human TA muscle

The typical number N of MUs was estimated for the TA muscle from cadaveric studies (Feinstein et al., 1955), statistical methods (Trojaborg et al., 2002), decomposed-enhanced spike-triggered-averaging (DESTA) approaches (Van Cutsem et al., 1997; McNeil et al., 2005; Boe et al., 2009; Power et al., 2010; Hourigan et al., 2015; Piasecki et al., 2016), and adapted multiple point stimulation methods (Xiong et al., 2008) in 20-80-year-old human subjects. Because of method-specific limitations (Gooch et al., 2014), results across methods varied substantially, with estimates for N of, respectively, 445, 194, 190, 188 and 300 MUs for the TA muscle. DESTA methods systematically underestimate the innervation ratio due to the limited muscle volume covered by the surface electrodes. Cadaveric approaches rely on samples of small size and arbitrarily distinguish alpha from gamma MNs. Twitch torque measurements are an indirect method for estimating N . Accounting for these limitations, it is

taken in the following the potentially conservative $N = 400$ MUs in a typical adult TA muscle. Assuming 200,000 fibres in the TA muscle (Henriksson-Larsén et al., 1983; Henriksson-Larsén, 1985), $N = 400$ yields a mean of 500 fibres per TA MU, consistently with previous findings (Henriksson-Larsén, 1985).

3.1.2.2. Distribution of MU force recruitment thresholds in the MU pool

Three studies measured in the human TA muscle *in vivo* the force thresholds F^{th} at which the MUs are recruited, given as a percentage of the maximum voluntary contraction (% MVC) force, for 528 (Van Cutsem et al., 1997), 256 (Van Cutsem et al., 1998), and 302 (Feiereisen et al., 1997) MUs. Other studies investigated TA MU pools but reported small population sizes (Desmedt & Godaux, 1977) and/or did not report the recruitment thresholds (Andreassen & Arendt-Nielsen, 1987; Vander Linden et al., 1991; Connelly et al., 1999).

The scatter plot in Figure 3 in Van Cutsem et al. (1997) was digitized using the online tool WebPlotDigitizer (Ankit, 2020). The normalized MU population was partitioned into 10%-ranges of the values of F^{th} (in % MVC), as reported in (Feiereisen et al., 1997; Van Cutsem et al., 1998). The distributions obtained from these three studies were averaged. The normalized frequency distribution by 10% MVC-ranges of the F^{th} quantities hence obtained was mapped to a pool of N MUs, providing a step function relating each j^{th} MU in the MU population to its 10%-range in F^{th} . This step function was least-squares fitted by a linear-exponential trendline (EQ. 3-1) displayed in Figure 3-1A, providing a continuous frequency distribution of TA MU recruitment thresholds in a MU pool of the form $F^{th}(x) = ax + bc^{(x^d)}$ that reproduces the available literature data. In this equation, j is a real number between 0 and 1 expressing the normalized index of a MU in the pool of MUs ranked in the ascending order of force recruitment thresholds.

$$F^{th}(j) = 0.50 \cdot \left(58.12 \cdot \frac{j}{N} + \Delta_F \left(\frac{j}{N} \right)^{1.83} \right), j \in \llbracket 1; N \rrbracket \quad (\text{EQ. 3-1})$$

Simpler trendlines, such as $F^{th}(j) = k_1 \cdot \Delta_F \left(\frac{j}{N} \right)^{k_3}$ (Fuglevand et al., 1993), returned fits of lower r^2 values. According to the three studies and to (Heckman & Enoka, 2012), a $\Delta_F = 120$ -fold range in F^{th} was set for the TA muscle, yielding $F^{th}(N) = 90\% \text{ MVC} = \Delta_F \cdot F^{th}(1)$, with $F^{th}(1) = 0.75\% \text{ MVC}$. With this distribution, 231 TA MNs, i.e. 58% of the MN pool is recruited below 20% MVC, as identified

with blacked dashed lines in Figure 3-1A, which is consistent with previous conclusions (Heckman & Enoka, 2012).

3.1.2.3. Approximation of the distribution of MU tetanic forces in the MU pool

MU twitch forces f^{tw}

Following the same approach, a typical continuous distribution of the MU twitch force normalized to the muscle maximum isometric force $\overline{f^{tw}}$ in the pool of N TA MUs was obtained in (EQ. 3-2) from published experimental TA data (Andreassen & Arendt-Nielsen, 1987; Van Cutsem et al., 1997; Van Cutsem et al., 1998). A recent review (Duchateau & Enoka, 2022) suggests from Figure 3a in Van Cutsem et al. (1997), where the non-normalized data from ten subjects are merged, that a 250-fold range exists for the f^{tw} values in a MU pool. Here, it is rather considered a 16.3-fold range for the f^{tw} values, as displayed in Figure 3a in Van Cutsem et al. (1997) for a single subject. In this equation, i is an integer between 0 and N expressing the index of a MU in the pool of MUs ranked in the ascending order of twitch forces.

$$\overline{f^{tw}}(i) = 6.07 \cdot \left(4.52 \cdot \frac{i}{N} + 11.96 \left(\frac{i}{N} \right)^{4.66} \right), \quad i \in \llbracket 1; N \rrbracket \quad (\text{EQ. 3-2})$$

MU maximum isometric forces f_0^{MU}

The Hill-type MU models in the MN-driven model introduced in Chapter 1 must be scaled by their specific MU maximum tetanic forces f_0^{MU} . However, these were never measured in human muscles, and the normalized $\overline{f_0^{MU}}(i)$ continuous distribution in (EQ. 3-3) was here approximated from the $\overline{f^{tw}}$ distribution in (EQ. 3-2). To do so, a 11-fold range was set for the $\overline{f_0^{MU}}$ values in the MU pool, consistently with twitch-tetanus ratios obtained for slow and fast fibres in the rodent literature (Celichowski & Grottel, 1993). The normalized $\overline{f_0^{MU}}(i)$ distribution was finally scaled to Newton units, as displayed in Figure 3-1B, under the constraint $F_0^M = \sum_{i=1}^N f_0^{MU}(i)$, where F_0^M is the subject-specific value of TA maximum isometric force derived in the later section 3.3.1.4 and reported in Table 3-2.

$$\overline{f_0^{MU}}(i) = 0.82 \cdot \left(3.00 \cdot \frac{i}{N} + 8.20 \left(\frac{i}{N} \right)^{5.29} \right), \quad i \in \llbracket 1; N \rrbracket \quad (\text{EQ. 3-3})$$

According to the Henneman's size principle (Henneman, 1957; Henneman et al., 1965a; Henneman et al., 1965b; Henneman, 1981; Henneman, 1985) and the discussion in Chapter 4, it is admitted in the

following that the MUs in a MU pool are ranked in the same order of force threshold and maximum isometric forces (EQ. 3-4).

$$\forall j, k \in \llbracket 1; N \rrbracket, j < k \Leftrightarrow F^{th}(j) < F^{th}(k) \Leftrightarrow f_0^{MU}(j) < f_0^{MU}(k) \quad (\text{EQ. 3-4})$$

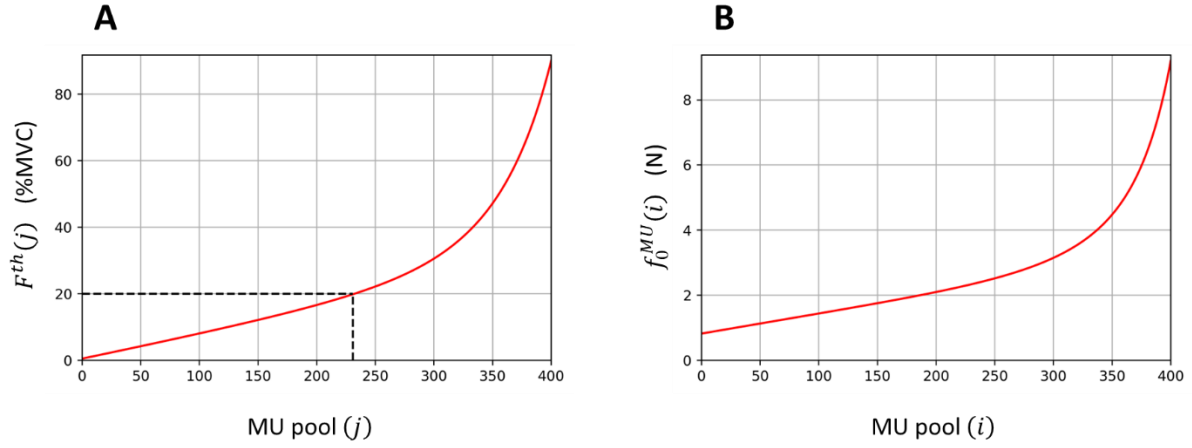


Figure 3-1: Distribution according to the literature of the MU-specific force recruitment thresholds $F^{th}(j)$ (A) and maximum isometric forces $f_0^{MU}(i)$ (B) in a pool of $N = 400$ MUs constituting a typical human TA muscle. For reasons given in the text, the MUs are assumed to be ranked in the same order in both plots, from low-threshold low-force MUs ($i = j = 1$) to high-threshold high-force MUs ($i = j = 400$). The dashed line (A) identifies the portion of the MU pool, i.e., the 231 lowest-threshold MUs of the MU pool, theoretically recruited at 20%MVC. The $f_0^{MU}(i)$ distribution was scaled to the subject-specific F_0^M value in Table 3-2.

3.2. MN spike trains from decomposed HDEMG signals: a size-density sensitivity analysis

3.2.1. Introduction

Experimental individual MN spike trains are required to control the individual Hill-type MUs constituting the neuromuscular model introduced in Chapter 1 and developed in this thesis. As opposed to bipolar surface EMG recordings, which lump the individual MU discharge activities into a single signal usually assimilated as the neural drive to muscle, the decomposition of HDEMG or intramuscular EMG signals (Holobar & Farina, 2014; Negro, Muceli et al., 2016) allows the in vivo decoding in human muscles of the firing activity of individual active MNs during voluntary contractions. HDEMG decomposition techniques provide a direct window on the internal dynamics of MN pools and have recently advanced our physiological understanding of the neurophysiology of human MU pools and of the interplay between the central nervous system and the muscle contractile machinery (Del Vecchio et al., 2019; Oliveira & Negro, 2021). Yet, HDEMG decomposition techniques only identify the activity of small portions of the active MU pool. High-yield decomposition commonly detects with 64 EMG electrodes 5-25MNs, up to 30-40 MNs in the TA muscle (Del Vecchio et al., 2020), i.e., less than 10% of the pool according to Section 3.1.2.1. As discussed later in Section 3.2.4, the small samples of MN spike trains identified with HDEMGs are typically not representative of the continuous distribution of the MN electrophysiological properties in the complete MN pool with a bias towards the identification of mainly high-threshold MUs. Importantly, the few identified spike trains cannot accurately estimate the true neural drive to muscle; the small samples of HDEMG-recorded MN spike trains typically obtained in the literature are consequently not reliable for controlling MU-scale neuromuscular models.

Here, it is shown that it is possible to identify more MNs, up to 81 for one subject, than typically proposed in the literature by increasing the number of EMG electrodes covering the TA muscle. Using seven surface grids of different size and electrode density involving 19 to 256 electrodes, it is concluded that the number and the quality of the identified spike trains, the relative number of ‘small’ identified MNs, and the accuracy of the experimentally estimated neural drive all increase with increasing grid size and density. However, it is shown that, despite improvements, the experimental spike trains alone remain inaccurate indicators of the real neural drive to muscle.

3.2.2. Methods

3.2.2.1. Experimental setup

Participants

Six healthy participants (age: 27 ± 3 years; height: 175 ± 8 cm; bodyweight: 68 ± 15 kg) volunteered to participate in the study. To maximise the number of identified MUs, only lean male subjects were considered. They had no history of lower limb injury or pain during the months preceding the experimental session. The Ethics Committee at Imperial College London reviewed and approve all procedures and protocols. All participants provided their written informed consent before the beginning of the experiment.

Experimental tasks

The experimental session consisted of a series of isometric ankle dorsiflexion performed at 30% and 50% of the maximal voluntary torque (MVC) during which HDEMG signals were recorded over the TA muscle. The participants sat on a massage table with the hips flexed at 30° , 0° being the hip neutral position, and the knees fully extended. The foot of the dominant leg (i.e., right leg for all participants) was fixed onto the pedal of a commercial dynamometer (OT Bioelettronica, Turin, Italy) positioned at 30° in the plantarflexion direction, 0° being the foot perpendicular to the shank. The thigh was fixed to the massage table with an inextensible three-centimetre-wide Velcro strap. The foot was fixed to the pedal with inextensible straps positioned around the proximal phalanx, metatarsal and cuneiform. Force signals $F^{transd}(t)$ were recorded with a force transducer (CCT Transducer s.a.s, Turin, Italy) in-series connected to the pedal using the same acquisition system as for the HDEMG recordings (EMG-Quattrocento; OT Bioelettronica). The dynamometer was positioned accordingly to the participant's lower limb length and secured to the massage table to avoid any motion during the contractions.

All experiments began with a warm-up, consisting of brief and sustained ankle dorsiflexion performed at what they considered, subjectively, 50% and 80% of their MVC. During the warm-up, all participants learnt to produce isometric ankle dorsiflexion without co-contracting the other muscles crossing the hip and knee joints. At the same time, the tightening and the position of the straps were iteratively adjusted to maximize the comfort of the participant. Then, each participant performed two 3-to-5s MVC with 120s of rest in between. The peak force value was calculated using a 250ms moving average window, and then used to set the target level during the submaximal contractions. After 2 min of rest, each participant performed two trapezoidal contractions at 30% and 50% MVC with 2 min of rest in between, consisting of linear ramps up and down performed at 5% MVC/s and a plateau maintained

for 20s and 15s at 30% and 50% MVC, respectively (Figure 3-2). The order of the contractions was randomized.

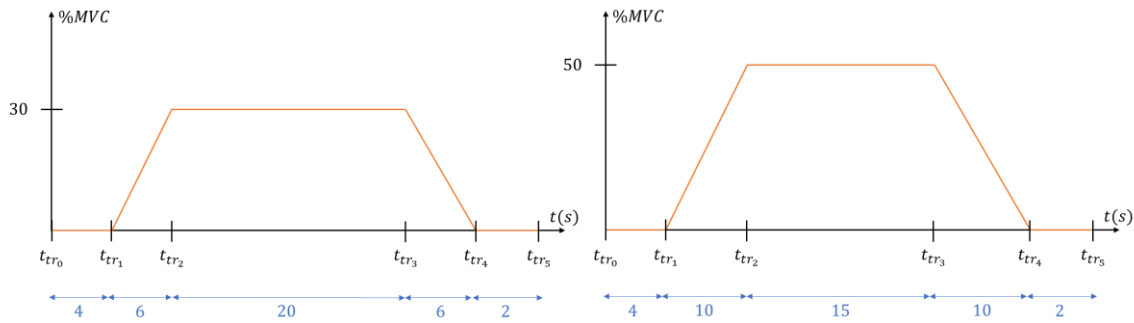


Figure 3-2: Trapezoidal contractions up to 30% MVC (left) and 50% MVC (right) performed by the participants. The ramps were performed at 5% MVC/sec.

HDEMG

Four adhesive grids of 64 electrodes (13 x 5; gold coated; 1-mm diameter; 4-mm interelectrode distance; OT Bioelettronica) were placed over the belly of the TA. The grids were carefully positioned side-to-side with a 4 mm-distance between the electrodes at the edges of adjacent grids (Figure 3-3). The 256 channels were centred to the muscle belly and laid within the muscle perimeter identified through palpation. The skin was shaved, abraded and cleansed with 70% ethyl alcohol. Electrode-to-skin contact was maintained with a biadhesive perforated foam layer filled with conductive paste. The grids were wrapped with tape and elastic bands to secure the contact with the skin. The four 64- pre-amplifiers were connected in-series with stackable cables to a wet reference band placed above the medial malleolus of the same leg. HD-EMG signals were recorded in monopolar mode with a sampling frequency of 2,048 Hz, amplified (x150), band-pass filtered (10–500 Hz), and digitised using a 400 channels acquisition system with a 16-bit resolution (EMG-Quattrocento; OT Bioelettronica).

3.2.2.2. Description of the channel configurations

The myoelectric activity of the TA was recorded with a total of 256 electrodes covering 36 cm² of the muscle surface (10 cm x 3.6 cm, 4 mm interelectrode distance, Figure 3-3A). To investigate the effect of the electrode density, rows and columns of electrodes were successively discarded from analysis to artificially generate grids covering the same area with interelectrode distances of 8 mm, 12 mm, and 16 mm. This led to grids of 256, 64, 35, and 20 channels for interelectrode distances of 4 mm, 8 mm, 12 mm, and 16 mm, respectively (Figure 3-3B-D). It is noteworthy that the 8 mm and 16 mm grids covered a surface of 32cm² because they include an odd number of rows and columns. To investigate the effect of the size of the grid, peripheral electrodes were removed to generate grids of 63, 34 and 19 channels of 4 mm interelectrode distance covering 7.7, 3.8 and 2 cm² of the muscle surface (Figure 3-3E-G). Note that these grid sizes were chosen to match the number of electrodes used in the density

analysis, thus making it possible to compare grids with the same number of electrodes but different density and size (in Figure 3-3, B versus E, C versus F, and D versus G).

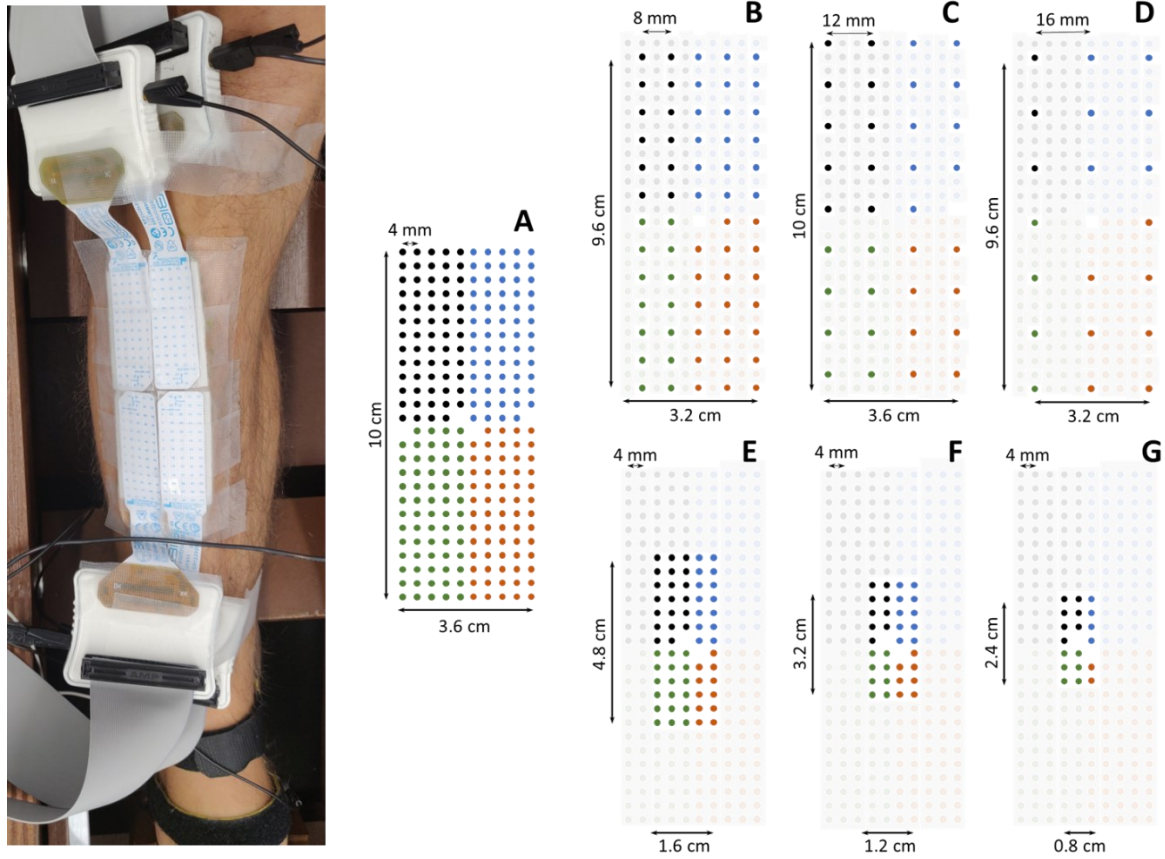


Figure 3-3: (Left) Experimental setup involving the four 64-channel HDEMG grids. (Right) The seven grid configurations considered in this study. All child grids (B-H) were obtained from the parent grid of 256 channels (A, grid size: 36cm^2 , inter-electrode distance: 4 mm) by discarding the relevant channels. The child grids consist in three 36cm^2 shallower grids of inter-electrode distance 8 mm (B), 12 mm (C), and 16 mm (D), three 4 mm-dense smaller grids of grid size 7.7cm^2 (E), 3.6cm^2 (F), and 2cm^2 (G).

3.2.2.3. HDEMG decomposition

All the conditions were decomposed using the same parameters and procedure using a Matlab implementation developed by Dr. Avrillon of a convolutive blind-source separation approach previously described (Negro, Muceli et al., 2016). It is noteworthy that the value of some parameters (number of iterations, SIL threshold, number of extended channels) was iteratively refined to optimize the number of outputs. First, the monopolar EMG signals were bandpass filtered between 20-500 Hz with a second-order Butterworth filter in Matlab. After visual inspection, channels with low signal-to-noise ratio or artifacts were discarded for all the conditions. The HDEMG signals were then decomposed into MU spike trains using convolutive blind-source separation, as previously described (Negro, Muceli et al., 2016). In short, the EMG signals were first extended to reach 1000 channels and

whitened, also referred as convolutive sphering of the signals (Thomas et al., 2006). Thereafter, a fixed-point algorithm that maximized the sparsity was applied to identify the sources embedded in the EMG signals, i.e., the MU spike trains. MU spike trains can be considered as sparse sources with most samples being 0 (i.e., absence of spikes) and a few samples being 1 (i.e., spikes). In this algorithm, a contrast function was iteratively applied to the EMG signals to estimate the level of sparsity of the identified source, and the convergence was reached once the level of sparsity did not vary when compared to the previous iteration, with a tolerance fixed at 10^{-4} . Although square contrast functions converge faster to identified MUs, a logcosh contrast function was here preferred to better track the low-threshold MNs (see Holobar et al. (2008) and Negro, et al. (2016) for the definition of the detailed contrast functions). At this stage, the estimated source contained high peaks (i.e., the spikes from the identified MU) and low peaks from other MUs and noise. High peaks were separated from low peaks and noise using peak detection and K-mean classification with two classes. The peaks from the class with the highest centroid were considered as the spikes of the identified MU. In order to maximize the number of identified MUs, a large number of iterations (600) was allowed to the algorithm and a low Safety Integrity Level (SIL) of 0.5 was set. A subsequent analysis of the effect of the number of iterations on the number of identified MNs revealed that only few, i.e., zero to three additional MUs were identified after the 250th iteration for Subjects S1 and S4. A second algorithm, used sequentially, refined the estimation of the discharge times by iteratively recalculating the MU filter and repeating the steps with peak detection and K-mean classification until the coefficient of variation of the inter-spike intervals was minimized. It was previously observed that this second iterative procedure improved the estimation of the MU discharge trains by preventing the algorithm from converging to unreliable estimates, favouring the assumption of regular discharge patterns (Negro et al., 2016). This decomposition procedure has been previously validated using experimental and simulated signals (Negro et al., 2016). After the automatic identification of the MUs, duplicates were removed, and all the MU spike trains were visually checked for false positives and false negatives (Del Vecchio et al., 2020). This manual step is highly reliable across operators (Hug et al., 2021). Only the MUs which exhibited a pulse-to-noise ratio (PNR) > 28 dB (Holobar et al., 2014) were retained for further analysis.

It is noteworthy that the four grids of 64 electrodes were also decomposed separately before removing the duplicates between grids. The idea behind this analysis was to test whether decomposing subsets of electrodes within a dense grid may increase the number of identified MUs.

3.2.2.4. Evaluation metrics

The number of identified MUs and their PNR was first reported as box plots providing median, interquartile distance, 95%-range, and outliers across participants for each grid configuration. In the text, the coefficient of variation (CoV), calculated as the standard deviation divided by the mean, is

also used. For each participant, the number of identified MUs was then normalized to the maximal number of MUs found across all grid configurations, yielding normalized numbers of identified MUs $\bar{N}$ in %. For each condition, the mean and standard deviation of the $\bar{N}$ values across participants was calculated. To investigate the effects of density and size of the grid, logarithmic trendlines of the form $y = a + b \cdot \log(x)$ were fitted to the relationships between the averaged $\bar{N}$ values and interelectrode distance and grid size. A logarithmic trendline was also fitted to the average $\bar{N}$ values and their corresponding number of channels, in which case the conditions involving the same number of electrodes, but different grid size and density were given a weight of 0.5 in the cost function of the trendline. The r-squared for each regression trendline was reported. To infer the type of MU dominantly identified in each condition, a normalized version (EQ. 3-5) of the continuous F^{th} distribution, given in (EQ. 3-1) and reproduced here, was used. In this equation, j is a real number between 0 and 1 expressing the normalized index of a MU in the pool of MUs ranked in the ascending order of force recruitment thresholds.

$$F^{th}(j) = 0.50 \cdot (58.12 \cdot j + 120j^{1.83}), j \in [0; 1] \quad (\text{EQ. 3-5})$$

This relationship suggests that 75% and 89% of the MU pool is active at 30% and 50% MVC respectively. Based on their measured force recruitment threshold, the identified MUs were classified to the first ('small') or second ('large MNs') half of the active pool ranked in the ascending order of F^{th} .

3.2.2.5. Assessment of the neural drive to muscle from the experimental MN spike trains

In the following of this study, all signals and vectors of values are discrete and sampled at 2048 Hz. For each experimental spike train where the discharge times f_t were identified, the corresponding binary spike train $sp(t)$, was mathematically described (EQ. 3-6).

$$\begin{cases} sp(t = f_t) = 1 \\ sp(t \neq f_t) = 0 \end{cases} \quad (\text{EQ. 3-6})$$

For each condition and each subject, the cumulative spike train (CST) was obtained as the temporal binary summation of the binary spike trains $sp(t)$ obtained for this dataset (EQ. 3-7).

$$CST = \sum_i sp_i(t) \quad (\text{EQ. 3-7})$$

The effective neural drive (eND) to the muscle was estimated for the 36- 64- and 256-channel cases (conditions A, B, C, E, and F in Figure 3-3) by low-pass filtering the CST in the bandwidth $[0; 4]Hz$ relevant for force generation (Negro et al., 2016). The computed eND traces were normalized to the

average of the eND values during the plateau of force. Finally, the normalized eND were compared with calculation of the nRMSE and r^2 metrics against the normalized experimental force trace $\overline{F^{transd}}(t)$, which is an indicator of the true neural drive to muscle in isometric contractions (Farina & Negro, 2015).

In the following, the datasets obtained from different subjects, for difference force levels, and with different grid configurations were distinguished with specific names. For example, the set of spike trains obtained for the subject S1 during the 30% MVC trapezoidal contraction with the grid configuration B in Figure 3-3 was identified as ' $S1_{30}^B$ '. The 87 other possibilities were identified with the same type of notation. Furthermore for the configuration A, because the 256 recorded signals were either decomposed at once or independently grid-by-grid, these two approaches were distinguished as conditions ' AM ' and ' AI ', respectively.

3.2.3. Results

3.2.3.1. Preliminary results

For the six subjects, HDEMG signals and transducer force trajectories were successfully recorded for the two trapezoidal tasks (Figure 3-2), except for participant S2 at 50% MVC, and the seven grid configurations displayed in Figure 3-3 were successfully produced. For the subjects S1, S3, S5, and S6, less than 5% of the channels were discarded for their poor signal quality in all seven grid configurations at both contraction levels (except for S6, 10% removed channels from the 12 mm-grid (Figure 3-3C)). For subjects S2 and S4, 6 to 12% of the channels were discarded in 15 of the 24 grid configurations. The discarded signals were mainly recorded by edge electrodes close to the muscle contour and at the interface with adjacent grids, which sometimes pinched during muscle contraction because of a reduced muscle area. For subjects S1, S3, and S6, the average baseline noise across the non-discarded HDEMG channels was below the advised threshold of $10\mu V$ for optimal signal decomposition. Acceptable baseline noise of 14 to $20\mu V$ was obtained for the remaining participants.

3.2.3.2. Number of identified MNs

1788 MNs were identified across all conditions and intensities with PNR values of $34.6 \pm 2.2\text{dB}$. All the spike trains were visually checked and carefully edited when a missing spikes or an identified artifact were observed. The highest number of identified MNs, displayed in Figure 3-4, was reached with the separate decompositions of the four grids of 64 electrodes with inter-electrode distance of 4 mm, yielding 56 MNs (CoV = 0.25; PNR = 34.2 ± 1.1) and 45 MNs (CoV = 0.22; PNR = 34.0 ± 0.9) in average across participants at 30% MVC and 50% MVC, respectively (Table 3-1). 25% and 50% less MUs were obtained when the 256 channels were decomposed simultaneously. On average, an additional 50% MNs were identified at 30% MVC than at 50% MVC for the group of participants (197 vs 138), with ratios from 1.1 to 1.9 for individuals. For all participants, more MNs were also systematically identified at 30% MVC than at 50% MVC for the same grid configuration.

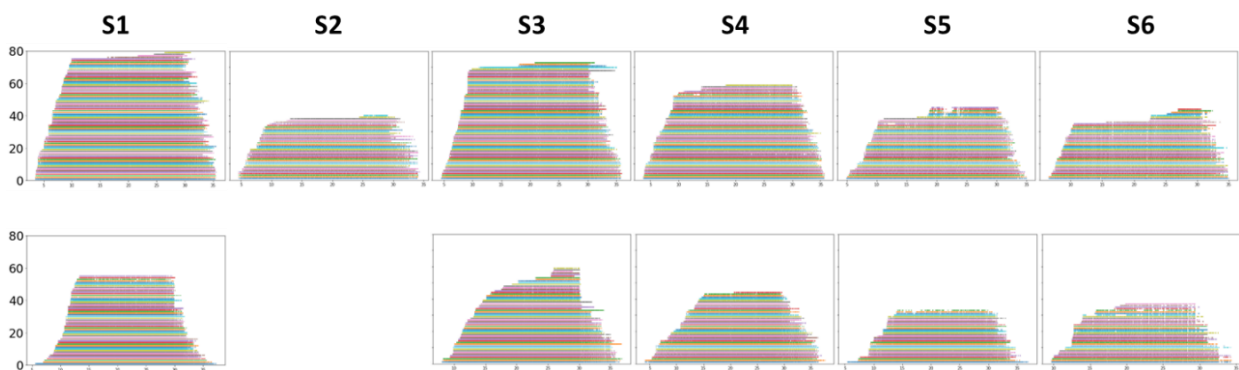


Figure 3-4: MN spike trains identified at 30% MVC (Top) and 50% MVC (Bottom) with the 'AI' condition for the six participants.

Table 3-1: Number *N* of identified MNs with the eight conditions (Figure 3-3) for the six participants S1-S6 at 30% and 50% MVC. As described in the Methods section, the signals recorded with the 256-channel configuration were processed in two different manners, either the four 64-channels grids were decomposed separately (AI), or the 256 channels were decomposed as a unique dataset (AM).

	30% MVC									50% MVC								
	AI	AM	B	C	D	E	F	G	Total	AI	AM	B	C	D	E	F	G	Total
S1	79	58	32	14	2	30	18	8	241	55	28	15	3	2	22	5	2	132
S2	40	38	32	21	6	18	11	4	170									
S3	73	51	30	16	6	31	15	6	228	59	36	26	11	3	24	13	6	178
S4	59	51	43	19	4	19	11	1	205	44	26	25	9	3	15	7	1	130
S5	45	37	23	7	2	21	14	4	153	33	18	16	6	3	13	9	5	103
S6	44	24	20	4	2	25	8	4	131	37	17	17	5	0	19	13	7	115
Mean	56	43	30	13	3	24	12	4	188	45	25	19	6	2	18	9	4	132
CoV	0.25	0.26	0.23	0.46	0.33	0.21	0.25	0.50	0.21	0.20	0.24	0.21	0.30	0.50	0.22	0.33	0.50	0.19

It is noteworthy that at least 82% of the MNs identified in any grid configuration were also identified in the conditions involving larger grid size and/or electrode density; 91-to-100% of the MNs obtained in the other conditions were also identified with the 256-channel configuration (4 mm interelectrode distance, 36 cm² size).

Sensitivity to interelectrode distance

When considering the effect of electrode density, the lowest number of MNs was found with 16 mm interelectrode distance, identifying 3 MNs (CoV = 0.33) and 2 MNs (CoV = 0.5) at 30% MVC and 50% MVC, respectively (Table 3-1). More MNs were gradually identified with higher electrode density (Figure 3-5AC). The highest number of MNs was observed with 4 mm interelectrode distance, respectively identifying 56 or 43 MNs (CoV = 0.25 or 0.26) and 45 or 25 MNs (CoV = 0.22 or 0.24) at 30% MVC and 50% MVC, depending on the decomposition procedure (Figure 3-5AC). Finally, a logarithmic relationship was found between the average normalized number of MNs for each participant and the interelectrode distances, with r-squared of 1.0 ($p = 2.5 \cdot 10^{-5}$) and r-squared of 0.99 ($p = 0.001$) at 30% MVC and 50% MVC, respectively (Figure 3-5BD).

Sensitivity to grid size

When considering the effect of the size of the grid (interelectrode distance fixed at 4 mm), the lowest number of MNs was found with a grid of 2 cm², identifying 4 MNs (CoV = 0.5) and 4 MNs (CoV = 0.5) at 30% MVC and 50% MVC, respectively (Table 3-1). More MNs were gradually identified with larger grid size (Figure 3-6AC). The highest number of MNs was observed with a grid of 36 cm², identifying 56 or 43 MNs (CoV = 0.25 or 0.26) and 45 or 25 MNs (CoV = 0.22 or 0.24) at 30% MVC and 50% MVC

respectively, depending on the decomposition procedure (Figure 3-6AC). A logarithmic relationship was also found between the average normalized number of MNs for each participant and the size of the grid, with r-squared of 0.99 ($p = 3.0 \cdot 10^{-4}$) and r-squared of 0.98 ($p = 0.001$) at 30% MVC and 50% MVC, respectively (Figure 3-6BD). It is noteworthy that, in both density and size cases, the parameters of the fits were very close at 30% MVC and 50% MVC.

Sensitivity to the number of channels

Because high inter-subject variability was observed (CoV values typically above 0.25 in Table 3-1), with for example twice more MUs identified in the Subject S1 than S6 in most conditions, N was normalized ($N \rightarrow \bar{N}$) to the maximum number of MNs identifiable in each subject. Except for the 64- and 35-channel conditions, $\bar{N}$ mostly showed low inter-subject variability (see standard deviation error bars in Figure 3-5BD and Figure 3-6BD). As both the density and the size of the grids impact the number of channels, the relationship between the normalized number of MNs $\bar{N}$ and the number of channels was finally fitted. As observed previously, a significant logarithmic relationship was observed with r-squared of 0.98 ($p = 0.018$) and r-squared of 0.95 ($p = 0.016$) at 30% MVC and 50% MVC, respectively (Figure 3-7). A plateau is expected from this logarithmic relationship to be reached with highly dense grids with 2mm and 1mm interelectrode distances, with a prediction of 50% and 90% of additional MNs identified. This plateauing tendency was confirmed with recently shared experimental results obtained with an ultra-dense grid of 400 dry electrodes (2 mm interelectrode distance) (Caillet et al., 2023). However, the redundancy of information between adjacent channels due to highly correlated motor unit action potentials limits the number of additional MNs identified. This was confirmed experimentally, as only 23% of additional MUs were identified with the grid of 2 mm interelectrode distance compared to the grid of 4 mm interelectrode distance (Caillet et al., 2023).

Interestingly, relatively more MNs were obtained with the 64-channel 'B' condition (large size, medium density, Figure 3-3B) than with the 63-channel 'E' condition (medium size, high density, Figure 3-3E) for the group of participants. This suggests that grid size, electrode density, and number of channels all three determine the number of identified MNs.

It must be noted that some of the results previously reported rely on boxplot analyses and significant trendline regressions. However, some of the conclusions previously reported on the preferable choice of grid configurations remain limited until an analysis of the statistical difference between these approaches is performed from the results of a larger pool of participants.

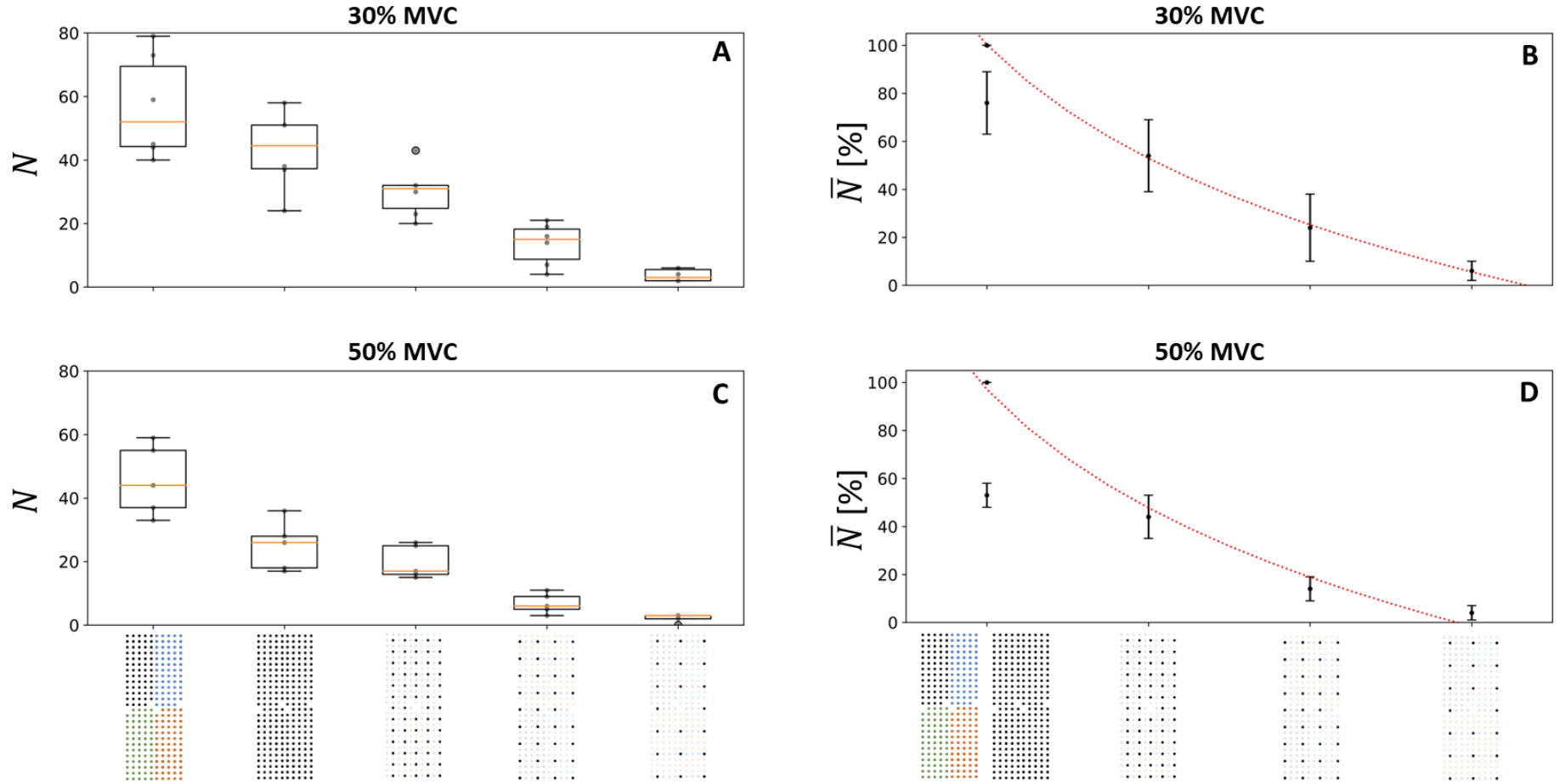


Figure 3-5: Effect of the electrode density on the number of identified MNs at 30% MVC (A,B) and 50% MVC (C,D). The boxplots in the left column report the absolute number N of identified MNs per participant (grey dots) and the median, quartiles, and 95%-range across participants. In the right column, the normalized number of MNs $\bar{N}$ logarithmically decreases with interelectrode distance d (4, 8, 12, and 16 mm in abscissa) as $\bar{N} = 195 - 68 \log(d)$ ($r^2 = 1.0, p = 2.5 \cdot 10^{-5}$) at 30% MVC (B) and $\bar{N} = 196 - 71 \log(d)$ ($r^2 = 0.99, p = 0.001$) at 50% MVC (D). The AI (not AM) configuration was used to produce the trendline. The standard deviation of $\bar{N}$ across subjects is displayed with vertical bars.

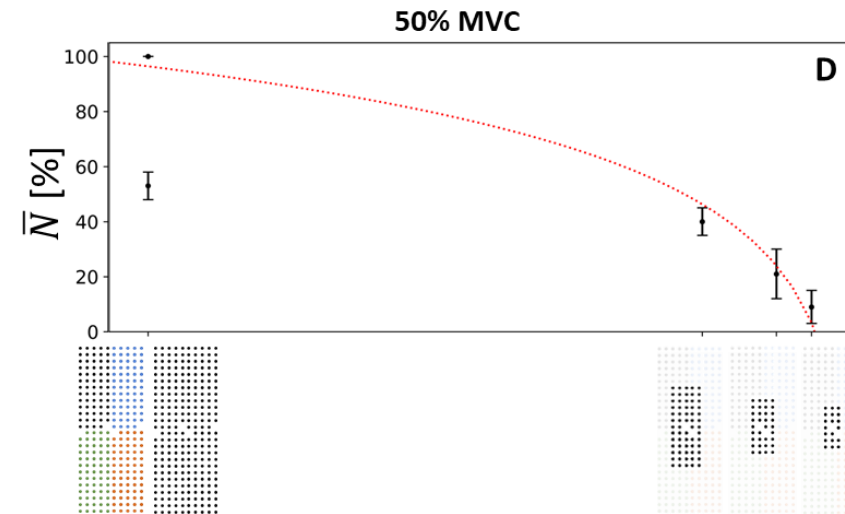
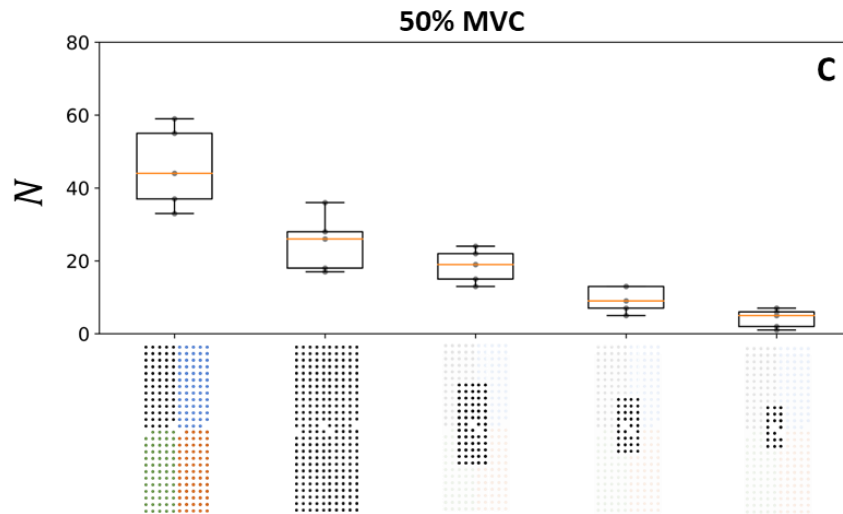
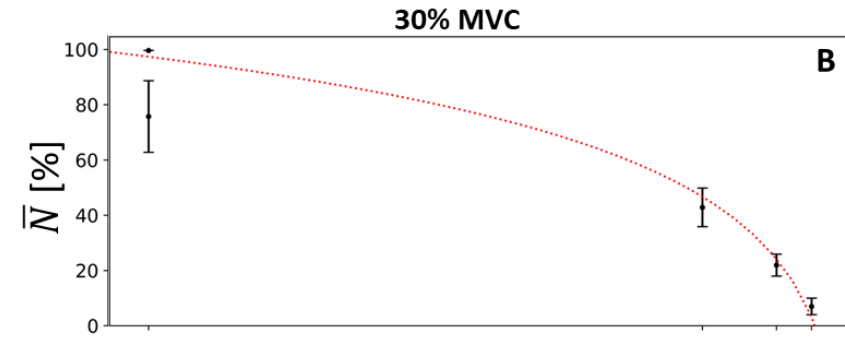
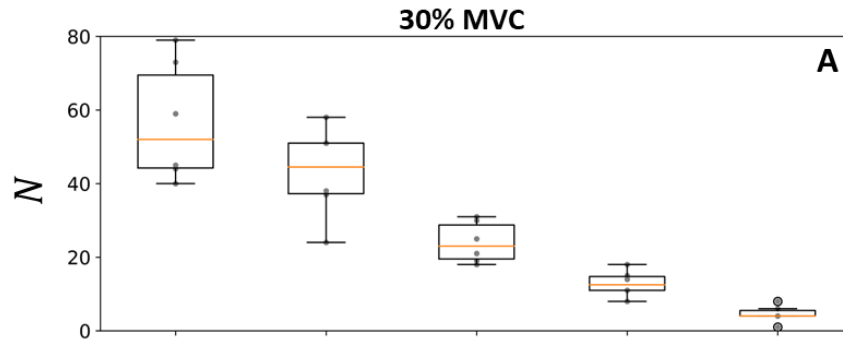


Figure 3-6: Effect of the size of the grid on the number of identified MNs at 30% MVC (A,B) and 50% MVC (C,D). The boxplots in the left column report the absolute number N of identified MNs per participant and the median, quartiles, and 95%-range across participants. In the right column, the normalized number of MNs $\bar{N}$ logarithmically decreases with the size of the grid s (2, 3.8, 7.7, and 36 cm² in abscissa) as $\bar{N} = -20 + 33 \log(s)$ ($r^2 = 0.99, p = 3.0 \cdot 10^{-4}$) at 30% MVC (B), and $\bar{N} = -19 + 32 \log(s)$ ($r^2 = 0.98, p = 0.001$) at 50% MVC (D). The standard deviation of $\bar{N}$ across subjects is displayed with vertical bars.

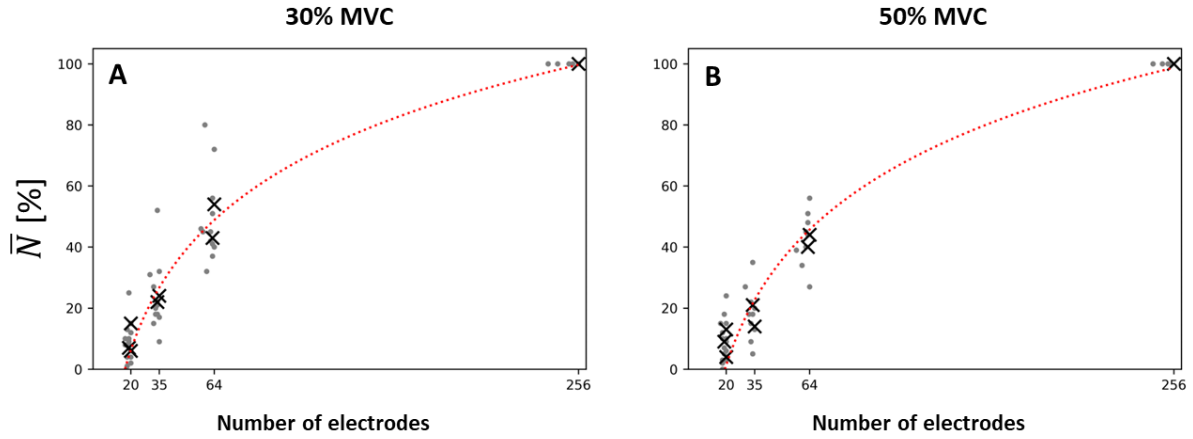


Figure 3-7: Effect of the number c of channels on the normalized number $\bar{N}$ of identified MNs at 30% (left) and 50% MVC (right). The discrete results per participant are displayed with grey data points. The average $\bar{N}$ values across participants per condition are displayed with black crosses. Weighted logarithmic trendlines were fitted to the data and returned (left) $\bar{N} = -104 + 37 \log(c)$ ($r^2 = 0.98, p = 0.018$), and (right) $\bar{N} = -113 + 38 \log(c)$ ($r^2 = 0.95, p = 0.016$).

3.2.3.3. Distribution of the type of identified MNs

The properties of the additional MNs identified with larger and denser grids were further investigated by computing the distribution of the MNs according to their type. Specifically, the MNs were classified in two ‘small’ and ‘large’ classes according to their recruitment threshold. A clear effect of the electrode density was first observed on this distribution at 30% MVC, with a percentage of small MNs increasing from 11% (CoV = 0.81) with 12 mm interelectrode distance to 29% (CoV = 0.48) with 4 mm interelectrode distance (Figure 3-8A). Such differences were not observed at 50% MVC, where the percentage of ‘small’ MNs stayed below 10% for all the conditions (Figure 3-8C). Contrary to the density, the size of the grid did not appear to impact the distribution of the type of the identified MNs. Similar values were observed between conditions, with the percentage of ‘small’ MNs ranging from 20% (CoV = 0.50) to 29% (CoV = 0.48) at 30% MVC (Figure 3-8B). Again, a low percentage of small MNs below 10% was observed at 50% MVC for all the conditions (Figure 3-8D). In all cases, the percentages of small MNs were not calculated, and not reported in Figure 3-8, when 10 or less MNs were identified. Supporting the conclusions made at 30% MVC that the type of identified MNs depends on density and not size, Figure 3-9 shows, in average across participants, that the MNs only identified in the E configuration (63 channels, 4 mm interelectrode distance, 7.7cm^2) are typically smaller than the MNs only identified with the B condition (64 channels, 8 mm interelectrode distance, 32cm^2).

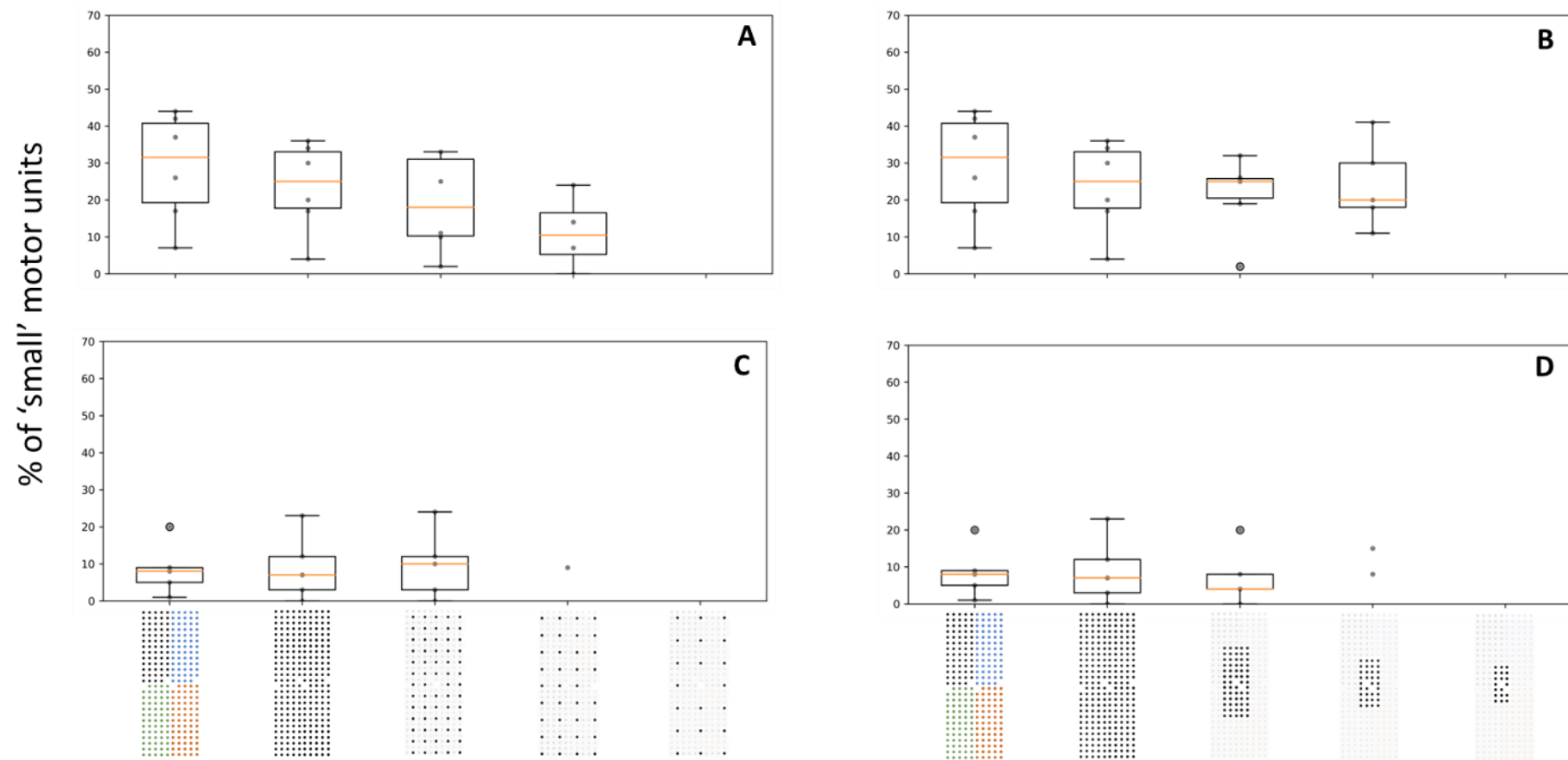


Figure 3-8: Effect of the electrode density (Left) and of the grid size (Right) on the percentage of 'small' MNs identified in the datasets at 30% MVC (Top) and 50% MVC (Bottom).

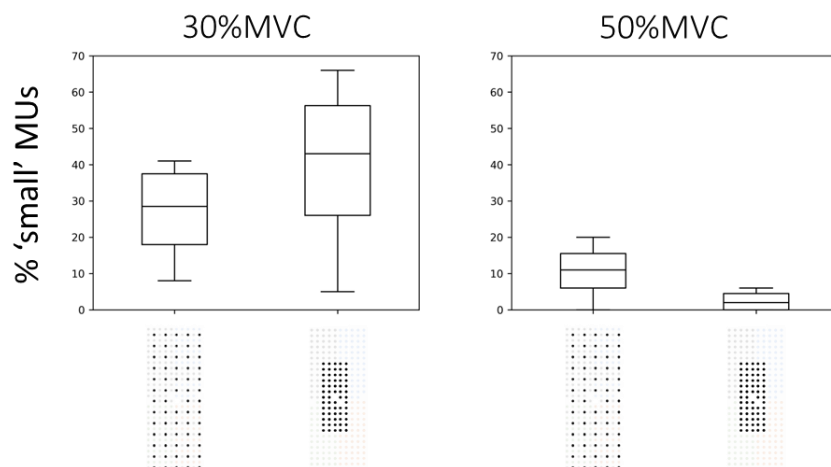


Figure 3-9: Comparison between the 64-channel and 63-channel grids, at 30% MVC and 50% MVC, of the proportion of ‘small MNs’ among the MNs identified only in one of the two grids. At 30% MVC, denser grids can identify relatively more ‘small’ MNs than larger but shallower grids of same number of channels.

3.2.3.4. Accuracy of the identified MN spike trains

The quality of HDEMG decomposition relies on the accuracy of the identified motor unit spike train, which is classically measured with the PNR. This accuracy will also dramatically impact the time needed for manually checking and editing the motor unit spike trains.

Sensitivity to electrode density

The PNR increased together with the density of the grid. In Figure 3-10AC, the lowest PNR values were observed with 16 mm interelectrode distance, i.e., 30 dB (CoV = 0.06) at 30% MVC and 29 dB (CoV = 0.05) at 50% MVC. The highest values of pulse-to-noise ratio were observed with 4 mm interelectrode distance and 256 electrodes, i.e., 36 dB (CoV = 0.02) at 30% MVC and 37 dB (CoV = 0.02) at 50% MVC, enabling the operators to quickly edit the identified MNs.

Sensitivity to grid size

Similar trends were observed in Figure 3-10BD with the effect of the size of the grid. The lowest pulse to noise ratios were observed with a grid of 2 cm² (31 dB (CoV = 0.03) at 30% MVC and 30 dB (CoV = 0.03) at 50% MVC). The highest pulse to noise ratios were observed with a grid of 36 cm² (36 dB (CoV = 0.02) at 30% MVC and 37 dB (CoV = 0.02) at 50% MVC). Across all conditions, high PNR values in the range 33-35 dB were obtained for the MNs only identified in the 256-channel condition (Figure 3-11AB).

Comparison of the 'AI' condition against the other configurations

The MN filters were finally recalculated in all the conditions using the discharge times from all the MNs identified in the 'AI' condition. The application of these filters enabled us to even evaluate the density and size effects on the PNRs from MNs non identified in the other conditions. When considering the effect of density, the pulse to noise ratio gradually dropped from 36 dB and 37 dB at 30% MVC and 50% MVC, respectively, with 4 mm inter electrode distance to 22 dB and 22 dB at 30% MVC and 50% MVC, respectively, with 16 mm inter electrode distance (Figure 3-11CD). When considering the effect of the size of the grid, the pulse to noise ratio gradually dropped from 36 dB and 37 dB at 30% MVC and 50% MVC, respectively, with a grid of 2 cm² to 19 dB and 20 dB at 30% MVC and 50% MVC, respectively, with a grid of 36 cm² (Figure 3-11CD). Interestingly, the MNs only identified in the AI condition at 30% MVC were dominantly 'small', suggesting that higher density, larger size, and larger number of electrodes increase the likelihood of identifying low-threshold MNs (Figure 3-11E).

3.2.3.5. Assessment of the neural drive to muscle from the experimental MN spike trains

The neural drive to muscle was estimated for the six subjects from the spike trains obtained with the AI, B, C, E, and F conditions of 256, 64, 35, 63, and 34 channels (Figure 3-3). Figure 3-12 compares for the $S1_{30}$, $S1_{50}$, and $S3_{30}$ datasets the normalized experimental force traces $\overline{F^{transd}}$ (black curves) against the computed eND (red dotted lines). The curves compare with $nRMSE$ and r^2 metrics, the average and standard deviation values of which across the six subjects at 30% MVC are reported in Figure 3-13. Figure 3-13 shows that the neural drive was best approximated in all three datasets with the AI condition, with average $r^2 = 0.96$ (0.02 standard deviation) and $nRMSE = 10\%$ (3% standard deviation). The accuracy of the neural drive approximations decreased with the number of channels, yielding $r^2 = 0.94$ (0.04 standard deviation) and $nRMSE = 16\%$ (4% standard deviation) for the condition C. Despite rather large inter-subject variability (Figure 3-13), similar tendencies were observed for the individual subjects. In more details, Figure 3-12 shows that an increasing number of channels decreases the noise of the estimated neural drive during the plateau of force, hence decreasing the $nRMSE$ values. Also, the neural drive was in all cases underestimated during the ramps of force, with increasing discrepancy when less channels and lower density were involved. Both these observations were accentuated at 50% MVC (Figure 3-12, $S1_{30}$ versus $S1_{50}$), where the onset of the neural drive is moreover wrongly estimated with up to six second onset error for 10s-long ramps. Interestingly, between two grids involving the same number of electrodes, the neural drive was more accurately predicted by the denser condition (Figure 3-13, B versus E, and C versus F).

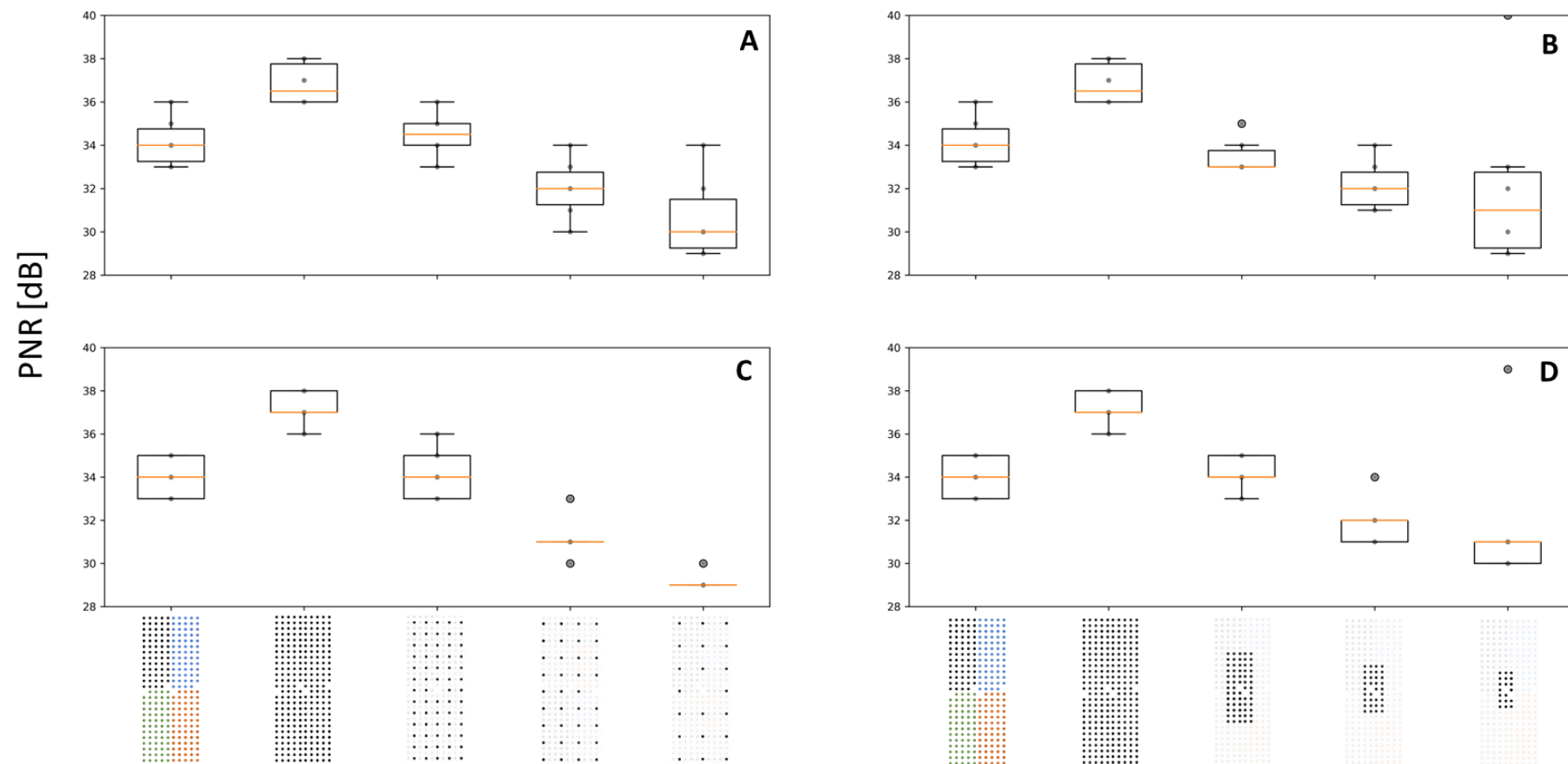


Figure 3-10 Effect of the electrode density (Left) and of the grid size (Right) on the PNR values at 30% MVC (Top) and 50% MVC (Bottom).

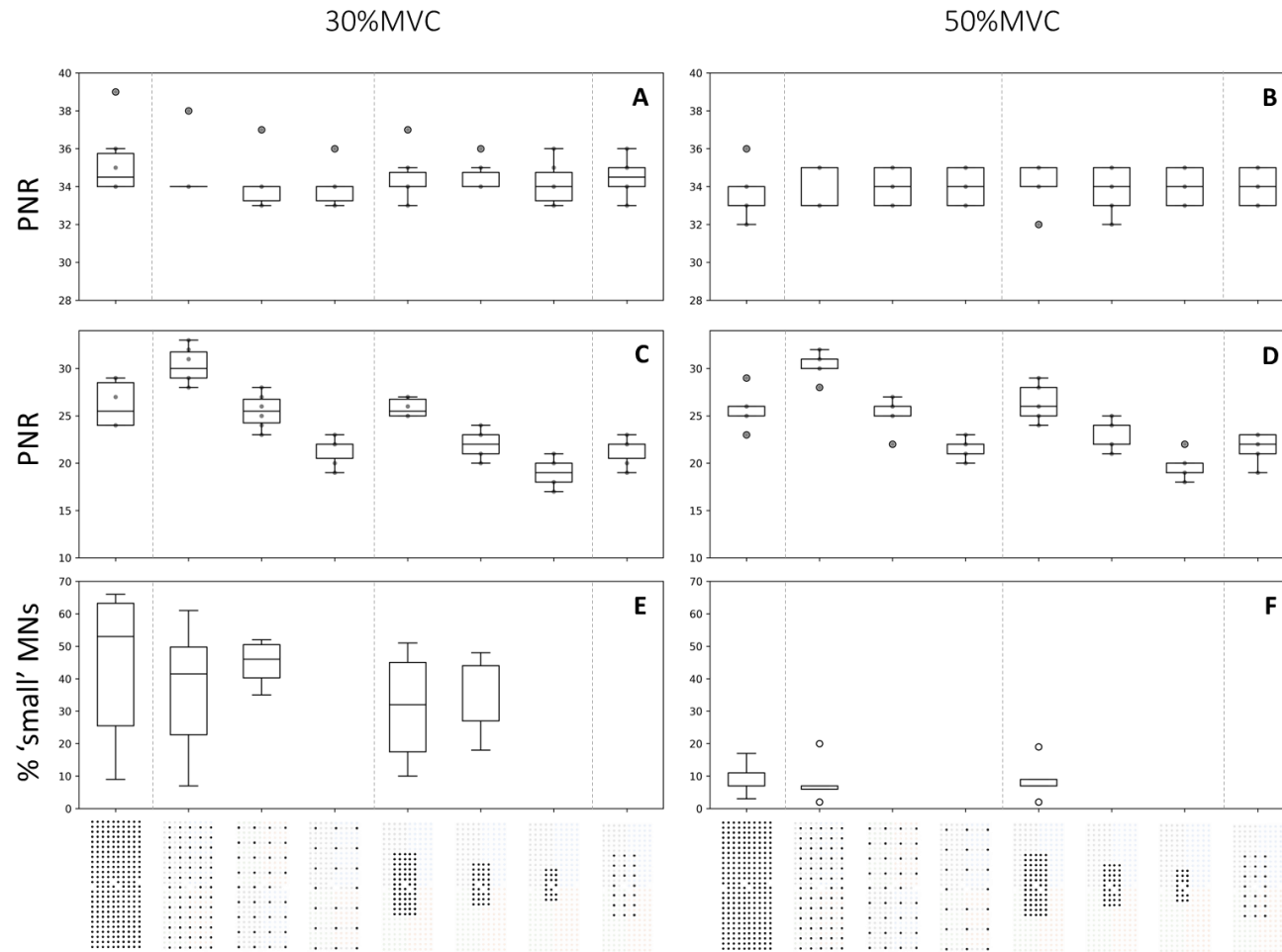


Figure 3-11: (A,B) PNR values of the spike trains only identified in the 'AI' configuration. (C,D) The MN filters were recalculated in all the conditions using the discharge times from all the MNs identified in the AI condition, and the recalculated PNR values are displayed. (E,F) Percentage of 'small' MNs among the MNs only identified with the 'AI' condition.

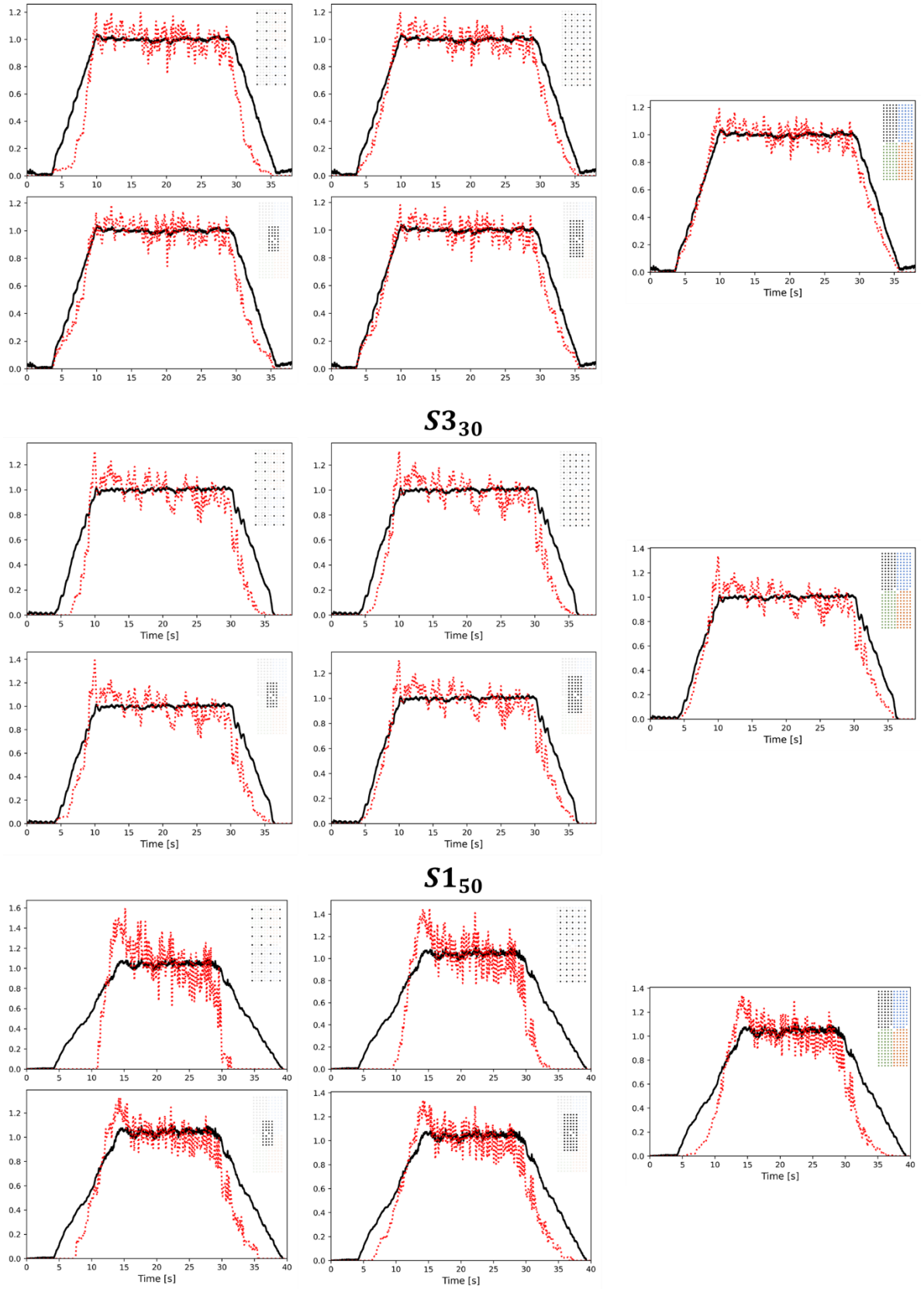


Figure 3-12: Comparison of the normalized recorded transducer forces (black traces) against the normalized neural drives (red traces) computed for the $S1_{30}$, $S3_{30}$, and $S1_{50}$ trials. For each trial, the neural drive was computed from the MN spike trains obtained with 256 EMG channels (right plots), 64 channels (middle plots), and 36 channels (left plots), where the large low-density grids were used for the top plots, and the smaller higher-density grids were used for the bottom plots.

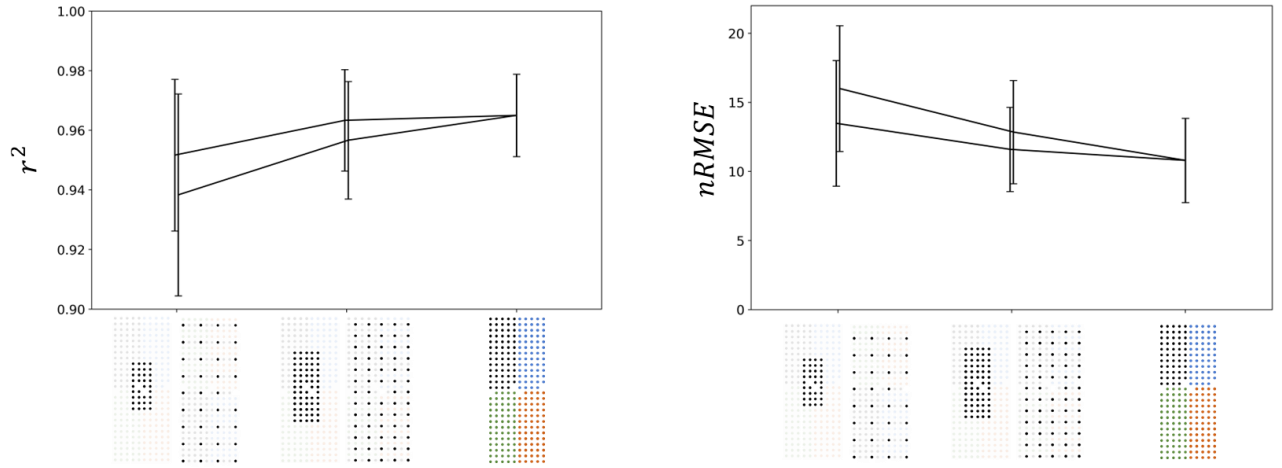


Figure 3-13: Evolution of the r^2 (Left) and $nRMSE$ (Right) metrics between normalized experimental force (up to 30% MVC) and normalized estimated eND for grids of increasing size and density that involve increasing number of EMG channels. The metrics are averaged across the six subjects and the calculated standard deviations are reported as error bars.

3.2.4. Discussion

In this Section 3.2, MN spike trains were obtained for six subjects from the decomposition of HDEMG signals recorded during trapezoidal isometric ankle dorsiflexion up to 30% and 50% MVC. To assess the sensitivity of the output to grid size, electrode density, and total number of electrodes, seven grid configurations (Figure 3-3) were used, of 2-36cm² surface area, 4-16 mm interelectrode distance, and 19-256 electrodes.

Number of identified MN spike trains

The 64-channel conditions B and E (Figure 3-3) mimicked the experimental approach commonly taken in the literature and returned 18-43 identified MUs (Table 3-1) for the six subjects at 30% MVC, which is in the upper range of the values reported in the literature. When large and dense grids of 256 electrodes were considered, higher values were obtained, as 40-to-81 MU spike trains were identified with the *AI* approach (Table 3-1). For each subject, and in average across the participant, more MU spike trains were shown to be identified when higher electrode density (Figure 3-5), larger grids (Figure 3-6), and/or higher number of electrodes (Figure 3-7) were considered. The increase followed in average across participants a logarithmic tendency in all three approaches and at both contraction levels. This logarithmic trendline suggested that the number of identified MUs may reach a plateau beyond 256 electrodes. Because the B-G conditions could not identify spike trains other than those identified in the *AI* condition, it is therefore believed that the maximum number of identifiable MUs with the current knowledge in HDEMG decomposition and available commercial technical equipment was obtained for the TA of the six subjects in this study.

Quality of the identified spike trains and type of identified MNs

Beyond the higher number of identified spike trains, the 256-channel approach showed two additional advantages. First, because the computed PNR increased with increasing grid size and electrode density (Figure 3-10), the edited spike trains obtained with the *AI* approach were highly reliable, with average PNR values of 36dB. This result underlined the importance of the redundancy in the EMG source in accurately identifying the spike trains, as observed in the reevaluation of the *AI* spike trains with the signals of the AM-G configurations, which always led to a drop in mean PNR values (Figure 3-11CD). Second, high percentages of 'small' MUs of low recruitment thresholds were identified at 30% MVC when the number of active electrodes, electrode density, and grid size increased. According to Figure 3-8 with 256 electrodes at 30% MVC, it can be expected that 8-44% (average 29%) of the identified MNs fall into the first half of the active MU pool ranked in the order of increasing F^{th} , against 2-33%

(average 22%) with 64-channel conditions B and E. In this case, electrode density played a key role, as more ‘small’ MUs were identified in the denser E than in the shallower B condition (Figure 3-9).

Neural drive

Overall, the accuracy of the estimated neural drive to muscle increased with both the number of active channels and electrode density (Figure 3-13) for two main reasons. First, the higher number of identified MNs decreased the noisiness of the estimated *eND* during the plateau of force (Figure 3-12). Second, because higher percentages of ‘small’ low-threshold MUs were identified in the high-density conditions, the *eND* was more accurately estimated during the ramps of force, where these types of MUs are recruited. This explained why the *eND* estimated at 50% MVC, where dominantly high-threshold MUs are identified (Figure 3-8CD), showed important onset errors in Figure 3-12, that are defined by the recruitment time of the lowest-threshold identified MU.

Relevance for controlling neuromuscular models

In this section 3.2, empirical methods were developed to increase the quality of HDEMG outputs compared to the typical results obtained in the literature with 64-channel grids. Because they included relatively more low-threshold MUs, the 256-channel *AI* datasets were more representative of the MU pool and could provide more accurate estimates of the neural drive than the 64-channel conditions. For this reason, and also because more MN spike trains of higher quality were obtained with 256 electrodes, this section 3.2 is a step forward towards reliably controlling MN-driven multi-MU models with empirical neural inputs. However, the true neural drive could never be entirely explained with the *AI* condition at 30% MVC, with average *nRMSE* values above 7.7% for individuals and 10.5% for the group, with worse estimations obtained with the 64-channel conditions. Consequently, the experimental sets of MU spike trains obtained in this study, and more generally in the literature, are not sufficiently representative of the discharge behaviour of the complete MU pool to control MU-scale models of muscles because of the strong uncertainty propagation in these models. For these reasons, computational methods (Chapter 5) should be developed to infer the discharge activity of the complete MU pool from the samples of identified MU spike trains.

3.3. Subject-specific properties of a TA muscle-tendon system

3.3.1. Subject-specific musculoskeletal properties

To infer the force generated by the TA from the experimentally recorded transducer force $F^{transd}(t)$, and to obtain more accurate model predictions of muscle force according to Chapter 2, subject-specific geometries of the TA muscle and of the ankle joint were obtained from processed MRI scans of the shank for the Subject S1.

3.3.1.1. MRI scans

A 3D T1-weighted VIBE (volumetric interpolated breath-hold examination) sequence was used to acquire high resolution images from the knee joint to mid-foot of the subject S1's dominant right leg. Because of the length of the available coils, the full shank and ankle joint scans, in Figure 3-14, consisted in three blocks with an overlap of 50mm between adjacent blocks. The participant was asked not to move between the block acquisition to facilitate image processing and allow a successful merging of the adjacent blocks. Because the TA is known to have an unclear interface with adjacent tissues in lean patients, and because the tendon must be clearly identified in this study, high resolution images with 0.45x0.45mm pixel size, 1mm slice thickness, and 1mm slice increment were acquired. The images were obtained from the personal initiative of the subject S1 who freely shared them for research purposes.

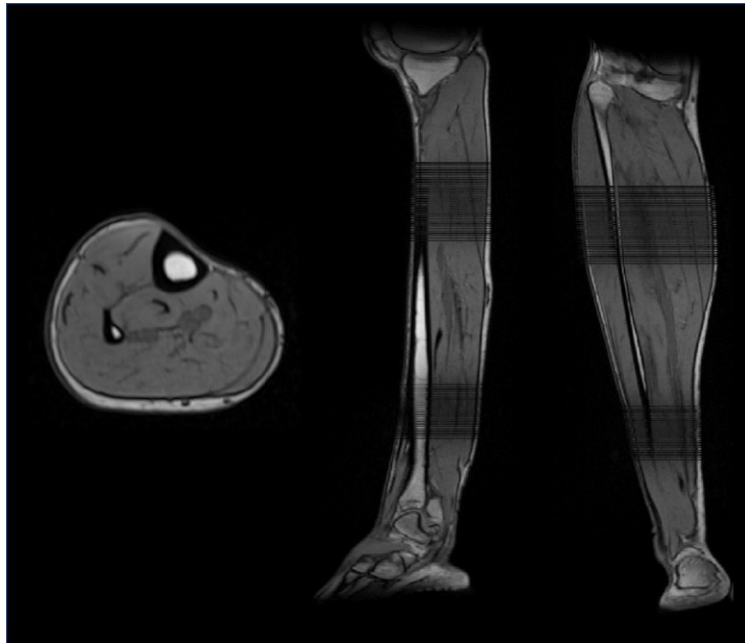


Figure 3-14: Overview of the MRI scans obtained for the subject S1 in the transverse, sagittal, and coronal planes.

3.3.1.2. Image processing

The MRIs in Figure 3-14 were segmented using the open-source tool ITK-SNAP (Yushkevich et al., 2006) to obtain volumetric shapes for the tibial, fibula, and foot bones (yellow, orange, and green in Figure 3-15), and for the TA muscle (red in Figure 3-15), the tendons of which (turquoise and white in Figure 3-15) were carefully identified. An expert radiologist confirmed the accuracy of the segmentation. When sufficient contrast was available, semi-automatic tools were used for the segmentation of the tibial and fibula bones. In all other cases, a manual segmentation was performed in the transversal plane using an anatomical atlas as support (Möller & Reif, 2007), and its quality was regularly assessed in the sagittal and frontal planes. The EDL and EHL muscles were not segmented because the interface between these muscles with other surrounding tissues was unclear; their properties will be inferred in the following from the literature. Once segmented, the shapes were exported to the open-source tool Netfabb where they were manually realigned and merged with a Boolean union to correct for the sections of MRI overlap displayed in Figure 3-14. The final shapes were smoothed with a Laplacian filter with Meshlab (Cignoni et al., 2008).

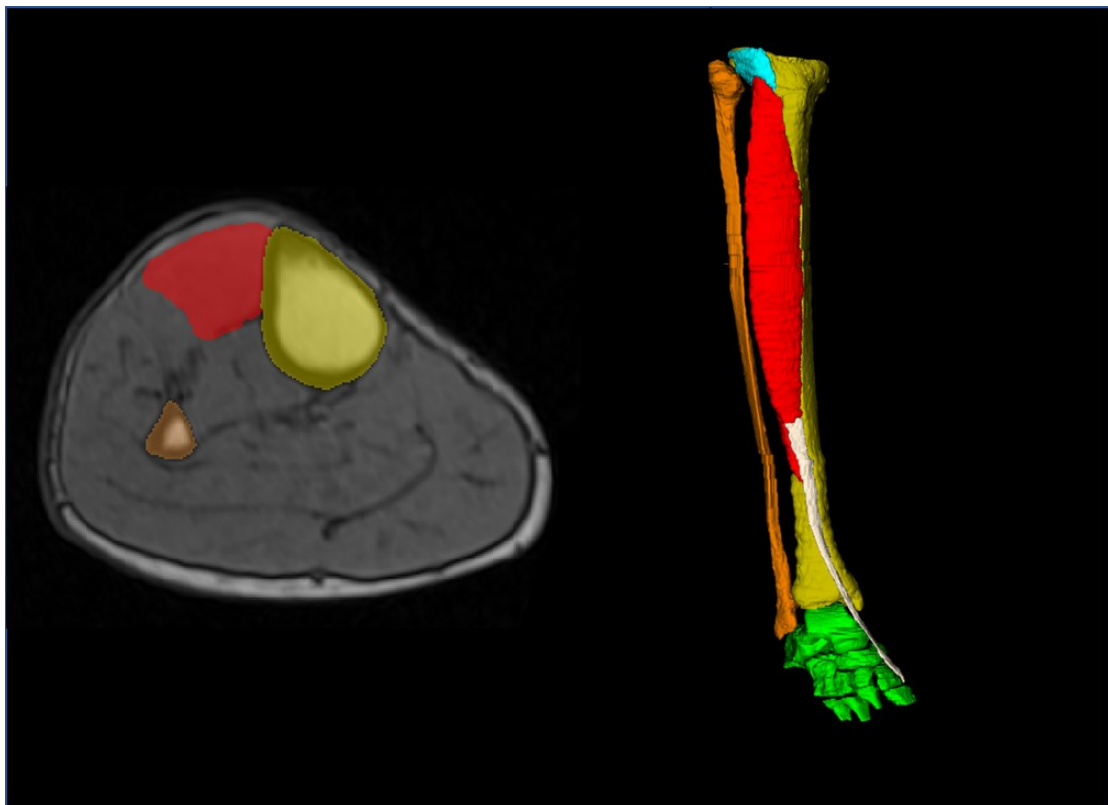


Figure 3-15: (Left) Manual segmentation of the TA muscle (red) and semi-automatic segmentation of the tibial and fibula bones (yellow and orange) in the transverse plane at mid-shank. (Right) Volumetric reconstruction of the segmented volumes. The muscle-tendon interface could be clearly identified.

3.3.1.3. Development of the subject-specific musculoskeletal model

The subject-specific bone volumes obtained in 3.3.1.2 were input to the open-source automated toolbox STAPLE (Modenese & Renault, 2021) to generate a skeletal model that is specific to the subject S1's geometries and is compatible with the OpenSim simulation platform (Delp et al., 2007; Seth, Ajay et al., 2011; Seth et al., 2018; Dembia et al., 2020). With this approach, the ankle joint was modelled as a pivot joint, the axis of rotation of which is determined by the shape of the segmented talus bone. Using another automated approach (Modenese & Kohout, 2020), the muscle belly of the TA muscle was described with $N = 400$ fibres (in red in Figure 3-16) that make the volumetric shape given by the segmented muscle geometry (in red in Figure 3-15). Clearly identified in Figure 3-15, the TA tendon was manually outlined in NMSBuilder (Valente et al., 2017) as two straight lines making attachments at the foot and tibia locations visible in Figure 3-16 and making the clear interface identified in Figure 3-15 with the muscle belly. This geometric description of the muscle-tendon system in Figure 3-16 deviates from the classic approach of describing the whole structure as a single unidirectional string.

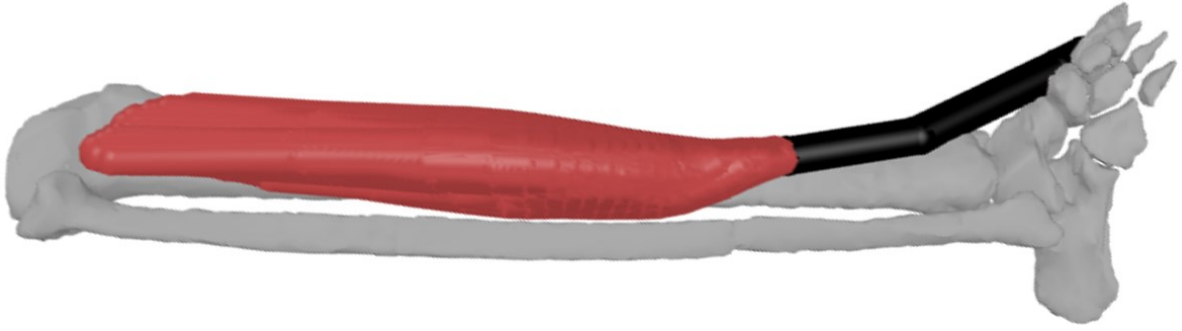


Figure 3-16: Subject-specific Opensim MSK model. The skeletal model was obtained with the STAPLE toolbox (Modenese & Renault, 2021). The muscle belly of the TA is described as $N = 400$ individual fibre-like strings making the volumetric shape defined by the segmentation step. The tendon was drawn on NMSBuilder (Valente et al., 2017) from the segmented geometries.

3.3.1.4. Derivation of subject-specific quantities of interest

To reproduce the experimental conditions of isometric TA contractions described in the later Section 3.2, the subject-specific MSK model was set to a 30° plantarflexion angle as displayed in Figure 3-16. At this angle, the subject-specific TA muscle-tendon system is measured with the OpenSim tools to have a length $l^{MT} = 319.1$ mm and the tendon makes a $L = 25.5$ mm moment arm with the ankle joint. While the personalised l^{MT} length is close to the one obtained when scaling the Rajagopal model to the subject's height ($l_{scaled}^{MT} = 326.5$ mm), the personalised moment arm is much lower than the scaled value ($L = 35.0$ mm). The generic muscle-specific values of muscle optimal length $l_{0,Raj}^M$, tendon slack length $l_{s,Raj}^T$ and muscle-tendon length l_{Raj}^{MT} proposed in the Rajagopal model (Rajagopal et al., 2016) were linearly scaled (EQ. 3-8) to the subject-specific muscle's optimal length l_0^M and the tendon slack length l_s^T .

$$\begin{cases} l_0^M = \frac{l^{MT}}{l_{Raj}^{MT}} \cdot l_{0,Raj}^M = 68.2mm \\ l_s^T = \frac{l^{MT}}{l_{Raj}^{MT}} \cdot l_{s,Raj}^T = 239.9mm \end{cases} \quad (\text{EQ. 3-8})$$

These quantities are very close, with less than 1% difference, to the values from the considered generic model (Rajagopal et al., 2016). The same quantities, reported in Table 3-2, were derived for the EDL and EHL muscles, the muscle-tendon lengths of which are scaled as, for example for the EDL muscle, $l_{EDL}^{MT} = \frac{l^{MT}}{l_R^{MT}} \cdot l_{EDL,R}^{MT}$, while the moment arms were obtained as $L_{EDL} = \frac{L}{L_R} \cdot L_{EDL,R}$.

Considering a specific tetanic tension of $\sigma = 60 \text{ N/cm}^2$ (Rajagopal et al., 2016), the subject-specific maximum isometric force F_0^M of the TA muscle was obtained from the segmented muscle volume V^M (EQ. 3-9).

$$F_0^M = \frac{V^M}{l_0^M} \cdot \sigma = 1046 \text{ N} \quad (\text{EQ. 3-9})$$

The subject-specific F_0^M is 15% lower than the generic value reported in Rajagopal's model (Rajagopal et al., 2016). For the EDL and EHL muscles, the V^M values in (EQ. 3-9) were inferred from anatomical relationships derived from cohort of segmented MRI scans (Handsfield et al., 2014), and the expected F_0^M values for these two muscles, calculated with (EQ. 3-9) adapted for the two muscles, are reported in Table 3-2. Because the personalised l^{MT} values are not available from segmentation for these two muscles, the l_0^M value in (EQ. 3-9) was obtained for these two muscles by linearly scaling the generic value reported in Rajagopal et al. (2016) with the scaling factor $k = \frac{l_0^M}{l_{0,Raj}^M} = 0.998$ obtained for the TA muscle.

Table 3-2: Summary of the subject-muscle-specific properties of the three extensor muscles (TA, EDL, and EHL) obtained from the processing of the subject-specific geometries in Figure 3-16. Subject-muscle-specific values were obtained by scaling the generic model described in Rajagopal et al. (2016) to the subject-specific geometries of the Subject S1 for the muscle maximum isometric force F_0^M and optimal length l_0^M , and the tendon slack length l_s^T , and for the moment arm L made between the tendon and the ankle joint, and the muscle-tendon length l^{MT} at 30° plantarflexion. Most of these subject-specific values will be used in Chapter 6 when developing the personalised MN-driven muscle model.

Muscle	L [mm]	F_0^M [N]	l^{MT} [mm]	l_0^M [mm]	l_s^T [mm]
TA	25.5	1046	319.1	68.2	239.9
EDL	22.6	519	444.7	67.4	360.7
EHL	24.1	232	413.3	73.3	319.6

3.3.2. Estimation of the TA force from the recorded transducer force

According to the load cell documentation, the quantity F^{transd} recorded by the load cell during the experimental session described in the Section 3.2.2.1 was given in Volts, and can be converted into a force F_N^{transd} in Newtons with the following (EQ. 3-10). The effects of gravity, including the rig weight, were cancelled by removing a measured offset during the experiments.

$$F_N^{transd}[N] = \frac{1000 \cdot F^{transd}[V] \cdot 100[kg] \cdot 9.81[N/kg]}{2[mV/V] \cdot 5[V] \cdot 100[V/V]} \quad (\text{EQ. 3-10})$$

$$= 981[N/V] \cdot F^{transd}[V]$$

From the geometry of the dynamometer shown in Figure 3-17 and guidelines provided by the documentation regarding the direction of the generated force during dorsiflexion tasks, the ankle joint torque $T[N \cdot m]$ was estimated from F_N^{transd} with (EQ. 3-11).

$$T = F_N^{transd}[N] \cdot \frac{\sqrt{10.5^2 + 13^2} \cdot 10^{-2}[m]}{\cos(30^\circ)} \quad (\text{EQ. 3-11})$$

$$= 0.170[m] \cdot F_N^{transd}[N]$$

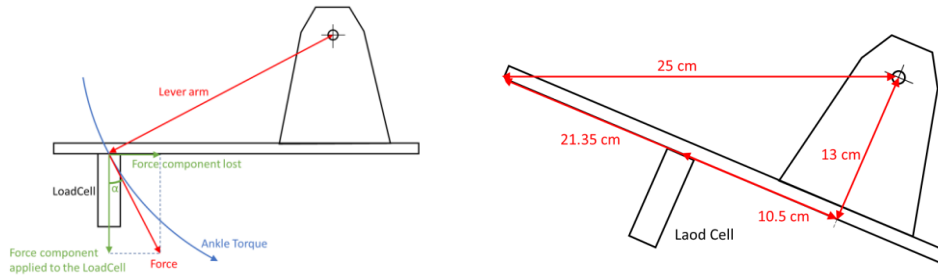


Figure 3-17: Geometry of the dynamometer used in the experimental protocol described in the Section 3.2.2.1.

(EQ. 3-11) yielded $T^{MVC} = 36.5 Nm$ at MVC for the Subject S1. To infer the TA force from the recorded torque T , the contribution to T of the agonistic EHL and EDL muscles must be estimated. In the following, it is assumed that only the three extensor muscles contribute to the recorded torque. With this assumption, the TA cannot take less than 52% of the maximum voluntary torque T^{MVC} according to (EQ. 3-12) where the EDL and EHL muscles develop their maximum isometric forces, the quantities specific to the subject S1 of which are given in Table 3-2.

$$\frac{T^{MVC} - (F_{0,EDL}^M \cdot L_{EDL} + F_{0,EHL}^M \cdot L_{EHL})}{T^{MVC}} = 52\% \quad (\text{EQ. 3-12})$$

Now, if the TA produced its maximum isometric force $F_{0,TA}^M$ when T^{MVC} is generated, it would explain 73% of the recorded T^{MVC} , according to (EQ. 3-13).

$$\frac{F_{0,TA}^M \cdot L_{TA}}{T^{MVC}} = 73\% \quad (\text{EQ. 3-13})$$

Finally, if the three extensors contributed to T^{MVC} with the same ratio of generated torque relative to maximum possible generated torque, the TA would take 61% of the recorded T^{MVC} (EQ. 3-14).

$$\frac{F_{0,EDL}^M \cdot L_{EDL} + F_{0,EHL}^M \cdot L_{EHL}}{F_{0,TA}^M \cdot L_{TA} + F_{0,EDL}^M \cdot L_{EDL} + F_{0,EHL}^M \cdot L_{EHL}} = 39\% \quad (\text{EQ. 3-14})$$

From these conclusions made at MVC, it was assumed that the TA contributed at each time instant to 73% of the recorded ankle torque T during submaximal contractions, for which the subjects had trained to focus on dominantly contracting the TA. Thus, the experimental TA force F_{TA}^M was estimated from the recorded transducer force with (EQ. 3-15).

$$F_{TA}^M [N] = \frac{0.73 \cdot T}{L_{TA}} = 28.6 [m^{-1}] \cdot T [N \cdot m] \quad (\text{EQ. 3-15})$$

The F_{TA}^M values obtained with this approach are acceptable, but their accuracy may be limited because of the important assumptions taken about the contribution of the agonistic extensor muscles to the recorded torque. To address this issue, the EDL and EHL myoelectric activity during submaximal contractions could be inferred, to some extent, through bEMG recordings, notwithstanding the crosstalk issues with the dominant and adjacent TA muscle. To allow a one-to-one validation and bypass the activity of agonistic muscles, the TA force could also be inferred from an estimation of the tendon stiffness from strength protocols and ultrasound recordings of the fascicle length, simultaneously obtained with HDEMg signals during isometric contractions as recently performed (Gerus et al., 2015; Dick et al., 2016; Zhang et al., 2020; Waasdorp et al., 2021; Zhang et al., 2021; Zhang et al., 2022).

3.3.3. Conclusion

In this section 3.3, subject-specific musculoskeletal quantities were obtained in Table 3-2 from processed MRI scans for the subject S1. The TA isometric force $F_{0,TA}^M$ was used to scale the distribution of MU maximum isometric forces $f_0^{MU}(i)$ in (EQ. 3-3). The TA-tendon length quantities l_0^M , l_s^T and l^{MT} , and the moment arm of the TA tendon with the ankle joint were used to infer similar muscle-specific quantities for the two agonistic EDL and EHL muscles. From there, the TA force F_{TA}^M developed during the trapezoidal contractions was estimated from the recorded transducer force. The force predictions

performed in Chapter 6 by the MU-scale model, scaled with the length quantities in Table 3-2, will be validated against F_{TA}^M .

Chapter 4

MATHEMATICAL RELATIONSHIPS BETWEEN SPINAL MOTONEURON PROPERTIES

NOTE: Aside minimal alterations, this content of this chapter has been published in the following journal article:

Caillet, A. H., Phillips, A. T., Farina, D., & Modenese, L. (2022). Mathematical relationships between spinal motoneuron properties. *eLife*, 11, e76489, doi: <https://doi.org/10.7554/eLife.76489>

Transition

In Chapter 3, large datasets of MN spike trains were obtained during trapezoidal isometric contractions for later controlling the MN-driven neuromuscular models developed in this thesis (Chapter 6). Two-to-four times more discharging MNs were identified compared to typical literature results by increasing the EMG channel density and the covered muscle area and fine-tuning the decomposition methods. However, despite these improvements, Chapter 3 showed that the resulting datasets undersampled the low-threshold MNs in the MN population, and that the resulting cumulative spike train computed from the experimental discharging activity was not an accurate approximation of the neural drive to muscle for most datasets. In this Chapter 4, it is quantitatively demonstrated that the MN electrophysiological properties are MN specific and inter-related in a MN and are monotonically related to the MN force recruitment threshold. From these conclusions, one therefore understands that the experimental MNs identified in Chapter 3 provide an incomplete window into the distribution of MN electrophysiological properties in the complete MN pool. The experimental sets of identified firings MNs are therefore not representative of the discharge activity of the complete MN pool, which explains the poor neural drive predictions in Chapter 3. The mathematical relationships derived in Chapter 4 between MN anatomical and electrophysiological properties are used in Chapter 5 to solve this limitation intrinsic to the HDEMG approach.

Chapter summary

In this Chapter 4, mathematical relationships are derived for the first time between eight key MN electrophysiological and anatomical properties, including MN size (soma diameter and MN surface area), MN membrane input resistance, specific resistance, capacitance, time constant, and afterhyperpolarization period, axonal conduction velocity, and current threshold for spike initiation. To derive these relationships, the data from 40 experimental studies in adult cat, rat and mouse MN preparations was digitized, processed, merged, fitted, and transformed. This validated mathematical framework supports the past findings that the MN membrane properties are all related to each other and quantifies the nature of these associations. The mathematical relationships also provide a quantification of the Henneman's size principle and are consistent with Rall's cable theory. First obtained from cat data, they are finally extrapolated to rodents to assess their scalability to other mammals including humans.

4.1. Introduction

Assessing the morphological and electrophysiological properties of individual spinal alpha-motoneurons (MNs) is crucial for understanding the recruitment and discharge behaviour of individual MNs and of the complete MN population. As measured in individual spinal MNs in experimental studies and reported in review papers (Henneman, 1981; Burke, 1981; Binder et al., 1996; Powers & Binder, 2001; Kernell, 2006; Heckman & Enoka, 2012), significant correlations exist in mammalian MN pools between the MN morphological and/or electrophysiological properties reported in Table 4-1. For example, the soma size (D_{soma}) and the current threshold for spike initiation (I_{th}) of a MN are both positively correlated to the axonal conduction velocity (ACV) and afterhyperpolarization period (AHP). Also, I_{th} decreases with increasing input resistance (R) and AHP , R is negatively correlated to ACV and D_{soma} , and ACV varies inversely with AHP . Besides supporting and sequentially leading to extensions of the Henneman's size principle (Henneman, 1957; Wuerker et al., 1965; Henneman et al., 1965a; Henneman et al., 1965b; Henneman et al., 1974; Henneman, 1981; Henneman, 1985), these empirical associations find strong consistency with Rall's theoretical approach of representing the soma and the dendritic trees of MNs as an equivalent cylinder (Rall, 1977; Rall et al., 1992; Powers & Binder, 2001). In such case, MNs behave like resistive-capacitive (RC) circuits, as successfully simulated with computational RC models (Izhikevich, 2004; Dong et al., 2011; Negro et al., 2016; Teeter et al., 2018), that rely on further property associations, such as the MN membrane time constant τ precisely equalling the product of the MN membrane specific capacitance C_m and resistivity R_m ($\tau = R_m C_m$).

Yet, because the empirical correlations between MN properties were obtained from scattered data from individual experimental studies, the quantitative mathematical associations between the MN properties reported in Table 4-1, beyond their aforementioned global relative variations, remain unclear. For example, the negative correlation between R and ACV was described in the literature with linear (Fleshman et al., 1981), exponential (Burke, 1968) or power relationships (Kernell, 1966; Kernell & Zwaagstra, 1981; Ulfhake & Kellerth, 1984; Gustafsson & Pinter, 1984a), while Kernell & Zwaagstra (1980) reported for this association a slope twice greater than Gustafsson & Pinter (1984a) in the double-logarithmic space. This makes it difficult to reconcile the conclusions of multiple empirical studies that investigated different property associations. For example, Burke et al. (1968) and Zwaagstra et al. (1980) respectively proposed exponential $R - ACV$ and linear $ACV - AHP$ associations, suggesting a hybrid exponential relationship between R and AHP , that is different from the power $R - AHP$ relationship directly reported by Ulfhake & Kellerth (1984). These divergences between studies are a major limitation for our understanding of the associations and the distribution of MN properties in a MN pool, which cannot be directly investigated experimentally either, as

measuring multiple MN morphometric properties *in vitro* and electrophysiological properties *in vivo* for a large sample of MNs is challenging.

Here, the published data from 19 available experimental studies in adult cat preparations were reprocessed and merged to derive and validate a unique set of mathematical power relationships between all pairs of the MN morphometric and electrophysiological cat properties listed in Table 4-1. The significance of these quantitative relationships, which are consistent with the conclusions from Rall's cable theory, supports the notion that the properties reported in Table 4-1 are all associated to each other. The uniqueness of these mathematical relationships tackles the aforementioned inter-study variability in describing the data and clarifies our understanding of the quantitative association between spinal MN properties, including for pairs of properties never simultaneously measured in experiments. These relationships also provide a convenient mathematical framework for modelers for the derivation of appropriate and consistent MN profiles of MN-specific morphometric and electrophysiological properties for the realistic scaling of pools of computational models of MNs, improving the interpretability of model predictions. Using the relationships, experimenters can readily complete their datasets by deriving MN-specific values, that are representative of the literature, for the MN properties that were not experimentally measured.

After deriving the mathematical framework from cat data, the normalized cat relationships were then demonstrated to apply to other mammals by validating them with data from 9 adult rat and mouse electrophysiological studies *in vivo*. This approach helps better understanding the inter-mammalian-species variations in MN properties. Finally, using additional correlations obtained between some MN and muscle unit (mU) properties from 14 mammal studies, the empirical relationships obtained between MN properties were discussed in the context of the Henneman's size principle.

Table 4-1. The motoneuron (MN) and muscle unit (mU) properties investigated in this study with their notations and SI base units. S_{MN} is the size of the MN. As reproduced in Table 4-2, the MN size S_{MN} is adequately described by measures of the MN surface area S_{neuron} and the soma diameter D_{soma} . R and R_m define the MN-specific electrical resistance properties of the MN and set the value of the MN-specific current threshold I_{th} (Binder et al., 1996; Powers & Binder, 2001; Heckman & Enoka, 2012). C and C_m (constant among the MN pool) define the capacitance properties of the MN and contribute to the definition of the MN membrane time constant τ (Gustafsson & Pinter, 1984a; Zengel et al., 1985). ΔV_{th} is the amplitude of the membrane voltage depolarization threshold relative to resting state required to elicit an action potential. I_{th} is the corresponding electrical current causing a membrane depolarization of ΔV_{th} . AHP is defined in most studies as the duration between the action potential onset and the time at which the MN membrane potential meets the resting state after being hyperpolarized. ACV is the axonal conduction velocity of the elicited action potentials on the MN membrane. S_{mU} is the size of the mU. As indicated in Table 4-2, the mU size S_{mU} is adequately described by measures of (1) the sum of the cross-sectional areas (CSAs) of the fibres composing the mU CSA^{tot} , (2) the mean fibre CSA CSA^{mean} , (3) the innervation ratio IR, i.e. the number of innervated fibres constituting the mU, and (4) the mU tetanic force F^{tet} . F^{tw} is the MU twitch force.

	Properties	Notation	Unit
Motoneuron (MN) properties	Size:	S_{MN}	
	Neuron surface area	S_{neuron}	$[m^2]$
	Soma diameter	D_{soma}	$[m]$
	Resistance	R	$[\Omega]$
	Specific resistance per unit area	R_m	$[\Omega \cdot m^2]$
	Capacitance	C	$[F]$
	Specific capacitance per unit area	C_m	$[F \cdot m^{-2}]$
	Time constant	τ	$[s]$
	Rheobase (current recruitment threshold)	I_{th}	$[A]$
	Voltage threshold	ΔV_{th}	$[V]$
	Afterhyperpolarization period	AHP	$[s]$
	Axonal conduction velocity	ACV	$[m \cdot s^{-1}]$
Muscle unit (mU) properties	Size:	S_{mU}	
	Total fibre cross-sectional area (CSA)	CSA^{tot}	$[m^2]$
	Mean fibre CSA	CSA^{mean}	$[m^2]$
	Innervation ratio	IR	$[]$
	Tetanic force	F^{tet}	$[N]$
	Twitch force	F^{tw}	$[N]$

4.2. Methods

4.2.1. Selected studies reporting processable adult mammal data

4.2.1.1. Identification of the selected studies

To optimize inter-study consistency, only studies that concurrently measured at least two of the morphometric and/or electrophysiological properties reported in Table 4-1 in individual spinal alpha-motoneurons of healthy adult cats, rats and mice were selected. Because measurements of the parameters in Table 4-1 were never performed in humans *in vivo* (Highlander et al., 2020), and the values of some of these parameters (*ACV*, *AHP*) were only indirectly inferred in humans, this study focuses on experiments performed in mammals, the neurophysiological dynamics of which being considered to be the closest to humans', although this study is part of a larger work focused on modelling the neuromuscular dynamics during human voluntary contraction. The extension of the conclusions drawn in this study to the human machinery is investigated in this study. To build an extensive set of relevant studies so that the mathematical relationships derived in this study describe a maximum of the data published in the literature, the output of the systematic analysis provided in Highlander et al. (2020) was screened and used for a further search by reference lists. Among the retrieved studies, larval and postnatal specimens were disregarded because of the pronounced age-related variance (Highlander et al., 2020) in the mean values of the electrophysiological properties listed in Table 4-1, which corroborates the non-extrapolability of neonatal neuronal circuitry to older ages (Song, Z-M et al., 2006; Carp et al., 2008; Nakanishi & Whelan, 2010; Mitra & Brownstone, 2012).

Due to the nonlinear inter-species scalability of the spinal alpha-MN electrophysiological and morphometric properties (Manuel et al., 2019) and a pronounced variance in the inter-species mean values (Highlander et al., 2020), non-mammalian species were also disregarded, while the retained data from cats, rats and mice were processed separately. Highlander et al. (2020) also report an 'unexplained' variance in the mean electrophysiological property values reported between studies investigating specimens of the same species, sex and age, which may be explained by differences between *in vivo* and *in vitro* protocols (Carp et al., 2008). For this reason, only studies measuring the electrophysiological properties *in vivo* were considered, while most *in vitro* studies had already been disregarded at this stage as dominantly performed on neonatal specimens due to experimental constraints (Manuel et al., 2009; Mitra & Brownstone, 2012). As all but two of the remaining selected studies focused on lumbosacral MNs, the final set of considered publications was constrained to MNs innervating hindlimb muscles to improve inter-study consistency.

From a preliminary screening of the final set of selected studies, it was finally found that relatively more cat studies (19) were obtained than rat (9) and mouse (4) studies, while the cat studies also investigated more pairs of morphometric and electrophysiological properties. The mathematical relationships sought in this paper between MN properties were thus derived from the cat data reported in the selected studies, and then validated for extrapolation to rat and mouse data.

4.2.1.2. Selected studies providing cat data – experimental approaches

The 19 selected studies focusing on cats that were included in this work were published between 1966 and 2001. They applied similar experimental protocols to measure the morphometric and electrophysiological properties reported in Table 4-1. All the selected studies performing morphometric measurements injected *in vitro* the recorded MNs intracellularly with horseradish peroxidase (HRP) tracer by applying a continuous train of anodal current pulses. The spinal cord was eventually sliced frozen or at room temperature with a microtome or a vibratome and the MN morphometry was investigated. All morphometric measurements were manually performed, and the MN compartments (soma, dendrites, axon) approximated by simple geometrical shapes, yielding some experimental limitations discussed later in the Methods section and Appendix B.2.

To measure the electrophysiological properties, animals were anesthetized, immobilized, and kept under artificial breathing. Hindlimb muscles of interest were dissected free, and their nerves were mounted onto stimulating electrodes, while maintaining body temperature between 35°C and 38°C. After a laminectomy was performed over the lumbosacral region of the spinal cord, the MNs were identified by antidromic invasion following electrical stimulation of the corresponding muscle nerves. All selected cat studies reported the use of electrodes having stable resistances and being able to pass currents up to 10 nA without evident polarization or rectification. Some aspects of the experimental protocol however diverged between the selected studies, such as the age and the sex of the group of adult cats, the size population of cats and recorded MNs, the muscles innervated by the recorded MNs, the means of anaesthesia, the level of oxygen, and whether the spinal dorsal roots were severed (Kernell, 1966; Burke, 1968; Barrett & Crill, 1974; Fleshman et al., 1981; Gustafsson & Pinter, 1984a; Gustafsson & Pinter, 1984b; Zengel et al., 1985; Foehring et al., 1987), the complete surrounding hindlimbs were denervated (Burke, 1968; Kernell & Zwaagstra, 1981) or the spinal cord was transected (Burke, 1968; Krawitz et al., 2001).

In all the selected cat studies, *ACV* was calculated as the ratio of the conduction distance and the antidromic spike latency between the stimulation site and the ventral root entry. The studies however

did not report how the nerve length was estimated. *AHP* was measured using brief suprathreshold antidromic stimulations, as the time interval between the spike onset to when the voltage deflection of the hyperpolarizing phase returned to the prespike membrane potential. In all studies, I_{th} was obtained as the minimal intensity of a 50-100ms-long depolarizing rectangular current pulse required to produce intermittent discharges. I_{th} was obtained with a trial-and-error approach, slowly increasing the intensity of the pulse. The selected studies did not report any relevant source of inaccuracy in measuring *ACV*, *AHP* and I_{th} . The studies measured R using the spike height method (Frank & Fuortes, 1956). In brief, small (1-5nA) depolarizing and/or hyperpolarizing current pulses (I_{in}) of 15-100ms duration were injected through a Wheatstone bridge circuit in the intracellular microelectrode amplifier, and the subsequent change in the amplitude of the membrane voltage potential (V_m) was reported in steady-state $V_m - I_{in}$ plots. In all studies, R was obtained by calculating the slope of the linear part of the $V_m - I_{in}$ plots near resting membrane potential, by analogy with Ohm's law. It is worth noting for inter-study variability that depolarizing pulses return slightly higher R values than hyperpolarizing currents (Sasaki, 1991). To measure the membrane time constant τ , all the selected studies analysed the transient voltage responses (V_m) to weak (1-12nA) brief (0.5ms) or long (15-100ms) hyperpolarizing current pulses. Both brief- and long-pulse approaches were reported to provide similar results for the estimation of τ (Ulfhake & Kellerth, 1984). Considering MNs as equivalent isopotential cables, the membrane time constant τ was identified in all studies as the longest time constant when modelling the measured voltage transient response to a current pulse as the sum of weighted time-dependent exponential terms (Rall, 1969; Rall, 1977; Powers & Binder, 2001). Semi-logarithmic plots of the time-history of the voltage transient V_m or of its time-derivative $\frac{dV_m}{dt}$ were drawn, and a straight line was fit by eye to the linear tail of the resulting plot (Fleshman et al., 1988), the slope of which was τ . A graphical 'peeling' process (Rall, 1969) was undertaken to recover the first equalizing time constant τ_1 , required to estimate the electronic length (L) of Rall's equivalent cylinder representation of the MN and the MN membrane capacitance with Rall's equations (Rall, 1969; Rall, 1977; Gustafsson & Pinter, 1984b; Powers & Binder, 2001):

$$L \approx \left[\frac{\pi}{\left(\frac{\tau}{\tau_1} - 1 \right)} \right]^{\frac{1}{2}} \quad (\text{EQ. 4-1})$$

$$C = \frac{\tau}{R} \cdot \frac{L}{\tanh(L)}$$

These electrophysiological measurements were reported to be subject to three main experimental sources of inaccuracy. First, a variable membrane 'leak' conductance, that can be estimated from the

ratio of two parameters obtained from the ‘peeling’ process (Gustafsson & Pinter, 1984b), arises from the imperfect seal around the recording micropipette (Ulfhake & Kellerth, 1984; Gustafsson & Pinter, 1984b; Pinter & Vanden Noven, 1989). As reviewed in Powers & Binder (2001) and in Kernell (2006), this ‘leak’ can affect the measurements of all the properties, notably underestimating the values of R and overestimating those of τ and C . However, this ‘leak’ is probably not of major significance in the cells of large spike amplitudes (Gustafsson & Pinter, 1984b), on which most of the selected studies focused. Importantly, the membrane ‘leak’ is also not expected to affect the relations between the parameters (Gustafsson & Pinter, 1984b).

Secondly, some nonlinearities (Ito & Oshima, 1965; Burke & Ten Bruggencate, 1971; Ulfhake & Kellerth, 1984) in the membrane voltage response to input current steps arise near threshold because of the contribution of voltage-activated membrane conductances to the measured voltage decay (Fleshman et al., 1988). This contradicts the initial assumption of the MN membrane remaining passive to input current steps (Rall, 1969; Burke & Ten Bruggencate, 1971). Because of this issue, the ends of the $V_m - I_{in}$ plot are curvilinear and can affect the estimation of R , while the transient voltage response to an input current pulse decays faster than exponentially (it undershoots) at the termination of the current injection, which makes it impossible to plot the entire course of $\ln(V(t))$ and challenges the graphical procedure taken to estimate τ (Fleshman et al., 1988). Therefore, some of the selected studies discarded all the MNs that displayed obvious large nonlinearity in the semi-logarithmic V-t or $\frac{dV}{dt}$ -t plots (Burke & Ten Bruggencate, 1971). The membrane nonlinearities can be corrected by adding a constant voltage to the entire trace (Fleshman et al., 1988) or by considering the three time-constant model of Ito & Oshima (1965) to approximate and subtract to the voltage signal the membrane potential change produced by the current steps. However, none of the selected studies performed such corrections, potentially yielding a systematic underestimation of the values of R and τ (Gustafsson & Pinter, 1984b; Zengel et al., 1985; Powers & Binder, 2001). Yet, this systematic error for R , which should not contribute to inter-study variability, is expected to remain low because the selected studies used input current pulses of low strength (Ulfhake & Kellerth, 1984) and measured R from the linear part of the I-V plots near the resting membrane potential where no membrane nonlinearity is expected to occur. This systematic error may also not affect the distribution of recorded R values, as displayed in Zengel et al. (1985). Zengel et al. (1985) besides corrected for the membrane nonlinearities with the three time-constant model approach of Ito & Oshima (1965) when measuring τ , and returned values of τ around 40% higher than the other selected studies. However, this systematic discrepancy is expected to disappear with the normalization of the datasets described in the following, as the reported normalized distributions of τ are very similar between the selected studies (see density histogram in Appendix Figure B-1 for the $\{\tau; R\}$ dataset). Thirdly, the highest

source of inaccuracy and inter-study variability arises from the subjective fit ‘by eye’ of a straight line to the transient voltage when estimating τ (Pinter & Vanden Noven, 1989).

Overall, all the selected studies used similar experimental approaches to measure ACV , AHP , I_{th} , R , C and τ , and little sources of inaccuracy and inter-study variability were identified.

4.2.2. Relationships between MN properties

For convenience, in the following, the notation $\{A; B\}$ refers to the pair of morphometric or electrophysiological MN properties A and B , with $A, B \in \{S_{MN}; ACV; AHP; R; I_{th}; C; \tau\}$, defined in Table 4-1. The selected studies generally provided clouds of data points for pairs $\{A; B\}$ of concurrently measured MN properties through scatter graphs. These plots were manually digitized using the online tool WebPlotDigitizer (Ankit, 2020). When a study did not provide such processable data – most reported the mean $\pm SD$ property values of the cohort of measured MNs – the corresponding author was contacted to obtain the raw data of the measured MN properties, following approval of data sharing. Upon reception of data, datasets of all possible $\{A; B\}$ pairs were created and included into the study.

4.2.2.1. Normalized space and choice of regression type

The sets of data retrieved from different cat studies for each property pair $\{A; B\}$ were merged into a ‘global’ dataset dedicated to that property pair. The $\{A; B\}$ data was also normalized for each study and transformed as a percentage of the maximum property value measured in the same study and normalized ‘global’ datasets were similarly created. A least squares linear regression analysis was performed for the $\ln(A) - \ln(B)$ transformation of each global dataset yielding relationships of the type $\ln(A) = a \cdot \ln(B) + k$, which were then converted into power relationships of the type $A = k \cdot B^a$. The adequacy of these global power trendlines and the statistical significance of the correlations were assessed with the coefficient of determination r^2 (squared value of the Pearson’s correlation coefficient) and a threshold of 0.01 on the p-value of the regression analysis, respectively. For each $\{A; B\}$ pair, the normalized global datasets systematically returned both a higher r^2 and a lower p-value than the datasets of absolute $\{A; B\}$ values, in agreement with the ‘unexplained’ inter-experimental variance reported in (Highlander et al., 2020). It was therefore decided to investigate the $\{A; B\}$ pairs of MN properties in the normalized space using the normalized global datasets to improve the cross-study analysis. For this preliminary analysis and the rest of the study, power regressions were preferred to linear ($A = k + a \cdot B$) or exponential ($A = k \cdot e^{a \cdot B}$) regressions to maintain consistency with the mathematical structure of the equations from Rall’s cable theory (Rall, 1957; Rall, 1959; Rall,

1960; Powers & Binder, 2001) and of other well-defined relationships, such as $R = k * I_{th}^{-1}$. Also, a least squares linear regression analysis was preliminary performed for each normalized experimental dataset and for its $\ln(A) - \ln(B)$ and $\ln(A) - B$ transformations, yielding linear, power, and exponential fits to the data. The power regressions overall returned $r^2 > 0.5$ for more experimental datasets than the linear and exponential fits (see Appendix Table B-1). To avoid bias, power regressions were the only type of fit used in this study, despite a few datasets being more accurately fitted by linear or exponential regressions (the difference in the quality of the fit was very small in all cases). Other regression types, such as polynomial fits, were not justified by previous findings in the literature.

4.2.2.2. Global datasets and data variability

The data variability between the studies constituting the same global dataset $\{A; B\}$ was assessed by analysing the normalized distributions of the properties A and B with four metrics, that were the range of the measured data, the mean of the distribution, the coefficient of variation (CoV) calculated as the standard deviation (sd) divided by the mean of the distribution, and the ratio $\frac{Me}{Md}$ of the median and the mean of the distribution. The inter-study data variability in the $\{A; B\}$ global dataset was assessed by computing the $mean_g \pm sd_g$ across studies of each of the four metrics. Low variability between the data distributions from different studies was concluded if the normalized distributions (in percentage maximum value) returned similar values for the range, mean, CoV and $\frac{Me}{Md}$ metrics, i.e. when $sd_g < 10\%$ and $\frac{sd_g}{mean_g} < 0.15$ were obtained for all four metrics. In such case, the data distributions would respectively span over similar bandwidth length of the MU pool, be centred around similar mean values, display similar data dispersion and similar skewness. The relative size of the experimental datasets reported by the selected studies constituting the same global datasets was also compared to assess their relative impact on the regression curves fitted to the global datasets. Then, the inter-study variability in associating the distributions of properties A and B was assessed by computing the 95% confidence interval of the linear model fitted to the global dataset $\{A; B\}$ in the log-log space, that yields the power regression $A = k \cdot B^a$. Low inter-study variability was considered if the value of a varied less than 0.4 in the confidence interval.

The variability of the data distribution of a property A between multiple global datasets $\{A; B\}$ was then assessed by computing the same four metrics as previously (range, mean, CoV, $\frac{Me}{Md}$) for each global dataset, and then computing the $mean_G \pm sd_G$ of each of the four metrics across the global datasets $\{A; B\}$. Low variability between the global datasets in the distribution of property A was considered if $sd_G < 10\%$ and $\frac{sd_G}{mean_G} < 0.15$ were obtained for all four metrics. If the inter-study and

inter-global datasets variability was low, the global datasets were created and processed to derive the mathematical relationships between MN properties according to the procedure described below.

Size-dependent normalized relationships

From inspection of the considered cat studies, most of the investigated MN property pairs comprised either a direct measurement of MN size, noted as S_{MN} in this study, or another variable well-admitted to be strongly associated to size, such as ACV , AHP or R . Accordingly, and consistently with Henneman's size principle, S_{MN} was identified as the reference MN property with respect to which relationships with the electrophysiological MN properties in Table 4-1 were investigated. To integrate the data from all global normalized datasets into the final relationships, the MN properties in Table 4-1 were processed in a step-by-step manner in the order $ACV, AHP, R, I_{th}, C, \tau$, as reproduced in Figure 4-1 for two arbitrary properties, to seek a 'final' power relationship of the type eq.1 between each of them and S_{MN} . For each electrophysiological property A and each $\{A; B\}$ dataset, two cases were considered. If $B = S_{MN}$, the global dataset was not further processed as the electrophysiological property A was already related to MN size S_{MN} from experimental measurements. If $B \neq S_{MN}$, the global $\{A; B\}$ dataset was transformed into a new $\{A; S_{MN}\}$ dataset by converting the discrete values of B with the trendline regression $S_{MN} = k_d \cdot B^d$, which was obtained at a previous step of the data processing. With this dual approach, as many MN size-dependent $\{A; S_{MN}\}$ intermediary datasets as available $\{A; B\}$ global datasets were obtained for each property A . These size-dependent datasets were merged into a 'final' $\{A; S_{MN}\}$ dataset to which a least squares linear regression analysis was performed for the $\ln(A) - \ln(S_{MN})$ transformation, yielding relationships of the type $\ln(A) = c \cdot \ln(S_{MN}) + k_c$, which were converted into the power relationships (EQ. 4-2).

$$A = k_c \cdot S_{MN}^c \quad (\text{EQ. 4-2})$$

The adequacy of the final power trendlines and the statistical significance of the correlations were assessed identically to the $\{A; B\}$ relationships derived directly from normalized experimental data, using the coefficient of determination r^2 (squared value of the Pearson's correlation coefficient) and a threshold of 0.01 on the p-value of the regression analysis, respectively. If $r^2 > 0.3$ (i.e. $r > 0.55$) and $p < 0.01$, another power trendline $S_{MN} = k_d \cdot A^d$ was fitted to the final dataset to describe the inverse relationship for $\{S_{MN}; A\}$ and used in the processing of the next-in-line property.

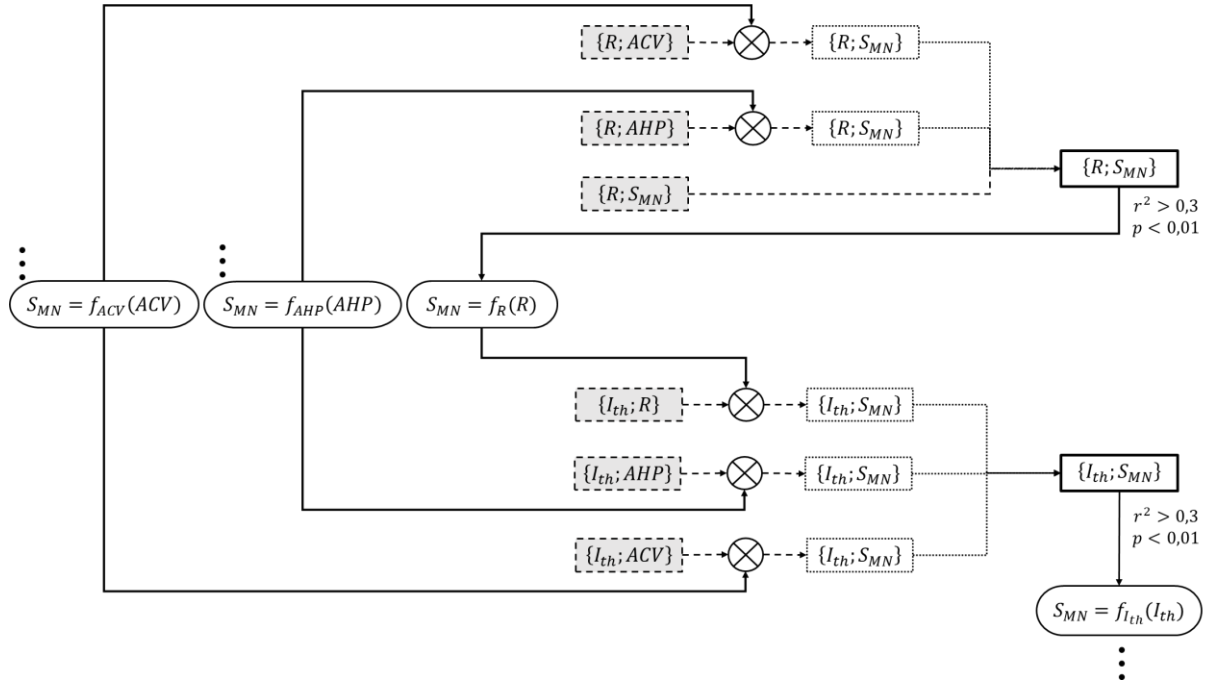


Figure 4-1. Detailed example for the process adopted to successively create the two final $\{R; S_{MN}\}$ and $\{I_{th}; S_{MN}\}$ datasets (right-side thick-solid contour rectangular boxes). These final datasets were obtained from respectively three and three normalized global datasets of experimental data obtained from the literature (dashed-contour grey-filled boxes) $\{R; ACV\}, \{R; AHP\}, \{R; S_{MN}\}$ and $\{I_{th}; R\}, \{I_{th}; AHP\}, \{I_{th}; ACV\}$. The $\{R; ACV\}$ and $\{R; AHP\}$ datasets were first transformed ($\otimes$ symbol) into two intermediary $\{R; S_{MN}\}$ datasets (dotted-contour boxes) by converting the ACV and AHP values to equivalent S_{MN} values with two ‘inverse’ $S_{MN} = f_{ACV}(ACV)$ and $S_{MN} = f_{AHP}(AHP)$ power relationships (oval boxes with triple dots), that had been previously obtained from two unshown steps that had yielded the final $\{ACV; S_{MN}\}$ and $\{AHP; S_{MN}\}$ datasets. The two intermediary $\{R; S_{MN}\}$ datasets were merged with the remaining global $\{R; S_{MN}\}$ dataset to yield the final $\{R; S_{MN}\}$ dataset, to which a power relationship of the form $R = k \cdot S_{MN}^e$ was fitted. If $r^2 > 0.3$ and $p < 0.01$, an ‘inverse’ power relationship $S_{MN} = f_R(R)$ (oval box) was further fitted to this final dataset. In a similar approach, the three normalized global datasets $\{I_{th}; R\}, \{I_{th}; AHP\}$ and $\{I_{th}; ACV\}$ were transformed with the three ‘inverse’ relationships into intermediary $\{I_{th}; S_{MN}\}$ datasets, which were merged to yield the final $\{I_{th}; S_{MN}\}$ dataset. An ‘inverse’ $S_{MN} = f_{I_{th}}(I_{th})$ power relationship was further derived to be used in the creation of the final $\{C; S_{MN}\}$ and $\{\tau; S_{MN}\}$ datasets in the next taken steps.

4.2.2.3. Normalized mathematical relationships between electrophysiological properties

When a final relationship with S_{MN} was obtained for two MN properties A and B (EQ. 4-2), i.e., $A = k_{cA} \cdot S_{MN}^{c_A}$ and $B = k_{cB} \cdot S_{MN}^{c_B}$, one of the expressions was mathematically inverted and a third empirical relationship (EQ. 4-3) was derived for the property pair $\{A; B\}$.

$$A = \frac{k_{cA}}{(k_{cB})^{c_B/c_A}} \cdot (B)^{c_A/c_B} = k_e \cdot B^e \quad (\text{EQ. 4-3})$$

This procedure was applied to all possible $\{A; B\}$ pairs in Table 4-1.

4.2.2.4. Validation of the normalized relationships

The normalized relationships were validated using a standard five-fold cross-validation procedure (Chollet, 2021). The data in each $\{A; B\}$ global normalized dataset was initially randomized and therefore made independent from the studies constituting it. Each shuffled global dataset was then split into five non-overlapping complementary partitions, each containing 20% of the data. Four partitions including 80% of the data were taken to constitute a training set from which the normalized relationships were built as described previously. The latter equations were validated against the last data partition, that includes the remaining 20% of the data and which is called test set in the following. To perform this validation, the final size-related relationships $A = k_c \cdot S_{MN}^c$ and relationships between electrophysiological properties $A = k_e \cdot B^e$ obtained with the training set were applied to the S_{MN} or B data in each test set, yielding predicted values A . It was then assessed to what extent the mathematical relationships predicted the test data A by calculating the normalized maximum error (nME), the normalized root mean squared error ($nRMSE$) and the coefficient of determination r_{pred}^2 between predicted and control values of A in each test set. This process was repeated for a total of 5 times by permutating the five data partitions and creating five different pairs of one training set and one test set. The nME , $nRMSE$ and r_{pred}^2 values were finally averaged across the five permutations. For each global dataset, the average r_{pred}^2 was compared to the r_{exp}^2 obtained from the power trendlines least-squared fitted to the control data in the log-log space. Once validated with this five-fold cross-validation method, the final normalized size-dependent relationships $A = k_c \cdot S_{MN}^c$ and relationships between electrophysiological properties $A = k_e \cdot B^e$ were computed for the complete global datasets.

4.2.2.5. Scaling of the normalized relationships

The $A = k_c \cdot S_{MN}^c$ and $A = k_e \cdot B^e$ normalized relationships were finally scaled to typical cat property values in three steps. First, it was assessed from the literature the fold-range over which each MN property was to vary. S_{MN} varied over a q_S^E -fold range, taken as the average across cat studies of the ratios of minimum and maximum values measured for S_{MN} (EQ. 4-4).

$$q_S^E = \frac{1}{N_{studies}} \sum_{i=1}^{N_{studies}} \left[\frac{(S_{MNi})_{max}}{(S_{MNi})_{min}} \right] \quad (\text{EQ. 4-4})$$

An experimental ratio q_A^E was similarly obtained for each electrophysiological property A . As $A = k_c \cdot S_{MN}^c$, any electrophysiological property A could theoretically vary over a $q_A^T = (q_S^E)^{|c|}$ -fold range, which was compared to q_A^E for consistency in the results. Then, a theoretical range $[A_{min}; A_{max}]$ of values were derived for each electrophysiological property A . Defining A_{min}^E and A_{max}^E as the

respective average of the minimum and maximum A -values across studies, it was enforced $\frac{A_{max}}{A_{min}} = q_A^T$ and $\frac{A_{min}+A_{max}}{2} = \frac{A_{min}^E+A_{max}^E}{2}$ so that the $[A_{min}; A_{max}]$ theoretical ranges were consistent with the normalized size-dependent relationships while best reproducing the experimental data from cat literature. A theoretical range for S_{MN} $[(S_{MN})_{min}; (S_{MN})_{max}]$ was similarly built over the q_S^E -fold range previously derived. Finally, the intercept k_c in the size-dependent relationships $A = k_c \cdot S_{MN}^c$ was scaled as defined in (EQ. 4-5).

$$\begin{cases} k_c = \frac{A_{min}}{(S_{MN})_{min}^c} & ; \text{ if } c > 0 \\ k_c = \frac{A_{max}}{(S_{MN})_{min}^c} & ; \text{ if } c < 0 \end{cases} \quad (\text{EQ. 4-5})$$

A similar approach was used to mathematically derive the intercept k_e and scale the relationships $A = k_e \cdot B^e$ between electrophysiological properties.

4.2.2.6. Extrapolation to rats and mice

It was finally assessed whether the $A = k_c \cdot S_{MN}^c$ and $A = k_e \cdot B^e$ scaled relationships derived from cat data accurately predicted rat and mouse quantities reported in other experimental studies. These mathematical relationships were applied to the S_{MN} or B data in each $\{A; B\}$ global dataset of absolute values obtained for rats and mice independently. The accuracy in predicting the quantity A was assessed for each available dataset with the same three validation metrics used to validate the normalized power trend lines: the normalized maximum error (nME), the normalized root-mean-square error ($nRMSE$) and the coefficient of determination r^2 between predicted and experimental values of A .

4.2.3. Definitions of MN Size S_{MN} and mU size S_{mU}

4.2.3.1. MN Size S_{MN}

The selected studies that performed both electrophysiological and morphometric measurements on the same MNs dominantly measured the MN soma diameter D_{soma} as an index of MN size. Therefore, $S_{MN} = D_{soma}$ was chosen in the described methodology. In the literature on spinal MNs in adult mammals, the size of a MN S_{MN} is also related to the measures of the somal cross-sectional area CSA_{soma} (Gao et al., 2009; Friesse et al., 2009; Deardorff et al., 2013; Mierzejewska-Krzyżowska et al., 2014; Dukkupati et al., 2018), soma surface area S_{soma} (Ulfhake & Kellerth, 1984; Brandenburg et al., 2020), axonal diameter D_{axon} (Cullheim, 1978), individual dendrite diameter $D_{dendrite}$ (Amendola & Durand, 2008; Carrascal et al., 2009; Mantilla et al., 2018), individual dendritic surface area $S_{dendrite}$ (Li, Yan et al., 2005; Obregón et al., 2009; Carrascal et al., 2009; Filipchuk & Durand, 2012; Kanjhan et

al., 2015), total dendritic surface area (Ulfhake, Brun & Cullheim, 1988; Amendola & Durand, 2008; Brandenburg et al., 2020) or total MN surface area S_{neuron} (Burke et al., 1982) defined as the summed soma and dendritic surface areas.

In the references (Zwaagstra & Kernell, 1981; Ulfhake & Kellerth, 1981), a linear correlation between D_{soma} and the average diameter of the stem dendrites $D_{dendrite_{mean}}$ has been reported ($r > 0.62$, population size in {40; 82} cells). Moreover, a linear or quasi-linear correlation has been found (Cullheim et al., 1987; Ulfhake, Brun & Cullheim, 1988; Moschovakis et al., 1991; Prakash et al., 2000; Li, Yan et al., 2005; Obregón et al., 2009; Mantilla et al., 2018) between the stem dendrite diameter $D_{dendrite}$ and the membrane surface area of the corresponding dendritic tree $S_{dendrite}$ ($r > 0.78$, population size in [33; 342] dendrites). Therefore, from these studies, an approximate linear correlation was assumed between D_{soma} and the average dendritic surface area $S_{dendrite_{mean}}$. Moreover, the number of dendritic trees $N_{dendrites}$ per cell increases with increasing soma surface S_{soma} (Brandenburg et al., 2018), and a linear correlation between D_{soma} or $S_{dendrites}$ with $N_{dendrites}$ has been observed (Zwaagstra & Kernell, 1981; Ulfhake, Brun & Cullheim, 1988; Amendola & Durand, 2008) ($r > 0.40$, population size in {14; 32; 87} cells). It was therefore assumed that D_{soma} and total dendritic surface area $S_{dendrite}^{tot}$ are linearly correlated. This assumption/approximation is consistent other conclusions of a linear correlations between D_{soma} and $S_{dendrite}^{tot}$ (Amendola & Durand, 2008; Brandenburg et al., 2020). Then, according to typical values of D_{soma} , S_{soma} and $S_{dendrites}^{tot}$ obtained from the studies previously cited, yielding $S_{dendrites}^{tot} \gg S_{soma}$, it was also assumed $S_{dendrites}^{tot} \approx S_{neuron}$. It is thus concluded that D_{soma} is linearly related to total neuron membrane area S_{neuron} , consistently with results by Burke et al. (1982) ($r = 0.61$, 57 cells).

For the above reasons and assumptions, the mathematical relationships $A = k_c \cdot S_{MN}^c$ derived previously, with $S_{MN} = D_{soma}$, were extrapolated with a gain to relationships between A and $S_{MN} = S_{neuron}$, notably permitting the definition of surface specific resistance R_m and capacitance C_m . Following the same method as described above, theoretical ranges $[(S_{neuron})_{min}; (S_{neuron})_{max}]$ were derived from additional morphometric studies on adult cat spinal alpha-motoneurons. The new relationships were extrapolated as $A = k_c \cdot D_{soma}^c = \frac{(S_{neuron})_{min}}{(D_{neuron})_{min}} \cdot S_{neuron}^c$.

It must however be highlighted that the conclusion of a linear correlation between D_{soma} and S_{neuron} , while plausible, is crude. Morphometric measurements of individual MNs are indeed difficult and suffer many limitations. MN staining, slice preparations and MN reconstruction and identification are complex experimental procedures requiring a large amount of work; the results from most of the cited studies thus rely on relatively small pools of investigated MNs. Most cited studies did not account for tissue shrinkage after dehydration, while some may have failed to assess the full dendritic trees from

MN staining techniques (Brandenburg et al., 2020), thus underestimating dendritic membrane measurements. Moreover, before automated tools for image segmentation, landmark mapping and surface tracking (Amendola & Durand, 2008; Obregón et al., 2009; Mierzejewska-Krzyżowska et al., 2014) were available, morphometric measurements were performed from the manual reproduction of the cell outline under microscope, yielding important operator errors reported to be of the order of $\sim 0.5\mu m$, i.e. $\sim 20\%$ stem diameter. In these studies, morphologic quantities were besides obtained from geometrical approximations of the 2D MN shape, such as modelling the individual dendritic branches as one (Cullheim et al., 1987) or more (Prakash et al., 2000) equivalent cylinders for the derivation of $S_{dendrite}$. Morphometric measurement approaches also varied between studies; for example, oval or circle shapes were best fitted by sight onto the soma outline and equivalent D_{soma} quantities were derived either as the mean of measured maximum and minimum oval diameters or as the equivalent diameter of the fitted circle. In these studies, CSA_{soma} and S_{soma} were therefore derived by classical equations of circle and spheric surface areas, which contradicts the results by Mierzejewska-Krzyżowska et al. (2014) obtained from surface segmentation of a linear relationship between D_{soma} and CSA_{soma} ($r = 0.94$, 527 MNs). Finally, no correlation between soma size and $N_{dendrites}$ was found (Ulfhake & Kellerth, 1981; Cullheim et al., 1987). Therefore, the linear correlation between D_{soma} and S_{neuron} is plausible but crude and requires awareness of several important experimental limitations and inaccuracies. Conclusions and predictions involving S_{neuron} should be treated with care in this study as these morphometric measurements lack the precision of the measures performed for the electrophysiological properties listed in Table 4-1.

4.2.3.2. Muscle unit size S_{mU}

To enable future comparisons between MN and mU properties, potential indices of the mU size S_{mU} suggested in the literature were here assessed. The size of a mU (S_{mU}) can be defined as the sum CSA^{tot} of the cross-sectional areas CSAs of the innervated fibres composing the mU. CSA^{tot} depends on the mU innervation ratio (IR) and on the mean CSA (CSA^{mean}) of the innervated fibres: $S_{mU} = CSA^{tot} = IR \cdot CSA^{mean}$. CSA^{tot} was measured in a few studies on cat and rat muscles, either indirectly by histochemical fibre profiling (Burke & Tsairis, 1973; Dum & Kennedy, 1980; Burke, 1981), or directly by glycogen depletion, periodic acid Schiff (PAS) staining and fibre counting (Burke et al., 1982; Rafuse et al., 1997). The mU tetanic force F^{tet} is however more commonly measured in animals. As the fibre mean specific force σ is considered constant among the mUs of one muscle in animals (Lucas et al., 1987; Enoka, 1995), the popular equation $F^{tet} = \sigma \cdot IR \cdot CSA^{mean}$ (Burke, 1981; Enoka, 1995) returns a linear correlation between F^{tet} and $IR \cdot CSA^{mean} = S_{mU}$ in mammals. Experimental results (Burke & Tsairis, 1973; Bodine et al., 1987; Chamberlain & Lewis, 1989; Kanda & Hashizume, 1992; Rafuse et al., 1997; Hegedus et al., 2007) further show a linear correlation between F^{tet} and IR

and between F^{tet} and CSA^{mean} . Consequently, F^{tet} , IR and CSA^{mean} are measurable, consistent, and linearly related measures of S_{mU} in animals, as summarized in Table 4-2.

Table 4-2: Measurable indices of MN and mU sizes in mammals. S_{MN} and S_{mU} are conceptual parameters which are adequately described by the measurable and linearly inter-related quantities reported in this table.

MN size (S_{MN})	mU size (S_{mU})
S_{neuron}	F^{tet}
D_{soma}	IR
	CSA^{mean}
	CSA^{tot}

4.2.4. Relationships between MN and mU properties

To assess whether the size-dependent relationships $A = k_c \cdot S_{MN}^c$ derived in this study were in accordance with Henneman's size principle of motor unit recruitment, a set of experimental studies was identified that concurrently measured a MN property B_{MN} (Table 4-1) and a muscle unit (mU) property A_{mU} for the same MU. The normalized global datasets obtained for the pairs $\{A_{mU}; B_{MN}\}$ were fitted with power trendlines, as previously described for MN properties, yielding $A_{mU} = k \cdot B_{MN}^b$ relationships. Using both the definition of S_{mU} (Table 4-2) and the $A = k_c \cdot S_{MN}^c$ relationships derived previously, the $A_{mU} = k \cdot B_{MN}^b$ relationships were then mathematically transformed into $S_{mU} = k \cdot S_{MN}^c$ relationships. If all c -values obtained from different $\{A_{mU}; B_{MN}\}$ pairs were of the same sign, it was concluded that mU and MN sizes were monotonically related. Considering the limited data available obtained from the literature, data obtained for MNs innervating different hindlimb muscles were merged. Also, cat, rat and mouse studies were processed independently but the resulting c -values were compared without regards to the related species.

4.3. Results

19, 6 and 4 studies were identified to respect our desired criteria on cats, rats and mice that reported processable experimental data for the morphometric and electrophysiological properties listed in Table 4-1. Additional publications including some from the past 10 years were identified but could not be included in this work as no processable data could be recovered. An additional 14 studies were found to perform concurrent MN and mU measurements on individual motor units. From the selected cat studies, the 17 pairs of MN properties and the 8 pairs of one MN and one mU property represented in the bubble diagram of Figure 4-2A were investigated.

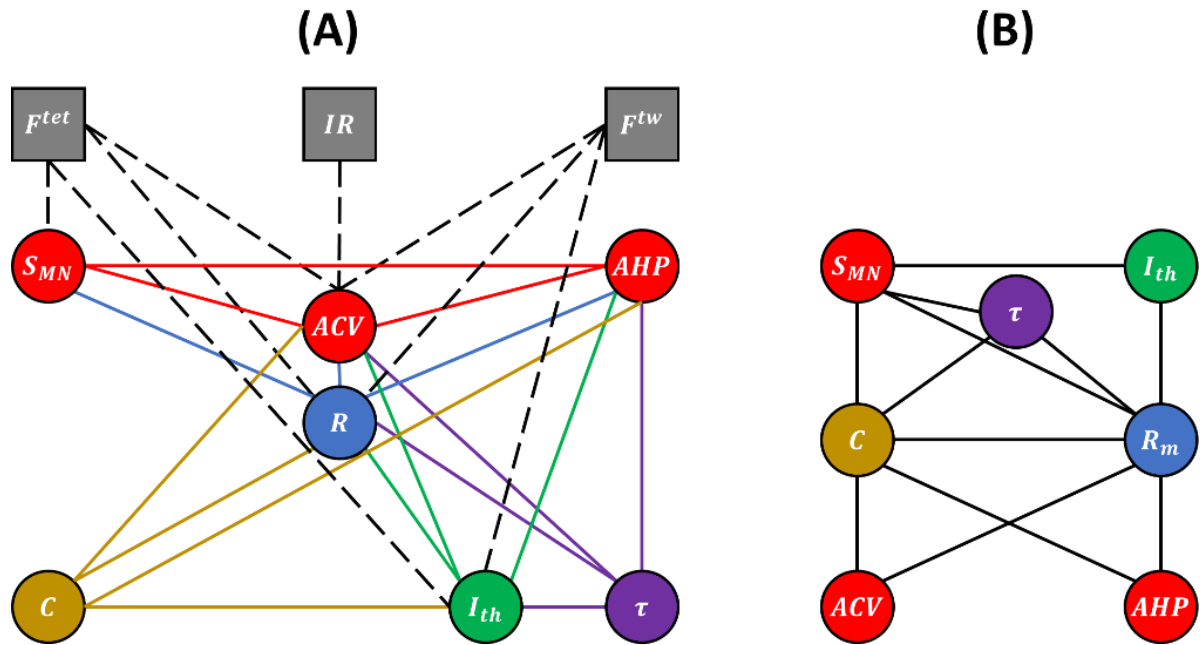


Figure 4-2: Experimental (A) and unknown (B) relations between MN and mU properties. (A) Bubble diagram representing the pairs of MN and/or mU properties that could be investigated in this study from the results provided by the 40 studies identified in our web search. MN and mU properties are represented in circle and square bubbles, respectively. Relationships between MN properties are represented by coloured connecting lines; the colours red, blue, green, yellow, and purple are consistent with the order $ACV, AHP, R, I_{th}, C, \tau$ in which the pairs were investigated (see Table 4-3 for mathematical relationships). Relationships between one MN and one mU property are represented by black dashed lines. (B) Bubble diagram representing the mathematical relationships proposed in this study between pairs of MN properties for which no concurrent experimental data is available.

4.3.1. Relationships between MN properties

4.3.1.1. Global datasets and data variability

The experimental data retrieved from the selected studies for the 17 pairs of MN properties drawn in Figure 4-2A were merged into 17 normalized global datasets plotted in Figure 4-3. Eight global datasets ($\{ACV; S_{MN}\}, \{AHP; ACV\}, \{R; S_{MN}\}, \{R; ACV\}, \{R; AHP\}, \{I_{th}; R\}, \{I_{th}; ACV\}, \{\tau; R\}$) included the data from two or more experimental studies.

These studies reported in each global dataset similar normalized property distributions, as visually displayed in the frequency histograms in Appendix Figure B-1. This was confirmed by the $mean_g \pm sd_g$ calculations described in the Methods of the four metrics range, mean, CoV, and $\frac{Me}{Md}$ displayed as error bars in Appendix Figure B-2. Out of the 64 $mean_g \pm sd_g$ calculations (four metrics for 16 property distributions of the 8 global datasets), 55 (86%) returned $sd_g < 10\%$ and $\frac{sd_g}{mean_g} < 0.15$. Otherwise, the distributions of only three, two and four properties showed sensibly higher inter-study variability in the global datasets for the range, mean and CoV metrics respectively, however still verifying $sd_g < 15\%$ and $\frac{sd_g}{mean_g} < 0.33$. Additional details are provided in Appendix B.1.

Then, as displayed in Figure 4-3 and reported in Table 4-3, power trendlines and their 95% confidence interval were fitted to the global datasets. All 17 trendlines were statistically significant and adequately represented by a power model $A = k_a \cdot B^a$ (p-value $< 10^{-5}$ and $r^2 \in [0.34; 0.72]$ for 15 datasets; p-value $< 10^{-5}$ and $r^2 \in \{0.24; 0.17\}$ for the $\{C; AHP\}$ and $\{C; CV\}$ datasets respectively). As displayed in green dotted lines in Figure 4-3, the confidence interval remained narrow for all datasets with the value of power a varying less than 0.4, except for the $\{I_{th}; ACV\}$ dataset, suggesting a low inter-study variability in associating the distributions of two properties. As displayed in Appendix Figure B-3, the selected studies however reported datasets of different sizes. Yet, out of the 35 experimental datasets constituting the 8 global datasets identified previously, only one and seven experimental datasets were identified to respectively constitute more than 50% and less than 10% of the global dataset they were included in. Therefore, despite a general imbalance towards the over-appearance of the work from Gustafsson & Pinter (1984ab) and a tendency towards overlooking some small datasets (Kernell, 1966; Burke & Ten Bruggencate, 1971; Sasaki, 1991), the remaining studies all played a significant role in the procedure to constitute the final datasets. Therefore, the inter-study data variability was globally low, and the data distributions reported in the experimental studies were confidently merged into the global datasets plotted in Figure 4-3. It is worth noting that three research groups provided 40%, 27% and 15% of the 2717 data points describing the $S_{MN}, ACV, AHP, R, I_{th}$ and τ property distributions in this study, while the C -related data was provided by one only research group, leading to potential group-specific methodological bias.

The merged property distributions obtained in the 17 global datasets showed low variability between global datasets, as displayed in Appendix Figure B-4. Indeed, $sd_g < 10\%$ and $\frac{sd_g}{mean_g} < 0.15$ was obtained for all four metrics for all the investigated properties across the global datasets they appeared in. Therefore, the property distributions were similar enough between global datasets to apply the process displayed in Figure 4-1 and derive the final size-dependent datasets.

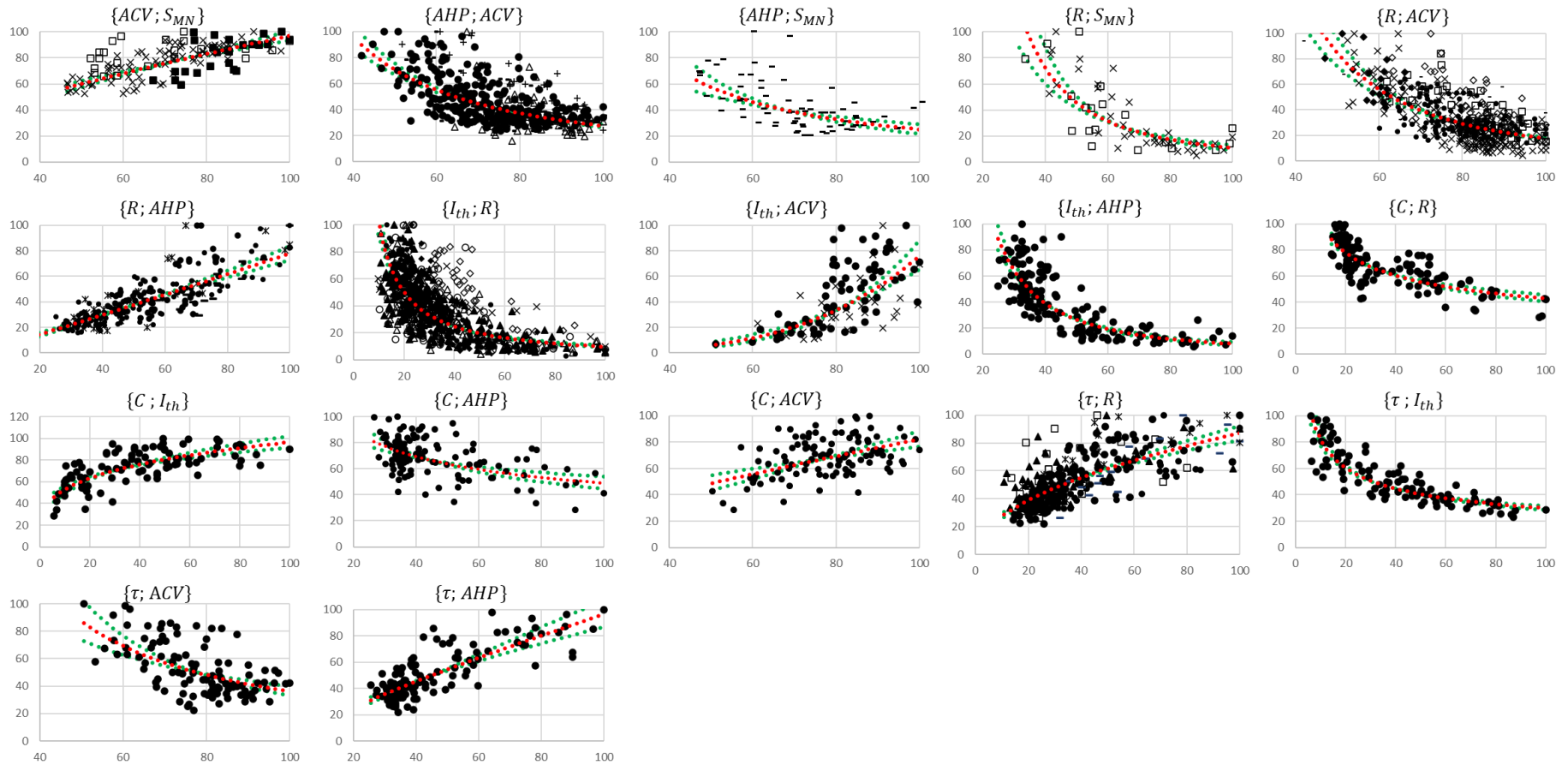


Figure 4-3. Normalized global datasets. These are obtained from the 19 studies reporting cat data that measured and investigated the 17 pairs of MN properties reported in Figure 4-2A. For each $\{A; B\}$ pair, the property A is read on the y axis and B on the x axis. For information, power trendlines $A = k \cdot B^a$ (red dotted curves) are fitted to the data of each dataset and reported in Table 4-3. The 95% confidence interval of the regression is also displayed for each dataset (green dotted lines). The studies are identified with the following symbols: $\bullet$ (Gustafsson, B., 1979; Gustafsson & Pinter, 1984a; Gustafsson & Pinter, 1984b), $\circ$ (Munson et al., 1986), $\blacktriangle$ (Zengel et al., 1985), $\triangle$ (Foehring et al., 1987), $\blacksquare$ (Cullheim, 1978), $\square$ (Burke, 1968; Burke & Ten Bruggencate, 1971; Burke et al., 1982), $\blacklozenge$ (Krawitz et al., 2001), $\diamond$ (Fleshman et al., 1981), $+$ (Eccles et al., 1958), $\times$ (Kernell, 1966; Kernell & Zwaagstra, 1981; Kernell & Monster, 1981), $-$ (Zwaagstra, B. & Kernell, 1980), $-$ (Sasaki, 1991), $\star$ (Pinter & Vanden Noven, 1989). The axes are given in % of the maximum retrieved values in the studies consistently with the Methods section.

4.3.1.2. Size-dependent normalized relationships

The 17 normalized global datasets were processed according to the procedure described in Figure 4-1 to derive normalized mathematical relationships between the electrophysiological properties listed in Table 4-1 and MN size S_{MN} . First, the normalized size-dependent relationship $ACV = 4.0 \cdot S_{MN}^{0.7}$, reported in Table 4-3, was derived from the trendline fitting of the normalized $\{ACV; S_{MN}\}$ global dataset, which was obtained from 3 studies (Cullheim, 1978; Kernell & Zwaagstra, 1981; Burke et al., 1982) and is represented in the upper-left panel in Figure 4-3. This resulted in a statistically significant relationship ($r^2 = 0.58$, $p\text{-value} < 10^{-5}$). A statistically significant ($r^2 = 0.59$, $p\text{-value} < 10^{-5}$) inverse relationship $S_{MN} = k_d \cdot ACV^d$ was also fitted to this dataset. Then, the $\{AHP; ACV\}$ dataset, represented in the second panel upper row in Figure 4-3 and obtained from 4 studies (Eccles et al., 1958; Zwaagstra, B. & Kernell, 1980; Gustafsson & Pinter, 1984b; Foehring et al., 1987) was transformed into a new $\{AHP; S_{MN}\}_1$ dataset by applying the priority derived $S_{MN} = k_d \cdot ACV^d$ relationship to the list of ACV -values. This transformed $\{AHP; S_{MN}\}_1$ dataset was then merged with the $\{AHP; S_{MN}\}_2$ dataset (third panel upper row in Figure 2) obtained from (Zwaagstra, B. & Kernell, 1980), yielding the final $\{AHP; S_{MN}\}_f$ dataset of $N = 492$ data points shown in Figure 4-4, second panel. A statistically significant ($r^2 = 0.58$, $p\text{-value} < 10^{-5}$) power trendline $AHP = 2.5 \cdot 10^4 \cdot S_{MN}^{-1.5}$ was fitted to the $\{AHP; S_{MN}\}_f$ dataset and is reported in Table 4-3. As before, a statistically significant ($r^2 = 0.38$, $p\text{-value} < 10^{-5}$) inverse relationship $S_{MN} = k_d \cdot AHP^d$ was also fitted to this dataset for future use. A similar procedure was applied to derive the normalized final relationships between $\{R, I_{th}, C, \tau\}$ and S_{MN} reported in the last column of Table 4-3. A statistically significant ($p\text{-value} < 10^{-5}$) correlation was obtained between each electrophysiological property $\{ACV, AHP, R, I_{th}, C, \tau\}$ and S_{MN} as reported in Table 4-3. With $r^2 \in [0.28; 0.58]$, it was obtained that $\{I_{th}, C, CV\}$ and $\{R, AHP, \tau\}$ respectively increased and decreased with increasing MN sizes S_{MN} , with slopes reported in Table 4-3 and plotted in Figure 4-5.

Table 4-3: Fitted experimental data of pairs of MN properties and subsequent normalized final size-related relationships. For information, the r^2 , p-value and the equation $A = k_a \cdot B^a$ are reported for each fitted global dataset. The normalized MN-size dependent relationships $A = k_c \cdot S_{MN}^c$ are mathematically derived from the transformation of the global datasets and from the power trendline fitting of the final datasets (N data points) as described in the Methods. The minimum and maximum values of k_a, k_c, a and c defining the 95% confidence interval of the regression are also reported in parenthesis for each global and final dataset. The r^2 values reported in this table are consistent with the r^2 values obtained when directly fitting the normalized experimental datasets with power regressions (see Appendix Table B-1).

MN property	$A = k_a \cdot B^a$ (normalized global datasets)					$A = k_c \cdot S_{MN}^c$ (final MN-size dependent datasets)				
A	Relationship	k_a	a	r^2	p-value	Reference studies	k_c	c	r^2	p-value N points
ACV	$ACV = k_a \cdot S_{MN}^a$	4.0 (2.5; 6.4)	0.7 (0.6; 0.8)	0.58	$p < 10^{-5}$	(Cullheim, 1978; Kernell & Zwaagstra, 1981; Burke et al., 1982)	4.0 (2.5; 6.4)	0.7 (0.6; 0.8)	0.58	$p < 10^{-5}$ 109
AHP	$AHP = k_a \cdot S_{MN}^a$	$6.1 \cdot 10^3$ ($1.2 \cdot 10^3$; $3.2 \cdot 10^4$)	-1.2 (-1.6; -0.8)	0.34	$p < 10^{-5}$	(Zwaagstra, B. & Kernell, 1980)	$2.5 \cdot 10^4$ ($1.2 \cdot 10^4$; $5.0 \cdot 10^4$)	-1.5 (-1.7; -1.3)	0.41	$p < 10^{-5}$ 492
	$AHP = k_a \cdot ACV^a$	$1.5 \cdot 10^4$ ($7.4 \cdot 10^3$; $2.9 \cdot 10^4$)	-1.4 (-1.5; -1.2)	0.41	$p < 10^{-5}$	(Eccles et al., 1958; Zwaagstra, B. & Kernell, 1980; Gustafsson & Pinter, 1984b; Foehring et al., 1987)				
R	$R = k_a \cdot S_{MN}^a$	$1.5 \cdot 10^5$ ($2.7 \cdot 10^4$; $7.9 \cdot 10^5$)	-2.1 (-2.5; -1.7)	0.61	$p < 10^{-5}$	(Kernell & Zwaagstra, 1981; Burke et al., 1982)	$9.6 \cdot 10^5$ ($4.1 \cdot 10^5$; $2.3 \cdot 10^6$)	-2.4 (-2.6; -2.2)	0.37	$p < 10^{-5}$ 745
	$R = k_a \cdot ACV^a$	$6.3 \cdot 10^5$ ($1.9 \cdot 10^5$; $2.1 \cdot 10^6$)	-2.3 (-2.6; -2.0)	0.38	$p < 10^{-5}$	(Kernell, 1966; Burke, 1968; Barrett & Crill, 1974; Kernell & Zwaagstra, 1981; Fleshman et al., 1981; Gustafsson & Pinter, 1984b; Sasaki, 1991)				
	$R = k_a \cdot AHP^a$	$6.2 \cdot 10^{-1}$ ($4.1 \cdot 10^{-1}$; $9.2 \cdot 10^{-1}$)	1.1 (0.9; 1.2)	0.65	$p < 10^{-5}$	(Gustafsson, B., 1979; Gustafsson & Pinter, 1984b; Foehring et al., 1987; Pinter & Vanden Noven, 1989; Sasaki, 1991)				
I_{th}	$I_{th} = k_a \cdot R^a$	$1.1 \cdot 10^3$ ($0.8 \cdot 10^3$; $1.3 \cdot 10^3$)	-1.0 (-1.1; -0.9)	0.37	$p < 10^{-5}$	(Kernell, 1966; Fleshman et al., 1981; Gustafsson & Pinter, 1984a; Zengel et al., 1985; Munson et al., 1986; Foehring et al., 1987; Krawitz et al., 2001)	$9.0 \cdot 10^{-4}$ ($4.7 \cdot 10^{-4}$; $1.7 \cdot 10^{-3}$)	2.5 (2.4; 2.7)	0.37	$p < 10^{-5}$ 722
	$I_{th} = k_a \cdot ACV^a$	$3.2 \cdot 10^{-6}$ ($1.3 \cdot 10^{-7}$; $8.2 \cdot 10^{-5}$)	3.7 (3.0; 4.4)	0.37	$p < 10^{-5}$	(Kernell & Monster, 1981; Gustafsson & Pinter, 1984a)				
	$I_{th} = k_a \cdot AHP^a$	$2.5 \cdot 10^4$ ($1.3 \cdot 10^4$; $4.8 \cdot 10^4$)	-1.7 (-1.9; -1.6)	0.60	$p < 10^{-5}$	(Gustafsson & Pinter, 1984a)				
C	$C = k_a \cdot R^a$	$2.4 \cdot 10^2$ ($2.0 \cdot 10^2$; $3.9 \cdot 10^2$)	-0.4 (-0.4; -0.3)	0.57	$p < 10^{-5}$	(Gustafsson & Pinter, 1984b)	1.2 (0.7; 2.0)	1.0 (0.9; 1.2)	0.28	$p < 10^{-5}$ 444
	$C = k_a \cdot I_{th}^a$	$2.9 \cdot 10^1$ ($2.4 \cdot 10^1$; $3.5 \cdot 10^1$)	0.3 (0.2; 0.3)	0.51	$p < 10^{-5}$	(Gustafsson & Pinter, 1984a)				
	$C = k_a \cdot AHP^a$	$2.8 \cdot 10^2$ ($1.8 \cdot 10^2$; $4.4 \cdot 10^2$)	-0.4 (-0.5; -0.3)	0.24	$p < 10^{-5}$	(Gustafsson & Pinter, 1984b)				
	$C = k_a \cdot ACV^a$	2.5 (0.7; 8.4)	0.8 (0.5; 1.0)	0.17	$p < 10^{-5}$	(Gustafsson & Pinter, 1984b)				
τ	$\tau = k_a \cdot R^a$	8.7 (7.2; 10.6)	0.5 (0.4; 0.6)	0.52	$p < 10^{-5}$	(Burke & Ten Bruggencate, 1971; Barrett & Crill, 1974; Gustafsson, B., 1979; Gustafsson & Pinter, 1984b; Zengel et al., 1985; Pinter & Vanden Noven, 1989; Sasaki, 1991)	$2.6 \cdot 10^4$ ($1.5 \cdot 10^4$; $4.5 \cdot 10^4$)	-1.5 (-1.6; -1.4)	0.46	$p < 10^{-5}$ 649
	$\tau = k_a \cdot AHP^a$	2.2 (1.3; 3.5)	0.8 (0.7; 1.0)	0.63	$p < 10^{-5}$	(Gustafsson & Pinter, 1984b)				
	$\tau = k_a \cdot I_{th}^a$	$2.3 \cdot 10^2$ ($1.9 \cdot 10^2$; $2.7 \cdot 10^2$)	-0.4 (-0.5; -0.4)	0.72	$p < 10^{-5}$	(Gustafsson & Pinter, 1984a)				
	$\tau = k_a \cdot ACV^a$	$1.2 \cdot 10^4$ ($2.2 \cdot 10^3$; $6.6 \cdot 10^4$)	-1.3 (-1.7; -0.9)	0.30	$p < 10^{-5}$	(Gustafsson & Pinter, 1984b)				

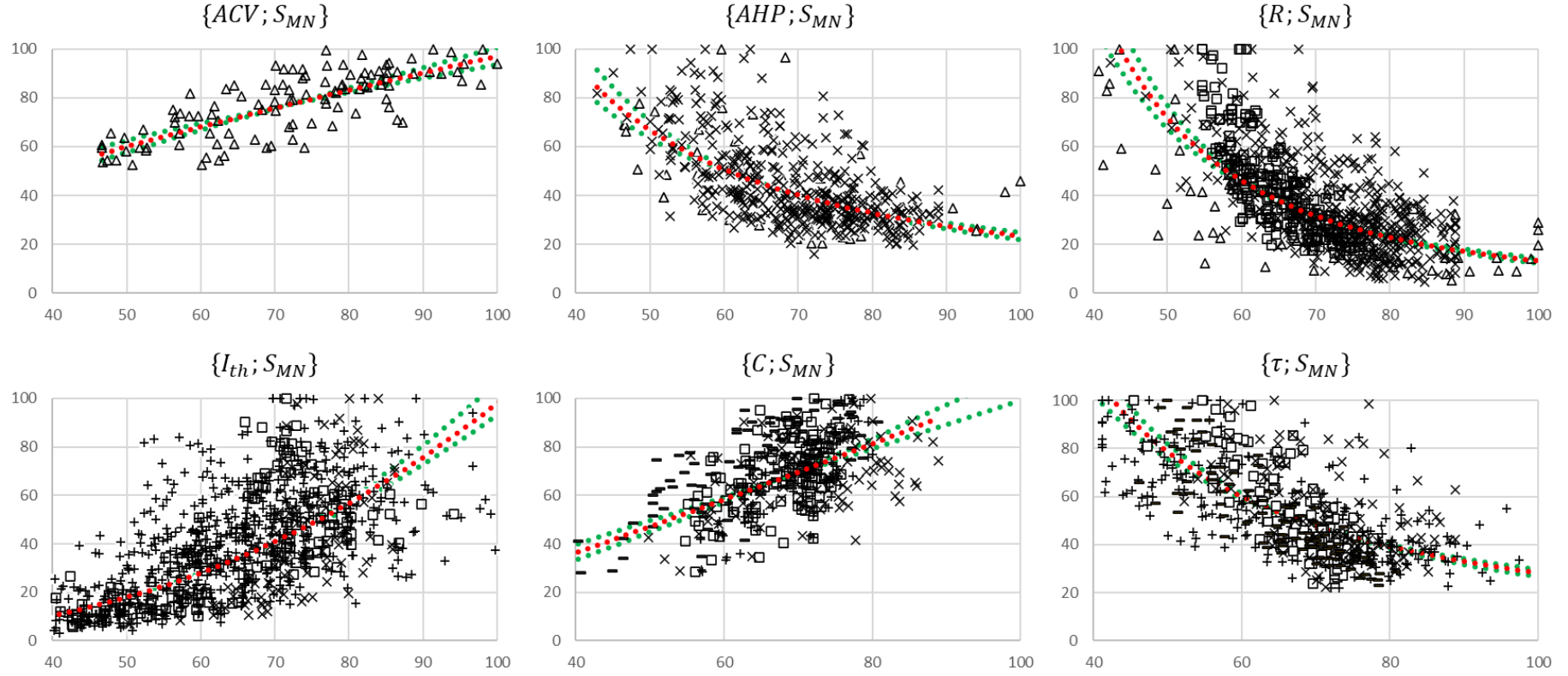


Figure 4-4. Normalized MN size-related final datasets. These are obtained from the 19 studies reporting cat data that concurrently measured at least two of the morphometric and electrophysiological properties listed in Table 4-1. For each $\{A; S_{MN}\}$ pair, the property A is read on the y axis and S_{MN} on the x axis. The power trendlines $A = k_c \cdot S_{MN}^c$ (red dotted curves) are fitted to each dataset and are reported in Table 4-3. The 95% confidence interval of the regression is also displayed for each dataset (green dotted lines). For each $\{A; S_{MN}\}$ plot, the constitutive sub-datasets $\{A; S_{MN}\}$ that were obtained from different global $\{A; B\}$ datasets are specified with the following symbols identifying the property B : $S_{MN} \leftrightarrow \Delta$, $ACV \leftrightarrow \times$, $AHP \leftrightarrow \square$, $R \leftrightarrow +$, and $I_{th} \leftrightarrow -$.

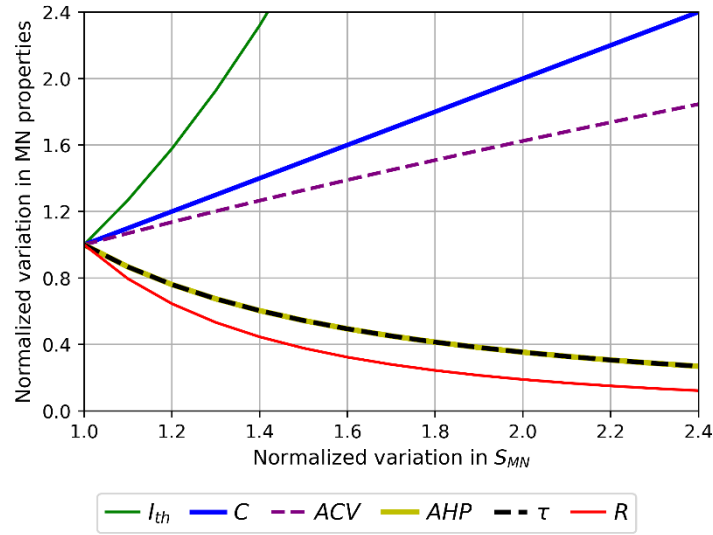


Figure 4-5. Normalized size-dependent behaviour of the MN properties ACV , AHP , R , I_{th} , C and τ . For displaying purposes, the MN properties are plotted in arbitrary units as power functions (intercept $k = 1$) of S_{MN} : $A = S_{MN}^C$ according to Table 4-3. The larger the MN size, the larger ACV , C and I_{th} in the order of increasing slopes, and the lower AHP , τ and R in the order of increasing slopes.

4.3.1.3. Normalized mathematical relationships between electrophysiological properties

The final size-dependent relationships reported in Table 4-3 were mathematically combined to yield normalized relationships between all electrophysiological properties listed in Table 4-1. As an example, $I_{th} = 9.4 \cdot 10^{-4} \cdot S_{MN}^{2.5}$ and $R = 9.6 \cdot 10^5 \cdot S_{MN}^{-2.4}$ were obtained in Table 4-3. When mathematically combined (with the latter mathematically inverted as $S_{MN} = 2.9 \cdot 10^2 \cdot R^{-0.4}$), these relationships yielded the normalized relationship between rheobase and input resistance $I_{th} = 1.5 \cdot 10^3 \cdot R^{-1}$. All normalized relationships between MN electrophysiological properties hence obtained are provided in Appendix Table B-2.

4.3.1.4. Validation of the normalized relationships

The normalized relationships reported in Appendix Table B-2 were obtained from the complete global datasets represented in Figure 4-3. A five-fold cross-validation of these relationships was performed, as described in the Methods section, and assessed with calculations of the nME , $nRMSE$ and r_{pred}^2 validation metrics averaged across the five permutations. As displayed in Figure 4-6, the normalized relationships obtained from the 17 training datasets predicted, in average across the five permutations, the experimental data from the test datasets with average nME between 52% and 300%, $nRMSE$ between 13% and 24%, and coefficients of determination r_{pred}^2 between 0.20 and 0.74 and of the same order of magnitude as the experimental r_{exp}^2 values.

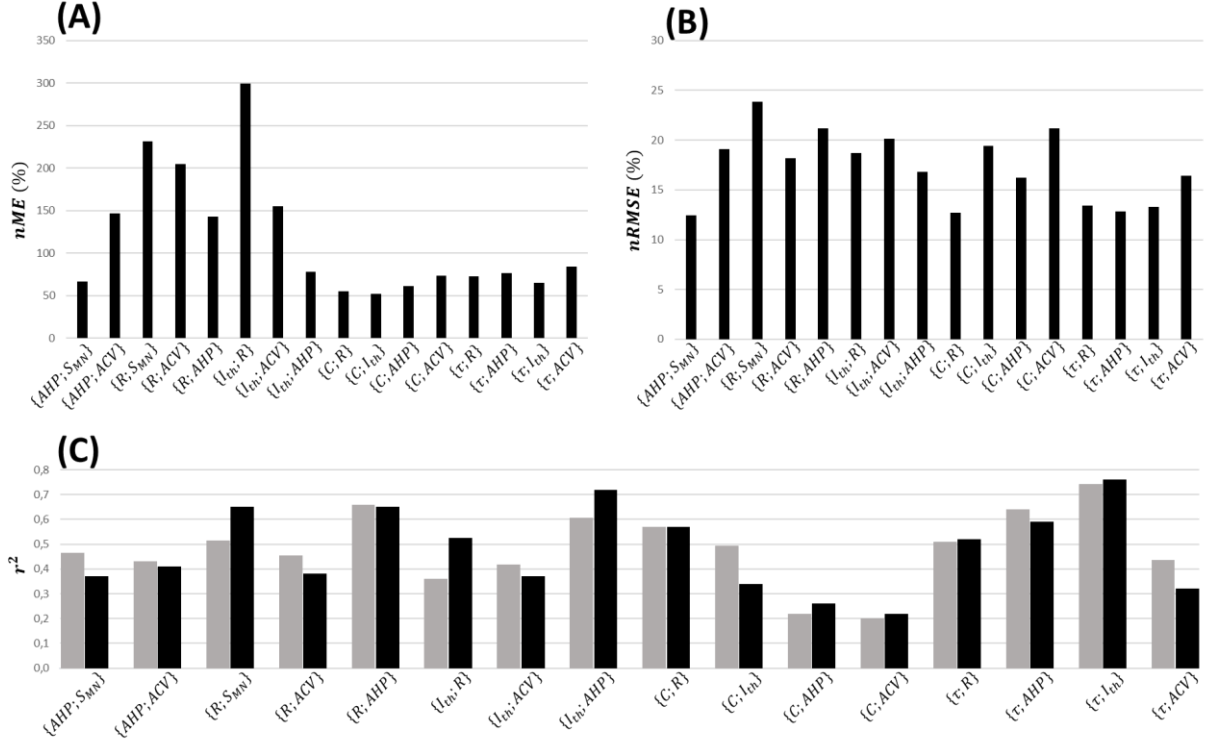


Figure 4-6. Five-fold cross-validation of the normalized mathematical relationships. Here are reported for each dataset the average values across the five permutations of (A) the normalized maximum error (nME), (B) the normalized root-mean-square error ($nRMSE$), and (C) coefficient of determination (r^2_{pred} , grey bars) which is compared with the coefficient of determination (r^2_{exp} , black bars) of the power trendline fitted to the log-log transformation of global experimental datasets directly.

4.3.1.5. Scaling of the normalized relationships

The normalized relationships provided in Appendix Table B-2 were finally scaled to typical cat data obtained from the literature following the procedure described in the Methods section. D_{soma} and S_{neuron} were found to vary in cats over an average $q_S^E = 2.4$ -fold range according to a review of the morphometric studies reported in the two first lines of Appendix Table B-3. q_S^E may however be underestimated for reasons discussed in Appendix B.2. Then, the empirical q_A^E and theoretical q_A^T ratios, defined in the Methods, were calculated for each MN electrophysiological property A and are reported in Appendix Table B-4. For example, MN resistance R was found to vary over an average $q_R^E = 10.9$ -fold range in a MN pool according to the literature, while the theoretical fold range $q_R^T = (q_S^E)^{|c_R|} = 2.4^{2.4} = 8.4$ was obtained from the $R = 9.6 \cdot 10^5 \cdot S_{MN}^{-2.4}$ normalized relationship previously derived when a 2.4-fold range is set for S_{MN} . As shown in Appendix Table B-4, $\frac{q_A^E}{q_A^T} \in [0.8; 1.3]$ for the MN properties ACV, AHP, C and τ , while the theoretical ranges for R and I_{th} span over a narrower domain than expected from experimental results ($q_R^T = 8.4 \ll 10.9 = q_R^E$ and $q_{I_{th}}^T = 9.1 \ll 16.3 = q_{I_{th}}^E$). This suggests that the range of variation of R and I_{th} is not entirely explained by the

variation in MN size S_{MN} , and that MN excitability is not only determined by S_{MN} , as reviewed in Powers & Binder (2001).

The normalized relationships were finally scaled using the theoretical ranges reported in the last column of Appendix Table B-4. Taking the $\{I_{th}; R\}$ pair as example, as $I_{th} = 1.4 \cdot 10^3 \cdot R^{-1}$ (normalized relationship derived previously and provided in Appendix Table B-2), $R \in [0.5; 4.0] \cdot 10^6 \Omega$ and $I_{th} \in [3.9; 35.0] \cdot 10^{-9} A$ (Appendix Table B-4), it was directly obtained from the Methods that $I_{th} = \frac{2.7 \cdot 10^{-2}}{R}$ in SI base units. A similar approach yielded the final mathematical relationships reported in Table 4-4 between all MN electrophysiological and morphometric properties $ACV, AHP, R, I_{th}, C, \tau$ and S_{MN} . All constants and relationships are given in SI base units.

4.3.1.6. Predicting correlations between MN properties

With this approach, the correlations between MN properties that were never concurrently measured in the literature are predicted, as displayed in Figure 4-2B. Such unknown relationships were indirectly extracted from the combination of known relationships (Table 4-3) and typical ranges of values obtained from the literature for these properties. Due to the prior five-fold cross-validation of the relationships in Table 4-4, these findings are reliable as indirectly consistent with the literature data processed in this study.

Table 4-4. Mathematical empirical cat relationships between the MN properties S_{neuron} , D_{soma} , R , R_m , C , τ , I_{th} , AHP and ACV . Each column provides the relationships between one and the eight other MN properties. If one property is known, the complete MN profile can be reconstructed by using the pertinent line in this table. All constants and properties are provided in SI base units (meters, seconds, ohms, farads and amperes). The relationships involving R_m were obtained from theoretical relationships involved in Rall's cable theory (See Discussion).

	$S_{neuron}[m^2]$	$D_{soma}[m]$	$R[\Omega]$	$R_m[\Omega \cdot m^2]$	$C[F]$	$\tau[s]$	$I_{th}[A]$	$AHP[s]$	$ACV[m \cdot s^{-1}]$
$S_{neuron}[m^2]$		$D_{soma} = 1.8 \cdot 10^2 \cdot S_{neuron}$	$R = \frac{1.7 \cdot 10^{-10}}{S_{neuron}^{2.4}}$	$R_m = \frac{1.0 \cdot 10^{-10}}{S_{neuron}^{1.4}}$	$C = 1.3 \cdot 10^{-2} \cdot S_{neuron}$	$\tau = \frac{1.0 \cdot 10^{-12}}{S_{neuron}^{1.5}}$	$I_{th} = 3.8 \cdot 10^8 \cdot S_{neuron}^{2.5}$	$AHP = \frac{1.0 \cdot 10^{-11}}{S_{neuron}^{1.5}}$	$ACV = 3.0 \cdot 10^6 \cdot S_{neuron}^{0.7}$
$D_{soma}[m]$	$S_{neuron} = 5.5 \cdot 10^{-3} \cdot D_{soma}$		$R = \frac{5.1 \cdot 10^{-5}}{D_{soma}^{2.4}}$	$R_m = \frac{1.7 \cdot 10^{-7}}{D_{soma}^{1.4}}$	$C = 7.9 \cdot 10^{-5} \cdot D_{soma}$	$\tau = \frac{2.3 \cdot 10^{-9}}{D_{soma}^{1.5}}$	$I_{th} = 7.8 \cdot 10^2 \cdot D_{soma}^{2.5}$	$AHP = \frac{2.7 \cdot 10^{-8}}{D_{soma}^{1.5}}$	$ACV = 8.1 \cdot 10^4 \cdot D_{soma}^{0.7}$
$R[\Omega]$	$S_{neuron} = \frac{9.5 \cdot 10^{-5}}{R^{0.4}}$	$D_{soma} = \frac{1.7 \cdot 10^{-2}}{R^{0.4}}$		$R_m = 5.7 \cdot 10^{-5} \cdot R^{0.6}$	$C = \frac{1.5 \cdot 10^{-6}}{R^{0.4}}$	$\tau = 9.6 \cdot 10^{-7} \cdot R^{0.6}$	$I_{th} = \frac{2.7 \cdot 10^{-2}}{R}$	$AHP = 1.3 \cdot 10^{-5} \cdot R^{0.6}$	$ACV = \frac{4.9 \cdot 10^3}{R^{0.3}}$
$R_m[\Omega \cdot m^2]$	$S_{neuron} = \frac{1.5 \cdot 10^{-7}}{R_m^{0.7}}$	$D_{soma} = \frac{2.6 \cdot 10^{-5}}{R_m^{0.7}}$	$R = 6.8 \cdot 10^6 \cdot R_m^{1.7}$		$C = \frac{1.8 \cdot 10^{-9}}{R_m^{0.7}}$	$\tau = 1.4 \cdot 10^{-2} \cdot R_m$	$I_{th} = \frac{2.2 \cdot 10^{-9}}{R_m^{1.8}}$	$AHP = 2.2 \cdot 10^{-1} \cdot R_m^{1.1}$	$ACV = \frac{5.4 \cdot 10^1}{R_m^{0.5}}$
$C[F]$	$S_{neuron} = 1.2 \cdot 10^2 \cdot C$	$D_{soma} = 2.1 \cdot 10^4 \cdot C$	$R = \frac{1.5 \cdot 10^{-15}}{C^{2.5}}$	$R_m = \frac{1.7 \cdot 10^{-13}}{C^{1.5}}$		$\tau = \frac{8.6 \cdot 10^{-16}}{C^{1.5}}$	$I_{th} = 6.5 \cdot 10^{13} \cdot C^{2.6}$	$AHP = \frac{7.7 \cdot 10^{-15}}{C^{1.6}}$	$ACV = 8.2 \cdot 10^7 \cdot C^{0.7}$
$\tau[s]$	$S_{neuron} = \frac{8.4 \cdot 10^{-9}}{\tau^{0.7}}$	$D_{soma} = \frac{1.5 \cdot 10^{-6}}{\tau^{0.7}}$	$R = 7.1 \cdot 10^9 \cdot \tau^{1.6}$	$R_m = 3.6 \cdot 10^1 \cdot \tau$	$C = \frac{1.6 \cdot 10^{-10}}{\tau^{0.7}}$		$I_{th} = \frac{1.6 \cdot 10^{-12}}{\tau^{1.7}}$	$AHP = 1.7 \cdot 10^1 \cdot \tau$	$ACV = \frac{7.5}{\tau^{0.5}}$
$I_{th}[A]$	$S_{neuron} = 4.0 \cdot 10^{-4} \cdot I_{th}^{0.4}$	$D_{soma} = 7.1 \cdot 10^{-2} \cdot I_{th}^{0.4}$	$R = \frac{3.1 \cdot 10^{-2}}{I_{th}}$	$R_m = \frac{7.4 \cdot 10^{-6}}{I_{th}^{0.6}}$	$C = 5.9 \cdot 10^{-6} \cdot I_{th}^{0.4}$	$\tau = \frac{1.2 \cdot 10^{-7}}{I_{th}^{0.6}}$		$AHP = \frac{1.5 \cdot 10^{-6}}{I_{th}^{0.6}}$	$ACV = 1.3 \cdot 10^4 \cdot I_{th}^{0.3}$
$AHP[s]$	$S_{neuron} = \frac{5.5 \cdot 10^{-8}}{AHP^{0.7}}$	$D_{soma} = \frac{9.8 \cdot 10^{-6}}{AHP^{0.7}}$	$R = 7.5 \cdot 10^7 \cdot AHP^{1.6}$	$R_m = 2.5 \cdot AHP^{0.9}$	$C = \frac{9.7 \cdot 10^{-10}}{AHP^{0.6}}$	$\tau = 6.2 \cdot 10^{-2} \cdot AHP$	$I_{th} = \frac{1.8 \cdot 10^{-10}}{AHP^{1.7}}$		$ACV = \frac{2.7 \cdot 10^1}{AHP^{0.5}}$
$ACV[m \cdot s^{-1}]$	$S_{neuron} = 4.6 \cdot 10^{-10} \cdot ACV^{1.4}$	$D_{soma} = 8.3 \cdot 10^{-8} \cdot ACV^{1.4}$	$R = \frac{8.3 \cdot 10^{12}}{ACV^{3.5}}$	$R_m = \frac{2.3 \cdot 10^3}{ACV^{2.1}}$	$C = 9.0 \cdot 10^{-12} \cdot ACV^{1.4}$	$\tau = \frac{7.4 \cdot 10^1}{ACV^{2.1}}$	$I_{th} = 1.1 \cdot 10^{-15} \cdot ACV^{3.6}$	$AHP = \frac{1.4 \cdot 10^3}{ACV^{2.2}}$	

4.3.1.7. Extrapolation to rats and mice

It was assessed whether the scaled relationships obtained from cat data and reported in Table 4-4 could be successfully applied to rat and mouse data. A global $\{I_{th}; R\}$ dataset was obtained from 5 studies focusing on rats reported in Appendix Table B-5, while four mice studies reported in Appendix Table B-5 provided processable data for the pairs $\{I_{th}; R\}$, $\{\tau; R\}$ and $\{C; R\}$. Only data from control groups of wild-type animals were considered while data on mutated, operated, or trained animals were disregarded. Because the information on the age distribution of the retrieved datasets is not available, data from adults rats and mice of unknown age were used, despite well-known age-related variations in the MN electrophysiological properties in these animals (Highlander et al., 2020; Huh et al., 2021). Like the cat data, low variability was observed between the R and I_{th} distributions reported by different studies in the rat and mouse $\{I_{th}; R\}$ datasets. Figure 4-7 reports the global datasets for all 4 pairs of properties and the predictions obtained with the cat relationships reported in Table 4-4. As displayed in Appendix Table B-5, r_{pred}^2 and r_{exp}^2 values were close for all 4 datasets, with a maximum 12.5% difference for $\{\tau; R\}$ in mice. $nRMSE$ values remained low in both rat and mouse $\{I_{th}; R\}$ datasets, while being substantially higher in both $\{\tau; R\}$ and $\{C; R\}$ datasets in mice because of an inter-species-specific offset in the relationships.

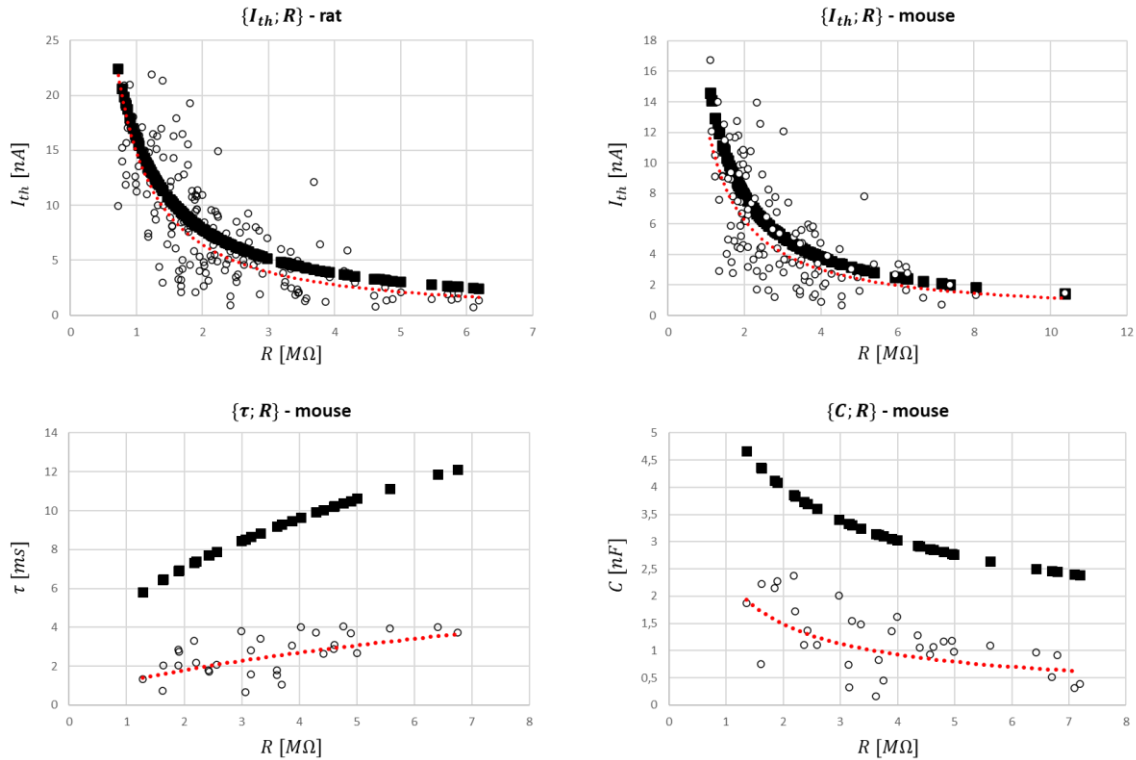


Figure 4-7. Global datasets for rat and mouse species and predictions of the MN properties with the cat mathematical relationships (Table 4-4). They were obtained from the 5 studies reporting data on rats and the 4 studies presenting data on mice reported in Appendix Table B-5 that measured the $\{I_{th}; R\}$, $\{\tau; R\}$ and $\{C; R\}$ pairs of MN electrophysiological properties. I_{th} , R , τ , C are given in nA , $M\Omega$, ms and nF respectively. The experimental data ($\circ$ symbol) is fitted with a power trendline (red dotted curve) and compared to the predicted quantities obtained with the scaled cat relationships in Table 4-4 ($\blacksquare$ symbol).

4.3.2. Relationships between MN and mU properties

As shown in Figure 4-2A, eight pairs of one MN and one mU property were investigated in 14 studies in the literature in cats and rats. As remarked by Heckman & Enoka (2012) and Manuel et al. (2019), most recent studies were performed on mice, while most dated were dominantly performed on cats. One study on the rat gastrocnemius muscle (Kanda & Hashizume, 1992) indicated no correlation between IR and CV . However, after removing from the dataset 2 outliers that fell outside two standard deviations of the mean IR data, a statistically significant correlation ($p < 0.05$) between IR and CV was successfully fitted with a power trendline ($r^2 = 0.43$). Eight studies, dominantly focusing on the cat soleus and medial gastrocnemius muscles, found a strong correlation between F^{tet} and CV , while one study showed a significant correlation for the pair $\{F^{tet}; R\}$ in both the cat tibialis anterior and extensor digitorum longus muscles. One study (Burke et al., 1982) on the cat soleus, medial and lateral gastrocnemius muscles inferred a statistically significant correlation for $\{F^{tet}; S_{MN}\}$. In mice, both R and I_{th} were significantly related to muscle tetanic and twitch forces. As F^{tet} , F^{tw} and IR are reliable indices of S_{mU} as discussed in the Methods, the MN size-dependent relationships in Table 4-3 return eight $S_{mU} = k \cdot S_{MN}^c$ relationships between mU and MN properties (Table 4-5). A 2.8-fold range in positive c -values $c \in [2.4; 6.6]$ (mean 3.8 ± 1.5 s.d.) was obtained between studies. This result infers that mU and MN size are positively related in cats, mice and potentially in rats.

Table 4-5. Fitted experimental data of pairs of one mU and one MN property and subsequent $S_{mU} \propto S_{MN}^c$ relationships. The r^2 , p-value and the equation $A \propto B^c$ of the trendlines are reported for each pair of properties. In the last column, prior knowledge is used to derive $S_{mU} \propto S_{MN}^c$ relationships.

Species	$A = k \cdot B^c$ (fitted relationships)					S_{mU} $= k \cdot S_{MN}^c$ (final relationships)
	Relationship	c	r^2	p-value	Reference studies	c
Rat	IR $= k \cdot CV^c$	3.4	0.45	6 $\cdot 10^{-3}$	(Kanda & Hashizume, 1992)	2.4
	F^{tw} $= k \cdot CV^c$	9.4	0.43	p $< 10^{-5}$	(Knott et al., 1971)	6.6
Cat	F^{tet} $= k \cdot CV^c$	7.2	0.37	p $< 10^{-5}$	(McPhedran et al., 1965; Wuerker et al., 1965; Appelberg & Emonet-Dénand, 1967; Proske & Waite, 1974; Bagust, 1974; Jami & Petit, 1975; Stephens & Stuart, 1975; Burke et al., 1982; Emonet-Dénand et al., 1988)	5.0
	F^{tet} $= k \cdot R^c$	-1.3	0.27	6 $\cdot 10^{-5}$	(Dum & Kennedy, 1980)	3.2
	F^{tet} $= k \cdot S_{MN}^c$	2.0	0.21	2 $\cdot 10^{-2}$	(Burke et al., 1982)	2.0
Mouse	$F^{tw} = k \cdot R^c$	-2.1	0.42	p $< 10^{-5}$	(Manuel & Heckman, 2011; De Lourdes Martínez-Silva et al., 2018)	5.1
	F^{tw} $= k \cdot I_{th}^c$	1.3	0.64	2 $\cdot 10^{-4}$	(Manuel & Heckman, 2011)	3.3
	F^{tet} $= k \cdot I_{th}^c$	1.0	0.80	6 $\cdot 10^{-2}$	(Manuel & Heckman, 2011)	2.5
Mean $\pm$sd						3.8 $\pm$ 1.5

4.4. Discussion

The normalized data (Figure 4-3) from previous cat experimental studies was processed to extract mathematical relationships (Table 4-4) between several MN electrophysiological and morphological properties (Table 4-1), providing a clear summary of previously known qualitative inter-relations between these MN properties. Table 4-4 is a new convenient tool for neuroscientists, experimenters, and modelers. Besides obtaining significant quantitative correlations between MN electrophysiological properties and MN size (Table 4-3, Figure 4-4, Figure 4-5), it was established (Table 4-4) and validated (Figure 4-6) a comprehensive mathematical framework that models an extensive set of experimental datasets available in the literature, quantitatively links all the pairs of the MN properties in Table 4-1, and is consistent with Rall's cable theory, as discussed in the following. This framework is directly applicable, although with limitations, to other mammalian species (Figure 4-7), and is used along with other measurements of mU properties (Table 4-5) to discuss the Henneman's size principle.

4.4.1. Consistency of the relationships with previous empirical results and Rall's theory

Agreement with other experimental findings

The mathematical relationships derived in Table 4-4 are first in strong agreement with the relative variations between MN properties that were reported in review papers and are listed in the Introduction section. Some combinations of the relationships in Table 4-4 are besides consistent with other cat experimental data that were not included in the global datasets and were not used for deriving the relationships. For example, the three relationships in (EQ. 4-6), when combined as (EQ. 4-7), are consistent with both the relationship $\frac{I^{th}}{C} = k \cdot \tau^{-1}$ experimentally observed by Gustafsson & Pinter (1985) and $\frac{1}{R \cdot S_{MN}} = k \cdot ACV^{1.9}$ measured by Kernell & Zwaagstra (1981).

$$\begin{aligned} I^{th} &= k \cdot \tau^{-1.7} \\ \frac{1}{S_{MN}} &= k \cdot ACV^{-1.4} \\ \frac{1}{R} &= k \cdot ACV^{3.5} \end{aligned} \tag{EQ. 4-6}$$

$$\begin{aligned} \frac{I^{th}}{C} &= k \cdot \tau^{-1} \\ \frac{1}{R \cdot S_{MN}} &= k \cdot ACV^{2.1} \end{aligned} \tag{EQ. 4-7}$$

The stronger-than-linear inverse relationship $R = k \cdot S_{MN}^{-2.4}$ is also consistent with the phenomenological conclusions by Kernell & Zwaagstra (1981).

Agreement with Rall's cable theory - R_m

Some standard equations resulting from Rall's cable theory can be derived with a combination of the relationships in Table 4-4, assuming the MNs as equivalent cylinders with spatial uniformity of both C_m and R_m . For example, taking $\frac{\tanh(L)}{L} = 0.5$, which is in the typical [0.48; 0.76] range reported in the literature (Powers & Binder, 2001), the relation $C = \frac{\tau}{R} \cdot \frac{L}{\tanh(L)}$ (eq.1. in Gustafsson & Pinter (1984a)) is obtained when combining the $R - S_{neuron}$, $C - S_{neuron}$ and $\tau - S_{neuron}$ relationships. Also, taking $\frac{\tanh(L)}{L} = 0.6$ (standard value provided in Powers & Binder (2001)), the $R - S_{neuron}$ equation in Table 4-4 applied to $R = \frac{R_m}{S_{neuron}} \cdot \frac{L}{\tanh(L)}$ in Rall (1977) yields $R_m = \frac{1.0 \cdot 10^{-10}}{S_{neuron}^{1.4}}$. This $R_m - S_{neuron}$ relationship is consistent with the observations in Powers & Binder (2001) that smaller MNs have larger R_m values. Also, considering that $q_S^E = 2.4$ and $S_{neuron} \in [0.18; 0.44] \text{ mm}^2$ (Appendix Table B-3), $R_m = \frac{1.0 \cdot 10^{-10}}{S_{MN}^{1.4}}$ makes R_m vary over the 3.5-fold theoretical range [0.12; 0.44] $\Omega \cdot m^2$ in the MN pool. This is highly consistent both with the $q_A^E = 3.2$ -fold range and the $[A_{min}^E; A_{max}^E] = [0.16; 0.59] \Omega \cdot m^2$ mean range previously reported in the literature (Albuquerque & Thesleff, 1968; Barrett & Crill, 1974; Burke et al., 1982; Gustafsson & Pinter, 1984b). Also, $R_m = \frac{1.0 \cdot 10^{-10}}{S_{neuron}^{1.4}}$ yields $R_m = 2.5 \cdot AHP^{0.9}$ from the relationships in Table 4-4, which is consistent with the indirect conclusion of a positive correlation between R_m and AHP in Gustafsson & Pinter (1984b). Finally, $R = \frac{0.6 R_m}{S_{neuron}}$ and $R_m = \frac{1.0 \cdot 10^{-10}}{S_{neuron}^{1.4}}$ support the statement that the variations in R_m in the MN pool may be as important as cell size S_{neuron} in determining MN excitability (Gustafsson & Pinter, 1985), although the variation of R in a MN pool may be also related to cell geometry (Gustafsson & Pinter, 1984b), which was not investigated in this study. This is consistent with the results in Appendix Table B-4, which demonstrate that the range of variation q_S^E of S_{MN} does not entirely explain the ranges of variation q_R^E and $q_{I_{th}}^E$ of R and I_{th} , and that MN excitability is not only determined by MN size.

Mathematical relationships for R_m

Because of the aforementioned consistency with previous findings and with Rall's theory, the remaining relationships between R_m and the other MN properties were calculated from $R_m = \frac{1.0 \cdot 10^{-10}}{S_{neuron}^{1.4}}$, following the same Methods as before, and added to Table 4-4. Importantly, R_m might however not be uniform across the somatodendritic membrane, according to results from completely

reconstructed MNs (Fleshman et al., 1988), and might be positively correlated to the somatofugal distance (Fleshman et al., 1983), with dendritic R_m being higher than somatic R_m (Barrett & Crill, 1974; Powers & Binder, 2001; Kernell, 2006) by a factor 100-300 (Clements & Redman, 1989). This contradicts the assumptions of membrane isopotentiality necessary to apply Rall's cable theory. The relationships in Table 4-4, which were obtained from measurements in the soma where R_m is mainly constant, may thus not directly extrapolate to the dendritic regions of the MNs. Yet, the dimensionless variations of the average across MN surface of R_m with the other MN properties may still be valid (Kernell, 2006).

Agreement with Rall's cable theory - C

The equations in Table 4-4 support the notion that MNs behave like resistive-capacitive systems, as demonstrated by the combination of the $R - \tau$ and $C - \tau$ relationships in Table 4, which yields $\tau^{0.9} = 0.8 \cdot RC$, close to the theoretical equation $\tau = RC \cdot \frac{\tanh(L)}{L}$ derived from Rall's theory. Also, Table 4-4 directly yields, from the combination of $R_m = \frac{1.0 \cdot 10^{-10}}{S_{neuron}^{1.4}}$ with the other relationships, $\tau = 1.4 \cdot 10^{-2} \cdot R_m$, which is exactly eq.2.15 in Rall (1977) ($\tau = R_m C_m$) with a constant value for $C_m = 1.4 \cdot 10^{-2} F \cdot m^{-2}$ that is consistent with the literature.

The relationship $C = 1.3 \cdot 10^{-2} \cdot S_{neuron}$ obtained in Figure 4-4 and reported in Table 4-4 is in agreement with the widely accepted proportional relationship between MN membrane capacitance and surface area, when spatial uniform values of R_m and C_m are assumed. It is worth noting that this proportional relationship was obtained in this study without simultaneous empirical measurements of S_{MN} and C . The values of C were obtained from Rall's $C = \frac{\tau}{R} \cdot \frac{L}{\tanh(L)}$ equation (Gustafsson & Pinter, 1984b) and the corresponding values of S_{MN} were indirectly obtained from the final datasets $\{ACV; S_{MN}\}$, $\{AHP; S_{MN}\}$, $\{R; S_{MN}\}$ and $\{I_{th}; S_{MN}\}$ that involved the data from 17 selected studies as described in Figure 4-1. Although consistent with typical values reported in the literature (Lux & Pollen, 1966; Albuquerque & Thesleff, 1968; Barrett & Crill, 1974; Adrian & Hodgkin, 1975; Sukhorukov et al., 1993; Major et al., 1994; Solsona et al., 1998; Thurbon et al., 1998; Gentet et al., 2000), the $C_m = 1.3 \cdot 10^{-2} F \cdot m^{-2}$ constant value reported in this study is slightly higher than the widely-accepted $C_m = 1.0 \cdot 10^{-2} F \cdot m^{-2}$ value (Ulfhake & Kellerth, 1984; Gustafsson & Pinter, 1984b). This high value is consistent with the conclusions from Gustafsson & Pinter (1984a) who obtained slightly conservative values for S_{neuron} when approximating the MN surface area as $S_{neuron} = \frac{C}{C_m}$ with $C_m = 1.0 \cdot 10^{-2} F \cdot m^{-2}$.

Agreement with other experimental findings

Finally, the empirical relationship $I_{th} = \frac{2.7 \cdot 10^{-2}}{R^{1.0}}$ (Table 4-4) provides $\Delta V_{th} = 27mV$, consistently with typical reported values (Brock et al., 1952; Eccles, J. C. et al., 1958), despite uncertainties in the value of the membrane resting potential (Heckman & Enoka, 2012). This supports the conclusions that the relative voltage threshold ΔV_{th} may be assumed constant within the MN pool in first approximation (Coombs et al., 1955; Gustafsson & Pinter, 1984a; Powers & Binder, 2001), and that Ohm's law is followed in MNs for weak subthreshold excitations (Glenn & Dement, 1981; Ulfhake & Kellerth, 1984; Spruston & Johnston, 1992; Powers & Binder, 2001; Kernell, 2006), as discussed in the Methods Section. However, voltage thresholds ΔV_{th} tend to be lower in MNs of large R and long AHP (Gustafsson & Pinter, 1984a), suggesting that ΔV_{th} might be inversely correlated to MN size S_{MN} . Moreover, because of the voltage-activation of persistent inward currents near threshold which add to the external stimulating current and increases the MN input resistance (Fleshman et al., 1988), with a prominent effect on small MNs (Powers & Binder, 2001), the experimental values of ΔV_{th} are generally greater than that directly predicted from the product $I_{th} \cdot R$ (Gustafsson & Pinter, 1984b), and a variant of Ohm's law such as $\Delta V_{th}(V) = I_{th} \cdot R(V)$ should be considered during MN discharging events. This voltage-dependent variation in the values of R might partly explain why the range of the values of R reported in the literature for weak subthreshold currents (Appendix Table B-4) is generally lower than that of I_{th} . While ΔV_{th} may be always size-dependent, and size-voltage-dependent close to threshold and during firing events, no correlation between $\frac{\Delta V_{th}}{R \cdot I_{th}}$ and C , AHP or ACV was found and reported in Table 4-4 in subthreshold conditions, consistent with measurements performed by Gustafsson & Pinter (1984b) and Ulfhake & Kellerth (1984), substantiating that the dynamics of MN recruitment dominantly rely on R , I_{th} and ΔV_{th} (Heckman & Enoka, 2012).

4.4.2. Relevance of the relationships

Table 4-4 provides the first quantitative description of an extensive set of cat experimental data available in the literature. These inter-related relationships were validated, successfully reconcile the conclusions formulated by the selected experimental studies and are in strong agreement with the theoretical equations describing Rall's cable theory. This robust framework advances our general understanding of the MN neurophysiology by inferring quantitative relationships for pairs of MN properties that were never concurrently measured in experiments (Figure 4-2B) by crossmatching other relationships involving these properties. For example, $I_{th} = 3.8 \cdot 10^8 \cdot S_{neuron}^{2.5}$ (Table 4-4) quantitatively supports the size-dependency of I_{th} that was predicted by Powers & Binder (2001) from Rall's equations. Furthermore, modelers can directly and realistically tune with Table 4-4 the physiological parameters of phenomenological MN models, such as leaky fire-and-integrate models,

and/or build pools of MNs displaying a continuous distribution of realistic MN profiles of MN-specific electrophysiological and morphometric properties, as it has been previously attempted (Dong et al., 2011; Negro et al., 2016). Models tuned with Table 4-4 can for example better replicate the discharging behaviour of MNs, obtained from decomposed high-density EMGs, than models scaled with generic property values (Caillet et al., 2022). In this view, such tuned models display MN-specific behaviours that should be easier to interpret. The mathematical relationships in Table 4-4 can also support future experimental investigations performed on spinal MNs in adult mammals *in vivo* by completing experimental datasets for which properties that are difficult to measure were not obtained, such as the MN membrane time constant. Experimenters can therefore choose to reduce the workload of measuring all the MN properties in Table 4-1 to favour the identification of a maximum number of MNs and obtain extensive datasets describing larger MN populations that provide an informative window on the continuous distribution of MN properties in a MN pool. The unknown electrophysiological MN properties, typically obtained *in vivo*, of cadaveric specimens can also be estimated with the size-related relationships in Table 4-4 from *in vitro* measurements of the somal diameter D_{soma} in a slice preparation of the spinal cord. The mathematical relationships in Table 4-4 describe the experimental data published in the literature and provide a convenient metric for experimenters to compare their measurements to previous findings.

4.4.3. Extrapolation of the relationships to other mammals

It is demonstrated in Figure 4-7 and with the calculation of the r^2 values in Appendix Table B-5 that the mathematical equations in Table 4-4 obtained from cat data adequately predict the normalized associations between pairs of MN properties in rats and mice. However, the scaling factor K applied to the intercept k_e to scale the normalized relationships $A = K \cdot k_e \cdot B^e$ is species-specific, being for example around 3 times smaller in mice than in cat for the $\{\tau; R\}$ and $\{C; R\}$ pairs (Figure 4-7) and explaining the large $nRMSE$ values in these cases. Absolute values of the $\{I_{th}; R\}$ pair were nevertheless accurately predicted in both rats and mice from the cat relationships, as explained by the larger values of R which counterbalances the respectively lower I_{th} values in mice and rats than in cats (Manuel et al., 2019; Highlander et al., 2020). Despite the age-related data variability (Highlander et al., 2020) in the rodent dataset displayed in Figure 4-7, these findings advance our understanding of the systematic inter-mammalian-species variations in MN properties. In light of these results in the normalized space, and because of the absence of available *in vivo* human data in the literature to scale those relationships to humans, the cat relationships in Table 4-4 were used to model the dynamics of the human neuromuscular system in the following chapters 5 and 6.

4.4.4. Henneman's size principle of motor unit recruitment

Table 4-5 reports statistically significant power relationships of positive power values between MN and mU indices of size. These results substantiate the concept that S_{MN} and S_{mU} are positively correlated in a motor unit (MU) pool and that large MNs innervate large mUs (Henneman, 1981; Heckman & Enoka, 2012), a statement that has never been demonstrated from the concurrent direct measurement of S_{MN} and S_{mU} . Besides, considering the positive $I_{th} - S_{MN}$ correlation (Table 4-4), and that the mU force recruitment threshold F^{th} is positively correlated to F^{tet} (Heckman & Enoka, 2012) and thus to S_{mU} (Table 4-2), larger MUs have both larger current and force recruitment thresholds I_{th} and F^{th} than relatively smaller MUs, which are thus recruited first, consistently with the Henneman's size principle of MU recruitment (Henneman, 1957; Wuerker et al., 1965; Henneman et al., 1965a; Henneman et al., 1965b; Henneman et al., 1974; Henneman, 1981; Henneman, 1985). The terminologies 'small MU', 'low-force MU' and 'low-threshold MU' are thus equivalent. Henneman's size principle thus relies on the amplitude of the MN membrane resistance (Binder et al., 1996; Powers & Binder, 2001; Heckman & Enoka, 2012). Finally, the relationships $S_{MN} \propto CV^{1.4} \propto \tau^{-0.7} \propto AHP^{-0.7}$ (Table 4-4) suggest that high-threshold MUs rely on relatively faster MN dynamics, which might partially explain why large MNs can attain relatively larger firing rates than low-thresholds MNs, for example during ballistic contractions or during events close to maximum voluntary contractions (Powers & Binder, 2001; Heckman & Enoka, 2012).

It has been repeatedly attempted to extend Henneman's size principle and the correlations between the MU properties in Table 4-1 to the concept of 'MU type' (Burke & Ten Bruggencate, 1971; Burke, 1981; Bakels & Kernell, 1993; Powers & Binder, 2001). While a significant association between 'MU type' and indices of MU size has been observed in some animal (Fleshman et al., 1981; Burke et al., 1982; Zengel et al., 1985) and a few human (Milner-Brown et al., 1973; Stephens & Usherwood, 1977; Garnett et al., 1979; Andreassen & Arendt-Nielsen, 1987) studies, it has however not been observed in other animal studies ((Bigland-Ritchie et al., 1998) for a review) and in the majority of human investigations (Sica & McComas, 1971; Goldberg & Derfler, 1977; Yemm, 1977; Young, J. L. & Mayer, 1982; Thomas et al., 1990; Nordstrom & Miles, 1990; Elek et al., 1992; Macefield et al., 1996; Van Cutsem et al., 1997; Mateika et al., 1998; Fuglevand et al., 1999; Keen & Fuglevand, 2004). Moreover, the reliability of these results is weakened by the strong limitations of the typical MU type identification protocols. Sag experiments are irrelevant in humans (Buchthal & Schmalbruch, 1970; Thomas et al., 1991; Bakels & Kernell, 1993; Macefield et al., 1996; Bigland-Ritchie et al., 1998; Fuglevand et al., 1999), and lack consistency with other identification methods (Nordstrom & Miles, 1990). MU type identification by twitch contraction time measurements is limited by the strong sources of inaccuracy involved in the transcutaneous stimulation, intramuscular microstimulation,

intraneural stimulation, and spike-triggered averaging techniques (Taylor et al., 2002; Keen & Fuglevand, 2004; McNulty & Macefield, 2005; Negro et al., 2014; Dideriksen & Negro, 2018). Finally, as muscle fibres show a continuous distribution of contractile properties among the MU pool, some MUs fail to be categorized in discrete MU types in some animal studies by histochemical approaches (Reinking et al., 1975; Totossy de Zepetnek et al., 1992). Owing to these conflicting results and technical limitations, MU type may not be related to MN size and the basis for MU recruitment during voluntary contractions (McNulty & Macefield, 2005; Duchateau & Enoka, 2011).

4.4.5. Limitations

The mathematical relationships derived Table 4-4 and the conclusions drawn in the Discussion present some limitations, most of which were previously discussed and are summarised here. A first limitation relates to the experimental inaccuracies in measuring the MN properties in Table 4-1. As discussed, the true values of R and τ , and thus of R_m and C , may be underestimated because of a membrane leak conductance around the electrode and some voltage-activated membrane nonlinearities. Yet, the selected studies reduced the impact of these phenomena, which besides may not affect the association between MN properties (Gustafsson & Pinter, 1984a). Also, τ , C and R_m were estimated assuming that the MN membrane is isopotential. This is valid in the soma, where τ , C and R_m were measured, and the relationships in Table 4-4 are adequate for a MN simplified to one equivalent cable. However, as the membrane resistivity may increase with somatofugal distance, as discussed previously, the relationships in Table 4-4 may be offset towards larger R values in dendritic areas, which should be accounted for when modelling the MN dendrites in compartmental MN models or completely reconstructed dendritic trees. Finally, the selected cat studies are relatively dated, as observed in Manuel et al. (2019). Thus, recent computer-assisted techniques of MN morphometric measurements were notably not applicable in those studies, yielding important sources of inaccuracies, as discussed in Appendix B.2. Therefore, the relationships involving S_{neuron} are subjected to a higher level of inaccuracy. A second limitation arises from the inter-study variability of the experimental datasets reported in the selected studies. As discussed, animal preparations slightly diverged between the selected studies. Also, because of the scarcity of available data in the literature, measurements obtained from MNs innervating different muscles from adult animals of different sex and age were merged into unique datasets. This inter-study variability, added to the ‘unexplained’ variance reported in Highlander et al. (2020), was however reduced by the normalization of the experimental datasets, as investigated in Appendix Figure B-2 and C-4. The normalization approach is however only valid if the experimental studies identified the same largest MN relatively to the MN populations under investigation. This cannot be verified but is likely as most studies returned similar normalized distributions of their measured parameters, as displayed in Appendix Figure B-1, inferring that a similar

portion of the MN pools was identified in the selected studies. Finally, the results proposed in this study may be affected by methodological bias from the three research groups that provided most of the data points processed in this study, even if all the selected studies reported similar methods to measure the properties, as discussed in the Methods section.

A third limitation is due to the limited processable experimental data available in the literature, especially in rats and mice, as also discussed by Heckman & Enoka (2012). The extrapolation of the relationships in Table 4-4 to rat and mouse species could only be assessed against a small set of studies and for three associations only. The conclusions on the associations between S_{mU} and S_{MN} also relied on few studies providing few measurements, due to the considerable amount of work required to measure both MN and mU properties in single MUs. In cats, some pairs of MN properties were investigated in only one study, preventing inter-study comparisons, and stressing the usefulness of Table 4-4 for reconciliating the data available in the literature. Within the experimental datasets, the scarcity of data points in some regions of the data distributions (see the skewed I_{th} and R distributions in the density histograms in Appendix Figure B-1 for example), transfers during processing to the final size-dependent datasets (Figure 4-4). This data heterogeneity may affect the reliability of the relationships in Table 4-4 for the extreme MN sizes (see, in Figure 4-4, the 95% confidence interval of the regressions widening for small S_{MN} in the $\{AHP; S_{MN}\}$ and $\{R; S_{MN}\}$ datasets and for large S_{MN} in the $\{I_{th}; S_{MN}\}$ dataset). In the validation procedure, the highest nME values reported in Figure 4-6 were obtained for these regions of limited data for most of the global datasets. This heterogeneous density of the data may be explained by the natural skewness of the MN property distributions in the MN pool towards many small MNs and few large MNs (Heckman & Enoka, 2012) and/or a systematic experimental bias towards identifying and investigating relatively large MUs.

A fourth limitation arises from the relatively low r^2 values obtained from the power regressions in Table 4-3. Although these power trendlines are statistically significant (see p-values in Table 4-3), and globally provide a better description of the data than other fits (see Appendix Table B-1), they cannot entirely describe the associations between MN property distributions, in line with the conclusions reported in the selected studies. According to these first four limitations, the mathematical relationships in Table 4-4 best reproduce published cat data but include the level of inaccuracies of the original experimental approaches.

A fifth limitation is the restriction of the study to the MN properties reported in Table 4-1. Other MN-specific properties, such as the electronic length, the relationship between discharge rate and excitatory current and its hysteresis, or the amplitude of the inhibitory and excitatory postsynaptic potentials were omitted because they were rarely measured or reported along with

S_{MN} , ACV , AHP , R , I_{th} , C and τ in the selected studies. Moreover, as the cell geometry was not investigated in this study, other MN properties (Clements & Redman, 1989) such as the effects of the distributed synaptic integration along the dendritic tree were overlooked. Finally, apart from I_{th} , ACV and AHP , all the MN properties investigated in this study are passive properties, i.e. base-line properties of the cell at rest (Powers & Binder, 2001), as they were measured using weak sub-threshold current pulses. Therefore, other MN active properties, such as voltage-dependent ionic channel-related properties including persistent inward currents (PICs), which activate close or above threshold, were not considered in this study. While this limits the applicability of Table 4-4 to a functional context or in comprehensive Hodgkin-Huxley-like models of MNs, some relationships in Table 4-4 remain pertinent in those conditions if they are linked with other observations in the literature on MN active properties, e.g., MNs of small S_{MN} having longer lasting total PICs of greater hyperpolarized activation than MNs of large S_{MN} (Heckman & Enoka, 2012). Despite the aforementioned limitations, the relationships in Table 4-4 can be directly used in phenomenological RC approaches like LIF models (Izhikevich, 2004; Teeter et al., 2018), which rely on the properties reported in Table 4-1, to derive profiles of inter-consistent MN-specific properties and to describe realistic continuous distributions of the MN properties R , I_{th} , C , ΔV_{th} and τ in the MN pool, as attempted in previous works (Dong et al., 2011; Negro et al., 2016).

As a final limitation, the relationships in Table 4-4 were obtained from a regression analysis and therefore provide correlations between some MN properties in a MN pool but cannot be used to draw conclusions on the causality behind these associations.

Chapter 5

ESTIMATION OF THE FIRING BEHAVIOUR OF A COMPLETE MOTONEURON POOL BY COMBINING ELECTROMYOGRAPHY AND REALISTIC MOTONEURON MODELLING

NOTE: a large portion of this chapter has been published in the following journal article, where the results obtained from open-source datasets of decomposed HDEMG signals were replaced with new results obtained with the experimental data from Chapter 3:

Caillet, A. H., Phillips, A. T., Farina, D., & Modenese, L. (2022). Estimation of the firing behaviour of a complete motoneuron pool by combining electromyography signal decomposition and realistic motoneuron modelling. PLOS Computational Biology, 18(9), e1010556, doi:

<https://doi.org/10.1371/journal.pcbi.1010556>

Transition

In Chapter 4, mathematical relationships were derived between several MN-specific electrophysiological properties that characterize the MNs' specific recruitment and discharge behaviour during muscle contraction. Because these relationships support Henneman's size principle, they explain why the neural drive to muscle was poorly predicted by the HDEMG spike trains in Chapter 3. With an over-sampling of high-threshold identified MNs, the electrophysiological properties and discharge activity of the low-threshold MN populations were under-represented in the experimental data. This experimental limitation hinders the reliable use of HDEMG data for controlling MN-driven neuromuscular models. Chapter 5 combines the knowledge from Chapters 3 and 4 to tackle this limitation and reconstruct the discharge activity of the complete MN population.

Chapter summary

This Chapter 3 fulfils the Objective (5) listed in Section 1.2. In this chapter 5, a novel computational method is developed for accurately predicting the discharge activity of entire pools of N motoneurons in humans from available HDEMG data and realistic MN modelling. The method complements the datasets of N_r spike trains identified from HDEMG signals, which do not provide sufficient information to predict the true effective neural drive to muscle, by inferring the discharge activity of the $N - N_r$ non-identified MN spike trains. Consequently, the method tackles the intrinsic limitations related to decomposed HDEMG data stressed in Chapter 3 and provides reliable inputs for controlling MN-driven neuromuscular models.

To achieve this aim, the MN pool is described as a cohort of N single-compartment leaky fire-and-integrate (LIF) models of MNs. To scale the N LIF models with MN-specific parameters, physiologically realistic distributions of MN electrophysiological properties are derived by using the mathematical relationships obtained in Chapter 4 in combination with the experimental knowledge provided by the spike trains of the N_r identified MNs derived in Chapter 3.

The method is successfully validated in a two-step approach for four subjects, two muscles and two levels of force and is consequently proven reliable to blindly replicate the individual MN spike trains identified experimentally with high accuracy, and to accurately predict the effective neural drive to muscle. Importantly, the method is not sensitive to the number N_r of experimentally identified MNs, provided they are sufficiently homogeneously spread in the MN pool.

5.1. INTRODUCTION

The datasets of MN spike trains obtained from decomposed HDEMG signals in Chapter 3 are not suitable candidates for controlling MN-driven neuromuscular models, because they poorly approximate the effective neural drive to muscle for standard experimental setups (Chapter 3). The unknown discharge behaviour of the low-threshold MNs that were not identified from the HDEMG signals and are therefore missing in the experimental datasets can be modelled with mathematical frameworks and computational models of populations of individual MNs that some studies have developed for investigating the MN-specific neurophysiological mechanisms of the complete MN population. These MN pool models have provided relevant insights for interpreting experimental data (Fuglevand et al., 1993; Dideriksen et al., 2010), investigating the MN pool properties and neuromechanics (Cisi & Kohn, 2008; Negro & Farina, 2011; Farina et al., 2014), neuromuscular mechanisms (Watanabe et al., 2013; Potvin & Fuglevand, 2017), and the interplay between muscle machinery and spinal inputs (Dideriksen et al., 2011). However, none of these MN pool models have been tested with experimental input data, instead either receiving artificial gaussian noise (Negro & Farina, 2011), sinusoidal (Farina et al., 2014) or ramp (Fuglevand et al., 1993) inputs, inputs from interneurons (Cisi & Kohn, 2008) or feedback systems (Dideriksen et al., 2010). These MN pool models have therefore never been tested in real conditions of voluntary muscle contraction. The forward predictions of MN spike trains or neural drive to muscle obtained in these studies were consequently not or indirectly validated against experimental recordings.

The MN-specific recruitment and firing dynamics of these MN pool models are usually described with comprehensive or phenomenological models of MNs. The biophysical approaches (Cisi & Kohn, 2008; Negro & Farina, 2011; Watanabe et al., 2013; Farina et al., 2014), which rely on a population of compartmental Hodgkin-Huxley-type MN models provide a comprehensive description of the microscopic MN-specific membrane mechanisms of the MN pool and can capture complex nonlinear MN dynamics (Röhrle, Oliver, Yavuz et al., 2019). However, these models are computationally expensive and remain generic, involving numerous electrophysiological channel-related parameters for which adequate values are difficult to obtain in mammalian experiments (Bondarenko et al., 2004; Fohlmeister, 2009) and must be indirectly calibrated or extrapolated from animal measurements in human models (Smit et al., 2008). On the other hand, phenomenological models of MNs (Dideriksen et al., 2010; Raikova et al., 2018; Carriou et al., 2019) provide a simpler description of the MN pool dynamics and rely on a few parameters that can be calibrated or inferred in mammals including humans. They are inspired from the Fuglevand's formalism (Fuglevand et al., 1993), where the output MN firing frequency is the gaussian-randomized linear response to the synaptic drive with a MN-specific gain. However, these phenomenological models cannot account for the MN-specific nonlinear

mechanisms that dominate the MN pool behaviour (Heckman & Enoka, 2012; Powers & Heckman, 2017; Röhrle, Oliver, Yavuz et al., 2019; Binder et al., 2020). MN leaky integrate-and-fire (LIF) models, the parameters of which can be defined by MN membrane electrophysiological properties for which mathematical relationships are available (Chapter 4), are an acceptable trade-off between Hodgkin-Huxley-type and Fuglevand-type MN models with intermediate computational cost and complexity, and accurate descriptions of the MN macroscopic discharge behaviour (Teeter et al., 2018) without detailing the MN's underlying neurophysiology (Burkitt, 2006). While repeatedly used for the modelling and the investigation of individual MN neural dynamics (Izhikevich, 2004; Dong et al., 2011; Negro et al., 2016), MN LIF models are however not commonly used for the description of MN pools.

There is no systematic method to record the firing activity of all the MNs in a MN pool, or to estimate from a sample of experimental MN spike trains obtained from signal decomposition the firing behaviour of MNs that are not recorded in the MN pool. There is no mathematical model of a MN pool that (1) was tested with experimental neural inputs and investigated the neuromechanics of voluntary human muscle contraction, (2) involves a cohort of MN models that relies on MN-specific profiles of inter-related MN electrophysiological properties, (3) is described by a physiologically-realistic distribution of MN properties that is consistent with available experimental data. This limits our understanding of the neural strategies of the whole MN pool and of how the firing activity of each MN contributes to the neural drive to muscle.

In this study, a novel four-step approach is designed to predict, from the neural information of N_r MN spike trains obtained from HDEMG signal decomposition, the recruitment and firing dynamics of the $N - N_r$ MNs that were not identified experimentally in the investigated pool of N MNs. The model of the MN pool was built upon a cohort of N single-compartment LIF models of MNs. The LIF parameters are derived from the available HDEMG data, are MN-specific, and account for the inter-relations existing between mammalian MN properties (Chapter 4). The distribution of N MN input resistances hence obtained defines the recruitment dynamics of the MN pool. The MN pool model is driven by a common synaptic current, which is estimated from the available experimental data as the filtered cumulative summation of the N_r identified spike trains. The MN-specific LIF models phenomenologically transform this synaptic current into accurate discharge patterns for the N_r MNs experimentally identified and predict the MN firing dynamics of the $N - N_r$ unidentified MNs. The blind predictions of the spike trains of the N_r identified MNs and the effective neural drive to the muscle, computed from the firing activity of the complete pool of N virtual MNs, are both successfully validated against available experimental data.

Neuroscientists can benefit from this proposed approach for inferring the neural activity of MNs that cannot be observed experimentally and for investigating the neuromechanics of MN populations. Experimenters can use this approach for better understanding their experimental dataset of N_r identified discharging MNs. Most importantly in this thesis, this method realistically infers the discharge activity of the complete MN pool from the limited experimental data and provides the necessary neural inputs for controlling the MN-driven multi-MU model developed in this thesis.

The Python code used to implement the method is available in the open-source Github repository at: https://github.com/ArnaultCAILLET/Caillet-et-al-2022-PLOS_Comput_Biol.

Remark: for readability, the MN surface area (Size) is described in this chapter with the notation ' S ' and not ' S_{MN} ' like in Chapter 4. To avoid later confusion, $S1$ refers to 'Subject 1' and S_1 refers to the size of the first MN.

5.2. METHODS

5.2.1. Overall approach

The spike trains $sp_j^{sim}(t)$ elicited by the entire pool of N MNs were inferred from a sample of N_r spike trains $sp_i^{exp}(t)$ experimentally identified in Chapter 3 with a 4-step approach displayed in Figure 5-1. The N_r experimentally-identified MNs were allocated to the entire MN pool according to their recorded force recruitment thresholds F_i^{th} (step 1). The common synaptic input to the MN pool was estimated from the experimental spike trains $sp_i^{exp}(t)$ of the N_r MNs (step 2) and was linearly scaled for simplicity to the total postsynaptic membrane current $I(t)$ responsible for spike generation. A cohort of N_r single-compartment leaky-and-fire (LIF) models of MNs, the electrophysiological parameters of which were mathematically determined by the unique MN size parameter S_i^{MN} , transformed the input $I(t)$ to simulate the experimental spike trains $sp_i^{exp}(t)$ after calibration of the S_i parameter (step 3). The distribution of the N MN sizes $S(j)$ in the entire MN pool, which was extrapolated by regression from the N_r calibrated S_i quantities, scaled the electrophysiological parameters of a cohort of N LIF models. The N calibrated LIF models predicted from $I(t)$ the spike trains $sp_j^{sim}(t)$ of action potentials elicited by the entire pool of N virtual MNs.

According to the conclusions made in Chapter 4, the following assumptions were made. (1) The MU pool is idealized as a collection of N independent MUs that receive a common synaptic input and possibly MU-specific independent noise. (2) In a pool of N MUs, N MNs innervate N muscle units (mUs). (3) In our notation, the pool of N MUs is ranked from $j = 1$ to $j = N$, with increasing recruitment threshold. For the N MNs to be recruited in increasing MN and mU size and recruitment thresholds according to Henneman's size principle (Henneman, 1957; Henneman et al., 1965a; Henneman et al., 1965b; Henneman et al., 1974; Henneman, 1981; Henneman, 1985; Heckman & Enoka, 2012), the distribution of morphometric, threshold and force properties in the MN pool follows the series of equivalences in (EQ. 5-1).

$$\begin{aligned} \forall j, k \in [1; N], (j < k) \\ \Rightarrow (S_j < S_k \Leftrightarrow I_j^{th} < I_k^{th} \Leftrightarrow IR_j < IR_k \Leftrightarrow F_j^{th} < F_k^{th} \Leftrightarrow f_{0,j}^{MU} < f_{0,k}^{MU}) \end{aligned} \quad (\text{EQ. 5-1})$$

In (EQ. 5-1), using the same notations as in Chapters 3 and 4, S is the MN surface area, I^{th} the MN current threshold for recruitment, IR the MU innervation ratio defining the MU size, F^{th} is the MU force recruitment threshold, and f_0^{MU} is the MU maximum isometric force. (4) Because the MN-specific

electrophysiological properties are mathematically defined by the MN size S (Chapter 4)(Caillet et al., 2022), (EQ. 5-1) extends to (EQ. 5-2).

$$\forall j, k \in [1; N], (j < k) \Rightarrow (S_j < S_k \Leftrightarrow C_j < C_k \Leftrightarrow R_j > R_k \Leftrightarrow \tau_j > \tau_k) \quad (\text{EQ. 5-2})$$

In (EQ. 5-2), C is the MN membrane capacitance, R the MN input resistance and τ the MN membrane time constant.

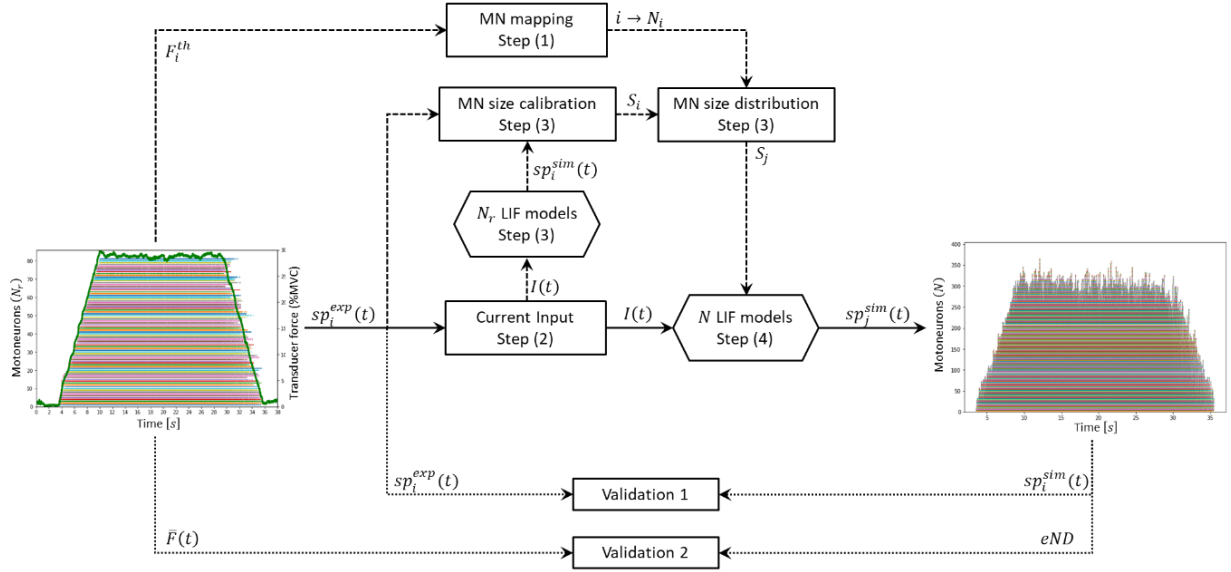


Figure 5-1: Four-step workflow predicting the spike trains $sp_j^{sim}(t)$ of the entire pool of N MNs (right figure) from the experimental sample of N_r MN spike trains $sp_i^{exp}(t)$ (left figure). Step (1): according to their experimental force thresholds F_i^{th} , each MN, ranked from $i = 1$ to $i = N_r$ following increasing recruitment thresholds, was assigned the N_i^{th} location in the complete pool of MNs ($i \rightarrow N_i$ mapping). Step (2): the current input $I(t)$ common to the MN pool was derived from the N_r spike trains $sp_i^{exp}(t)$. Step (3): using $I(t)$ as input, the size parameter S_i of a cohort of N_r leaky-and-fire (LIF) MN models was calibrated by minimizing the error between predicted and experimental filtered spike trains. From the calibrated S_i and the MN $i \rightarrow N_i$ mapping, the distribution of MN sizes S_j in the entire pool of virtual MNs was obtained by regression. Step (4): the S_j distribution scaled a cohort of N LIF models which predicted the MN-specific spike trains $sp_j^{sim}(t)$ of the entire pool of MNs (right). The approach was validated by comparing experimental and predicted spike trains (Validation 1) and by comparing normalized experimental force trace $\bar{F}(t)$ (Left figure, green trace) with normalized effective neural drive (Validation 2). In both figures, the MN spike trains are ordered from bottom to top in the order of increasing force recruitment thresholds.

5.2.2. Experimental data

The four-step workflow displayed in Figure 5-1 was tested with ten experimental datasets, named as reported in the first column of Table 5-1, that provide the time-histories of recorded MN spike trains $sp_i^{exp}(t)$ and whole muscle force trace $F(t)$ (left panel in Figure 5-1). Eight of the ten datasets were those obtained in Chapter 3, and the other two were taken from the open-source supplementary material from the studies (Hug et al., 2021a; Hug et al., 2021b), one of which was acquired from the Gastrocnemius Medialis (GM) human muscle. In all cases, the HDEMG signals were recorded with a sampling rate $f_s = 2048\text{Hz}$. The ten datasets were obtained from trapezoidal contractions, as described in Figure 5-2, as a zero force in $[t_{tr0}; t_{tr1}]$, a ramp of linearly increasing force in $[t_{tr1}; t_{tr2}]$, a plateau of constant force in $[t_{tr2}; t_{tr3}]$, a ramp of linearly decreasing force in $[t_{tr3}; t_{tr4}]$, and a zero force in $[t_{tr4}; t_{tr5}]$. The $t_{tr0 \rightarrow 5}$ times are reported in Table 5-1. For the TA muscle, the four-step workflow in Figure 5-1 was tested at two force levels, i.e. 30-35% MVC with six datasets ($HTA_{35}, S1_{30}^{AI}, S1_{30}^B, S1_{30}^E, S3_{30}^{AI}, S5_{30}^{AI}$) and at 50% MVC with three datasets ($S1_{50}^{AI}, S3_{50}^{AI}, S5_{50}^{AI}$). The test group was restricted to the Subjects S1, S3 and S5 for conciseness, and because their datasets were observed to be representative of the spread of results obtained in Chapter 3. Besides the 256-channel AI cases, the 64-channel datasets $HTA_{35}, S1_{30}^B$ and $S1_{30}^E$ were also considered in this analysis both for appreciating the behaviour of the workflow when 64-channels HDEMG grids are used and 20-35 MNs are identified, as commonly observed in the literature, and investigating the sensitivity of the performance of the method to the number N_r of identified MNs. A second AI-setup-trial of S1, named $S1_{30,2}^{AI}$, reported in Table 5-1, and obtained from the same experimental and processing procedures $S1_{30}^{AI}$ on the same subject was used for validation.

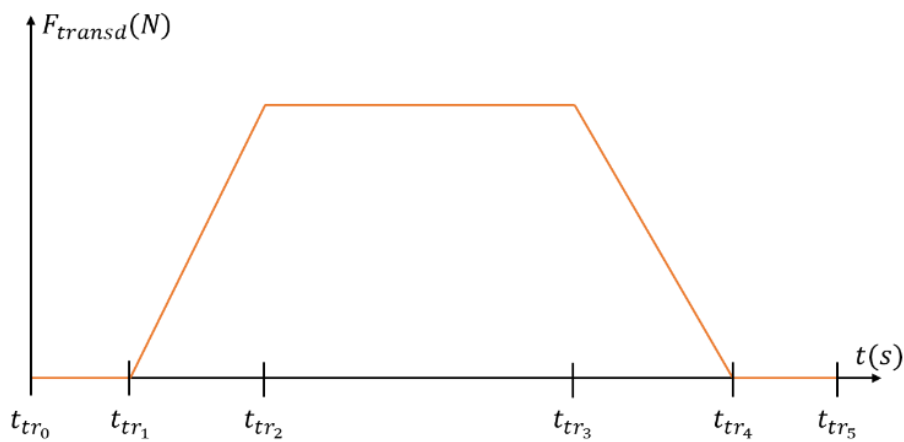


Figure 5-2: Definition of times t_{tr_i} that describe the trapezoidal shape of the muscle isometric contraction.

Table 5-1: The ten experimental datasets processed in this study. N_r spike trains are identified per dataset during trapezoidal contractions of the Tibialis Anterior (TA) or Gastrocnemius Medialis (GM) muscles. The trapezoidal force trace is described by times t_{tr_i} in seconds up to a dataset-specific level of maximum voluntary contraction (% MVC).

Dataset	Muscle	% MVC	t_{tr0}	t_{tr2}	t_{tr3}	t_{tr5}	N_r	Source
HGM_{30}	GM	30	0	9.1	28	107	27	(Hug et al., 2021; Hug et al., 2021)
HTA_{35}	TA	35	0	10.5	20.5	33	21	
$S1_{30}^{AI}$	TA	30	0	10	30	38	81	Chap.3
$S1_{30}^B$			0	10	30	38	35	
$S1_{30}^E$			0	10	30	38	28	
$S3_{30}^{AI}$			0	10.2	39.8	39	74	
$S5_{30}^{AI}$			0	10.6	30	36.6	42	
$S1_{50}^{AI}$		50	0	14	29	40	58	
$S3_{50}^{AI}$			0	15	29	41	48	
$S5_{50}^{AI}$			0	14.3	29.3	40	34	
$S1_{30,2}^{AI}$		30	0	9	28.5	37	79	

Like in Chapter 3, the experimental N_r MNs were ranked from $i = 1$ to $i = N_r$ in the order of increasing recorded force recruitment thresholds F_i^{th} , i.e. $\forall i \in [1; N_r], F_i^{th} < F_{i+1}^{th}$. Already presented in Chapter 3 (EQ. 3-6) and reproduced here, the sample time of the k^{th} firing event of the i^{th} identified MN is noted as ft_i^k , and the binary spike train of the i^{th} identified MN was mathematically defined with (EQ. 5-3).

$$\begin{cases} sp_i^{exp}(t = ft_i^k) = 1 \\ sp_i^{exp}(t \neq ft_i^k) = 0 \end{cases} \quad (\text{EQ. 5-3})$$

Also, the train of instantaneous discharge frequency $IDF_i(t)$ of the i^{th} identified MN was computed between firing times ft_i^k and ft_i^{k+1} with (EQ. 5-4).

$$\begin{cases} IDF_i(t = ft_i^k) = \frac{1}{ft_i^{k+1} - ft_i^k} \\ IDF_i(t \neq ft_i^k) = 0 \end{cases} \quad (\text{EQ. 5-4})$$

Already reported in Chapter 3, the continuous filtered instantaneous discharge frequencies (FIDFs) for all N_r identified MNs were obtained by moving-average filtering the IDFs by convolution with a Hanning window of length 400ms (De Luca, C. J. et al., 1982).

5.2.3. Step (1): MN mapping

In the first step of the approach overviewed in Figure 5-1, the N_r experimentally identified MNs were allocated to the entire pool of N MNs according to their recorded force recruitment thresholds F_i^{th} . Recalling the typical TA-specific frequency distribution of the MN force recruitment thresholds F^{th} in the MN pool derived in Chapter 3 (EQ. 3-1) and reproduced here in (EQ. 5-5), the equation $F^{th}(N_i) = 0.50 \cdot \left(58.12 \cdot \frac{N_i}{N} + \Delta_F \left(\frac{N_i}{N} \right)^{1.83} \right) = F_i^{th}$ was solved for the variable N_i for all N_r identified MUs for which the experimental threshold F_i^{th} was recorded.

$$F^{th}(j) = 0.50 \cdot \left(58.12 \cdot \frac{j}{N} + \Delta_F \left(\frac{j}{N} \right)^{1.83} \right), j \in \llbracket 1; N \rrbracket \quad (\text{EQ. 5-5})$$

The N_r identified MUs were thus assigned the N_i^{th} locations of the complete pool of N MUs ranked in order of increasing F^{th} with the transformation in (EQ. 5-6).

$$i \in \llbracket 1; N_r \rrbracket \rightarrow N_i \in \llbracket 1; N \rrbracket \quad (\text{EQ. 5-6})$$

When considering a 100-ms electromechanical delay between MN recruitment time and onset of muscle unit force, the mapping $i \in \llbracket 1; N_r \rrbracket \rightarrow N_i \in \llbracket 1; N \rrbracket$ did not substantially change. It was therefore simplified that a MN and its innervated muscle unit were recruited at the same time, and the $i \in \llbracket 1; N_r \rrbracket \rightarrow N_i \in \llbracket 1; N \rrbracket$ mapping derived for muscle units was extrapolated to MNs.

Considering that typically less than 30 MUs (5% of the GM MU pool) can be currently identified by HDEMG decomposition in GM muscles (Del Vecchio et al., 2020), and that the few papers identifying GM MUs with intramuscular electrodes either did not report the MU F^{th} (Garnett et al., 1979; Vieira et al., 2012; Kallio et al., 2013) or identified less than 24 MUs up to 100% MVC (Ballantyne et al., 1993; Héroux et al., 2014), a GM-specific $F^{th}(j)$ distribution could not be obtained from the literature for the GM muscle. The $F^{th}(j)$ distribution obtained for the TA muscle was therefore used for the simulations performed with the GM muscle, which is acceptable as an initial approximation based on visual comparison to the scattered data provided in these studies.

5.2.4. Step (2): Current input $I(t)$

In the second step of the approach in Figure 5-1, the common synaptic input to the MN pool was first estimated from the experimental data. As performed in Chapter 3 and briefly reproduced here, the cumulative spike train (CST) was obtained as the temporal binary summation of the N_r experimental spike trains $sp_i^{exp}(t)$ (EQ. 5-7). The effective neural drive (eND) to the muscle was then estimated by low-pass filtering the CST in the bandwidth $[0; 10]Hz$ relevant for force generation (Negro et al., 2016).

$$CST = \sum_i sp_i^{exp}(t) \quad (\text{EQ. 5-7})$$

As the pool of MNs filters the MN-specific independent synaptic noise received by the individual MNs and linearly transmits the common synaptic input (*CSI*) to the MN pool (Farina et al., 2014; Farina & Negro, 2015), the *CSI* was taken as a linear transformation of the *eND* in arbitrary units (EQ. 5-8). The common synaptic control (*CSC*) signal was obtained by low pass filtering the *CSI* in $[0; 4]Hz$.

$$CSI(t) = k \cdot eND(t) \quad (\text{EQ. 5-8})$$

This approach, which estimates the *CSI* from the N_r experimental spike trains is only valid if the sample of N_r MNs is ‘large enough’ and ‘representative enough’ of the complete MN pool for the linearity properties of the population of N_r MNs to apply (Farina & Negro, 2015). To assess if this approximation holds with the N_r MNs obtained experimentally, the following two validations were performed. (1) The coherence $coher_{\frac{N_r}{2}} \in [0; 1]$, averaged in $[1; 10]Hz$, was calculated between two cumulative spike trains $CST_{\frac{N_r}{2},1}$ and $CST_{\frac{N_r}{2},2}$ computed from two complementary random subsets of $\frac{N_r}{2}$ MNs. This was repeated 20 times for random permutations of complementary subsets of $\frac{N_r}{2}$ MNs, and the $coher_{\frac{N_r}{2}}$ values were average yielding $coher_{\frac{N_r}{2},mean} \in [0; 1]$. The coherence $coher_{N_r}$ between the complete experimental sample of N_r MNs and a virtual sample of N_r non-identified MNs was finally estimated by reporting the pair $\left(\frac{N_r}{2}; coher_{\frac{N_r}{2},mean}\right)$ similarly to Figure 2A in (Negro et al., 2016). (2) The time-histories of the normalized force $\bar{F}(t)$ and common synaptic control $\overline{CSC}$, which should superimpose if the linearity properties apply (see Figure 6 in (Farina & Negro, 2015)), were compared with calculation of the normalized root-mean-square error (nRMSE) and coefficient of determination r^2 . If $coher_{N_r} > 0.7$, $r^2 > 0.7$ and $nRMSE < 30\%$, it was assumed that the sample of N_r MNs was large and representative enough of the MN pool for the linearity properties to apply, and the *eND* was confidently assimilated as the *CSI* to the MN pool in arbitrary units. It must be noted that if $coher_{N_r} < 1$, the linearity properties do not fully apply for the sample of N_r MNs, and the *CSI* computed from the N_r MNs is expected to relate to the true *CSI* with a coherence close but less than $coher_{N_r}$.

The *CSI* hence obtained reflects the net excitatory synaptic influx common to the MN population, which triggers the opening of voltage-dependent dendritic channels and the activation of intrinsic membrane currents, such as persistent inward currents (PICs), which contribute to the total dendritic membrane current $I(t)$ responsible for spike generation. As the single-compartment LIF model considered in this study does not describe the dendritic activity, the PIC-related nonlinearity in the *CSI* – I transformation was excluded from the model and $I(t)$ was simplified to be linearly related to *CSI* with a constant gain G across the MN pool. To avoid confusion, $I(t)$ is referred as ‘current input’

in the following. The limitations of this simplification are investigated in the Limitations Section (Limitation 3). To identify G , it must be noted that the CSI , which was computed from a subset of the MN pool, does not capture the firing activity of the MNs that are recruited before the smallest identified N_1^{th} MN, which is recruited at time $ft_{N_1}^1$. It non-physiologically yields $CSI(t < ft_{N_1}^1) = 0$. Accounting for this experimental limitation, $I(t)$ was defined in (EQ. 5-9) to remain null until the first identified MN starts firing at $ft_{N_1}^1$, and to non-continuously reach $I_{N_1}^{th}$ at $t = ft_{N_1}^1$.

$$I(t) = \begin{cases} 0 & \text{if } t < ft_{N_1}^1 \\ I_{N_1}^{th} + G \cdot CSI(t) & \text{else} \end{cases} \quad \text{with} \quad G = \frac{I_{N_r}^{th} - I_{N_1}^{th}}{CSI(ft_{N_r}^1) - CSI(ft_{N_1}^1)} \quad (\text{EQ. 5-9})$$

In (EQ. 5-9), the rheobase currents of the first and last identified MNs $I_{N_1}^{th}$ and $I_{N_r}^{th}$ were estimated from a typical distribution of rheobase in a MN pool $I^{th}(j) = k_1 \cdot \Delta_I \left(\frac{j}{N}\right)^{k_3}$, obtained the following procedure as for the distribution of F^{th} in Chapter 3, from normalized experimental data from populations of hindlimb alpha-MNs in adult rats and cats in vivo (Kernell, 1966; Fleshman et al., 1981; Kernell & Monster, 1981; Gustafsson & Pinter, 1984a; Zengel et al., 1985; Foehring et al., 1987; Bakels & Kernell, 1993; Gardiner, 1993; Krawitz et al., 2001). It is worth noting that the experimental I^{th} were obtained by injecting current pulses directly into the soma of identified MNs, thus bypassing the dendritic activity and most of the aforementioned PIC-generated nonlinear mechanisms (Binder et al., 2020). A $\Delta_I = 9.1$ -fold range in MN rheobase in $[3.9; 35.5]nA$ was taken (Manuel et al., 2019; Caillet et al., 2022), while a larger Δ_I is also consistent with the literature ((Caillet et al., 2021), Table 4) and larger values of I^{th} can be expected for humans (Manuel et al., 2019).

5.2.5. Step (3): LIF model – parameter tuning and distribution of electrophysiological properties in the MN pool

In the third step of the approach in Figure 5-1, single-compartment LIF MN models with current synapses are calibrated to mimic the discharge behaviour of the N_r experimentally identified MNs.

5.2.5.1. MN LIF model: description

A variant of the LIF MN model was chosen in this study for its relative mathematical simplicity and low computational cost and its adequacy in mimicking the firing behaviour of MNs. The LIF model described in (EQ. 5-10) describes the discharge behaviour of a MN of rheobase I^{th} and input resistance R as a capacitor charging with a time constant τ and instantaneously discharging at time ft when the membrane voltage V_m meets the voltage threshold V_{th} , after which V_m is reset and maintained to the membrane resting potential V_{rest} for a time duration called ‘inert period’ IP in this study. For simplicity

without loss of generalisation, the relative voltage threshold was defined as $\Delta V_{th} = V_{th} - V_{rest} > 0$, and V_{rest} was set to 0.

The model is described by the following set of equations (EQ. 5-10):

$$\begin{cases} \tau \frac{dV_m}{dt} = R \cdot I(t) - V_m \\ \tau = RC \\ V_m(ft) = \Delta V_{th} \\ \lim_{t \rightarrow ft^+} V_m = 0 \\ \forall t \in [ft; ft + IP], V_m = 0 \end{cases} \quad (\text{EQ. 5-10})$$

The differential equation was solved with a time step $dt \leq 0.001s$ as (EQ. 5-11):

$$\begin{cases} V_m(0) = 0 \\ \forall n \in \mathbb{N}^*, V_m[nT] = e^{-\frac{T}{\tau}} V_m[(n-1)T] + R \frac{T}{\tau} \cdot I(nT) \end{cases} \quad (\text{EQ. 5-11})$$

This model includes 5 electrophysiological parameters: R , C , τ , ΔV_{th} and IP . The passive parameters R and C were mathematically related to the MN surface area S as $R = \frac{k_R}{S^{2.43}}$ and $C = C_m \cdot S$ after an extensive meta-analysis of published experimental data on hindlimb alpha-MNs in adult cats in vivo (Chapter 4), that sets the constant value of C_m to $1.3\mu F \cdot cm^2$, supports the equality $\tau = RC$ and the validity of Ohm's law in MNs as $I^{th} = \frac{2.7 \cdot 10^{-2}}{R} = \frac{\Delta V_{th}}{R}$, setting the constant value $\Delta V_{th} = 27mV$ in this study. The model was thus reduced to the MN size parameter S and to the IP parameter.

5.2.5.2. MN LIF model: the IP parameter

Single-compartment LIF models with no active conductances receiving current inputs can predict non-physiologically large values of MN firing frequency (FF) because of a linear FF-I gain at large but physiological current inputs $I(t)$ (Burkitt, 2006). The saturation in the FF-I relation typically observed in the mammalian motor unit firing patterns is primarily mediated by the voltage-dependent activity of the PIC-generating dendritic channels (Binder et al., 2020), that was overlooked in the $CSI - I$ transformation in Step (2), and which decreases the driving force of the synaptic current flow as the dendritic membrane depolarizes. While a physiological modelling of this saturation could be achieved with a LIF model with conductance synapses, as discussed in Limitation 3 in the Limitations section, this nonlinear behaviour could also be captured in a simple approach by tuning the phenomenological IP parameter in (EQ. 5-10). Considering a constant supra-threshold current $I \gg I^{th}$ input to a LIF MN, the steady-state firing frequency FF predicted by the LIF model is reduced to (EQ. 5-12).

$$FF(I) = \frac{1}{IP - RC \ln\left(1 - \frac{\Delta V_{th}}{RI}\right)} \xrightarrow{I \gg I^{th}} FF(I) \approx \frac{1}{IP + \frac{C \cdot \Delta V_{th}}{I}} \quad (\text{EQ. 5-12})$$

As I and C typically vary over a 10-fold and 2.4-fold range respectively (Chapter 4), the FF predicted by the LIF model is dominantly determined by the value of IP as the input current increasingly overcomes the MN current threshold: $FF(I \gg I^{th}) \approx \frac{1}{IP}$.

In the previous phenomenological models of MNs (Fuglevand et al., 1993; Callahan et al., 2013; Carriou et al., 2019), a maximum firing rate $FF^{\max}$ was defined and a non-derivable transition from $FF(I)$ to constant $FF^{\max}$ was set for increasing values of $I(t)$. Here, IP was integrated to the dynamics of the LIF model and was derived from experimental data to be MN-specific in the following manner. For each of the N_r identified MNs, the time-course of the MN instantaneous discharge frequencies (IDFs) was first smoothed, as performed in (Hug et al., 2021), with a sixth-order polynomial trendline (IDF_{trend}) to neglect any unexplained random noise in the analysis. The mean M of the trendline values during the plateau of force $[t_{r_2}; t_{r_3}]$ was then obtained. If $\exists t \in [t_{r_1}; t_{r_2} - 1]$, $IDF_{trend}(t) > 0.9M$, i.e. if the MN reached during the ramp of $I(t)$ in $[t_{r_1}; t_{r_2} - 1]$ an IDF larger than 90% of the IDF reached one second before the plateau of force, the MN was identified to ‘saturate’. Its IP parameter was set to $IP = \frac{1}{\max(IDF_{trend})}$, which constrains the MN maximum firing frequency for high input currents to $FF^{\max} \approx \frac{1}{IP} = \max(IDF_{trend})$. A power trendline $IP(j) = a \cdot j^b$ was finally fitted to the pairs $(N_i; IP_{N_i})$ of saturating MNs and the IP_{N_i} values of the non-saturating MNs were predicted from this trendline. To account for the residual variation in FF observed to remain at high $I(t)$ due to random electrophysiological mechanisms, the IP parameter was randomized at each firing time, taking the value $IP + o$, where o was randomly obtained from a normal gaussian distribution of $\frac{IP}{10}$ standard deviation.

5.2.5.3. MN LIF model: MN size parameter calibration

The remaining unknown parameter - the MN size S - defines the recruitment and firing dynamics of the LIF model. The size S_i of the i^{th} identified MN was calibrated by minimizing over the time range $[t_{tr_0}; \frac{t_{tr_2} + t_{tr_3}}{2}]$ (Table 5-1) the cost function $J(S_i)$ computed as the root-mean-square difference between experimental $FIDF_i^{exp}(t)$ and LIF-predicted $FIDF_i^{sim}(t)$ filtered instantaneous discharge frequencies (EQ. 5-13).

$$\left\{ \begin{aligned} J(S_i) &= \sqrt{\frac{dt}{\frac{t_{tr_2} + t_{tr_3}}{2} - t_{r_0}} \cdot \sum_{t_k = t_{r_0}}^{\frac{t_{tr_2} + t_{tr_3}}{2}} \left(FIDF_i^{sim}(t_k) - FIDF_i^{exp}(t_k) \right)^2} \\ \min_{S_i} J(S_i), i \in \llbracket 1; N_r \rrbracket \end{aligned} \right. \quad (\text{EQ. 5-13})$$

To assess how well the calibrated LIF models can replicate the available experimental data, the normalized RMS error (nRMSE) (%) and coefficient of determination r^2 between $FIDF_i^{exp}(t)$ and $FIDF_i^{sim}(t)$, and the error in seconds between experimental and predicted recruitment times $\Delta f t_i^1 = f t_i^{1,sim} - f t_i^{1,exp}$ were computed for the N_r MNs. Finally, a power trendline in (EQ. 5-14) was fitted to the pairs $(N_i; S_{N_i})$, and the continuous distribution of MN sizes in the entire pool of N MNs was obtained.

$$S(j) = k_1 \cdot \Delta_S^{\left(\frac{j}{N}\right)^{k_3}} \quad (\text{EQ. 5-14})$$

$\Delta_S = 2.4$ was taken from Chapter 4. The $S(j)$ distribution defines the continuous distribution of the MN-specific electrophysiological properties across the MN pool (Table 4-4 in Chapter 4).

5.2.5.4. MN LIF model: parameter identification during the derecruitment phase

The time-range $[t_{r_3}; t_{r_5}]$ over which the MNs are being derecruited was not considered in the S calibration in (EQ. 5-13) because the MN's current-voltage relation presents a hysteresis triggered by long-lasting PICs, as discussed in (Lee & Heckman, 1998; Binder et al., 2020). This hysteresis, which leads MNs to be derecruited at lower current threshold than at recruitment, can be phenomenologically interpreted in the scope of a single-compartment LIF model with no active conductance as an increase in the 'apparent' MN resistance R during derecruitment to a new value R^d . To determine R^d , a linear trendline $I^{dth} = k_{th}^{dth} \cdot I^{th}$ ($k_{th}^{dth} < 1$) was fitted to the association of experimental MN recruitment I^{th} and recruitment I^{dth} current thresholds for the N_r recruited MNs, and the distribution of MN input resistance $R = \frac{k_R}{S^{2.43}}$ was increased over the derecruitment time range $[t_{r_3}; t_{r_5}]$ according to (EQ. 5-15).

$$R^d = \frac{k_R^d}{S^{2.43}} \text{ with } k_R^d = \frac{k_R}{k_{th}^{dth}} > k_R \quad (\text{EQ. 5-15})$$

As previously reviewed (Heckman & Enoka, 2012; Binder et al., 2020), the current-voltage hysteresis also explains the typically lower MN discharge rate observed at derecruitment than at recruitment. For purely modelling perspectives, this phenomenon was phenomenologically captured by increasing over

the time-range $[t_{r_3}; t_{r_5}]$ the value of the C_m property, which is the main parameter influencing the predicted MN FF in (EQ. 5-10) for current inputs close to I^{th} . The simulated $FIDF_i^{sim}$ traces were iteratively simulated for the N_r MNs over the time-range $[t_{r_3}; t_{r_5}]$ with the N_r $S_i - IP_i$ –calibrated LIF models obtained from Step (3), for $0.1\mu F \cdot cm^2$ incremental changes in the value of the membrane specific capacitance C_m . The $FIDF_i^{sim}$ results were compared to the $FIDF_i^{exp}$ traces with nRMSE and r^2 values. The ‘apparent’ C_m value returning the lowest output for (EQ. 5-16) was retained and was renamed C_m^d .

$$J(C_m) = \frac{\frac{nRMSE}{100} - \overline{r^2}}{2} \quad (\text{EQ. 5-16})$$

In the following, the individual spike trains $sp_i^{sim}(t)$ were predicted with C_m over the $[t_{r_0}; t_{r_3}]$ time range and C_m^d over the $[t_{r_3}; t_{r_5}]$ time range.

It must be noted that, although this approach can accurately capture the observed MN nonlinear input-output behaviour and is coherent with the modelling constraints imposed by the LIF formulation in (EQ. 5-10), as the C_m and R parameters mainly affect the discharge and recruitment properties of the LIF model respectively in this model, the R and C_m parameters are passive MN properties which remain independent from the MN synaptic and membrane activity in actual MNs, while the R -to- R^d and C_m -to- C_m^d transformations are merely phenomenological interpretations of complex neurophysiological mechanisms, and do not provide any insights into the working principles underlying MN recruitment. This limitation is further discussed in Limitation 3.

5.2.5.5. Step (4): Simulating the MN pool firing behaviour

The firing behaviour of the complete MN pool, i.e. the MN-specific spike trains $sp_j(t)$ of the N virtual MNs constituting the MN pool, was predicted with a cohort of N LIF models receiving the common synaptic current $I(t)$ as input. The inert period IP_j and MN size S_j parameters scaling each LIF model were obtained from the distributions $IP(j)$ and $S(j)$ previously derived at Step (3).

5.2.6. Validation

5.2.6.1. Validation 1

It was assessed whether the N_r experimental spike trains $sp_i^{exp}(t)$ were accurately predicted by this 4-step approach (Figure 5-1). Steps (2) and (3) (Figure 5-1) were iteratively repeated with samples of $N_r - 1$ spike trains, where one of the experimentally recorded MNs was not considered. At each i^{th} iteration of the validation, the i^{th} identified spike train $sp_i^{exp}(t)$ was not used in the derivation of the synaptic current $I(t)$ (step 2) and in the reconstruction of the MN size and IP distributions $S(j)$ and $IP(j)$ in the MN pool (step 3). As in Step (4), the $FIDF_i^{sim}$ of the i^{th} MN was finally predicted with a LIF model, which was scaled with the parameters $S(N_i)$ and $IP(N_i)$ predicted from the $S(j)$ and $IP(j)$ distributions. For validation, $FIDF_i^{sim}$ was compared to the experimental $FIDF_i^{exp}$ with calculation of Δft_i^1 , r^2 and nRMSE metrics, that aim to evaluate the key features of the MN discharge behaviours. For each blindly simulated motoneuron, the Δft_i^1 metric assesses the accuracy in predicting its current recruitment threshold and first discharge time, the nRMSE metric evaluates the accuracy in predicting the amplitude of its filtered instantaneous discharge frequencies, while r^2 evaluates the time-course of the variation of its discharge behaviour. This validation was iteratively performed for all the N_r identified MN spike trains.

5.2.6.2. Validation 2

It was assessed whether the N MN spike trains $sp_j^{sim}(t)$, predicted in Step 4 for the entire MN pool from the N_r identified trains $sp_i^{exp}(t)$, accurately predicted the effective neural drive (eND_N) to the muscle. The eND_N was computed as the $[0; 4]$ Hz low-pass filtered cumulative spike train of the N predicted spike trains, $CST_N = \sum_{j=1}^N sp_j^{sim}(t)$. As suggested for isometric contractions (Farina & Negro, 2015), the normalized eND_N was compared for validation against the normalized experimental force trace $\bar{F}(t)$ with calculation of the nRMSE and r^2 metrics. $\bar{F}(t)$ was also compared with nRMSE and r^2 to the normalized effective neural drive eND_{N_r} computed directly from the N_r experimentally identified MN spike trains. The added value of the presented workflow (Steps 1 to 4) in predicting the neural drive to muscle was finally assessed by comparing the (nRMSE, r^2) pairs obtained for the eND_{N_r} and eND_N traces. Considering the uncertainty on the value of N in the literature, as discussed in Chapter 3, the performance of the method with reconstructed populations of $N = \{N_r, 100, 200, 300, 400\}$ MNs was also investigated. This sensitivity analysis provides different scaling factors N for the normalized MN mapping (Step 1) and IP and S_{MN} parameter distributions (Step 3) and constrains the number of firing MNs involved in the computation of eND_N (Step 4).

5.2.6.3. Validation 3

Validations 1 and 2 do not provide any indication whether the IP and S parameters identified from a first trapezoidal force profile would generalize to another trapezoidal force profile for the same subject at the same level of force and same experimental conditions and provide accurate predictions of the N_r experimental spike trains and of the eND_N . To perform such validation, a second experimental dataset $S1_{30,2}^{AI}$ was obtained for subject S_1 following the same experimental and processing protocols as for $S1_{30}^{AI}$ and described in Chapter 3. The dataset $S1_{30,2}^{AI}$ provides a new set of N_r^{new} spike trains, which are used to derive the new current input $I^{new}(t)$ (Step 2). $I^{new}(t)$ drives a cohort of N MN models (Step 4), the characteristics of which are defined by the IP and S distributions (Step 3) derived with the first contraction profile $S1_{30}^{AI}$, to predict the new effective neural drive eND_N^{new} . Like Validation 2, eND_N^{new} is validated against the normalized experimental force profile $F^{new}(t)$ recorded in $S1_{30,2}^{AI}$. Using the MN mapping (Step 1), the N_r identified spike trains $sp_i^{exp}(t)$ were compared against the corresponding N_r predicted MN spike trains.

5.3. RESULTS

In the following (Figure 5-3 to Figure 5-9), the intermediary results output by the four-step method (Figure 5-1), other than the validation results, were only displayed for the dataset $S1_{30}^{AI}$ for improved readability.

5.3.1. Experimental data

The $N_r = 81$ MN spike trains, identified in the dataset $S1_{30}^{AI}$ across the complete TA pool of $N = 400$ MNs, are represented in Figure 5-3A in the order of increasing force recruitment thresholds F_i^{th} . The N_r MNs were globally derecruited at relatively lower force thresholds (Figure 5-3B) and generally discharged at a relatively lower firing rate (Figure 5-3C) at derecruitment than at recruitment. Similar observations were made for the nine other datasets reported in Table 5-1.

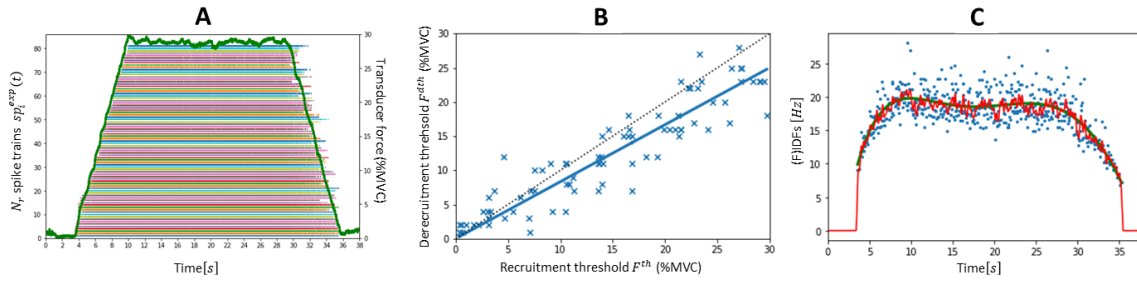


Figure 5-3: Experimental data obtained from HDEMG signal decomposition in the dataset $S1_{30}^{AI}$. (A) Time-histories of the transducer force trace in % MVC (green curve) and of the $N_r = 81$ MN spike trains identified from HDEMG decomposition and ranked from bottom to top in the order of increasing force recruitment thresholds F_i^{th} . (B) Association between force recruitment and derecruitment threshold, fitted by a linear trendline $y = 0.83 \cdot x$ ($r^2 = 0.85$). Identity is displayed as a black dotted line. (C) Time-histories of the MN instantaneous discharge frequencies (IDFs, blue dots) smoothed by convolution with a Hanning window of length 400ms (red curve) and with a sixth-order polynomial trendline (green curve) of the lowest-threshold identified (1st) MN.

5.3.2. Step (1): MN mapping

From the $F^{th}(j)$ distribution in (EQ. 5-5), the $N_r = 81$ MNs identified in the dataset $S1_{30}^{AI}$ were allocated (blue crosses in Figure 5-4B) to the entire pool of $N = 400$ MNs according to their recruitment thresholds F_i^{th} (% MVC) (ordinates in Figure 5-3B). As already observed in Chapter 3 and shown in Figure 5-4C, the N_r MNs identified experimentally in the dataset $S1_{30}^{AI}$ were rather homogeneously spread in the entire MN pool ranked in the order of increasing force recruitment thresholds, as 22% of the identified MNs fell in the first quarter of the recruited MN pool, 20% in the second quarter, 21% in the third quarter and 33% in the fourth quarter. In most other datasets, this spread is heterogeneous with a bias towards identifying high-thresholds MNs. For example, the datasets $S5_{30}^{AI}$, $S1_{30}^B$ and HTA_{35} respectively distribute as 5%-24%-26%-45%, 14%-14%-26%-46% and 5%-10%-14%-71% across the four quarters of the recruited MN pool. Also, no MN was identified in the first quarter of the MN pool in the dataset HGM_{30} (second column of Table 5-2).

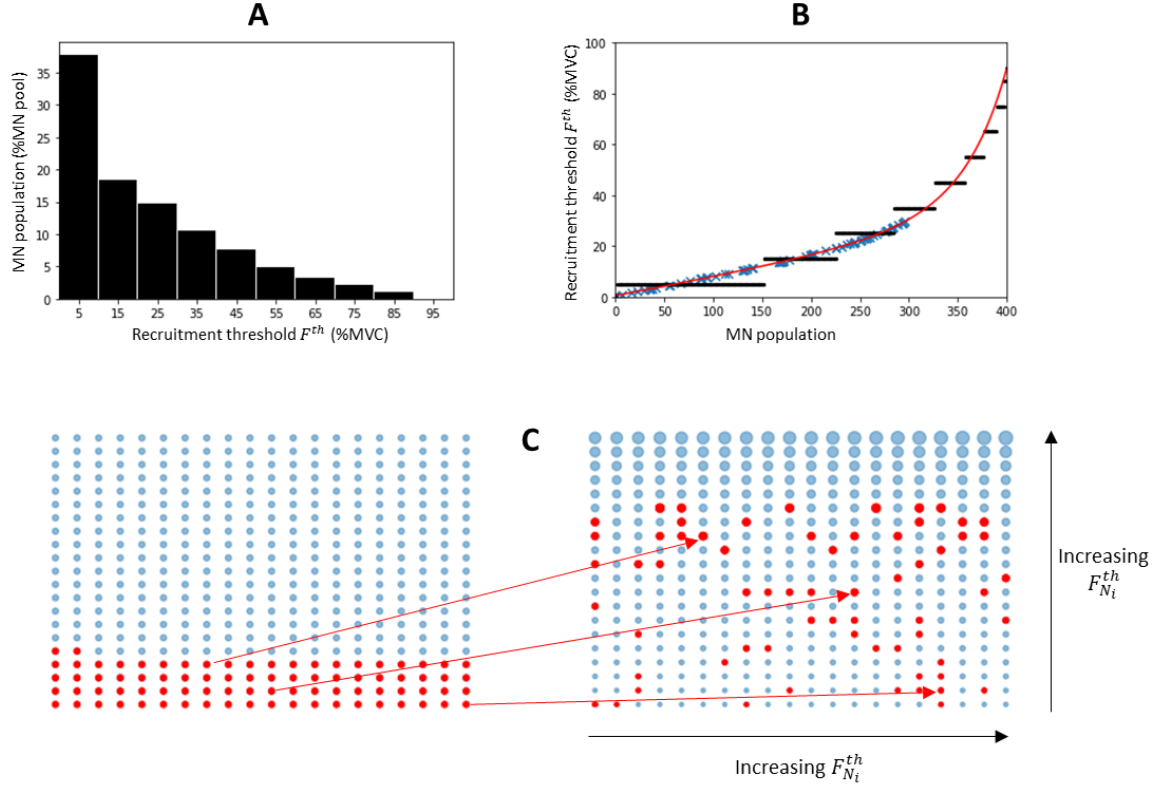


Figure 5-4: Distribution of force recruitment thresholds F^{th} in the human Tibialis Anterior (TA) muscle and mapping of the N_r identified MNs to the complete MN pool. (A) Typical partition obtained from the literature of the TA MN pool in 10% increments in normalized F^{th} . (B) Equivalent F^{th} stepwise distribution (black dots) in a TA pool of $N = 400$ MNs, approximated by the continuous relationship $F^{th}(j)$ (red curve). The mapping (blue crosses) of the $N_r = 81$ MNs identified in the dataset $S1_{30}^{AI}$ was obtained from the recorded F_i^{th} (Figure 5-3B). (C) $N_r = 81$ MNs (red dots) of unknown properties (Left) are mapped (Right) to the complete MN pool from the $F^{th}(j)$ distribution, represented by the blue dots of increasing sizes. The MNs represented here are numbered from left to right and bottom to top from $j = 1$ to 400.

5.3.3. Step (2): Common current input $I(t)$

To approximate the common synaptic input $CSI(t)$ to the MN pool, the CST and the eND to the MN pool were obtained in Figure 5-5 from the N_r MN spike trains identified experimentally (EQ. 5-7) and (EQ. 5-8). After 20 random permutations of $\frac{N_r}{2}$ complementary populations of MNs, an average coherence of $coher_{\frac{N_r}{2}, mean} = 0.81$ was obtained between $\frac{N_r}{2}$ -sized CSTs of the $S1_{30}^{AI}$ dataset. From Figure 2 in Negro et al. (2016), a coherence of $coher_{N_r} = 0.91$ is therefore expected between the CST in Figure 5-5A and a typical CST obtained with another virtual group of $N_r = 81$ MNs, and by extension with the true CST obtained with the complete MN pool. The normalized eND and force traces (black and green curves respectively in Figure 5-5B) compared with $r^2 = 0.97$ and $nRMSE = 12.6\%$ for the $S1_{30}^{AI}$ dataset. With this approach, $coher_{N_r} > 0.7$ (Table 5-2), $r^2 > 0.7$ and $nRMSE < 30\%$ (Table 5-5) was obtained for all ten datasets, with the exception of HGM_{30} for which $coher_{N_r} < 0.7$ (third column of Table 5-2). For the TA datasets, the sample of N_r identified MNs was therefore concluded to be

sufficiently representative of the complete MN pool for its linearity property to apply, and the eND (red curve in Figure 5-5B for the dataset DTA_{35}) in the bandwidth $[0,10]Hz$ was confidently identified to be the common synaptic input (CSI) to the MN pool. As observed in Figure 5-5B, a non-negligible discrepancy, which partially explains why $coher_{N_r} \neq 1$, was however systematically obtained between the eND and force traces in the regions low forces, where mostly small low-threshold MNs are recruited. As hinted in Chapter 3 and discussed in the Discussion Section, this discrepancy reflects the undersampling of small MUs identified from decomposed HDEMG signals and a bias towards mainly identifying the large high-threshold units which are recruited close to the plateau of force. This discrepancy was therefore observed to be accentuated in the datasets obtained with 64 channels ($HTA_{35}, S1_{30}^B, S1_{30}^E$) and at 50% MVC ($S1_{50}^{AI}, S3_{50}^{AI}, S5_{50}^{AI}$), and $coher_{N_r}$ was overall lower in these datasets (Table 5-2). From Figure 2 in (Negro et al., 2016), it is worth noting that the computed CSI in Figure 5-5B (red curve) accounts for 60% of the variance of the true synaptic input, which is linearly transmitted by the MN pool, while the remaining variance is the MN-specific synaptic noise, which is assumed to be filtered by the MN pool and is neglected in the computation of the eND in this workflow.

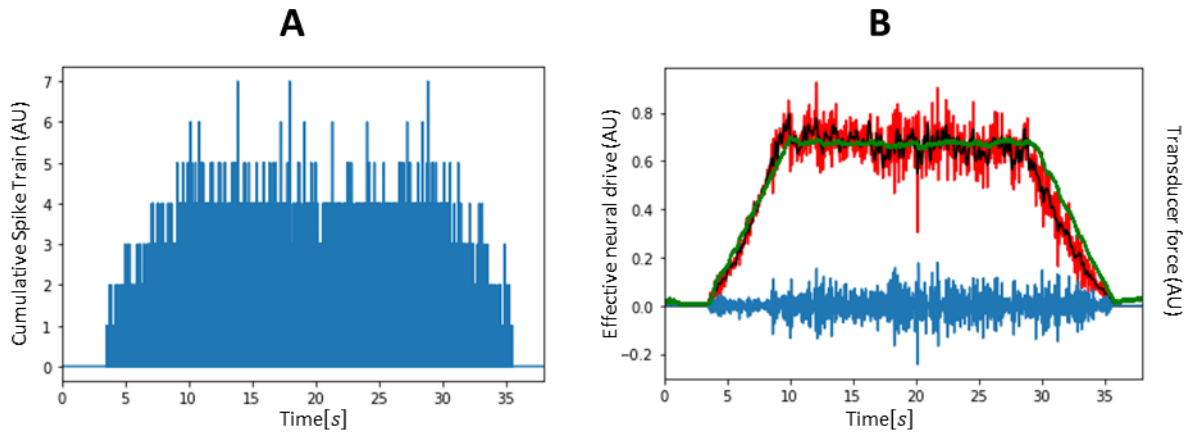


Figure 5-5: Neural drive to the muscle derived from the N_r identified MN spike trains in the dataset $S1_{30}^{AI}$. (A) Cumulative spike train (CST) computed by temporal binary summation of the N_r identified MN spike trains. (B) Effective neural drive – Upon applicability of the linearity properties of subsets of the MN pool, the effective neural drive is assimilated as the synaptic input to the MN pool. The normalized common synaptic input (red), control (black) and noise (blue) are obtained from low-pass filtering the CST in the bandwidths relevant for muscle force generation. The experimental force trace (green curve, arbitrary units) is displayed for visual purposes.

As discussed in the Methods section, the normalized CSI (red curve in Figure 5-5B) was simplified to be linearly related to the total dendritic membrane current $I(t)$. To do so, the scaling factor was determined with a typical distribution of the MN membrane rheobase $I^{th}(j)$ in a cat MN pool (EQ. 5-17), which was obtained (Figure 5-6A) from the literature (Fleshman et al., 1981; Kernell & Monster, 1981; Gustafsson & Pinter, 1984a; Zengel et al., 1985; Foehring et al., 1986; Munson et al., 1986; Foehring et al., 1987; Kernell & Zwaagstra, 1989).

$$I^{th}(j) = 3.9 \cdot 10^{-9} \cdot \Delta_1 \left(\frac{j}{N}\right)^{1.18}, j \in \llbracket 1; N \rrbracket \quad (\text{EQ. 5-17})$$

Using (EQ. 5-9), the time-history of the current input $I(t)$ was obtained (Figure 5-6B) with (EQ. 5-18).

$$I(t) = \begin{cases} 0 & \text{if } t < ft_{N_1}^1 \\ 3.9 \cdot 10^{-9} + 6.0 \cdot 10^{-8} \cdot CSI(t) & \text{else} \end{cases} \quad (\text{EQ. 5-18})$$

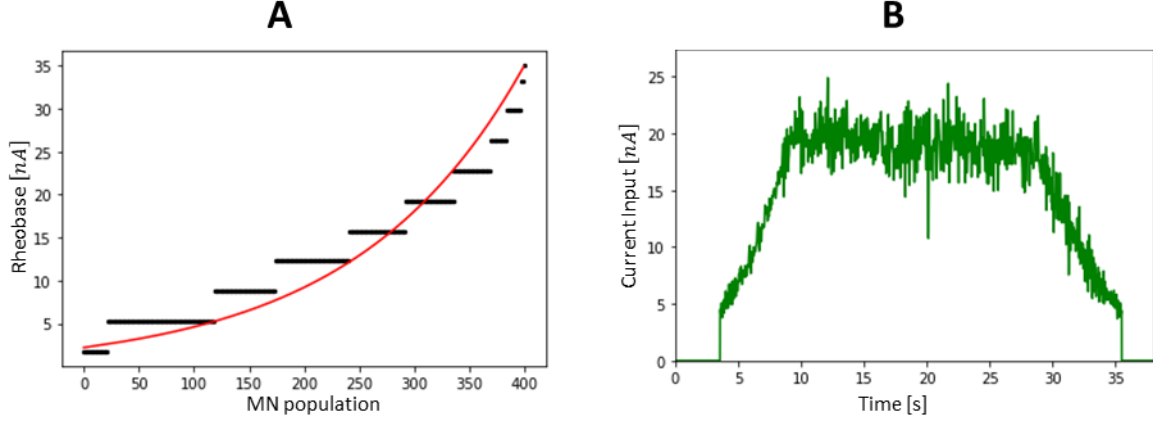


Figure 5-6: (A) Typical distribution of MN current recruitment threshold $I^{th}(N_i)$ in a cat MN pool according to the literature. (B) Current input $I(t)$, taken as a non-continuous linear transformation of the common synaptic input (red curve in Figure 5-5B) for the dataset $S1_{30}^{AI}$.

5.3.4. Step (3): LIF model – MN size calibration and distribution

Because of the modelling choices made for our MN LIF model, the MN inert period (IP) and the MN size S parameters entirely define the LIF-predictions of the MN firing behaviour. The IP_i parameters of the N_r MNs in the dataset $S1_{30}^{AI}$ (Figure 5-7) were obtained from the maximum firing frequency of the 62 MNs identified to ‘saturate’, from which the distribution of IP values in the entire pool of N MNs was obtained: $IP(j)[s] = 0.05 \cdot j^{0.01}$. With this approach, the maximum firing rate assigned to the first recruited and unidentified MN is $\frac{1}{IP_1} = \frac{1}{0.05 s} = 20Hz$. The IP distributions obtained with this approach for the ten datasets are reported in the fourth column of Table 5-2. The IP distributions for the seven datasets obtained at 30% MVC all returned physiological approximations for the maximum firing rate for the unidentified lowest -threshold MN $\frac{1}{IP_1}$ in the range $[20; 33]Hz$ and respected the onion-skin theory with positive power values. The IP distributions for the three datasets at 50% MVC, which lack the information of too large a fraction of the MN pool for accurate extrapolations to be performed, were non-physiological, with predicted $\frac{1}{IP_1}$ values of 500Hz ($S1_{50}^{AI}$) or 11Hz ($S3_{50}^{AI}$).

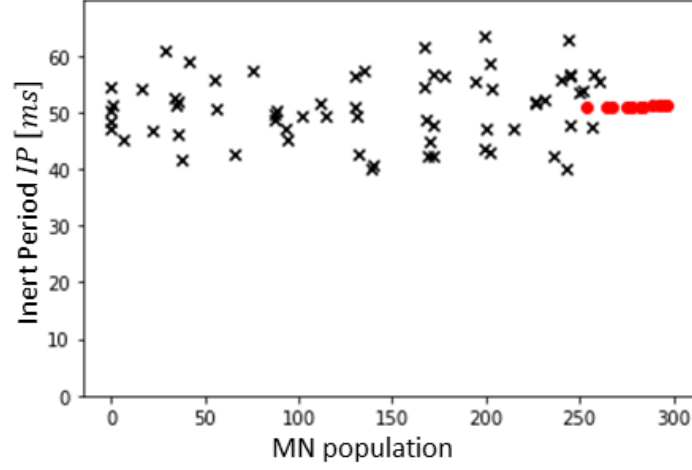


Figure 5-7: MN Inert Periods (IPs) in ms obtained from the experimental measurements of IDFs in dataset $S1_{30}^{AI}$. The 62 lowest-threshold MNs are observed to ‘saturate’ as described in the Methods and their IP (black crosses) is calculated as the inverse of the maximum of the trendline fitting the time-histories of their instantaneous firing frequency. The IPs of the 19 highest-threshold MNs (red dots) are obtained by trendline extrapolation.

Table 5-2: Intermediary results obtained for the datasets DTA_{35} , HTA_{35} , HTA_{50} and HGM_{30} from the three first steps of the approach. For each dataset are reported (1) the locations in the complete pool of N MNs of the lowest- (N_1) and highest-threshold (N_{N_r}) MNs identified experimentally, (2) the $coher_{N_r}$ value between the experimental and virtual cumulative spike trains (CST), and the coefficients defining the distributions in the complete MN pool of (3) the inert period (IP) parameter and of (4) the MN size (S). For the TA and GM muscles, $N = 400$ and $N = 550$ respectively.

	Identified population		CST	$IP(j)[s] = a \cdot j^b$		$S(j)[m^2] = S_{min} \cdot \Delta_S \left(\frac{j}{N}\right)^c$	
Dataset	N_r	$i \rightarrow N_i$	$coher_{N_r}$	a	b	$S_{min}[m^2]$	c
HTA_{35}	21	72-313	0.71	0.04	0.05	$1.73 \cdot 10^{-7}$	2.76
HGM_{30}	27	162-412	0.65	0.03	0.20	$1.20 \cdot 10^{-7}$	0.57
$S1_{30}^{AI}$	81	1-296	0.91	0.05	0.01	$1.37 \cdot 10^{-7}$	0.67
$S1_{30}^B$	35	15-294	0.82	0.05	0.01	$1.33 \cdot 10^{-7}$	0.90
$S1_{30}^E$	28	1-294	0.78	0.05	0.00	$1.60 \cdot 10^{-7}$	1.67
$S3_{30}^{AI}$	74	13-293	0.88	0.05	0.02	$1.50 \cdot 10^{-7}$	1.43
$S5_{30}^{AI}$	42	55-291	0.79	0.03	0.15	$1.40 \cdot 10^{-7}$	0.88
$S1_{50}^{AI}$	58	110-339	0.96	0.002	0.60	$1.69 \cdot 10^{-7}$	3.21
$S3_{50}^{AI}$	48	212-354	0.88	0.09	-0.14	$1.50 \cdot 10^{-7}$	1.69
$S5_{50}^{AI}$	34	79-351	0.83	0.06	-0.03	$1.64 \cdot 10^{-7}$	2.48

The size parameter S_i of the N_r LIF models was calibrated using the minimization function in (EQ. 5-13) so that the LIF-predicted filtered discharge frequencies $FDIF_i^{sim}(t)$ of the N_r MNs replicated the

experimental $FDIF_i^{exp}(t)$, displayed in Figure 5-8A and B (blue curves). As reported in Table 5-3, it was obtained on average for the N_r identified MNs $\Delta ft^1 < 300ms$ for all the datasets except HTA_{35} , HGM_{30} and $S3_{50}^{AI}$. It was also obtained average $nRMSE < 15\%$ for all datasets, and $r^2 > 0.90$ for all the datasets, except HGM_{30} and HTA_{35} . In more details for the dataset $S1_{30}^{AI}$, as shown in Figure 5-8C, the recruitment time ft^1 of 80% of the N_r identified MNs was predicted with an error less than 250ms. The calibrated LIF models were also able to accurately mimic the firing behaviour of the 67 lowest-threshold MNs as experimental and LIF-predicted FIDF traces compared with $r^2 > 0.8$ and $nRMSE < 15\%$ (Figure 5-8D and E). The scaled LIF models reproduced the firing behaviour of the 14 highest-threshold MNs ($N_i > 270$) with moderate accuracy, with Δft^1 and $nRMSE$ values up to -1.5s and 22.1%, which explains the large σ values reported in Table 5-3. Similar observations were made, and similar results were obtained for the nine other datasets. Consequently, despite modelling limitations discussed in the Discussion section, the global results in Figure 5-8 and Table 5-3 confirm that the two-parameters calibrated LIF models can accurately reproduce the firing and recruitment behaviour of the N_r experimental MNs. It must be noted that the Δft^1 and r^2 metrics were not included in the calibration procedure and the $(\Delta ft^1, r^2)$ values reported in Figure 5-8 were therefore blindly predicted.

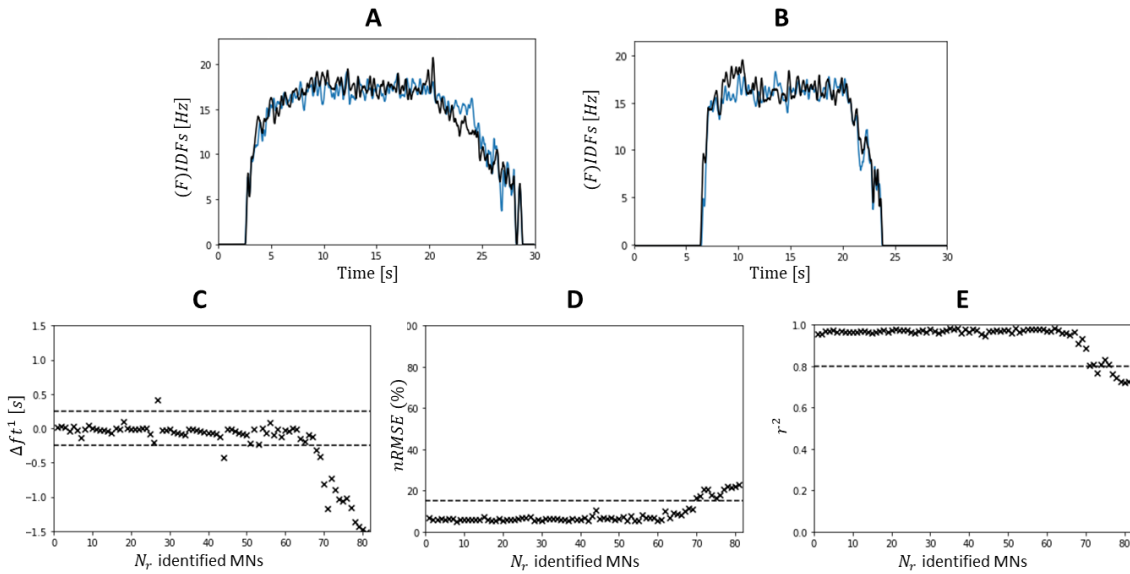


Figure 5-8: Calibration of the MN size S_i parameter. (A and B) Time-histories of the experimental (black) versus LIF-predicted (blue) filtered instantaneous discharge frequencies (FIDFs) of the 1st (A) and 47th (B) MNs identified in the $S1_{30}^{AI}$ dataset after parameter calibration. (C) Absolute error Δft^1 in seconds in predicting the MN recruitment time with the calibrated LIF models. The accuracy of LIF-predicted FIDFs is assessed for each MN with calculation of the nRMSE (D) and r^2 (E) values. (C-E) The metrics are computed for the $[t_{r0}; t_{r3}]$ time range only. The dashed lines represent the $\Delta ft^1 \in [-250; 250]ms$, $nRMSE \in [0; 15]\%MVC$ and $r^2 \in [0.8, 1.0]$ intervals of interest respectively.

Table 5-3: For all ten datasets, the average and standard deviation σ across the N_r identified MNs of the Δft^1 , $nRMSE$ and r^2 metrics obtained after size calibration.

Dataset	Δft^1 (s)		$nRMSE$ (%)		r^2	
	mean	σ	Mean	σ	mean	σ
HTA₃₅	0.58	1.15	9.7	4.8	0.85	0.24
HGM₃₀	0.54	1.64	10.8	5.4	0.89	0.16
S1^{AI}₃₀	0.23	0.40	8.6	4.9	0.94	0.07
S1^B₃₀	0.28	0.40	9.1	4.7	0.94	0.06
S1^E₃₀	0.29	0.39	8.7	4.3	0.94	0.06
S3^{AI}₃₀	0.28	0.39	7.8	3.8	0.96	0.05
S5^{AI}₃₀	0.29	0.38	9.4	3.4	0.93	0.06
S1^{AI}₅₀	0.29	0.36	7.5	3.0	0.96	0.04
S3^{AI}₅₀	0.4	0.77	9.0	5.0	0.94	0.09
S5^{AI}₅₀	0.18	0.30	7.3	2.9	0.95	0.10

The cloud of N_r pairs $\{N_i; S_{N_i}\}$ of data points (black crosses in Figure 5-9A), obtained from MN mapping (Figure 5-4B and C) and size calibration for the dataset $S1_{30}^{AI}$, were least-squares fitted (red curve in Figure 5-9A, $r^2 = 0.96$) by the power relationship in (EQ. 5-19), with $\Delta_S = 2.4$.

$$S(j) = 1.37 \cdot 10^{-7} \cdot \Delta_S \left(\frac{j}{N}\right)^{0.67} \quad (\text{EQ. 5-19})$$

As reported in the last column of Table 5-2, the minimum MN size (in m^2) obtained by extrapolation of this trendline was in the range $[1.20, 1.73] \cdot 10^{-1} mm^2$ for all the datasets, with expected maximum MN size (in m^2) in the range $[2.88, 4.15] \cdot 10^{-1} mm^2$ which is consistent with typical cat data (Barrett & Crill, 1974; Ulfhake & Kellerth, 1981; Burke et al., 1982; Ulfhake & Kellerth, 1984; Gustafsson & Pinter, 1984b; Ulfhake, Brun & Cullheim, 1988; Caillet et al., 2022), even if slightly in the lower range. Most datasets returned similar values for the c coefficient in the range $[0.57; 1.43]$, suggesting a mild skewness in MN size distribution. The datasets where mostly high-threshold large MNs are identified ($S1_{50}^{AI}, S3_{50}^{AI}, S5_{50}^{AI}$) and those of small size ($S1_{30}^E, HTA_{35}$) returned larger c values up to 3.21 and suggest a potentially non-physiologically skewed distribution of MN size in the MN pool.

As displayed in Figure 5-8A and B, the phenomenological adjustment of the R and C_m parameters described in (EQ. 5-15) and (EQ. 5-16) successfully captured the main effects of the hysteresis mechanisms involved with MN derecruitment, where MNs discharge at lower rate and stop firing at lower current input than at recruitment. In all ten datasets, $J(C_m) = \frac{nRMSE - r^2}{2}$ was parabolic for

incremental values of C_m and a global minimum was found for $C_m^d \in [1.6; 3.0]\mu F \cdot cm^2$. Seven of the ten datasets yielded $C_m^d = 2.6\mu F \cdot cm^2 = 2 \cdot C_m$. Most importantly, the same value of C_m^d was obtained from the datasets $S1_{50}^{AI}, S1_{50}^B$ and $S1_{50}^E$ which were obtained from the same experiment. These values for the parameter C_m^d were retained for the final simulations (Figure 5-11 and Figure 5-12) over the $[t_{r_3}; t_{r_5}]$ time range.

5.3.5. Step (4): Simulating the MN pool firing behaviour

As displayed in Figure 5-9B for the dataset $S1_{50}^{AI}$, the $S(j)$ distribution determined the MN-specific electrophysiological parameters (input resistance R and membrane capacitance C) of a cohort of $N = 400$ LIF models, which predicted from $I(t)$ the spike trains of the entire pool of N MNs (Figure 5-9C). As displayed in the plot of smoothed FIDFs in Figure 5-9C, the output behaviour of the reconstructed MN pool agrees with the Onion-skin scheme (De Luca, Carlo J. & Hostage, 2010) where lower-threshold MNs start discharging at higher frequencies and reach higher discharge rates than larger recruited units.

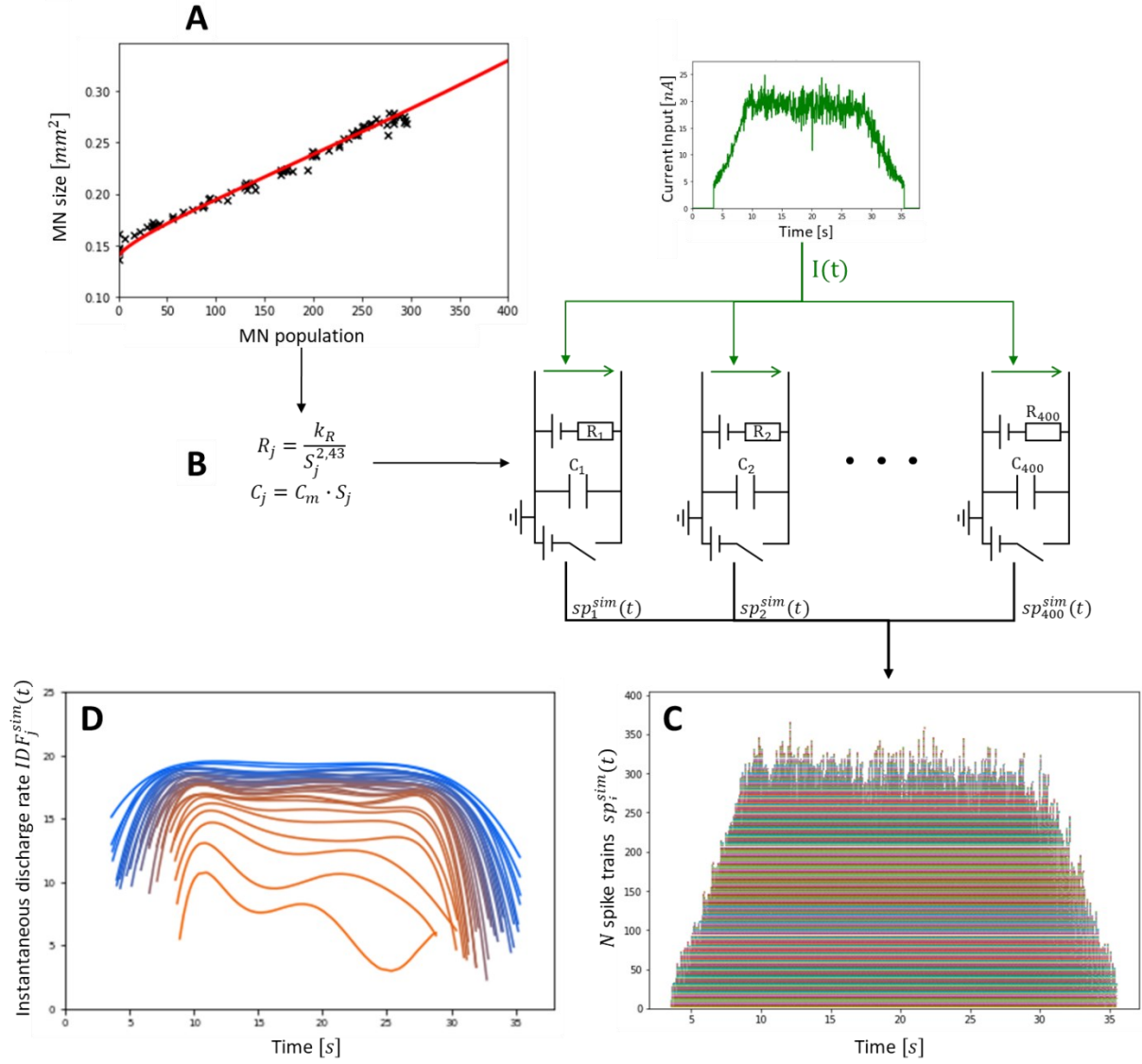


Figure 5-9: Reconstruction of the firing behaviour and recruitment dynamics of the complete MN pool for the dataset $S1_{50}^{AI}$. (A) The N_r calibrated MN sizes (black crosses) are least-squares fitted by the power trendline

$S(j)[\text{m}^2] = 1.37 \cdot 10^{-7} \cdot \Delta_S \left(\frac{j}{N}\right)^{0.67}$, which reconstructs the distributions of MN sizes in the complete MN pool. (B) The $S(j)$ distribution determines the MN-specific R and C parameters of a cohort of N LIF models, which is driven by the current input $I(t)$ and predicts (C) the spike trains $sp_j^{sim}(t)$ of the N virtual MNs constituting the complete MN pool. (D) The MN instantaneous discharge frequencies (IDF) were computed from the simulated spike trains $sp_j^{sim}(t)$ and were smoothed with a sixth-order polynomial (Hug et al., 2021). One out of ten IDFs is displayed for clarity. The blue-to-red gradient for small-to-large MNs shows that the output behaviour of the reconstructed MN population is in agreement with the Onion-Skin scheme.

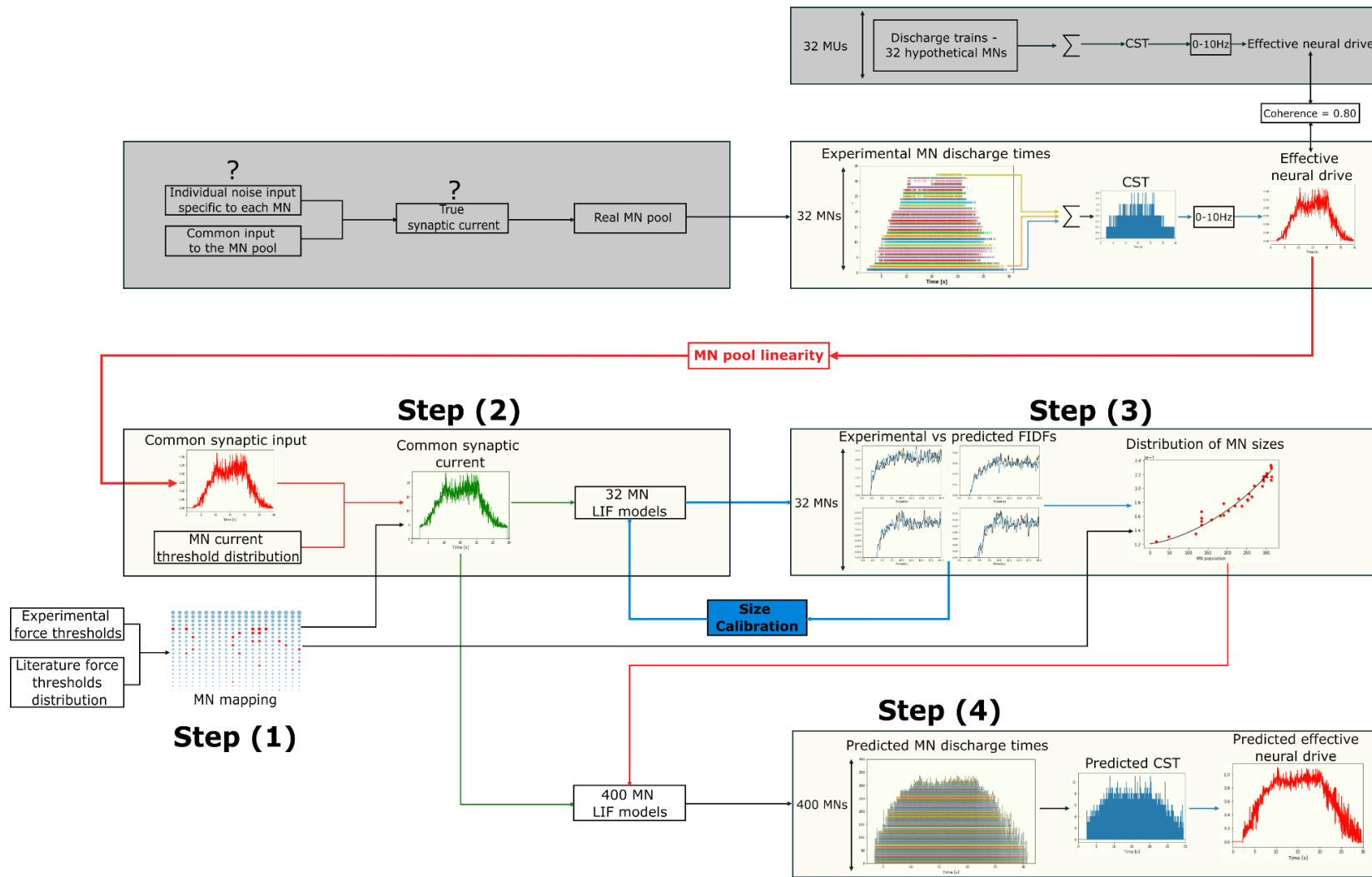


Figure 5-10: Detailed description of the 4-step workflow applied to the HTA_{35} dataset. The firing activity of a fraction of the MN pool is obtained from decomposed HDEMG signals. These N_r experimental spike trains provide an estimate of the effective neural drive to muscle and explain most of the MN pool behaviour ($coherence = 0.8$). From a mapping of the N_r identified MNs to the complete MN pool (Step (1)), literature knowledge on the typical distribution of I^{th} in a mammalian MN pool, and using the linearity properties of the population of N_r MNs, the current input $I(t)$ is estimated (Step (2)). $I(t)$ is input to N_r LIF models of MN to derive, after a one-parameter calibration step minimizing the error between experimental and LIF-predicted FIDFs, the distribution of MN sizes across the complete MN pool (Step (3)). The distribution of MN sizes, which entirely describes the distribution of MN-specific electrophysiological parameters across the MN pool, scales a cohort of $N = 400$ LIF models which transforms $I(t)$ into the simulated spike trains of the N MNs of the MN pool (Step (4)). The effective neural drive to muscle is estimated from the N simulated spike trains.

5.3.6. Validation

The four-step approach summarized in Figure 5-1 is detailed in Figure 5-10 and was validated in three ways.

5.3.6.1. Validation 1

The simulated spike trains were validated for the N_r MNs by comparing experimental and LIF-predicted FIDFs, where the experimental information of the investigated MN was removed from the experimental dataset and not used in the derivation of the $IP(j)$ and $S(j)$ distributions of the synaptic current $I(t)$. Table 5-4 reports for the ten datasets the average across the N_r identified MNs of the Δft^1 , $nRMSE$ and r^2 metrics described in the Methods section. Apart from the two open-source datasets and two datasets at 50% MVC for which large values of standard deviation σ were also obtained, the first discharge time of the experimental MNs was blindly predicted in average with less than 300ms error in the remaining datasets. The experimental and predicted FIDFs compared with $nRMSE < 10\%$ and $r^2 > 0.93$ in all ten datasets, except the two open-source datasets ($r^2 = 0.85$ and 0.89 for HTA_{35} and HGM_{30} respectively). Importantly, according to the results returned by the $S1_{30}^{AI}$, $S1_{30}^B$, $S1_{30}^E$, $S3_{30}^{AI}$ and $S5_{30}^{AI}$ datasets, the predictions are not strictly sensitive to the number N_r of identified MNs, but to their representativity of the complete MN pool. In more details, Figure 5-11 reports for the N_r MNs identified in the datasets $S1_{30}^{AI}$, $S1_{30}^B$, HTA_{35} , HGM_{30} and $S1_{50}^{AI}$ the absolute error Δft^1 in predicting the MN recruitment time (1st row) and the comparison between experimental and LIF-predicted filtered instantaneous discharge frequencies (FIDFs) with calculation of $nRMSE$ (%) (2nd row) and r^2 (3rd row) values over the $[t_{r_0}; t_{r_5}]$ time range. In these and the five remaining datasets, the recruitment time of 45-to-70% of the N_r identified MNs was predicted with an absolute error less than $\Delta ft^1 = 250ms$. In all datasets, the LIF-predicted and experimental FIDFs of more than 80% of the N_r MNs compared with $nRMSE < 20\%$ and $r^2 > 0.8$, while 75% of the N_r MN experimental and predicted FIDFs compared with $r^2 > 0.8$ in the dataset HGM_{30} . These average and MN-specific results confirm that the four-step approach summarized in Figure 5-1 is valid for all ten datasets for blindly predicting the recruitment time and the firing behaviour of the N_r MNs recorded experimentally. The identified MNs that are representative of a large fraction of the complete MN pool, i.e. which are the only identified MN in the range $[N_i - \frac{N}{10}; N_i + \frac{N}{10}]$ of the entire MN pool, such as the 1st MN in the dataset $S1_{50}^{AI}$, are some of the MNs returning the highest Δft^1 and $nRMSE$ and lowest r^2 values. As observed in Figure 5-11, ignoring the spike trains $sp_i^{exp}(t)$ of those ‘representative’ MNs in the derivation of $I(t)$, $IP(j)$ and $S(j)$ in steps (2) and (3) therefore affects the quality of the predictions more than ignoring the information of MNs that are representative of a small fraction of the MN population. In all datasets, $nRMSE > 20\%$ and $r^2 < 0.8$ was mainly obtained for the last-recruited

MNs (4th quarter of each plot in Figure 5-11) that exhibit recruitment thresholds close to the value of the current input $I(t)$ during the plateau of constant force in the time range $[t_{tr_2}; t_{tr_3}]$. The datasets where high-threshold MNs were dominantly identified (HTA_{35} , HGM_{30} , $S1_{50}^{AI}$ in Figure 5-11) are those which returned the poorest average results in Table 5-4. In all datasets, the predictions obtained for all other MNs that have intermediate recruitment thresholds and are the most identified MNs in the datasets, were similar and the best among the pool of N_r MNs.

Table 5-4: For all ten datasets, the average and standard deviation σ across the N_r identified MNs of the Δft^1 , $nRMSE$ and r^2 metrics obtained from blind predictions during Validation 1.

Dataset	Δft^1 (s)		$nRMSE$ (%)		r^2	
	mean	σ	Mean	σ	mean	σ
HTA_{35}	0.58	1.15	9.9	5.1	0.85	0.24
HGM_{30}	0.54	1.64	10.8	5.4	0.89	0.16
$S1_{30}^{AI}$	0.23	0.40	8.6	4.9	0.94	0.07
$S1_{30}^B$	0.28	0.40	9.1	4.7	0.94	0.06
$S1_{30}^E$	0.29	0.39	8.7	4.3	0.94	0.06
$S3_{30}^{AI}$	0.28	0.39	7.8	3.8	0.96	0.05
$S5_{30}^{AI}$	0.29	0.38	9.4	3.4	0.93	0.06
$S1_{50}^{AI}$	0.34	0.36	7.5	3.0	0.96	0.04
$S3_{50}^{AI}$	0.49	0.77	9.0	5.0	0.94	0.09
$S5_{50}^{AI}$	0.18	0.30	7.3	2.9	0.95	0.10

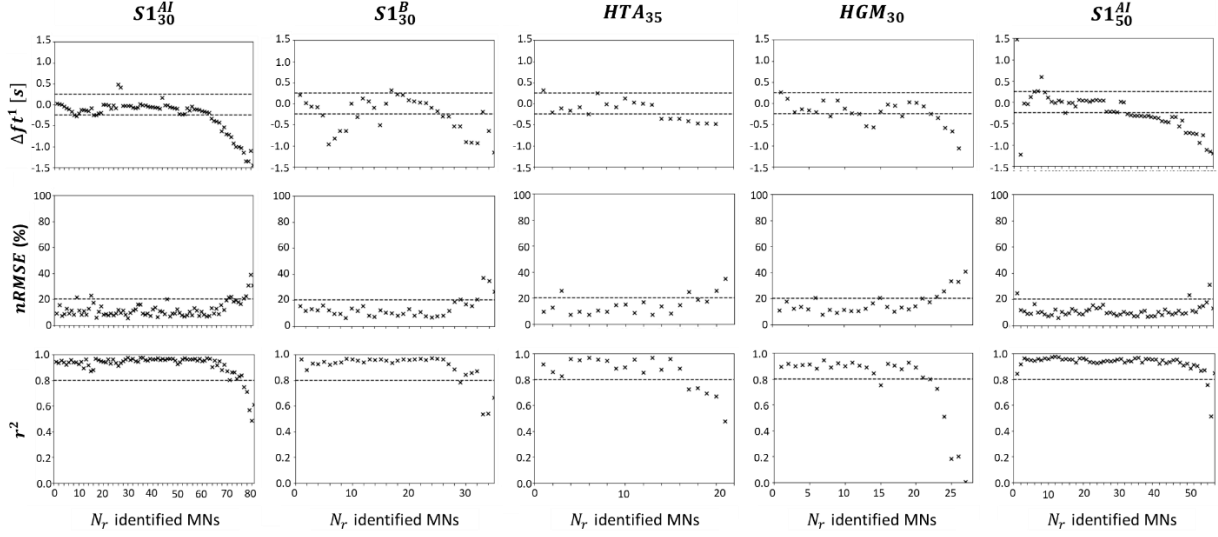


Figure 5-11: Validation of the MN recruitment and firing behaviour predicted with the 4-step approach (Figure 5-1) for the N_r MNs experimentally identified in the four datasets $S1_{30}^{AI}$, $S1_{30}^B$, HTA_{35} , HGM_{30} and $S1_{50}^{AI}$ described in Table 5-1. For the validation of each i^{th} predicted MN spike train, the experimental information of the i^{th} identified MN was omitted when deriving the current input $I(t)$ and the $IP(j)$ and $S(j)$ distributions (steps 2 and 3), from which the IP_i and S_i parameters are obtained without bias for the i^{th} MN. The spike train $sp_i^{sim}(t)$ of the i^{th} MN is then predicted with an IP_i, S_i -scaled LIF model receiving $I(t)$ as input. The absolute error in predicting the experimental recruitment time Δft_i^1 (s) is reported for each of the N_r MNs in the first row of the figure. Experimental and filtered instantaneous discharge frequencies $FIDF_i^{exp}(t)$ and $FIDF_i^{sim}(t)$, computed from $sp_i^{exp}(t)$ and $sp_i^{sim}(t)$ are compared with calculation of $nRMSE$ (%) and r^2 in the second and third rows of the figure. The dashed lines represent the $\Delta ft_i^1 \in [-250; 250]ms$, $nRMSE \in [0; 15]\%MVC$ and $r^2 \in [0.8, 1.0]$ intervals of interest respectively.

5.3.7. Validation 2

The effective neural drive predicted by the 4-step approach summarized in Figure 5-1 was validated by comparing for isometric contractions, the normalized eND_N (orange traces in Figure 5-12) computed from the N predicted spike trains $sp_j^{sim}(t)$ to the normalized force trace $\bar{F}(t)$ (green trace in Figure 5-12). The eND_N was accurately predicted for all nine TA datasets with $r^2 \geq 0.95$ and $nRMSE < 10\%$ (Table 5-5). No significant difference in accuracy was observed between the six TA datasets at 30%, suggesting that the four-step method can accurately reconstruct the MN pool from $N_r = 21$ to 81 identified MNs for 30% MVC trapezoidal contractions, provided the N_r identified MNs are sufficiently spread in the MN pool. Less accurate results were obtained for the three datasets obtained at 50% MVC. In the datasets $S1_{50}^{AI}$ and $S3_{50}^{AI}$, a null eND was predicted until the muscle builds 10-15%MVC, after which the eND was overestimated for the rest of the (de)recruitment phase (orange trace, Figure 5-12). As discussed previously and detailed in the discussion, the latter is explained by an inadequate $IP(j)$ distribution (Table 5-2), due to a lack of experimental information in those datasets (third column in Table 5-2), which returns non-physiological maximum firing rates for the low-thresholds MNs. The results obtained from the dataset HGM_{30} returned $r^2 = 0.89$ and $nRMSE = 18.2\%$ (Table 5-5),

accurately predicting the eND_N for the positive ramp and first half of the plateau of force and then underestimating the true neural drive (HGM_{30} , Figure 5-12). With two-to-three times lower $nRMSE$ and higher r^2 values for all ten datasets (Table 5-5), the eND_N predicted from the 4-step approach (orange dotted traces in Figure 5-12) was systematically a more accurate representation of the real effective neural drive than the eND_{N_r} (blue dotted traces in Figure 5-12) computed from the N_r experimental spike trains, especially in the phases of MN (de)recruitment where the real effective neural drive was underestimated by eND_{N_r} . It is worth noting that eND_N was more accurate than eND_{N_r} even for the datasets $S1_{30}^{AI}$ and $S3_{30}^{AI}$ where numerous homogeneously-spread MNs were experimentally identified. Consequently, this four-step approach is valid for accurately reconstructing the eND to a muscle produced by a collection of N simulated MN spike trains, which were predicted from a sample of N_r experimental spike trains. It is worth noting that the accuracy of the eND_N predictions, performed in Table 5-5 and Figure 5-12 for $N_{TA} = 400$ and $N_{GM} = 550$ MNs as discussed in the Methods Section, was not sensitive to the size N of the reconstructed MN population. For example, for the $S1_{30}^{AI}$ dataset, the eND_N computed with populations of $N = \{32, 100, 200, 300, 400\}$ MNs systematically compared to the real effective neural drive with $r^2 = 0.98$, and respectively returned $nRMSE = 8.7, 7.8, 7.0, 6.8$ and 6.4% , with slightly more accurate predictions of eND_N with an increasing N at low force during derecruitment.

Table 5-5: For all ten datasets, the r^2 and $nRMSE$ values obtained for the comparison of the time-histories of the normalized experimental force trace against the effective neural drive (1) eND_{N_r} and (2) eND_N computed from the spike trains of (1) the N_r identified MNs and (2) the N virtual MNs.

	N_r MNs (experimental)		N MNs (simulated)	
Dataset	r^2	$nRMSE$ (%)	r^2	$nRMSE$ (%)
HTA_{35}	0.85	26.8	0.97	7.9
HGM_{30}	0.85	28.8	0.89	18.2
$S1_{30}^{AI}$	0.97	12.6	0.98	6.4
$S1_{30}^B$	0.95	15.3	0.98	6.8
$S1_{30}^E$	0.96	13.6	0.98	5.2
$S3_{30}^{AI}$	0.93	21.9	0.97	10.0
$S5_{30}^{AI}$	0.92	17.6	0.97	7.5
$S1_{50}^{AI}$	0.87	22.9	0.95	8.6
$S3_{50}^{AI}$	0.89	21.0	0.95	9.6
$S5_{50}^{AI}$	0.90	20.5	0.97	7.2

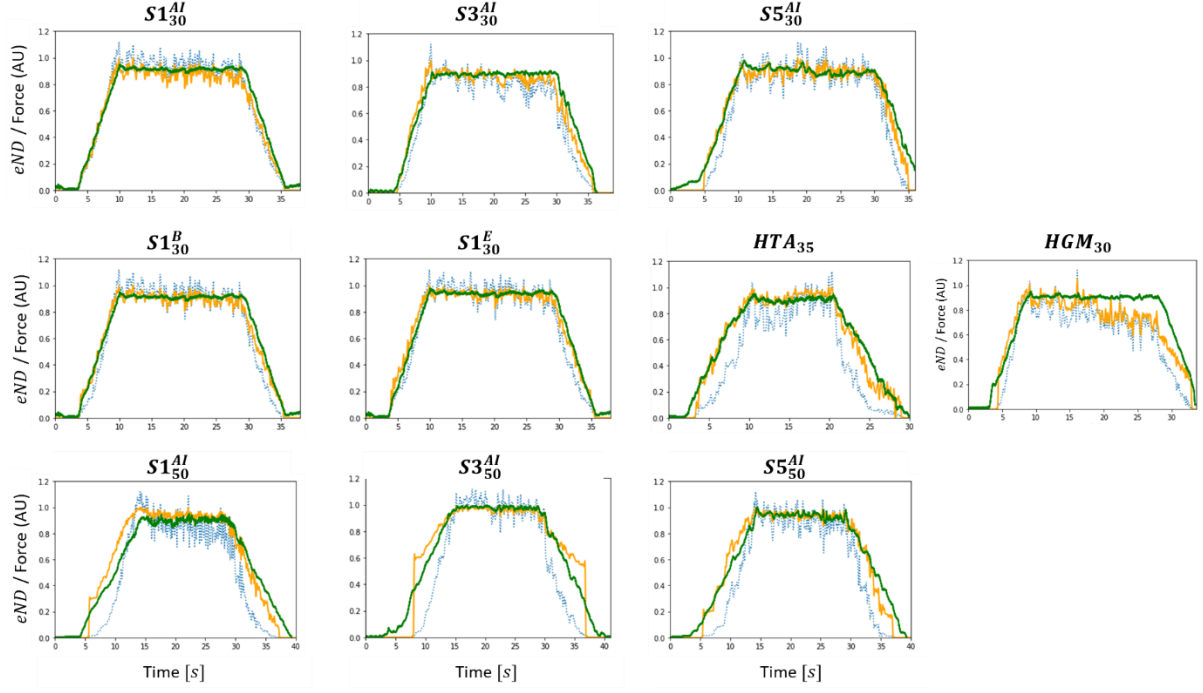


Figure 5-12: For the ten datasets, validation against the normalized force trace $\bar{F}(t)$ (green trace) of the normalized effective neural drive to muscle (eND_N) produced by the complete MN pool (orange trace) and computed from the N MN spike trains $sp_j^{sim}(t)$ predicted with the 4-step approach. For comparison, the eND_{N_r} directly computed from the N_r identified MNs is also reported (blue dashed trace). First row: HDEMG data acquired with 256 channels at 30% MVC. Second row: HDEMG data acquired with 64 channels at 30-35% MVC. Third row: HDEMG data acquired with 256 channels at 50% MVC.

5.3.1. Validation 3

In Validation 3, it was assessed whether the S and IP parameters calibrated with $S1_{30}^{AI}$ would generalize to the data obtained from another similar 30% MVC trapezoidal contraction performed by subject S1 ($S1_{30,2}^{AI}$) with similar experimental and processing conditions. The current input $I^{new}(t)$ derived from the activity of the $N_{r,2} = 81$ MNs identified in $S1_{30,2}^{AI}$ drove N LIF models, which were scaled with the S and IP parameter distributions obtained with the $S1_{30}^{AI}$ dataset: $S(j) = 1.37 \cdot 10^{-7} \cdot \Delta_S \left(\frac{j}{N}\right)^{0.67}$ and $IP(j) = 0.05 \cdot j^{0.01}$ (Table 5-2). The activity of the N LIF models produced the eND_N displayed in Figure 5-13A (orange trace), which compares to the normalized muscle force (green trace) with $nRMSE = 10.1\%$ and $r^2 = 0.98$. These results are like those obtained for Validation 2 in Figure 5-12 and Table 5-5. As before, the four-step workflow improved the accuracy in predicting the effective neural drive provided by the N_r MN spike trains alone, as eND_{N_r} returned $nRMSE = 15.2\%$ and $r^2 = 0.96$. Like in Validation 1 and as displayed in Figure 5-13BCD, 60% of the identified MNs returned $|\Delta ft^1| \leq 250ms$ and 90% returned $r^2 > 0.8$, although only two thirds of the MNs returned $nRMSE < 20\%$. The lesser accurate results for $nRMSE$ are explained by the higher firing rate of the high-

threshold MNs in $S1_{30,2}^{AI}$ than in $S1_{30}^{AI}$, that the IP distribution derived for $S1_{30}^{AI}$ could not predict. More advanced models, detailed in the Discussion section could solve this problem. In Figure 5-13, only 58 of the $N_r = 81$ identified MNs of $S1_{30,2}^{AI}$ are displayed, because $2 \cdot 21$ MNs were located to the same locations into the MN pool according to their force thresholds. In each case, only one MN was randomly kept for analysis.

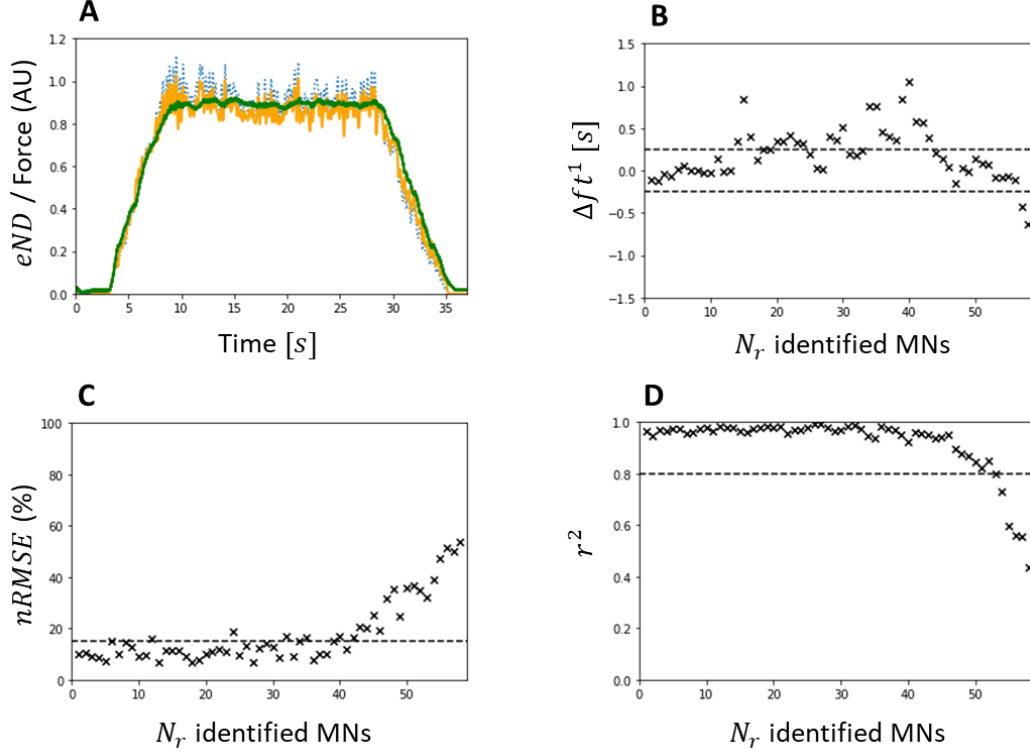


Figure 5-13: Results for Validation 3, where the experimental results from $S1_{30,2}^{AI}$ are input to the four-step workflow scaled with the S and IP distributions derived for $S1_{30}^{AI}$. Like Validations 1 and 2, the eND_{N_r} , eND_N , Δft^1 , $nRMSE$ and r^2 metrics are computed. For additional information, please refer to Figure 5-11 and Figure 5-12.

5.4. DISCUSSION

This study reports a novel four-step approach, summarized in Figure 5-1 and displayed in detail in Figure 5-10, to reconstruct the recruitment and firing behaviour of a complete human pool of N MNs from a sample of N_r experimental spike trains obtained from the decomposition of HDEMG or intramuscular recordings during voluntary contractions. This approach can help neuroscientists, experimentalists, and modelers to investigate MN pool neuromechanics, better understand experimental datasets, and, most importantly in this thesis, control more detailed MN-driven neuromuscular models to advance our understanding of the neural strategies taken by the human central nervous system to control voluntary muscle contractions.

The three first steps of our approach identify from a sample of N_r experimental spike trains a distribution of the MN electrophysiological properties across the MN pool. The N_r MN spike trains are used to approximate the common synaptic input $CSI(t)$ to the complete MN pool (Figure 5-6). For simplicity, the CSI is linearly related to the post-synaptic total dendritic membrane current $I(t)$, which is input to a cohort of N_r single-compartment LIF models with current synapses. The LIF firing behaviour is entirely described by the phenomenological MN Inert Period parameter IP_i , derived from the experimental data (Figure 5-7), and by the MN size S_i parameter to which all the MN electrophysiological properties are related, according to the relations provided in Chapter 4. After calibration of the S_i parameter, the N_r LIF models accurately mimic the filtered discharge behaviour and accurately predict the recruitment dynamics of the N_r experimental MNs (Figure 5-8). The N_r MNs are allocated into the complete MN pool (Figure 5-4B and C) according to their recorded force recruitment thresholds F_i^{th} (Figure 5-3B) and a typical species- and muscle-specific $F^{th}(j)$ distribution (Figure 5-4). From the previous findings, the continuous distribution of MN sizes $S(j)$ (Figure 5-9A) is derived for the complete pool of N MNs. $S(j)$ defines the electrophysiological properties (Chapter 4) of the MNs constituting the complete MN pool. The neural behaviour of the complete pool of N MNs is predicted in the 4th step with a cohort of N S_j - IP_j -scaled LIF models and the application of the current drive $I(t)$ (Figure 5-9).

5.4.1. Validation of the approach

The approach was successfully validated both for individual MNs and for the complete pool of N MNs. To do so, eight experimental datasets built in Chapter 3 and two datasets obtained from the literature were considered (Table 5-1).

5.4.1.1. Validation 1

By blindly scaling the LIF models in steps (1-3) with permutations of $N_r - 1$ input experimental spike trains, the filtered discharge behaviour and the recruitment dynamics of the N_r individual MNs were accurately predicted for the ten investigated datasets (Figure 5-11 and Table 5-4). In average, lower accuracy (Table 5-4) was observed with the open-source data than with the datasets obtained in Chapter 3, potentially because they returned in average higher Pulse-to-Noise ratios. In all the datasets, the first discharge time was accurately predicted and the $nRMSE$ and r^2 remained respectively low and high for the low-threshold MNs, while less accurate predictions were obtained for the high-threshold MNs recruited close to or after t_{tr_1} (Figure 5-2). In this respect, the predictions obtained at 50% MVC and from the open-source datasets, where mainly high-threshold MNs are identified, were less accurate than the 30% MVC cases obtained from Chapter 3. The datasets $S1_{30}^{AI}$, $S1_{30}^B$ and $S1_{30}^E$ returned similar results.

5.4.1.2. Validation 2

The effective neural drive (eND_N) to muscle elicited by the complete pool of N MNs was also accurately predicted by the 4-step approach for the ten datasets (Figure 5-12, Table 5-5). The latter result suggests that the recruitment dynamics and the normalized distribution of firing rates across the firing MN pool were accurately predicted for the non-identified population of $N - N_r$ MNs. According to the $nRMSE$ and r^2 metrics obtained for eND_N in Table 5-5, and emphasized with the $S1_{30}^{AI}$, $-S1_{30}^B - S1_{30}^E$ comparison, the method provides similar performance for predicting eND_N irrespective of the number N_r of identified MNs. It is worth noting that the lower N_r , the larger the improvement in accuracy between eND_{N_r} and eND_N , with up to three times lower $nRMSE$ values. As for Validation 1, less accurate results were obtained for the datasets, like $S3_{50}^{AI}$, where large partitions of the MN pool were not identified experimentally. Additionally, the accuracy of the eND_N predictions was not sensitive to the size N of the reconstructed population, with acceptable eND_N predictions ($nRMSE < 20\%$, $r^2 > 0.9$) obtained with as few as $N = 10$ distributed MNs for the dataset $S1_{30}^{AI}$. This suggests that the MN mapping (Step 1) and the derivation of the MN properties distribution (Step 3) in Figure 5-7 and Figure 5-9A are the key contributions for describing the complete MN pool behaviour from the input experimental data.

5.4.1.3. Validation 3

According to Figure 5-13, the method accurately extrapolates to other but similar contraction profiles for the same subject. The N LIF models, scaled with the S and IP distributions obtained from $S1_{30}^{AI}$, and receiving the experimental input from $S1_{30,2}^{AI}$, accurately predicted eND_N and returned values for the Δft^1 , $nRMSE$ and r^2 metrics in line with those obtained with Validation 1. In average, Δft^1 and $nRMSE$ were slightly higher for Validation 3 than for Validation 1, which is explained by the high-threshold MNs firing at higher discharge frequencies in $S1_{30,2}^{AI}$ than in $S1_{30}^{AI}$, thus limiting the predictive capacity of the $S1_{30}^{AI}$ -derived IP distribution.

5.4.1.4. Other validations

Despite the modelling limitations, discussed in (Burkitt, 2006) and in the Limitations Section, inherent to single-compartment LIF models with current synapses and related to the phenomenological IP , C^d and R^d parameters, the LIF models scaled following the methodology described in Step (3) can capture the distribution of MN discharging characteristics across the MN population. For example, the firing activity of the reconstructed MN population agrees with the onion-skin scheme (Figure 5-9D), as expected for slow low-force trajectories in human muscle contraction. While the MN size parameter calibration (Figure 5-8) only relies on the minimization of the root-mean square difference between experimental and predicted FIDFs, the normalized time-history of the FIDFs (high r^2 in Figure 5-11) and the MN recruitment times (low Δft^1 values in Figure 5-11) of the N_r MNs are both accurately mimicked (Figure 5-8) and blindly predicted (Figure 5-11) for all ten datasets. Some realistic aspects of the MN neurophysiology are also maintained. For example, the range of MN surface areas $[0.15; 0.36]mm^2$ and input resistances $[0.5; 4.1]M\Omega$ obtained from the $S(j)$ distribution falls into the classic range of MN properties for cats (Manuel et al., 2019; Caillet et al., 2022), which is consistent with the distribution of rheobase values used in Step (2) that was obtained from cat data. The distribution of MN input resistance in the MN pool, obtained from the calibrated sizes $S(j)$, defines the individual MN rheobase thresholds and predicts that 80% of the human TA MU pool is recruited at 35% MVC (Figure 5-9C), which is consistent with previous findings in the literature (Van Cutsem et al., 1997; Heckman & Enoka, 2012).

5.4.1.5. Conclusion on the performance of the method

In conclusion, the four-step approach described in Figure 5-1 accurately predicts the discharge behaviour of the $N - N_r$ MNs not identified experimentally. In all eleven cases, eND_N is more accurate than eND_{N_r} , with increased incremental accuracy as N_r is low. In all eleven cases, the discharge behaviour of the low-threshold MNs are accurately blindly predicted, with lower agreement for the few high-threshold MNs recruited around the plateau of force. The method extrapolated well to

another similar contraction profile. The method was insensitive to the MN pool size N and to the number of identified MNs N_r , although less accurate predictions were made when the discharge behaviour of large partitions of the MN pool were experimentally unknown.

5.4.2. Benefits of the approach

5.4.2.1. Benefit 1 – Better understanding the MN pool firing behaviour inferred from decomposed HDEMG outputs

The workflow provides a method to better understand datasets of N_r experimental spike trains obtained from signal decomposition. HDEMG decomposition techniques commonly identify samples of few tens of MNs (Del Vecchio et al., 2020) at most, i.e. typically less than 10-25% of the MU pool. As discussed in Chapter 3, the mapping in Figure 5-4 and Figure 5-9A demonstrates that these N_r -sized samples of MNs currently are not representative of the complete MN population, as supported by the $[0.65; 0.96]$ $coher_{N_r}$ values in Table 5-2. EMG decomposition shows a bias towards under-sampling small low-threshold MUs and identifying large high-threshold MUs because of their larger electric signal amplitude detected by the HDEMG electrodes. Consequently, the CST_{N_r} computed from the N_r identified MNs is not an accurate representation of the true muscle neural drive (Figure 5-12, Table 5-5). The method in Figure 5-10 identifies credible values for some MN-specific electrophysiological and morphometric properties, including MN membrane surface area, input resistance, capacitance, rheobase and time constant, for the dataset of N_r experimental MNs and for the $N - N_r$ MNs that cannot be recorded. Using a realistic distribution of these properties, the eND_N produced by the reconstructed MN population is accurately estimated, insensitive to the value of N , because it includes the firing activity of the complete fraction of the smaller MNs that were underrepresented in the experimental dataset, especially during the force ramps in $[t_1; t_2]$ and $[t_3; t_4]$ (Figure 5-2), where the eND_{N_r} can reach 70% normalized maximum error. The eND_N besides includes the activity of the complete population of high-threshold MNs, which reduces noise (orange vs blue trace in Figure 5-12) during the plateau of force in $[t_2; t_3]$. The eND_N also filters the local non-physiological variations displayed by the eND_{N_r} such as the decrease in eND_{N_r} in $[10; 15]$ s in HTA_{35} (Figure 5-12), the sudden rise and drop in eND_{N_r} at $t = 10$ s and $t = 30$ s in $S3_{50}^{AI}$ (Figure 5-12) or the steeply decreasing eND_{N_r} from $t = 28$ s in HGM_{30} . Understanding and accounting for these experimental limitations is important and has critical implications in HDEMG-driven neuromuscular modelling when the CST_{N_r} is input to a muscle model (Thompson et al., 2018; Kapelner et al., 2020).

The workflow also provides a method to support future experimental investigations. The signal decomposition process can be refined and more MNs can be identified by iteratively running this workflow and using the $N - N_r$ simulated spike trains of the non-identified MNs as a model for

identifying more MNs from the recorded signals. The method moreover provides simple phenomenological two-parameter-scaled LIF models described in step (3) to accurately replicate the recruitment and firing behaviour of the N_r identified MNs (Figure 5-8) for further investigation.

5.4.2.2. Benefit 2 – Advances in MN pool modelling

This four-step approach advances the state-of-the-art in MN pool modelling. As discussed in the introduction, using a sample of N_r experimental MN spike trains permits for the first time in human MN pool modelling to (1) estimate the true time-course of the common synaptic input to the MN pool, which cannot be measured experimentally, (2) approximate with a one-parameter calibration, available HDEMG data and knowledge from the literature, the MN-specific electrophysiological properties of all the MNs in the MN pool and a realistic distribution of these properties across the pool, which could, to date, only be obtained in specialized experimental and MN pool modelling studies in animals (Raikova et al., 2018), and (3) validate the forward predictions of MN spike trains and effective neural drive to muscle to human experimental data. The pool of LIF models, the maximum firing frequency of which is obtained from the available experimental data, intrinsically accounts for the onion-skin theory (De Luca, Carlo J. & Hostage, 2010) (Figure 5-9D), and better replicates the MN membrane dynamics of the MN pool than Fuglevand-type phenomenological models (Fuglevand et al., 1993; Carriou et al., 2019), where the MN-specific firing rates are predicted as a linear function of the current input $I(t)$. Moreover, single-compartment current-input LIF models can credibly replicate most of the MN membrane dynamics (Teeter et al., 2018) and allowed accurate predictions of the MN pool behaviour (Figure 5-11, Figure 5-12), while they provide a simpler modelling approach and a more convenient framework for MN electrophysiological parameter assignment in the MN pool than comprehensive Hodgkin-Huxley-type MN models (Huxley & Niedergerke, 1954; Huxley, 1957; Cisi & Kohn, 2008; Negro & Farina, 2011). This four-step approach demonstrated to be robust to systematic differences in the input experimental datasets. For example, it accurately predicted the individual MN firing behaviour of the N_r MNs for all ten datasets (Figure 5-11) despite the latter being obtained at different levels of muscle contractions, on different subjects and muscles and in different experimental approaches. The approach accurately predicted the eND_N in most cases from $N_r = 21$ to 81 identified MNs respectively (Table 5-5), suggesting that the method is not sensitive to the number N_r of identified MNs, providing that the N_r identified MNs and their properties are homogeneously spread in the MN pool, as in the S_{30} -like datasets where at least one MN is identified in the each 10%-range of the rheobase domain. The accurate prediction of eND_N for the dataset HGM_{30} in the time range $[4.7; 7.2]$ s (Figure 5-12 bottom-right plot) also suggests that the quality of the predictions is not sensitive to the hindlimb muscle investigated, providing that the F^{th} distribution is representative of the investigated muscle.

5.4.3. Limitations of the approach and potential improvements

Despite the aforementioned good performance of the four-step workflow, the method presents 4 main limitations, for some of which potential improvements are proposed in the following.

5.4.3.1. Limitation 1 – Model validation

The three validations of the approach (Figure 5-11 to Figure 5-13) present some limitations. The local validation in Figure 5-11 only ensures that the method accurately estimates the MN firing behaviour for the fractions of the MN pool that were experimentally identified. This local validation alone does not inform on the accuracy of the predictions for the non-identified regions of the MN pool and must be coupled with a global validation of the MN pool behaviour by validating the predicted eND_N . While the local validation was successful for the $S3_{50}^{AI}$ dataset (Figure 5-11), where less than 40% of the active MN pool is represented, it is inferred in Figure 5-12 that the individual MN firing rates were overestimated for the first half of the non-recorded MN pool. This local validation would be self-sufficient for experimental samples like the $S1_{30}$ datasets that contain a homogeneously spread population of identified MNs, obtained from decomposed fine-wire intramuscular electrode and HDEMG grid signals. The validation of the eND_N is performed in this study against an experimental force recorded by a transducer (green traces in Figure 5-12), which accounts for the force-generating activity of both the muscle of interest and the synergistic and antagonistic muscles crossing the ankle. The experimental force trace measured in the TA datasets may be an acceptable validation metric as the TA muscle is expected to explain most of the recorded ankle torque in dorsiflexion. The TA muscle is indeed the main dorsiflexor muscle with a muscle volume and maximum isometric force larger than the flexor hallucis longus muscle, which moment arm is besides not aligned with the dorsiflexion direction and mainly acts for ankle inversion. However, the gastrocnemius lateralis, soleus, and peroneus muscles acts synergistically with the GM muscle for ankle plantarflexion and the experimental torque recorded in dataset HGM_{30} may not be representative of the GM muscle force-generation activity and may not be a suitable validation metric for eND_N . In Figure 5-12, the decreasing eND_N and eND_{N_r} (orange and blue dotted traces) may accurately describe a gradually increasing sharing of the ankle torque between synergistic muscles initially taken by the GM muscle, which the constant validation trace does not capture. To answer such limitations, the predicted eND_N should be validated against a direct measure of muscle force, which can be performed as in other recent studies (Dick et al., 2017; Martinez-Valdes et al., 2021; Waasdorp et al., 2021) with ultrasound measurements of the muscle fascicle or of the muscle tendon concurrently obtained with (HD)EMG recordings of the muscle activity. Finally, Validation 3 successfully extrapolates the method only to a similar contraction profile up to the same level of force. It does not provide any indication whether the parameters calibrated with a trapezoidal force profile would generalize, for the same subject, to another force

trajectory or a trapezoidal force profile up to another force level and provide accurate predictions of the N_r experimental spike trains and of the eND_N .

5.4.3.2. Limitation 2 – Sensitivity of the method to input HDEMG data

While the method predicts a list of simulated spike trains $sp_j^{sim}(t)$ and a eND_N that more accurately describes the global MN pool behaviour than the experimental $sp_i^{exp}(t)$ and eND_{N_r} , as discussed previously, the accuracy of these predictions (Figure 5-11 and Figure 5-12) remains sensitive to the distribution in the entire MN pool of the N_r MNs identified experimentally, reported in the third column of Table 5-2, for two main reasons. (1) Because of the definition of the current input $I(t)$, the eND_N onset is defined by the recruitment time $tf_{N_1}^1$ of the lowest-threshold MN N_1 identified experimentally, as shown in Figure 5-12, while the unknown firing behaviour for $t < tf_{N_1}^1$ of the non-identified MNs of rheobase lower than $I_{N_1}^{th}$ is not captured. This is not an issue in samples of homogeneously distributed MNs like dataset $S1_{30}^{AI}$, where the smallest MNs are identified (Figure 5-4BC), Table 5-2) and the eND obtained from the smallest MNs is predicted during the initial time range. However, the heterogeneous or incomplete samples like the open-source datasets HTA_{35} and HGM_{30} and those obtained at 50% MVC return a zero muscle neural drive $eND = 0$ for long time periods. For example in the $S3_{50}^{AI}$ dataset, the lowest-threshold (212th, Table 5-2) identified MN identified is recruited at $t = tf_{N_1}^1 = 7.9s$ and the approach wrongly return $eND = 0$ over $[3.1; 7.9]s$ where the muscle actually builds 24% MVC during the ramp of contraction (bottom-left plot in Figure 5-12). To tackle this issue, the normalized force trace, which is non-zero for $t < tf_{N_1}^1$ and representative of the CSI in this time region, could be scaled and used in lieu of the current definition of $I(t)$. However, this approach, which may not be suitable for non-isometric conditions, is not applicable in forward predictions of unknown whole muscle force from neural inputs. (2) Experimental samples of homogeneously distributed MNs are also required to derive realistic $S(j)$ and $IP(j)$ distributions with the four-step approach. As observed in Figure 5-11, ignoring in the derivation of $I(t)$, $IP(j)$ and $S(j)$ the spike trains $sp_i^{exp}(t)$ of the MNs that are representative of a large subset of the MN pool affects the accuracy of the predicted recruitment and firing behaviour of the MNs falling in that subset (Figure 5-11). More specifically, the non-physiological distribution $IP(j)[s] = 0.09 \cdot j^{-0.14}$ was fitted to the experimental data of the 48 high-threshold MNs identified in dataset $S3_{50}^{AI}$, where the neural information of the 50% smallest MNs of the MN pool (Table 5-2) is lacking. Such $IP(j)$ distribution suggests that high-threshold MNs discharge at higher rates than low-threshold MNs, which contradicts the onion-skin theory. Therefore, it overestimates the LIF-predicted maximum firing frequency of high-threshold MNs, which explains the overestimation of the eND_N in Figure 5-12 (bottom-left plot, orange curve). Such IP distribution could be corrected by enforcing the IP trendline

to meet specific conditions obtained from the literature or other experimental results, such as $\frac{1}{IP_1}$ being in the range $[25; 40]Hz$ at 50% MVC. Consequently, the method performs less accurately for datasets providing relatively large numbers of high-thresholds MNs, typical of datasets obtained at high % MVC and of high pulse-to-noise ratios.

5.4.3.3. Limitation 3 – LIF MN modelling

The LIF MN model described from (EQ. 5-10) to (EQ. 5-16) was shown, with credible sets of inter-related parameters S_{MN} , R , C and τ after Chapter 4, to accurately mimic (Figure 5-8) and blindly predict (Figure 5-11) most of the key phenomenological features of the N_r firing MNs, including their FIDFs and time of first discharge, as well as nonlinear behaviours such as firing rate saturation (Figure 5-7) and hysteresis (Figure 5-3B and C). However, this MN model is mostly phenomenological and does not provide a realistic description of the actual mechanisms underlying the dynamics of action potential firing for several reasons. First, this single-compartment approach neglects the activity of the MN dendrites, which account for more than 95% of the total MN surface area and gather most of the post-synaptic receptors and MN PIC-generating channels responsible for the MN nonlinear input-output functions (Binder et al., 2020). The inherent difference in membrane voltage dynamics between the dendrites and the soma, also partially mediated by hundred-fold differences in the value of passive electrophysiological parameters such as R which increases with somatofugal distance (Fleshman et al., 1983) is therefore neglected in this point model approach. Then, the nonlinear dendritic activity being overlooked, the Common Synaptic Input $CSI(t)$ in (EQ. 5-8), which is the common net excitatory synaptic influx, is non-physiologically assimilated with a constant gain to the post-synaptic total dendritic membrane current $I(t)$ that depolarizes the MN soma and is responsible for spike generation. With this linear $CSI - I$ scaling in (EQ. 5-9), the approach neglects the realistic description of the voltage-driven dynamics of the PIC-generating channels that are responsible for some important nonlinear mechanisms, such as firing rate saturation (Powers & Heckman, 2017) and hysteresis in the MN's current-voltage relation (Lee & Heckman, 1998; Lee & Heckman, 1998), which dictate the nonlinear $CSI - I$ transformation (Binder et al., 2020). In this study, the firing rate saturation, which in real MNs is due to a decrease in driving force for synaptic current flow as the dendrites become more depolarized (Powers & Heckman, 2017), is captured by the phenomenological IP parameter. The observed hysteresis between the recruitment and decruitment thresholds (Figure 5-3B) and in the current-firing rate relation between recruitment and decruitment phases (Figure 5-3C), explained by an hysteresis in the MN's current-voltage relation that arises from the activity of long-lasting PICs (Heckman & Enoka, 2012), are successfully but non-physiologically addressed by a tuning during decruitment of the R and C_m parameters respectively, although the values of the passive property R and of C_m , biological constant for the MN population (Gentet et al., 2000), should be independent

from the MN activity (Burkitt, 2006). For these reasons, even if the distributions of the S_{MN} , R , C and τ parameters at recruitment take values consistent with the literature, as discussed, the MN LIF model described in (EQ. 5-10) to (EQ. 5-16) is not suitable for investigating the neurophysiology and the neuromechanics of individual MNs. The phenomenological tuning of the IP , R and C_m parameters, although symptomatic of underlying nonlinear mechanisms, does not provide any insight into the true MN neurophysiology. If a more realistic MN model was considered in lieu of the LIF model, such as multiple-compartments Hodgkin-Huxley-based approaches (Kim et al., 2014), the four-step workflow would be an adequate tool for testing neurophysiological hypotheses and investigating some mechanisms and properties of the complete MN pool that cannot be directly observed experimentally in conditions of human voluntary contractions. A possible trade-off would be considering a single-compartment LIF model with active conductances (Burkitt, 2006), in which case (EQ. 5-7) is re-written as (EQ. 5-20), where $g_R = \frac{1}{R}$ is the constant MN passive conductance, $E_+ > \Delta V_{th}$ is the constant reversal potential for excitatory channels, $g(t, CSI)$ the time-varying total active excitatory synaptic conductance, and $I_{ps}(t, CSI, V_m) = g(t, CSI) \cdot (E_+ - V_m)$ the post-synaptic total dendritic membrane current induced by the synaptic drive CSI , the driving force of which is decreased by the term $(E_+ - V_m)$ when the dendritic membrane depolarizes, as discussed.

$$\frac{dV_m}{dt} = \frac{1}{C} \cdot [g(t, CSI) \cdot (E_+ - V_m) - g_R \cdot V_m] \quad (\text{EQ. 5-20})$$

In the literature, $g(t, CSI) = g_{max} \cdot T(t, CSI)$ where g_{max} is the maximum active conductance of the synapse and $T(t, CSI)$ can be interpreted as the fraction of bound synaptic receptors (Cisi & Kohn, 2008) or of opened ionic channels in the range $[0; 1]$ or as a train of synaptic pulses (Burkitt, 2006). Because setting $T(t, CSI) = CSI(t)$, with $CSI(t)$ as defined in Figure 5-5B, does not meet the requirements in firing rate saturation for large CSI input, the saturation in dendritic ionic channel activation for large CSI (Powers & Heckman, 2017) must be accounted for and $T(t, CSI)$ should be a nonlinear saturating function of CSI. In this approach, the MN-specific hysteresis and firing rate behaviour could be obtained with a tuning during recruitment and derecruitment and a distribution across the MN pool of the g_{max} parameter and of the shape parameter of the S function based on the experimental data. Although this approach more realistically describes the neurophysiological mechanisms underlying the nonlinear MN behaviour during discharge events, it is more challenging to scale using the experimental information. Considering that this study focuses on the phenomenological behaviour of individual MNs and on the overall activity of reconstructed MN populations, the LIF model defined in (EQ. 5-7) was judged an adequate trade-off between accuracy and modelling complexity, considering the overall accurate predictions of the MN firing behaviours

(Figure 5-11) and its low computational cost, and no other modelling approaches such as (EQ. 5-20) were yet pursued.

It must be noted that, whatever the MN modelling approach, the experimental drive to the MN currently considered in the four-step method is the CSI, which disregards the MN-specific synaptic noise $SN(t)$, which is responsible for most of the inter-spike variability (ISV). Considering that the MN pool and the MU neuromechanical mechanisms are expected to filter the MN-specific $SN(t)$, this simplification is adequate for accurate predictions of the eND_N and of the whole muscle force (Farina & Negro, 2015). Sometimes modelled as a random Gaussian-like event (Fuglevand et al., 1993), the ISV can be obtained as the response to a $SN(t)$ white noise added to the current definition of the synaptic input as $CSI(t) + SN(t)$. The relative proportion of CSI and SN in % of the variance of the total synaptic current can be approximated from Figure 2A in (Negro et al., 2016). Accounting for $SN(t)$ might improve the accuracy of the predicted firing behaviour of the largest MNs, for which the largest Δft^1 and $nRMSE$ and lowest r^2 values were obtained in Figure 5-11 for all ten datasets, and the firing behaviour and recruitment dynamics of which are dominantly dictated by random fluctuations of $SN(t)$.

5.4.3.4. Limitation 4 – Limited experimental data in the literature

The four-step approach is constrained by the limited knowledge in the literature of the characteristics of the human MU pool. Therefore, the LIF parameters and the MN rheobase distribution in the MN pool (Figure 5-6A) are tuned with typical cat data from various hindlimb muscles (Chapter 4) while the experimental MN spike trains were obtained from the human TA muscle. While the normalized mathematical relationships relating the MN electrophysiological parameters of the LIF model ($R, C, \tau, \Delta V_{th}$) can be extrapolated between mammals (Chapter 4) and thus to humans, no experimental data is yet available to scale these relationships to typical human data.

Also, the typical $F^{th}(j)$ and $I_{th}(j)$ threshold distributions, derived for mapping the N_r identified MNs to the complete MN pool (Figure 5-4) and for scaling the CSI to physiological values of $I(t)$ (Figure 5-6), were obtained from studies which relied on experimental samples of MN populations of small size. These source studies therefore did not ensure that the largest and lowest values were identified and reported, and that the identification process was not biased towards a specific subset of the MN pool, such as larger MNs. In such cases, the true threshold distributions would be more skewed and spread over a larger range of values, as discussed in Chapter 4, than the distributions reported in Figure 5-4 and Figure 5-6. The $F^{th}(j)$ distribution is besides muscle-specific (Heckman & Enoka, 2012) with large hindlimb muscles being for example recruited over a larger range of MVC than hand muscles. However, enough data is reported in the literature to build the $F^{th}(j)$ distributions for the TA and first

dorsal interossei human muscles only. For these limitations, the two first steps of this approach could be made subject- species- and muscle-specific by calibrating the $F^{th}(j)$ and $I^{th}(j)$ described as the 3-parameter power functions defined in this study.

Chapter 6

DEVELOPMENT AND VALIDATION OF MOTONEURON-DRIVEN MOTOR UNIT-SCALE NEUROMUSCULAR MODEL

Transition

In Chapter 5, a four-step method was developed to augment the neural experimental data obtained in Chapter 3. From the N_r experimental spike trains obtained from decomposed HDEMG signals (Chapter 3), the method accurately inferred the firing activity of the complete pool of N MNs (Chapter 5). Importantly, the reconstructed MN pool more accurately approximated the effective neural drive to muscle than the sole experimental population, primarily because the reconstructed population accounted for the electrical activity of the small MNs and their specific electrophysiological properties (Chapter 4) that were underrepresented in the experimental datasets (Chapter 3). The set of artificial spike trains derived in Chapter 5 is used in this Chapter 6 to control the MN-driven multi-MU model introduced in Chapter 1. This novel model is scaled and validated with the experimental results from Chapter 3 and tackles three key limitations identified in the systematic review in Chapter 2.

Chapter summary

This Chapter 6 fulfils the final Objective (6) listed in Section 1.2. This chapter provides a detailed description and a validation of the MN-driven multi-MU neuromuscular model introduced in Chapter 1. The model is MN-driven, as vectors of MN spike trains control its pool of Hill-type MUs. Careful attention was given to the modelling of the MU excitation-contraction coupling properties and of the distribution of the MU properties in the MU pool. Scaled with the subject-specific quantities from Chapter 3, the model was validated against the experimental force traces derived in that chapter. To assess the importance of the method developed in Chapter 5, neuromuscular force was predicted from both the experimental and reconstructed vectors of MN spike trains obtained in Chapters 3 and 5, respectively. This introductory model addresses three key limitations in Hill-type modelling identified in Chapter 3, opens avenues for modelling the MU dynamics during human voluntary contractions, and provides strong foundations for future iterative improvements.

6.1. Introduction

Chapter 2 showed that most Hill-type models in the literature used a single force generator (FG) to describe the active properties of the whole muscle. In doing so, these models suffered from the limitations of the MU-to-muscle multiscale simplification and could not distinguish the dynamics of MN recruitment from the dynamics of MN discharge in their description of the neural drive to muscle. As a consequence of these limitations, the distribution of the MU properties in the MU pool and their independent dynamics were typically overlooked, and the muscle's excitation and activation dynamics were systematically lumped into simple mathematical descriptions of the muscle excitation-contraction coupling properties, the behaviour of which was therefore difficult to interpret and tune (Hussein et al., 2022). Also, no Hill-type-like neuromuscular model in the literature was ever controlled by the real spiking behaviour of identified discharging MNs in the simulation of human voluntary contractions. These limitations hinder the investigation of human neuromuscular control with computational tools.

To address these limitations, a novel MN-driven multi-MU neuromuscular model, introduced in Chapter 1 (Figure 1-1) and detailed in Figure 6-1 below, was developed and validated in this chapter. First, the rheological structure, the scaling of the model to subject-specific properties, the interplay between the MU Contractile Element (CE) and Neuromechanical Element (NE) for MU force generation, the modelling assumptions and simplifications, and the mathematical methods to solve the neuromuscular dynamics were described and discussed. Then, focus was given on the derivation of advanced models of the MU excitation and activation dynamics. Finally, simulations of the human voluntary isometric TA contractions were performed with either input vectors of N_r , experimental (Chapter 3) or N simulated (Chapter 5) MN spike trains. The resulting predictions of TA muscle force were validated against the experimental data reported in Chapter 3. The chapter concludes with a list of future works to improve this introductory MN-driven multi-MU model.

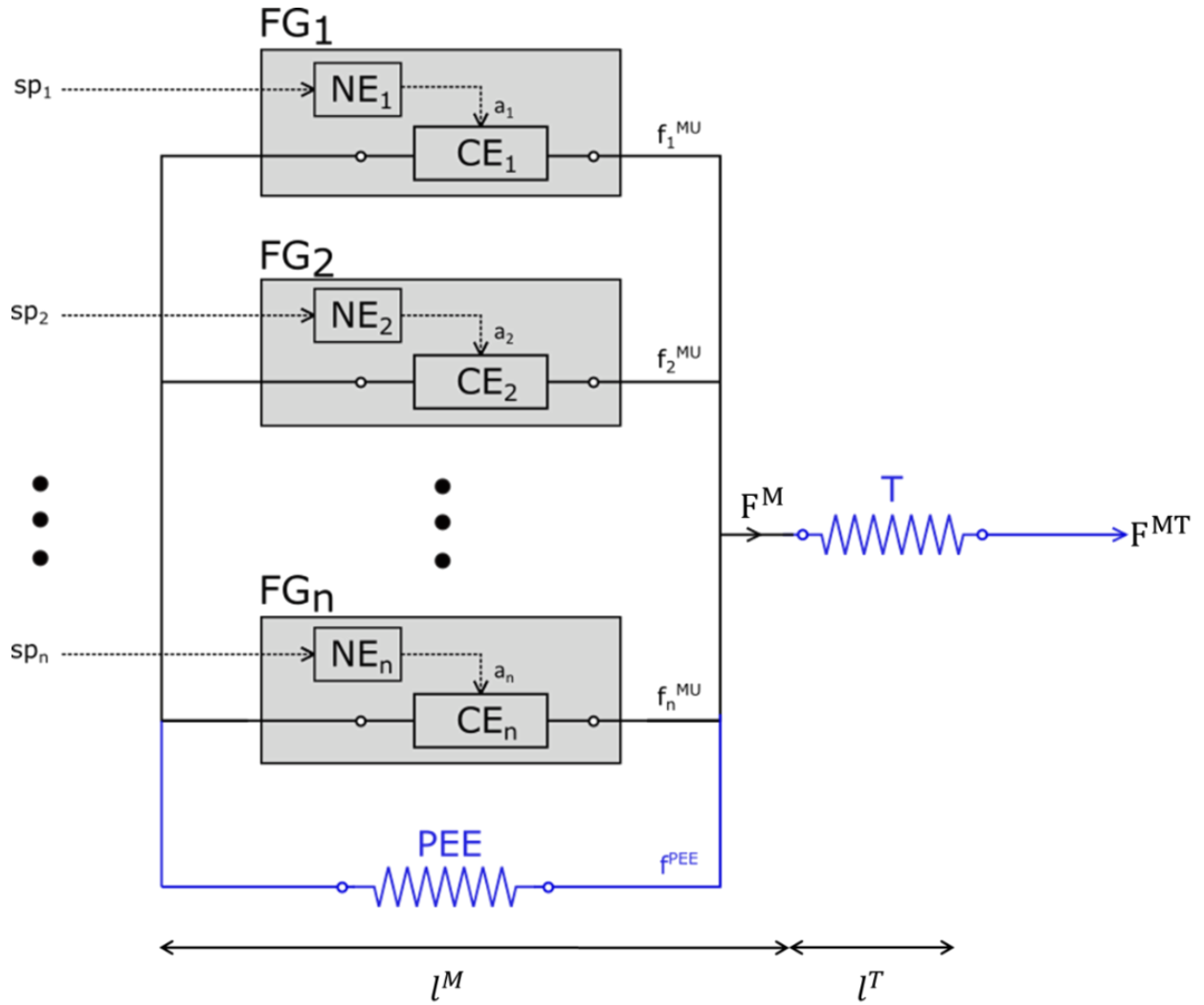


Figure 6-1: Rheological arrangement of the MN-driven multi-MU neuromuscular model developed in this Chapter 6. The muscle-tendon force F^{MT} , passively transmitted by the Tendon Element (T) builds as the linear sum of muscle-related passive forces F^{PEE} and a distribution of MU-specific active forces f_i^{MU} , with $i \in \llbracket 1; n \rrbracket$. Each f_i^{MU} is produced by a MU-specific Force Generator FG_i , which includes a Contractile Element (CE_i) and a Neuromechanical Element (NE_i), and is controlled by the spike train of its innervating MN (sp_i). For simplicity, the in-parallel MUs have the same length l^M , making zero pennation with the tendon of length l^T . As discussed, the tendon is assumed rigid in this chapter, i.e. l^T is constant, and the behaviour of the tendon and PEE (in blue) is neglected in isometric contractions at optimal length for simplicity.

6.2. Methods

6.2.1. Global overview - Rheological description – Preliminary simplifications

Overview of the rheological structure of the neuromuscular model

The model developed in this chapter includes n FGs, a passive elastic element (PEE), and a common tendon T (Figure 6-1). Additional passive elements could be considered according to Chapter 2, although the focus was given in this chapter to the active muscle properties. The cohort of FGs describes the active force-generating dynamics of the individual MUs constituting the muscle, while the PEE accounts for the passive stretch-resisting properties of the muscle tissue. The size n of the MU pool represented in Figure 6-1 depends on the vector of available MN spike trains (N_r or N) controlling the muscle model. As shown in Figure 6-1, the FGs were mathematically placed in-parallel and individually develop the MU active forces f_i^{MU} , $i \in \llbracket 1; n \rrbracket$, which linearly sum to yield the total active force F^{MU} generated by the MU pool (EQ. 6-1).

$$F^{MU} = \sum_{i=1}^n f_i^{MU} \quad (\text{EQ. 6-1})$$

This approach neglects both the geometrical orientation of the individual MNs and the lateral force transmission between MUs which decrease F^{MU} . The FGs were mathematically placed in-parallel with a single muscle-scale PEE and in-series with the common tendon. At this stage, the MUs have specific lengths l_i^{MU} .

Focus on the rheological arrangement of the PEE

In Figure 6-1, the PEE accounts for two scales of passive muscle tissues. It includes the muscle aponeurosis, that provides the dominant contribution to the total passive force in the muscle tissue (Chapter 2). It also describes the additional passive activity of the subscale passive structures, that was sometimes modelled with a SEE2 in-series with the FG, although it should be mathematically in-parallel with the active muscle tissue at supra-molecular scales (Herzog, 2019). Because MU-specific passive properties cannot be measured in human, a generic passive property common to all MUs and independent from their size was placed in-parallel to the pool of active MU FGs and merged with the stress-strain properties of the muscle aponeurosis. Consequently, a single fibre-to-muscle multiscale PEE, which passively develops the force F^{PEE} under stretch, was placed in-parallel with the population of MU FGs in Figure 6-1. Because of unresolved multiscale limitations discussed in Chapter 2, the passive titin and cross-bridge elasticities were neglected. From there, the total force developed by the muscle tissue was calculated with (EQ. 6-2).

$$F^M = \sum_i f_i^{MU} + F^{PEE} = F^{MU} + F^{PEE} \quad (\text{EQ. 6-2})$$

Muscle-tendon Equilibrium

Because their relative inclinations were overlooked, the MUs were simplified to make with the tendon the same pennation angle α . In the TA muscle, $\alpha = 11.2^\circ$ (Rajagopal et al., 2016). Because $\cos(11.2^\circ) = 0.98$, the pennation angle was neglected, yielding a potential underestimation of F^M of only 2%, and putting the in-series muscle and tendon forces F^M and F^T in equilibrium (EQ. 6-3).

$$F^T = F^M = \sum_i f_i^{MU} + F^{PEE} \quad (\text{EQ. 6-3})$$

Model normalization

As previously advised (Zajac, 1989), the model in Figure 6-1 was normalized with subject-muscle-specific parameters and made generic. The PEE, tendon, and MU maximum isometric forces were normalized to the muscle maximum isometric force F_0^M (EQ. 6-4).

$$\begin{aligned} \overline{F^{PEE}} &= \frac{F^{PEE}}{F_0^M} \\ \overline{F^T} &= \frac{F^T}{F_0^M} \\ \overline{f_{i,0}^{MU}} &= \frac{f_{i,0}^{MU}}{F_0^M} \end{aligned} \quad (\text{EQ. 6-4})$$

The MU forces were also normalized to their specific maximum isometric force $f_{i,0}^{MU}$ (EQ. 6-5).

$$\overline{f_i^{MU}} = \frac{f_i^{MU}}{f_{i,0}^{MU}} \quad (\text{EQ. 6-5})$$

Finally, the MU lengths were normalized to their specific optimal lengths $l_{0,i}^{MU}$, and the tendon length to the tendon slack length l_s^T (EQ. 6-6)

$$\begin{aligned} \overline{l_i} &= \frac{l_i^{MU}}{l_{0,i}^{MU}} \\ \varepsilon_T &= \frac{l^T - l_s^T}{l_s^T} \end{aligned} \quad (\text{EQ. 6-6})$$

Dynamics of the FGs

The individual normalized MU force $\overline{f}_i^{MU}$ produced by a FG was modelled to be the linear product (EQ. 6-7) of four terms:

1. the MU normalized maximum isometric force $\overline{f}_{i,0}^{MU}$,
2. the MU active state $a_i(\overline{l}_i, type_i)$,
3. the MU normalized force-length scaling factor $f_{FL,i}(a_i, \overline{l}_i)$, and
4. the MU normalized force-velocity scaling factor $f_{FV,i}(a_i, \overline{l}_i, type_i)$.

The a_i , $f_{FL,i}$, and $f_{FV,i}$ factors were modelled to nonlinearly dependent to the MU active state a_i , normalized length $\overline{l}_i$, normalized velocity $\overline{v}_i$, and type $type_i$ according to Chapter 2.

$$\begin{aligned} \overline{f}_i^{MU}(a_i, \overline{l}_i, \overline{v}_i, type_i) \\ = \overline{f}_{i,0}^{MU} \cdot a_i(\overline{l}_i, type_i) \cdot f_{FL}(a_i, \overline{l}_i) \cdot f_{FV}(a_i, \overline{l}_i, \overline{v}_i, type_i) \end{aligned} \quad (EQ. 6-7)$$

Solving the muscle-tendon dynamics – Model simplifications

To solve the dynamics of the neuromuscular model in Figure 6-1, five key assumptions and simplifications identified with {1-5} brackets were made. To provide some background, when the muscle-tendon system is described at the whole muscle scale with a single FG in the normalized space, (EQ. 6-3) and (EQ. 6-7) reduce to (EQ. 6-8) and (EQ. 6-9) as the i subscripts disappear.

$$\overline{F}^M(a, \overline{l}, type) = a(\overline{l}, type) \cdot f_{FL}(a, \overline{l}) \cdot f_{FV}(a, \overline{l}, \overline{v}, type) \quad (EQ. 6-8)$$

$$\overline{F}^T = \overline{F}^M + \overline{F}^{PEE} \quad (EQ. 6-9)$$

Rearranging (EQ. 6-8) gives (EQ. 6-10) for whole muscle Hill-type models.

$$f_{FV}(a, \overline{l}, \overline{v}) = \frac{\overline{F}^M(a, \overline{l})}{a(\overline{l}) \cdot f_{FL}(a, \overline{l})} \quad (EQ. 6-10)$$

Inverting the FV relationship and isolating the muscle contraction velocity $\overline{v}$, the combination of (EQ. 6-9) and (EQ. 6-10) gives (EQ. 6-11), which defines the muscle contraction dynamics and is typically solved for the muscle normalized length $\overline{l}$ at each time instant t from the known $\overline{l}^{MT}$ and neural sp (Figure 6-1) input variables.

$$\bar{v} = \frac{d\bar{l}}{dt} = f_v^{-1} \left(\frac{\bar{F}^T(t, \bar{l}^{MT}, \bar{l}) - \bar{F}^{PEE}(t, \bar{l})}{a(t, sp, \bar{l}) \cdot f_{FL}(t, a, \bar{l})} \right) \quad (\text{EQ. 6-11})$$

Following the same method for the MU-scale model in Figure 6-1, the combination of (EQ. 6-3) and (EQ. 6-7) yields (EQ. 6-12).

$$\bar{F}^T - \bar{F}^{PEE} = \sum_i \bar{f}_{l,0}^{MU} \cdot \left(a_i(\bar{l}_i, type_i) \cdot f_{FL}(a_i, \bar{l}_i) \cdot f_{FV}(a_i, \bar{l}_i, \bar{v}_i, type_i) \right) \quad (\text{EQ. 6-12})$$

Because the relationship between the normalized MU lengths $\bar{l}_i$ and the muscle-tendon length $\bar{l}^{MT}$ is unknown, (EQ. 6-12) is indeterminate. Although it is later discussed how the volumetric muscle representation in Chapter 3 can address this issue, it was here first simplified {1} that all the MUs in the MU pool share a common normalized length $\bar{l}$ and by extension a common normalized contraction velocity $\bar{v}$, as $\bar{v} = \frac{d\bar{l}}{dt}$. Hence, (EQ. 6-12) was reduced to (EQ. 6-13) in the normalized space. Moreover, because the optimal length of individual MUs $l_{i,0}^{MU}$ cannot be measured in human for a complete MU pool, it was further simplified {2} that all the MUs in the MU pool shared the same optimal MU length l_0^{MU} , which was taken as the subject-specific muscle optimal length l_0^M obtained in Chapter 3. It yields that the MUs share the same length l^{MU} , reported as l^M in Figure 6-1.

$$\bar{F}^T - \bar{F}^{PEE} = \sum_i \bar{f}_{l,0}^{MU} \cdot \left(a_i(\bar{l}, type_i) \cdot f_{FL}(a_i, \bar{l}) \cdot f_{FV}(a_i, \bar{l}, \bar{v}, type_i) \right) \quad (\text{EQ. 6-13})$$

To isolate $\bar{v} = \frac{d\bar{l}}{dt}$ and rearrange (EQ. 6-13) as a differential equation of $\bar{l}$ like in (EQ. 6-11), the MU FV relationship was simplified {3} to be independent from the fibre type of the muscle unit (MU type) and active state, in which case (EQ. 6-13) became (EQ. 6-14).

$$\bar{v} = \frac{d\bar{l}}{dt} = f_v^{-1} \left(\frac{\bar{F}^T - \bar{F}^{PEE}}{\sum_i \bar{f}_{l,0}^{MU} \cdot \left(a_i(\bar{l}, type_i) \cdot f_{FL}(a_i, \bar{l}) \right)} \right) \quad (\text{EQ. 6-14})$$

As discussed later, (EQ. 6-14) can be numerically solved for $\bar{l}$, although its implementation is a work in progress. In this Chapter 6, two further simplifications were made. First, the tendon was assumed to be rigid {4}. With a $\frac{l_s^T}{l_0^M}$ ratio of 3.5 for the subject S1's TA muscle (Chapter 3), this assumption was judged acceptable for low-to-medium TA isometric contractions, even though the tendon of the TA is long. For comparison, $\frac{l_s^T}{l_0^M} = 1$ describes a 'very stiff' tendon in the literature (Zajac, 1989). With this assumption, both $\bar{l}^T$ and $\bar{l}^{MT}$ are constant during the isometric contractions in Chapter 3, yielding the constant normalized MU length $\bar{l} = \bar{l}^{MT} - \bar{l}^T$. In this fixed-length condition, the FV relationship was

disregarded. Second, considering that $l^{MT} = 31.9$ cm, $l_s^T = 24.0$ cm and $l_0^M = 6.8$ cm for the subject S1 (Chapter 3), it was approximated {5} that the FGs worked at optimal length l_0^M in this ‘stiff tendon unique-length’ scenario. Consequently, $\bar{l} = 1$ in the following and the activity of the PEE was neglected.

With these five key simplifications, the model was simplified to the black-line rheological structure in Figure 6-1, the dynamics of which reduced to (EQ. 6-15).

$$\begin{aligned} \overline{F^T} = \overline{F^{MU}} &= \sum_i \overline{f_i^{MU}} = \sum_i \overline{f_{i,0}^{MU}} \cdot (a_i(\bar{l}, type_i) \cdot f_{FL}(a_i, \bar{l})) \\ \bar{l} &= 1 \end{aligned} \quad (\text{EQ. 6-15})$$

Because this introductory fixed-length model gives foundations for upcoming iterative improvements, the first of which is solving (EQ. 6-14) and addressing the simplifications {4,5}, detailed models for the for now neglected FV, PEE and tendon dynamics, as well as nonlinear the $\bar{l}$ -dependencies of the MU activation dynamics were still developed in the following for future perspectives.

6.2.2. Description of the Force Generators in Model 1

6.2.2.1. Overview of the MU excitation, activation, and contraction dynamics

In Figure 6-1, the excitation, activation and contraction dynamics of each of the n modelled MUs were described with a MU-specific FG_i , that includes a Neuromechanical Element (NE_i) and a Contractile Element (CE_i) (see Chapter 2), with $i \in \llbracket 1; n \rrbracket$. For improved readability, the subscript i was discarded in the following to refer to a representative MU of the pool.

Figure 6-2 shows the chain of events that define the behaviour of the MU’s NE and CE. The NE is controlled by the input spike train $sp(t)$ and predicts the MU active state $a(t)$ with advanced models for the MU excitation-contraction coupling properties that define the MU excitation and activation dynamics. In more details, $sp(t)$ controls, in a cascading manner, the time-course of (1) the MN Action Potentials (APs), (2) the muscle Units (mU) APs, (3) the free calcium concentration in the sarcoplasm ($[Ca^{2+}]$), (4) the concentration of the Calcium-Troponin complex ($[Ca^{2+} - Tn]$), and (5) the MU active state, described by the cascading state variables $e(t), u(t), c(t), T(t)$ and $a(t)$. The MU contraction dynamics are lumped into the CE, the behaviour of which is entirely defined by the MU Force-Length (FL) relationship, because of the simplifications described in 6.2.1, which predicts the normalized MU force-length scaling factor $f_{FL}(t)$. Importantly, the MU activation dynamics (steps (3) to (5)) are nonlinearly dependent to the normalized MU length $\bar{l}(t)$ and MU ‘type’, while the FL relationship

nonlinearly scales with the MU active state $a(t)$, as indicated with dotted arrows in Figure 6-2. This nonlinear dependency to the state variables $a(t)$ and $\bar{l}(t)$ of the MU activation and contraction dynamics ensures a physiological interplay between the neural and mechanical compartments of the neuromuscular model, as advised in Chapter 2 and contrary to most approaches proposed in the literature.

The MU excitation, activation and contraction dynamics are therefore entirely described by the system of six transformations in (EQ. 6-16). The mathematical equations describing the $f_{sp \rightarrow e}$, $f_{e \rightarrow u}$, $f_{u \rightarrow c}$, $f_{c \rightarrow T}$, $f_{T \rightarrow a}$, and f_{FL} neuromechanical properties are detailed in the following sections.

$$\begin{cases} e(t) = f_{sp \rightarrow e}(t, sp) \\ u(t) = f_{e \rightarrow u}(t, e) \\ c(t) = f_{u \rightarrow c}(t, u, \bar{l}, type) \\ T(t) = f_{c \rightarrow T}(t, c, \bar{l}, type) \\ a(t) = f_{T \rightarrow a}(t, T, type) \\ f_{FL}(t) = f_{FL}(t, a, \bar{l}) \end{cases} \quad (\text{EQ. 6-16})$$

As discussed, and reproduced in (EQ. 6-17), the normalized MU force $\overline{f^{MU}}$ is finally obtained with a linear combination of the a and f_{FL} state variables in the constant-length stiff-tendon conditions described previously:

$$\overline{f^{MU}}(t, a, \bar{l}, type) = a(t, \bar{l}, type) \cdot f_{FL}(t, a, \bar{l}) \quad (\text{EQ. 6-17})$$

To be consistent with the experimental conditions in Chapter 3, it is noteworthy that the data used to tune the properties in (EQ. 6-15) was obtained from experiments on individual human MUs or bundles of fibres. When such data was not available in the literature, were in turn and decreasing order of preference considered data from human fibres, cat fibres, rodent fibres, rodent muscles, and amphibian fibres or muscles. Because it is assumed in the following that a MU behaves like one of its representative fibre and/or sarcomere, any experimental quantity and dynamics obtained at the sarcomere and/or fibre scales were automatically assumed acceptable and extrapolated to the MU scale in the following.

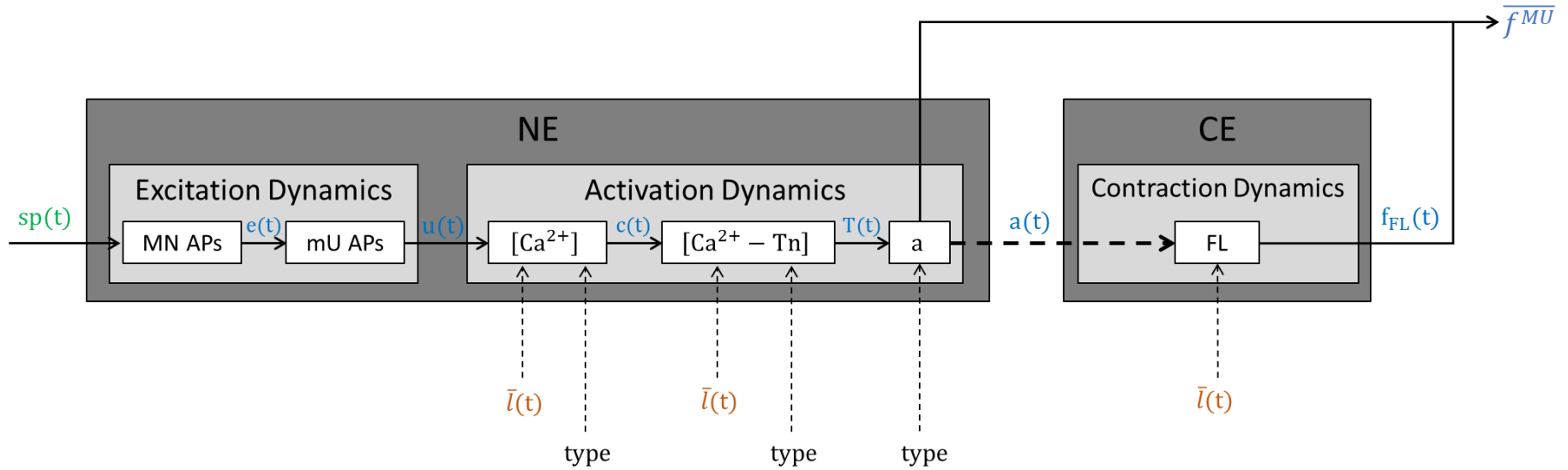


Figure 6-2: For a representative MU in Figure 6-1, description of the interplay between the neuromechanical properties defining the behaviour of the MU Neuromechanical Element (NE) and Contractile Element (CE) to predict the normalized MU force $\bar{f}^{MU}$. The NE is controlled by the experimental spike train $sp(t)$ (green font), which defines in the chain of events the time-course of (1) the MN Action Potentials (APs), (2) the muscle Units (mU) APs, (3) the free calcium concentration in the sarcoplasm ($[Ca^{2+}]$), (4) the concentration of the Calcium-Troponin complex ($[Ca^{2+} - Tn]$), and (5) the MU active state, yielding the cascading (plain arrows) state variables $e(t)$, $u(t)$, $c(t)$, $T(t)$ and $a(t)$ for the neural excitation, muscle excitation, free calcium concentration, calcium-troponin concentration, and active state, respectively. As represented with dotted arrows, the NE's activation dynamics are nonlinearly dependent to the normalized MU length $\bar{l}(t)$ and the 'MU type'. The behaviour of the CE is, in muscle isometric conditions, entirely defined by the Force-Length (FL) relationship, which is nonlinearly scaled by the MU active state $a(t)$, and $\bar{l}(t)$ and produces the state variable $f_{FL}(t)$. The normalized MU force $\bar{f}^{MU}$ is calculated with (EQ. 6-17).

6.2.2.1. Properties of the MU pool

Backed by the results obtained in Chapters 3 to 5, the pool of n MUs was modelled to follow the extended Henneman's size principle described in (EQ. 6-18), consistently with (EQ. 5-1).

$$\begin{aligned} \forall j, k \in [1; n], (j < k) \\ \Rightarrow (S_j < S_k \Leftrightarrow I_j^{th} < I_k^{th} \Leftrightarrow IR_j < IR_k \Leftrightarrow F_j^{th} < F_k^{th} \Leftrightarrow f_j^{tw} < f_k^{tw} \Leftrightarrow f_{0,j}^{MU} < f_{0,k}^{MU}) \end{aligned} \quad (\text{EQ. 6-18})$$

In (EQ. 6-18), S is the size of the MN and I^{th} its current recruitment threshold, IR the innervation ratio of the MU, and F^{th} , f^{tw} , and f_0^{MU} are the MU force recruitment threshold, twitch force, and maximum isometric force. (EQ. 6-18) means that the larger the MN (S), the higher number of muscle fibres (IR) it innervates, the higher its twitch and maximum isometric tetanic forces (f^{tw} , f_0^{MU}), and the higher its current and force recruitment thresholds (I^{th} , F^{th}). For convenience, the MU pool is always ranked in the following in the ascending order of F^{th} .

Beyond (EQ. 6-18), human muscles display a continuous distribution of slow-to-fast MU types in the MU pool (Chapter 4 - Discussion Section). MUs of different types do not display the same neuromechanical properties, which explains the nonlinear type-dependency of the $[Ca^{2+}]$, $[Ca^{2+} - Tn]$, and a properties in Figure 6-2. Let us derive a MU type distribution for the MU pool displayed in Figure 6-1. In a human TA of $N = 400$ MUs (Chapter 3), 28% of the $N_f^{tot} = 200,000$ TA fibres (Chapter 3) (Feinstein et al., 1955; Henriksson-Larsén et al., 1983) are Type 2 'fast' fibres (Henriksson-Larsén et al., 1983). Considering that the innervation ratio IR of a MU can be inferred from the amplitude of the MU force twitch f^{tw} ($\overline{f^{tw}}(i) \propto IR(i)$ - Chapter 4), the $\overline{f^{tw}}$ distribution derived in Chapter 3 yielded (EQ. 6-19). From the cumulative distribution of the MU IR s derived from Figure 6-3B, it was finally obtained that the 41 MUs in the MU pool that have the largest innervation ratios account for 28% of N_f^{tot} and are of the 'fast' type.

$$IR(i) = \frac{\overline{f^{tw}}(i)}{\sum_{j=1}^{400} \overline{f^{tw}}(j)} \cdot N_f^{tot} \quad (\text{EQ. 6-19})$$

Although it is debated whether the MU type, currently determined by the MU twitch force, is also correlated or not to the MU size, i.e., to the MU Force recruitment threshold in humans (Chapter 4), it was here assumed that the small low-threshold MUs were of the 'slow' type, and the large high-threshold MUs of the 'fast' type. Therefore, the 354 lowest-threshold MUs, identified with the dashed lines in Figure 6-3, were considered of the 'slow' type in the following.

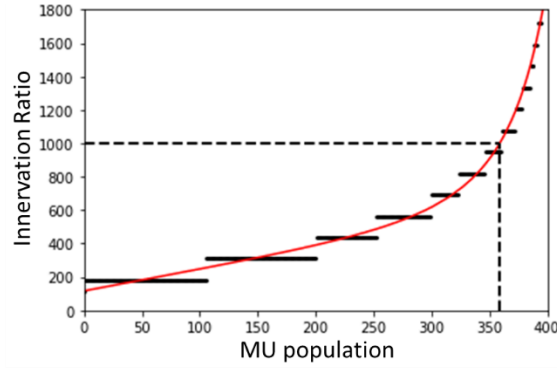


Figure 6-3: Distribution of MU innervation ratios ($IR(i), i \in \llbracket 1; 400 \rrbracket$) (red curve) in the human TA muscle. As identified by the dashed lines, the 354 smallest lowest-threshold MUs account for 72% of the muscle fibres that are ‘slow’, and the 41 largest highest-threshold MUs gather 28% of the muscle fibres that are ‘fast’.

6.2.2.2. Neuromechanical Element - Modelling the MN Excitation Dynamics

MN Action Potentials

The NE first transforms the input binary train of MN spikes $sp(t)$ into a train $e(t)$ of MN APs ($e(t) = f_{sp \rightarrow e}(t, sp)$) in (EQ. 6-16). Because $sp(t)$ defines the time-course of the MN’s firing frequency FF and discharge times, this input intrinsically accounts for the physiological mechanisms of AP elicitation. Because a window into these mechanisms was not required in this study, the MN APs were phenomenologically modelled as analytical sine waves (Hatze, 1977) of half period $\frac{T}{2}$ and amplitude V_e (EQ. 6-20). The waves are triggered at the discharge times t_i when $sp(t = t_i) = 1$, while the MN membrane remains at equilibrium ($e(t) = 0$) for $t \notin [t_i; t_i + \frac{T}{2}]$.

$$\begin{cases} e(t) = V_e \sin \left[\frac{2\pi}{T} (t - t_i) \right] & \text{for } t_i \leq t \leq t_i + \frac{T}{2} \\ e(t) = 0 & \text{otherwise} \end{cases} \quad (\text{EQ. 6-20})$$

The individual AP shapes recorded in anesthetized and dissected cats in the literature reviewed in Chapter 4 were fitted with (EQ. 6-20), yielding $V_e = 90mV$ and $T = 1.4ms$. T is here shorter than the amphibian $T = 2ms$ (Ebashi & Endo, 1968) previously used (Hatze, 1977). Although gaussian shapes provided a better fit to the experimental data, the negligible improvement it brought did not justify adding a third parameter to this modelling step.

Neuromuscular junction

The nerve-to-fibre transmission of the train of MN APs $e(t)$ through the neuromuscular junction (NMJ) is a complex process, the modelling of which is usually not included in the excitation dynamics of neuromuscular Hill-type models. During exocytosis, the MN AP triggers the release of acetylcholine

(Ach) neurotransmitters in the gap (synaptic cleft) of the NMJ, through which they diffuse, gradually disappear by connecting to the buffer acetylcholinesterase (AChE), and eventually bind to nicotinic Ach receptors (nAChRs) on the postsynaptic membrane. The nAChR channels open, and postsynaptic membrane voltage, called end-plate potential (EPP) rises and triggers a muscle AP. A phenomenological model of this process was developed. In this model, the transients of the Ach concentration in the NMJ, taken from a finite element modelling study (Smart & McCammon, 1998), controlled the ratio of activated nAChR receptors at each time step, using typical Ach-nAChR binding rate constants (Land et al., 1981; Mathie et al., 1991), from which the time-history of the EPP was derived with experimental values for the mU membrane extracellular resistivity (Elmqvist et al., 1964) and inward ion current per opened nAChR channel (Cull-Candy et al., 1979), up to the voltage threshold for mU AP initiation (Elmqvist et al., 1964; Boyd et al., 1978; Wood & Slater, 2001). Yet, the neuromuscular transmission is a robust process in skeletal muscles, i.e., a MN AP always triggers a mU AP (Bigland-Ritchie et al., 1982; Wood & Slater, 2001; Kovyazina et al., 2010), and the aforementioned modelling approach was eventually not adopted, but remains available with the rest of the computational implementation of the model.

Muscle Unit Action Potentials

The train of the mU APs ($u(t)$ in Figure 6-2) that travel along the fibre membranes was modelled with the previously proposed (Hatze, 1977) 2nd-order ODE in (EQ. 6-21) as the response to the train $e(t)$ of MN APs ($u(t) = f_{e \rightarrow u}(t, e)$ in (EQ. 6-16)).

$$\frac{d^2u}{dt^2} = b_1 e(t) - b_2 u - b_3 \frac{du}{dt} \quad (\text{EQ. 6-21})$$

In (EQ. 6-21), when the fibre receives an isolated MN AP, b_1 determines the peak-to-peak amplitude A_p of the mU AP, while b_2 and b_3 mainly determine its time-to-peak duration t_{tp} and decay time constant τ_d respectively. t_{tp} is the duration between the time instant the AP reaches 1% of its maximum value, and the time instant when the AP peak value is reached. τ_d is the duration between the peak of the AP and the time instant when the AP has decreased to 37% of its peak value. The b_i coefficients were tuned, so that the mU APs simulated with (EQ. 6-21) matched typical experimental cat values for A_p (77 mV), t_{tp} (0.6 ms) and τ_d (0.6 ms) (Farmer et al., 1960; Brooks & Hongdalarom, 1968; Ludin, 1969; Kopec et al., 1978; Wallinga-De Jonge et al., 1985; Wolters et al., 1994; Balog et al., 1994; Dillmann et al., 1996; Dumitru & King, 1999; Arabadzhev et al., 2005; Kim, G. et al., 2013). With $b_1 = 9 \cdot 10^7$, $b_2 = 5 \cdot 10^7$, and $b_3 = 2 \cdot 10^4$, mammalian mU APs five times shorter than previously proposed for amphibians at low temperature (Hatze, 1977) were simulated (Figure 6-4).

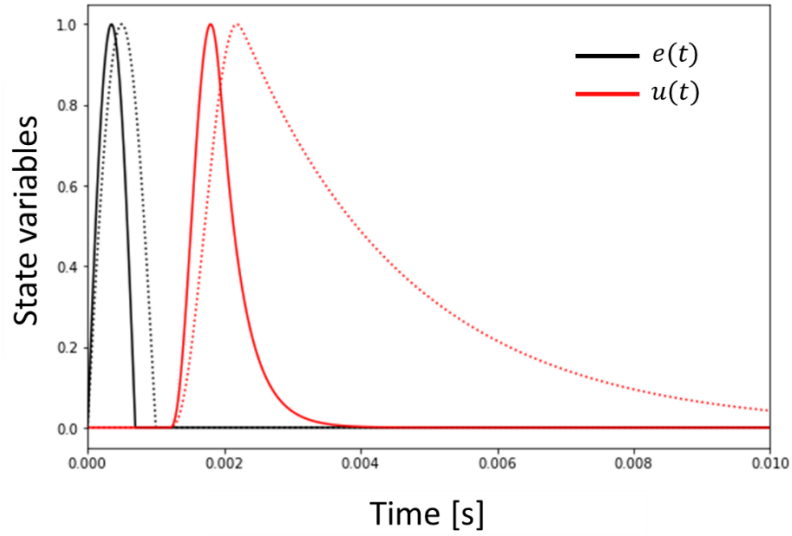


Figure 6-4: Time-history of the simulated MN (black curves) and mU (red curves) normalized APs obtained with (EQ. 6-20) and (EQ. 6-21) respectively. Plain lines were obtained with the b_i and T coefficients fitted to cat experimental data, while dotted lines were obtained from amphibian data in Hatze's work (Hatze, 1977).

6.2.2.3. Neuromechanical Element – Modelling the MU Activation Dynamics

Dynamics of calcium concentration

The MU excitation state $u(t)$ controls, as indicated in Figure 6-2, the level $c(t)$ of calcium concentration in the MU sarcoplasm ($c(t) = f_{u \rightarrow c}(t, u, \bar{l}, type)$ in (EQ. 6-16). According to the results of Chapter 2, only a few Hill-type models in the literature, including 16 (30%) eligible studies, proposed an explicit description of the dynamics of the free calcium ions in the sarcoplasm (property 'ADC' in Chapter 2). According to the inheritance diagrams in Appendix A.10, most of these ADC properties were modelled after (Hatze, 1980) with a 1st-order ODE. The calcium transients were here phenomenologically modelled with the more flexible 2nd-order ODE in (EQ. 6-22) (Hatze, 1977), that transforms the train of mU APs $u(t)$ into the time-course of the free calcium concentration $c(t)$.

$$\frac{d^2c}{dt^2} = c_3 \cdot u(t) - \frac{1}{f_1(\bar{l})} \left(c_1 \cdot \frac{dc}{dt} + c_2 \cdot f_2(\bar{l}) \cdot c \right) \quad (\text{EQ. 6-22})$$

In (EQ. 6-22), when the fibre receives an isolated mU AP $u(t)$, c_3 determines the peak-to-peak amplitude A_c of the $[Ca^{2+}]$ twitch, and c_1 and c_2 its time-to-peak duration t_{tp} and decay time constant τ_d . The equation proposed in Hatze (1977) was modified to introduce the length-dependent scaling factors $f_1(\bar{l})$ and $f_2(\bar{l})$ that respectively make A_c and τ_d nonlinearly dependent to the normalized MU length $\bar{l}$.

Despite some disagreements (Haugen, 1991), it has been repeatedly observed in *in vitro* experiments on amphibian fibres from the semitendinosus, iliofibularis and TA muscles (Blinks et al., 1978; Baylor et al., 1983; Close & Lännergren, 1984; Konishi et al., 1991) that the peak value of the calcium concentration nonlinearly varies with the sarcomere length l^s . The experimental data provided by these studies was manually digitized and normalized to the highest peak $[Ca^{2+}]$ value retrieved in each study and to the optimal sarcomere length l_0^s ($\bar{l} = \frac{l^s}{l_0^s}$), which is typically $2.1\mu m$ in these amphibian muscles. A piecewise linear trendline $f_1(\bar{l})$ given in (EQ. 6-23) and displayed in Figure 6-5 was fitted to the normalized data, which reveals that the peak $[Ca^{2+}]$ value is obtained between 1.15 and $1.30 l_0^s$.

$$\begin{cases} f_1(\bar{l}) = 0.8 & \text{if } \bar{l} \leq 1.0 \\ f_1(\bar{l}) = 0.8 + 1.33(\bar{l} - 1.0) & \text{if } \bar{l} \leq 1.15 \\ f_1(\bar{l}) = 1.0 & \text{if } \bar{l} \leq 1.30 \\ f_1(\bar{l}) = 1.0 - 0.6(\bar{l} - 1.3) & \text{if } \bar{l} > 1.30 \end{cases} \quad (\text{EQ. 6-23})$$

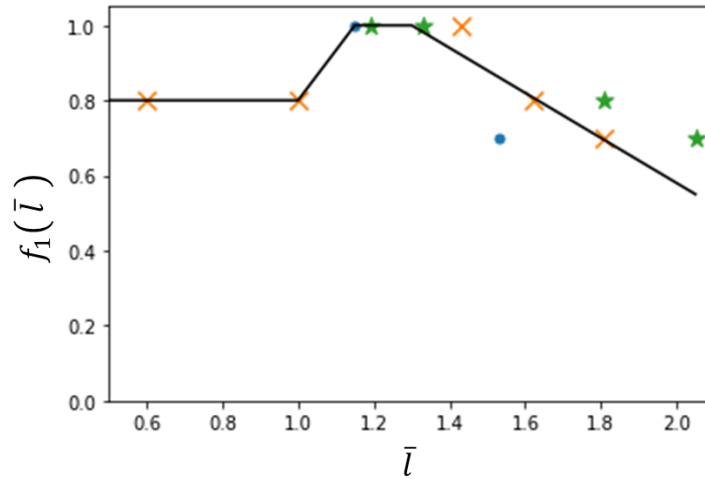


Figure 6-5: Nonlinear dependency of the peak Ca^{2+} concentration in muscle fibres from amphibian muscles to the normalized sarcomere length, directly scaled to fibre length. Blue dots (Blinks et al., 1978), green stars (Konishi et al., 1991), orange crosses (Close & Lännergren, 1984).

In a similar approach, the $f_2(\bar{l})$ scaling factor described in (EQ. 6-24) models the nonlinear decrease in τ_d with shorter normalized fibre lengths $\bar{l}$ (Konishi et al., 1991).

$$\begin{cases} f_2(\bar{l}) = 1.0 & \text{if } \bar{l} \leq 1.15 \\ f_2(\bar{l}) = 1.0 - 0.4 \cdot (\bar{l} - 1.15) & \text{if } \bar{l} > 1.15 \end{cases} \quad (\text{EQ. 6-24})$$

In the literature of Hill-type muscle models, the coefficients scaling the ADC property (here c_1, c_2, c_3) are typically tuned to match dated experimental observations of calcium waves obtained *in vitro* from

amphibian muscle fibres in low-temperature baths. Yet, the calcium dynamics in the sarcoplasm are strongly species-dependent, which transients twice longer in amphibians than in mammals (Hollingworth et al., 1996), and temperature-dependent (Blinks et al., 1978; Miledi et al., 1982; Eusebi et al., 1983; Hirota et al., 1989; Raju et al., 1989; Konishi et al., 1991; Delbono & Stefani, 1993; Delbono et al., 1995; Zhao et al., 1996; Capote et al., 2005; Hollingworth, Stephen et al., 2008; Baylor, Stephen M. & Hollingworth, 2011; Hollingworth, Stephen et al., 2012). Moreover, these dated experimental observations were typically obtained with chemical indicators (murexide, aequorin and arsenazo III for example) that have a high affinity to components other than calcium ions in the myoplasm, such as Mg ions, and wrongly report calcium twitches of too low peak values and delayed transients. Because of these limitations, rather than considering the values proposed in Hatze's work (Hatzé, 1977) for the c_1, c_2, c_3 coefficients, which are typical of frog data at 9°C obtained with the aequorin indicator, the c_1, c_2, c_3 coefficients were here tuned to match the experimental data from two experimental studies (Hollingworth et al., 1996; Baylor & Hollingworth, 2003), where the experimental $[Ca^{2+}]$ waves are observed at 28-35°C in rodent fibres with the reliable (Hirota et al., 1989; Konishi et al., 1991; Hollingworth et al., 1996; Zhao et al., 1996; Baylor & Hollingworth, 2003; Hollingworth, Stephen et al., 2008) mag-fura-2 indicator. Lacking human data in the literature, it was believed that the calcium dynamics in rodents at 35°C were the most representative for the human species at body temperature. Because both studies (Hollingworth et al., 1996; Baylor & Hollingworth, 2003) performed their experiments at the same sarcomere length $l^s = 3.8\mu m \approx 1.6l_0^s$ (Rassier et al., 1999), the length-dependent scaling factors were set to $f_1(1.6) = 0.82$ and $f_2(1.6) = 0.82$ when tuning the c_1, c_2, c_3 coefficients.

As reported in Figure 6-6A for fast fibres, the calcium transients simulated with the coefficients in Table 6-1 compared well with the experimental trace at 125Hz stimulation from Hollingworth et al. (1996) once steady-state was reached. The 10th simulated and experimental calcium twitch returned $t_{tp} = 2.0ms$, $\tau_d = 6.6ms$, and $A_c = 12\mu M$ up to a sustained $20\mu M$ with less than 5% difference. Because the 2nd-order ODE does not account for some physiological mechanisms, such as receptor saturation and the CASQ-Traidin-RYR1 action, the typically larger A_c and τ_d of the first experimental calcium transients (Baylor & Hollingworth, 2003) were underpredicted.

Table 6-1: c_i coefficients scaling (EQ. 6-22) that defines the $[Ca^{2+}]$ transients in the myoplasm. These coefficients are obtained to match typical dynamics observed in slow and fast fibres in rodents at body temperature (35°C) and fibre length $1.6l_0^s$.

MU type	c_1	c_2	c_3
Slow	$2.5 \cdot 10^3$	$1.5 \cdot 10^5$	0.4
Fast	$2.4 \cdot 10^3$	$4.3 \cdot 10^5$	0.9

Data for slow type rodent fibres were available in Baylor & Hollingworth (2003) at 16°C, but not at 35°C. In Hollingworth et al. (1996), A_c , t_{tp} and τ_d respectively increased by 26%, remained constant, and decreased by 56% when increasing the temperature from 16°C to 35°C. The 16°C data from Baylor 2003 were interpolated to 35°C using the same scaling factors. As shown in Figure 6-6B, the simulated calcium transients at 35°C for slow fibres at $1.6l_0^s$ length under 67Hz stimulation compared logically with the experimental data obtained at 16°C. The simulated and experimental steady-state transients respectively reach 14 and 11 μM , and have similar t_{tp} , and τ_d of 19 and 44ms.

Figure 6-6CD compares at $l^s = l_0^s$ the simulated traces obtained with the coefficient values in Table 6-1 to the outputs from Hatze's model (Hatze, 1977) calibrated on frog data at 9°C, which returned similar t_{tp} , but up to ten times longer half-widths and 2.3 times higher amplitudes for the calcium twitches.

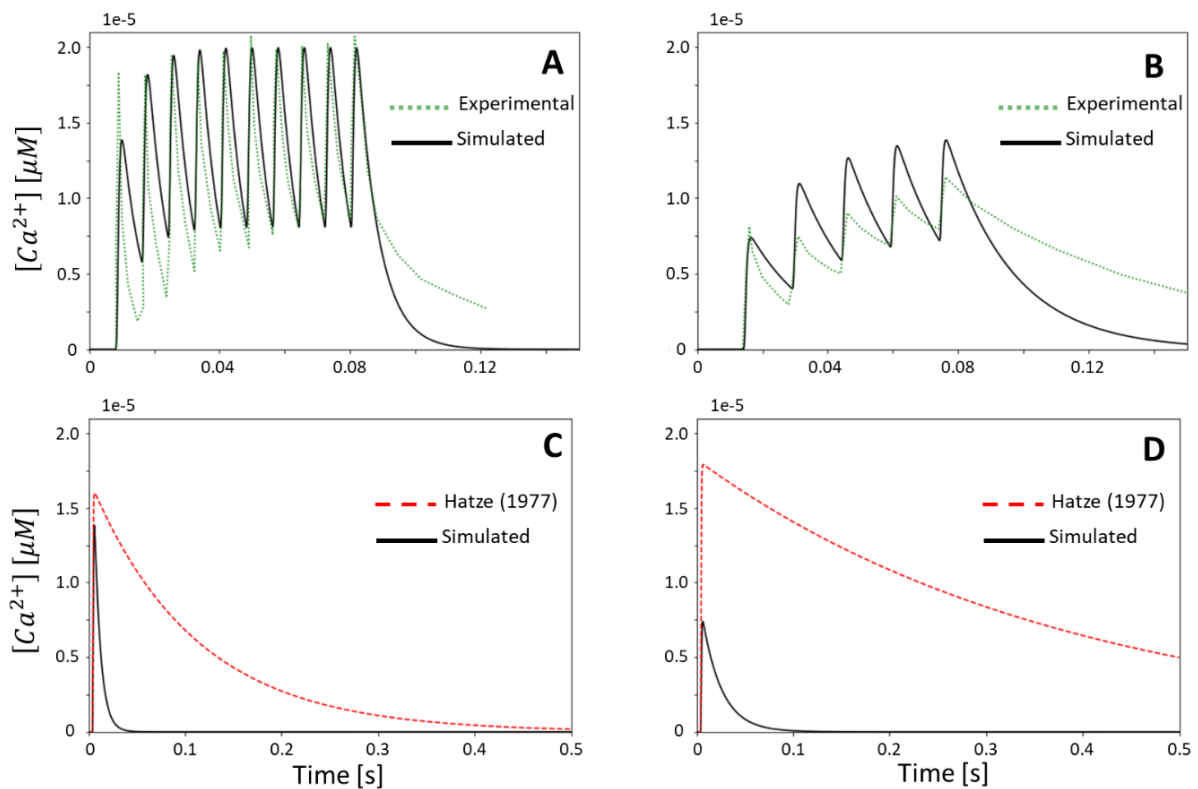


Figure 6-6: Time-course of the Calcium concentration $[Ca^{2+}]$ in μM for fast (A, C) and slow (B, D) rodent fibres. In A,B, $l^s = 1.6l_0^s$ and in C,D, $l^s = l_0^s$. The $[Ca^{2+}]$ simulated with (EQ. 6-22) and the c_i coefficients from Table 6-1 are displayed in plain dark lines. A, B: comparison of the simulated and experimental (Hollingworth et al., 1996; Baylor & Hollingworth, 2003) traces at 125Hz (A) and 67Hz (B) stimulation at 35°C, except for the experimental data in B obtained at 26°C. C,D: comparison for fast (C) and slow (D) fibres of the simulated rodent calcium transients against those proposed in Hatze's work (Hatze, 1977) for amphibians (red dotted curves).

Dynamics of Calcium-Troponin complex formation

In the literature of Hill-type modelling, only a few studies phenomenologically modelled the calcium-troponin (Ca-Tn) binding process in the myoplasm. While some studies proposed steady-state sigmoidal descriptions of the phenomenon (Hatze, 1980; Ding et al., 2000), another approach (Wexler et al., 1997) relied on a physiological description of the Ca-Tn binding-unbinding process to model the time-course of the concentration T of Ca-Tn structures in the myoplasm. This approach is here modified in (EQ. 6-25) to account for the nonlinear length-dependency of the $[Ca - Tn]$ transients.

$$\frac{dT}{dt} = \frac{k_1}{f_3(\bar{l})} \cdot \left(\frac{T_0}{f_4(\bar{l})} - T \right) \cdot c^2 - \frac{k_2}{f_5(\bar{l})} \cdot T \quad (\text{EQ. 6-25})$$

In (EQ. 6-25), T_0 is the total Troponin concentration in the myoplasm, and k_1, k_2 are respectively the forward and backward reaction rates of the Ca-Tn binding-unbinding process. The T_0, k_1, k_2 coefficients, originally calibrated to match experimental force data with a neuromuscular model (Wexler et al., 1997), were tuned to match contemporary experimental data (Baylor & Hollingworth, 2003; Hollingworth, Stephen et al., 2008). When triggered by a unique AP, $\frac{T_0}{f_4(\bar{l})}$ scales the peak-to-peak amplitude A_t of the $[Ca - Tn]$ twitch, and $\frac{k_1}{f_3(\bar{l})}$ and $\frac{k_2}{f_5(\bar{l})}$ determine its time-to-peak t_{tp} and decay constant τ_d .

As previously reported (Matsuo et al., 2010), cross-bridge formation increases the Ca^{2+} affinity of troponin and slows down Ca^{2+} detachment from troponin. When triggered by a single AP, the decay rate constant τ_d of the $[Ca - Tn]$ twitch therefore strongly decreases as the filamentary overlap decreases. In other terms, when $\bar{l}$ increases, $[Ca - Tn]$ decays more rapidly. Consequently, the mag-2-fura measurements of $[Ca - Tn]$, which overlook this affinity to cross-bridge formation, are not reliable at the sarcomere lengths for which filamentary overlap occurs, and the trajectory of the intensity of the meridional reflection from troponin at $1/38.5\text{nm}^{-1}$ must be used instead (Matsuo et al., 2010).

In Matsuo's work (Matsuo et al., 2010), experiments were performed in frogs at 16°C , in muscles of dominantly fast fibres at sarcomere lengths $l^s = 1.9l_0^s$ (nonoverlap state) and $l^s = 1.3l_0^s$ (overlap state). The experimental $[Ca - Tn]$ time traces were digitized and the t_{tp} and τ_d values were measured at both lengths l^s . To obtain $[Ca - Tn]$ values for rodents and slow fibres, the available experimental data (frog, fast fibre, 16°C) was extrapolated, in first approximation, to rodent data at 35°C in slow fibres by using the same species- temperature- and fibre type-scaling factors as used with the $[Ca^{2+}]$ transients previously investigated. In three steps, the t_{tp} and τ_d frog quantities were therefore gradually multiplied by 0.5, 0.44, and 2.5. Because the t_{tp} and τ_d values, initially measured

at $l^S = 1.9l_0^S$ and $l^S = 1.3l_0^S$, depend on the number of formed cross-bridges, piecewise linear length-dependent relationships $t_{tp}(\bar{l})$ and $\tau_d(\bar{l})$ were inferred (Table 6-2) from a typical piecewise-linear force-length shape (Rassier et al., 1999) corrected for rodent optimal sarcomere lengths $l_0^S = 2.4\mu m$. Finally, the $[Ca - Tn]$ peak-to-peak amplitude was set to $120\mu M$ and $220\mu M$ for slow and fast fibres respectively (Baylor & Hollingworth, 2003; Baylor, Stephen M. & Hollingworth, 2007; Baylor, Stephen M. & Hollingworth, 2012).

As before, the $f_3(\bar{l}), f_4(\bar{l}), f_5(\bar{l})$ length-dependent scaling factors and the k_1, k_2, T_0 parameters, respectively reported in Table 6-3 and Table 6-2, were tuned so that the output of the differential equation in (EQ. 6-25) matched the length-dependent t_{tp} and τ_d , and A_t quantities in the first column of Table 6-2 with less than 5% error. As reported in Table 6-3, the adjusted T_0, k_1, k_2 coefficients are consistent with typical coefficient values reported in the literature.

Table 6-2: (Left) Typical t_{tp}, τ_d and A_t values of the Ca-Tn concentration twitch in rodent fast and slow fibres, and their length-dependencies. (Right) Description of the length-dependent scaling factors in (EQ. 6-25).

$[Ca - Tn]$ metrics	Length-dependent scaling factors
$t_{tp,fast}(\bar{l}) = \begin{cases} 6ms & \text{if } \bar{l} < 0.7 \\ 2 + 5\bar{l} & \text{if } \bar{l} \in [0.7; 0.97] \\ 7ms & \text{if } \bar{l} \in [0.97; 1.02] \\ 12 - 5\bar{l} & \text{if } \bar{l} \in [1.02; 1.6] \\ 4ms & \text{if } \bar{l} > 1.6 \end{cases}$	$\begin{cases} f_3(\bar{l}) = 0.33 & \text{if } \bar{l} \leq 0.7 \\ f_3(\bar{l}) = 0.33 + 2.47(\bar{l} - 0.75) & \text{if } \bar{l} \leq 0.97 \\ f_3(\bar{l}) = 1.0 & \text{if } 0.97 \leq \bar{l} \leq 1.02 \\ f_3(\bar{l}) = 8.9 - 11.73\bar{l} + 3.90\bar{l}^2 & \text{if } 1.02 \leq \bar{l} \leq 1.6 \\ f_3(\bar{l}) = 0.125 & \text{if } \bar{l} > 1.6 \end{cases}$
$\tau_{d,fast}(\bar{l}) = \begin{cases} 15ms & \text{if } \bar{l} < 0.7 \\ -1 + 23\bar{l} & \text{if } \bar{l} \in [0.7; 0.97] \\ 21ms & \text{if } \bar{l} \in [0.97; 1.02] \\ 44 - 23\bar{l} & \text{if } \bar{l} \in [1.02; 1.6] \\ 8ms & \text{if } \bar{l} > 1.6 \end{cases}$	$\begin{cases} f_5(\bar{l}) = 0.75 & \text{if } \bar{l} \leq 0.7 \\ f_5(\bar{l}) = 0.75 + 0.94(\bar{l} - 0.75) & \text{if } \bar{l} \leq 0.97 \\ f_5(\bar{l}) = 1.0 & \text{if } 0.97 \leq \bar{l} \leq 1.02 \\ f_5(\bar{l}) = 0.9 + 0.84\bar{l} - 0.75\bar{l}^2 & \text{if } 1.02 \leq \bar{l} \leq 1.6 \\ f_5(\bar{l}) = 0.34 & \text{if } \bar{l} > 1.6 \end{cases}$
$A_{t,fast} = 220\mu M$	$\begin{cases} f_4(\bar{l}) = 1.09 & \text{if } \bar{l} \leq 0.7 \\ f_4(\bar{l}) = 1.09 - 0.32(\bar{l} - 0.75) & \text{if } \bar{l} \leq 0.97 \\ f_4(\bar{l}) = 1.0 & \text{if } 0.97 \leq \bar{l} \leq 1.02 \\ f_4(\bar{l}) = -4.4 + 8.24\bar{l} - 2.84\bar{l}^2 & \text{if } 1.02 \leq \bar{l} \leq 1.6 \\ f_4(\bar{l}) = 1.46 & \text{if } \bar{l} > 1.6 \end{cases}$
$t_{tp,slow}(\bar{l}) = \begin{cases} 11ms & \text{if } \bar{l} < 0.7 \\ 4 + 10\bar{l} & \text{if } \bar{l} \in [0.7; 0.97] \\ 14ms & \text{if } \bar{l} \in [0.97; 1.02] \\ 24 - 10\bar{l} & \text{if } \bar{l} \in [1.02; 1.6] \\ 8ms & \text{if } \bar{l} > 1.6 \end{cases}$	$\begin{cases} f_3(\bar{l}) = 0.75 & \text{if } \bar{l} \leq 0.7 \\ f_3(\bar{l}) = 0.75 + 0.93(\bar{l} - 0.75) & \text{if } \bar{l} \leq 0.97 \\ f_3(\bar{l}) = 1.0 & \text{if } 0.97 \leq \bar{l} \leq 1.02 \\ f_3(\bar{l}) = 1.2 - 0.01\bar{l} - 0.22\bar{l}^2 & \text{if } 1.02 \leq \bar{l} \leq 1.6 \\ f_3(\bar{l}) = 0.67 & \text{if } \bar{l} > 1.6 \end{cases}$

$\tau_{d,slow}(\bar{l}) = \begin{cases} 38ms & \text{if } \bar{l} < 0.7 \\ -3 + 57\bar{l} & \text{if } \bar{l} \in [0.7; 0.97] \\ 52ms & \text{if } \bar{l} \in [0.97; 1.02] \\ 110 - 57\bar{l} & \text{if } \bar{l} \in [1.02; 1.6] \\ 19ms & \text{if } \bar{l} > 1.6 \end{cases}$	$\begin{cases} f_5(\bar{l}) = 0.66 & \text{if } \bar{l} \leq 0.7 \\ f_5(\bar{l}) = 0.66 + 1.27(\bar{l} - 0.75) & \text{if } \bar{l} \leq 0.97 \\ f_5(\bar{l}) = 1.0 & \text{if } 0.97 \leq \bar{l} \leq 1.02 \\ f_5(\bar{l}) = 4.2 - 4.28\bar{l} - 1.11\bar{l}^2 & \text{if } 1.02 \leq \bar{l} \leq 1.6 \\ f_5(\bar{l}) = 0.21 & \text{if } \bar{l} > 1.6 \end{cases}$
$A_{t,slow} = 120\mu M$	$\begin{cases} f_4(\bar{l}) = 1.06 & \text{if } \bar{l} \leq 0.7 \\ f_4(\bar{l}) = 1.06 - 0.23(\bar{l} - 0.75) & \text{if } \bar{l} \leq 0.97 \\ f_4(\bar{l}) = 1.0 & \text{if } 0.97 \leq \bar{l} \leq 1.02 \\ f_4(\bar{l}) = 4.5 - 5.09\bar{l} + 1.62\bar{l}^2 & \text{if } 1.02 \leq \bar{l} \leq 1.6 \\ f_4(\bar{l}) = 0.52 & \text{if } \bar{l} > 1.6 \end{cases}$

Table 6-3: Coefficients scaling (EQ. 6-25) that defines the $[Ca - Tn]$ transients in the myoplasm in both fast and slow fibres. The values obtained for coefficients are consistent with the literature (Baylor & Hollingworth, 2003; Hollingworth, Stephen et al., 2008).

	$k_1[M^{-2} \cdot s^{-1}]$	$k_2[s^{-1}]$	$T_0[M]$
Slow fibres	$6 \cdot 10^{12}$	21	$1.7 \cdot 10^{-4}$
Fast fibres	$1 \cdot 10^{12}$	41	$3.8 \cdot 10^{-4}$
Literature	$3.6 \cdot 10^{12}$	100	$2.2 \cdot 10^{-4}$

Modelling the MU active state

In Chapter 2, it was proposed to define the MU active state as the normalized instantaneous ratio of formed cross-bridges in the force-generating state in a population of myosin heads from a representative sarcomere, irrespective of the degree of filamentary overlap, relative inter-filamentary sliding speed and other external mechanical mechanisms. Without comprehensively detailing the cross-bridge dynamics like in Huxley-type models, it is still possible to use semi-phenomenological models of cross-bridge attachments (Zot & Hasbun, 2016) to infer the MU active state from the Ca-Tn concentration. Yet, as such models involve many parameters, are computationally expensive, and are difficult to tune, including them in the current workflow is a work in progress. Here, a phenomenological model (EQ. 6-26) of the MU activation dynamics controlled by the Ca-Tn concentration was considered (Ding et al., 2000).

$$\frac{da}{dt} = d_1 \cdot T - \frac{a}{d_2 + d_3 \cdot T} \quad (\text{EQ. 6-26})$$

In (EQ. 6-26), a is the active state, T is the Ca-Tn concentration, and d_1, d_2 and d_3 are scaling coefficients, which respectively control the amplitude A_a of the activation twitch, its time-to-peak t_{tp} and its time of half-relaxation $t_{0.5}$. Because the physiological length-dependency of the cross-bridge

attachment dynamics at known Ca-Tn concentration T is unclear in the literature, the d_1 , d_2 and d_3 parameters were modelled constant at all lengths and were calibrated at optimal length $\bar{l} = 1.0$.

The d_i coefficients were tuned so that the predicted MU active state produced by a Ca-Tn twitch matched the normalized experimental twitch torque or force $\overline{f^{tw}}$ recorded in individual human MUs in the TA muscle (Andreassen & Arendt-Nielsen, 1987; Van Cutsem et al., 1997; Van Cutsem et al., 1998). Although the experimental $\overline{f^{tw}}$ also accounts for mechanical dynamics other than the activation dynamics, such as the muscle-tendon interplay or muscle co-contraction and may not be a precise indicator of the MU active state, it remains the only available metric for a in the human literature. Although it is debated in the literature, as discussed in Chapter 4, whether the MU type is correlated to t_{tp} and $t_{0.5}$, it was assumed for simplicity that the slow-type MUs had the longest time-to-peak and half relaxation time values in the MU pool. Considering that 12% of the MUs are of the fast type in the MU pool (Figure 6-3), and assuming that 12% of the MUs identified in (Andreassen & Arendt-Nielsen, 1987; Van Cutsem et al., 1997; Van Cutsem et al., 1998) were of the fast type, it was derived from the t_{tp} and $t_{0.5}$ bar plot distributions in these studies that the slow and fast type MUs respectively had t_{tp} values in the $[35; 90]ms$ and $[20; 35]ms$ ranges, of weighted average 50ms and 30ms, and $t_{0.5}$ values in the $[50; 90]ms$ and $[40; 50]ms$ ranges, of weighted average 65 and 42ms. Finally, d_1 was tuned so that the active state reached 1.0 at 50 Hz stimulation, as firing frequencies above 50Hz were never reported in the human literature. Because of the MU type-specific $[Ca^{2+}]$ and $[Ca^{2+} - Tn]$ dynamics obtained in the previous sections, similar values for the tuned d_1 , d_2 and d_3 coefficients (Table 6-4) were obtained for both slow and fast MUs, with less than 7% error for the A_t , t_{tp} and $t_{0.5}$ parameters.

Table 6-4: Values of the coefficients d_1 , d_2 and d_3 scaling (EQ. 6-26) for both slow and fast-type MUs.

MU type	d_1	d_2	d_3
Slow / Fast	$1.00 \cdot 10^5$	0.026	270

6.2.2.4. Contractile Element – Modelling the MU contraction dynamics

MU Force-Length relationship

The MU normalized length-dependent isometric force f_{FL} was defined with a Force-Length (FL) relationship in the CE (Figure 6-2). To the author's knowledge, no experimental FL relationship was measured at the MU scale in humans. At the muscle-scale, only torque-length and torque-angle relationships were measured in humans; those account for the muscle-tendon dynamics and the co-contraction activity of other muscles and cannot be considered for describing with accuracy the force-length dynamics of a single muscle. Although FL relationships were obtained for whole muscles and

fibre bundles in cat, rabbit and rat specimens, and despite the limitations related to the multiscale approach discussed in Chapter 2, it was considered more suitable in this study to consider a human FL relationship obtained from skinned fibres than a FL relationship from another species that display different optimal sarcomere lengths (Burkholder & Lieber, 2001). The FL relationship hence considered in this study was measured *in vitro* on skinned fibres from the gastrocnemius muscle in fixed-end conditions where the sarcomere length was monitored by laser diffraction and the fibre stimulation obtained with baths of varying calcium concentration (Gollapudi & Lin, 2009). This fibre force – sarcomere length relationship is acceptable at the MU scale, considering that muscles modelled as a scaled sarcomere for their normalized isometric force-length properties can provide accurate Hill-type model predictions for rabbit whole muscles (Winters et al., 2011).

Rather than a piecewise linear relationship consistent with the sliding filament theory at the sarcomere scale (Gordon et al., 1966), a gaussian mathematical description in (EQ. 6-27) was fitted to the experimental data (red curve and black dots in Figure 6-7) to account for the smoother relationship with larger plateau observed at larger scales (Hatze, 1980; Moo et al., 2020).

$$f_{FL}(\bar{l}, a) = \exp\left(-\left(\frac{\bar{l} - \bar{l}_0(a)}{0.45}\right)^2\right) \quad (\text{EQ. 6-27})$$

For higher physiological credibility (Chapter 2) and improved prediction accuracy (Sun et al., 2018), the FL relationship was made nonlinearly dependent to the activation state $a(t)$ (Lloyd & Besier, 2003) in (EQ. 6-28) at submaximal contraction levels (dashed lines in Figure 6-7).

$$\bar{l}_0(a) = 0.15(1 - a) + 1 \quad (\text{EQ. 6-28})$$

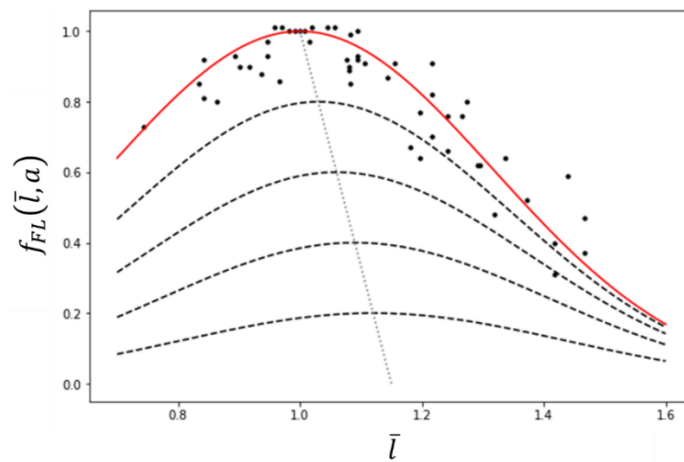


Figure 6-7: Force-Length (FL) relationship considered in this study to describe the MU normalized isometric force f_{FL} . The experimental data points (black dots), obtained from human skinned fibres (Gollapudi & Lin, 2009), are fitted (red curve) with a gaussian mathematical expression (EQ. 6-27). f_{FL} is nonlinearly dependent on the active state a at submaximal levels (dashed lines), where the apparent optimal MU length $\bar{l}_0^M$ increases by up to 15% (dotted line)

MU Force-Velocity relationship

Because of the simplifications made in 6.2.1, the constant-length MUs develop a normalized velocity-dependent force $f_{FV} = 1$, and the FV relationship was ignored when describing their dynamics in Figure 6-2. An advanced FV relationship is here described for future purposes (see (EQ. 6-14). Variants (van Soest & Bobbert, 1993; John et al., 2013) of Hill's (Hill, 1938) (Type 2 FVC, see Appendix A.10 for acronym) and Mashima's (Mashima et al., 1972) (Type 3 FVE, see Appendix A.10 for acronym) hyperbola were considered to describe the muscle's concentric and eccentric contractions, respectively.

The FVC relationship was made nonlinearly dependent on the MU length $\bar{l}$, the MU activation state a and the MU type in two steps. First, the muscle maximum shortening velocity v_{max} depends on the type of recruited MUs, as high-threshold MUs exhibit fast contraction velocities, and on other geometrical considerations related to the muscle activation level and not yet well understood. This is modelled in the literature as $v_{max}(a) = f_v(a) \cdot v_{MAX}$ with v_{MAX} the maximum muscle shortening velocity at full muscle activation and $f_v(a) = \alpha + \beta \cdot a$ with $\alpha + \beta = 1$ and $\{\alpha; \beta\} = \left\{ (0.35; 0.65), (0.25, 0.75), (0.5, 0.5), \left(\frac{3}{7}; \frac{4}{7}\right) \right\}$ (Otten, 1987; Thelen, 2003; Günther et al., 2007; John et al., 2013). Here, it was modelled in (EQ. 6-29) that fast-type MUs ($k_{MU} = 1$) reach maximum shortening velocities twice those of slow-type MUs ($k_{MU} = \frac{1}{2}$), while $f_v(a)$ was arbitrary reduced to $f_v(a) = 0.8 + 0.2 \cdot a$ to account for other activation-related mechanisms independent from the type of recruited MUs. Moreover, v_{max} decreases with sub-optimal shorter MU lengths by the scaling factor $g(\bar{l}) = \begin{cases} f_{FL}(\bar{l}) & ; \quad \bar{l} < 1 \\ 1 & ; \quad else \end{cases}$ (van Soest & Bobbert, 1993; Kistemaker et al., 2006; Günther et al., 2007).

$$v_{max}(a, \bar{l}, type) = k_{MU}(type) \cdot f_v(a) \cdot g(\bar{l}) \cdot v_{MAX} \quad (\text{EQ. 6-29})$$

Second, the curvature of the FVC relationship is fibre type dependent, with a_f values typically 1.5-to-2.5 times higher in rodent and monkey fast-type fibres and rodent and cat muscles of dominantly fast-type fibres (Spector et al., 1980; Otten, 1987; Bottinelli et al., 1991; Fitts et al., 1998). a_f also linearly increases by a factor two between fibre- and muscle-scales (compare (Ranatunga, 1982; McDonald et al., 1994; Roots et al., 2007) for works on rat skinned fibres, bundles of fibres, and whole muscles). Using these observations in combination with experimental a_f values obtained in human skinned fibres and whole lower limb muscles (Andersen et al., 2005; de Brito Fontana et al., 2014; Hauraix et al., 2015; Dada et al., 2018), it was chosen $a_f(slow) = 0.20$ for slow-type MUs and $a_f(fast) = 0.40$ for fast-type MUs in the human TA muscle, which is consistent with previous reviews (Wakeling et al., 2012; Dick et al., 2017). Of note, the higher a_f the lower the FVC curvature. The debated length-

dependency of a_f was not considered here. $a_f(j)$ and $k_{MU}(j)$ distributions across the MU pool could have been considered by using the $F^{th}(j)$ distribution as index.

Similar nonlinearities apply for the FVE relationship during eccentric contractions. A typical value obtained for young human adults was considered for the maximum normalized force attainable in eccentric contractions: $\overline{F^{max}} = 1.4$ (Thelen, 2003).

The MU contraction velocity $v = \frac{dl}{dt}$ was finally normalized to the MU maximum shortening contraction velocity obtained at full activation $\bar{v} = \frac{v}{v_{MAX}}$. The final FV relationship is given in (EQ. 6-30) and (EQ. 6-31) and plotted in Figure 6-8.

$$f_{FV}(\bar{v}, \bar{l}, a) = \begin{cases} \frac{1 + \frac{\bar{v}}{K}}{1 + \frac{1}{a_f(type) \cdot K}} & ; \quad \bar{v} < -1 \\ \frac{1 + \frac{\bar{v}}{K}}{1 - \frac{\bar{v}}{a_f(type) \cdot K}} & ; \quad -1 \leq \bar{v} < 0 \\ \frac{1 + \overline{F^{max}} \frac{\bar{v}}{K \cdot b}}{1 + \frac{\bar{v}}{K \cdot b}} & ; \quad 0 \leq \bar{v} \end{cases} = f_v(\bar{v}, \bar{l}, a) \quad (\text{EQ. 6-30})$$

$$\begin{cases} K(type, \bar{l}, a) = k_{MU}(type) \cdot f_v(a) \cdot g(\bar{l}) \\ k_{MU}(type) = \begin{cases} 0.5 & ; \text{ slow} \\ 1 & ; \text{ fast} \end{cases} \\ f_v(a) = 0.8 + 0.2 \cdot a \\ g(\bar{l}) = \begin{cases} f_{FL}(\bar{l}) & ; \quad \bar{l} < 1 \\ 1 & ; \text{ else} \end{cases} \\ a_f(type) = \begin{cases} 0.20 & ; \text{ slow} \\ 0.40 & ; \text{ fast} \end{cases} \\ b = \frac{\overline{F^{max}} - 1}{2 + \frac{2}{a_f}} \end{cases} \quad (\text{EQ. 6-31})$$

Useful for solving the muscle-tendon dynamics, the FV relationship $f_v(\bar{v}, \bar{l}, a)$ in (EQ. 6-30) is inverted in (EQ. 6-32).

$$\bar{v} = f_v^{-1}(f_{FV}, \bar{l}, a) = K \cdot \begin{cases} f_{FV} \cdot \left(1 + \frac{1}{a_f(type) \cdot K}\right) - 1 & ; \quad f_{FV} < 0 \\ \frac{f_{FV} - 1}{\frac{f_{FV}}{a_f(type)} + 1} & ; \quad 0 \leq f_{FV} < 1 \\ b(type) \cdot \frac{f_{FV} - 1}{\overline{F^{max}} - f_{FV}} & ; \quad 1 \leq f_{FV} \end{cases} \quad (\text{EQ. 6-32})$$

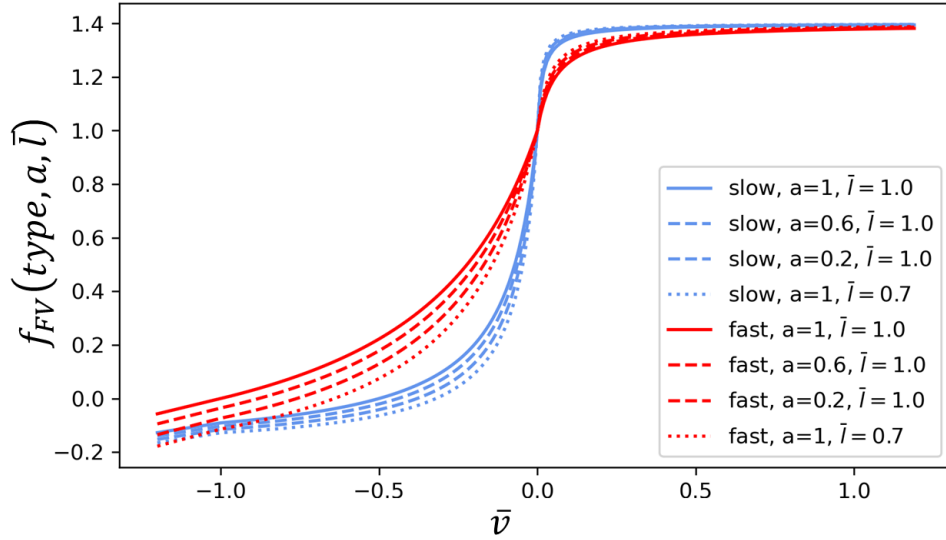


Figure 6-8: The FV relationship (EQ. 6-30) considered in this study, displayed for fast (red) and slow-type (blue) MUs, at optimal MU length $\bar{l} = 1$ and full activation $a = 1$ (plain traces), for submaximal activations (dashed lines), and suboptimal lengths (dotted lines).

6.2.3. Description of the Passive Elements in Model 1

Because of the simplifications made in 6.2.1, the PEE and tendon dynamics were ignored in this Chapter 6. Two PEE and tendon properties are here described for future modelling perspectives (see (EQ. 6-14)).

PEE

In Figure 6-2, the PEE produces the normalized passive elastic force $\overline{F}^{PEE}$, which is modelled, with $k_1 = 5$ and $k_2 = 0.6$ for human young adults (Thelen, 2003), with the well-accepted (EQ. 6-33).

$$\overline{F}^{PEE}(\bar{l}) = \begin{cases} 0 & ; \quad \bar{l} < 1 \\ \frac{e^{\frac{k_1(\bar{l}-1)}{k_2}} - 1}{e^{k_1} - 1} & ; \quad \bar{l} \geq 1 \end{cases} \quad (\text{EQ. 6-33})$$

Tendon

In Figure 6-2, the tendon element T builds the normalized passive elastic force $\overline{F}^T$, which is modelled with $k_1 = 3$, $k_2 = 0.025$, $k_3 = 42.8$, and $k_4 = 0.33$ with the well-accepted (EQ. 6-34) (Giat et al., 1993; John et al., 2013).

$$\overline{F}^T(\varepsilon) = \begin{cases} \frac{1 + \varepsilon}{10^3} & ; \quad \varepsilon < 0 \\ \frac{e^{\frac{k_1\varepsilon}{k_2}} - 1}{e^{k_1} - 1} & ; \quad 0 < \varepsilon < k_2 \\ k_3(\varepsilon - k_2) + k_4 & ; \quad \varepsilon > k_2 \end{cases} \quad (\text{EQ. 6-34})$$

6.2.4. Solving the dynamics of the MU pool

First, the cascading ODEs in (EQ. 6-21), (EQ. 6-22), (EQ. 6-25), and (EQ. 6-26), that describe the MU-specific excitation and activation dynamics, were solved at each time step for all considered n MUs. For each MU, the derived activate state was linearly combined to the MU-specific f_{FL} scaling factor to predict the normalized MU force $\overline{f}^{MU}$ with (EQ. 6-17). It is noteworthy that the MU force $\overline{f}^{MU}$ builds upon the discrete forces of the $IR_i = N_f^{tot} \cdot \overline{f}_{0,i}^{MU}$ fibres of the MU, that non-simultaneously receive the same MN AP in a $\Delta t = 20ms$ time range. This range was estimated from the geometrical arrangement of the fibres in the muscle, the different locations of the NMJ and the finite MN axon and mU fibre conduction velocity. To account for the non-simultaneous fibre activities, the calculation of the normalized MU force $\overline{f}^{MU}$ was updated with (EQ. 6-35), where the delay α_j is fibre-specific and randomly taken in $\left[-\frac{\Delta t}{2}; \frac{\Delta t}{2}\right]$. (EQ. 6-35) acts as a low-pass filter.

$$\overline{f}_i^{MU}(t) = \frac{1}{IR_i} \cdot \sum_{j=0}^{IR_i} \overline{f}_i^{MU}(t + \alpha_j) \quad (\text{EQ. 6-35})$$

The simulated MU $\overline{f}_i^{MU}(t)$ traces were finally scaled with the $f_0^{MU}(i)$ from Chapter 3 and linearly summed to predict the whole muscle force trace with (EQ. 6-15).

6.2.5. Model validation

Datasets of input MN spike trains

The whole muscle force $F^M(t)$ was predicted with the neuromuscular model in Figure 6-1 from two types of neural inputs. In one case, the neuromuscular model was directly controlled by the N_r experimental spike trains identified from decomposed HDEMGs, in which case $F_{N_r}^M(t)$ was predicted. Otherwise, $F_N^M(t)$ was predicted from the N simulated spike trains of the completely reconstructed MN population derived in Chapter 5. Three Force-HDEMG datasets were used, including the $S1_{30}^{AI}$ and $S1_{30}^B$ datasets obtained in Chapter 3, for which subject-specific musculoskeletal data is also available for validation, and the open-source HTA_{35} dataset introduced and used in Chapter 5, which represents typical data found in the literature. With these three datasets, in which $N_r = 81, 35$ and 21 discharging MNs were respectively identified, with homogeneous, rather homogeneous, and rather heterogeneous distributions in the MN pool (see Chapters 3 and 5), it was assessed the sensitivity of the neuromuscular model predictions to the quality of the experimental input data. When the neuromuscular model was controlled by the vectors of N_r experimental spike trains, two approaches were considered for scaling the N_r simulated normalized MU forces $\overline{f}_i^{MU}$ with the $f_0^{MU}(i)$ distribution (EQ. 3-3). First, a ‘blind’ prediction of muscle force ($F_{N_r,1}^M(t)$) was obtained by assigning a

homogeneous distribution of the $f_{0,i}^{MU}$ values to the N_r MUs, disregarding their location into the MN pool. In this 'naïve' approach (EQ. 6-36), among the N_a MUs supposed to be active according to the $F^{th}(i)$ distribution in Chapter 3, each of the N_r identified MUs was assigned a maximum isometric force equal to the sum of the f_0^{MU} values of the $\left\lfloor \frac{N_a}{N_r} \right\rfloor$ MUs surrounding it, using the $f_0^{MU}(i)$ distribution from Chapter 3.

$$\begin{aligned}
 i &\rightarrow N_i = i \cdot \left\lfloor \frac{N_a}{N_r} \right\rfloor, \quad i \in \llbracket 1; N_r \rrbracket \\
 N_{i,1} &= \frac{N_{i-1} + N_i}{2} \\
 N_{i,2} &= \frac{N_i + N_{i+1}}{2} \\
 f_0^{MU}(i) &= \sum_{j=N_{i,1}}^{N_{i,2}} f_0^{MU}(j)
 \end{aligned} \tag{EQ. 6-36}$$

In a second approach where $F_{N_r,2}^M(t)$ was predicted, the true location of the N_r identified MUs into the MN pool was obtained from their recorded Force thresholds $F^{th}(i)$ (Chapter 3), and the portions of the MU pool represented by the N_r representative MUs were not of equal length $\left\lfloor \frac{N_a}{N_r} \right\rfloor$ anymore, but adapted to the identified MU locations.

Principle of validation

In total, three predictions $F_N^M(t)$, $F_{N_r,1}^M(t)$, and $F_{N_r,2}^M(t)$ of the whole muscle force $F^M(t)$ were obtained for the three datasets $S1_{30}^{AI}$, $S1_{30}^B$, and HTA_{35} with the neuromuscular model in Figure 6-1 of N , N_r and N_r in-parallel FGs. The time-course of these predictions was validated against the experimental TA force $F_{exp}^M(t)$, estimated in Chapter 3, with calculation of the normalized maximum error (nME), $nRMSE$ and r^2 validation metrics, that aim to evaluate the key features of the predicted whole muscle forces. nME assesses the maximum error in predicting the whole muscle force, expected, according to Chapter 5, to arise in the regions of low force production. The $nRMSE$ metric evaluates the accuracy in predicting the amplitude of the predicted whole muscle force, which relies on both the distribution of MU tetanic forces (EQ.3-3) and the accurate prediction of the MU active states, while r^2 evaluates the time-course of the variation of the muscle force, which mainly relies on the distribution of the MU neuromechanical properties across the MU pool. It is worth noting that the Δft_i^1 metric, used in Chapter 5, was not used here. This values of Δft_i^1 are determined by the first discharge time of the lowest-threshold motor unit identified experimentally, both when the experimental data (Chapter 3) and the discharge behaviour of the completely reconstructed MU pool

(Chapter 5) are input to the neuromuscular model. The accuracy in predicting the force onset with the Δft_i^1 metric was therefore already investigated in Chapter 5 and was not reproduced here.

To constrain the nME values to interpretable ranges, the nME was only calculated for the time instants t when $F^M(t) > 3\%MVC$. The $F^M(t)$ predictions made with the HTA_{35} dataset were validated in the normalized space for the lack of subject-specific musculoskeletal quantities for this participant.

6.3. Results

6.3.1. Simulation and validation of the MU activation dynamics

When receiving a single neural spike, the NE of a slow-type Hill-type MU at optimal fibre length produced the MN AP, mU AP, free calcium twitch, Ca-Tn twitch and activation twitch displayed in Figure 6-9A. The time-to-peak and decay time constant of these quantities closely reproduced experimental measurements or estimations made on cats or rodents at body temperature. Physiological delays consistent with the literature were added between the onsets of the successive dynamics. Figure 6-9B displays the time-course of these five state-variables when the MU received an excitatory spike train of constant 30Hz firing frequency. Steady-state was reached at 30Hz after a 250ms delay.

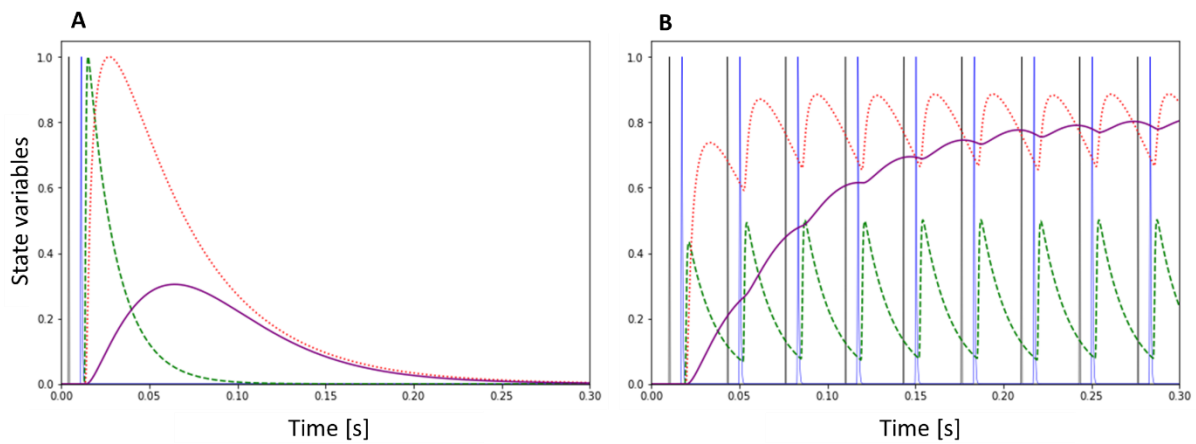


Figure 6-9: Time-course of the five state variables describing the MU excitation and activation dynamics when the MU receives a unique nerve impulse (A) and a train of nerve impulses at 30Hz (B). Black curve: MN AP $e(t)$. Blue curve: mU AP $u(t)$. Green dashed curve: free calcium concentration $c(t)$. Red dotted curve: Ca-Tn concentration $T(t)$. Purple curve: MU active state $a(t)$. Except the MU activate state, the quantities were normalized for visual purposes.

Because the internal MU electrical and chemical dynamics cannot be measured in humans, only the dynamics of the MU active state a was validated against experimental measurements of human muscle force or joint torque. As shown in Figure 6-10A for slow-type MUs, the simulated activation twitches fused for MN firing frequencies above 2Hz. Consistent with previous findings (Milner-Brown et al., 1973), the additional gain provided at steady-state by every simulated activation twitch to the MU active state exponentially decreased with increasing MN discharge frequency. In Figure 6-10A, every new elicited activation twitch increased the MU active state by 100% at 5Hz, and only by 1% at 50Hz. As supported by previous findings in mammals (Heckman & Enoka, 2012), the initial rate of increase of the MU active state was independent from discharge frequency, while the 0-to-80% rate of increase increased with higher firing frequencies from 3.5/s at 15Hz and 5.8/s at 50Hz (Figure 6-10A).

Figure 6-10B displays the simulated steady-state activation-frequency relationship for both slow-type MUs (blue stars) and fast-type MUs (red stars). Steady-state twitch-tetanus ratios of 0.30 and 0.19 were obtained for the simulated slow and fast-type MU active states, respectively. These ratios are of the same order of magnitude as those experimentally obtained for human hand muscles (range 0.1-0.3, (Thomas et al., 1991; Macefield et al., 1996; Fuglevand et al., 1999)). The activation-frequency results for the simulated slow-type MUs compared relatively well with the findings from these experimental studies with a maximum difference of 30% at 15Hz with the EDB muscle data (Macefield et al., 1996) and less than 10% at all physiological frequencies above 5Hz with the thenar muscle data (Thomas et al., 1991). However, the simulated rate of increase in a with firing frequency largely underestimated the available experimental for the fast-type MUs (red dots in Figure 6-10B) with up to 200% difference. Moreover, rodent studies (Celichowski & Grottel, 1993) suggest that the twitch-tetanus ratio for fast MUs is 1.4 times larger than for slow MUs, and not 1.6 times lower as simulated in Figure 6-10B.

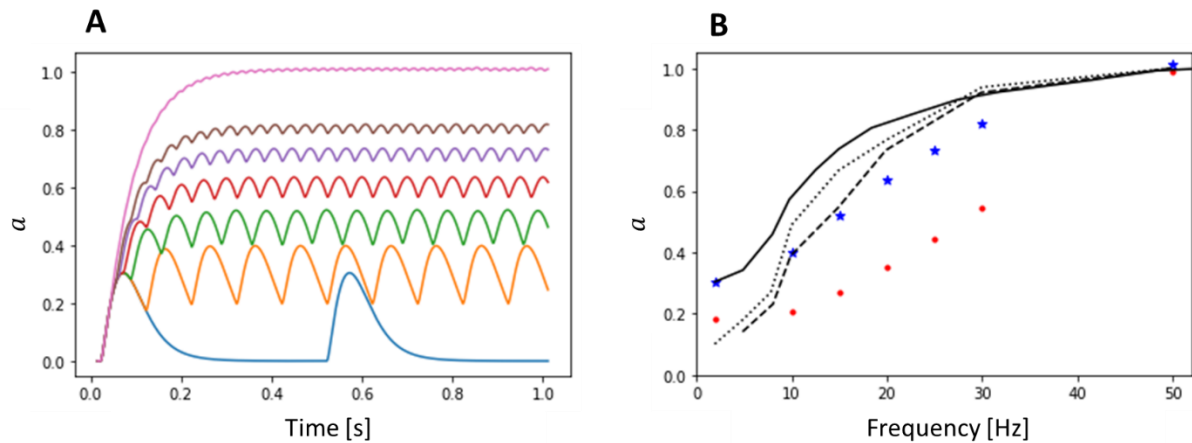


Figure 6-10: Relationship between the amplitude of the simulated active state a and the MN firing frequency in Hz. (A): for slow-type MUs, time-history of the active state $a(t)$ with increasing MN firing frequency at 2 (blue), 10 (orange), 15 (green), 20 (red), 25 (purple), 30 (brown) and 50Hz (rose) where the activation twitches fuse to reach 1.0 at steady-state. (B) Activation-Frequency relationship in the steady-state. Simulations for slow and fast-type MUs are displayed in blue stars and red dots respectively. For information, experimental Activation-Frequency relationships obtained from FDI (dotted trace, (Fuglevand et al., 1999)), EDB (plain trace, (Macefield et al., 1996)) and thenar (dashed trace, (Thomas et al., 1991)) muscle fibres are superimposed.

6.3.2. Force validation

Figure 6-11 compares for three experimental datasets the time evolutions of the experimental recorded muscle force $F_{exp}^M(t)$ (black curve) and the whole muscle forces predicted with the MN-driven muscle model described in Figure 6-1. As a reminder, the three considered datasets are $S1_{30}^{AI}$ (256 electrodes, 81 identified MNs) and $S1_{30}^B$ (64 electrodes, 35 identified MNs) from Chapter 3, and HTA_{35}

(64 electrodes, 21 identified MNs) from the literature and introduced in Chapter 5. For each dataset, the whole muscle force was predicted from the N simulated spike trains obtained from Chapter 5 ($F_N^M(t)$, red curves in Figure 6-11) and from the sole N_r experimental spike trains in two approaches described previously ($F_{N_r,1}^M(t)$, blue dotted curves, and $F_{N_r,2}^M(t)$, green dashed curves in Figure 6-11).

For all three datasets, the most accurate predictions of the experimental force were obtained from the N reconstructed spike trains, with $nME \in [40\% - 79\%]$, $nRMSE \in [8\% - 11\%]$, and $r^2 \in [0.95 - 0.98]$ (Table 6-5). Predictions of close accuracy were obtained from the experimental N_r -vectors when the identified MNs were located into the MN pool and assigned realistic representative f_0^{MU} values, $nME \in [46\% - 72\%]$, $nRMSE \in [8\% - 14\%]$, and $r^2 \in [0.95 - 0.97]$, which explains why the green dashed traces overlap with the $F_N^M(t)$ red trace in Figure 6-11. For all three datasets, the least accurate predictions of $F_{exp}^M(t)$ were obtained from the N_r reconstructed spike trains with the 'naïve' approach of homogeneous MN across the MN pool, with $nME \in [60\% - 78\%]$, $nRMSE \in [12\% - 25\%]$, and $r^2 \in [0.80 - 0.97]$. Figure 6-11 shows that $F_{exp}^M(t)$ was better approximated by $F_N^M(t)$ and $F_{N_r,2}^M(t)$ than $F_{N_r,1}^M(t)$ in the regions of low forces where dominantly low-thresholds MUs are activated. These results stress the importance of carefully describing the continuous distribution of both the MU electrophysiological and mechanical properties across the MU pool.

With all three types of inputs, the simulated forces $F_{N_r,1}^M(t)$, $F_{N_r,2}^M(t)$, and $F_N^M(t)$ were the most accurate representations of $F_{exp}^M(t)$ for the $S1_{30}^A$ dataset with $nME \in [40\% - 60\%]$, $nRMSE \in [8\% - 12\%]$, and $r^2 \in [0.97 - 0.98]$ (Table 6-5). Least accurate results were obtained with the open-source HTA_{35} dataset, with $nME \in [71\% - 79\%]$, $nRMSE \in [11\% - 25\%]$, and $r^2 \in [0.80 - 0.95]$. Results of intermediate accuracy were obtained with the $S1_{30}^B$ dataset. These results stress the importance of obtaining experimental datasets of MN spike trains of sufficient size and quality, which representatively describe the discharge activity of the complete MN pool.

In all cases and for all datasets, $F_{exp}^M(t)$ was not accurately predicted in the frequency domain during the plateau of force, when large high-threshold MUs are recruited close to their recruitment threshold.

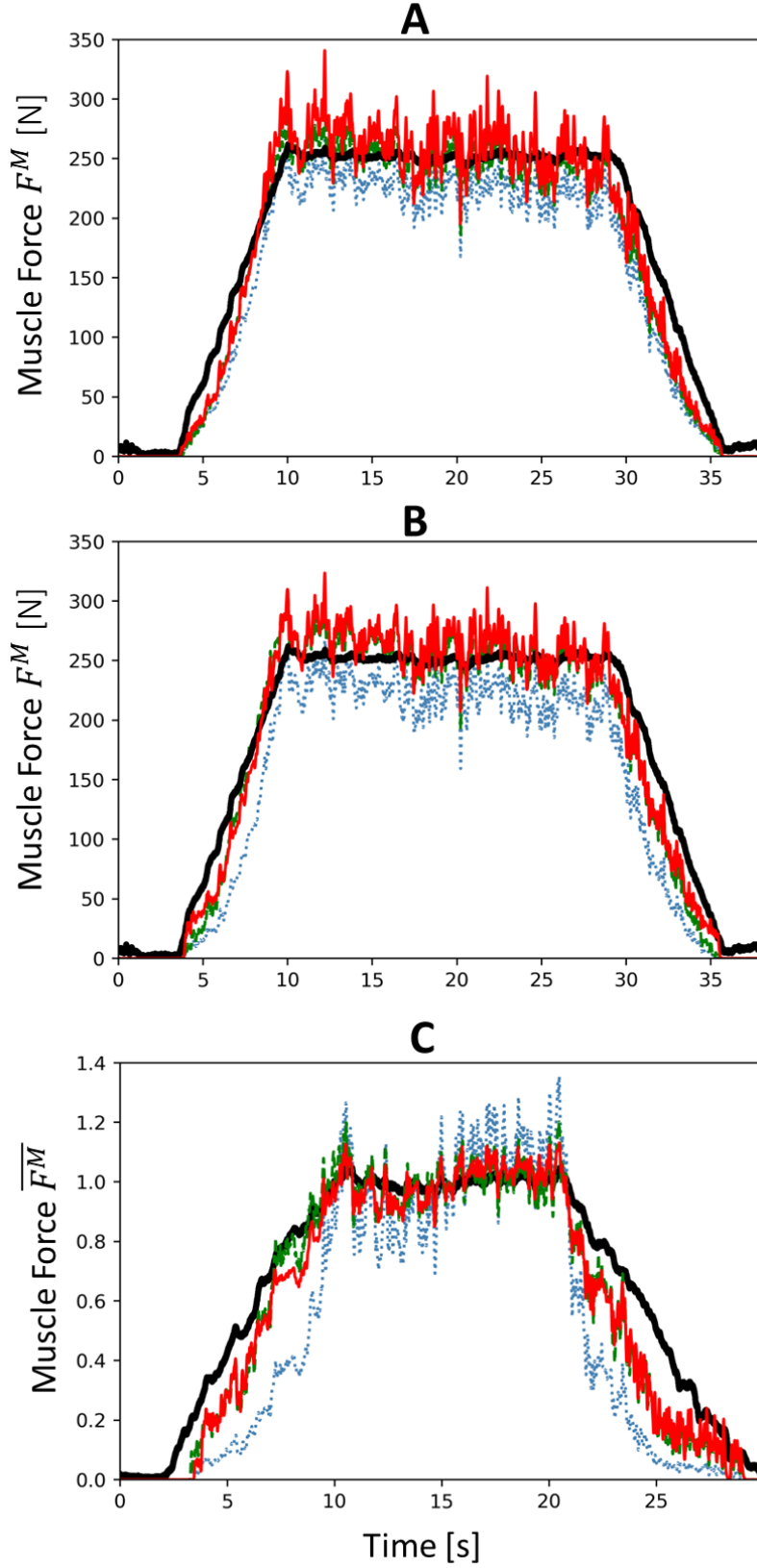


Figure 6-11: Experimental (black traces) and simulated whole muscle forces for the three datasets $S1_{30}^{AI}$ (A), $S1_{30}^B$ (B), and HTA_{35} (C). The simulated forces $F_{Nr,1}^M(t)$, $F_{Nr,2}^M(t)$, and $F_N^M(t)$ are represented with dotted blue, dashed green and plain red lines respectively. Forces are given in N in (A,B) and are normalized to the absolute mean value computed during the plateau of force in (C).

Table 6-5: Validation with the nME , $nRMSE$ and r^2 metrics of the whole muscle forces $F^M(t)$ predicted by the neuromuscular model in Figure 6-1 against the recorded experimental muscle force $F_{exp}^M(t)$ for the three datasets $S1_{30}^{AI}$, $S1_{30}^B$, and HTA_{35} . For each dataset, three predictions are performed. $F_{N_{r,1}}^M(t)$ is blindly predicted from the N_r experimental spike trains assuming they are homogeneously distributed across the MU pool. $F_{N_{r,2}}^M(t)$ is predicted from the N_r experimental spike trains knowing their location into the MU pool. $F_N^M(t)$ is predicted from the N simulated spike trains reconstructed for the complete MU pool in Chapter 5.

	Predicted Forces	nME [%]	$nRMSE$ [%]	r^2
$S1_{30}^{AI}$	$F_{N_{r,1}}^M$	60	12	0.97
	$F_{N_{r,2}}^M$	50	8	0.97
	F_N^M	40	8	0.98
$S1_{30}^B$	$F_{N_{r,1}}^M$	78	23	0.94
	$F_{N_{r,2}}^M$	46	10	0.97
	F_N^M	46	8	0.97
HTA_{35}	$F_{N_{r,1}}^M$	71	25	0.80
	$F_{N_{r,2}}^M$	72	14	0.95
	F_N^M	79	11	0.95

6.4. Discussion

This Chapter developed and validated the MN-driven multi-MU neuromuscular model introduced in Chapter 1, for which neural controls were designed in Chapters 3 to 5 to allow the simulation of human muscle voluntary contraction. The neuromuscular model (Figure 6-1) was built as a collection of n Hill-type MUs which collectively produced the active muscle force $F^M(t)$, that was validated against the experimental TA force reported in Chapter 3.

6.4.1. Model validation

6.4.1.1. Active state validation

The active state transients simulated by the slow-type and fast-type Hill-type MUs constituting the model were compared in the steady-state in Figure 6-10 against experimental data of isometric forces obtained in human hand muscles (Thomas et al., 1991; Macefield et al., 1996; Fuglevand et al., 1999). The simulated twitch-tetanus ratios of 0.30 and 0.19 obtained for the slow and fast-type MU active states agreed with the experimental range 0.1-0.3, (Thomas et al., 1991; Macefield et al., 1996; Fuglevand et al., 1999). The three experimental force-frequency relationships compared well with the simulated activation-frequency for the slow-type MU. This was not the case for the fast-type MUs, the initial simulated rate of increase of which largely underestimated the experimental recordings. This slow rate supports the observations that the simulated twitch-tetanus ratio for fast MUs was 1.6 times lower than for slow MUs, although experiments in rodents suggest 1.4 times higher ratios (Celichowski & Grottel, 1993). These results may suggest that the twitch gain-frequency relationship may be too steep for the simulated fast MUs and that the resulting predicted active state may be underestimated for fast MUs for most of the physiological range of frequencies. It is however noteworthy that the comparisons were made against forces from muscles of dominantly slow-type fibres. Also, this shortcoming did not impact the results displayed in Figure 6-11, as a maximum 343 MUs were recruited over the course of the trapezoidal contractions at 30-35% MVC, according to the $F^{th}(i)$ distribution in Chapter 3, and these were all of the slow type according to Section 6.2.2.1. It is noteworthy that these conclusions remain limited for two reasons. First, the simulated active state was compared to an experimental force that accounts for mechanisms other than those of the MU excitation-contraction coupling properties. Second, the results of the TA simulations were compared to experimental data from other muscles that display drastically different neuromechanical properties (Duchateau & Enoka, 2022), because no force-frequency relation was measured for the human TA muscle, as stressed in a recent review (Duchateau & Enoka, 2022).

6.4.1.2. Force validation

The experimental force F_{exp}^M recorded during trapezoidal isometric contractions of the TA at 30-35% MVC was predicted by the neuromuscular model with experimental input vectors of $N_r = 21, 32$, and 81 MN spike trains and reconstructed input vectors of $N = 400$ MN spike trains for three datasets, including two datasets developed in Chapter 3 ($S1_{30}^{AI}$ and $S1_{30}^B$) and one from the literature (HTA_{35}). For all three datasets, F_{exp}^M was most accurately predicted from the reconstructed vectors of N spike trains obtained from Chapter 5. These results strengthen the previous conclusions that the reconstructed MN pool predicted reliable approximations of the real neural drive to muscle (Chapter 5), and that the activation and contraction dynamics developed in this model can accurately recreate the process of whole muscle force generation from the individual MU dynamics when the complete MU pool is modelled. Importantly, excellent predictions were obtained in the low-force regions, suggesting that the contractile activity of the low-threshold small MUs were successfully accounted for. For all three datasets, the least accurate predictions of F_{exp}^M were obtained when the neuromuscular model was controlled by the experimental vector of N_r MN spike trains and the N_r MUs forces were scaled with evenly distributed MU maximum isometric forces f_0^{MU} . With important underestimation of F_{exp}^M in the low-force regions, these results confirm the conclusions made in Chapters 3 and 5 that the outputs from decomposed HDEMG signals should not blindly control multi-MU neuromuscular models, because of the bias of HDEMG recording and decomposition techniques towards oversampling high-threshold MUs. Interestingly, the vectors of N_r experimental inputs provide very accurate predictions, close to those made with the completely reconstructed pool of N MUs, when realistic f_0^{MU} values are assigned to the N_r modelled MUs. This strengthens the importance of interpreting the decomposed HDEMG data and assigning the identified MNs to realistic location into the MN pool.

The results in Table 6-5 and Figure 6-11 also stress the importance of the quality of the input experimental data for accurate force predictions. First, obtaining subject-specific musculoskeletal quantities to scale the neuromuscular model and infer the TA force from the recorded joint torque allowed, in combination with realistic distribution of the TA-specific MU properties, excellent predictions of the muscle force amplitude (in N). This was achieved without any mechanical parameter calibration, with less than 10% overestimation of the plateau of force for both the $S1_{30}$ datasets. Second, the best predictions with all three types of inputs were obtained with the dataset $S1_{30}^{AI}$, that provides more MN spike trains of better quality and a better explanation of the continuous distribution of the MN electrophysiological properties than the two other $S1_{30}^B$ and HTA_{35} datasets. Although number seems to play a secondary role, the homogeneous distribution of the identified MNs is crucial,

whether or not the complete MN pool is reconstructed, to accurately predict the time of force onset, account for the dynamics of MU recruitment, and simulate the specific MN firing dynamics.

6.4.2. Relevance for neuromuscular modelling

This neuromuscular model addresses several limitations in the field of Hill-type muscle modelling identified with the systematic review in Chapter 2.

Firstly, the neural control to the neuromuscular model in Figure 6-1 is a vector of experimental spike trains that is a more easily interpretable and a more detailed description of the muscle neural drive than the rectified-normalized-filtered envelopes of recorded bEMGs (Hof & Van den Berg, 1981; Zajac, 1989; Lloyd & Besier, 2003) used in single-input muscles models. Most of the information provided by the HDEMG data was used, contrary to previous HDEMG-driven approaches, in which the MN spike trains were lumped into a unique CST-like neural control to a single actuator (Sartori et al., 2017; Thompson et al., 2018; Kapelner et al., 2020).

Secondly, despite advanced multi-scale approaches in modelling whole muscles as a single equivalent MUs (Hatze, 1977; Hatze, 1980; Dorgan & O'Malley, 1997; Song et al., 2008; Dick et al., 2017; Hussein et al., 2021), the neuromuscular model in Figure 6-1 was built as a collection of in-parallel MUs. Describing the discrete composition of the MU pool allowed a precise assignment of the input MN spike trains and an explicit description of the dynamics of MU recruitment, that are intrinsic to the set of neural controls. The multi-MU description also provided a useful structure to precisely identify the taken multiscale assumptions and simplifications. Moreover, it provided a convenient framework for partitioning the MU pool into slow- and fast-type MUs, that would display different neuromechanical behaviours, and for assigning to the MU pool continuous distributions of MU mechanical properties. Finally, the multi-MU structure made it easier to advance the modelling of the MU excitation and activation dynamics with explicit description for the dynamics of the free calcium and Ca-Tn transients. Thirdly, only a few other multi-MU models were proposed in the literature to predict whole muscle forces with collections of N MU Hill-type (Legreneur et al., 1996; Callahan et al., 2013) Hill-Huxley-type (Carriou et al., 2019) or twitch-type (Raikova et al., 2018) muscle models. Because they lacked experimental control at the level of the individual MN, these studies modelled artificial dynamics of MU recruitment, mostly with the Fuglevand's formalism (Fuglevand et al., 1993), and controlled the modelled MUs with artificial inputs. Consequently, the force predicted with these models was indirectly validated against results from other models and not against synchronously recorded experimental data as performed in this Chapter 6. The model proposed in this chapter is therefore the first in the literature to perform MN-driven forward predictions of human muscle voluntary contractions from recorded MN controls, and to be validated against a recorded experimental force trace.

6.4.3. Current limitations and future iterating improvements

The novel neuromuscular model presented in this Chapter 6 provides a state-of-the-art tool for investigating and modelling the dynamics of the MU pool in forward simulations of human voluntary muscle contraction. However, this introductory work suffers from limitations, some of which will be addressed with future iterative improvements of the model.

Predicted muscle forces and model validation

Even though some gold standards were followed by using subject-specific MSK quantities to scale the recorded experimental force (Chapter 3) and the muscle model (Chapter 6), the validation of the neuromuscular model remains incomplete or limited for several reasons.

First, some dynamics simulated by the model, such as the calcium and Ca-Tn dynamics, could not be validated against available human experimental data because these quantities cannot be measured in human muscle *in vivo*. This however stresses the importance of such detailed neuromuscular models for investigating the human muscle's neuromechanics.

Then, because of experimental constraints for obtaining the neural controls, the neuromuscular model was only tested in isometric trapezoidal contractions. While decomposition techniques were recently developed for quasi-isometric (Oliveira & Negro, 2021) and some dynamic (Glaser & Holobar, 2018) contractions, the model should first be tested for isometric trajectories different from trapezoidal contractions.

Furthermore, the performance of the neuromuscular model was not compared to the predictions from well-established models in the literature. In a later development of the model, the parameters of both the neuromuscular model presented in Chapter 6 and the muscle model generated by the open-source CEINMS toolbox (Pizzolato et al., 2015) will be calibrated with the torque-HDEMG data from the dataset $S1_{30,1}^{AI}$. The torque predictions of the two calibrated models will be assessed and compared with the second trapezoidal 30% MVC dataset $S1_{30,2}^{AI}$. The neural control to the CEINMS model will be obtained as the summation of the cumulative spike train computed from the $S1_{30}^{AI}$ spike trains and of the residual unexplained EMG signals (Sartori et al., 2017). It is noteworthy that beyond the quality of the muscle force predictions, the MN-driven MU-scale model from Chapter 6 give a level of understanding of the internal muscle neuromechanics that the CEINMS model cannot provide.

Then, it was not assessed whether the neuromuscular model would adapt well to other neuromuscular systems that display different neuromechanical properties, like hand muscles. In this Chapter 6, besides the subject-specific MSK geometries, only the subject-specificity of the muscle maximum isometric force was used to scale the distribution of MU maximum isometric forces in the MU pool, which was obtained from human TA-specific data from the literature. Because MRI scans are not

always available to derive subject-specific MSK properties, and because muscle-specific data on the distribution of the MU mechanical properties is available in the literature for only a few muscles (FDI and gastrocnemius medialis mainly), some model parameters could be tuned with calibration routines. For example, the shape factors defining the f_0^{MU} distribution, as well as the l_0^M , l_s^T , and F_0^M quantities could be calibrated to match recorded joint torques.

Also, validating the predicted muscle force against an assumed muscle force derived from the experimental ankle torque remains a key limitation typical of static optimization approaches. As discussed in Chapter 3, it is assumed here that the joint torque is only shared between the synergistic muscles following a distribution of the load sharing that was assumed at MVC and extrapolated at 30% MVC between the TA, EHL and EDL muscles. The experimental setup proposed in this work does not provide the means to validate whether this distribution is valid or not. Consequently, the validation of the absolute values in N of the TA force predicted with the model remains limited.

Moreover, although the Δft_1 , nME , $nRMSE$, and r^2 metrics used for validating the whole muscle force predictions in this Chapter are well-admitted and commonly used in the literature and individually evaluate some of the key features of the force predictions (onset, time-course trajectory, or amplitude of force production), other suitable metrics could have been considered. To stress the importance of the activity of the low-threshold MU population, a comparison of the slopes of the ramps of contraction between experimental and simulated trajectories could be performed. Also, a more accurate evaluation of the error in predicting the whole muscle force during the ramps of contraction could be obtained by low-pass filtering the simulated output in the [0-4 Hz] frequency range to reduce the contribution of the simulated force oscillations during the plateau of force to the $nRMSE$ values.

Finally, it remains to be solved the discrepancy between experimental and predicted muscle forces in the frequency domain (Figure 6-11). The only other model that predicted in the literature the muscle force from the summation of the individual MU twitches of the complete MU pool did not encounter this issue because of non-physiologically long simulated activation twitches (Hatze, 1980). The physiological mechanisms responsible for the low-pass filtering effects of the muscle-tendon system should be identified and added to the model. One explanation may be the contribution to the muscle force of the noisily-activated high-force MUs, which may be overestimated in this model. This could be solved with lower twitch-tetanus ratios or maximum isometric forces for these MUs. Another solution would be to consider a passive damping element in-parallel with each individual MU.

Neuromuscular modelling

The neuromusculoskeletal model proposed in this Chapter 6 will be refined in four main steps. First, the computational method will be extended to account for the muscle-tendon dynamics with a non-

rigid tendon, the dynamics of which are described with (EQ. 6-34). To account for the passive activity of the compliant tendon, the differential equation in (EQ. 6-14) must be simultaneously solved at each time step for the n MUs constituting the model to maintain the muscle-tendon equilibrium. In a non-rigid tendon scenario, the MU lengths are not constant anymore over the course of muscle-tendon isometric contractions, and the PEE (EQ. 6-33) in Figure 6-1 is not neglected anymore.

In a second step diverging from the same-length simplification, the MUs will be assigned specific lengths. The combination of the volumetric muscle representation proposed in Chapter 3 and an experimental mapping of the MUs (Lubel et al., 2022) will provide the necessary MU-tendon length $l_i - l^T$ relationships necessary to lift the indeterminacy in (EQ. 6-12). The individual optimal MU lengths $l_{0,i}^{MU}$ will be determined using the same scaling method as proposed in Chapter 3 for l_0^M to obtain the updated MU-specific normalized lengths $\bar{l}_i$.

Third, because these steps will add up to the already high computational cost of the model, strategies to obtain a computationally cheaper actuator will be investigated. As shown in Chapter 5, smaller reconstructed MN pools can be considered without loss of accuracy in the estimated neural drive to muscle. Chapter 6 also suggests that, provided the experimental data is representative enough of the complete MN pool and the assignment of the MU maximum isometric forces is correctly applied, the reconstruction of the MN pool in Chapter 5 may be avoided for whole muscle predictions without loss of accuracy. Therefore, one could pick a maximum number of rather evenly spaced identified MNs from a 'good' experimental dataset (leaving some high-threshold MUs out) or reconstruct with Chapter 5 a small 50-MU population of MUs and assign to those subsets of MUs realistic f_0^{MU} values, hence decreasing the overall computational cost by at least 8. Furthermore, because the experimental and reconstructed spike trains describe both the MN and MU electric activity, and because two APs never overlap, the 2nd-order ODE defining the MU excitation dynamics could be traded for a computationally cheaper analytical description of firing APs.

Finally, although they advance the state-of-the-art of modelling the muscle activation dynamics by detailing the ion transients in the sarcoplasm, the models of calcium transients in Chapter 6 are mostly phenomenological. Equivalent models relying on physiological mechanisms of chemical reactions and mass action principle will be developed. Because they will have a physiological meaning, the parameters scaling these models will be easier to assign to a continuous distribution of the MU properties in the MU pool, rather than the non-physiological discrete partition of the pool between slow and fast MUs currently used in the model.

To conclude, once iterative improvements and further validations are performed, this neuromuscular model will find crucial applications in investigating the neuromechanics of human voluntary neuromuscular control, with important applications in human-machine interfacing.

Chapter 7

GENERAL DISCUSSION – LIMITATIONS – FUTURE PERSPECTIVES

Chapter summary

In this thesis, a novel MN-driven multi-MU neuromuscular model was developed. This model received empirical MN spike trains and predicted the whole muscle force in forward simulations of human voluntary muscle contraction from the simulated discrete dynamics of the MU pool. This model, introduced in Chapter 1 and detailed in Chapter 6, addressed some current limitations in the field of Hill-type muscle actuators, that were identified with the systematic review in Chapter 2. This thesis focused, in Chapters 3, 4, and 5, on developing novel experimental and computational methods to realistically control the MU-scale model with individual MN spike trains derived from decomposed HDEMG signals. Because this MN-driven MU-scale model provides a window onto the internal MU dynamics, it can advance our understanding of the human muscles' neuromechanics during voluntary contractions, with for example important subsequent applications in human-machine interfacing. This final Chapter 7 first outlines the most important achievements of this thesis, by summarizing the key findings and conclusions of each chapter. Then, the main limitations of the presented work are reviewed, to finally open to the author's perspectives on future work.

7.1. Summary of achievements

Chapter 2 of this thesis proposed the first systematic review of the field of Hill-type neuromuscular modelling. From the analysis of 55 eligible studies that described a Hill-type actuator, multiple objectives were achieved. First, the content of the field of Hill-type neuromuscular modelling, which is very dense and has lacked comprehensive reviews for the past 25 years, was clarified by clearly identifying the main trends and gold standard in Hill-type modelling. Second, these gold standards were challenged, and alternatives were proposed. Third, the state-of-the-art was clearly outlined by identifying the neuromuscular properties that are rarely or not modelled in the field. Fourth, a list of good practices was reported about model validation, reusability, and calibration, and about modelling strategy. Because the 55 eligible studies are altogether representative of the published strategies in Hill-type modelling, this review helps researchers navigating the field and choosing the ‘right’ available muscle model for their study, using the scoring results as support. To help in the generation of new Hill-type models, Appendix A.10 provides, along with inheritance diagrams, the most commonly published mathematical relationships proposed in the literature for describing the key six excitation-contraction and mechanical properties of muscles. Modelers should also adhere to the good practices outlined in the review, and consider the 61 additional studies reported in Appendix A.7 that propose sensitivity analyses, reviews of the field, model comparisons, and further modelling approaches. This review opens the discussion on the necessity of more regular reviews and global recommendations in the field of Hill-type modelling, with an emphasis on model reusability for optimal transmission of knowledge. Most importantly for this thesis, the review identified key gaps in the field of neuromuscular modelling that this thesis aimed to fill. No model was ever developed to mimic the dynamics of the muscle’s MUs during human voluntary contractions. Most models in the field described the whole muscle with a single Hill-type actuator and either neglected the dynamics of MU recruitment entirely or considered those dynamics to be lumped with the MN firing frequencies in the bEMG signals in EMG-driven approaches. Besides usually overlooking the continuous distribution of the MU properties in the muscle, these whole-muscle modelling approaches relied on multiscale assumptions, did not provide a window onto the muscle’s internal excitation-activation mechanisms, and further restrained more physiological models of the muscle’s multiscale excitation-contraction coupling properties. Seeking more realistic descriptions for these dynamics, some studies proposed Hill-type models of individual MUs, but either investigated single MUs, or controlled the individual MUs of the reconstructed MU pool with artificial inputs. These MU-scale models were not validated against ad hoc experimental recordings. These identified limitations motivated the development of a novel MN-driven neuromuscular model described as a population of Hill-type MUs that would receive neural controls from individual MNs to reproduce human voluntary contraction tasks.

Chapter 3 of this thesis provided the necessary experimental data to control, personalise, and validate the neuromuscular model. HDEMG signals from the TA muscle and ankle torques were simultaneously recorded for six subjects performing voluntary trapezoidal isometric ankle dorsiflexion tasks. First, the HDEMG signals were decomposed using finely-tuned blind-source separation algorithms to identify a maximum number experimental MN spike trains, later neural inputs to the neuromuscular model. The novelty proposed in Chapter 3 was the use of a large high-density array of 256 EMG electrodes covering the TA muscle. A sensitivity analysis showed that more MN spike trains, of higher quality, and relatively more low-threshold MNs were obtained with the 256-channel array than with smaller or less dense grids that would involve 64 or less electrodes. In this respect, sets of up to 81 MN spike trains were obtained in this work, which is substantially more than the sets of 15-40 trains obtained in the literature for the TA muscle with 64-channel setups. The largest obtained sets of spike trains provided the best estimates of the neural drive to muscle and may constitute the most possible detailed experimental representation of the MU pool behaviour during human voluntary contractions that can currently be obtained with this empirical approach and in these conditions. Because some inaccuracy in estimating the neural drive remained, it was concluded that these experimental sets of spike trains, despite significant improvement, were not reliable enough to alone control the MU-scale neuromuscular model developed in this thesis. In a second part, subject-specific MSK geometries were obtained for one subject from the segmentation of MRI scans of the subject's shank and foot. Using state-of-the-art automated tools, it was obtained a MSK model specific to the subject, for whom subject-specific MSK quantities were derived. These quantities were used to scale the whole muscle's properties of the neuromuscular model, and the distribution of MU maximum isometric forces in the MU pool, and to estimate from the recorded ankle torque the force developed by the TA muscle for later model validation.

In Chapter 4, mathematical relationships between eight key MN electrophysiological and morphological properties were derived, including the MN size (soma diameter and MN surface area), MN membrane input resistance, specific resistance, capacitance, time constant, and afterhyperpolarization period, axonal conduction velocity, and current threshold for spike initiation. From the Henneman's size principle, Rall's cable theory, and multiple experimental studies, reviews had established that correlations existed between these MN properties, and it was known the monotonical variations between them. In chapter 4, the data from 40 experimental studies was processed to propose a quantitative description for these correlations with a set of mathematical relationships between the investigated MN properties. The derived mathematical relationships were strongly consistent with the conclusions from both the Henneman's size principle and Rall's cable theory, and extrapolate to some extent from cat to other mammalian species. These relationships are

useful for modelers, experimenters, and neuroscientists, as any of the seven properties can be directly calculated if the eighth property is known, within a level of accuracy consistent with the one empirically reported. With these relationships, Chapter 4 underlined that low- and high-threshold MNs display very different electrophysiological properties, and therefore discharge behaviour, which physiologically explains the inaccuracy of the empirically estimated neural drives in Chapter 3.

Chapter 5 presented a novel computational method to infer the discharge activity of the complete MN pool from the known firing behaviour of the MNs identified experimentally. This method aimed to extend the experimental datasets obtained in Chapter 3, in order to obtain neural controls to the neuromuscular model that are more representative of the real behaviour of the complete MN pool innervating the muscle. The synaptic current common to the MNs of the MN population was first estimated from the experimental data obtained in Chapter 3. The synaptic current drove single-compartment models of MN, which were scaled with electrophysiological properties, the inter-relation of which were given in Chapter 4. To mimic the discharge behaviour of the experimental MNs, these properties were calibrated and made MN-specific. Because the location of the experimental MNs into the MN pool was obtained according to the MNs' recorded force recruitment thresholds and the TA-specific properties from Chapter 3, the calibration step output a continuous subject-muscle-specific distribution of the MN electrophysiological properties into the MN pool. In a final step, a cohort of MN models were scaled with the property distribution and received the experimental common input current to simulate the spike trains of all the individual MNs constituting the complete MN population. This method was validated at the level of the individual MNs. The discharge behaviour of the reconstructed pool more accurately estimated the neural drive to muscle than the sole experimental data, especially in the low-force regions where the low-threshold recruited MNs were undersampled in the experimental datasets. Finally, the cohort of MN models calibrated with one trial accurately predicted the neural drive to muscle for a second trial where a similar muscle contraction profile was performed. Provided large portions of the MN pool were not overlooked in the input experimental dataset, the method was proven robust to the number of spike trains experimentally determined, with similar MN pool reconstructions obtained from the 64-channel and 256-channel data. Consequently, the method in Chapter 5 created reliable neural inputs to the neuromuscular model, that were representative of the experimental data, and performed well with experimental datasets classically obtained in the literature with 64-channel HDEMG grids.

Chapter 6 described the MN-driven multi-MU neuromuscular model introduced in Chapter 1. This model was built as a collection of n in-parallel Hill-type models, that described the properties and dynamics of the individual MUs constituting the whole muscle. The Hill-type MUs were individually controlled by MN spike trains and built the whole muscle force in forward predictions. To perform the

simulations, two approaches were considered, where the neuromuscular model either received the experimental spike trains obtained in Chapter 3, or the simulated spike trains of the completely reconstructed MN population from Chapter 5. This dual analysis was performed twice with the neural data obtained from the 64- and 256-channel configurations (Chapter 3) for the subject for whom subject-specific MSK data was available, and a third time with an open-source Force-HDEMG dataset from the literature. In all configurations, the MN-driven MU-scale neuromuscular model more accurately predicted the experimental TA force, estimated in Chapter 3, with the reconstructed MN pool than with the sole experimental data. Accurate predictions were obtained with all the reconstructed MN populations, irrespective of the initial number of identified experimental spike trains. When the neuromuscular model was controlled with the sole experimental MN datasets, the whole muscle force was underestimated in the regions of low force and the best predictions were obtained with the 256-channel data. Besides providing accurate force predictions, the MU-scale definition of the muscle model offered an adequate structure to model the distribution of the MU properties across the MU pool. In this respect, specific maximum isometric forces were assigned to the individual MUs from typical TA data (Chapter 3), and the MUs were assigned a ‘slow’ or ‘fast’ type according to their force recruitment thresholds. It is noteworthy that the experimental neural inputs derived in Chapters 3 and 5 intrinsically account for the distribution across the MU pool of the MUs’ firing characteristics and dynamics of recruitment. With this MU-scale structure, careful multiscale assumptions could be made, and advanced models for the excitation-contraction muscle properties were proposed, hence addressing another limitation identified in the field of Hill-type neuromuscular modelling in Chapter 2. The MU active states were physiologically derived as the summation of activation twitches predicted from new phenomenological models of the free calcium and calcium-troponin transients in the sarcoplasm. Importantly, these dynamics were tuned to mimic experiment data obtained in humans or mammals, at body temperature, in fibre bundle, fibre, or muscle preparations. To account for the interplay between the neuromechanical and contractile elements discussed in Chapter 2, the MU activations and contraction dynamics were respectively made MU length and MU activation-dependent. Finally, it was discussed the several reasons why this MN-driven multi-MU neuromuscular model advanced the field of Hill-type neuromuscular modelling.

7.2. Current limitations of the work and future perspectives

Although the workflow presented in the Chapters 3 to 6 addressed several current limitations in the field of Hill-type modelling (Chapter 2) and fulfilled the objectives of this thesis, it met a few key limitations already mostly discussed in the previous chapters and reported here. How to address some of these limitations is also recalled in this section.

Experimental neural control

The applicability of MN-driven neuromuscular models controlled by decomposed HDEMG data remains constrained by experimental limitations. First, obtaining individual MN spike trains from decomposed HDEMG signals remains a challenging task. Although individual spike trains provide a decisive window onto the neuromechanics of the MN pool, acquiring this neural data is more challenging than obtaining processed bEMG signals, for which automated filtering-rectification computational methods are available. Until automated machine learning methods are established for HDEMG approaches, good understanding of the concepts of blind-source separation are required to optimally tuning the decomposition algorithms, while training is also required for the false-positive false-negative manual edition of the spike trains, which is moreover extremely time-consuming. It took around five hours of processing to an experienced operator to obtain one 256-channel dataset of MN spike trains. For these reasons, it remains currently easier to perform EMG-driven than MN-driven neuromuscular simulations from electromyographic data.

Second, the MN spike trains obtained from decomposed HDEMG signals are usually not representative of the discharge activity of the complete MN pool. While the deep MUs that lay far from the surface electrodes may be entirely overlooked, the low-threshold MUs that theoretically have low innervation ratios are 'hidden' by the important electrical activity of the active high-threshold MNs, which are therefore over-represented in the experimental datasets. Because the experimental datasets could only provide fair to unacceptable estimates of the neural drive to muscle, depending on the number, quality, and type of the identified spike trains, this empirical neural data obtained from HDEMGs cannot be directly used as input to neuromuscular models. Although this limitation can, to some extent, be empirically addressed, and more low-threshold MNs can be identified by increasing the density or the number of electrodes covering the muscle (Chapter 3) at level of contractions up to 30% MVC, only high-threshold MUs are identified at 50% MVC. It is believed that additional low-threshold MNs could be detected by recording longer trapezoidal tasks and decompose the HDEMG signals with multiple decomposition windows across the plateau, every time recalculating the MU filters and reevaluating the signals, although it would further lengthen the processing time. Another solution would be combining surface HDEMGs with intramuscular high-density electrode arrays (Muceli et al.,

2022). However, because of the novelty and the high cost of the equipment, and the challenging and uncomfortable procedures involved with intramuscular insertion, such approach is not yet conceivable for large subject populations and remains exceptional. A computational approach was presented in Chapter 5 to infer the activity of the missing low-threshold MNs. While mostly successful, this method remained sensitive to the type of MNs identified experimentally. When significant portions of the MN pool were empirically overlooked, such as in the 50% MVC trials, the method was unable to reconstruct a realistic distribution of the MN properties across the MN pool, in which case the discharge behaviour inferred for the overlooked MNs was non-physiological. To address this limitation, a new version of the method may be released, where physiological constraints will be enforced to the *S* and *IP* trendlines.

Third, only isometric contractions were considered in this thesis because of experimental constraints. In the past four years, new computational approaches were developed to decompose HDEMG signals recorded during quasi-static muscle contractions (Glaser & Holobar, 2018; Oliveira & Negro, 2021). Yet, during dynamics muscle contractions, the muscle geometry changes and the surface electrodes, which remain static on the skin, cannot track the electrical activity of the moving muscle fibres, which affects the spatial sampling of the MU APs. Consequently, typically less MNs are identified in dynamics tasks than during isometric contractions, with on average 7-20 identified MNs, depending on the investigated muscle. As suggested by the sensitivity analysis in Chapter 3, these figures remain too low to reliably control neuromuscular models. Moreover, most of the contractions performed in these studies remained quasi-static in these dynamic studies, with joint velocities of 3-to-20°/sec. Until machine learning methods overcome these limitations, neuromuscular models driven by decomposed HDEMG data will remain constrained to isometric or low-velocity contractions, preventing daily life applications such as locomotion or running.

Model validation

As thoroughly discussed in Chapter 6, although several gold standards were followed, the validation of the neuromuscular computational models developed in this thesis was somewhat limited or incomplete. The main reasons why are briefly repeated here. First, some of the muscle's dynamics, like the transients of free calcium and Ca-Tn concentrations, could not be validated because of the lack of available experimental human data in the literature for these quantities. Second, because of the aforementioned experimental limitations, the model was not tested in dynamic conditions. Nevertheless, the model's behaviour will be assessed with isometric force trajectories different from trapezoidal contractions. Third, the neuromuscular model was not yet extrapolated to other muscles. As seen in Chapter 5 with the Gastrocnemius Medialis muscle, accounting for the muscle-specific neuromechanics is crucial. To allow the application of the neuromuscular model to other muscles for

which subject-specific MSK quantities and/or literature neuromechanical data would be missing, calibration procedures will be proposed to tune some of the key model parameters to the muscle's specificities. Yet, such calibration procedures will inherently face the load distribution problem, which is a classic limitation in current EMG-driven approaches. Fourth, the performance of the MN-driven multi-MU model will be compared to the predictions made by the well-established CEINMS single-actuator neuromuscular model (Pizzolato et al., 2015). Finally, the experimental TA force, inferred in Chapter 3 from the measured transducer force and against which the model predictions are validated, may be fairly inaccurate because of the unknown contribution to the recorded ankle torque of the other muscles spanning the ankle joint. In chapter 3, the EDL and EHL co-contributions were inferred from their subject-specific maximum isometric forces and moment arms in three scenarios of minimal, medium, and maximal activity. Beside affecting the force validation, the conclusions taken in Chapters 3 and 5 about the estimation of the neural drive to muscle, which was compared to the normalized transducer force, should be interpreted in the light of this limitation. To address this issue, the EDL and EHL myoelectric activity during submaximal contractions could have been inferred, to some extent, through bEMG recordings, notwithstanding the crosstalk issues with the dominant and adjacent TA muscle. To allow a one-to-one validation and bypass the activity of agonistic muscles, the TA force could also be inferred from an estimation of the tendon stiffness from strength protocols and ultrasound recordings of the fascicle length, simultaneously obtained with HDEMG signals during isometric contractions as recently performed (Gerus et al., 2015; Dick et al., 2016, 2017; Zhang et al., 2020; Waasdorp et al., 2021; Zhang et al., 2021; Zhang et al., 2022).

Neuromusculotendon modelling

The computational methods in Chapters 5 and 6 will be refined following strategies thoroughly discussed in those chapters and briefly reported here. The four-step workflow in Chapter 5 was specifically developed for trapezoidal isometric contractions. Although the calibrated pools of MN models proved to accurately blindly predict the neural drive to muscle for other trapezoidal contractions at same MVC, it is unknown whether they could extrapolate to other force trajectories. Additional modelling complexity may be required to accurately capture the time-dependent variations of MN maximum firing frequencies. In this respect, active-conductance LIF models will be further investigated, so that the amplitude of the MN firing frequencies is determined by the realistic MN membrane dynamics rather than the phenomenological IP parameter fitted to the data.

Furthermore, the neuromusculoskeletal model proposed in Chapter 6 will be refined in four main ways. First, the computational methods will be developed to solve the muscle-tendon dynamics in a scenario where the tendon is compliant. In this more realistic approach, the dynamics of the tendon and the PEE, the MU FV relationships, and the length-dependencies of the MU activation dynamics will

play a so far neglected role in the whole muscle force generation. Second, by using the volumetric muscle representation to lift the identified indeterminacy, the MUs will be assigned specific lengths and optimal lengths, thus diverging from the current same-length simplification. Third, computational cost will be reduced by considering smaller MU pools and simplifying the current model of excitation dynamics. Finally, the proposed phenomenological models of the MU activation dynamics will be further refined to be more representative of the MUs' physiological mechanisms, allowing a continuous distribution of the MU properties in the MU pool, rather than the non-physiological discrete partition of the pool between slow and fast MUs currently used in the model.

Once some of these limitations are addressed, the workflow developed in this thesis will find crucial applications in investigating the neuromechanics of human voluntary neuromuscular control, with important applications in human-machine interfacing.

PUBLICATIONS

Published journal articles

- (1) Caillet, A. H., Phillips, A. T., Farina, D., & Modenese, L. (2022). Mathematical relationships between spinal motoneuron properties. *eLife*, 11, e76489, doi: <https://doi.org/10.7554/eLife.76489>
- (2) Caillet, A. H., Phillips, A. T., Farina, D., & Modenese, L. (2022). Estimation of the firing behaviour of a complete motoneuron pool by combining electromyography signal decomposition and realistic motoneuron modelling. *PLOS Computational Biology*, 18(9), e1010556, doi: <https://doi.org/10.1371/journal.pcbi.1010556>

Preprints

Caillet, A. H., Phillips, A. T., Carty, C., Farina, D., & Modenese, L. (2022). Hill-type computational models of muscle-tendon actuators: a systematic review. *bioRxiv*,
doi: <https://doi.org/10.1101/2022.10.14.512218>

Conference podium presentations

- (1) **Caillet, A. H.**, Phillips, A. T., Del Vecchio A., Farina, D., & Modenese, L. (2021). Development of a high-density EMG-driven Hill-type muscle model, *XXVII Congress of the International Society of Biomechanics* - **David Winter Young Investigator Award (podium)**
- (2) Modenese, L., **Caillet, A. H.**, Phillips, A. T., Farina, D., & (2022). A novel neuromechanical model for predicting muscle force from motoneuron spike trains. *27th Congress of the European Society of Biomechanics*
- (3) **Caillet, A. H.**, Phillips, A. T., Farina, D., & Modenese, L. (2022). Prediction of the firing behaviour of the motoneuron population for motoneuron-driven muscle modelling. *9th World Congress of Biomechanics*

Appendix **A**

SUPPLEMENTARY MATERIAL FOR THE SYSTEMATIC REVIEW IN CHAPTER 2

A.1. Genesis of Hill-type modelling

The definition of a Hill-type muscle model has emerged from preliminary studies published in the first half of the 20th century. While skeletal muscles were described as rheological arrangements of purely elastic and viscoelastic elements in the late 19th century, Hill proposed in 1922 (Hill, 1922) a new metabolic energy-based force generating element, later called contractile element (CE), that Levin et al. further connected in 1927 (Levin & Wyman, 1927) to an undamped series elastic element. Hill proposed in 1938 (Hill, 1938) in a milestone study the bases of Hill-type modelling with the derivation of Hill's Force-Velocity (FV) hyperbolic relationship, further developed (Katz, 1939), a definition of the active state and a description of the muscle with three elements including an active CE and passive series and parallel elements in a Maxwell rheological configuration (Maxwell, 1867). The Wilkie-Ritchie-Abbott-Jewell research group then extended Hill's work in 1950-1960 and derived most of the current gold standards in modelling methods. They validated and calibrated Hill's FV hyperbolic relationship for several muscles, species and fibre types (Wilkie, 1949; Jewell & Wilkie, 1958; Wells, 1965), compared the physiological accuracy of the Maxwell and Kelvin-Voigt rheological structures including a CE (Jewell & Wilkie, 1958), provided a first physiological definition of the active state (Wilkie, 1956), derived a Force-Length (FL) relationship linearly scaled by the active state (Wilkie, 1956; Ritchie & Wilkie, 1958), made Hill's hyperbolic curve active state and length nonlinearly dependent (Abbott & Wilkie, 1953; Wilkie, 1956), and inverted the FV relationship to unveil a first-order differential equation in muscle length relating variations in muscle length with time (Wilkie, 1956; Ritchie & Wilkie, 1958). After focusing on cardiac muscles in 1960-1970 (Fry et al., 1964; Sonnenblick, 1965; Brady, 1967), the Hill-type modelling approach was extended by the emerging sliding filament theory (Huxley & Niedergerke, 1954) and cross-bridge cycle mechanism (Huxley & Hanson, 1954). The current cross-bridge-based definition of the active state was proposed in 1968 (Ebashi & Endo, 1968), while Gordon et al. reconciled in 1966 (Gordon et al., 1966) the phenomenological FL relationship to the degree of sarcomere filament overlap, leading to the first sarcomere-to-muscle multiscale approach. Hill-based models gradually became in 1960-1970 a class of muscle models with emerging terminology such as 'Hill's three-element / component model' (Buchthal & Rosenfalck, 1960; Bahler et al., 1967), and 'Hill-type model' (Grood et al., 1974) and the first computational Hill-type model relying on solving time-dependent differential equations of muscle lengths to predict muscle quantities (Bahler, 1968a). In 1970-1980 were proposed the first accurate mathematical descriptions of the FV relationship in eccentric contractions (Mashima et al., 1972; FitzHugh, 1977), while Hatze derived and validated in 1976 the force of the contractile element as obtained from the linear product of the active state and the instantaneous forces as obtained from the FL and FV relationships, and developed the first Hill-type muscle driven musculoskeletal simulation additionally using processed EMGs as inputs

(Hatze, Herbert, 1976). In 1990-2000, four milestone reviews assessed the state-of-the-art of Hill-type MT modelling to propose a robust theoretical scheme encouraging personalized Hill-type modelling (Zajac, 1989), a didactic introduction to Hill-type modelling (Yamaguchi, 2001), and to summarize the field to provide consensus in terminology and definitions relating theory and physiology (Epstein, 1998), and compare the numerous modelling approaches (Winters, 1990) that had increasingly emerged since the 1980s.

A.2. Methods – PRISMA checklist

This checklist was taken from (Moher et al., 2009).

PRISMA item n°	Respected [Y/N]	Comments if relevant
1	Y	
2	Y	
3	Y	
4	Y	Not completely applicable as defined as not a clinical systematic review, though 3 distinct objectives for the systematic review are defined
5	Y	This is the first systematic review on Hill-type modelling though systematic reviews on muscle modelling-related fields are referred to in the text. No review protocols exist.
6	Y	Eligibility criteria: Journal article in English for the 1938-2021 period, specified in the Methods section
7	Y	
8	Y	Most details are provided in Appendix A.3. along with the key term search string for the PubMed database
9	Y	
10	N	Not applicable as not a clinical systematic review relying on data
11	Y	23 properties and 10 questions around methodological practices are defined for which data is sought
12	Y	The study inclusion and the scoring were performed by two independent reviewers. A consensus was reached after discussion in case of different conclusions
13	Y	The review is mainly focused on trends, for which simple ratio measurements are suitable objective criteria

14	N	Not applicable
15	Y	This is discussed in the limitations of the study in the Discussion section. The chosen eligibility criteria 4 to 6 and the choice of scoring schemes bring mild bias to the conclusions
16	N	Not applicable
17	Y	The tree diagram of study selection with step-by-step details is provided in Appendix A.5
18	N	Not applicable
19	N	Not applicable
20	Y/N	The resulting marks for completeness and modelling assessments and global scores were provided for each study
21	N	Not applicable
22	N	Not applicable
23	Y	Trends in Hill-type modelling approaches, their time-evolution and trends in methodological practices are unveiled
24	Y	
25	Y	
26	Y	
27	Y	

A.3. Methods – Key terms for the systematic search

The keyterms are grouped into 4 concepts. Within each concept, the keyterms are joined with the OR Boolean operator. The systematic search is performed with the string Concept 1 AND Concept 2 AND Concept 3 NOT Concept 4.

PubMed was performed with the following string:

((hill[tiab] or musculotendon[tiab] or myocybernetic[tiab])) **AND**

(muscl*[tiab] or muscu*[tiab] or tendon[tiab] or myocybernetic[tiab])) **AND**

(model*[tiab] or actuat*[tiab] or system*[tiab] or "contractile element"[tiab] or complex*[tiab] or unit*[tiab] or simulat*[tiab] or dynamics*[tiab] or optimization[tiab] or "EMG-driven"[tiab] or "Muscle-driven"[tiab] or control*[tiab] or predict*[tiab] or estimat*[tiab])) **NOT**

(smooth[tiab] or cardiac*[tiab] or myocard*[tiab] or papillary[tiab] or heart*[tiab] or myocyt*[tiab] or virus*[tiab])

Filter: English, Journal article

And updates were enabled with a weekly frequency to update the review with the most recent published studies.

Appendix Table A-1: Keyterms used for the systematic search, grouped into concepts and free-text and mesh terms

	Concept 1: Hill-type musculotendon models	Concept 2: the skeletal muscle and the tendon	Concept 3: model – actuator - simulation	Concept 4: excluding cardiac and smooth muscles
Free- text terms	Hill	Muscl*, muscu*	Model*	Cardiac* Myocard* Papillary Heart* Myocyt*
	Musculotendon	Tendon	Actuat*, System*, Contractile element*, complex*, unit*	Smooth
	Myocybernetic	myocybernetic	Simulat* Dynamics	

			Optimization EMG-driven, Muscle-driven Control Predict*, estimate*	
Mesh Terms		Muscle, Skeletal / physiology		

A.4. Methods - Details of the scoring scheme of the Modelling Assessment

In order to yield an objective scoring and a robust comparison between studies for the two reviewers, a detailed scoring scheme was developed. Each paper would score 0, 1, or 2 to the 10 questions following precise criteria. These criteria were derived before the systematic search according to a preview of the literature of a hundred Hill-type studies chosen randomly in the literature.

As detailed in the main text of the study:

- (I): Model validation
- (II): Model reusability
- (III): Modelling choices and strategy
- (IV): Model calibration.

The 10 questions are provided in the core text of the review.

Appendix Table A-2: Details on the scoring scheme of the Modelling Assessment

Benchmarks	Question number	Scoring	Scoring scheme
(I)	1	0	• No validation of the accuracy of the model, or • Comparison with user-defined target quantity, or • Comparison with results from other models, or • Comparison with inconsistent experimental results from the literature (different muscle, species, protocol of contraction)
		1	Predicted results are compared against experimental data from the literature, the experimental conditions of which are carefully reproduced (same species, same muscle, same stimulation, and length-varying protocol)
		2	Validation against ad hoc experimental data, or quantities derived from ad hoc experimental data
	2	0	Question 1 scored 0
		1	The calculated and experimental quantities which are compared for validation are: • Joint torques, or • Limb motion, or • Muscle activation profiles, or • Muscle energy consumption

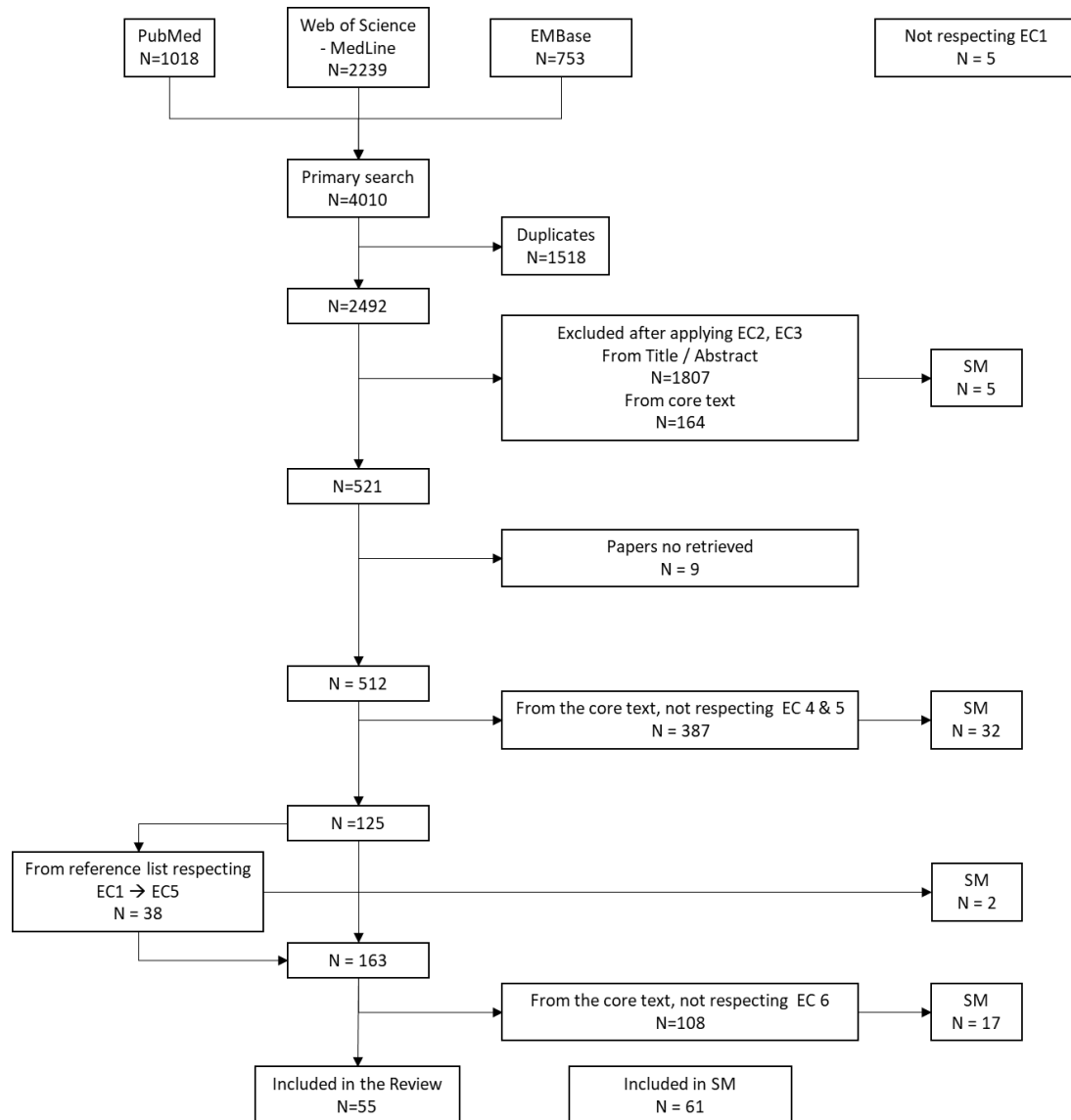
	3	2	Validation of the simulated force output against experimental force profiles (fibre, fibre bundles, muscle, or muscle-tendon complex)
		0	Qualitative evaluation of the results: no quantitative conclusions, or qualitative comparison of curves
		1	Use of basic quantitative objective criteria (percentages, ratios, ranges...)
		2	• Use of advanced quantitative objective criteria: RMS error, or • Use of statistical analysis (Pearson coefficient, ANOVA...)
(II)	4	0	The model is not fully re-implementable: • Missing mathematical expressions describing the material properties, or • No parameter values are provided explicitly or by references
		1	The model is almost fully re-implementable and requires personal effort to be reproduced: • All mathematical expressions describing the material properties are reproducible (explicitly provided or referenced), and • Most parameter values are provided • Yet, any of ○ Some mathematical equations describing the material properties are only provided through references, or ○ Mathematical relationships between material properties are not provided, or ○ Some parameter values are not provided, or ○ No indication on the implementation of the model is provided
		2	The model is easily and fully re-implementable • The implementation is provided in supplementary material, or • All mathematical expressions and parameter values defining the material properties are explicitly provided, and • The rheological structure and / or the mathematical relationships between material properties are provided, and • There is help provided in re-implementing the model and / or in reproducing the results of the study
	5	0	No
		1	Only remarks are done on the computational efficiency of Hill-type model in general
		2	Yes, the model includes at least one voluntary alteration that improves the model stability, computational speed, or avoids numerical stiffness, singularities or unnecessary model complexity
(III)	6	0	The taken simplifications are not explicitly stated.
		1	At least one simplification on the physiology or on the geometrical model is stated. Physiological simplifications:

			• Massless model • Frictionless / Incompressible / Isotropic material Geometrical simplifications: • Straight-line model, or Inclusion / exclusion of the pennation angle • All fibres are coplanar, parallel, of same length and straight
		2	Explicit multiscale approach, and justified by homogeneous material properties between contractile sub-scale elements
	7	0	Incomplete and unjustified modelling choices of the rheological model and its material properties
		1	• The arrangement of the rheological elements in the mechanical structure is explicitly stated • Each rheological element and its constitutive mathematical expression are explicitly described, and • Yet, no reason given for these modelling choices (inclusion / exclusion of a rheological element, or experimental reasons behind the mathematical expression), and no link to physiological mechanisms
		2	• The arrangement of the rheological elements in the mechanical structure is explicitly stated, and • Each rheological element and its constitutive mathematical expression are explicitly described, and • The modelling choices are justified with references, or considering the aim of the study, or • Elements and properties are critically commented and linked back to physiological mechanisms, or • There is no unnecessary unjustified overshooting complexity to the model
	8	0	No concluding remarks on the strengths and limitations of the model
		1	• There are concluding remarks on the strengths and limitations of the model, and • Hill-type modelling is not the main topic of the study, and / or • The analysis is only qualitative and not supported by references and findings from other studies
		2	• Quantitative analysis, and / or • Qualitative analysis supported by references and findings from other studies
(IV)	9	0	• Arbitrary parameter values, or • Parameter values imported from a previous Hill-type model, or • Parameter values taken from non-consistent literature (different species, muscle-tendon complex, or scale)
		1	Significant effort in obtaining some parameters from: • The literature for the same species, muscle-tendon complex and scale (experimental studies, or studies including a Hill-type model built from ad hoc experiments), and / or • A review of the literature
		2	At least one parameter obtained from: • The literature for the same species, muscle-tendon complex and scale, and scaled for the subject

			• A complete experimental database from a single study for the same species, muscle, and scale (providing input conditions, output force profiles, and experimentally derived parameters) • An ad hoc sensitivity study • Ad hoc muscle-specific experiments • Parameter calibration by minimizing an objective function
	10	0	No
		1	• Using at least one scaling parameter • Parameter obtained from the literature and is species-consistent
		2	• Using at least one scaling parameter • Parameter obtained from ○ A scaled or processed parameter from the literature ○ Parameter calibration ○ Ad hoc experiments

A.5. Results - Flowchart of paper selection

This flowchart is a detailed version of the one provided in the main text of the review. In Appendix Figure A-1 are detailed the steps taken to identify the eligible studies, according to the eligibility criteria defined in the main text of the study.



Appendix Figure A-1: Flowchart of the selection of the eligible papers after application of EC1 to EC6. N corresponds to the number of retained or discarded studies when and after the relevant tasks are performed. SM depicts the contributory studies that were retained from the search for a parallel assessment for completeness.

A.6. Results – Reasons why the eligible models meet EC6

Appendix A.6 details the reasons why the eligible studies fulfilled EC6, and their modelling contributions to the field of Hill-type modelling are gathered in Appendix Table A-3. It is recalled that these studies fulfilled EC6 if they were considered to be innovative, i.e., they proposed a modelling approach to describe at least one of the properties of the Hill-type model in a way that had not been proposed yet in any of the previous eligible studies. To limit the number of eligible studies was disregarded for fulfilment of EC6 any innovation in the shape of the rheological structure, the method for parameter calibration, optimization of computational runnability, improvements in MSK simulation-related topics and the mathematical description of the FL relationship and PEE.

Appendix Table A-3: Eligible studies and their modelling contribution to the field of Hill-type modelling

N°	Eligible study	Modelling contribution to the field
1	(Bahler, 1968a)	First computational Hill-type model First Hill study proposing the widely-used $f_l(\Delta l) = \exp\left(-\left(\frac{\Delta l}{W}\right)^2\right)$ gaussian mathematical expression for the FL relationship
2	(Hatze, 1977)	Detailed model of ED Detailed model of ADC and ADL, inspiring many subsequent studies First inclusion of an FVE, PDE and PEE2 in a computational Hill-type model First Hill study proposing the widely-used $0; k_1\left(e^{k_2 l^M} - 1\right)$ piecewise mathematical expression for the PEE and the SEE
3	(Hatze, 1978)	First phenomenological model of MURD
4	(Wyss & Pollak, 1984)	Modelling the ED as processed EMGs Models the AD as a twitch event

		FVL Using torque angle relationship
5	(Pierrynowski & Morrison, 1985)	Custom model of MURD Custom AD Inclusion of a SEE2
6	(Otten, 1987)	Modelling the ED as processed EMGs Custom ADC
7	(Bobbert & van Ingen Schenau, Gerrit Jan, 1990)	First eligible paper modelling the AD with a lump 1st-order ODE (inspired from (Clark & Stark, 1974; Winters & Stark, 1988) which are not eligible studies) First Hill study proposing the widely-used $0; k(l - l_s^T)^2$ piecewise mathematical expression for the SEE
8	(Giat et al., 1993)	First trigonometric form for a C2 FVC-FVE relationship Mass Fatigue
9	(van Soest & Bobbert, 1993)	Custom FVL, FVA C2 FVE-FVE junction

		First Hill study proposing the widely-used $1 + c - 2cl^M + cl^{M^2}$ mathematical expression for the FL relationship First Hill study proposing the widely-used $0; k(l - l_s^T)^2$ piecewise mathematical expression for the PEE
10	(Durfee & Palmer, 1994)	Custom AD
11	(Happee, 1994)	Custom model of ED
12	(Winters, 1995)	Important source in modelling the AD with an active state-dependent (de)activation rate FLA Custom FVL, FVA Activation-dependent PEE2
13	(Caldwell, 1995)	Custom steady-state AD
14	(Van Zandwijk et al., 1996)	Custom steady-state part of ADC
15	(Dorgan & O'Malley, 1997)	Custom model of MURD
16	(Nussbaum & Chaffin, 1998a)	Custom model of steady-state AD
17	(Shue & Crago, 1998)	Mass Simple model of MURD ADV Short-range stiffness
18	(Curtin et al., 1998)	ADC Double-hyperbolic FVC

19	(Brown et al., 1999)	ADL ADV Sag Yield
20	(Cheng et al., 2000)	Custom MURD Mass
21	(Ettema & Meijer, 2000)	FD RFE
22	(DeWoody et al., 2001)	Considers muscle mass but then eliminates it to reduce system dimension
23	(Gallucci & Challis, 2002)	First Hill study introducing the widely-used $1 - \frac{\Delta l^2}{W^2}$ mathematical expression for the FL relationship, this does not look sufficient
24	(Lloyd & Besier, 2003)	Custom model of ED involving EMG processing Custom AD FLA
25	(Thelen, 2003)	Custom FVA
26	(McLean et al., 2003)	Custom FVA
27	(Stelzer, Maximilian & Von Stryk, 2006)	Custom modelling of the PEE, for which there is a typo Wobbling masses (soft tissue mass dynamically shifting relative to the bone)
28	(Kistemaker et al., 2006)	Custom FVL, FVA
29	(Mavritsaki et al., 2007)	Custom ED, MURD, ADC, AD
30	(Günther et al., 2007)	Custom FVL, FVA

		First SDE among eligible studies
31	(Gömmel et al., 2007)	Custom FVL, FVA Mass
32	(Song et al., 2008)	Custom ED and MURD
33	(Menegaldo & Oliveira, 2009)	Custom FVC
34	(Moody et al., 2009)	Mass
35	(Rengifo et al., 2010)	Custom FV relationship
36	(Proctor & Holmes, 2010)	Comprehensive model of ED
37	(Tsianos et al., 2011)	Custom ED and MURD
38	(Mörl et al., 2012)	SDE
39	(Maceri et al., 2012)	Custom model of ED Multiscale tendon model
40	(Wang, J. et al., 2012)	Custom modelling of the PEE2 Mass
41	(Callahan et al., 2013)	Custom FVL, FVA MURD inspired from Fuglevand 1993
42	(Blümel et al., 2012)	Custom AD Custom FVA
43	(Millard et al., 2013)	First introduction of quintic Bezier splines
44	(John et al., 2013)	Custom FVA
45	(Lee et al., 2013)	Custom model of ED and MURD from EMG processing and analysis 2 CE-approach for fast and slow fibres
46	(Elias et al., 2014)	Custom steady-state AD

47	(Hamouda et al., 2016)	Custom MURD Tackles the limitations of the FL relationship (instability of descending limb)
48	(Dick et al., 2017)	Custom model of ED and MURD from EMG processing and analysis 2 CE-approach for fast and slow fibres First ultrasound approach for derivation of muscle-tendon lengths and stiffness
49	(Marcucci et al., 2017)	Custom AD FE approach for analysis of the validity of the multiscale approach
50	(Ross, Nigam et al., 2018)	SDE Mass
51	(Siebert et al., 2018)	Transversal PEE
52	(Kim et al., 2015; Kim & Kim, 2018)	Custom comprehensive ED ADC / ADL
53	(Rockenfeller, R. et al., 2020)	Custom ADL
54	(Warner et al., 2020)	Custom AD
55	(Hussein et al., 2022)	Custom ADC

A.7. Results – Non-eligible studies with significant contributions to the field

Appendix Table A-4, Appendix Table A-5, and Appendix Table A-6 list the non-eligible studies that were identified with the systematic search and were observed to provide important contributions to the field of Hill-type modelling while not meeting all the eligibility criteria EC1 to EC6. Appendix Table A-4 focuses on milestone Hill-type models and review studies that shaped the field. Appendix Table A-5 focuses on the studies that investigated the field of Hill-type modelling, including sensitivity studies, studies comparing the behaviour of various muscle and Hill-type models, or studies about the optimization of the numerical cost of Hill-type models. Appendix Table A-6 gathers all the non-eligible studies that provide a modelling contribution to the field: these studies usually did not meet EC4 but did meet EC6. The completeness assessment was performed for the non-eligible studies in Appendix Figure A-5. Overall, the eligible studies are more complete than this set of non-eligible studies. As concluded for the eligible studies, the AD, FL, FVC, FVE, PEE and SEE properties are relatively more frequently modelled than the remaining 17 other properties.

Appendix Table A-4: Milestone and review studies in Hill-type modelling, important sources of inspiration for later studies

Studies	Important features	Reason why not eligible
(Winters & Stark, 1985)	Important source in modelling the AD with a 1 st order ODE	Joint angle state variable (EC5) No explicit AD (EC4)
(Zajac, 1989)	Most cited study in Hill-type modelling	Does not include mathematical descriptions for the CE and passive element properties (EC4)
(Winters, 1990)	First narrative review on the properties of Hill-type models	Does not propose a final Hill-type model (EC3)
(Delp et al., 1990)	First Hill-type model implemented in a simulation platform (SIMM) Important source of standard parameter values for human subjects	PhD thesis, not a Journal article (EC1) No AD (EC4)
(Kaufman et al., 1991)	Review on the theoretical formulation of Hill-type models Work on the index of architecture	Does not propose a final Hill-type model (EC3)
(He et al., 1991)	Important source in modelling the AD with a 1 st order ODE	No explicit mathematical description of the CE properties (EC4). They are obtainable in He's PhD thesis which is not publicly available
(Schutte, Lisa & Margaret, 1993)	Important source of inspiration for subsequent models in the field Implemented in OpenSim	PhD thesis, not a Journal article (EC1)
(Van Leeuwen, 1992)	Narrative review	Book chapter, not a journal article (EC1)

(Epstein, 1998)	Narrative review on theoretical models of Skeletal muscle with a focus on Hill-type models	Book chapter, not a journal article (EC1)
(Yamaguchi, 2001)	Narrative review on modelling muscle and tendon with a Hill-type approach	Book chapter, not a journal article (EC1)
(Günther et al., 2018)	Review / modelling approach on linking microscopic and macroscopic scales including a Hill-type modelling approach	Does not build a final Hill-type model (EC3)
(Schmitt et al., 2019)	Narrative review on the Hill-type modelling of the dynamics of skeletal muscles	Does not build a final Hill-type model (EC3)

Appendix Table A-5: Studies investigating the Hill-type modelling approach

Studies	Important features	Reason why not eligible
(Audu & Davy, 1985)	Milestone study about the influence of Hill-type modelling complexity on predicted quantities	No modelling contribution (EC6), mainly inspired from (Hatze, 1978)
(Brown, I. E. et al., 1996)	Comparison between modelling approaches for FL, FV, PEE, SEE and PEE2 and final model validation	Does not include AD (EC4)
(Perreault et al., 2003)	Assesses the link between Hill model prediction accuracy and MU firing rate level	No explicit description of the Hill-type model (EC4)
(Scovil & Ronsky, 2006)	Sensitivity study	No modelling contribution (EC6)
(Albracht & Arampatzis, 2006)	Sensitivity study	No modelling contribution (EC6)
(Redl et al., 2007)	Sensitivity study	Does not include AD (EC4)
(Siebert et al., 2008)	Comparison between Maxwell and Voigt approaches	No modelling contribution (EC6)
(Xiao & Higginson, 2010)	Sensitivity study	No explicit description of the Hill-type model (EC4)
(Blümel et al., 2012)	Sensitivity study	Another study from the same year used as eligible model
(Hasson & Caldwell, 2011)	Sensitivity study	No modelling contribution (EC6)
(Romero & Alonso, 2016)	Comparison of three models (van Soest & Bobbert, 1993; Thelen, 2003; Silva & Ambrosio, 2003)	No modelling contribution (EC6)

(Lemaire et al., 2016)	Comparison of the validity of Hill and Huxley-type muscle tendon models	No modelling contribution (EC6)
(Carbone et al., 2016)	Sensitivity study	No explicit description of the Hill-type model (EC4)
(Bayer et al., 2017)	Sensitivity study	No modelling contribution (EC6)
(Hamburger, 2017)	Comparison between Hill- and Huxley-type models	MSc dissertation
(Bujalski et al., 2018)	Sensitivity study	No explicit description of the Hill-type model (EC4)
(Sun et al., 2018)	Assessing the impact of FLA on model predictions	No modelling contribution (EC6)
(Harischandraid et al., 2019)	Comparison of 5 modelling approaches of the ADs	Does not build a full Hill-type model (EC3)

Appendix Table A-6: Non-eligible Hill studies but presenting a modelling contribution to the field

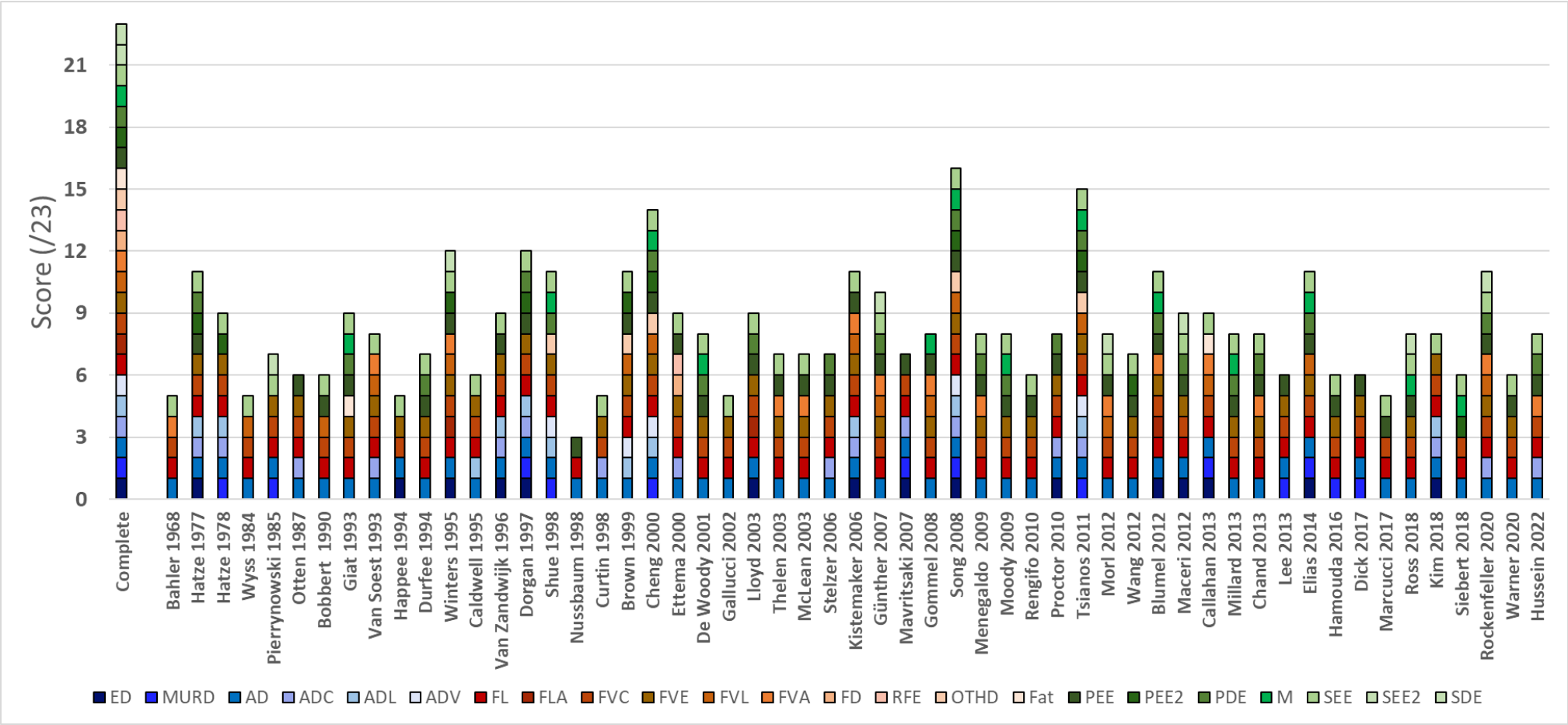
Studies	Important features	Reason why not eligible
(Clark & Stark, 1974)	First Hill study proposing a 1 st order ODE expression for the AD	Joint angle state variable (EC5)
(Hof & Van den Berg, 1981)	First Hill-type model reported to use processed EMGs as neural inputs	Joint angle state variable (EC5)
(Woittiez et al., 1984)	Index of architecture and 3D volumetric representation of a muscle Hill-type model	No explicit AD (EC4)
(Winters & Stark, 1988)	Proposes a fibre type-dependent expression for the FVC relationship	No explicit mathematical description of the CE properties (EC4)
(van Ingen Schenau, G J et al., 1988)	First Hill-type study modelling the in-series viscoelastic properties with an SDE	Does not include AD (EC4)
(Van Ruijven & Weijs, 1990)	Interesting approach with EMGs	No modelling contribution (EC6)
(Legreneur et al., 1996)	Connects a phenomenological model of MURD and ED to a Hill-type model	No explicit mathematical description of the controller (EC4)
(Wexler et al., 1997)	Custom model of AD and ADC Includes an SDE	Does not include a CE with CE properties (EC4)
(Meijer et al., 1998)	Includes an SDE First reported Hill model including Force Depression	Does not include AD (EC4)

(Forcinito et al., 1998)	First reported 'Hill model' including Residual Force Enhancement Includes Force Depression	Does not include AD or CE properties (EC4)
(Riener et al., 1996; Riener & Fuhr, 1998)	Fatigue First Hill-time model involving splines MURD from FES	
(Rosen et al., 1999)	First reported Hill-type model connected to a model of neural network FVA	Does not include AD (EC4)
(Wang, L. & Buchanan, 2002)	Development of the neural network – Hill-type model approach	No explicit mathematical description of the CE properties (EC4)
(Till et al., 2008)	Models RFE	Does not include AD (EC4)
(Rode et al., 2009)	Models FD and RFE	Does not include AD (EC4)
(Geyer & Herr, 2010)	Open-source implementation	No modelling contribution (EC6)
(Van Den Bogert et al., 2011)	Implicit formulation of musculoskeletal dynamics leading to new numerical methods and optimal control Formulation of a new lump state variable	No modelling contribution (EC6)
(Kosterina et al., 2012)	Phenomenological models of FD and RFE	No explicit mathematical description of the Hill model (EC4)
(Gerus et al., 2012)	Subject-specific SEE relationship obtained from in vivo ultrasound measurements – first reported study to connect this with a Hill-type model	No explicit mathematical description of the CE properties (EC4)
(Wakeling et al., 2012)	Derivation of a Hill rheological structure with 2 CEs to account for fibre type recruitment pattern	No complete description of the model (EC4)
(Nowshiravan Rahatabad et al., 2012)	Use of a fuzzy-genetic Hill-type model	Does not include AD (EC4)
(McGowan, Craig P. et al., 2009; McGowan, C. P. et al., 2012)	Models FD and RFE	Does not include AD, and does not provide explicit description of the remaining properties (EC4)
(Haeufle et al., 2012)	Open-source implementation Advanced model of the SDE Explicit FVE relation for the eligible model from Günther et al. (2007)	No explicit mathematical description of the AD (EC4)

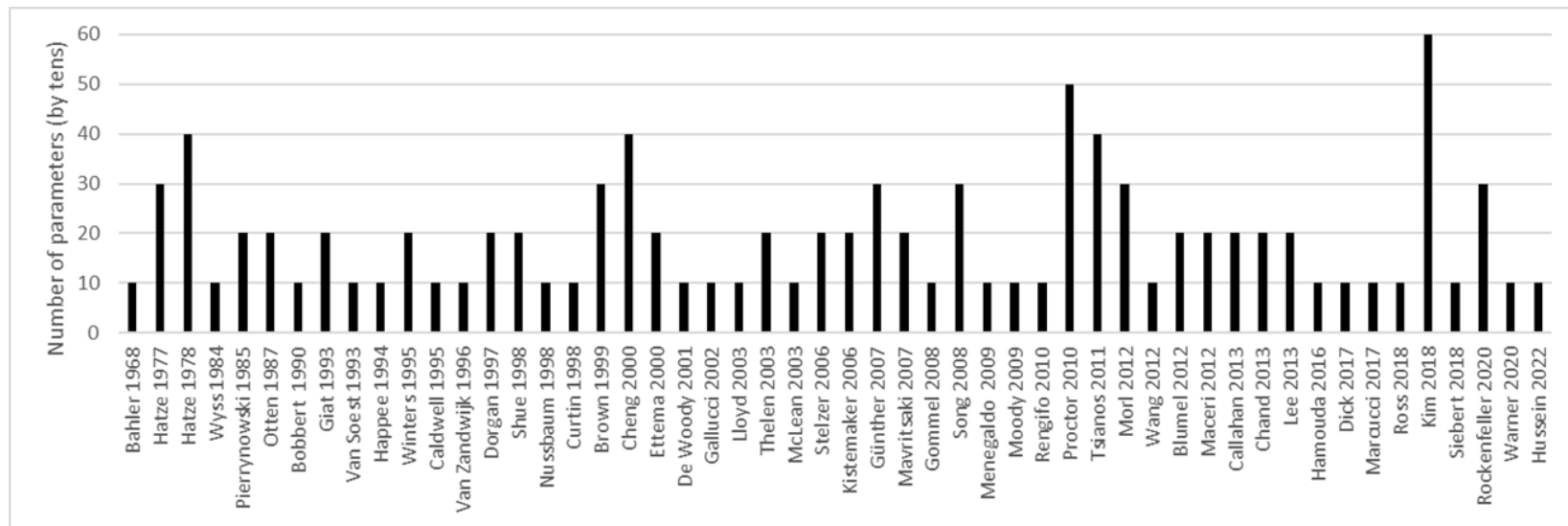
(Smith, S. L. & Hunter, 2013; Smith, S. L. & Hunter, 2014)	Proposes an Ogden material PEE FVL, FVA	Does not include AD (EC4)
(Siebert et al., 2014)	Introductory work to (Siebert et al., 2018) on the inclusion of transversal loads in Hill-type models	Improved in (Siebert et al., 2018) by the same author (EC6)
(Ovesy et al., 2016)	Proposes an equivalent linear damping characterization in linear and nonlinear force-stiffness models	No modelling contribution (EC6)
(De Groote et al., 2017)	Considers short-range stiffness	No explicit mathematical description of the Hill model (EC4)
(Sartori et al., 2017)	First Hill-type model receiving processed HDEMGs as neural inputs	No modelling contribution (EC6)
(Lai et al., 2018)	Mass Investigation of the improved accuracy brought by 2 CEs for slow and fast fibres	No explicit mathematical description of the Hill model (EC4) No modelling contribution (EC6)
(Penasso & Thaller, 2018)	Proposes a new modelling approach in making model parameters fatigue-dependent	No modelling contribution (EC6)
(Heinen et al., 2019)	Fibre type-dependent FV relationship	Does not include AD (EC4)
(Guo et al., 2020)	Introduction to mass-flowing Hill-type muscle models	No modelling contribution (EC6)

A.8. Results – Detailed results for the completeness assessment

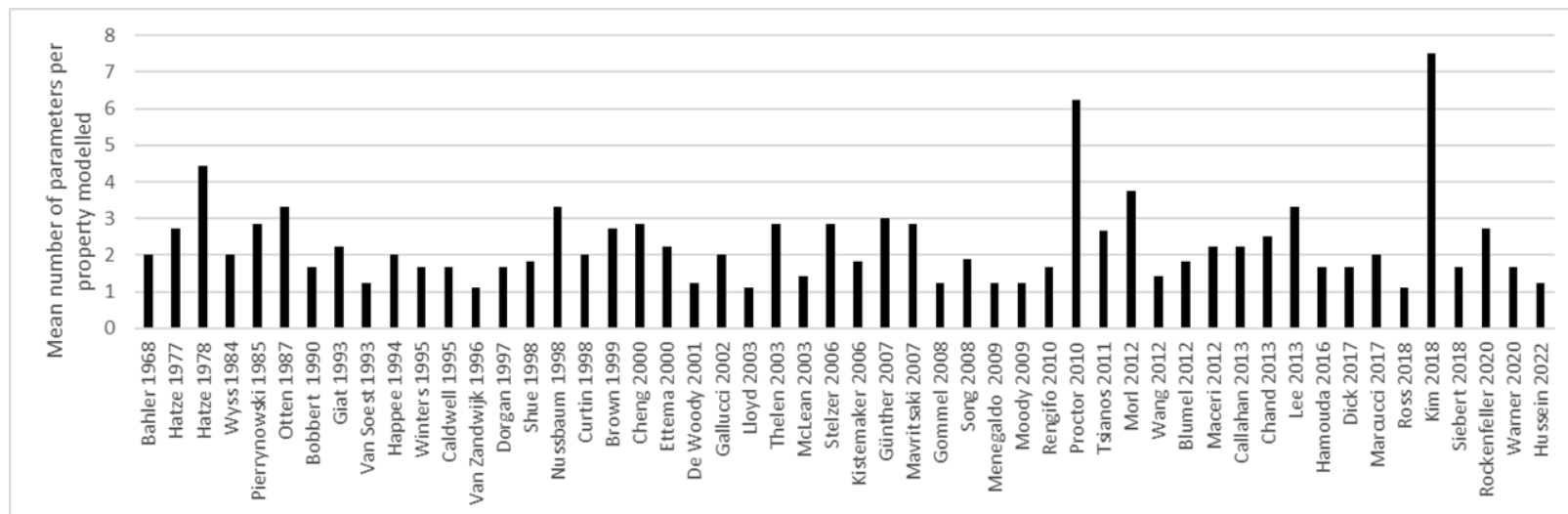
The results of the binary scoring of the Completeness assessment are provided in the bar graph Appendix Figure A-2. A first bar is provided for comparison, representing the scoring of a complete Hill-type model. All three types of properties (excitation-contraction, CE and passive element properties) are represented with shades of blue, red and green respectively. The graph in Appendix Figure A-2 can be used for inter-study comparison and to quickly assess the properties modelled in a specific study. Figures 3 and 4 detail the absolute number and relative number per modelled property of parameters necessary to scale the Hill-type model of each study.



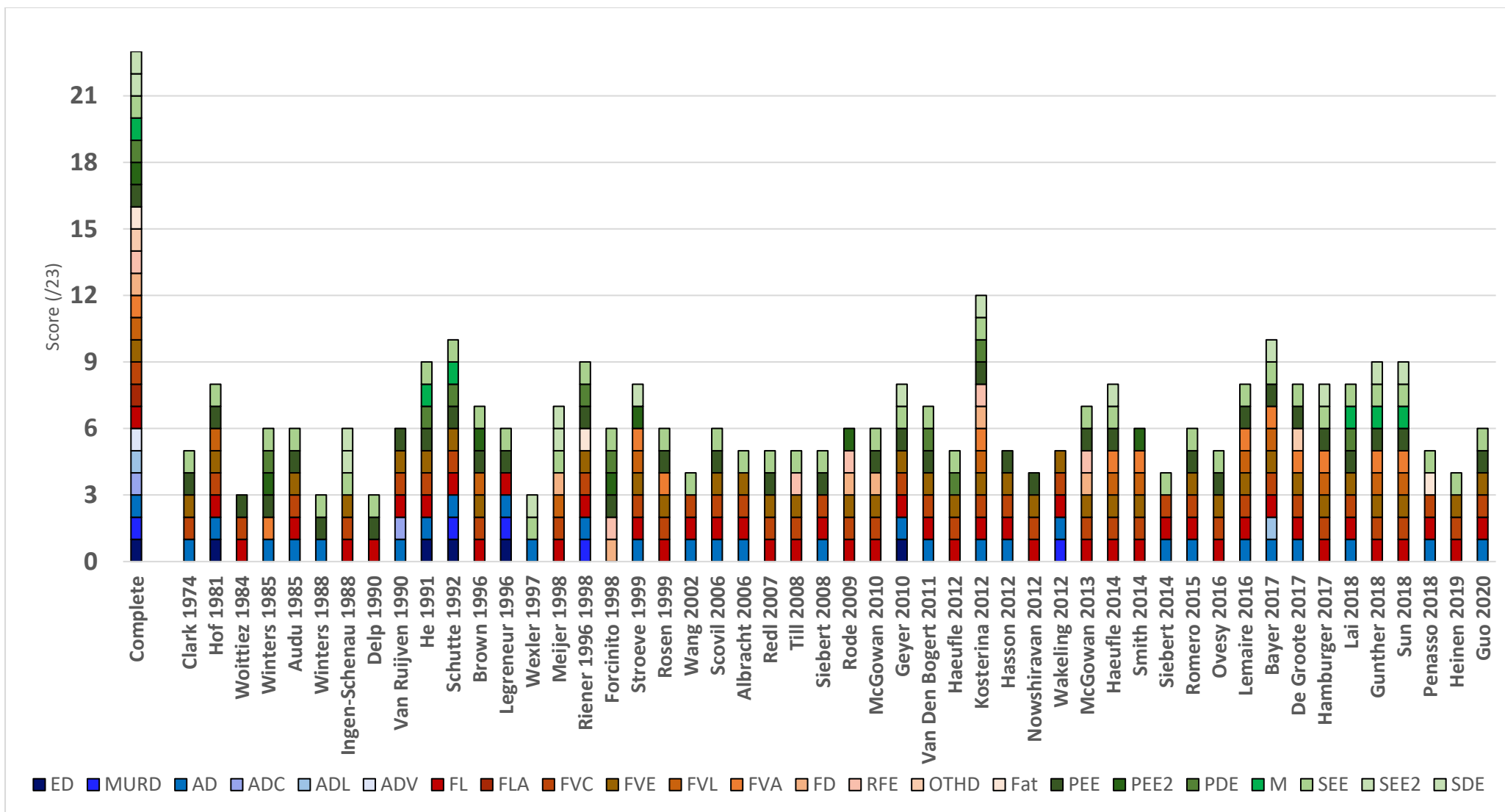
Appendix Figure A-2: Bar graph of the completeness of the eligible studies. Studies are reported in a chronological order. Shades of blue: NE, of red: CE, of green: passive elements.



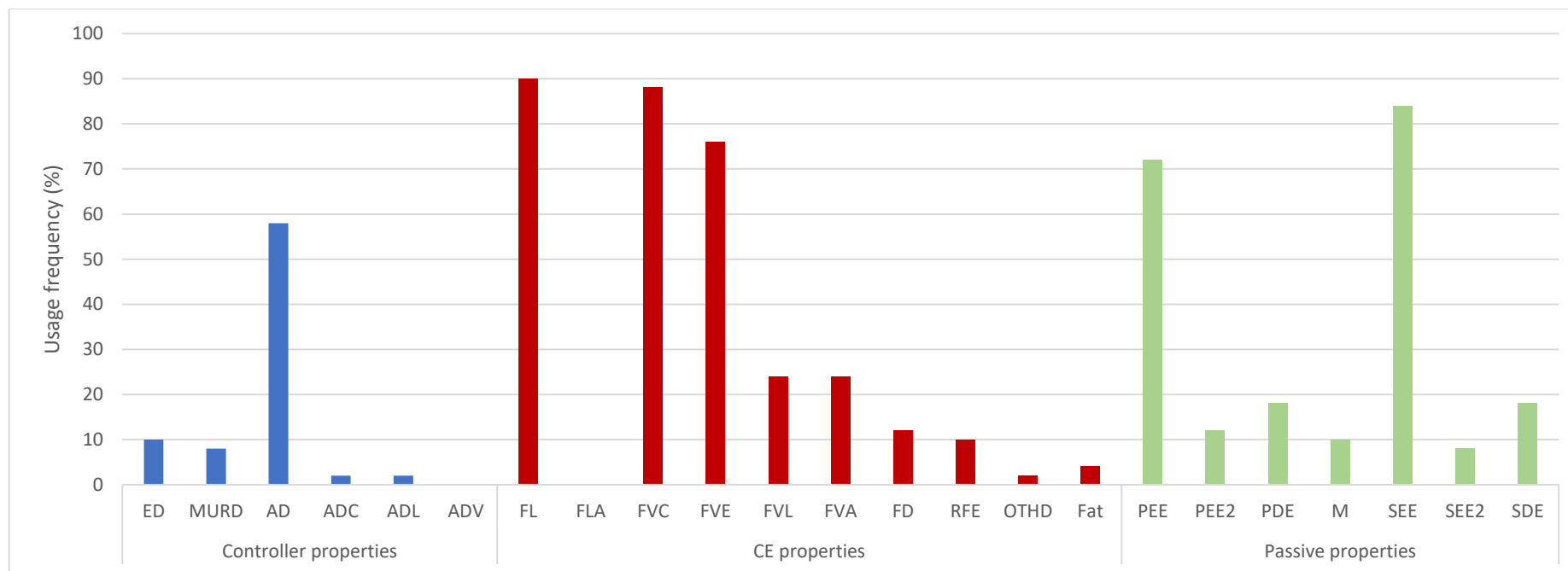
Appendix Figure A-3: Order of magnitude of the number of parameters used to define the Hill-type model in each eligible study. A paper including N parameters as read in the graph will include between N-4 and N+4 parameters. Are considered the parameters defining the properties of the model, and the three scaling parameters counted once even in case of a multi-muscle MSK model



Appendix Figure A-4: Average number of parameters used to describe one property of the model.

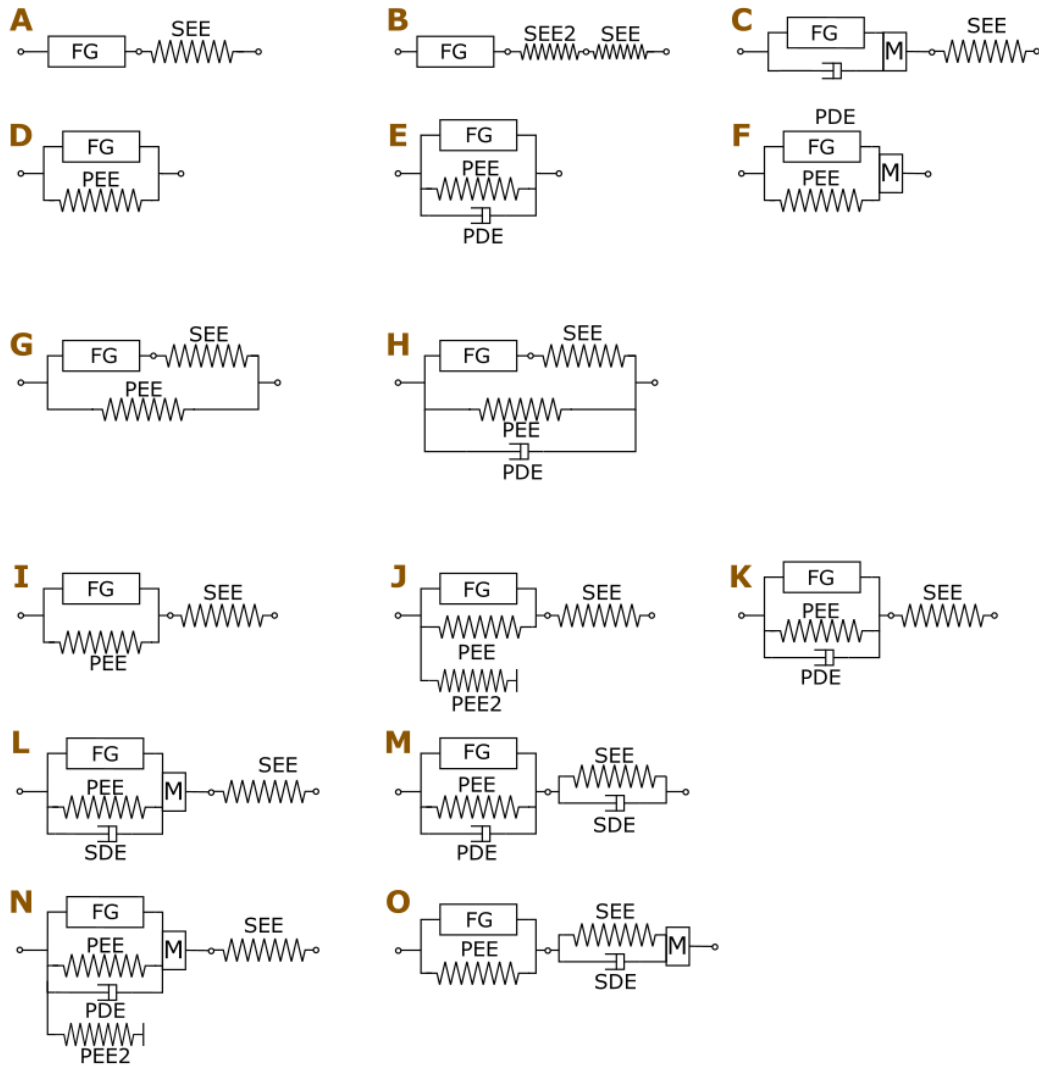


Appendix Figure A-5: Bar graph of the completeness of non-eligible contributory Hill-type models



Appendix Figure A-6: Frequency of property modelling among the non-eligible studies in Appendix Table A-6. As for the eligible studies, the five standard properties (AD, FL, FV, PEE, SEE) are the most widely modelled in the studies.

A.9. Results - Rheological structures proposed in the eligible studies



Appendix Figure A-7: Rheological structures proposed in the eligible studies. (A) (Wyss & Pollak, 1984; van Soest & Bobbert, 1993; Happee, 1994; Caldwell, 1995; Curtin et al., 1998; Gallucci & Challis, 2002; Callahan et al., 2013; Kim et al., 2015; Kim & Kim, 2018), (B) (Pierrynowski & Morrison, 1985), (C) (Shue & Crago, 1998), (D) (Otten, 1987; Van Ruijven & Weijs, 1990; Nussbaum & Chaffin, 1998a; Mavritsaki et al., 2007; Lee et al., 2013; Dick et al., 2017), (E) (Stelzer, Maximilian & Von Stryk, 2006; Proctor & Holmes, 2010), (F) (Gömmel et al., 2007), Maxwell-Hill-type structures (G) (Ettema & Meijer, 2000; Hamouda et al., 2016; Marcucci et al., 2017) and (H) (Hatze, 1977; Hatze, 1978; Durfee & Palmer, 1994; Dorgan & O'Malley, 1997), Kelvin-Voigt-Hill-type structures (I) (Bahler, 1968a; Bobbert et al., 1986; Winters, 1995; Legreneur et al., 1996; Van Zandwijk et al., 1996; McLean et al., 2003; Thelen, 2003; Kistemaker et al., 2006; Rengifo et al., 2010; Mörl et al., 2012; De Groote et al., 2017; Warner et al., 2020), (J) (Brown et al., 1999; Wang, J. et al., 2012), (K) (Riener et al., 1996; Riener & Fuhr, 1998; Lloyd & Besier, 2003; Menegaldo & Oliveira, 2009; John et al., 2013; Millard et al., 2013; Lai et al., 2018), (L) (Giat et al., 1993; DeWoody et al., 2001; Moody et al., 2009; Blümel et al., 2012; Elias et al., 2014), (M) (Günther et al., 2007; Kosterina et al., 2012; Mörl et al., 2012; Maceri et al., 2012; Rockenfeller, R. et al., 2020), (N) (Cheng et al., 2000; Song et al., 2008; Tsianos et al., 2011), (O) (Ross, Nigam et al., 2018). For clarity, pennation angles are not represented

A.10. Results - Inheritance diagrams and mathematical descriptions of the properties of the eligible models

In this section, inheritance diagrams are proposed for the AD, FV and FL relationships and the properties of the PEE and the SEE. Only the eligible models are considered in this section. These diagrams are useful for tracking the modelling approaches taken in a study back to their source study. In the diagrams, the red colour is used for the source studies. The orange colour is used for studies that take inspiration from the source studies but slightly adapted the mathematical equations. White is for the studies that used the modelling approach described in the source study. For each property, when a main trend is observable, the corresponding mathematical expressions are reproduced. In the following, the excitation, calcium concentration and active states are referred to as $u(t)$, $\gamma(t)$ and $a(t)$. All other parameters are constants consistent with the notations of the corresponding studies.

A.10.1. Activation dynamics

A.10.1.1. Calcium-dependent activation dynamics - ADC

In the following, the equations for calcium transient depict the time evolution of the representative calcium concentration in the sarcoplasm of the muscle fibres. The steady-state transfer function embodies the sigmoid steady-state experimental relationship F-pCa between isometric tetanic force and calcium concentration in the muscle fibre. The transient attachment dynamics represent the transient phenomenon of cross-bridge attachment.

Hill-type studies in the literature dominantly modelled the ADC after two studies.

(Hatze, 1977) - Model 2:

- Calcium transient:

$$\frac{d\gamma}{dt} = m(cv - \gamma) \quad (1)$$

- Steady-state transfer function:

$$a = \frac{a_0 + [\rho_n(l^M)\gamma]^n}{1 + [\rho_n(l^M)\gamma]^n} \quad (2)$$

$$\rho_n(l^M) = k_n \frac{k - 1}{\frac{k}{l^M} - 1}$$

(Otten, 1987):

- Calcium transient:

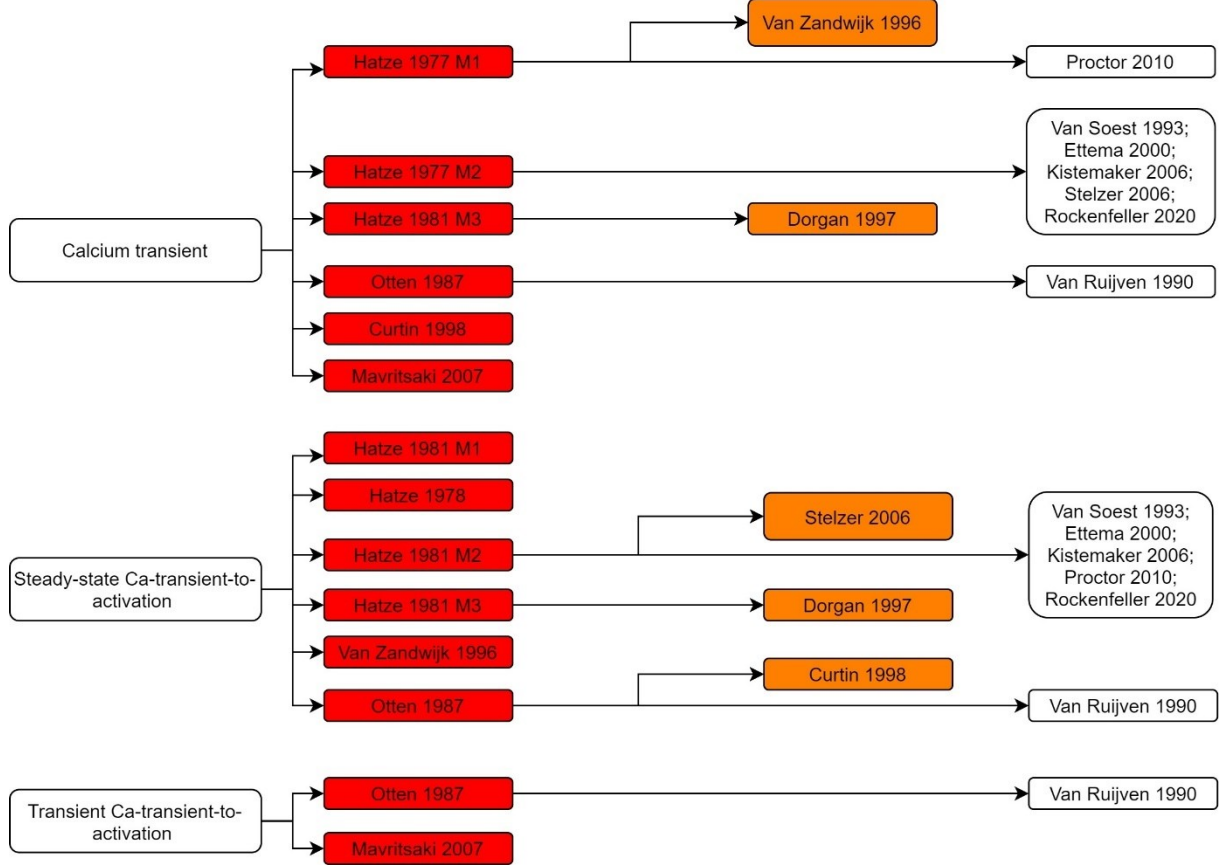
$$\frac{d\gamma}{dt} = c(\gamma_{max}u - \gamma) \quad (3)$$

- Steady-state transfer function:

$$a_{ss} = \frac{\gamma^{2.6}}{\gamma_{0.5}^{2.6} + \gamma^{2.6}} \quad (4)$$

- Transient attachment dynamics:

$$\frac{da}{dt} = c_2 \left(1 + \frac{a - a_{ss}}{2}\right) (a_{ss} - a) \quad (5)$$

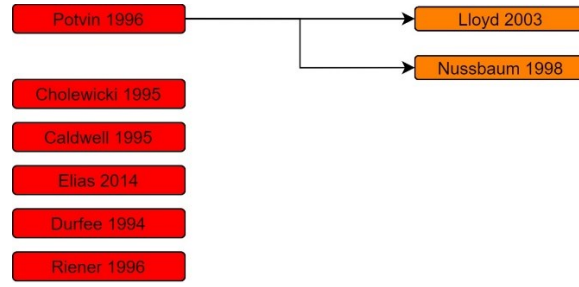


Appendix Figure A-8: Inheritance tree diagram for the Calcium-dependent models of Activation Dynamics among the eligible studies

A.10.1.2. Activation Dynamics as Steady-state transfer functions

In case of EMG-driven simulation, the most common trend is to input the excitation state obtained from processed and filtered EMGs to a steady-state experimental transfer function accounting for the linear or non-linear EMG-to-force relationship. The most observed steady-state transfer function in the field is from (Lloyd & Besier, 2003):

$$a(t) = \frac{e^{Au(t)} - 1}{e^A - 1} \quad (6)$$



Appendix Figure A-9: Inheritance tree diagram for the Activation Dynamics modelled as a steady-state transfer function among the eligible studies

A.10.1.3. Activation Dynamics as 1st-order ODEs

Most Hill-type studies have been lumping the mechanisms occurring during the ADs (calcium release from the sarcoplasmic reticulum, calcium diffusion in the sarcoplasm, Calcium-troponin binding, cross-bridge attachment and detachment, cross-bridge power stroke...) with a single first-order ODE. Various mathematical expressions have been proposed for it. In the following are replicated the most common mathematical expressions along with their source study.

- (Winters & Stark, 1988):

$$\begin{cases} \frac{da}{dt} = \frac{1}{\tau_a}(u - a) & ; \quad u > a \\ \frac{da}{dt} = \frac{1}{\tau_{dea}}(u - a) & ; \quad u \leq a \end{cases} \quad (7)$$

- (Zajac, 1989):

$$\frac{da}{dt} = \frac{u}{\tau_a} - a \left[\frac{1}{\tau_a} \left(\frac{\tau_a}{\tau_{dea}} + \left(1 - \frac{\tau_a}{\tau_{dea}} \right) u \right) \right] \quad (8)$$

- (He et al., 1991):

$$\frac{da}{dt} = (u - a)(c_1 u + c_2) \quad (9)$$

With similar notations, these three modelling approaches can be re-written as:

- (Winters & Stark, 1988): $\frac{da}{dt} = c(u - a)$
- (Zajac, 1989): $\frac{da}{dt} = c(u - a(c_1 u + c_2))$
- (He et al., 1991): $\frac{da}{dt} = (u - a)(c_1 u + c_2)$

Some studies in the field also use active state dependent (de)activation time constants (Winters, 1995):

$$\begin{cases} \frac{da}{dt} = \frac{1}{f(a)}(u - a) \\ \tau(a) = \tau_a(k + a) & ; \quad u > a \\ \tau(a) = \frac{\tau_{dea}}{(k + a)} & ; \quad u \leq a \end{cases} \quad (10)$$

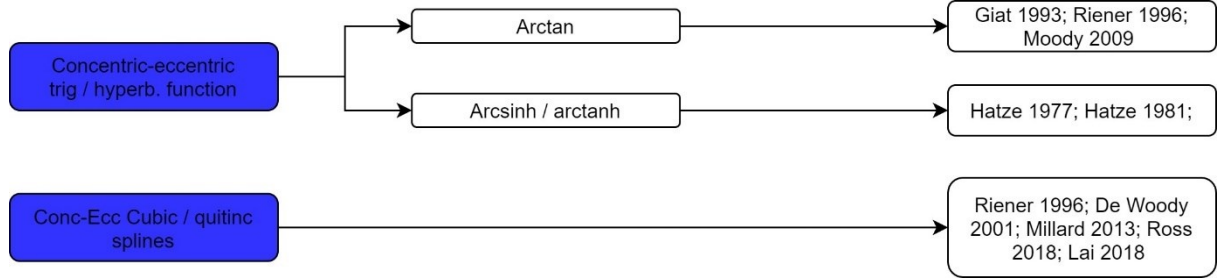


Appendix Figure A-10: Inheritance tree diagram for the Activation Dynamics modelled as a 1st-order differential equation among the eligible studies

A.10.2. FV relationships

A.10.2.1. Continuous Force-Velocity relationships

A few studies modelled both the FVC and the FVE branches of the FV relationship with a single continuous mathematical expression, either with a trigonometric function such as arctan, hyperbolic function such as Arcsinh, or with Bezier splines.



Appendix Figure A-11: Inheritance tree diagram for the complete FV relationship modelled as a continuous function among the eligible studies

A.10.2.2. Concentric Force-Velocity relationship - FVC

The FV relationship for concentric contractions is dominantly modelled with Hill's hyperbolic formula (equation (11) below) as shown in Figure 2-7. To be consistent with a majority of the studies in the field, v is defined negative for shortening contractions:

$$(F + a)(b - v) = b(F_0^M + a) \Leftrightarrow (F + a)v = b(F - F_0^M) \quad (11)$$

With

$$\frac{b}{v_m} = \frac{a}{F_0^M} > 0 \quad (12)$$

Let $f = \frac{F}{F_0^M}$ be the normalized force as obtained by the FV relationship, $\bar{v} = \frac{v}{v_m}$ the normalized contraction velocity, and $a_f = \frac{a}{F_0^M}$ the coefficient that varies the curvature of the hyperbola according to the species and the fibre type under consideration at fixed .

Type 2 FVC relationship

$$F = \frac{b(F_0^M + a)}{b - v} - a = f_v(v) \quad (13)$$

$$F = \frac{bF_0^M + av}{b - v} \quad (14)$$

Equations (13) and (14) describe the unnormalized Hill's FV relationship which is named 'Type 2' FVC relationship in the following.

Type 1 FVC relationship

Equation (13) can be normalized as

$$\begin{aligned} f &= \frac{1 + a_f \frac{v}{b}}{1 - \frac{v}{b}} = f_v(v) \\ &= \frac{1 + \bar{v}}{1 - \frac{\bar{v}}{a_f}} = f_v(\bar{v}) \end{aligned} \quad (15)$$

This normalized form of the FVC relies on the single parameter a_f and is named 'Type 1' FVC relationship in the following.

In some studies, the FVC relationship is inverted to display a first-order ordinary differential equation of the form $v = \frac{dl^M}{dt} = f(l^M)$. In this respect, the Type 2 relationship (equation 14) can be equivalently written as:

$$\begin{aligned} v &= b \frac{F - F_0^M}{F + a} = f_v^{-1}(F) \\ v &= b \frac{f - 1}{f + a_f} \\ v &= \frac{b}{a_f} \cdot \frac{f - 1}{\frac{f}{a_f} + 1} = f_v^{-1}(f) \end{aligned} \quad (16)$$

And the Type 1 relationship (equation 15) can be written as:

$$\bar{v} = \frac{f - 1}{\frac{f}{a_f} + 1} = f_v^{-1}(f) \quad (17)$$

Some studies propose a nonlinear **activation-dependency and/or length-dependency of Hill's hyperbolic relationship**. In this case, the reader is invited not to make confusion between an inverted nonlinear activation-dependency and/or length-dependency of Hill's hyperbolic relationship which writes $v = f_v^{-1}(F, l^M, a)$ and an inverted classic FV velocity-dependent relationship that includes the description of the whole CE dynamics, which in this case writes $v = f_v^{-1}(F^{CE}) = f_v^{-1}(a \cdot f_l \cdot F)$ with f_l the isometric force obtained from the FL relationship here and in the following.

In the following, $l = \frac{l^M}{l_0^M}$ is the normalized fibre length. Most studies further develop the expression of Hill's FV relationship in imposing a zero or low constant force boundaries for shortening speed exceeding the maximum shortening velocity. These regions of high shortening speed are not investigated in the following.

Length-dependent FVC:

- (Brown et al., 1999; Cheng et al., 2000; Song et al., 2008; Tsianos et al., 2011; Elias et al., 2014):

$$f = \frac{1 + \bar{v}}{1 - f_c(l)\bar{v}} = f_v(\bar{v}, l) \quad (18)$$

$$f_c(l) = (c_1 + c_2 l)$$

- (Wyss & Pollak, 1984; Hamouda et al., 2016):

In this approach, the maximum isometric force F_0^M is replaced by the value of the isometric force as given by the FL relationship. This approach is only partially valid as stated previously (Epstein, 1998): the maximum shortening velocity becomes length-dependent: $v_m(l) = \frac{f_l(l) \cdot b}{a}$ which is contradictory with other experimental findings of a constant maximum shortening velocity at all lengths (Edman, 1979).

$$f = \frac{f_l(l) + \frac{a_f}{b} v}{1 - \frac{v}{b}} = f_v(v, l)$$

Or

$$f = \frac{f_l(l) + \bar{v}}{1 - \frac{\bar{v}}{a_f}} = f_v(\bar{v}, l) \quad (19)$$

Or

$$v = \frac{b}{a_f} \frac{f - f_l(l)}{\frac{f}{a_f} + 1} = f_v^{-1}(f, l)$$

Or

$$\bar{v} = \frac{f - f_l(l)}{\frac{f}{a_f} + 1} = f_v^{-1}(f, l)$$

Activation-dependent FVC:

(Otten, 1987; McLean et al., 2003; Thelen, 2003; John et al., 2013; De Groote et al., 2017): these studies make the maximum shortening velocity activation-dependent to reflect on the recruitment order of faster fibre types.

$$f = \frac{1 + \frac{\bar{v}}{f(a)}}{1 - \frac{\bar{v}}{a_f \cdot f(a)}} = f_v(\bar{v}, a)$$

$$(Otten, 1987): f(a) = (0.35 f^M(a) + 0.65) \quad (20)$$

$$(Thelen, 2003 ; De Groote et al., 2017): f(a) = 0.25 + 0.75a$$

$$(McLean et al., 2003): f(a) = 1 - e^{-3.82a} + ae^{-3.82}$$

$$(John et al., 2013): f(a) = 0.5(1 + a)$$

Activation-length-dependent FVC:

- (Winters, 1995):

$$f = \frac{1 + \frac{\bar{v}}{g(a, l)}}{1 - \frac{\bar{v}}{a_f \cdot g(a, l)}} = f_v(\bar{v}, a, l) \quad (21)$$

$$g(a, l) = 0.5(1 + a \cdot f_l(l))$$

- (van Soest & Bobbert, 1993; Kistemaker et al., 2006; Callahan et al., 2013):

$$v = f(a)b \frac{f - 1}{f + \frac{a}{g(l)}} = f_v^{-1}(f, a, l) \quad (22)$$

Or

$$f = \frac{1 + \frac{\bar{v}}{f(a) \cdot g(l)}}{1 - \frac{\bar{v}}{a_f \cdot f(a)}} = f_v(\bar{v}, a, l)$$

(Callahan et al., 2013): $g(l) = f_l(l) \quad ; \quad f(a) = a^{0.3}$

(Van Soest & Bobbert, 1993): $g(l) = \begin{cases} f_l(l) & ; \quad l < 1 \\ 1 & ; \quad else \end{cases} \quad ; \quad f(a) = \min(1, 3.33a)$

(Kistemaker et al., 2006): $g(l) = \begin{cases} f_l(l) & ; \quad l < 1 \\ 1 & ; \quad else \end{cases} \quad ; \quad f(a) = b(1 - 0.9Q)^2 ;$

$$\begin{cases} Q = \frac{a - a_{crit}}{a_0 - a_{crit}} & ; \quad a < a_{crit} \\ 1 & ; \quad else \end{cases}$$

- (Günther et al., 2007; Kosterina et al., 2012; Mörl et al., 2012; Rockenfeller, R. et al., 2020):

$$v = f_B(a)b \frac{f - 1}{f + a \frac{f_A(a)}{g(l)}}$$

Or

$$f = \frac{1 + \frac{f_A(a) \cdot \bar{v}}{f_B(a) \cdot g(l)}}{1 - \frac{\bar{v}}{a_f \cdot f_B(a)}} = f_v(\bar{v}, a, l)$$

(23)

$$f_A(l) = \begin{cases} f_l(l) & ; \quad l < 1 \\ 1 & ; \quad else \end{cases}$$

$$f_A(a) = \frac{0.25}{a} (1 + 3a)$$

$$f_B(a) = \frac{1}{7} (3 + 4a)$$

One study (Curtin et al., 1998) proposed a **double-hyperbolic FV relationship**:

$$f = \begin{cases} \frac{1}{m} \cdot \frac{1 + \bar{v}}{1 - \frac{\bar{v}}{a_f}} & ; \quad 0 < f < f_1 \\ 1 - k_1 \ln\left(1 - \frac{\bar{v}}{k_2}\right) & ; \quad f_1 < f < 1 \end{cases} \quad (24)$$

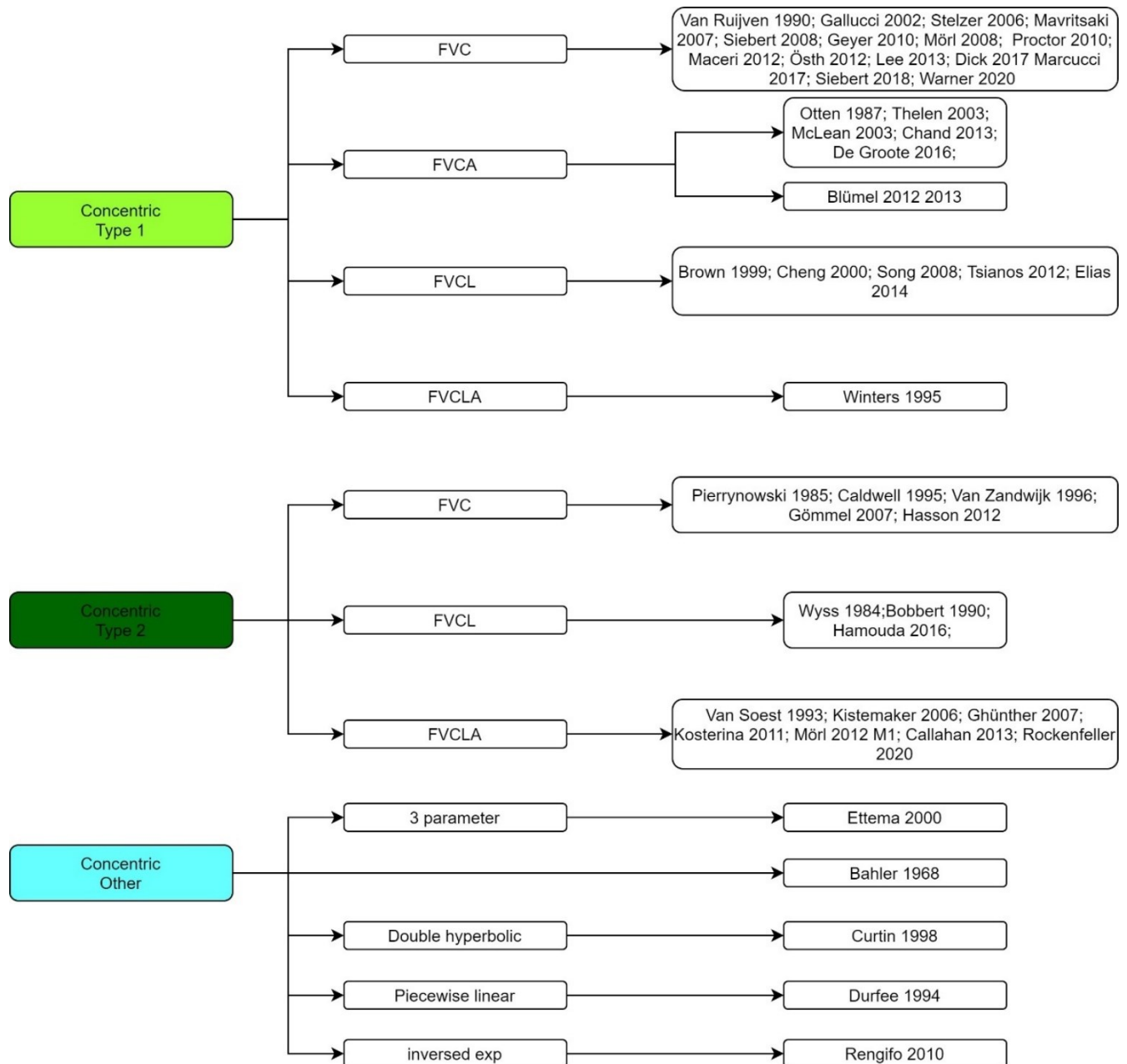
Two studies fitted the curvature of Hill's relation with mathematical expressions according to the fraction of fast and slow fibres in the muscle into consideration, or to the relative fraction of recruited MUs. In the following, $\%_{fast}$ represents the relative fraction of fast fibres composing the muscle under consideration, and $n_{MU}(t)$ the relative number of recruited MUs at time instant t .

(Winters & Stark, 1988):

$$a_f = 0.1 + 0.4 * \%_{fast} \quad (25)$$

(Callahan et al., 2013):

$$a_f = 0.2 - 0.3n_{MU}(t) \quad (26)$$



Appendix Figure A-12: Inheritance tree diagram for the complete FVC relationship among the eligible studies. Please refer to the text for the definition of Type 1 and Type 2 relationships.

A.10.2.3. Eccentric Force-velocity relationship - FVE

Most FVE relationships are described in the eligible studies as a nonlinear curve up to a certain lengthening velocity at which the relationship becomes mainly linear or constant. The following only focuses on the nonlinear region of the relationship.

The most common mathematical description taken for the FVE property is derived from two source studies (Mashima et al., 1972; FitzHugh, 1977) and is named ‘Type 3’ relationship in the following. Eccentric relationships are defined for positive contraction speed v . In the following, $\overline{F}^{max}$ is the

normalized maximum force achievable in eccentric contractions, ranging in [1.1; 1.25] for humans up to 1.8 for other species.

This inversed hyperbolic Type 3 form is obtained from (Mashima et al., 1972):

$$(2F_0^M - F + a')(b' - \bar{v}) = b'(F_0^M + a') \quad (27)$$

Which can be re-written very similar to Type 1 equation as the normalized:

$$f = \frac{1 + \overline{F^{max}} \frac{\bar{v}}{b'}}{1 + \frac{\bar{v}}{b'}} = f_v(\bar{v}) \quad (28)$$

$$b' = a'_f \frac{\overline{F^{max}} - 1}{a'_f + 1}$$

Or as:

$$f = \overline{F^{max}} - (\overline{F^{max}} - 1) \frac{1 - \bar{v}}{1 + \frac{\bar{v}}{K}} = \frac{1 + K_F \bar{v}}{1 + \frac{\bar{v}}{K}} = f_v(\bar{v}) \quad (29)$$

$$K_F = \overline{F^{max}} - 1 + \frac{\overline{F^{max}}}{K}$$

When written in its inversed form:

$$\bar{v} = b' \frac{f - 1}{k - f} = f_v^{-1}(f) \quad (30)$$

Some studies use a length-dependent and/or activation-dependent Type 3 relationship.

Length-dependent FVE:

- (Brown et al., 1999; Cheng et al., 2000; Song et al., 2008; Tsianos et al., 2011; Elias et al., 2014):

$$f = \frac{1 + f_e(l) \frac{\bar{v}}{b}}{1 + \frac{\bar{v}}{b}} \quad (31)$$

$$f_e(l) = (a_1 + a_2 l + a_3 l^2)$$

Activation-dependent FVE:

- (Thelen, 2003; John et al., 2013):

$$\frac{1 + \overline{F^{max}} \frac{\bar{v}}{f(a)b'}}{1 + \frac{\bar{v}}{f(a)b'}} \quad (32)$$

$$b' = \frac{\overline{F^{max}} - 1}{2 + \frac{2}{A}}$$

(Thelen, 2003): $f(a) = 0.25 + 0.75a$

(John et al., 2013): $f(a) = 0.5(1 + a)$

Activation-length-dependent FVE:

- (Winters, 1995):

$$f = \frac{1 + \frac{\bar{v}}{g(a,l)}}{1 + \frac{\bar{v}}{g(a,l)(\overline{F^{max}} - 1)c}} \quad (33)$$

$$g(a, l) = 0.5(1 + a \cdot f_l(l))$$

A.10.2.4. $\mathcal{C}^2$ continuity between concentric and eccentric branches

Numerous studies use a hyperbolic shape to describe the FVE relationship and enforce a $\mathcal{C}^2$ condition at the point of isometric contraction that joins the concentric and eccentric branches, following the work from Van Soest & Bobbert (1993).

The eccentric branch is written as a 3-parameter c_1, c_2, c_3 expression:

$$\bar{v} = -\frac{c_1}{f + c_2} - c_3 = \frac{-c_1 - c_2 c_3 - c_3 f}{c_2 + f} \Leftrightarrow f(\bar{v}) = \frac{-(c_1 + c_2 c_3) - c_2 \bar{v}}{\bar{v} + c_3} \quad (34)$$

The 3 parameters are obtained to respect the following conditions:

1. f_v^{conc} and f_v^{ecc} are continuous for $\bar{v} = 0$
2. At $\bar{v} = 0$, the ratio between the eccentric and concentric derivatives is specified by the df coefficient: $\frac{df_{ecc}}{d\bar{v}} = sf * \frac{df_{conc}}{d\bar{v}}$
3. $\lim_{\bar{v} \rightarrow \infty} f_v^{ecc} = \overline{F^{max}}$

From condition 3, $c_2 = -\overline{F^{max}} * f_l(l)$.

From condition 2, $c_1 = \frac{b*(f_l(l)+c_2)^2}{(a+f_l(l))*sf}$

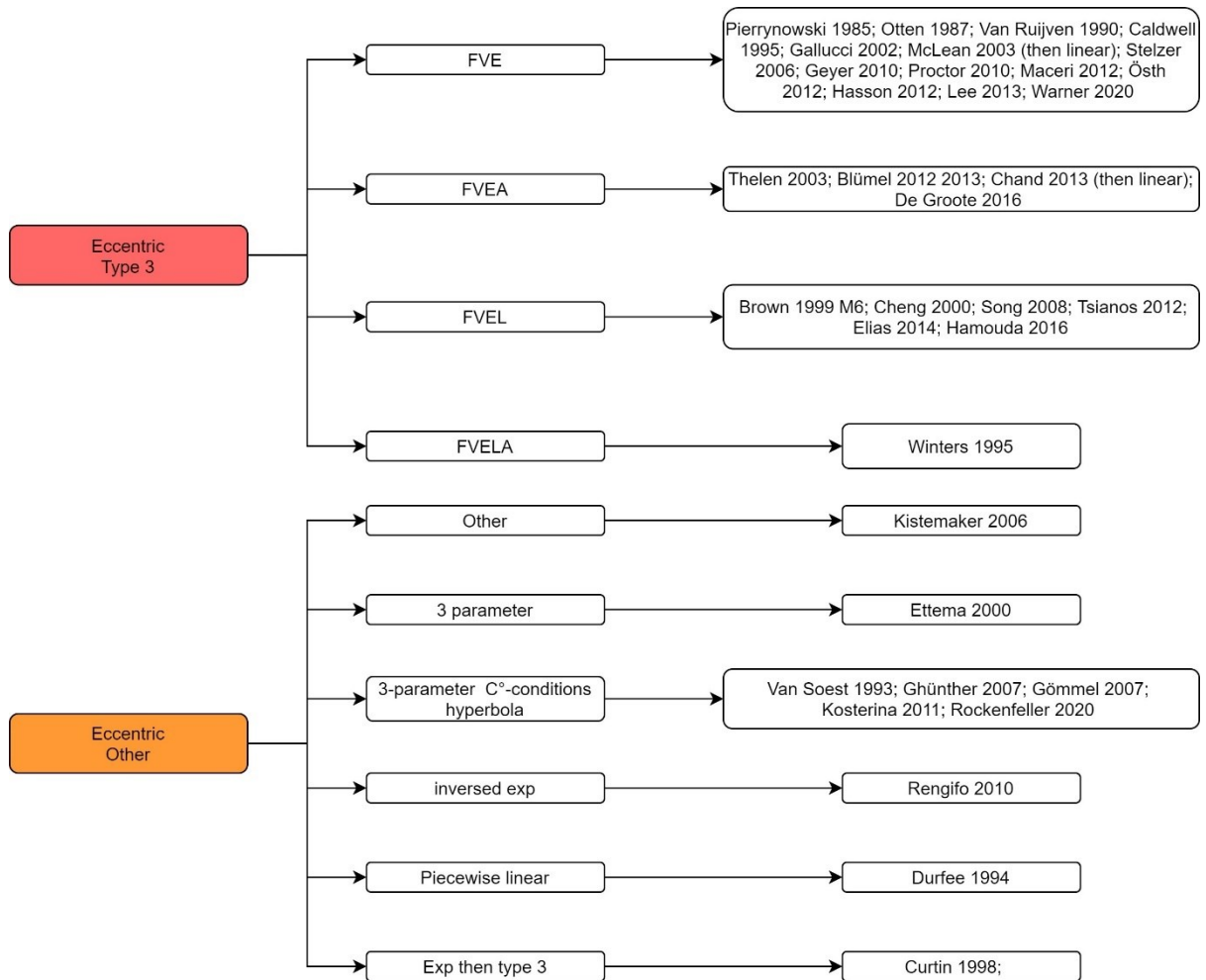
From condition 1, $c_3 = \frac{c_1}{f_l(l)+c_2}$

Reinserting the initial expression, $\bar{v} = c_1 \left[\frac{1}{1-\bar{F}^{max}} - \frac{1}{f-\bar{F}^{max}} \right] = \frac{b(\bar{F}^{max}-1)}{sf(1+a)} \frac{f-1}{\bar{F}^{max}-f} = d_1 \frac{f-1}{\bar{F}^{max}-f}$

Inverting the last expression, a Type 3 expression can be observed:

$$f(\bar{v}) = \frac{1 + \bar{F}^{max} \frac{\bar{v}}{d_1}}{1 + \frac{\bar{v}}{d_1}} \quad (35)$$

$$d_1 = \frac{b(\bar{F}^{max} - 1)}{sf(1 + a)}$$



Appendix Figure A-13: Inheritance tree diagram for the complete FVE relationship among the eligible studies. Please refer to the text for the definition of the Type 3 relationship.

A.10.3. Force-Length relationships - FL

All the eligible studies considered an instantaneous FL relationship between fibre length and isometric force, except in the work from Hamouda et al. (2016), where the issue of the instability of the descending limb of the relationship is accounted for by accounting for the fibre length recorded at the onset of the contraction. In the following, $\Delta l = \frac{l^M - l_0^M}{l_0^M}$ is the normalized deviation of the fibre length around the optimal fibre length and $l = \frac{l^M}{l_0^M}$ is the normalized fibre length. Numerous different mathematical expressions have been proposed to describe the FL relationship as shown in Appendix Figure A-14 and reviewed in (Rockenfeller, R. & Günther, 2017), the most popular of which are:

- The quadratic expressions from

- (van Soest & Bobbert, 1993):

$$f_l(\Delta l) = 1 + c - 2c \cdot \Delta l + c \cdot (\Delta l)^2 \quad (36)$$

- (Caldwell, 1995):

$$f_l(l) = k_1 + k(l - k_1)^2 \quad (37)$$

- (Gallucci & Challis, 2002):

$$f_l(\Delta l) = 1 - \left(\frac{\Delta l}{W}\right)^2 \quad (38)$$

- The gaussian-like expressions from:

- (Bahler, 1968a):

$$f_l(\Delta l) = \exp\left(-\left(\frac{\Delta l}{W}\right)^2\right) \quad (39)$$

- (Otten, 1987):

$$f_l(l) = \exp\left(-\left|\frac{l^b - 1}{W}\right|^c\right) \quad (40)$$

Three studies include an **active-state dependency of the optimal fibre length** to their FL relationship:

- (Winters, 1995):

$$f_l(l, a) = \exp\left(-\left(\frac{l - l_0(a)}{W}\right)^2\right) \quad (41)$$

$$l_0(a) = 1.05 + \frac{l_1}{l_0^M}(1 - a)$$

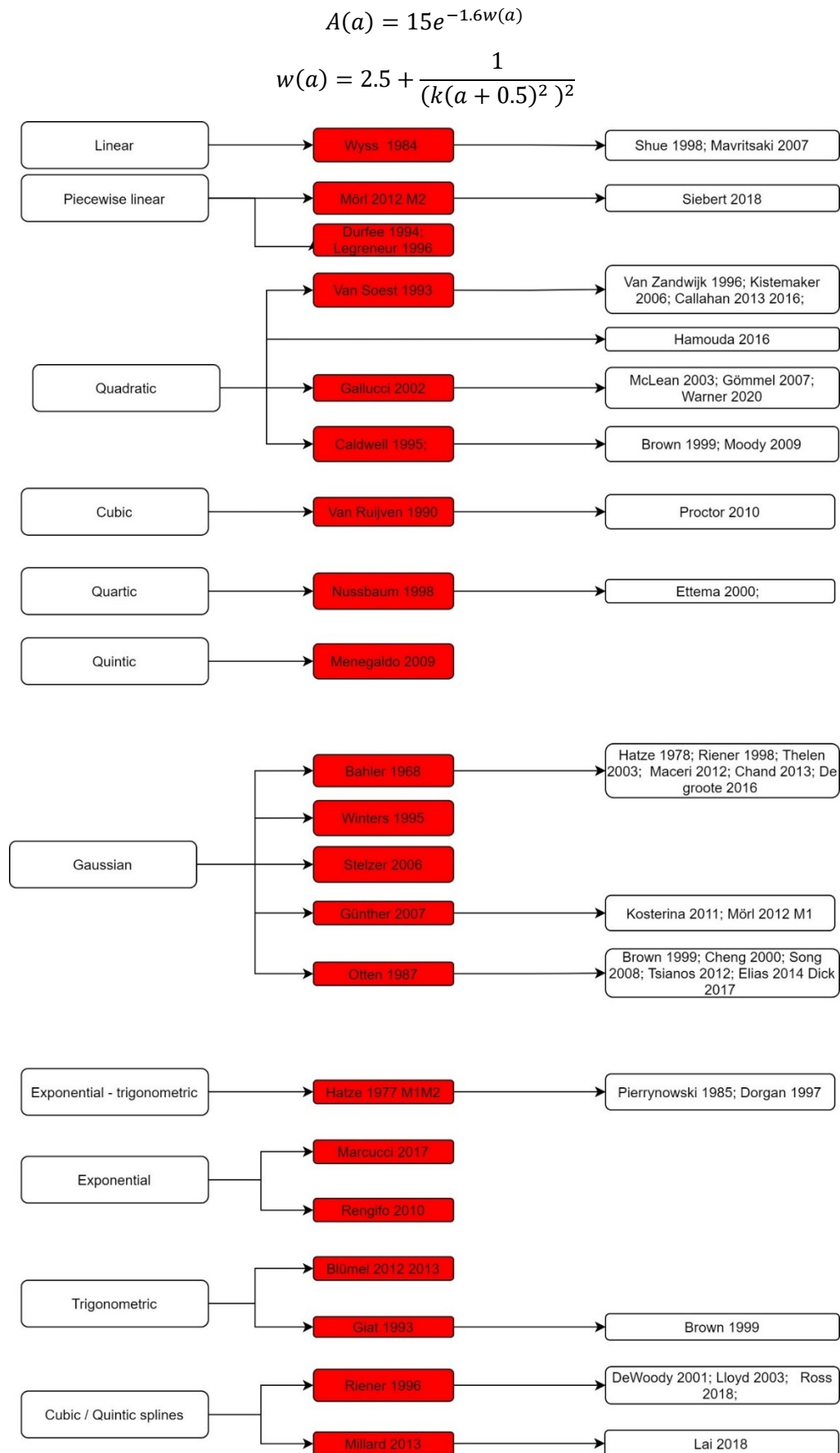
- (Lloyd & Besier, 2003):

$$\text{Cubic spline interpolation} \quad (42)$$

$$l_0(a) = 1 + 0.15(1 - a)$$

- (Blümel et al., 2012):

$$f_l(l, a) = A(a) \cdot \left[\frac{1 + \sin\left(wl - \left(\frac{\pi}{2} + 2.7w\right)\right)}{2} \right] \quad (43)$$



Appendix Figure A-14: Inheritance tree diagram for the FL relationship among the eligible studies.

A.10.4. Parallel Elastic Element - PEE

The behaviour of the PEE is represented as an instantaneous stress-strain relationship in all the eligible studies, while one study proposed a PEE described with an Odgen material (De Groote et al., 2017). In the following, it is assumed that the slack length of the PEE is the optimal fibre length l_0^M and that $\Delta l = \frac{l^M - l_0^M}{l_0^M}$ is the normalized deviation of the PEE length around the optimal fibre length.

Four main mathematical descriptions of the PEE are encountered in the literature as shown in Appendix Figure A-15. PEE force is set to 0 or to a low value for SEE lengths below l_0^M . The equations below are defined for $l^{PEE} > l_0^M$.

(van Soest & Bobbert, 1993):

$$f^{PEE}(\Delta l) = k(\Delta l)^2 \quad (44)$$

(Van Ruijven & Weijs, 1990):

$$f^{PEE}(\Delta l) = k_1 \exp(k_2 \Delta l) \quad (45)$$

(Hatze, 1977):

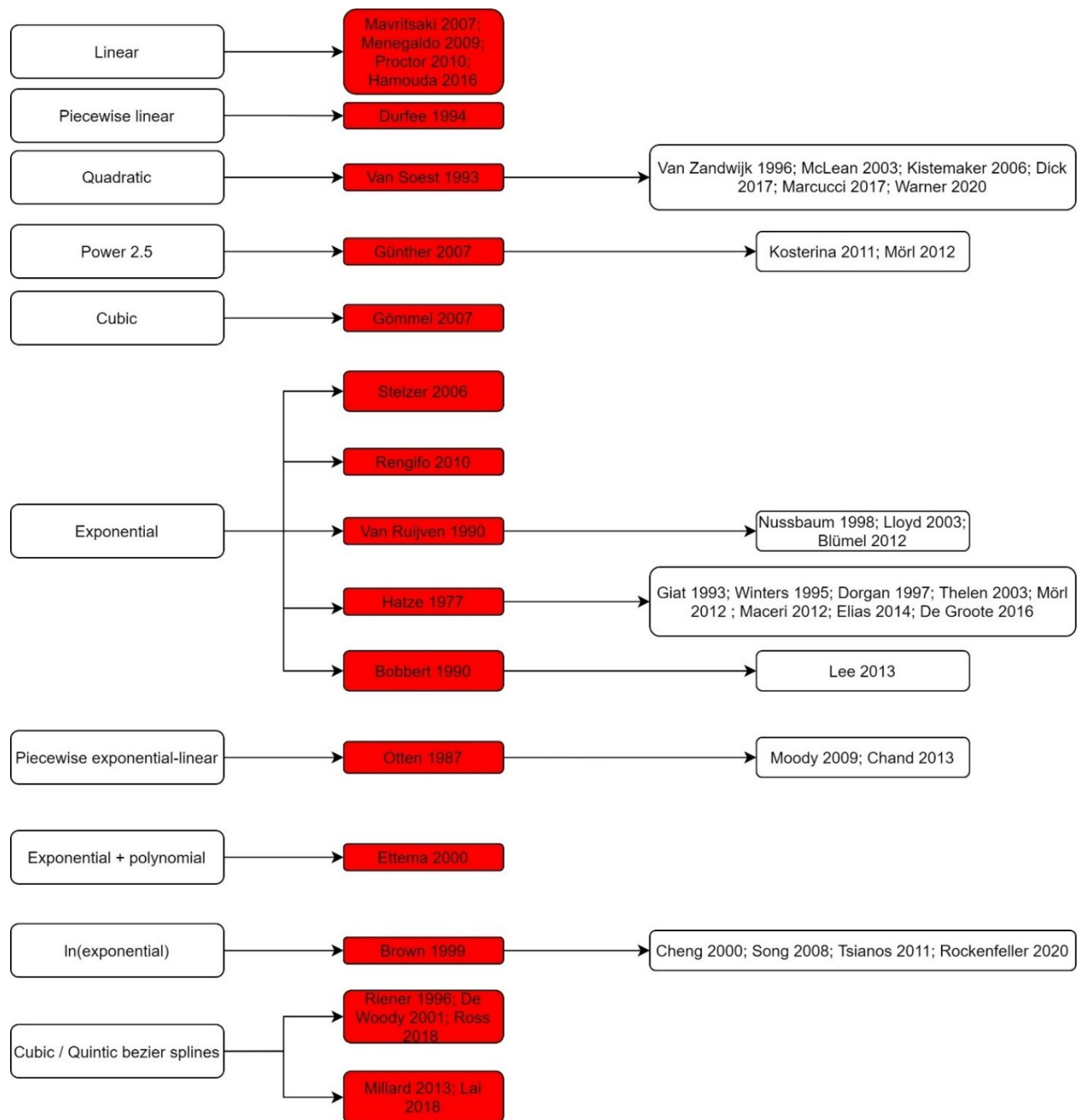
$$f^{PEE}(\Delta l) = k(e^{k_1 \Delta l} - 1)$$

Or

$$f^{PEE}(\Delta l) = \frac{e^{\frac{k}{k_1} \Delta l} - 1}{e^k - 1} \quad (46)$$

(Brown et al., 1999):

$$f^{PEE}(\Delta l) = c_1 \cdot k \cdot \ln \left[e^{\frac{l^M - l_1}{k}} + 1 \right] \quad (47)$$



Appendix Figure A-15: Inheritance tree diagram for the modelling of the properties of the PEE among the eligible studies.

A.10.5. Series Elastic Element - SEE

The behaviour of the SEE is described with an instantaneous stress-strain relationship in all the eligible studies, with mathematical expressions similar to the ones used for describing the PEE. One study proposed a multiscale description of the SEE (Maceri et al., 2012), and one study considered the cross-sectional area of the tendon to derive its stiffness (Pierrynowski & Morrison, 1985). In the following, $\varepsilon = \frac{l^{SEE} - l_s^{SEE}}{l_s^{SEE}}$ is the strain of the SEE as defined by the normalized variation of SEE length around the SEE slack length, often called tendon slack length in the studies.

Four main mathematical descriptions of the SEE are encountered in the literature. The force transmitted by the SEE is set to 0 or to a low value for SEE lengths below l_s^{SEE} . The equations below are defined for $l^{SEE} > l_s^T$.

(Bobbert & van Ingen Schenau, Gerrit Jan, 1990):

$$f^{SEE}(\varepsilon) = k\varepsilon^2 \quad (48)$$

(Hatze, 1977; Winters & Stark, 1988):

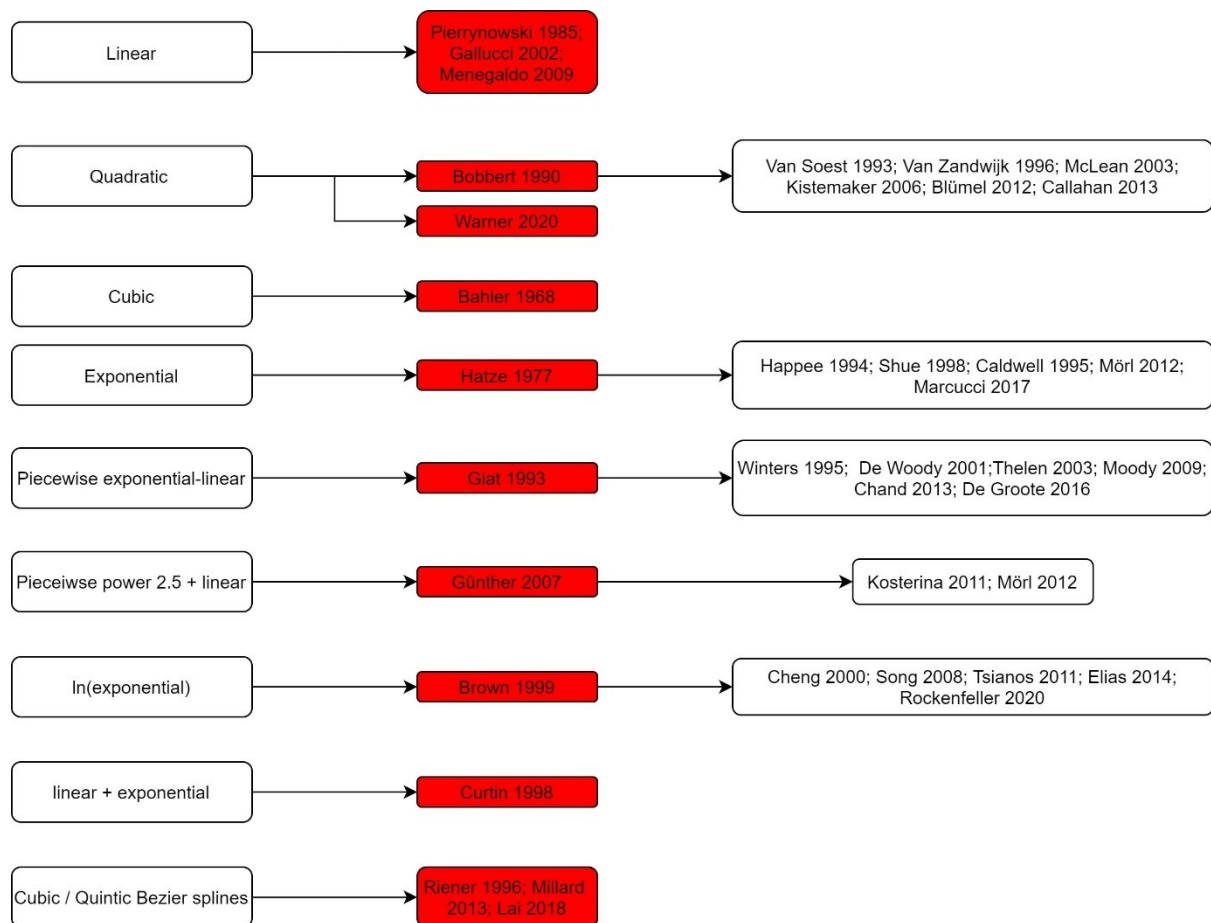
$$f^{SEE}(\varepsilon) = \frac{e^{\frac{k}{k_1}\varepsilon} - 1}{e^k - 1} \quad (49)$$

(Giat et al., 1993):

$$f^{SEE}(\varepsilon) = \begin{cases} k(e^{k_1\varepsilon} - 1) & ; \varepsilon < \varepsilon_0 \\ k_2(\varepsilon - \varepsilon_0) + f^{SEE}(\varepsilon_0) & ; \varepsilon > \varepsilon_0 \end{cases} \quad (50)$$

(Brown et al., 1999):

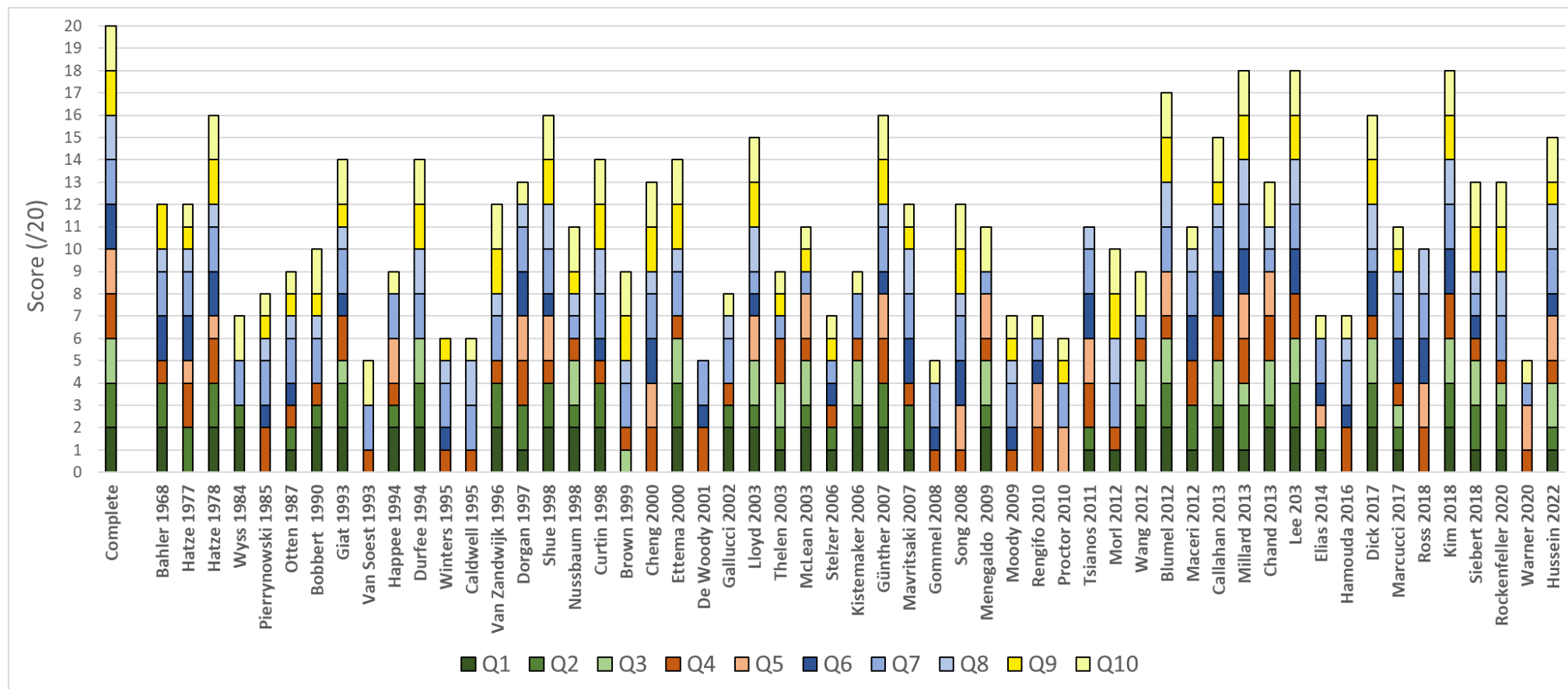
$$f^{SEE}(\varepsilon) = c_1 \cdot k \cdot \ln \left[e^{\frac{\varepsilon}{k}} + 1 \right] \quad (51)$$



Appendix Figure A-16: Inheritance tree diagram for the modelling of the properties of the SEE among the eligible studies.

A.11. Results - Detailed results on the modelling assessment of each eligible model

The results of the scoring of the Completeness assessment are provided in the bar graph in Appendix Figure A-17. The excel spreadsheet gathering the detail of the scoring for each question for each model is also provided online. In Appendix Figure A-17, studies are chronologically listed. A first bar is provided for comparison, representing the scoring of a Hill-type model that fulfils all requirements for model validation, reusability, strategy, and calibration, respectively represented by shades of green, red, blue and yellow.



Appendix Figure A-17: Bar graph presenting the detailed results of the modelling assessment. Shades of green: model validation, of red: model reusability, of blue: modelling choices and strategy, of yellow: parameter calibration. Each question could score 0, 1, or 2.

A.12. Results – Trends in the methodological practices presented in the eligible models

Appendix Table A-7: Details on the methodological practices among the 54 eligible studies. For each of the ten questions, the most popular and/or the most advanced methodological approach is retrieved, and the corresponding references are provided.

Q n°	Feature	Additional precisions	Percentage of eligible studies	References
1	Ad hoc experimental data for model validation		46%	
		Ad hoc animal experiments	19%	(Bahler, 1968b; Durfee & Palmer, 1994; Van Zandwijk et al., 1996; Curtin et al., 1998; Shue & Crago, 1998; Ettema & Meijer, 2000; Günther et al., 2007; Blümel et al., 2012; Lee et al., 2013; Kim & Kim, 2018)
		Ad hoc human experiments	28%	(Hatze, 1978; Wyss & Pollak, 1984; Bobbert & van Ingen Schenau, Gerrit Jan, 1990; Giat et al., 1993; Happee, 1994; Nussbaum & Chaffin, 1998b; Gallucci & Challis, 2002; McLean, S. G. et al., 2003; Lloyd & Besier, 2003; Kistemaker et al., 2006; Menegaldo & Oliveira, 2009; Wang, J. et al., 2012; Callahan et al., 2013; John et al., 2013; Dick et al., 2017)
	Validation using a complete experimental dataset and simulating the experimental protocol available in the literature		24%	(Marcucci et al.,; Otten, 1987; Dorgan & O'Malley, 1997; Thelen, 2003; Stelzer, M. & von Stryk, 2006; Mavritsaki et al., 2007; Tsianos, G. A. et al., 2012; Mörl et al., 2012; Maceri et al., 2012; Millard et al., 2013; Elias et al., 2014; Siebert et al., 2018; Rockenfeller, R. et al., 2020)
	No validation / Validation against data from the literature		30%	All remaining studies
2	Validation against ad hoc animal individual muscle force		19%	(Anonymous ; Bahler, 1968b; Durfee & Palmer, 1994; Van Zandwijk et al., 1996; Curtin et al., 1998; Shue & Crago, 1998; Günther et al., 2007; Blümel et al., 2012; Lee et al., 2013; Kim & Kim, 2018)

	Validation against in vivo human muscle force either via ultrasound-measurements of tendon length or from a specific experimental protocol and interpretation of muscle-induced joint moment	4%	(Hatze, 1978; Dick et al., 2017)
		22%	
	Validation against human joint torque	17%	(Hatze, 1978; Wyss & Pollak, 1984; Bobbert & van Ingen Schenau, Gerrit Jan, 1990; Giat et al., 1994; Nussbaum & Chaffin, 1998b; Gallucci & Challis, 2002; Lloyd & Besier, 2003; Menegaldo & Oliveira, 2009; Callahan et al., 2013)
	Obtained from inverse dynamics analysis	7%	(Happee, 1994; Lloyd & Besier, 2003; Wang, J. et al., 2012; John et al., 2013)
	Validation against human joint angle	6%	(McLean, S. G. et al., 2003; Kistemaker et al., 2006; Wang, J. et al., 2012)
3		30%	
	Objective criterion used for the analysis of the results	19%	(Durfee & Palmer, 1994; Nussbaum & Chaffin, 1998b; McLean, S. G. et al., 2003; Lloyd & Besier, 2003; Kistemaker et al., 2006; Menegaldo & Oliveira, 2009; Blümel et al., 2012; Lee et al., 2013; John et al., 2013; Kim & Kim, 2018)
		7%	(Nussbaum & Chaffin, 1998b; Lloyd & Besier, 2003; Menegaldo & Oliveira, 2009; Lee et al., 2013)
		9%	(McLean, S. G. et al., 2003; Thelen, 2003; Wang, J. et al., 2012; Callahan et al., 2013; Siebert et al., 2018)
		9%	(Durfee & Palmer, 1994; Nussbaum & Chaffin, 1998b; Lloyd & Besier, 2003; Menegaldo & Oliveira, 2009; Blümel et al., 2012)
		13%	(Anonymous ; Lloyd & Besier, 2003; Kistemaker et al., 2006; Menegaldo & Oliveira, 2009; Blümel et al., 2012; Lee et al., 2013; Dick et al., 2017)

		proportions and the use of mean against muscle-specific parameter values.		
		Paired student's t- or Wilcoxon signed-rank tests for assessing the effects of model physiological correctness, parameter optimization and type of muscle model	6%	(Lloyd & Besier, 2003; Kistemaker et al., 2006; Blümel et al., 2012)
		Qualitative analysis of the results	61%	
4	Provided enough information for the full re-implementation of the Hill-type model		35%	
		Open-source implementation are provided	13%	(Cheng et al., 2000; Thelen, 2003; Mavritsaki et al., 2007; Song et al., 2008; John et al., 2013; Millard et al., 2013; Kim & Kim, 2018)
		Sufficient material provided for the full reproduction of the results	2%	(Millard et al., 2013)
		Missing equations, rheological arrangement of the elements, parameter values	65%	
5	Optimized numerical stability and computational speed, notably in avoiding singularities and numerical stiffness		31%	
		C1 or C2 continuity for all properties (C1-C2 functions or Bezier interpolation splines)	15%	(Van Soest & Bobbert, ; Happee, 1994; Gömmel et al., 2007; Günther et al., 2007; Rengifo et al., 2010; Millard et al., 2013; Ross, Nigam et al., 2018; Rockenfeller, R. et al., 2020)
		Avoiding singularities in the inverted FV relationship: enforcing non-zero minimal values for the active state, the isometric muscle force and the tendon force, preventing a 90° pennation angle or non-physiological large stretching or shortening, or adding a damper or a muscle mass element to the rheological structure	22%	(Happee, 1994; Cheng et al., 2000; McLean, S. G. et al., 2003; Lloyd & Besier, 2003; Günther et al., 2007; Song et al., 2008; Menegaldo & Oliveira, 2009; Rengifo et al., 2010; Tsianos, G. A. et al., 2012; Millard et al., 2013; John et al., 2013; Warner et al., 2020)
		Adding a SDE to prevent unstable non-physiological mass oscillations.	9%	(Günther et al., 2007; Mörl et al., 2012; Maceri et al., 2012; Ross, Nigam et al., 2018; Rockenfeller, R. et al., 2020)
		Models not optimized for stability and computational speed	69%	
6	Explicit geometrical or physiological assumptions	Massless, isotropic straight-line and fusiform model (pennation angle disregarded); fibres in parallel and not interacting with each other	59%	

	Justification of the multiscale assumptions taken to simplify the model as opposed to the architecture of real muscles		30%	
		Building a fibre-scale model	4%	(Marcucci et al.,; Hatze, Herbert, 1977; Cheng et al., 2000; Song et al., 2008; Tsianos, G. A. et al., 2012)
		Building a MU-scale model	9%	(Hatzel, Herbert, 1977; Hatzel, 1978; Dorgan & O'Malley, 1997; Mavritsaki et al., 2007; Callahan et al., 2013)
		Lumping a whole muscle as two fast and slow representative MUs	4%	(Lee et al., 2013; Dick et al., 2017)
		Assuming homogeneity in material properties, and lengths between sarcomeres, fibres or MUs further assumed to be excited at the same time instant, and describe the whole muscle as a representative scaled sarcomere, fibre or MU capable of generating a whole muscle force	24%	(Bahler, 1968b; Hatzel, Herbert, 1977; Hatzel, 1978; Dorgan & O'Malley, 1997; Cheng et al., 2000; Mavritsaki et al., 2007; Song et al., 2008; Tsianos, G. A. et al., 2012; Lee et al., 2013; Millard et al., 2013; Dick et al., 2017; Ross, Nigam et al., 2018; Kim & Kim, 2018)
		Simplifications and assumptions are not presented in the study	41%	
7	Modelled the force of the CE as product between active state and forces from the FL and the FV properties		96%	
	Activation and contraction dynamics are decoupled		56%	
	Using a Voigt-Kelvin-like rheological structure		54%	
	Using a Maxwell-like rheological structure		13%	
	Not using both PEE and SEE		33%	
8	Conclusions on the strengths and limitations of their Hill model regarding their results, the results from other studies and potential future modelling advances		26%	
9	Calibrating at least one property to be subject-specific		37%	
		Performing experimental protocols on animals or on human to derive experimental time profiles of a quantity then fit by mathematical expressions	17%	(Anonymous ; Bahler, 1968b; Durfee & Palmer, 1994; Curtin et al., 1998; Günther et al., 2007; Blümel et al., 2012; Lee et al., 2013; Dick et al., 2017; Kim & Kim, 2018)
		Parameter calibration (especially FV parameters) by minimization of a cost function between simulated and experimental force or torque profiles	11%	(Hatzel, 1978; Van Zandwijk et al., 1996; Shue & Crago, 1998; Lloyd & Besier, 2003; Millard et al., 2013; Rockenfeller, R. et al., 2020)
		Cat-to-human parameter scaling	2%	(Cheng et al., 2000)

10	Derivation of subject-specific muscle-specific scaling parameters (maximum isometric force F_0^M , tendon slack length l_s^T and optimal fibre length l_0^M)	From ad hoc experiments of individual muscle forces and curve fitting	22%	(Anonymous ; Bobbert & van Ingen Schenau, Gerrit Jan, 1990; Giat et al., 1993; van Soest & Bobbert, 1993; Zandwijk et al., 1996; Curtin et al., 1998; Günther et al., 2007; Blümel et al., 2012; Callahan et al., 2013; Lee et al., 2013; Dick et al., 2017; Kim & Kim, 2018)
		Parameter calibration by minimization of a cost function between simulated and experimental force or torque profiles	13%	(Hatze, 1978; Durfee & Palmer, 1994; Shue & Crago, 1998; Nussbaum & Chaffin, 1998b; Lloyd & Besier, 2003; Millard et al., 2013; Rockenfeller, R. et al., 2020)
		Scaling parameters combining subject-specific anthropometric measurements and literature data	15%	(Bobbert & van Ingen Schenau, Gerrit Jan, 1990; van Soest & Bobbert, 1993; Van Zandwijk et al., 1996; Cheng et al., 2000; Menegaldo & Oliveira, 2009; Wang, J. et al., 2012; John et al., 2013; Dick et al., 2017)
	Obtaining parameters from the literature for the same species		39%	

Appendix B

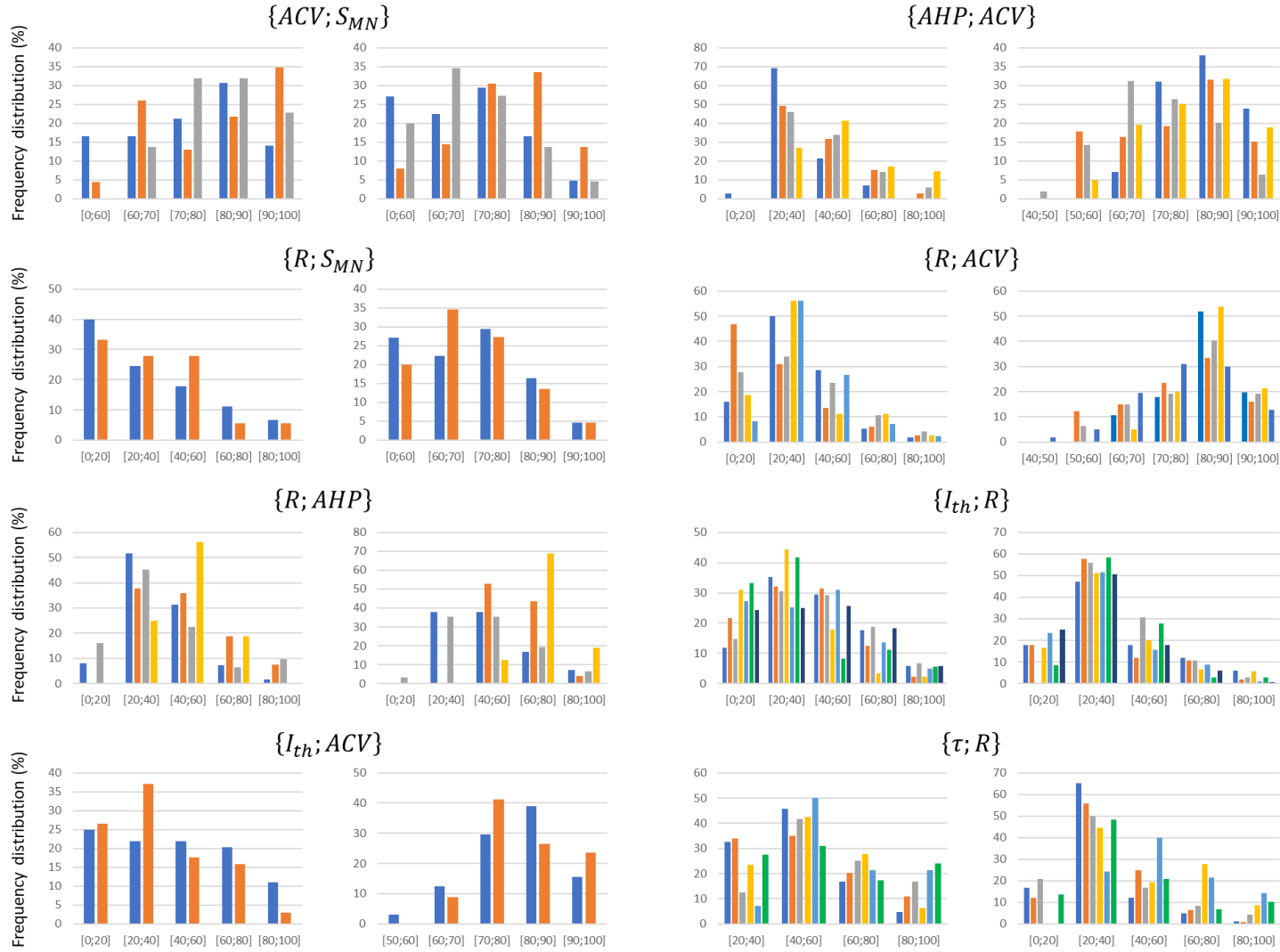
SUPPLEMENTARY MATERIAL FOR THE MATHEMATICAL RELATIONSHIPS BETWEEN MOTONEURON PROPERTIES IN CHAPTER 4

B.1. Inter-study data variability

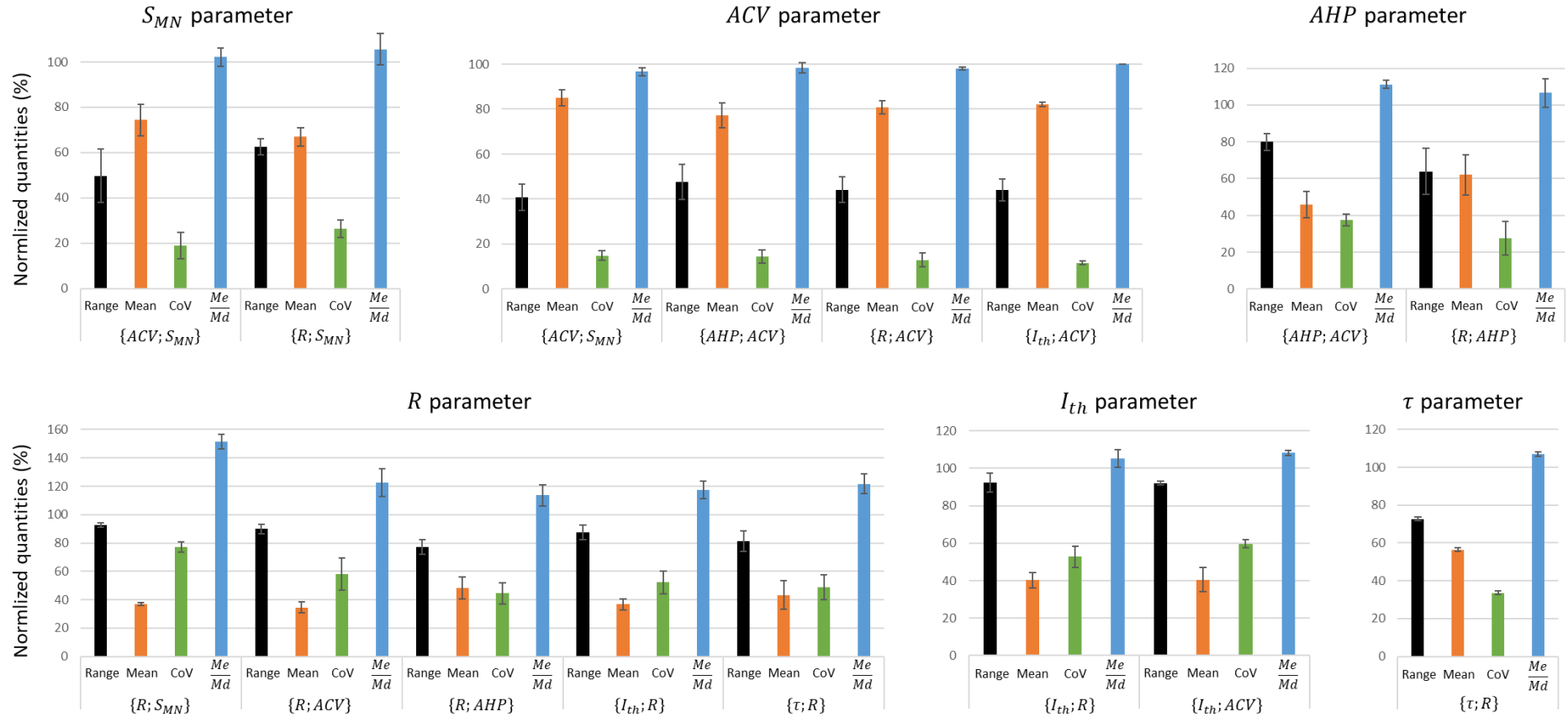
Inter-study variability greater than the threshold defined in the Methods section was observed in 3,2 and 4 property distributions for the metrics Range, Mean and CoV metrics, respectively. Indeed, sensibly different ranges of property values were observed to be reported between studies of the $\{ACV; S_{MN}\}$, $\{AHP; ACV\}$, and $\{R; AHP\}$ datasets for the S_{MN} , ACV , and AHP properties, respectively, with $sd_g \in [10; 15]\%$ and/or $\frac{sd_g}{mean_g} \in [0.15; 0.33]$. Also, the studies of the $\{R; AHP\}$ and $\{\tau; R\}$ datasets returned some variability in the mean of the reported data for properties AHP and R , respectively, with $sd_g \in [10; 11]\%$ and/or $\frac{sd_g}{mean_g} \in [0.15; 0.23]$. The $\{ACV; S_{MN}\}$, $\{R; ACV\}$ and $\{R; AHP\}$ global datasets showed variable data dispersion around the mean for properties S_{MN} , R and ACV , and AHP , respectively, with $sd_g \in [10; 11]\%$ and/or $\frac{sd_g}{mean_g} \in [0.15; 0.30]$.

B.2. Discussion on the potential underestimation of q_S^E

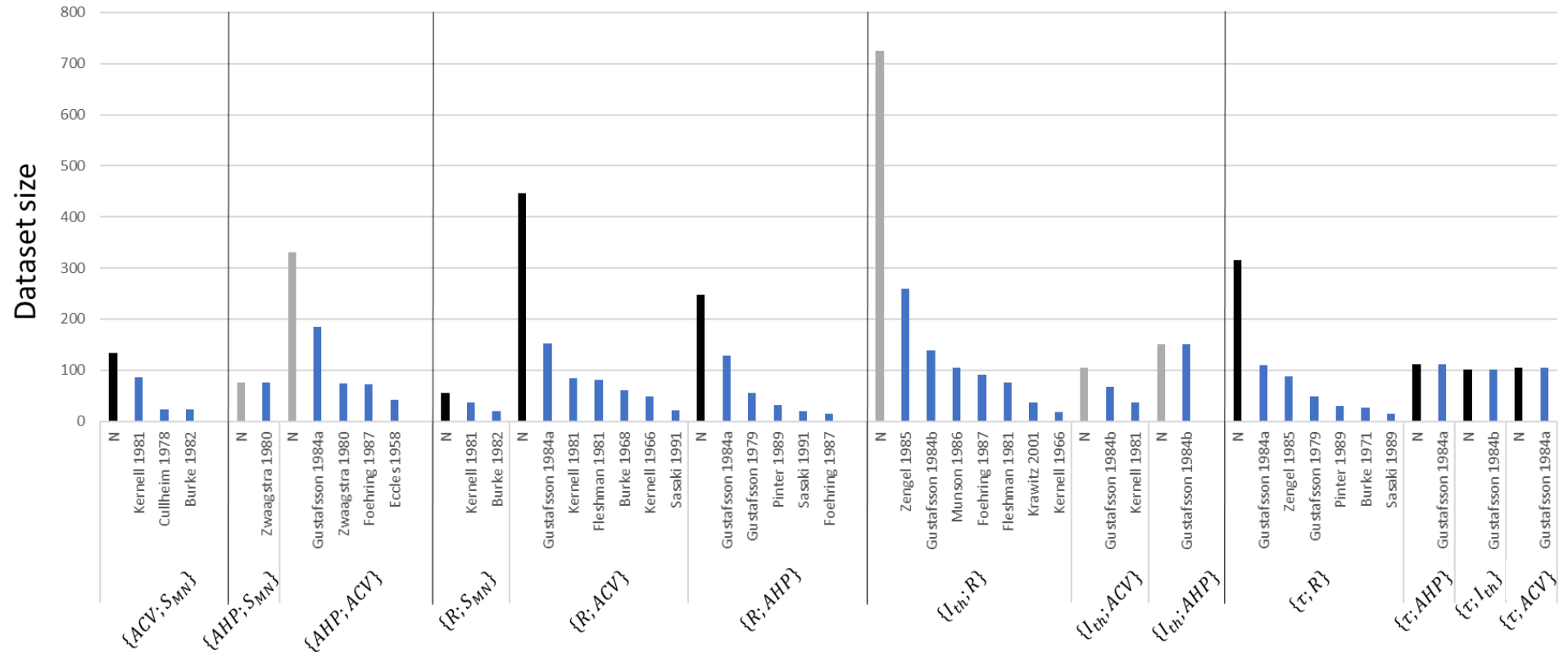
The minimum and maximum values of S_{neuron} and D_{soma} reported in Appendix Table B-3 may underestimate the true distribution of MN sizes in a MN pool for several reasons, and thus underestimate the true value of q_S^E . First, the studies reported in Appendix Table B-3 investigated MN populations of small size, so that the largest and smallest MNs of the investigated MN pools may not have been systematically identified and measured. Also, dendritic labelling by staining may only identify dendrites up to the 3rd dendritic branch (Issa et al., 2010; Fogarty et al., 2018) and full dendritic trees may not be identified, thus underestimating S_{neuron} for the largest MNs that exhibit the highest dendritic tree complexity (Fogarty et al., 2020). Finally, the distribution of gamma- and alpha-MN sizes is typically bimodal and the size distributions of the two populations are reported to overlap (Moschovakis et al., 1991; von Steyern et al., 1999; Friesen et al., 2009; Deardorff et al., 2013). However, most studies reported in Appendix Table B-3 identify the alpha MNs from the gamma MNs by visually splitting the bimodal distributions of MN sizes in two independent domains (Donselaar et al., 1986; Ishihara et al., 2001), thus neglecting the overlap. Similarly, some studies identify as gamma MNs any MN showing an axonal conduction velocity typically less than $\sim 60 m \cdot s^{-1}$ (Zwaagstra, B. & Kernell, 1980; Ulfhake & Kellerth, 1984). These approaches lead to overestimating the lower limit for the reported D_{soma} and S_{neuron} values. Consequently, true ranges in MN sizes may be larger than the theoretical ones reported in Appendix Table B-3. A similar comment can be made for the electrophysiological properties reported in Table TSM2. However, the $q_S^E = 2.4$ -fold range obtained for cats is consistent with the fold-ranges obtained from rat and mouse data for D_{soma} , as reported in Table TSM1, while it underestimates the 4.4-fold range in S_{neuron} values observed in mice. Appendix Table B-3 finally shows that typical cat MN sizes are larger than in rats and mice, as reviewed by Manuel et al. (2019).



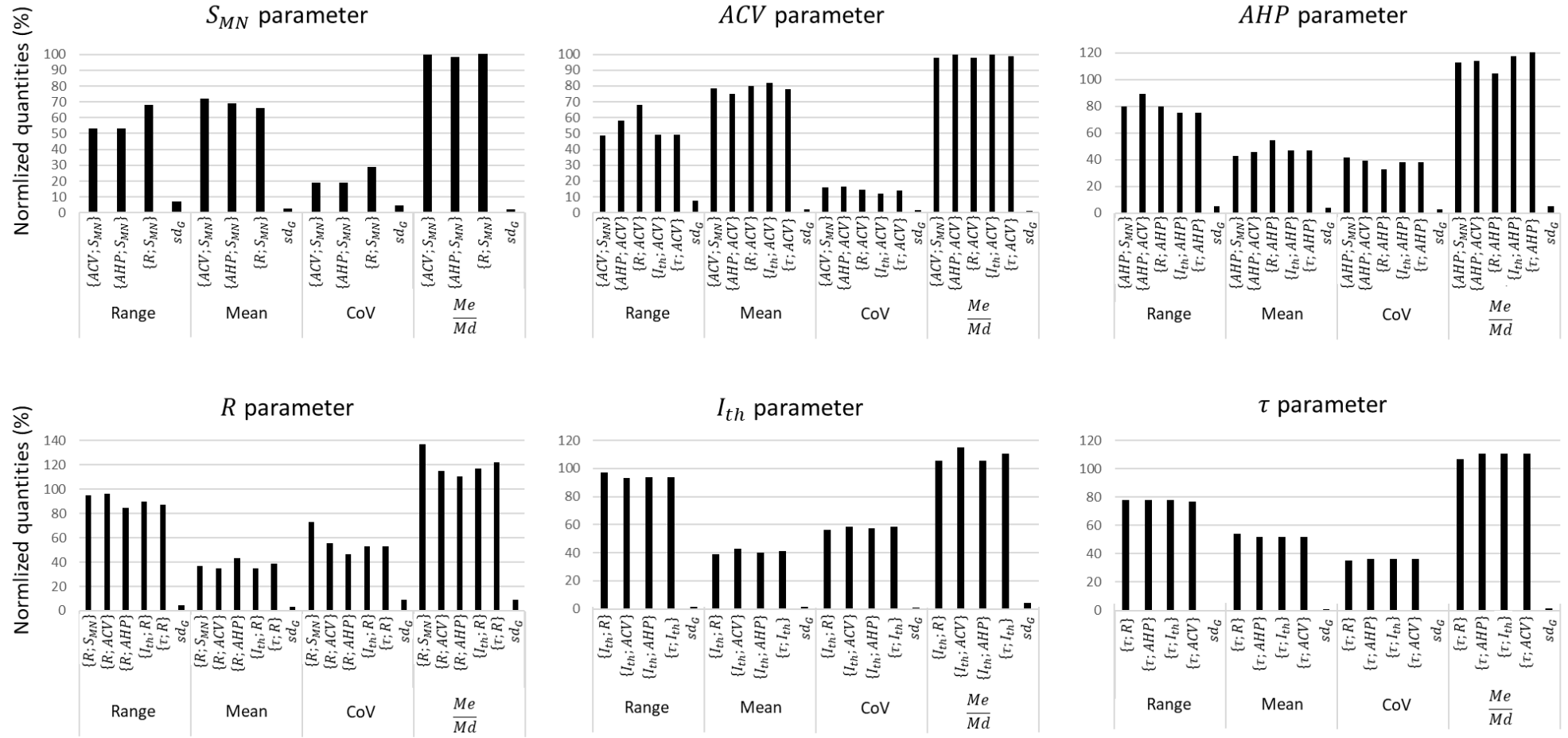
Appendix Figure B-1. Density histograms of the data distributions reported in the experimental studies included in the global datasets. Depending on the typical range over which each property spans, the distributions are divided in steps of 10% or 20%. The frequency distribution is provided in percentage of the total number of reported data points in a study. Different studies are displayed with different colours in each graph. For each dataset $\{A; B\}$, the frequency distributions of property A and B are provided in the left and right plot respectively.



Appendix Figure B-2. Assessment of data variability between the experimental studies that constitute the 8 global datasets that include at least two experimental studies. For each experimental study included in the global dataset $\{A; B\}$, the Range, Mean, coefficient of variation ($CoV = \frac{\text{standard deviation}}{\text{Mean}}$) and the ratio $\frac{Me}{Md} = \frac{\text{Mean}}{\text{Median}}$ of the experimental A values measured in this study were computed. Then, the average (bars) and standard deviation (error bars) across experimental studies of these four metrics (Range, Mean, CoV, $\frac{Me}{Md}$) were calculated for each global dataset independently. For example, the first bar in the ' S_{MN} parameter' plot represents the average across three studies (Kernell & Zwaagstra, 1981; Cullheim, 1978; Burke et al., 1982) of the range of S_{MN} values reported in these studies, for the global dataset $\{ACV; S_{MN}\}$. The computed standard deviations (error bars) express for each global dataset the inter-study variability of (1) the length of the identified bandwidth of the MN pool (Range metric), (2) the spread of values around the mean (CoV metric), (3) the skewness of the distributions, and (4) whether the distributions from different studies are centred (Mean metric). A global dataset $\{A; B\}$ reporting narrow error bars for parameter A for the four metrics infers that the experimental studies constituting this dataset measured similar distributions of property A .



Appendix Figure B-3. Distribution of the size of the experimental datasets constituting the global datasets for assessment of the variability in the input data. The histogram is divided between global datasets (half vertical lines), grouped as final size-dependent datasets (full vertical lines). For each global dataset, the total number of data points is reported (label: “N”), while the size of the constitutive experimental studies is reported in decreasing order.



Appendix Figure B-4. Assessment of data variability between the global datasets. This figure compares the distributions of the S_{MN} , ACV , AHP , R , I_{th} and τ properties between the normalized global datasets built in this study. For each property, the Range, Mean, coefficient of variation ($CoV = \frac{\text{standard deviation}}{\text{Mean}}$) and the ratio $\frac{Me}{Md} = \frac{\text{Mean}}{\text{Median}}$ of its distribution in a global dataset is compared to the other global datasets it appears in. For each property, the standard deviation sd_G between global datasets of these four metrics is computed. A low sd_G value reflects the low variability of the property distribution between global datasets.

Appendix Table B-1. r^2 values obtained for each experimental dataset $\{A; B\}$ when performing a linear regression analysis on $\{A; B\}$ directly ('Linear') and on the $\ln(A) - \ln(B)$ ('Power') and $\ln(A) - B$ ('Exponential') transformations of $\{A; B\}$. The r^2 values returned by the three types of regression cannot be directly compared to estimate the best model. However, the power fit returned $r^2 > 0.5$ for relatively more experimental datasets than the linear and exponential fits.

	Studies	Linear	Power	Exponential
$\{ACV; S_{MN}\}$	(Cullheim, 1978)	0,42	0,45	0,43
	(Kernell & Zwaagstra, 1981)	0,62	0,63	0,6
	(Burke et al., 1982)	0,23	0,26	0,24
$\{AHP; S_{MN}\}$	(Zwaagstra, B. & Kernell, 1980)	0,33	0,37	0,35
$\{AHP; ACV\}$	(Eccles, J. C. et al., 1958)	0,53	0,54	0,54
	(Zwaagstra, B. & Kernell, 1980)	0,42	0,44	0,42
	(Gustafsson & Pinter, 1984b)	0,71	0,68	0,7
	(Foehring et al., 1987)	0,15	0,14	0,14
$\{R; S_{MN}\}$	(Kernell & Zwaagstra, 1981)	0,68	0,71	0,7
	(Burke et al., 1982)	0,41	0,5	0,46
$\{R; ACV\}$	(Kernell, 1966)	0,70	0,65	0,64
	(Burke, 1968)	0,28	0,31	0,31
	(Kernell & Zwaagstra, 1981)	0,68	0,71	0,71
	(Fleshman et al., 1981)	0,3	0,21	0,2
	(Gustafsson & Pinter, 1984b)	0,45	0,44	0,42
	(Sasaki, 1991)	0,68	0,68	0,66
$\{R; AHP\}$	(Gustafsson, B., 1979)	0,43	0,41	0,42
	(Gustafsson & Pinter, 1984b)	0,71	0,68	0,67
	(Foehring et al., 1987)	0,33	0,32	0,33

	(Pinter & Vanden Noven, 1989)	0,66	0,53	0,59
	(Sasaki, 1991)	0,57	0,49	0,52
$\{I_{th}; R\}$	(Kernell, 1966)	0,52	0,52	0,68
	(Fleshman et al., 1981)	0,28	0,34	0,38
	(Gustafsson & Pinter, 1984a)	0,63	0,83	0,83
	(Zengel et al., 1985)	0,49	0,6	0,62
	(Munson et al., 1986)	0,34	0,42	0,47
	(Foehring et al., 1987)	0,34	0,47	0,47
	(Krawitz et al., 2001)	0,32	0,49	0,44
$\{I_{th}; ACV\}$	(Kernell & Zwaagstra, 1981)	0,23	0,24	0,24
	(Gustafsson & Pinter, 1984a)	0,4	0,52	0,5
$\{I_{th}; AHP\}$	(Gustafsson & Pinter, 1984a)	0,55	0,72	0,69
$\{C; R\}$	(Gustafsson & Pinter, 1984b)	0,55	0,57	0,57
$\{C; I_{th}\}$	(Gustafsson & Pinter, 1984b)	0,23	0,34	0,24
$\{C; AHP\}$	(Gustafsson & Pinter, 1984b)	0,25	0,26	0,26
$\{C; ACV\}$	(Gustafsson & Pinter, 1984b)	0,17	0,22	0,19
$\{\tau; R\}$	(Burke & Ten Bruggencate, 1971)	0,04	0,07	0,03
	(Gustafsson, B., 1979)	0,6	0,63	0,61
	(Gustafsson & Pinter, 1984b)	0,63	0,69	0,59
	(Zengel et al., 1985)	0,39	0,33	0,36
	(Pinter & Vanden Noven, 1989)	0,65	0,69	0,62
	(Sasaki, 1991)	0,68	0,72	0,64
$\{\tau; I_{th}\}$	(Gustafsson & Pinter, 1984a)	0,63	0,59	0,57
$\{\tau; ACV\}$	(Gustafsson & Pinter, 1984b)	0,69	0,76	0,76
$\{\tau; AHP\}$	(Gustafsson & Pinter, 1984b)	0,34	0,32	0,31

Appendix Table B-2. Mathematical empirical NORMALIZED relationships between the MN properties D_{soma} , R , C , τ , I_{th} , AHP and ACV . Each column provides the relationships between one and the six other MN properties. All constants and properties are normalized up to a theoretical 100% maximum value. To scale the normalized relationships $A = k \cdot B^c$ for a specific mammalian species, the normalized intercept k must be scaled with a pair of values for properties A and B obtained from the same motoneuron in that species.

	D_{soma}	R	C	τ	I_{th}	AHP	ACV
D_{soma}		$R = \frac{9.6 \cdot 10^5}{D_{soma}^{2.4}}$	$C = 1.2 \cdot D_{soma}$	$\tau = \frac{2.6 \cdot 10^4}{D_{soma}^{1.5}}$	$I_{th} = 9.0 \cdot 10^{-4} \cdot D_{soma}^{2.5}$	$AHP = \frac{2.5 \cdot 10^4}{D_{soma}^{1.5}}$	$ACV = 4.0 \cdot D_{soma}^{0.7}$
R	$D_{soma} = \frac{2.9 \cdot 10^2}{R^{0.4}}$		$C = \frac{2.7 \cdot 10^2}{R^{0.4}}$	$\tau = 5.8 \cdot R^{0.6}$	$I_{th} = \frac{1.5 \cdot 10^3}{R}$	$AHP = 4.7 \cdot R^{0.6}$	$ACV = \frac{2.0 \cdot 10^2}{R^{0.3}}$
C	$D_{soma} = 8.6 \cdot 10^{-1} \cdot C$	$R = \frac{1.4 \cdot 10^6}{C^{2.5}}$		$\tau = \frac{3.3 \cdot 10^4}{C^{1.5}}$	$I_{th} = 6.2 \cdot 10^{-4} \cdot C^{2.6}$	$AHP = \frac{3.1 \cdot 10^4}{C^{1.6}}$	$ACV = 3.6 \cdot C^{0.7}$
τ	$D_{soma} = \frac{9.5 \cdot 10^2}{\tau^{0.7}}$	$R = 5.6 \cdot 10^{-2} \cdot \tau^{1.6}$	$C = \frac{8.5 \cdot 10^2}{\tau^{0.7}}$		$I_{th} = \frac{2.9 \cdot 10^4}{\tau^{1.7}}$	$AHP = 7.8 \cdot 10^{-1} \cdot \tau$	$ACV = \frac{4.6 \cdot 10^2}{\tau^{0.5}}$
I_{th}	$D_{soma} = 1.6 \cdot 10^1 \cdot I_{th}^{0.4}$	$R = \frac{1.1 \cdot 10^3}{I_{th}}$	$C = 1.7 \cdot 10^1 \cdot I_{th}^{0.4}$	$\tau = \frac{4.2 \cdot 10^2}{I_{th}^{0.6}}$		$AHP = \frac{3.7 \cdot 10^2}{I_{th}^{0.6}}$	$ACV = 2.7 \cdot 10^1 \cdot I_{th}^{0.3}$
AHP	$D_{soma} = \frac{8.1 \cdot 10^2}{AHP^{0.7}}$	$R = 8.4 \cdot 10^{-2} \cdot AHP^{1.6}$	$C = \frac{7.3 \cdot 10^2}{AHP^{0.6}}$	$\tau = 1.3 \cdot AHP$	$I_{th} = \frac{1.9 \cdot 10^4}{AHP^{1.7}}$		$ACV = \frac{4.1 \cdot 10^2}{AHP^{0.5}}$
ACV	$D_{soma} = 1.4 \cdot 10^{-1} \cdot ACV^{1.4}$	$R = \frac{1.2 \cdot 10^8}{ACV^{3.5}}$	$C = 1.7 \cdot 10^{-1} \cdot ACV^{1.4}$	$\tau = \frac{5.0 \cdot 10^5}{ACV^{2.1}}$	$I_{th} = 5.9 \cdot 10^{-6} \cdot ACV^{3.6}$	$AHP = \frac{5.0 \cdot 10^5}{ACV^{2.2}}$	

Appendix Table B-3. Typical ranges of physiological values for D_{soma} and S_{neuron} in cat rat and mouse species. S_{MN} is found to vary over an average $q_S^E = 2.4$ -fold range, which sets the amplitude of the theoretical ranges. Absolute {min; max} reports the minimum and maximum values retrieved in the reference studies for D_{soma} and S_{neuron} , while average {min; max} is obtained as the average across reference studies of minimum and maximum values retrieved per study.

	Property	Unit	Absolute {min;max}	Average {min; max}	q_S^E	Reference studies	Theoretical range
Cat	D_{soma}	$[\mu m]$	{25.0; 90.0}	{36.7; 75.5}	2.2	(Kernell, 1966; Cullheim, 1978; Zwaagstra, B. & Kernell, 1980; Kernell & Zwaagstra, 1981; Ulfhake & Kellerth, 1981; Zwaagstra & Kernell, 1981; Burke et al., 1982; Donselaar et al., 1986; Destombes et al., 1992)	[33; 79]
	S_{neuron}	$[mm^2]$	{0.08; 0.64}	{0.17; 0.45}	2.7	(Barrett & Crill, 1974; Ulfhake & Kellerth, 1981; Burke et al., 1982; Ulfhake & Kellerth, 1984; Ulfhake, Brun & Cullheim, 1988; Moschovakis et al., 1991)	[0.18; 0.44]
Rat	D_{soma}	$[\mu m]$	{17.5; 66.0}	{23.9; 55.7}	2.4	(Swett et al., 1986; von Steyern et al., 1999; Copray & Kernell, 2000; Ishihara et al., 2001; Deardorff et al., 2013; Mierzejewska-Krzyżowska et al., 2014)	
Mouse	D_{soma}	$[\mu m]$	{14.0; 35.0}	{14.0; 35.0}	2.5	(von Steyern et al., 1999)	
	S_{neuron}	$[mm^2]$	{0.01; 0.08}	{0.01; 0.06}	4.4	(Amendola & Durand, 2008; Brandenburg et al., 2020)	

Appendix Table B-4. Typical ranges of physiological values for the MN properties R , R_m , C , τ , I_{th} , AHP and ACV . As described in the Methods section, q_A^E is the average among reference studies of the ratios of minimum and maximum values; the properties experimentally vary over a q_A^E -fold range. This ratio compares with the theoretical ratio $q_A^T = (q_S^E)^{|c|}$ (with c taken from Table 3 in manuscript), which sets the amplitude of the theoretical ranges. Absolute and average {min; max} are obtained as described in the main manuscript.

MN property	Unit	Absolute exp {min;max}	Average exp {min; max}	q_A^E	Reference studies	q_A^T	$\frac{q_A^E}{q_A^T}$	Theoretical range
R	$M\Omega$	{0.2; 8.1}	{0.4; 4.0}	10.9	(Kernell, 1966; Burke, 1968; Burke & Ten Bruggencate, 1971; Barrett & Crill, 1974; Gustafsson, B., 1979; Kernell & Zwaagstra, 1981; Fleshman et al., 1981; Burke et al., 1982; Ulfhake & Kellerth, 1984; Gustafsson & Pinter, 1984b; Zengel et al., 1985; Foehring et al., 1986; Munson et al., 1986; Foehring et al., 1987; Sasaki, 1991; Krawitz et al., 2001)	8.4	1.3	[0.5; 4.0]
C	nF	{2.2; 8.5}	{2.2; 8.5}	3.9	(Gustafsson & Pinter, 1984b; Gustafsson & Pinter, 1985)	2.3	1.6	[3.2; 7.5]
τ	ms	{2.0; 14.2}	{2.9; 10.2}	3.5	(Burke & Ten Bruggencate, 1971; Barrett & Crill, 1974; Gustafsson, B., 1979; Ulfhake & Kellerth, 1984; Gustafsson & Pinter, 1984b; Gustafsson & Pinter, 1985; Sasaki, 1991)	3.7	0.9	[2.8; 10.3]
I_{th}	nA	{1.7; 52.7}	{2.3; 36.6}	16.3	(Fleshman et al., 1981; Kernell & Monster, 1981; Ulfhake & Kellerth, 1984; Gustafsson & Pinter, 1984a; Zengel et al., 1985; Foehring et al., 1986; Munson et al., 1986; Foehring et al., 1987; Krawitz et al., 2001)	9.1	1.8	[3.9; 35.0]
AHP	ms	{10.6; 266.6}	{44.2; 158.7}	4.1	(Eccles, J. C. et al., 1957; Gustafsson, B., 1979; Dum & Kennedy, 1980; Zwaagstra, B. & Kernell, 1980; Ulfhake & Kellerth, 1984; Gustafsson & Pinter, 1984b; Zengel et al., 1985; Foehring et al., 1987; Sasaki, 1991)	3.8	1.1	[42.6; 160.2]
ACV	$\frac{m}{s^{-1}}$	{51.1; 127.2}	{65.3; 114.8}	1.8	(Eccles, J. C. et al., 1957; McPhedran et al., 1965; Kernell, 1966; Appelberg & Emonet-Dénand, 1967; Burke, 1968; Barrett & Crill, 1974; Proske & Waite, 1974; Bagust, 1974; Stephens & Stuart, 1975; Cullheim, 1978; Dum & Kennedy, 1980; Zwaagstra, B. & Kernell, 1980; Kernell & Zwaagstra, 1981; Fleshman et al., 1981; Glenn & Dement, 1981; Burke et al., 1982; Gustafsson & Pinter, 1984b; Zengel et al., 1985; Foehring et al., 1986; Foehring et al., 1987)	2.3	0.8	[63.5; 116.6]

Appendix Table B-5. Validation of the cat relationships (Table 6 in manuscript) against rat and mouse data. nME: normalized maximum error. nRMSE: normalized root-mean-square error. r_{pred}^2 : coefficient of determination between experimental and predicted quantities. r_{exp}^2 : coefficient of determination of the power trendline directly fitted to the experimental data.

Animal	Dataset	Reference studies	nME (%)	nRMSE (%)	r_{pred}^2	r_{exp}^2
Rat	$\{I_{th}; R\}$	(Gardiner, 1993; Bakels & Kernell, 1993; Lee & Heckman, 1998; Button et al., 2008; Turkin et al., 2010; Krutki et al., 2015)	352	16	0.53	0.54
		(Delestrée et al., 2014; De Lourdes Martínez-Silva et al., 2018; Huh et al., 2021)	385	16	0.46	0.46
Mouse	$\{\tau; R\}$	(Manuel et al., 2009)	1219	168	0.35	0.40
	$\{C; R\}$	(Manuel et al., 2009)	1915	98	0.38	0.38

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