Motor unit firing rate control of agonist skeletal muscle during voluntary isometric and shortening contractions with limb movement

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A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Kinesiology
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Abstract

Voluntary movements throughout mammalian lifespan require skeletal muscle contractions that are controlled by time- and recruitment-dependent firing rate patterns. Single motor unit (MU) activity reflects the final efferent neural drive to the muscle, yet the underlying neural control of movement at the MU level is not well understood. Single MU recordings using intramuscular electromyography evaluated relationships between voluntary contraction kinematics and MU firing rates in vivo, in groups of young and older adult participants.

The purpose of study one was to characterize how MU firing rates are differently scaled among muscles relative to voluntary contraction intensity. Across 12 different muscles, MU firing rates were compared between voluntary intensities and two adult age groups. Findings supported that firing rates are muscle and contraction intensity dependent. But with adult ageing, firing rates were more similar across muscles, although more dissimilar in response to contraction intensity.

The purpose of study two was to compare whether MU firing rates recorded from the anconeus muscle change with an age-related effect in both isometric and limb movement contractions. Results indicated that single MU firing rates were lower in older adults during isometric contraction but not during limb movements, supporting observations of greater descending drive during movements in older adults.

The purpose of study three was to investigate how MU firing rates recorded from the anconeus and triceps brachii muscles are related with elbow extension movement kinematics. Effects of time lag between comparisons were minimized by relating each firing-time to separated kinematic parameters. Through correlation analysis, results showed that firing rates of both muscles were directly related to limb torque, but not velocity or position, supporting that MU activity controls movements indirectly through torque.
In study four, MU firing rate trajectories of the anconeus and triceps brachii muscles were compared between isometric contractions and limb movements. During self-initiated contractions, results indicated that characteristics of the trajectory were dependent on the task. Whereas during reaction-cued contractions, MU firings occurred sooner relative to the go-cue, suggesting that descending inputs determine the trajectory, but the state of limb-movement further modifies trajectory timing.
Keywords

Discharge rate, dynamic contraction, electromyography, human, intramuscular, motoneurone, muscle function, lower motor neuron, skeletal muscle, spinal motor neuron
Summary for lay audience

Voluntary movements require direct control from the brain, brainstem, and spinal cord. At the smallest scale, the motor unit (MU) represents a single lower motor neuron located in the brainstem or spinal cord, its motor axon that travels into the periphery and all innervated skeletal muscle fibres acting in unison to produce a voluntary contraction. These contractions at the muscle can apply force to our external environment through the tendon and bone and are task specific. These forces are influenced by how and when MU activation occurs; including the number of MU activations per second (i.e., firing rate), how many MUs are activated (i.e., recruitment), and the MU structure. Despite considerable research of electrified muscle contractions, there remains few studies that have explored how movement is controlled at the single MU firing rate level during more ‘real-life’ movements. Therefore, in a series of experiments, this thesis sought to characterize and compare how the modulation of MU firing rate activity is different between muscles, and between voluntary contractions that permit the joint to move. Furthermore, MU firing rates differences were compared between groups of young and older adults, as ageing of older adults is accompanied by muscle slowness and weakness.

From our results, MU firing rates were contraction intensity, muscle, and age dependent during isometric contractions, but during limb movements, MU firing rates were more similar between age groups. Across different muscles, the firing rate range was broad during isometric contraction and generally lower in older adults. However, during limb movements, MU firing rates recorded from the anconeus muscle (accessory elbow extensor) were similar between age groups, suggesting that muscle weakness was explained by factors other than lower MU firing rates. In young adults, the underlying neural control of elbow extension movement was more closely related to limb torque and was different as compared to sustained isometric contractions. Although, when voluntary preparation and contraction profiles were controlled, firing rate patterns were similar between isometric contractions and movements, but occurred sooner during limb movement. This suggested that single MU firing rate trajectories are task dependent and can occur sooner shifted during the movement-related state.
Co-authorship statement

For all chapters, Eric A. Kirk was the first author and Charles L. Rice was the supporting senior investigator and co-author. In chapter 2, co-authors Anita D. Christie and Christopher A. Knight contributed to the formation of the aggregated dataset and editing the manuscript. In addition, several co-authors who collected data for the primary publications should be recognized for their indirect contribution and can be found in the descriptive table 2.1 in Chapter 2. In Chapter 3, co-author Kevin J. Gilmore contributed with data collection and editing the manuscript. All experimental data presented in this thesis were aggregated (chapter 2), collected (chapters 2-5), analyzed and interpreted by Eric A. Kirk.
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# Table of contents

Contents

Abstract ........................................................................................................................................ ii
Keywords ....................................................................................................................................... iv
Summary for lay audience .............................................................................................................. v
Co-authorship statement ................................................................................................................ vi
Acknowledgments ........................................................................................................................ vii
Table of contents .......................................................................................................................... viii
List of tables .................................................................................................................................. xi
List of figures .................................................................................................................................. xii
List of appendices ........................................................................................................................ xiv
List of abbreviations ..................................................................................................................... xvi
Glossary of terms .......................................................................................................................... xvii

Chapter 1 ........................................................................................................................................ 1

1 General introduction .................................................................................................................... 1

1.1 Brief overview: organization of the corticospinal pathway: voluntary control from brain-to-spine-to-muscle .......................................................... 2

1.2 Motor unit structure and function .......................................................................................... 8

1.3 From voluntary isometric contraction to movements ............................................................. 13

1.4 Brief overview: voluntary motor unit control throughout human lifespan ....................... 21

1.5 The elbow extensor muscle group ......................................................................................... 24

1.6 Goals of this thesis ................................................................................................................... 28

Chapter 2 ....................................................................................................................................... 32

2 Motor unit firing rates during constant isometric contraction: establishing and comparing an age-related pattern among muscles .............................................. 32
2.1 Introduction .................................................................................................................. 33
2.2 Methods ......................................................................................................................... 37
2.3 Results ............................................................................................................................ 44
2.4 Discussion ....................................................................................................................... 50
2.5 Limitations ..................................................................................................................... 55
2.6 Conclusions ................................................................................................................... 56

Chapter 3 ............................................................................................................................. 58
3 Anconeus motor unit firing rates during isometric and muscle shortening contractions comparing young and very old adults ................................................................. 58
3.1 Introduction ................................................................................................................... 59
3.2 Methods ......................................................................................................................... 62
3.3 Results ............................................................................................................................ 66
3.4 Discussion ...................................................................................................................... 78
3.5 Limitations .................................................................................................................... 84
3.6 Conclusions .................................................................................................................. 85

Chapter 4 ............................................................................................................................. 87
4 The relationship of agonist muscle single motor unit firing rates and elbow extension limb movement kinematics .......................................................................................... 87
4.1 Introduction ................................................................................................................... 87
4.2 Methods ......................................................................................................................... 91
4.3 Results ............................................................................................................................ 94
4.4 Discussion ..................................................................................................................... 100
4.5 Limitations ................................................................................................................... 104
4.6 Conclusions .................................................................................................................. 105

Chapter 5 ............................................................................................................................. 107
5 Agonist muscle motor unit firing rate trajectories underlying stereotype voluntary elbow extension contractions in humans .............................................................. 107

5.1 Introduction ................................................................................................. 108

5.2 Methods ....................................................................................................... 111

5.3 Results .......................................................................................................... 118

5.4 Discussion .................................................................................................... 134

5.5 Limitations ................................................................................................... 141

5.6 Conclusions ................................................................................................. 142

Chapter 6 .......................................................................................................... 143

6 Thesis conclusions .......................................................................................... 143

6.1 Thesis summary ............................................................................................ 143

6.2 Thesis limitations ......................................................................................... 146

6.3 Future labours .............................................................................................. 147

References ........................................................................................................... 150

 Appendices ......................................................................................................... 185

Curriculum Vitae ............................................................................................... 205
List of tables

Table 2.1 Dataset summary ........................................................................................................... 38
Table 2.3 Linear correlation of mean firing rate and contraction intensity. .................. 43
Table 3.1 Summary of measured motor unit samples during elbow extension ............ 67
Table 3.2 Average firing rate change as function of contraction time course. ............. 76
Table 4.1 Dataset summary of motor unit trains during voluntary elbow extension movements. ........................................................................................................... 95
Table 5.1 Summary dataset of MU trains in experiment one. ........................................ 119
Table 5.2 Comparing the firing rate trajectory for each MU train as half-life or linear. 124
List of figures

Figure 1.1 Schematic of basic concepts that activate and sustain motor unit (MU) activity during voluntary muscle contraction. ................................................................. 7

Figure 1.2 Cartoon representing a simplified account of the somatic nervous system spinal circuitry to an agonist muscle during voluntary contraction................................. 12

Figure 1.3 Simplified schematic of MU controls and feedback from an agonist muscle with question marks pertaining to unknown concepts.............................................. 20

Figure 2.1 Overview of voluntary contraction and intramuscular techniques.................. 36

Figure 2.2 Ensemble mean firing rate 95% confidence interval ranges dependent on contraction intensity for each muscle and age group, with an example of the analysis workflow in the FDI muscle at 100% of MVC............................................................... 46

Figure 2.3 The percentage of firing rate variance explained by voluntary isometric contraction intensity........................................................................................................ 49

Figure 3.1 Voluntary contractions. Including isometric and elbow extension limb movements (i.e., shortening contraction).......................................................................... 61

Figure 3.2 Summary MU firing rates for each participant and condition....................... 68

Figure 3.3 Empirical cumulative distribution plots depict summary firing rates measured from the anconeus muscle during isometric and unconstrained-velocity limb movements................................................................. 70

Figure 3.4 Empirical cumulative distribution plots depict summary firing rates measured from the anconeus muscle during constrained-velocity limb movements.............. 71

Figure 3.5 Representative data and summary relationships of MU firing rate activity from the anconeus muscle and elbow joint kinematics during isometric and unconstrained-velocity movements. ........................................................................................................ 73
Figure 3.6 Summary MU firing rate activity from the anconeus muscle and elbow joint kinematics during constrained-velocity movement (i.e., isokinetic). ........................................ 77

Figure 4.1 Relationship of agonist muscle single MU firing activity with measured limb kinematics during voluntary elbow extension movement. ........................................... 90

Figure 4.2 Representative from a female participant (21 years old). ........................................ 96

Figure 4.3 Aggregated data from all participants. ................................................................. 99

Figure 5.1 Voluntary contraction experimental set-up. ......................................................... 111

Figure 5.2 Voluntary self-initiated isometric contractions at targeted elbow extension torque ramps and MVCs. ................................................................. 120

Figure 5.3 Voluntary self-initiated limb movements at targeted elbow extension velocities. .............................................................................................................. 122

Figure 5.4 Linear estimate of the MU firing rate trajectory during self-initiated voluntary contractions. ............................................................................................... 126

Figure 5.5 Comparisons of summary MU firing rate trajectories that passed the statistical filter. .............................................................................................................. 129

Figure 5.6 Single MU firing rate trajectories of the anconeus muscle during reaction-cued voluntary elbow extension contractions. ............................................. 131

Figure 5.7 Summary MU firing rate trajectory analysis of the anconeus muscle during reaction cued voluntary elbow extension contractions. .............................................. 134
List of appendices

Appendix A: Chapter 3. Rate of torque development and time graphs. ....................... 185

Appendix B: Chapter 3. Firing rate and limb acceleration time graphs of the older group.
........................................................................................................................................ 186

Appendix C: Chapter 3. Firing rate and limb acceleration time graphs of the young group.
........................................................................................................................................ 187

Appendix D: Chapter 4. Principal component analysis of the aggregated dataset. .... 188

Appendix E: Chapter 4. Pearson’s correlation estimates.............................................. 189

Appendix F: Chapter 4. Associations of indirect relationships between MU firing rate and limb movement kinematics. .................................................................................. 190

Appendix G: Chapter 5. Torque and firing rate at motor unit firing recruitment threshold, from experiment one. ........................................................................................................ 191

Appendix H: Chapter 5. Comparing firing rates between isometric and movement conditions for each MU, from experiment two............................................................... 192

Appendix I: Chapter 5. Comparing the timing of firings relative to the go stimulus cue between isometric and movement conditions for each MU, from experiment two .... 193

Appendix J: Chapter 5. Anconeus MU firing rate and time graphs............................ 194

Appendix K: Chapter 5. Elbow extension kinematic and time graphs. ..................... 195

Appendix L: Chapter 5. Lateral head of the triceps brachii MU firing rate and time graphs................................................................................................................................. 196

Appendix M: Chapter 5. Elbow extension kinematic and time graphs. .................... 197

Appendix N: Chapter 5. Summary of MU samples 1-4 in experiment two. .............. 198
Appendix O: Chapter 5. Summary of MU samples 5-7 in experiment two. .................. 199

Appendix P: Chapter 5. Summary of MU samples 8-10 in experiment two. ............... 200

Appendix Q: Chapter 5, experiment 2. Calculating the fixed kernel bandwidths. ....... 201

Appendix R: Experimental set-up used in chapters 3-5............................................. 202

Appendix S: Research ethics initial approval notice. .................................................. 203

Appendix T: Research ethics continued approval. ..................................................... 204
List of abbreviations

EMG = electromyography
FR = firing rate
ISI = inter-spike-interval
MU = motor unit
MVC = maximum voluntary contraction
PIC = persistent inward current
ROM = range of motion
RT = recruitment threshold
sEMG = surface electromyography
V_{max} = maximum voluntary movement velocity
Glossary of terms

Agonist muscle – skeletal muscle that actively shortens during voluntary contraction and produces tension because of excitation-contraction coupling.

Firing rate – the number of instances per unit time that an action potential waveform is occurring (detected). The firing rate is often averaged due to large firing rate variability at small time increments. For this thesis, the firing rate is the inverse of the inter-spike-interval, in seconds, between successive muscle fibre action potentials.

Kinematics – mechanics concerned with limb motion without direct reference to the forces causing the motion. For the purpose of this thesis, limb kinematics are referred to as limb torque, velocity and position.

Motor unit – a single alpha motor neuron and all the muscle extrafusal fibres it innervates. There are also beta and gamma motor units but are not the focus of this thesis.

Motor unit recruitment threshold – from recording local field potentials of muscle fibres, the first firing of a single motor unit is related to kinematic parameters that occur at the same time point.

Reaction-cued voluntary contraction – voluntary contractions in response to a cued and controlled visual stimulus.

Self-initiated voluntary contraction – for the purpose of this thesis, these are voluntary contractions that occur without a direct experimental cue or defined external stimulus preceding contraction onset.

Sustained isometric contraction – voluntary skeletal muscle contraction of increasing or fixed contraction intensity that occurs without change of the limb and presumably intrinsic muscle length positions.

Voluntary contraction – is a skeletal muscle contraction that is activated by a goal-based decision and required motor commands. These motor commands produce muscle activation first from descending communications across brain to spinal to muscle levels.
Voluntary contraction intensity – relative to the set maximum, the gradation of voluntary contraction presumably provides different scaling of descending synaptic drive to the motor unit pool.
Chapter 1

1 General introduction

In vertebrates, voluntary interaction with the environment requires neural commands to wield muscle actions. Among many organisms, acting and responding through movement on the time scale of milliseconds to seconds requires a spectrum of time-dependent communications across cellular structures to perform different specialized behaviours (Grimby 1984; Petersen and Berg 2016; Srivastava et al. 2017). With movement, the evolved advantages and limitations are perhaps a central reason why neural tissue was required in the first place (and likely began as an involuntary reflex loop). In mammals, and specifically primates, an estimated boundary is hard to determine between voluntary and involuntary movement, as voluntary movement can be shaped by involuntary reflexes, while an involuntary movement may instigate a voluntary response. Perhaps unique to the act of voluntary movement, which is contrasted to pure involuntary reflex or central pattern generation during ambulation, are the conscious abilities with voluntary movements to remember, imagine, and prepare without the act being performed at the muscle. But when carried through to actually cause muscle contraction, the ultimate unit of control (Liddell and Sherrington 1925) is reduced to extrafusal muscle fibres that are grouped by their synaptic connection to a single lower alpha motor neuron, defined as a motor unit (MU). Understanding how the brain, sub-brain, spinal cord, and periphery integrate signals (i.e., information) to produce self-initiated movement behaviour is an area of considerable research. While many insights have been gained recently due to technological scaling of electrode arrays, and with aides of computational models and theory, the field is well positioned to make MU population-based inferences from hundreds of MUs simultaneously. Although, the neuromuscular (brain to spinal to muscle) control to permit and modify muscle contraction during ‘real-life’ voluntary movements remain unexplored at both the single and population levels of the MU pool.
1.1 Brief overview: organization of the corticospinal pathway: voluntary control from brain-to-spine-to-muscle

In self-initiated voluntary movement, corticospinal excitability is greater than cued or reaction-timed movements in humans (Chen et al. 1998). The anterior and lateral corticospinal tracts which terminate onto lower motor neurons and associated interneurons of the spinal cord are thought to be the main component involved in the duality of fast precise movements (Lemon 2008; Nicolas et al. 2001; Rothwell 2012). For each upper motor neuron in the primary motor cortex (M1), propagation of action potentials from the axon hillock (Colbert and Pan 2002) will transfer electrical impulses directly and indirectly through synapses onto lower motor neurons in the intact system (Brouwer and Ashby 1990; Churchland and Shenoy 2007a; Devanne et al. 1997; Evarts 1968; Fuglevand et al. 1993; Schieber and Rivlis 2005, 2007). Unique to humans (homo sapiens sapiens) and unlike other primates, ~20% of the lateral corticospinal (or corticobulbar) tract directly synapses onto lower motor neurons (Lemon 2008; Watson et al. 2009) and without pronounced effects of presynaptic inhibition (Nielsen and Petersen 1994). Although less studied during voluntary movements, there are many other ascending (i.e., spinal-to-brain, somatosensory) and descending spinal tracts which are not discussed (e.g., rubrospinal, reticulospinal, vestibulospinal, etc.), but should be appreciated (Watson et al. 2009). Overall, the spinal tracts controlling voluntary movements are evolved entities (Brownstone 2020; Nudo and Masterton 1988) and depending on the task, information to the muscle can be equally shaped by non-corticospinal pathways (Buford and Davidson 2004; Economo et al. 2018; Heckman 1994; Lawrence and Kuypers 1968a, 1968b; Lemon 2008; Taub 1976; Watson et al. 2009; Whishaw et al. 1998). For example, the rubrospinal tract is thought to be involved with fast velocity movements based on non-human primate lesion experiments (Watson et al. 2009; Whishaw et al. 1998), however, in human participants, this has been minimally explored (Rothwell 2012). Regardless of the spinal tract connectivity, outputs of the lower motor neuron pool occur through excitatory and inhibitory gradations of the effective synaptic currents that reach the soma of the lower motor neuron (Binder et al.
Generally, the recording of MU firing rate outputs therefore represents the summation and integration of such effective synaptic currents.

In the spinal cord, lower motor neuron columns correspond to the dedicated anatomical regions that they innervate. For example, from very few mapping studies in humans, the most inferior motor columns (spinal region L5-S3), reasonably innervate the foot (Sharrard 1964). Overall, during voluntary contractions, synaptic inputs from descending pathways are modelled to be evenly distributed throughout the lower motor neuron pool (Fuglevand et al. 1993), however recent evidence in non-human primates suggests a fraction of synaptic integrations from M1 neurons can synapse onto motor neuron pools that are fractionated, and over less-direct pathways that involve synchronization (Schieber and Rivlis 2005, 2007). Regardless of where summary synaptic inputs arise from the supraspinal motor system, what remains well described are the responses of the lower motor neuron, specifically alpha motor neurons during ‘steady-state’ conditions of sustained voluntary isometric contractions. Other types of lower motor neurons (beta and gamma) which are involved in the neuronal control of muscle spindle intrafusal fibers (i.e., fusimotor drive) are not discussed here, but should be appreciated that they too can be coupled under voluntary controls (Edin et al. 1998) and may affect MU activity during voluntary movements (Hunt 1951; Macefield and Knellwolf 2018). Indeed, during voluntary movements, single MU firing rate trajectories are very likely modulated from afferent proprioceptive feedback of the muscle spindle and Golgi tendon systems (Al-Falahe et al. 1991; Al-falahe et al. 1990; Burke et al. 1978; Day et al. 2017; Dimitriou and Edin 2010; Edin and Vallbo 1998; Macefield et al. 1993; Macefield and Knellwolf 2018; Matthews 1964; Merton 1953; Sergio et al. 2005; Vallbo 1971, 1974; Windhorst 2007), however, there exists few examples of direct evidence.

Like many branches of neuroscience, single neuronal firing rate activity is understood through the model of cellular action potential generation from the five seminal papers by Hodgkin and Huxley in 1952, that mathematically described the theory governing ion concentration dynamics during a nerve action potential (Hodgkin and Huxley 1952a, 1952b, 1952c, 1952d, 1952e). Since then, many experiments and mathematical models have been developed in relation to the nuances of input-output relationships at the lower
motor neuron (Binder et al. 2020; Heckman and Binder 1991; Heckman and Enoka 2012; Petersen and Berg 2016), but the general consensus continues to be that the neuronal resting potential is depolarized by extrinsic synaptic inputs until the threshold potential is reached (~ -50 mV), after which an action potential occurs. This binary electrical event, initiated by inward currents from sodium and calcium ions will propagate the efferent travelling action potential across axonal Nodes of Ranvier and throughout all axonal branches of the alpha lower motor neuron synapsing onto muscle fibres in the intact system, releasing vesicles of acetylcholine per neuromuscular-junction, therefore initiating and controlling the process of excitation-contraction coupling to produce intrinsic muscle tension (Edman 1979; Ford et al. 1977; Huxley 1957).

At the lower motor neuron and therefore the MU (figure 1.1), increasing synaptic inputs (via agonist stretch reflex) results in the size-ordered recruitment of the MU pool (Bawa et al. 1984; Henneman et al. 1964), where small neuronal cells with higher electrical resistance first reach firing recruitment threshold (RT), scaling to larger neuronal cells that innervate larger numbers of muscle fibres that have markedly different structural and contractile properties (Bakels and Kernell 1993a, 1993b; Brown and Loeb 1999; Gardiner and Kernell 1990; Gossen et al. 2003). This input-output relationship of extrinsic synaptic inputs to MU firing outputs throughout the MU pool (Devanne et al. 1997; Heckman and Binder 1991), has been shown at the level of single motor neurons to have firing rate gain dependent on the magnitude of the injected current (Granit et al. 1963a, 1966a; Heckman and Binder 1991). After reaching firing RT, the firing rate, is described to reside within three defined ranges dependent on the state of injected current, approximating the magnitude of synaptic input bombardment in vivo (Matthews 1996). These three ranges correspond to the subprimary, primary and secondary firing rate ranges, dependent on the firing-current relationship. In the subprimary range, MU firings have a sub-linear relationship to injected currents (Granit et al. 1966a; Kudina 1999; Manuel and Heckman 2011) and result in greater firing irregularity as the extrinsic synaptic inputs fluctuate above and below firing RT (Matthews 1996; Petersen and Berg 2016). While in the primary range, the input-output relationship is largely linear (Granit et al. 1966a) and has been assumed to be the range of most MU recordings in intact humans during ramp and sustained isometric contractions where the voluntary contraction intensity is increased or
held constant (Clamann 1969). Finally, the secondary firing range is described as a steeper (i.e., supra-linear) current-frequency slope relationship to extrinsic inputs resulting in greater firing rate gain (Granit et al. 1966b), and may have importance to generate a large tetanic effect at the muscle during fast limb movements (Harwood et al. 2011).

Motor neuron firing is activated by synaptic inputs that depolarize the intrinsic state above firing RT (Hounsgaard et al. 1988), then, spinal motor neuron persistent inward currents (PICs) that are largely comprised of inward calcium ion conductance on dendrites act to depolarize the resting potential state (Harvey et al. 2005; Schwindt and Crill 1977). However, in brainstem motor neurons (hypoglossal nuclei), there is evidence showing that inward calcium and sodium ion conductance on the dendrite (Powers and Binder 2003) and calcium conductance on the soma (Moritz et al. 2007; Westenbroek et al. 2005) act as PICs. In relation to the axon hillock (i.e., initial segment), the distribution of depolarizing PIC channels are located at relatively distanced sections along the motor neuron soma and mainly on dendrites (Alvarez et al. 1998; Heckman et al. 2008), altogether displaying the complexity of intrinsic processes that drive and effect firing rate behaviour (Binder et al. 2020; Powers et al. 2012; Revill and Fuglevand 2011). The importance of PICs is to act as an independent intrinsic process from extrinsic inputs, that affects the input-output relationship, working to amplify, alter or saturate the firing rate response from excitatory synaptic inputs (Binder et al. 2020; Lee et al. 2003a; Lee and Heckman 2001; Schwindt and Crill 1980). Indeed, PICs are likely required to achieve full recruitment and firing rate gain of MU pools during voluntary contractions, and are theorized to have importance during sustained isometric (i.e., postural) contractions (Heckman and Enoka 2012). However, other intrinsic effects of motor neuron accommodation and adaption likewise effect intrinsic conductance and the input-output relationship of resulted firing rates (Kernell 1965a; Miles et al. 2005; Powers et al. 2012; Revill and Fuglevand 2011; Sawczuk et al. 1995).

In the intact system, the effect of PICs are monoamine dependent (5-HT and norepinephrine), and these neuromodulators are released from the brainstem permeating MU pools (Heckman et al. 2008) with the hypothesized effects observed to occur in
human participants under pharmacological (methamphetamine) intervention (Udina et al. 2010). Controlling the gain effects of PICs during passive limb rotation has recently been shown in the decerebrate cat, providing evidence that during movements, PICs in lower motor neurons that innervate the agonist muscle can be reduced during antagonist muscle stretch from Ia reciprocal inhibition (Hyngstrom et al. 2007). These results suggest that PICs and reciprocal inhibition act as gain-control, push-pull mechanisms of MU pool excitability during movements between tightly coupled flexor-extensor muscles (Binder et al. 2020; Hyngstrom et al. 2008; Johnson and Heckman 2010, 2014). During voluntary contractions, this gain-control inhibition to the MU pool likely results in a more linear input-output relationship at the lower motor neuron, as shown in low threshold MU activity during voluntary contractions in human participants with inhibitory inputs provided through sural nerve stimulation (cutaneous afferent) (Revill and Fuglevand 2017). Therefore, during voluntary movements, extrinsic descending synaptic inputs provided to the MU pool that are modified by intrinsic lower motor neuron PIC effects would likely be dampened once the limb is moving, by short latency extrinsic inputs from the periphery (Johnson and Heckman 2010, 2014). However, the MU firing rate trajectory during voluntary movements is largely undescribed.
Figure 1.1 Schematic of basic concepts that activate and sustain motor unit (MU) activity during voluntary muscle contraction.

A1) Size ordered recruitment thresholds based on the relationship of somatic alpha lower motor neuron cellular resistance and excitatory synaptic input to the MU pool. A2) Firing rate gain relationships of each MU to net excitatory synaptic inputs, with the sub-primary (SPR), primary (PR) and secondary ranges (SR) illustrated by different linear slopes. These linear relationships are modified (to become non-linear) by effects of the dendritic persistent inward current (PIC) and its neuromodulators (serotonin and norepinephrine). A3) Schematic MUs for a variety of different muscles with the contractile tension controlled by spatial (i.e., recruitment) and temporal (i.e., firing rate) frequency control, based on the recruitment threshold (A1) and firing rate gain (A2) of each MU. B) Conceptualization of MU innervation ratios across different muscles.
1.2 Motor unit structure and function

Expanding into the periphery, the axon of the alpha lower motor neuron synapses onto extrafusal muscle fibres at the neuromuscular junction permitting excitation-contraction coupling. Therefore, MU firing activity acts as the efferent neural drive ultimately controlling muscle contraction. The MU comprises a lower motor neuron, its myelinated axon and innervated muscle fibres that are integrated within the extracellular connective matrix (Heckman and Enoka 2012; Reinking et al. 1975; Willingham et al. 2020). The size of MU pools have muscle-dependence, likewise, the ratio between the lower motor neuron and the number of innervated muscle fibres for each MU are also muscle dependent (Gath and Stålberg 1981; Goldberg et al. 1998; Heckman and Enoka 2012; Rafuse et al. 1997). At the motor endplate, acetylcholine binding to ligand-gated channels will produce inward sodium currents at the muscle fibre membrane that will ultimately initiate a muscle fibre action potential, propagating along the muscle membrane in opposing directions, to initiate the excitation-contraction coupling signal cascade at the T-tubules (Kuo and Ehrlich 2015). This results in an increase of the intrinsic muscle fibre calcium ion concentration by efflux into the cytosol from the higher concentration gradient in the sarcoplasmic reticulum. The increase of calcium ion in the cytosol results in conformational changes of troponin, freeing the active binding site for myosin cross-bridges on the actin molecule that together permit the shortening of sarcomeres and therefore sarcomere forces (tension) being produced (Herzog et al. 2012). Importantly, shortening velocity of the muscle fibre is dependent on cross-bridge cycling that is rate-limited due to myosin ATPase activity in the non-fatigued state in which ATP concentrations (and other nucleosides) are abundant. Furthermore, the phosphorylation of the regulatory light chain of sarcomeric myosin, is dependent on myosin light chain kinase activity that can permit changes to skeletal muscle function. This muscle potentiation is theorized to increase the rate of cross-bridge cycling force generation caused by increased calcium ion sensitivity of the contractile apparatus (Stull et al. 2011; Vandenboom et al. 2013), and may have critical importance during faster movements (Brown and Loeb 1999, 2000a). For the whole muscle, the velocity of muscle shortening is likely dependent on the fastest velocity fibres, as negative forces can increase
shortening velocity of slower type MUs (Edman 2014). The relationship between muscle force and excitation-contraction coupling which is dependent on tetanic neural inputs (i.e., firing rate) or time-dependent calcium ion inputs to the cytosol has been explored extensively in isometric contraction, showing an S-curve (i.e., sigmoidal) force-frequency relationship from whole muscle, single MU and single fibre preparations (Balnave and Allen 1996; Bigland-Ritchie and Woods 1984; Cooper and Eccles 1930; Ebashi and Endo 1968; Eccles et al. 1961; Edwards et al. 1977; Fuglevand et al. 1999; Huxley 1957; Thomas et al. 1991). However, in relation to movement, reduced preparations experiments that use isotonic or isovelocity controls, are limited by fixed tetanic frequency (Edman 1979; Edman and Josephson 2007; Hill 1964; Kristensen et al. 2018), and cooler experimental temperatures, with few exceptions (Brown et al. 1999; Brown and Loeb 1999, 2000b, 2000a).

Within each muscle, the MU pool which is comprised of tens to perhaps thousands of discrete MUs has many properties that are likely muscle dependent, as the distribution of fibres types are vast across muscles from human cadavers (Johnson et al. 1973). Despite differences of muscle fibre composition across muscles, the muscles are commonly controlled by the nervous system using two common mechanisms that first involve MU recruitment (i.e., spatial frequency control), and firing rate modulation (i.e., temporal frequency controls) acting to produce muscle tension throughout the muscle (figure 1.1). For each MU in the non-fatigued state, muscle tension when provided single twitch or tetanic inputs are dependent on the structure of the innervated muscle fibres (Barclay et al. 1993; Lewis et al. 1994), that are largely homogenous per MU and are characterized by three main fibre type classifications which are 1) histochemical staining for myosin adenosine triphosphatase (mATPase), 2) myosin heavy chain identification, and 3) biochemical identification of metabolic enzymes (Scott et al. 2001). Unlike some muscles from non-human species (Bakels and Kernell 1993a; Brown and Loeb 1999; Manuel et al. 2018; Sutlive et al. 1999), many human muscles have MU and fibre type heterogeneity (Johnson et al. 1973), indicating that the matched innervating MUs are also diverse to a certain extent in their electrophysiological properties (Bakels and Kernell 1993b, 1993a; Bellemare et al. 1983; Dum and Kennedy 1980; Eccles et al. 1958;

In upper limb nerves, sensory axons outnumber motor axons by a ratio of nine to one (Gesslbauer et al. 2017), therefore, sensorimotor feedback likely have great importance in the dynamic control of single MU activity during movements (Jami 1992). For the modulation of voluntary supraspinal inputs to the MU pool in humans, the influence of propriospinal and afferent feedback tracts/circuits should be appreciated (Watson et al. 2009; Windhorst 2007), and are depicted in an oversimplified schematic of an agonist muscle single lower motor neuron (figure 1.2, A). Upper motor neurons from the corticospinal tract can directly and indirectly synapse onto lower motor neurons (Gu et al. 2017; Ueno et al. 2018), with the majority of upper motor neurons synapsing onto interneuron populations (Watson et al. 2009), with propriospinal influences reflecting task specificity (Nicolas et al. 2001). In the spinal cord, some lower motor neuron (alpha and gamma) axons can also synapse onto Renshaw cells (figure 1.2), which have a recurrent inhibitory affect (Renshaw 1941; Windhorst 2007). Within the limb, there are proprioceptive feedback, including the muscle spindle system, Golgi tendon and kinesthetic afferents that provide direct and indirect feedback to the lower motor neuron (i.e., MU), in addition to cutaneous, propriospinal networks, sub-cortical and cortical regions that effect movement and muscle function (Fink et al. 2014; Heckman 1994; Jami 1992; McNulty and Macefield 2001; Partridge 1967; Pruszynski et al. 2011; Scott 2004; Weiler et al. 2019; Windhorst 2007). Of direct importance to short duration voluntary contractions, afferent feedback from the muscle spindle and Golgi tendon organ systems of antagonist muscles can effect lower motor neuron intrinsic excitability innervating the agonist muscle (Hyngstrom et al. 2007; Revill and Fuglevand 2017; Weiler et al. 2019), once the voluntary contraction has been initiated (Vallbo 1971). Likewise, afferent feedback from the agonist muscle (i.e., parent muscle or homonymous) can also modify lower motor neuron excitability through muscle spindle (Capaday and Stein 1987; Jankowska 1992; Meunier and Pierrot-deseilligny 1989; Pratt 1995) and Golgi tendon feedback (Houk and Henneman 1967).
At the single MU level, local field potentials of muscle fibres are easily accessible by electromyography (EMG) recordings, supporting the large component of present-day neuroscience informed by output characteristics of alpha lower motor neurons (Binder et al. 2020; Heckman and Enoka 2012). One reason has been attributed to the widespread use and early adoption of electricity to study physiological systems, with the muscle providing a window into the nervous system requiring only minimal hardware (voltage) amplification (Duchateau and Enoka 2011; Duchenne 1867; Galvani 1791). From some of the first intramuscular recordings during voluntary contractions by Wachholder (Wachholder 1928), and Adrian and Bronk (Adrian and Bronk 1928, 1929), the gradation of muscle force was shown to be caused by MU recruitment and firing rate modulation, however, these were largely done under lower contraction forces and during isometric contraction (constant joint angle). Since then, understanding of single MU activity during voluntary contraction remains largely informed from experiments during isometric contraction and at lower contraction forces (figure 1.2, B), despite almost every human behaviour requiring movements that involve muscle shortening and lengthening contractions of skeletal muscle to displace joints, facial sheaths, and skin.
Figure 1.2 Cartoon representing a simplified account of the somatic nervous system spinal circuitry to an agonist muscle during voluntary contraction.

Panel A, was based on diagrams from two prior reviews (Gandevia 2001; Windhorst 2007). The scale of the alpha motor neuron (a-MN) diameter can reach up to ~120 µm with muscle fibres at ~200 µm. A) Simplified spinal circuitry of the agonist muscle. The small, black-filled circles represent inhibitory interneurons, while unfilled circles represent excitatory interneurons. Dashed lines represent pre-synaptic inhibition. Abbreviated terms: a-MN, alpha motor neuron; b-MN, beta motor neuron; y-MN, gamma motor neuron; RC, Renshaw cell. There are many other factors not shown that alter motor neuron excitability including neuromodulators, supporting non-neuronal cells, smaller vesicles, extracellular fluid characteristics, etc. B) Simplified agonist muscle. At the top, the raw data depicts a tungsten intramuscular electromyography (EMG) recorded from a single motor unit potential train of the first dorsal interosseous (FDI) muscle of the hand during a sustained voluntary isometric contraction at ~10% of maximum finger abduction torque. In awake humans during voluntary contraction, the firing rate behaviour of single motor units is used to explore the corresponding firing rate properties of alpha motor neurons in the spinal cord or brainstem. In the middle, the cartoon depicts a muscle fascicle that is joined to connective tissue and an axis of rotation of a limb joint.
1.3 From voluntary isometric contraction to movements

The transient role of MU activity is to provide a controlled dose of tension within the muscle. If enough agonist muscle tension is achieved through MU recruitment and firing rate gain (figures 1.1 and 1.2), the resultant force will either remain isometric or overcome the summary moment of inertia. If the inertia is overcome, the result is a load-moving muscle shortening contraction and ultimately limb movement (figure 1.3). Here, we focus on studies of single MU activity from humans participants during voluntary contraction with some form of finger or limb movements.

In the neural control of voluntary isometric contraction, observations of both single and population-based MU activity are well established, utilizing intramuscular and surface EMG technologies. From these observations during simple voluntary contraction to produce joint torque, low threshold MU samples have been more extensively explored, with few comparisons across many muscles. In many human experiments, the measured muscle force is represented in the limb torque that is contributed from several agonist muscle groups and negated by antagonist muscles. In human experiments, targeted torque levels, associated with different voluntary contraction intensities are used as the independent variable to grade comparisons of firing rate responses (Kukulka and Clamann 1981; Monster and Chan 1977). Analogous to injected current preparations (Granit et al. 1966a, 1966b), the relationship between voluntary contraction intensity and descending synaptic inputs to the MU pool are presumed to be closely related (Devanne et al. 1997; Fuglevand et al. 1993; Heckman and Binder 1991; Todd et al. 2004). From human studies during isometric contraction, firing rate variance is expected to be closely related to muscle contractile properties of torque and rate of torque development (RTD) (Bellemare et al. 1983; Desmedt and Godaux 1977, 1978, 1979; Gossen et al. 2003; Del Vecchio et al. 2019), supported from the more extensive literature on the relationship between firing rate and muscle fibre contractile shortening speed in reduced isometric preparations (Bakels and Kernell 1993a, 1993b; Dum and Kennedy 1980; Eccles et al. 1958; Gardiner 1993; Gardiner and Kernell 1990; Hammarbarg and Kellerth 1975; Kernell 1979). However, this direct linear relationship between firing rate and contractile properties may be modified by the intrinsic state of the lower motor neuron, as low
threshold MUs have different firing rate behaviours dependent on imposed reflex inhibition (Fuglevand et al. 2015; Revill and Fuglevand 2017) and implicates the importance of intrinsic factors, such as PICs during sustained isometric contractions. Whereas during rapid maximal effort contraction, the population-based response of MU recruitment timing determines the peak torque during isometric conditions (Desmedt and Godaux 1977, 1978; Desmedt 1980; Del Vecchio et al. 2019), inferring a movement speed outcome. Under higher contraction intensity efforts, in which higher threshold MUs are active, many comparisons have been made between age groups (see section 1.4) and during different types of task-specific fatigue during isometric contractions (Bigland-Ritchie et al. 1983; Gandevia 2001; Peters and Fuglevand 1999), but not during movements. During isometric ramp contractions in the non-fatigued state without large effects of extrinsic inhibition, the non-linear relationship of firing rates as a function of increasing contraction intensity (i.e., torque) is observed across many muscles (Fuglevand et al. 2015; Kim et al. 2020; Revill and Fuglevand 2017; Wilson et al. 2015). However, during steady-torque isometric contractions, a central component of firing rate responses when the force is held constant (analogous to the concept of ‘steady state’) are that firing rates occur from an underlying Gaussian distribution, with the average firing rate assumed to be stationary (Clamann 1969). During isometric maximal voluntary contractions (MVC), one of the first studies to compare MU firings from three different muscles at relative contraction intensity was by Bellemare et al., in the lab of Bigland-Ritchie (Bellemare et al. 1983). They found that averaged firing rates were different by up to a four-fold range between three tested muscles and that absolute firing rates were theorized to be limited by characteristics of the MU pool. However, it remains unclear whether this stratified relationship of MU control occurs in all >600 uniquely innervated muscles of the human somatic nervous system (Kukulka and Clamann 1981) and if these relative differences among muscles persist throughout lifespan or are similarly shaped during muscle shortening or lengthening contractions.

In contrast, during voluntary shortening muscle contractions that result in movement, observations of single MU activity have been made very sparingly in humans using intramuscular or subcutaneous EMG techniques. Based on earlier work by Hill (Hill 1922; Lupton and Hill 1923). One of the first links between MU activity with contractile
tension, and MU activity with velocity in humans was made in the plantar flexors during constant tension ankle joint rotation at different movement speeds (Bigland and Lippold 1954b). In this approach, MU activity was measured by global surface and intramuscular EMG that could not differentiate between the contribution of MU recruitment or firing rate modulation. From this first view linking MU activity to limb kinematics, there were observed correlations only during iso-velocity muscle shortening contraction at different set velocities, as compared to lengthening, and in agreement with the application of Hill’s force-velocity and power curves (Hill 1922). Only later, were global EMG relationships compared between voluntary muscle shortening and lengthening in the plantar flexors (Nardone and Schieppati 1988). As compared to experiments during voluntary contractions in humans, some of the first and most extensive characterizations of the relationship between neural inputs to muscle, and muscle mechanical outputs, are from LD Partridge and his experiments on reduced preparation in cats. These experiments used electrical inputs to whole muscle to understand the mechanical responses (Partridge 1965, 1966, 1967) with muscles arranged in both series and parallel (Demieville and Partridge 1980). Findings showed that Newtonian mechanics were observed to explain many movement related inferences based on force and acceleration, and that the importance of relationships between firing rate inputs and muscle lag were dependent on the baseline inertial load (Partridge 1965, 1966). Again in reduced preparations, but at finer scale at the fascicle level, later theoretical models describing movements (Loeb et al. 2002) were based on integrated data from experiments at physiological temperatures in relation to post-activation potentiation (Brown and Loeb 1999), stimulus frequency (Brown et al. 1999), force enhancement and depression (Brown and Loeb 2000b) and activation dynamics (Brown and Loeb 2000a). Indeed, the integration of firing rate control of shortening muscle is complex and not fully understood. For example, it remains unknown how firing rates control the entirety of muscle mechanics as the shortening muscle itself is self-driving and self-limiting, having unique properties based on the inverse force-velocity relationship (Edman 1979; Hill 1938). Such that faster muscle shortening velocity under Huxley’s sliding filament model results in fewer attached cross-bridges (Huxley 1957), but with cross-bridge cycling ultimately determining the rate of muscle shortening (Edman 2014).
Bioenergetically, more heat is released during a shortening contraction as compared to an isometric contraction (Fenn 1923; Lupton and Hill 1923), and later revisions of the Hill-Huxley models showed that the constant of shortening muscle heat is dependent on both the shortening velocity and muscle tension (Hill 1964). To achieve this, shortening contractions required a two-stage process to explain the heat release (Huxley 1973), that first involves the attachment/detachment without energy use, and followed by the second stage of attachment resulting in force development. Altogether, this meant that the fraction of cross-bridges that cycle to expend ATP and produce tension are proportional to the squared time available for attachment, therefore resulting in greater shortening speeds that have more saturated heat release (Hill 1964; Huxley 1973), suggesting that muscle shortening velocity is independent of energy constraints in the unfatigued state.

In human participants, understanding how single MU activity is linked to and controls limb movement has occurred through observations of muscle shortening (i.e., concentric) and lengthening (i.e., eccentric) contractions in the upper limb from recordings in the hand (Desmedt and Godaux 1979; Gydikov et al. 1986; Howell et al. 1995), anconeus, biceps brachii, triceps brachii and tibialis anterior muscles (Cowling et al. 2016; Garland and Griffin 2000; Garland et al. 1996; Gydikov et al. 1986; Harwood et al. 2011, 2012; Harwood and Rice 2012, 2014; Ivanova et al. 1997; Kossev and Christova 1998; Oliveira and Nergo 2021; Del Valle and Thomas 2005), with fewer observations in the lower limb (Grimby 1984), but mainly during slow isokinetic shortening contractions (Kallio et al. 2013, 2014; Oliveira and Nergo 2021). From identification of distinct characteristics of MU potential waveforms, the MU recruitment order of the first dorsal interosseus muscle was not changed during voluntary contraction in isometric or load-moving conditions, but during rapid contractions (termed “ballistic” in this paper) shifted recruitment thresholds to be lower during isometric and load-moving contractions (Desmedt and Godaux 1979). However, defining a rapid isometric contraction as ballistic was criticized by a contemporary paper that studied rapid limb and finger movements (Gydikov et al. 1986), as the definition of ballistic in physics relates to an object in motion (i.e., a projectile in flight). From these prior experiments that only occurred during continued muscle shortening contractions, and therefore in the positive acceleration phase of movement, a next study that tracked distinct MU potentials from the lateral head of the triceps brachii
muscle found that the same MU recruitment order occurred in limb acceleration and
deceleration of elbow joint movement, and the number of MU counts, indicative of both
MU recruitment and firing rates was positively related to the magnitude of limb
acceleration (Garland et al. 1996; Ivanova et al. 1997). In other experiments that relied on
following the same MU potential waveform, averaged firing rates were calculated
throughout a large range of motion and were compared between movement speed
conditions (Harwood et al. 2011; Harwood and Rice 2012, 2014). From this approach,
MU recruitment thresholds were negatively correlated with peak limb movement speed
(Harwood and Rice 2012) and they found that higher averaged firing rates (defined as 50-
100 Hz in the anconeus muscle) were not related to MU recruitment thresholds (Harwood
and Rice 2014).

In tracking single MU firings throughout muscle shortening and lengthening contractions
of the agonist muscle, there is greater relative MU firing rate activity during muscle
shortening than isometric contractions (Harwood et al. 2011; Howell et al. 1995; Ivanova
Across targeted elbow extensions contractions at different velocities, averaged firing
rates were positively related to limb movement velocity (Harwood et al. 2011). However,
apart from averaging single MU firing rate activity during unconstrained-velocity limb
movements, none have systemically quantified changes of the firing rate trajectory
throughout contraction time course. From one of the first studies investigating single MU
activity in relation to movement, firing rates of the medial biceps brachii and medial
interosseus dorsalis were plotted during spring-resisted voluntary movements (Gydikov
et al. 1986), and were interpreted to be constant throughout contraction time course and
were velocity dependent during submaximal velocities. However, firing rates were
observed to quickly decrease during “very quick ballistic” limb or finger joint movements
(Gydikov et al. 1986). As these changes of firing rates were attributed to be dependent on
a velocity effect, there remains considerable room to further explore how firing rates may
be related to the magnitude of descending inputs, kinematic relationships, and afferent
feedback.
Movement by Newtonian definition occurs within a time-dependent domain, as motion of the limb (i.e., load) moves through space dependent on the force vector acting on it (i.e., acceleration). In relating MU activity to more naturalistic movements that occur on a daily basis, the velocity tracings of the limb are required to have unconstrained-velocity and are shown to be bell shaped (Garland et al. 1996; Hallett et al. 1975; Harris and Wolpert 1998; Harwood et al. 2011; Ivanova et al. 1997). As a function of contraction time course, single MU firing rate activity during voluntary movement has been most extensively studied in extra-ocular muscles that control eye movement (Van Gisbergen et al. 1981; Robinson 1970), and supported from electrical stimulation studies relating single MU and whole extra-ocular muscle responses (Goldberg et al. 1998). However, brainstem motor neurons that innervate eye muscles are likely different than spinal motor neurons that govern limb movement, for example, the electrophysiological responses of firing rates to injected currents are markedly different and higher in motor neurons of the hypoglossus (Tadros et al. 2016).

In limb movement of non-human primates, single and population-based neuronal activity from supplementary motor cortex (Russo et al. 2020), primary motor cortex (Churchland et al. 2012; Churchland and Shenoy 2007a; Evarts 1968; Russo et al. 2018) and the spinal cord of decerebrate turtles (Petersen and Berg 2016) are shown to have non-stationary firing rate activity during reaction-time and stereotypic reflex movements. When analyzing the population-based activity in a dynamical systems framework (Yu et al. 2009), there are observed rotational dynamics of neurons (i.e., motor and interneurons) that contribute towards the ultimate muscle contraction, with relationships interpreted across different studies to be predictive of muscle EMG responses, force, and movement kinematics (Evarts 1968; Sergio et al. 2005), or correspond to other features than just kinematic or muscle outputs (Churchland et al. 2012; Churchland and Shenoy 2007b; Russo et al. 2018). Although, to our knowledge, cortical motor neurons do not directly synapase onto the muscle, and therefore, the spinal motor neuron firing rate characteristics, representing the intermediary step of the pathway from brain-to-muscle has not been measured during these experiments. Likewise, during most voluntary contraction experiments that measure MU firing rates that represent lower motor neuron outputs, movement speed outcomes are inferred from the isometric state. From extra-
ocular muscles, the firing rate characteristics of brainstem motor neurons were shown to almost directly relate to movement speed as tension had a near instantaneous response to firing rate (Robinson 1970). This finding was largely supported from stimulated extra-ocular MU experiments finding the time delay between stimulated inputs and whole muscle twitch responses was on the order of milliseconds (Goldberg et al. 1998), but found MUs could be grouped by physiological responses that were frequency dependent. However, these relationships during eye movement, may not correspond to the speed of limb movement, as the phase lag between developing muscle tension and torque are greater in the limb in response to firing rate inputs (Baldissera et al. 1998; Buchthal and Rosenfalck 1960; Cavanagh and Komi 1979; Partridge 1966). Furthermore, evidence suggests that the contraction velocity of the fastest muscle fibres influence the shortening velocity of the whole muscle (Demieville and Partridge 1980; Edman 1979, 2014), providing additional rationale for how limb muscles, which comprise few fast-twitch MUs in human muscles (Johnson et al. 1973) with presumably higher innervation ratios (Rafuse et al. 1997) may be controlled differently to achieve fast limb movement speeds. Therefore, it may be critically important to understand how MU activity occurs as a function of contraction time course in the limb (figure 1.3) in relation to gradations of descending synaptic inputs and across different tasks, as efferent controls that produce changes of the muscle state represent an impetus for afferent feedback controls (Capaday and Stein 1987; Houk and Henneman 1967; Meunier and Pierrot-deseilligny 1989; Pratt 1995; Vallbo 1971).
Figure 1.3 Simplified schematic of MU controls and feedback from an agonist muscle with question marks pertaining to unknown concepts.

A) Descending inputs to the agonist muscle. B) Recruitment and firing rate modulation from the MU pool during isometric contraction and is largely unknown during limb movement. C) Afferent feedback (Ia, Ib, Aα/β, II, III & IV) and the individual and combined effects on single MU activity during movement are largely unknown in an integrated framework (Al-Falahe et al. 1991; Reschehtko and Pruszynski 2020; Vallbo 1971). Not shown but should be appreciated is the complexity of other inputs from sources of different spinal tracts, propriospinal, antagonist, and synergist muscles.
1.4 Brief overview: voluntary motor unit control throughout human lifespan

When we can no longer move quick enough, the *grim reaper* catches up (Stanaway et al. 2011). In this epidemiological analysis by Stanaway et al., walking faster than 3 km per hour resulted in a marked decrease in all-cause mortality, stressing how *health* is intricately linked to our ability to keep moving. In human participants, the comparison of voluntary contractions between young and aged groups provides researchers the ability to measure and contrast MU activity between two different neuromuscular states (Opie and Semmler 2014; Peinemann et al. 2001; Rowe et al. 2006; Wolpe et al. 2016) independent of the relative voluntary intensity (Jakobi and Rice 2002; Rozand et al. 2017).

For those participants lucky enough to survive into older age, the structure and function of MU morphology and activity in the aged system of old and very old adults (McNeil et al. 2005) has undergone a prolonged acclimatization process to an altered physiological environment. For example, the altered physiological environment can be understood as the broad insult of biochemical changes and cellular degradations (Adalbert and Coleman 2013; Delbono et al. 2021; Hepple and Rice 2016) and the relationship of lifestyle behaviours (social interaction, exercise, diet, air quality, etc.) influence the rate of insult (or resilience) impacting the quality and quantity of the MU pool (Booth et al. 2017; Chopek and Gardiner 2010; Christie et al. 2011; Hepple and Rice 2016; Narici et al. 2008). With sufficient ageing, these compounding insults in humans result in MU loss (Terao et al. 1996; Tomlinson and Irving 1977) and are supported from evidence of subsequent MU remodeling through the process of collateral reinnervation (Allen et al. 2013; Brown et al. 1988; Campbell et al. 1973; Chan et al. 2001; Dalton et al. 2008; Doherty and Brown 1993; Gilmore et al. 2016, 2017; Hourigan et al. 2015; Saboisky et al. 2014). In adult participants, electrophysiological recordings show that MU potentials from the survived MU pool are relatively larger in older adults as compared to young adults (Ling et al. 2009; Stålberg and Fawcett 1982). In humans, when morphological changes of the muscle (Overend et al. 1992b, 1992a) and MU loss from the effects of ageing occurs, subsequent MU remodeling at the muscle is associated with muscle fibre atrophy, grouping of similar muscle fibre type (Jakobsson et al. 1990; Larsson and Edström 1986; Lexell et al. 1988; Lexell and Downham 1991) and motor end plate
instability (Wokke et al. 1990). Functional consequences of these anatomical changes at the muscle therefore result in different twitch and tetanic responses of older adult muscle that are slower and have longer relaxation times as compared to young adults (Allman and Rice 2004; Vandervoort and McComas 1986).

In human newborns, MU activity (recorded from the tibialis anterior muscle) is largely synchronized during lower limb kicks and is hypothesized to be attributed to reflexive inputs (Vecchio et al. 2020) apart from voluntary controls via descending spinal tracts that are not yet fully myelinated (Watson et al. 2009). With comparisons between young and older adults, older adults are shown to have higher torque thresholds at MU recruitment (Kamen et al. 1995), have lower instances of doublet discharges (Christie and Kamen 2006, 2009) and have lower averaged firing rates (Connelly et al. 1999; Dalton et al. 2009, 2010; Erim et al. 1999; Kamen et al. 1995; Kamen and Knight 2004; Kirk et al. 2018, 2019; Roos et al. 1999). From these changes, a functional consequence of altered MU activity is the result of force unsteadiness during sub-maximal contractions in older adults (Christou and Enoka 2011; Galganski et al. 1993; Moritz et al. 2005; Tracy and Enoka 2002). Despite these many studies, few have compared MU activity across more than two muscles from one individual or more critically, have not examined MU activity during movements (i.e., shortening muscle contractions) in older adults with a comparison to a young adult group. The importance of such comparisons are to understand how changes of MU activity with ageing differently control voluntary movements, or may provide insights regarding how older adults are more fatigable during repeated movements (Dalton et al. 2015; McNeil and Rice 2007). From experiments of single MU activity in limb muscles during joint rotation (i.e., movement), we are aware of only one study that has made recordings in older adults. In the soleus muscle of older adult males (mean 69, standard deviation 5 years), MU firing rate were measured between isometric, shortening and lengthening contractions during slow isokinetic contractions (10° deg • s⁻¹) at different voluntary contraction intensities (Kallio et al. 2014). From this, averaged firing rates were observed to be higher during the shortening muscle contraction at 10, 20 and 40% of plantar flexion MVC, as compared to the isometric and lengthening contraction conditions (Kallio et al. 2014), but there was no direct comparison to a younger adult group so an age-related effect was not quantified. It
remains unknown how firing rates are different between age groups during limb movement, and if there are age-dependent or sex-dependent differences in MU control throughout different aspects of the contraction time course.
1.5 The elbow extensor muscle group

In three of the four chapters, the elbow extensor muscles were utilized (see the figure in appendix R), and specifically the anconeus muscle to probe how single MU activity related to movement. Therefore, this is a brief overview of the elbow extensor muscle group.

Although the most comprehensive studies between MU firing rates and movement have occurred in the extra-ocular muscles of non-human primates, there are several reports comparing averaged MU firing and recruitment threshold to different conditions of muscle shortening and lengthening contractions, and joint movement speed. In awake humans, recording MU activity during voluntary movement has occurred mainly in the agonist muscle during shortening contractions of the elbow extensors (Cowling et al. 2016; Garland and Griffin 2000; Garland et al. 1996; Harwood et al. 2011; Harwood and Rice 2012, 2014; Kossev and Christova 1998; Del Valle and Thomas 2005). Like most limb movements, the activity of several muscle agonists and synergists are required to initiate position change, while antagonist muscles act to stabilize the joint and support movement termination (Hallett et al. 1975; Ivanova et al. 1997). In the upper limb, the radial nerve innervates the elbow extensor muscle group which comprises the long, lateral, and medial heads of the triceps brachii and the anconeus muscle. Specifically, the spinal motor neuron column for the anconeus muscle resides in the C6-7 region of the human spinal cord (Levin et al. 1996). The long head of the triceps brachii muscle originates on the infraglenoid tubercle of the scapula, whereas the lateral and medial heads originate above and below the radial groove on the humerus, respectively. Only the long head crosses both the glenohumeral and elbow joints, with all three heads inserting on the olecranon process of the ulna bone. The anconeus muscle acts as both an elbow joint stabilizer and elbow extensor originating just posteriorly to the lateral epicondyle of the humerus and inserting along the proximal third of the posterior face of the ulna (Le Bozec and Maton 1987; Coriolano et al. 2009). Each elbow extensor muscle has a distinct fibre type composition, architectural properties, and force profiles (Le Bozec and Maton 1987; Coriolano et al. 2009; Davidson and Buford 2006; Davidson and Rice 2010; van Groeningen and Erkelens 1994; Harwood et al. 2013; Johnson et al. 1973; Klein et al.
2001; Miguel-Andres et al. 2017; Stevens et al. 2014; Wilson et al. 2015). From experiments by Le Bozec and Maton (Le Bozec et al. 1980; Le Bozec and Maton 1982, 1987; Maton et al. 1980), the fibre type composition was measured along with twitch properties and integrated surface EMG, with MU firings measured up to 30% of MVC. The anconeus was found to have a comparatively slower fibre type profile (~63% type I) and was speculated to have all constituent MUs recruited by a set torque of 30% of maximum (Le Bozec and Maton 1987). Furthermore, the anconeus was shown to aid in elbow joint movement precision (Le Bozec and Maton 1982), being estimated to be a weak extensor contributing a small amount of total elbow joint extension, likely ~5% of MVC or less (Le Bozec et al. 1980; Le Bozec and Maton 1982, 1987; Coriolano et al. 2009; Maton et al. 1980; Miguel-Andres et al. 2017). Unlike the anconeus, the fibre type composition of the triceps brachii muscles are predominantly faster twitch (~33% type I) (Johnson et al. 1973).

Specific to experiments of single MU activity of the elbow extensors during voluntary isometric contraction, MU firing rates, recruitment thresholds, the delta-F (an indirect estimate of the PIC effect on MU firing rates) and age-related comparisons up to MVC have investigated the lateral and medial heads of the triceps brachii (Le Bozec and Maton 1982, 1987; Dalton et al. 2010; Ivanova et al. 1997; Katz et al. 1991; Wilson et al. 2015). Whereas MU number estimates (MUNE) and MU potential characteristics have been investigated only in the anconeus muscle in young and very old adults (Gilmore et al. 2017; Stevens et al. 2013). In work by Harwood et al., synergist muscle activity was compared across the lateral and long head of the triceps brachii with the anconeus muscle during isometric ramp contractions up to 75% of MVC (Harwood et al. 2013) finding that limb position in addition to muscle dependent differences of MU firing rates and RT should be considered when making comparisons between muscles. Furthermore, the lateral head of the triceps brachii had higher averaged firing rates as compared to the anconeus muscle (Davidson and Rice 2010; Harwood et al. 2013). In elbow extension movements that would involve shortening contractions of the triceps brachii and anconeus muscles, MU activity recorded from the lateral (Garland and Griffin 2000; Garland et al. 1996; van Groeningen and Erkelens 1994; Ivanova et al. 1997) and medial (Kossev and Christova 1998) heads of the triceps brachii muscle have been investigated,
but the majority of movement-related experiments at higher contraction intensities have investigated the anconeus muscle. These experiments compared averaged firing rate for each contraction from MU trains followed between different targeted movement velocities up to maximum velocity (Harwood et al. 2011), have investigated firing rate in relation to torque RT (Harwood and Rice 2012), doublets and short inter-spike-intervals (Harwood and Rice 2014) and during moderately loaded movement fatigue in young adults (Cowling et al. 2016; Harwood et al. 2012).

Despite the small relative size and physiological role of the anconeus muscle, it has been used as a neuromuscular model for over 150 years contributing towards the study of limb movement (Harwood 2012; Stolworthy 2017), and described in some of the first experiments that electrically stimulated involuntary extension of the elbow joint (Duchenne 1867). In the cat, the anconeus muscle was also used in seminal work by Eccles comparing intracellular action potential profiles between fast and slow muscles, and compared the slower type anconeus muscle to faster type muscles (Eccles et al. 1958). In humans, the anconeus muscle has dual function as both an elbow joint stabilizer and weak extensor (Le Bozec and Maton 1982; Coriolano et al. 2009; Miguel-Andres et al. 2017), and undergoes relative fascicle length and pennation changes like the triceps brachii (Nelson et al. 2016; Stevens et al. 2014). It is likely that the anconeus muscle is active throughout elbow extension at all angles, forces, and velocities (Le Bozec et al. 1980; Maton et al. 1980), and activation precedes other elbow extensor synergists (Le Bozec and Maton 1987; Harwood et al. 2013).

The anconeus muscle morphology resembles a hemisected rectangular-based pyramid, with the base at the ulna and apex at the lateral epicondyle, and with the muscle fibres arising obliquely from the tendinous expansion where it inserts at the ulna. Thus, it has a penniform muscle fibre architecture that is able to produce more intrinsic force than it can displace (Coriolano et al. 2009). Furthermore, the unique anatomical qualities of the anconeus muscle being superficial, thin and undergoing relatively less muscle displacement (Coriolano et al. 2009; Grewal et al. 2019; Maselli et al. 1991; Stevens et al. 2014) with few estimated MUs (Gilmore et al. 2017; Stevens et al. 2013) are likely reasons why well-isolated intramuscular EMG recordings, using fine-wire techniques
(Basmajian and Stecko 1962), can differentiate single MU potentials during fast movement velocities in awake humans (Cowling et al. 2016; Harwood et al. 2011, 2012; Harwood and Rice 2014). As a model to probe how MU activity corresponds to movement in relation to measured kinematic parameters throughout voluntary contraction, and under different muscle states (i.e., ageing), the anconeus muscle serves to enable straightforward experimental recordings. However, the limitations of the anconeus muscle itself should be equally considered, with general functionality of its role as a supporting agonist muscle during elbow extensions.
1.6 Goals of this thesis

The purpose of this thesis was to gain insight as to how the somatic nervous system controls agonist muscle tension during conditions of isometric contractions in contrast to muscle shortening contractions that cause a limb joint to rotate. Prior studies in humans have investigated MU firing rates in relation to averaged comparisons between isometric and movement conditions (see section 1.3) but have not characterized MU firing rates during movements in relation to kinematic parameters or as a function of contraction time course. Because each movement requires a starting point and an end, the processes of motor control that are constrained by time-dependent factors require consideration for us to understand how the MU firing rate trajectory (i.e., neural drive to the muscle) can control contractions that result in movement. Likewise, in models that alter the motor system, such as normal adult ageing, the MU firing rate pattern is undescribed during movement. But such an experimental contrast to assess the same goal-directed voluntary contraction between two groups of vastly different ages, would provide valuable understanding to the role of the MU firing rate pattern throughout lifespan, despite having significant differences in absolute strength and movement speed. Thus, a central goal of this thesis was to generate new empirical observations of MU firing rates during limb movements. This was achieved by recording and analyzing single MU firing rate activity across several muscles in adult participants during voluntary contractions in vivo. Although technically difficult, and descriptive in nature, the aims of these studies were to provide direct recordings of ‘neural drive’ at the single MU level to test new and existing hypotheses regarding the neural control of voluntary movement in humans. Overall objectives were to: 1) compare MU firing rates across many different muscles in both young and older adult age groups; 2) compare how MU firing rates are different between young and older adult age groups during limb movements at maximal voluntary efforts; 3) explore through correlation analysis how MU firing rates are related to different kinematic parameters during limb movement; 4) to explore how the MU firing rate pattern is modified as a function of the contraction time course, consequently the MU firing rate trajectory; and 5) to explore how the MU firing rate trajectory is task
dependent between sustained isometric contractions and limb movements at a range of voluntary contraction efforts.

Below, I briefly summarize the objectives and general purpose for each chapter.

**Chapter 2** characterized MU firing rates associated with relative contraction intensities during voluntary isometric contractions across 12 distinct muscle groups. This was made possible by construction of an aggregated dataset of published and unpublished experimental data. In addition to muscle comparisons, an age-related comparison was made between a young and an older adult group. Firing rate ranges and firing rate variance was explained by muscle dependent and contraction intensity effects. With the addition of an age-related comparison, lower firing rate ranges across muscles was more dissimilar in relation to voluntary contraction intensity in the older group. The purpose was to characterize how MU firing rates are differently scaled among muscles relative to voluntary contraction intensity. This provided an initial framework to understand how MU firing rates are confined to absolute frequency ranges across muscles throughout lifespan during sustained isometric contractions.

**Chapter 3** measured single MU activity of the anconeus muscle followed throughout voluntary elbow extension contractions in young and very old adults during isometric contractions and limb movements. All efforts were at maximal voluntary contraction intensity (i.e., MVC), and single MU firing rate activity between age groups was compared. During isometric contraction, MU firing rates were lower in the older group. However, during limb movement, MU firing rates were not different between age groups. In comparison to the young during elbow extension limb movement, MU firing rates start lower at voluntary contraction onset but decrease at an attenuated rate throughout contraction time course in older adults. The purpose was to compare how MU firing rates recorded during maximal voluntary efforts are different between isometric contractions and limb movements in young and very old adults. This provided information regarding how single MU firing rates are not the limiting factor to explain voluntary movement weakness in the older group. Instead, during limb movements, MU
firing rates can be increased in both age groups as compared to isometric contractions, and function within a similar MU firing rate range during movement independent of age.

**Chapter 4** employed recordings of single MU firing rates in agonist muscles of the lateral head of the triceps brachii and anconeus during elbow extension limb movements at different velocities. Time-dependent effects were minimized between comparisons by instantaneously linking each MU firing rate to kinematic parameters throughout the contraction time course. Through correlation analysis, MU firing rates of the agonist muscles during elbow extension at different movement velocities were related to limb torque, but not movement velocity or position. The purpose was to define what parameters of limb movement are most closely related to MU firing rates in human participants. Considerable work has investigated the relationship of limb movement kinematics and surface EMG, however, surface EMG cannot discriminate between MU recruitment or firing rates during movement. Therefore, this chapter provided evidence in human participants that MU firing rates control elbow extension movements through the gradation of limb torque.

**Chapter 5** explored the relationship of single MU firing rates as a function of contraction time course between sustained isometric and limb movements during self-initiated and reaction-cued voluntary contractions. Characteristics of the MU firing rate trajectory, estimated through linear regression, were assessed using two experimental paradigms. These paradigms required voluntary contractions to be self-initiated (i.e., without a controlled go-cue) and reaction-cued by a visual stimulus. The reaction-cued paradigm also involved blinding the participant to the contraction condition that was isometric or load-moving. During self-initiated contractions at a range of voluntary intensities, the direction of the MU firing rate trajectory was related to the contraction task, that was either an isometric contraction or limb movement. Whereas during reaction-cued voluntary contractions, that involved the same preparation and initiation regardless of contraction condition, the MU firing rate trajectory was not different between contraction conditions. However, the timing of the MU firing rate trajectory relative to the go cue occurred sooner, and within a compressed time duration during limb movement as compared to the isometric contraction. The purpose was to study the
MU firing rate pattern as a function of contraction time course, therefore, estimating the underlying trajectory. We sought to explore what factors can change the MU firing rate trajectory, and designed experiments and analyses to make comparisons between isometric contractions and limb movements at a range of voluntary contraction intensities. In addition, the MU firing rate trajectory was compared between isometric and movement conditions from two types of voluntary contraction paradigms, that provided different states of descending motor commands to the spinal MU population. These consisted of 1) self-initiated contractions with the time prior to the voluntary contraction having no set external stimulus, and 2) reaction-cued contractions with the time prior to the voluntary contraction being determined by external visual stimuli. These approaches could measure the averaged MU firing rate trajectory in humans during movements that resemble contractions done on a regular basis outside the laboratory, and during the reaction-cued experiment the state of muscle contraction preparation and initiation was controlled. This chapter provided evidence that the MU firing rate trajectory, that represents the neural pattern of muscle activation is determined by descending motor commands that are task specific, but the trajectory can be further modified based on contraction intensity and during the movement related state.
Chapter 2

Motor unit firing rates during constant isometric contraction: establishing and comparing an age-related pattern among muscles

A version of this chapter has been published in the Journal of Applied Physiology (Kirk et al. 2021b), and is presented here with permission.

In comparison to young adults, motor unit (MU) firing rates are generally lower in aged adults at relative voluntary contraction intensities. However, from a variety of independent studies of disparate muscles, the age-related degree of difference in firing rates among muscles is unclear. Using a standardized statistical approach with data derived from primary studies, we quantified differences in firing rates across several muscles between younger and older adults. The dataset included 12 different muscles in young (18-35) and older adults (62-93 years) from 18 published and one unpublished study. Single MU activity was recorded from intramuscular electromyography during constant isometric contraction at different (step-like) voluntary intensities. For each muscle, firing rate ranges and firing rate variance explained by voluntary contraction intensity were determined using bootstrapping. Dissimilarity of firing rate variance among muscles was calculated by Euclidean distances. There were 3-fold differences in the absolute firing rate ranges across muscles in the young (soleus 8-16 and superior trapezius 20-49 Hz), but in the old, firing rate ranges were more similar and lower for 9 out of 12 muscles. In contrast, the explained firing rate variance from voluntary contraction intensity in the older group had 1.6-fold greater dissimilarity among muscles than the young (p < 0.001), with firing rate variance differences being muscle dependent. Therefore, differences between muscle firing rate ranges were not explained by how firing rates scale to changes in voluntary contraction intensity within each muscle. Instead, firing rates were muscle dependent but were more dissimilar among muscles in the older group in their responsiveness to voluntary contraction intensity.
2.1 Introduction

Skeletal muscles are comprised of different motor unit (MU) pools that have common neuromuscular controls of MU recruitment (Bawa et al. 1984; Henneman et al. 1964) and firing rate (firing rate) modulation. Despite general agreement on size-ordering of MU recruitment, there are multiple hypotheses about the mechanisms determining synaptic inputs and intrinsic amplifications that result in different firing rate ranges across different muscles (Le Bozec and Maton 1987; Calvin and Schwindt 1972; Chopek et al. 2013; Dum and Strick 2002; Eccles et al. 1958; Friede et al. 1984; McComas 1998; Schwindt and Crill 1995; Tadros et al. 2016; Wilson et al. 2015). Therefore, standardizing firing rate changes explained by voluntary isometric contraction among many muscles will contribute to understanding how different muscles are driven by the somatic nervous system.

In awake humans, electromyography (EMG) recordings of MU activity from muscle fibres closely approximates lower motor neuron firing (figure 2.1). Experimentally, synaptic inputs to the lower motor neuron cannot be quantified precisely in humans (Devanne et al. 1997), like what is done during step excitatory current preparations in non-human animals (Brownstone et al. 1992; Granit et al. 1966a; Kernell 1965a, 1965b; Schwindt and Crill 1982). However, the gradation of targeted isometric contractions is used as a proxy to step-like excitation from descending inputs to the MU pool (Devanne et al. 1997; Fuglevand et al. 1993). With such arrangements during steady-state isometric contraction, the voluntary synaptic input and firing rate outputs (Heckman and Binder 1991), are observed and statistically modelled to be stationary throughout contraction time with an underlying Gaussian distribution (Clamann 1969). From this, step-like gradation of the relative voluntary intensity is the independent factor to induce changes of the mean firing rate during isometric contraction (table 2.1; 6, 62, 71).

In comparing MU activity of muscles between two different age groups, the loss of force in older adults is independent to the absolute contraction intensity, as older adults can achieve voluntary activation levels during maximal voluntary contraction (MVC) like young adults (Connelly et al. 1999; Dalton et al. 2010; Erim et al. 1999; Jakobi and Rice
With ageing, MU loss and structural remodeling of the motor neuron and MU pool commonly reduce MU type diversity with expected larger and slower firing MUs in limb muscles (Brown et al. 1988; Campbell et al. 1973; Chan et al. 2001; Dalton et al. 2008; Doherty and Brown 1993; Gilmore et al. 2016, 2017; Jakobsson et al. 1990; Larsson and Edström 1986; Lexell et al. 1988; Ling et al. 2009; McNeil et al. 2005; Stålberg and Fawcett 1982; Terao et al. 1996; Walton et al. 1977). However, descriptions of MU function are based on age-related comparisons of firing rate between single muscles (Connelly et al. 1999; Dalton et al. 2010; Erim et al. 1999; Kamen et al. 1995; Kamen and Knight 2004; Kirk et al. 2016, 2018, 2019), with few direct comparisons made between 2-3 different muscles using the same analysis framework (Dalton et al. 2010; Kirk et al. 2016, 2018). The observation of lower firing rates during voluntary contraction has been attributed to changes in synaptic inputs received by the lower motor neuron (Maxwell et al. 2018; Rowe et al. 2006), and by decreases in conduction velocity and the rheobase of the cell membrane (Chase et al. 1985; Morales et al. 1987). However, understanding how ageing alters firing rates from recorded MU activity among muscles is limited and has not been compared using a systematic and standardized approach (table 2.1). Furthermore, it remains unclear whether firing rates are similar or different among muscles when compared throughout the full range of voluntary effort (Bellemare et al. 1983; Dalton et al. 2010; Kirk and Rice 2017; Kukulka and Clamann 1981), as firing rate changes dependent on voluntary contraction intensity may not be a generalized feature of all muscles (Kukulka and Clamann 1981), or throughout the lifespan.

The present analysis was based on studies (table 2.1) that recorded single MU trains using intramuscular EMG (Bellemare et al. 1983; Kamen et al. 1995) at various contraction intensities, including MVC (figure 2.1). As there was no current standard for comparison of single MU activity between many muscles, it was important to consider that intramuscular EMG techniques relied on random sampling of the MU pool with sample replacement (Rich et al. 1998). This provided analysis constraints related to different participant numbers, sample sizes, and unknown errors of MU resampling that may confound comparisons using classical statistical approaches (table 2.1). Therefore, to
enable consistent comparisons across muscles, the technique of bootstrapping was applied. Our purpose was to first characterize the firing rate range and firing rate variance explained by voluntary contraction intensity for each muscle. Specifically, 1) the firing rate range referred to the 95% confidence interval of mean firing rate dependent on different step-like voluntary isometric contraction intensities; and 2) the percentage of firing rate variance explained by voluntary contraction intensity was determined as the coefficient of determination (Pearson’s r-squared) between firing rate and contraction intensity, up to and including MVC. Our secondary purpose was to measure the dissimilarity of firing rate variance calculated among all muscle pairwise comparisons for each age group, and to test whether there was a significant age-related effect. We hypothesized there would be greater similarity in firing rate ranges and firing rate variance among muscles explained by voluntary contraction in the older group when compared with younger adults.
Figure 2.1 Overview of voluntary contraction and intramuscular techniques.

A) Depiction of a monopolar EMG recording from two motor units during different step intensities during voluntary isometric contraction. B) Monopolar (~31 gauge) (Bellemare et al. 1983) and quadrifilar (~25 gauge) (Kamen et al. 1995) needle techniques with an arbitrary targeted isometric contraction. The black arrow indicates the start of the steady-state aspect of an isometric contraction in which the monopolar electrode is moved slowly during contraction, while the quadrifilar electrode is maintained in a stationary position. In between contraction efforts, both electrodes are repositioned within the muscle or re-inserted. C) Raw data of three different isometric contraction intensities (25, 50 and 100% of MVC) normalized to the participant’s MVC. Monopolar EMG was recorded from the first dorsal interosseus (FDI) muscle in an older participant (86 years old). Asterisks (*) denote repositioning of electrodes within the muscle. D) Expanded
time view of the 100% of MVC contraction intensity in panel C with two discrete MU trains highlighted. E) From filtered EMG (high-pass 1 kHz) of the two MU trains in panel D, waveform overlays are presented: MU1 had 6 MU potentials firing at 26±3 Hz (mean ± standard deviation) with a coefficient of variation of the mean 12.5%; and MU2 had 29 MU potentials, 26±4 Hz, 18.2%, respectively.

2.2 Methods

Motor unit dataset construction

We collected and manually curated a dataset from published human MU studies (table 2.1) and added a new dataset from the first dorsal interossei (FDI) described below. For inclusion, studies must have: 1) occurred during targeted voluntary isometric contraction (targeted either force or torque); 2) occurred in a baseline state with rest periods between contractions to minimize the effects of fatigue or potentiation; 3) applied intramuscular EMG to clearly record single MU potentials from muscle fibres; 4) involved moving or reinserting the intramuscular electrode between each contraction; 5) used a template matching algorithm for MU potential shape and amplitude analysis; 6) at least 4 consecutive inter-spike-intervals within a MU potential train from which a mean firing rate and mean contraction intensity (% of MVC) statistic were derived; 7) had a mean firing rate for each MU potential train with a coefficient of variation of the mean <30%; and 8) relied on visual inspection and acceptance by an experienced investigator. For published data, we obtained information on each article’s experimental conditions, importantly considering participant characteristics, control measures taken to avoid fatigue and MVC repeatability criteria. Data were supplied from the original research groups and consisted minimally of descriptive firing rate statistics on each measured MU train.
### Table 2.1 Dataset summary.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>n</th>
<th>Mean age (years)</th>
<th>Number of MU trains</th>
<th>Sex</th>
<th>Isometric levels (% of MVC)</th>
<th>Intra-muscular technique</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biceps Brachii</td>
<td>6</td>
<td>24±1</td>
<td>718</td>
<td>M</td>
<td>10, 25, 50, 75, 100</td>
<td>Monopolar</td>
<td>(Dalton et al. 2010)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>83±4</td>
<td>950</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biceps Femoris</td>
<td>10</td>
<td>26±4</td>
<td>565</td>
<td>M</td>
<td>25, 50, 100</td>
<td>Monopolar</td>
<td>(Kirk et al. 2018; Kirk and Rice 2017)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>80±5</td>
<td>661</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First Dorsal Interossei</td>
<td>8</td>
<td>19±1 (18-21)</td>
<td>82</td>
<td>F</td>
<td>20</td>
<td>Quadrifilar</td>
<td>(Knight and Kamen 2007)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>73±7 (62-88)</td>
<td>91</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>28±5 (22-33)</td>
<td>698</td>
<td>M</td>
<td>25, 50, 100</td>
<td>Monopolar</td>
<td>Present study</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>85±6 (81-93)</td>
<td>582</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral Gastrocnemius</td>
<td>10</td>
<td>27±3 (23-33)</td>
<td>569</td>
<td>M</td>
<td>25, 50, 75, 100</td>
<td>Monopolar</td>
<td>(Kirk et al. 2016)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>81±4 (76-86)</td>
<td>485</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial Gastrocnemius</td>
<td>10</td>
<td>27±3 (23-33)</td>
<td>773</td>
<td>M</td>
<td>25, 50, 75, 100</td>
<td>Monopolar</td>
<td>(Kirk et al. 2016)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>81±4 (76-86)</td>
<td>558</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semimembranosus &amp; Semitendinosus</td>
<td>10</td>
<td>26±4</td>
<td>631</td>
<td>M</td>
<td>25, 50, 100</td>
<td>Monopolar</td>
<td>(Kirk et al. 2018; Kirk and Rice 2017)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>80±5</td>
<td>723</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soleus</td>
<td>6</td>
<td>24±3</td>
<td>576</td>
<td>M</td>
<td>25, 50, 75, 100</td>
<td>Monopolar</td>
<td>(Dalton et al. 2009)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>75±4</td>
<td>536</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tibialis Anterior</td>
<td>6</td>
<td>21±1 (20-22)</td>
<td>732</td>
<td>M</td>
<td>25, 50, 75, 100</td>
<td>Monopolar</td>
<td>(Connelly et al. 1999)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>82±2 (80-85)</td>
<td>864</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>22±4</td>
<td>227</td>
<td>F/M</td>
<td>10, 30, 50</td>
<td>Quadrifilar</td>
<td>(Christie and Kamen 2006)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>74±9</td>
<td>172</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>24</td>
<td>43</td>
<td>F</td>
<td>10</td>
<td>Quadrifilar</td>
<td>(Kamen et al. 2006)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>73</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Mean ± standard deviations are presented with a range (in brackets) when available. Summary statistics of MU trains were only accepted from baseline values during the steady-state aspect of isometric contraction. If from a muscle length study, values were taken from the joint position that elicited the highest MU firing rate profile. With exception of the FDI muscle collected for the current analysis, all other data were accessed from prior publication datasets. The semimembranosus and semitendinosus muscles (SS) were not able to be differentiated and they were combined in the original dataset. Therefore, 13 total muscles were included in this dataset, but only 12 separate muscles could be compared.

Experiment to record and measure motor unit firing rates of an intrinsic hand muscle

In the right hand, we performed an experiment to measure mean firing rate from record MU trains in the FDI muscle of healthy young (n = 5, 18-35 years) and older adult males (n = 5, 62-93 years). The method (figure 2.1) was like those of the published datasets (table 2.1). This study was approved by the local University’s research ethics board for human experimentation and in accordance with the latest revision of the declaration of Helsinki (appendices R and S). Subjects (n = 10) provided informed consent and were free from known neurological, metabolic, or orthopedic disease with no prior injury to the hand or upper limb.
Monopolar intramuscular EMG of the FDI muscle was recorded during steady-state isometric contraction at submaximal (25 and 50% of MVC) and maximal (MVC) intensities, following experimental methods from existing protocols (Bellemare et al. 1983; Kirk et al. 2019). Each contraction was between 5-10 s, with rest periods between MVC being >5 min. Force was measured as the calibrated voltage recorded in a strain gauge and custom-built dynamometer during isometric voluntary first digit abduction. The voltage signal was digitally converted (Power1401, Cambridge Electronics Design, UK) and sampled at 1 kHz. Maximal force in the young group was 44.0±7.4 N (mean±standard deviation) as compared to 38.8±5.1 N in the old group. For EMG, the intramuscular electrode (insulated tungsten wire, 250 µm diameter, 1 µm tip with 30-degree taper angle, Fredrick Haer, USA) raw voltage signal was amplified 200-500x and was bandpass filtered from 10 Hz and 10 kHz (Digitimer Ltd, UK), and digitized at 25 kHz. The surface reference and ground electrodes were placed over the FDI muscle and ulnar prominence, respectively. The converted signal was then digitally high pass filtered (IIR filter, 2nd order Butterworth) at 1 kHz and data analysis was performed in Spike2 (version 7.2, Cambridge Electronics Design, UK) using an algorithm that allowed individual MU potential waveform inspection and acceptance by the lead investigator (Connelly et al. 1999; Roos et al. 1999). For inclusion, each MU train must have consisted of at least 4 inter-spike-intervals with the mean having a coefficient of variation <30%. In total, 1,280 discrete MU trains were measured with 698 and 582 from the young and older group, respectively.

Analysis and statistics

Bootstrapping

Although not extensively utilized in MU electrophysiology (46, 85), the bootstrapping technique is well described (Efron 1979; Efron and Tibshirani 1991) and is applied with similar experimental reasoning, as intramuscular recordings rely on random sampling of a larger MU pool with replacement (Rich et al. 1998). Likewise, the resampling aspect of bootstrapping can be viewed as a stochastic process and required a higher number of iterations to be confident in presented results, and was based on prior workflows (Efron
and Tibshirani 1993). Thus, from the dataset (table 2.1), bootstrapping was applied to generate equal sized probability distribution estimates of: 1) ensemble mean firing rate values for each muscle, and 2) Pearson’s correlations (and therefore r-squared) between mean firing rate and mean contraction intensity (% of MVC) for each muscle.

Mean firing rate 95% confidence intervals

For each MU train, the calculated mean firing rate was dependent on graded contraction intensity (figure 2.1). Because each muscle comprised a composite of different MU trains at various contraction intensities and from different participants (table 2.1), a range for mean firing rates were determined for each muscle and age group. For each muscle, the number of MU trains for each binned contraction intensity was >50 (table 2.1), and the distribution was visually inspected to confirm they appeared continuous (were often positively skewed and with 99.8% of total mean firing rates in the dataset greater than 5 Hz). Targeted isometric contractions (10, 20, 25, 50, 75 and 100% of MVC) were normalized to each participant’s MVC (table 2.1), and contraction intensity bins that were present across muscle studies were determined. These included the: 25% bin containing MU trains that occurred between 15-35% of MVC, and the 50, 75 and 100% bins containing MU trains that occurred between 40-60, 65-85 and >85% of MVC, respectively. In some muscles, data were not available for some contraction intensity bins, and therefore could not be included, for example in the VL muscle (figure 2.2).

From the composite of MU trains, ensemble mean firing rate ranges were calculated for each contraction intensity bin, muscle, and age group. Probability distribution estimates of ensemble mean firing rates derived from bootstrap simulation were calculated, with two-tail 95% confidence intervals applied, and were bias-corrected and accelerated (BCa) (Efron 1987). For this and other bootstrapping approaches (see below), we used the boot package (Canty and Ripley 2019; Davison and Hinkley 1997) in R (version 3.6). For each bootstrap iteration, the draw size was common among muscles and therefore, each iteration consisted of an ensemble mean firing rate derived from 25 randomly sampled mean firing rate (each attributed to a distinct MU train) with sample replacement. This modest draw size (of 25) was chosen because it was on the lower end of values accepted
for some MU studies (table 2.1), with each bootstrap probability distribution estimate comprised of $1.0 \cdot 10^5$ iterations. The firing rate range therefore captured the variability of the ensemble mean firing rate based on the present dataset (table 2.1) and were scaled based on the absolute value of firing rates (figure 2.2). To determine significant differences between firing rate ranges, standard scores (Z-scores) were calculated between pairwise comparisons and the alpha was Bonferroni corrected and equal to $3.16 \cdot 10^{-6}$.

**Correlation estimates between firing rate and voluntary isometric contraction for each muscle**

Pearson’s correlations between the mean firing rate and mean contraction intensity (% of MVC) were determined through bootstrapping the available dataset of MU trains for each muscle. For each MU train, mean contraction intensity was the voluntary torque or force normalized to each participant’s MVC for that muscle and acted as the independent step in the voluntary gradation of the measured firing rate response (figure 2.1). Each bootstrap probability distribution estimate comprised $1.0 \cdot 10^3$ iterations from the composite of all available MU trains for each muscle (table 2.2). As a measure of the percentage of firing rate variance explained by voluntary contraction intensity, correlation values were squared and resulted in probability distribution of the Pearson’s r-squared statistic for each muscle, with the mean reported (figure 2.3, panel C).

Spearman’s rank correlations were used to test whether there was an ordered relationship between the mean firing rates at 100% of MVC and the percentage explained firing rate variance (Pearson’s r-squared) across muscles within each age group. To determine a significant difference in firing rate variance between age groups for each muscle, standard scores were calculated with the alpha equal to 0.05.
Table 2.2 Linear correlation of mean firing rate and contraction intensity.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Muscle</th>
<th>Mean (r)</th>
<th>Standard deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>BB</td>
<td>0.73</td>
<td>0.015</td>
<td>0.68</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>BF</td>
<td>0.53</td>
<td>0.035</td>
<td>0.41</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>FDI</td>
<td>0.71</td>
<td>0.023</td>
<td>0.63</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>LG</td>
<td>0.56</td>
<td>0.030</td>
<td>0.45</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>MG</td>
<td>0.64</td>
<td>0.017</td>
<td>0.58</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>0.52</td>
<td>0.033</td>
<td>0.40</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>SOL</td>
<td>0.65</td>
<td>0.025</td>
<td>0.57</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>TA</td>
<td>0.66</td>
<td>0.018</td>
<td>0.59</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>TB</td>
<td>0.70</td>
<td>0.018</td>
<td>0.63</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>ST</td>
<td>0.71</td>
<td>0.013</td>
<td>0.66</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>VM</td>
<td>0.74</td>
<td>0.015</td>
<td>0.69</td>
<td>0.78</td>
</tr>
<tr>
<td>Older</td>
<td>BB</td>
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<td>0.020</td>
<td>0.56</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>BF</td>
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<td>0.037</td>
<td>0.18</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>FDI</td>
<td>0.74</td>
<td>0.021</td>
<td>0.67</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>LG</td>
<td>0.56</td>
<td>0.025</td>
<td>0.47</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>MG</td>
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<td>0.027</td>
<td>0.39</td>
<td>0.57</td>
</tr>
<tr>
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<tr>
<td></td>
<td>SOL</td>
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<td>0.018</td>
<td>0.65</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>TA</td>
<td>0.68</td>
<td>0.018</td>
<td>0.62</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>TB</td>
<td>0.55</td>
<td>0.024</td>
<td>0.48</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>ST</td>
<td>0.61</td>
<td>0.019</td>
<td>0.56</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>VM</td>
<td>0.76</td>
<td>0.014</td>
<td>0.71</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Pearson’s correlations (r) between mean firing rate and mean contraction intensity linked for each MU train were calculated based on bootstrapping the composite of MU trains from each muscle and age group. The ensemble mean and standard deviation (i.e., standard error of the simulated distribution) of the probability distribution estimate are reported. Bias of the simulated distribution was low as compared to the original dataset and was <1.2 •10^3. See table 2.1. for muscle code abbreviations. The VL muscle was omitted from analysis because there was not enough variability in mean contraction intensity values to calculate correlations.

Euclidean distances between muscles

Among muscles, a dissimilarity metric for each age group was quantified based on the calculated Euclidean distance between muscle pairwise comparisons of the explained firing rate variance by contraction intensity (figure 2.3). For each muscle-to-muscle
comparison, the Euclidean distance between the distribution of r-squared values was determined, with the distribution standardized by bootstrap simulation. Each distribution was randomly ordered and of equal size. For statistical comparisons between age groups, each dissimilarity metric was vectorized, z-scaled, centered, and evaluated using the Kruskal-Wallis rank sum test, as variances were unequal between age groups (F test, \( F = 3.1, df = 54, p < 0.001 \)) with the alpha set to 0.05. For comparisons of Euclidean distances and therefore the dissimilarity metric, we assumed that distance values between age groups were equivalent, as the same analysis methods and muscle comparisons were employed in both groups.

**Code availability**

Analysis and code in R can be accessed by contact with the corresponding author. Data figures were created using ggplot2.

### 2.3 Results

The dataset (table 2.1) comprised 17,080 MU trains recorded from a total of 154 young (18-35 years) and 154 older (62-93 years) participants across studies from 12 muscles (the semimembranosus and semitendinosus muscles were grouped). In total, the studies had 81 female participants (26%) and 227 male participants, and in the older group there were 41 older females (27%). Each MU train was recorded from intramuscular EMG, and the mean firing rate from the steady-state aspect of voluntary isometric contraction (% of MVC) was measured (figure 2.1).

**Mean firing rates are muscle dependent and lower in the older group**

Targeted voluntary isometric contractions ranged from 10% to 100% of MVC and acted as independent gradations of step-like descending inputs to the MU pool, to compare the outcome of mean firing rates across muscles (figure 2.2). For each muscle, the absolute firing rate ranges (figure 2.2, panels D and E) were positively associated with relative contraction intensity, but it was clear that different muscles operate within different firing
rate frequencies when compared systematically. Across muscles, separations in firing rate ranges were muscle dependent at relative contraction intensities (figure 2.2). In figure 2.2, each firing rate range represents the 95% confidence interval, and overlap along the y-axis corresponds to a greater similarity between muscle firing rate frequencies, whereas a larger separation indicated a greater probability of firing rate ranges being different. For example, from MU activity at MVC (figure 2.2, panel D), there was a 3-fold difference in the mean firing rates between the soleus (SOL) and superior trapezius (ST) muscle in the young group (Z-score, \( p < 3.16 \times 10^{-6} \)). Likewise, absolute firing rate ranges during submaximal contractions had muscle dependence (figure 2.2, panel D). However, with some muscles that have similar anatomical location and function, like the gastrocnemii (MG and LG), the firing rate range had overlap and was similar at some but not all contraction intensities (Z-score, non-significant difference: 25% of MVC, \( p = 0.0001 \); 75% of MVC, \( p = 0.005 \)).

In the older group (figure 2.2, panel E), firing rate frequencies were lower for 9 out of 12 muscles (\( p < 3.16 \times 10^{-6} \)) and had greater firing rate range overlap as compared to the young, showing that absolute firing rates were to a lesser extent muscle dependent. However, three muscles (FDI, SOL and VM) did not have significantly lower firing rate frequencies in the older group as compared with the young (\( p \) value range = 0.06-0.18).
Figure 2.2 Ensemble mean firing rate 95% confidence interval ranges dependent on contraction intensity for each muscle and age group, with an example of the analysis workflow in the FDI muscle at 100% of MVC.

A) Representative data of two separate MU trains from a young participant (33 years old). From bottom channel to top: force from the FDI muscle abduction during MVC, intramuscular EMG, 1 kHz high pass filtered intramuscular EMG, firing rate graph of isolated waveform potentials and template overlays from two distinct MU trains. MU1 consisted of 27 MU potentials firing at 39±9 Hz (mean ± SD) with a coefficient of
variation of the mean 20.5%; MU2 consisting of 30 MU potentials, 31±6 Hz, 21.0%. B) Histogram of mean firing rate values from 83 distinct MU trains recorded during MVC in the young FDI muscle. The ensemble mean was 31 Hz. C) Histogram representing the 95% confidence interval of the bootstrapped probability distribution estimate of ensemble mean firing rates (from panel B), 2.5th percentile was 29.4 Hz and the 97.5th percentile was 33.5 Hz. D and E) Dot-plots depict the ensemble mean firing rate 95% confidence interval ranges for each isometric contraction intensity gradation (% of MVC), in the young (panel D) and older groups (panel E). See table 2.1 for muscle code abbreviations.

Linear relationship between mean firing rates and mean contraction intensity for each muscle

To quantify the percentage of firing rate dependence explained by isometric contraction throughout the available spectrum of graded voluntary intensity, firing rate variance (figure 2.3, panel B) was calculated based on linear correlation from the composite of MU trains for each muscle (table 2.2). Linear correlations were based on the expectation that: 1) voluntary isometric contractions acted as an in vivo approximation of graded descending synaptic inputs distributed to the MU pool (Fuglevand et al. 1993), analogous to step current inputs and mean firing rate outputs in non-human reduced preparations (Granit et al. 1966a; Kernell 1965a, 1965b; Schwindt and Crill 1982); and 2) the steady-state aspect of short duration isometric contraction (see MU train recordings in figures 2.1-3) had stable mean firing rate throughout the contraction duration (Clamann 1969).

In comparing the age-related effect of explained firing rate variance within each muscle (figure 2.3, panel C), six muscles were lower (range -13 to -20), as compared to four muscles (SOL, FDI, TA and VM) that were observed to have a smaller relative increase in the older group (range +2 to +8). For each age group, the explained firing rate variance (figure 2.3, panel C) was unrelated to the absolute mean firing rate across muscles at 100% of MVC (figure 2.2) as there was no significant ordered relationship in the young (Spearman’s rank = 0.3, p = 0.38) or older group (Spearman’s rank = 0.5, p = 0.12). For example, the soleus (SOL) muscle had the lowest firing rates in both young and old (figure 2.2) but had moderate firing rate variance explained by voluntary contraction
intensity, and supported that firing rate variance was not explained by a ranked effect of firing rate observed to be different across muscles (figure 2.2).

Among muscle comparisons between age groups

Among muscles, the dissimilarity metric for each age group represented all pairwise distance comparisons of firing rate variance explained by voluntary contraction intensity between muscles (figure 2.3, panels D and E). In the young group (figure 2.3, panel D), distances between upper limb muscles (BB, FDI, ST and TB) were lower (distance, range 1.2-1.7) as compared to larger distances observed in lower limb muscles (distance, range 1.7-8.9). In the lower limb, the largest distances were observed between anterior (VM and TA) and posterior (BF, LG, MG, SOL, SS) muscles (distance, range 3.9-8.9). In both age groups, the hamstring muscles had the largest absolute distance among muscles (figure 2.3), despite the large age-related change of lower firing rates in the older group (figure 2.2).

The older group had significantly greater dissimilarity (median 1.6-fold increase) of distances among muscle firing rate variances than the younger group (Kruskal-Wallis rank sum test, $X^2 = 11.2$, df = 1, $p < 0.001$), indicating that the percentage of firing rate variance explained by voluntary isometric contraction intensity was more different among muscles of older adults. Within each age group (figure 2.3, panels D and E) the young had lower distances of firing rate variance among muscles (median 3.6, range 1.1-8.9), whereas the older group had larger distances (distance, median 5.7, range 1.1-16.0). Among muscles in the older group, larger distances were due to four muscles having an age-related increase of the explained firing rate variance by contraction intensity (FDI, SOL, TA and VM), and seven muscles having an age-related decrease (BB, BF, MG, SS, ST and TB) as compared to the young group.
Figure 2.3 The percentage of firing rate variance explained by voluntary isometric contraction intensity.

A) For the corresponding point on the scatterplot in B, a MU train from a young adult participant (33 years) is recorded. From the bottom channel to top: force from the FDI muscle during voluntary isometric contraction targeting 25% of MVC, 1 kHz high pass filtered intramuscular EMG, firing rate graph of an isolated MU waveform. The MU train consisted of 55 MU potentials firing at 15±2.5 Hz (mean ± SD) with a coefficient of
variation of the mean 16.4%. B) Scatterplot of summary MU trains for the FDI muscle of
the young group with voluntary contraction intensity (% of MVC) normalized for each
participant. The simple linear regression line (blue) is depicted with the Pearson’s
correlation and r-squared. C) Bar plots depict the mean r-squared value for each muscle
and age group, see table 2.2. The standard deviation for each bar plot was <1%, and error
bars were not depicted. Only the LG muscle had a non-significant difference in firing rate
variance between age groups (Z-score, p > 0.05). D and E) Dissimilarity metric
representing Euclidean distances across muscle pairwise comparisons of the percentage
of firing rate variance explained by voluntary contraction intensity. In the young (panel
D) and older group (panel E), darker shading within each age group indicates larger
distances between muscles. See table 2.1. for muscle code abbreviations.

2.4 Discussion

From gradations of voluntary isometric contraction, the absolute firing rate ranges were
largely muscle dependent but were lower and with more firing rate range overlap in the
older group (figure 2.2). In comparing the percentage of firing rate variance explained by
voluntary contraction intensity among muscles (figure 2.3), the older group had 1.6-fold
greater dissimilarity when compared to the young, rejecting the null hypothesis of equal
similarity among muscles in the aged state (p < 0.001). Due to an age-related effect,
absolute firing rates from recorded MU activity is lower and more similar across muscles
(figure 2.2); however, firing rate response explained by voluntary contraction intensity
had greater dissimilarity among muscles in the older group as the age-related change of
firing rate variance was muscle dependent (figure 2.3).

In older adults, MU trains operated within lower firing rate ranges (figure 2.2) and are
generally matched with slowed muscle contractile responses (Allman and Rice 2004;
Vandervoort and McComas 1986). When considering that the effects of age-related MU
loss and remodeling result in a more homogenous structure across MUs throughout the
muscle, in which lower-threshold MUs can undergo collateral reinnervation and are
larger (Brown et al. 1988; Campbell et al. 1973; Chan et al. 2001; Dalton et al. 2008;
Doherty and Brown 1993; Gilmore et al. 2016, 2017; Jakobsson et al. 1990; Larsson and
Edström 1986; Lexell et al. 1988; Ling et al. 2009; McNeil et al. 2005; Saboisky et al.
2014; Stålberg and Fawcett 1982; Terao et al. 1996; Walton et al. 1977), it was unexpected that firing rate variances were more dissimilar to relative voluntary contraction intensity in the older group (figure 2.3). As explained, firing rate variance was only related to voluntary isometric contraction intensity for each muscle, the greater dissimilarity in the older group was due to some muscles having higher (FDI, SOL, TA and VM) or lower firing rate variance (BB, BF, MG, SS, ST and TB) relative to the young group. This muscle dependent age-related difference of firing rate variance, which quantified the percentage change of firing rate response to voluntary contraction intensity, may be explained by several other factors than MU loss and remodeling. Indeed, these differences may be more dependent on age-related changes from altered synaptic inputs to the motor neuron (Chase et al. 1985; Maxwell et al. 2018; Rowe et al. 2006) that may affect some but not all muscles (figure 2.3, panel C). Although there is likely no singular mechanism responsible for the observed age-related firing rate differences among muscles there are several possibilities to consider.

Considerations of firing rate muscle dependence

In humans, experimental arrangements measuring MU activity use voluntary contraction intensity as a proxy to grade descending inputs to the MU pool during isometric contractions (table 2.1) (Bellemare et al. 1983; Desmedt and Godaux 1977; Kamen et al. 1995; Kukulka and Clamann 1981; Monster and Chan 1977; Del Vecchio et al. 2019). Therefore, it was expected that summary excitatory synaptic inputs (Granit et al. 1966a) related to contraction intensity (i.e., descending inputs) represented a driving force of current (Fuglevand et al. 1993) where stronger currents facilitated higher mean firing rate outputs (Brownstone et al. 1992; Granit et al. 1966a; Kernell 1965a, 1965b; Schwindt and Crill 1982). Within each gradation, the firing rate range was based on MU activity measured from the brief (~5-10 s) steady-state aspect of voluntary isometric contraction (figure 2.1). Hence, mean firing rate activity for MU trains were observed to be largely stationary with contraction time (see data in figures 2.1-3) and scaled positively with gradations of voluntary contraction intensity. Unstable mean firing rate, likely attributed to technical errors related to waveform superimpositions were filtered from the dataset, as the coefficient of variation of the mean was required to be <30%. Because firing rate
were measured during steady-state isometric contraction (Clamann 1969; Heckman and Binder 1991), muscle lag properties (Baldissera et al. 1998; Partridge 1965) and nonlinear effects (Binder et al. 2020) close to initial MU firings or due to large changes in synaptic inputs (i.e., decreasing force ramps) were minimized. Likewise, as a large proportion of measured MU activity occurred at contraction intensities ≥10% of MVC, nonlinear effects attributed to fluctuating currents (Granit et al. 1966a; Lee et al. 2003b) and low recruitment thresholds (Bennett et al. 1998) were expected to be minimized.

From muscles compared in the present analysis, the range of mean firing rate frequencies (figure 2.2) at relative voluntary contraction intensities were muscle dependent, indicating muscles (each comprised of different MU pool) that received voluntary synaptic inputs at the same relative intensities have different firing rate outputs, however, the role of corticospinal and other spinal tract influences (Binder et al. 2002) may indeed be MU pool dependent (Schieber and Rivlis 2005, 2007). For example, at MVC, the superior trapezius muscle had a 3-fold higher firing rates (mean firing rate range 95\% confidence interval = 45.1-48.7 Hz) as compared to the soleus muscle in the young (95\% confidence interval = 15.6-17.5 Hz), whereas firing rate frequencies between the superior trapezius and biceps brachii muscles were closer with both >40 Hz (figure 2.2, panel D). Especially during higher contraction intensities (>50\% of MVC), non-linear firing rate reductions of motor neuron initial phase adaptation to strong injected currents (i.e., supraspinal descending inputs) were presumably minimized (Granit et al. 1963a; Kernell 1965b), as firing rate were recorded 1-5 seconds after voluntary contraction onset (figure 2.2, panel A).

There was no clear pattern to explain differences of firing rates based on anatomical location or basic function as flexors or extensors in either age group, despite compression and overlap of firing rate ranges in aged adults (figure 2.2, panel E). How firing rate are different across muscles during relative contraction intensity remains unclear, but may be explained by selective processes that enable behaviours that require high precision in motor output timing (Srivastava et al. 2017; Tadros et al. 2016). Equally, differences in structural properties among muscles that enable faster action potential generation and transfer are also possible, and related to MU type composition (i.e., fibre type), brainstem
versus spinal innervation (Kirk et al. 2019; Tadros et al. 2016), the amplitude and duration of afterhyperpolarization (Bakels and Kernell 1993b; Gardiner 1993; Gardiner and Kernell 1990; MacDonell et al. 2010), and structures related to axon length and caliber (Le Bozec and Maton 1987; Dum and Strick 2002; Eccles et al. 1958; Friede et al. 1984; McComas 1998; Tadros et al. 2016) – and likely are changed with age.

Between age groups, it was unexpected that firing rate frequencies of three muscles (FDI, SOL and VM) were not statistically lower in the aged adults when comparisons were standardized through bootstrapping (figure 2.2, panels D and E). It is unclear why some muscle in the hand and lower limb do not have largely different firing rate ranges between age groups. The current approach supports those original findings of no age-related effect at MVC in the SOL and VM (Dalton et al. 2008, 2009; Roos et al. 1999) but are contrary to prior findings in the FDI (Kamen et al. 1995). Furthermore, these three muscles (FDI, SOL and VM) each had a positive increase of the firing rate variance explained by contraction intensity in the older group (figure 2.3, panel C). This may indicate that in ageing, surviving MU pools of some muscles that can retain high MU firing rate activity like the young may become more responsive to descending voluntary inputs throughout the available spectrum of contraction effort.

Increased dissimilarity of explained firing rate variance among muscles in the older group

From the framework of step-like gradations of voluntary input (figures 2.2 and 3), the correlation coefficient calculated from the composite of MU trains between mean firing rate and voluntary contraction intensity (% of MVC) for each muscle, was comparable to the linearity of mean firing rate dependence during step excitatory current preparations in reduced preparations (Granit et al. 1966a; Kernell 1965a, 1965b; Schwindt and Crill 1982). Despite observing correlation differences across muscles, we expected linear relationships (figure 3, panel B) to be largely within the primary firing range, as raw data scatterplots did not clearly demonstrate a severe secondary slope (Granit et al. 1966b). A major difference between reduced preparation and observations from the intact system, especially during stronger voluntary efforts (i.e., MVC) is that descending inputs from supraspinal factors (Brouwer and Ashby 1992; Devanne et al. 1997; Lawrence and
Kuypers 1968a, 1968b; Steward and Preston 1967) may provide different timing of excitatory synaptic inputs as compared to stretch reflexes and high frequency local inputs (Granit et al. 1966a).

Across muscles, we observed no clear pattern in age-related changes of the firing rate range (figure 2.2) to predict an increased or decreased of firing rate dependence to voluntary contraction intensity (figure 2.3, panel C). For example, firing rate responses in the BB, FDI, MG, ST, TA, TB and VM muscles had >40% of the firing rate variance explained by voluntary contraction intensity in the young group (Kukulka and Clamann 1981), whereas the BF, LG and SS muscles were <40%, and were the lowest in the hamstring muscles of the older group (figure 2.3, panel C). As the explained firing rate variance was observed to be muscle dependent (figure 2.3, panel C) and not clearly related to the firing rate range across muscles (figure 2.2), this indicated that voluntary descending inputs throughout the spectrum of isometric contraction intensities, may directly relate to differences between muscles in the timing or spread of the synaptic inputs from supraspinal centres (Brouwer and Ashby 1992; Churchland et al. 2012; Schieber and Rivlis 2005, 2007), intrinsic inward currents (Heckman et al. 2008; Tadros et al. 2016; Wilson et al. 2015) or to extrinsic feedback which act to inhibit or alter firing rate variability (Fuglevand et al. 2015; Revill and Fuglevand 2017). An exception was observed in upper limb muscles from the young group (BB, FDI, and TB) that had closer distances between explained firing rate variance (all above 40%) indicating greater similarity in firing rate responses to voluntary contraction intensity but were lower (BB and TB) or higher (FDI) in older adults (figure 2.3, panels D and E), and may be attributed to the strength of corticospinal projections in the upper versus the lower limb (Brouwer and Ashby 1992). For example, in the lower limb, the greatest distances between firing rate variance were in the hamstring muscle in comparison to all other muscles (figure 2.3, panels D and E) and this disparity may be further attributed to length dependent muscle effects on firing rate in some lower limb muscles (Kirk and Rice 2017) that may be exacerbated with age.

Despite lower firing rates across muscles in the older group, there remained considerable separation between firing rate ranges (figure 2.2), and may correspond to the ‘speed-
matching’ between firing rate and contractile outputs (Bellemare et al. 1983; Eccles et al. 1958) throughout lifespan, as there were observable trends within each dissimilarity metric (figure 2.3, panels D and E). The firing rate responses to voluntary contraction intensity was more dissimilar among muscles in the older group as compared to the young (figure 2.3, panels D and E), and was unlikely that age-related changes corresponded directly to a matching of slowed contractile responses in the older group (Allman and Rice 2004; Connelly et al. 1999; Dalton et al. 2010; Larsson and Edström 1986; Vandervoort and McComas 1986), as four muscles were observed to have an age-related increase in explained firing rate variance (figure 2.3, panel C). Furthermore, the BB and TB muscles had age-related firing rate variance decreases but are observed to have minimal contractile slowing to evoked twitch responses in the original experiment (Dalton et al. 2010). In contrast, the SOL muscle which is predominately slow twitch with the lowest firing rate range (Dalton et al. 2009), had no significant firing rate decrease (figure 2.2) and the largest age-related increase in explained firing rate variance from contraction intensity (figure 2.3, panel C). Based on the SOL and other muscles that follow this observation, this could indicate an increased firing rate responsiveness to voluntary contraction intensity with ageing and may be attributed to the muscle’s functional role as being chronically active (23).

2.5 Limitations

The dataset (table 2.1) only included measures from techniques that directly recorded MU trains from intramuscular EMG, throughout all regions and depths of the muscle and during the full range of voluntary effort (Bigland and Lippold 1954a; Kamen et al. 1995). However, the invasive requirement of intramuscular EMG relied on variable MU sample sizes and from a relatively small numbers of participants (n = 5-30) for each muscle. We were unable to consistently group MU trains by participant across the many muscle datasets, and this possibly contributed to increased error between comparisons. The present analysis only studied ~3% of total human musculature during isometric contraction and provides a starting point for a neuromuscular framework in humans. We
included only healthy young and old participants that were recreationally active and thus comparisons were cross generational and the pattern among muscles (figure 2.3) did not represent very frail older adults. Furthermore, the aggregated dataset was largely comprised of measurements from males (table 2.1), and we were unable to investigate sex differences, as many muscles and studies did not have female participants. This limitation underscores the need in future studies to assess potential sex-related effects on MU properties in many adapted states. We compared relationships between mean firing rates dependent on voluntary contraction intensity (% of MVC) during the steady-state aspect of isometric contraction and relied on a modest number of MU trains per muscle. We were unable to characterize the MU type that was recorded and were unaware of the contribution of different MU types and sizes for each comparison. Furthermore, the same MU sample was not followed throughout different contraction intensities, but instead, these methods relied on random sampling of a larger number of MUs with replacement (Rich et al. 1998). It is recognized that linear correlation has limitations to understand firing rate relationships in the intact human, however it offers a simple and straightforward approach for comparison among muscles and age groups during the assumption of stationary firing rates measured against different gradations of voluntary isometric contraction intensity (21).

### 2.6 Conclusions

Our results show firing rates are lower and more similar among muscles with adult ageing, however, there was ~1.6-fold greater dissimilarity in firing rate variance explained by voluntary contraction intensity in the aged group. This occurred with seven of the 11 muscles assessed had an age-related decrease in firing rate variance explained by contraction intensity. Thus, firing rates are unchanged or lower in muscles of older adults, although the percentage of firing rate variance explained by voluntary contraction intensity is greater in some muscles but lower in other muscles. These divergent changes may correspond to how different MU pools with ageing integrate descending synaptic inputs to control tension at the muscle, suggesting that a relative increase of contraction
intensity results in different firing rate outputs that are dependent on the muscle. By providing a framework to contrast age-related differences among muscles, and providing a measure of dissimilarity, this comparison between age groups supports a more detailed description of MU activity throughout the somatic nervous system.

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Chapter 3

Anconeus motor unit firing rates during isometric and muscle shortening contractions comparing young and very old adults

A version of this chapter has been accepted for publication in the Journal of Neurophysiology (Kirk et al. 2021a) and is presented here with permission.

With effects of ageing, voluntary neural drive to the muscle, measured as motor unit (MU) firing rates, are lower in older adults during sustained isometric contractions as compared to young, but differences remain unknown during limb movements. Therefore, our purpose was to compare MU firing rates during both isometric and shortening contractions (i.e., limb movement) between two adult age groups. We analyzed intramuscular electromyography of single MU recordings in the anconeus muscle of young (n = 8, 19-33 years) and very old (n = 13, 78-93 years) male adults during maximal voluntary contractions (MVCs). In sustained isometric, and muscle shortening contractions during limb movement, MU trains were linked with elbow joint kinematic parameters throughout contraction time course. The older group was 33% weaker and 10% slower during movements than the young (p < 0.01). In isometric contractions, median firing rates were 42% lower (p < 0.01) in the older group (18 Hz) as compared with the young (31 Hz), but for both age groups during shortening contractions, firing rates were higher and not statistically different between groups. As a function of contraction time, firing rates at MU recruitment threshold were 39% lower in the older group but the firing rate decrease was attenuated 3-fold throughout shortening contraction as compared to the young. At the single MU level, age-related differences during isometric contractions (i.e., pre-movement initiation) do not remain constant throughout movement that comprises greater effects of muscle shortening. Results indicate neural drive is task dependent and in older adults during movement is decreased minimally.
3.1 Introduction

Voluntary movement is required throughout the lifespan, yet the effects of adult ageing on neural drive to the muscle during limb movement is poorly understood. In healthy ageing, structural changes of the MU pool results in MU loss (Terao et al. 1996; Tomlinson and Irving 1977) and subsequent remodeling through collateral reinnervation (Aare et al. 2016; Gutmann et al. 1971; Kung et al. 2014), with surviving MUs of limb muscles becoming larger (Brown et al. 1988; Campbell et al. 1973; Chan et al. 2001; Dalton et al. 2008; Doherty and Brown 1993; Ling et al. 2009; McNeil et al. 2005; Stålberg and Fawcett 1982). In limb muscles, a functional consequence of MU loss is muscle weakness, that is accompanied by lower neural drive across most (Connelly et al. 1999; Dalton et al. 2010; Erim et al. 1999; Kamen et al. 1995; Kamen and Knight 2004; Kirk et al. 2018), but not all muscles (Dalton et al. 2009; Roos et al. 1999). Voluntary neural drive to the muscle in humans can be quantified by assessing the firing rate from MU recordings, with the firing rate output dependent on factors that change lower motor neuron excitability (Heckman and Enoka 2012). In limb muscles, differences in firing rates between age groups have been compared only during isometric contraction, with existing studies in young adults showing that mean firing rates are greater during shortening contraction (i.e., concentric) as compared with isometric contraction (Gydikov et al. 1986; Harwood et al. 2011; Howell et al. 1995; Kallio et al. 2013, 2014; Oliveira and Nergo 2021; Pasquet et al. 2006). Therefore, the effects of ageing on MU firing rate changes during limb movements remains unknown, despite older adults being able to achieve full voluntary activation like the young (Rozand et al. 2017).

We are aware of one study that compared single MU activity between shortening and isometric contractions in limb muscles of older adults (Kallio et al. 2014). During shortening contraction with constrained-velocity movement (at 10 deg s⁻¹), mean firing rates measured from recorded MU trains in the soleus muscle were higher than during isometric contraction at the same relative intensity (Kallio et al. 2014), however, the age-related effect was not tested. In another experiment, that did not track single MU activity, but instead averaged all firings from intramuscular recordings of the soleus muscle, the older group was shown to have lower averaged firings across isometric, muscle
shortening and lengthening contractions, as compared to a group of young adults (Kallio et al. 2010). In the soleus muscle, the quantity and quality of MU properties may be less affected by ageing effects (Dalton et al. 2008, 2009) and these limited findings in slow (i.e., 10 deg \( \cdot \) s\(^{-1}\)) and constrained-velocity (i.e., isokinetic) shortening contractions of relatively ‘young’ (mean ± SD, \(70 ± 5\) years) older adults (Kallio et al. 2010, 2014) are inconclusive. To study MU firing rates during limb movement in a muscle that has undergone substantial age-related changes in MU properties, we utilized the anconeus muscle as a model. The anconeus undergoes fascicle length and pennation angle changes throughout elbow joint displacement (Stevens et al. 2014) and single MU activity can be reliably tracked during limb movement even at fast angular velocities of joint rotation with moderate resistance (Harwood et al. 2011; Harwood and Rice 2012, 2014). The anconeus muscle of very old adults (85 ± 4 years) has shown through electrophysiological estimates to undergo MU loss (Gilmore et al. 2017). Therefore, we hypothesized that older adults would have lower firing rates during both voluntary isometric and shortening contractions as compared with younger adults.

We compared differences in firing rates recorded from single MU trains in the anconeus muscle during voluntary elbow extension contractions in a group of young and older adults separated in age by \(50\) years. Elbow extension contractions occurred in three conditions that were isometric, unconstrained-velocity and constrained-velocity movements, and all contractions were self-initiated maximal voluntary efforts (Figure 3.1). For the comparison of firing rates between age groups, two analysis methods were used for each condition. Firstly, this involved minimizing the effect of contraction time course (i.e., phase lag) that occurs between firing rate and movement parameters (Baldissera et al. 1998; Cavanagh and Komi 1979; Partridge 1966) by instantaneously linking firing rates to separated limb kinematics. Secondly, firing rates for each MU train were compared as a function of contraction time course (Granit et al. 1963a; Partridge 1966) with the firing rate change measured as the slope of the simple linear regression. These methods were necessary to compare firing rates between age groups, as MU activity during limb movement requires a non-stationary assumption (Partridge 1966) that diverges from isometric contraction (Clamann 1969).
Figure 3.1 Voluntary contractions. Including isometric and elbow extension limb movements (i.e., shortening contraction).

A) The anconeus (Anc) and long head (LH) of the triceps brachii were agonist muscles recorded during elbow extension. During each maximal voluntary contraction (MVC), conditions for each participant were 1) isometric, 2) shortening contraction with unconstrained-velocity movement loaded at 20% maximum torque, and 3) shortening contraction with constrained-velocity movement at 25, 50 and 75% of maximum velocity (Vmax). B) Data of a single MU train recorded in the anconeus muscle from the oldest participant (93 years old) during unconstrained-velocity movement. For this contraction, the peak torque and velocity were 34 Nm and 157 deg • s⁻¹, respectively. Intramuscular EMG of the anconeus was amplified, and 1 kHz high pass filtered. The frequency based on the 29 isolated MU potential waveforms was 60 ± 13 Hz (mean ± standard deviation) with a coefficient of variation of the mean equal to 20.1%.
3.2 Methods

Participant characteristics. Twenty-one healthy male adults (eight young, 19-33 years; thirteen old, 78-93 years) participated in the current investigation. All participants were free of prior injury to the upper limb and any neurological, metabolic, or orthopedic illness that may confound neuromuscular measures apart from ageing. These experiments were approved by the local University’s research ethics board and conform to the latest revision of the Declaration of Helsinki (appendices R and S). Each participant gave their informed written consent.

Experimental set-up. Seated in an upright posture in a dynamometer (Humac Norm, Computer Sports Medicine Inc., MA, USA) the upper limb and hip were secured. The elbow was aligned to the axis of rotation and the range of motion (ROM) occurred along the sagittal axis with the wrist and hand in the neutral position. Elbow extension consisted of maximal voluntary contractions (MVC) for three different conditions (Figure 3.1). The first condition was isometric with the elbow joint fixed at 90-degrees. The second and third conditions were elbow joint extension movements throughout 60-degrees range of motion. The second condition consisted of muscle shortening contraction with unconstrained-velocity movement with the dynamometer loaded with 20% resistance of the peak isometric torque for each participant. The third condition was muscle shortening contraction with constrained-velocity movement (i.e., isokinetic), occurred at 25, 50 and 75% of the peak movement velocity (Vmax) for each participant that was achieved during unconstrained-velocity movement (the second condition). Signal acquisition of calibrated angular torque, velocity, and position from the dynamometer during elbow extensions were analog-digital converted (power-1401, Cambridge Electronic Design, Cambridge, UK) and each sampled at 1 kHz using Spike2 software (version 7.2, Cambridge Electronic Design).

Electromyography. Surface electrodes (0.9 cm diameter, Ag-AgCl, Kendall H59P, Covidien, MA, USA) were placed in a bi-polar configuration on the skin over the muscle belly of the long head of the triceps brachii with a 2 cm interelectrode distance. Surface EMG was hardware amplified (1k, Neurolog, Welwyn City, UK), hardware filtered (0.1-
10 kHz) and sampled at 2.5 kHz. For intramuscular electrodes, fine-wires (102 µm diameter, insulated stainless-steel wire, California Fine Wire Company, Grover Beach, CA, USA) were perpendicularly cut, hooked, and placed into a cannula of a 27-gauge needle. After sterilization, the assembled fine-wire electrodes were inserted into the anconeus muscle in a bi-polar configuration (Le Bozec and Maton 1987; Cowling et al. 2016; Harwood et al. 2011, 2012; Harwood and Rice 2014). A common ground was placed over the right clavicle. The intramuscular EMG was hardware amplified (0.1-0.5k), hardware filtered (0.1-10 kHz) and sampled at 25 kHz.

Experimental protocol. Each participant visited the lab for 1-3 sessions, with familiarization as part of the first visit. Each contraction was a maximal effort with visual feedback of torque and velocity provided. Once the contraction was initiated by the participant, strong verbal encouragement by study investigators was also provided. Conditions (Figure 3.1) were each repeated 4-9 times, with ~5 min rest provided between contractions to minimize effects of muscle potentiation and fatigue. Isometric contractions were 3-6 seconds in duration. Muscle shortening contractions with unconstrained-velocity movement were ~1 second duration. Constrained-velocity movements (i.e., isokinetic) were <3 seconds duration even at 25% of Vmax, with all movements occurring ≥40 deg • s⁻¹ (Flash and Hogan 1985; Hallett et al. 1975).

Data analysis. Surface EMG was band-pass filtered (infinite impulse response, IIR, second order) from 20-500 Hz, and the amplitude was root-mean-squared (RMS) and averaged with a time constant of 0.02 s. Intramuscular EMG was high pass filtered at 1 kHz (IIR, 2nd order) to identify near fibre MU potentials of the same MU train and to minimize non-local lower frequency effects from other MU trains (Stashuk 1999). Filtered MU potential waveforms were then sorted using the wavemark algorithm (Spike2 software). Using prior methods, each MU train was manually inspected based on template-matching as gradual amplitude changes dependent on the movement contraction cycle occurred (Cowling et al. 2016; Harwood et al. 2011, 2012; Harwood and Rice 2014). The decomposition envelope was set to individual MU potential characteristics (Figure 3.1, panel B). For inclusion, each distinct waveform, attributed to a single MU train must have been reliably tracked showing consistent and repeatable shape and
amplitude. From MU trains, inter-spike-interval (ISI) times were calculated based on occurrences of consecutive MU potential pairs and were converted to a measure of frequency for each firing rate. Kinematic parameters of angular torque (measure of force), velocity and position were measured based on relative change from resting baseline. Rate of torque development (RTD) and acceleration were calculated from the time of the first detected MU potential (i.e., firing recruitment threshold) for each contraction.

Comparisons minimizing contraction time course effects

For each MU train, MU potentials were aligned to the respective time coordinate of the contraction time course. At each MU potential time point, measures of elbow extension RTD, torque, acceleration, velocity, and position throughout contraction time course were linked, with a resolution of 1 ms based on the minimum sampling of 1 kHz. All statistical analysis and data Figures were performed in R (version 3.6).

Likelihood ratio test (LRT). For each condition (Figure 3.1) the effect of age on the variance of firing rate responses were tested between competing additive mixed-effects linear regression models using the package ‘lme4’ (Bates et al. 2015). First, firing rates (in Hz) were transformed by the natural logarithm (log Hz) to reduce the effect of leverage on linear models from firing rate skewness (Petersen and Berg 2016) and instances of high frequency firing rate doublets at contraction onset (Christie and Kamen 2006). From the aggregated dataset for each condition, intercept random effects were determined as the participant (Tenan et al. 2014) and MU sample, with fixed effects determined as elbow extension limb kinematics (RTD, torque, acceleration, velocity, and position), time course, contraction number and age group. To compare statistically, the LRT, which has equivalence to the F-test for testing the variance component of interest (Lu and Zhang 2010), was calculated between the negative-log likelihoods from the reduced model (no effect of age, i.e., the null) and full model (added effect of age, i.e., the alternative). Case bootstrapping was also performed to determine a measure of chi-square standard error in our dataset for each LRT. For significance, the alpha was equal to 0.05.
Comparisons as a function of contraction time course

Locally weighted scatterplot smoothing (LOESS). For each condition, kinematic parameters (i.e., torque, velocity, and position) and firing rate were plotted dependent on time. Data were LOESS fit using a simple linear regression statistic structured with weighted least squares (Cleveland et al. 1992), analogous to a time series moving average.

Firing rate change as a function of contraction time course. For each MU train, the firing rate change as a function of contraction time course (i.e., linear slope) provided a quantitative estimate to compare the magnitude of firing rate increase or decrease throughout contraction time course. It was important to statistically adjust for torque differences as mixed-effects models used in the LRT (above section) showed that both fixed-effects of torque and contraction time course had a significant effect on the explained firing rate variance (p < 0.001), as determined by t-tests using Satterthwaite’s method (Kuznetsova et al. 2017). In two steps, the firing rate change as a function of contraction time course for each MU train was determined as the slope coefficient from simple linear regression. First, residuals were calculated based on the firing rate variance explained by elbow extension torque. Subsequently, the firing rate responses as a function of contraction time course were determined through linear regression and adjusted based on torque-dependent residuals (from the prior step). For the firing rate change as a function of contraction time course for each MU (i.e., linear slope coefficient), the averaged residual sum of squared error (µSSE) from the linear regression was calculated. Within each condition, the age-related difference between age groups was determined by unpaired Wilcoxon rank sum tests. Due to multiple testing, the alpha was Bonferroni corrected and equal to 0.01.

Pearson’s correlation. Simulated probability distribution estimates of $1 \cdot 10^3$ correlation coefficients (r) were generated using the boot package (Canty and Ripley 2019; Davison and Hinkley 1997). Bootstrap sampling was stratified by the participant. Confidence intervals were bias-corrected and accelerated (BCa) and the correlation was determined
as non-significant when the 95% confidence interval spanned zero. To compare bootstrap correlation distributions between age groups, Z-scores were calculated with the one-tail alpha equal to 0.025.

3.3 Results

In a group of eight young (19-33 years) and thirteen very old adults (78-93 years), 30 distinct MU samples in the anconeus muscle were each followed across different isometric and muscle shortening contractions (table 3.1). Summary firing rates for each contraction condition are stratified by age of the participants (Figure 3.2) and was used as a measure of neural drive to the muscle that was compared between age groups and conditions. In the older group, peak elbow extension torque was 33% lower during isometric contraction. Likewise, the peak unconstrained-velocity movement was 10% slower in the older group when the movement resistance was normalized at 20% torque for each participant.
Table 3.1 Summary of measured motor unit samples during elbow extension.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Young group</th>
<th>Older group</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>Age (years)</td>
<td>28 ± 5 (19-33)</td>
<td>83 ± 5 (78-93) *</td>
</tr>
<tr>
<td>Peak isometric torque (Nm)</td>
<td>73 ± 15.4 (53-108)</td>
<td>49 ± 7 (40-62) *</td>
</tr>
<tr>
<td>Peak unconstrained-velocity movement (deg • s⁻¹)</td>
<td>201 ± 12 (179-214)</td>
<td>181 ± 13 (159-205) *</td>
</tr>
<tr>
<td>Followed MU samples (#)</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>(ISIs/ MU sample)</td>
<td>238 ± 90 (119-417)</td>
<td>199 ± 96 (56-418)</td>
</tr>
<tr>
<td>Isometric: (ISIs/ contraction)</td>
<td>57 ± 26 (14-138)</td>
<td>41 ± 29 (5-112)</td>
</tr>
<tr>
<td>Limb movement:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unconstrained-velocity</td>
<td>16 ± 5 (7-24)</td>
<td>14 ± 6 (6-27)</td>
</tr>
<tr>
<td>Constrained-velocity</td>
<td>17 ± 7 (5-38)</td>
<td>16 ± 8 (4-41)</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation (minimum – maximum) rounded to whole numbers. A total of 299 elbow extension contractions occurred in 21 participants. Each MU sample recorded in the anconeus muscle was followed for 2-8 contractions within each condition (Figure 3.1). One to three MU samples were recorded from each participant, with variance explained among participants adjusted for in mixed-effects linear regression models. A statistical difference (*) between age groups was determined from a two-tailed unpaired t-test, p < 0.05.
Figure 3.2 Summary MU firing rates for each participant and condition.

Grouped by participant and condition, each point in the jitter boxplot depicts a single firing rate. The horizontal line represents the median, and the vertical box dimensions represent the 1st and 3rd interquartile range. Outliers are depicted outside the vertical black lines. Each contraction was a MVC effort. The same participant (vertically linked across boxplots) contributed firing rates responses from following 1-3 MU samples across conditions, that were: A) isometric, B) unconstrained-velocity muscle shortening contraction, and C) constrained-velocity (i.e., isokinetic) muscle shortening contraction.

Comparisons minimizing contraction time course effects
In isometric contractions, the median of averaged firing rates per participant was 1.8-fold higher in the young (ensemble median, 1st-3rd inter-quartile range: 32.5, 28.7-38.6 Hz) as compared to the older group (18.2, 14.3-22.6 Hz). In isometric contraction (Figure 3.3, panel A), the age-related effect explained a significant difference in firing rates between age groups (negative log-likelihood, reduced model = -425.7, full model = -421.6; \(X^2 = 8.2; p = 0.004\)), and the difference was supported from bootstrap simulation of our dataset (1 • 10^3 bootstraps, \(X^2\) mean ± standard deviation, 8.1 ± 0.4).

During muscle shortening contractions with unconstrained-velocity, the firing rates were 1.2-fold higher in the young (37.9, 34.7-44.7 Hz) as compared to the older group (31.9, 19.2-35.3 Hz). However, unlike for isometric contraction, the age-related effect on firing rates during muscle shortening contraction with unconstrained-velocity movement was not significantly different between age groups (negative log-likelihood, reduced model = -312.2, full model = -310.4; \(X^2 = 3.5; p = 0.06\)), and bootstrap simulation supported that the probability of a difference between models was low (\(X^2\) mean ± standard deviation, 3.1 ± 0.8). Therefore, we accepted the null hypothesis, finding that the small difference of firing rate distributions (Figure 3.3, panel B) was not well explained by an age-related effect during unconstrained-velocity movement (Figure 3.5, panel B).
Figure 3.3 Empirical cumulative distribution plots depict summary firing rates measured from the anconeus muscle during isometric and unconstrained-velocity limb movements.

A) Isometric contraction, comparing the firing rate distribution between the young (n = 8) and older group (n = 13). In total, 1490 ISIs from 15 MU samples in the young, and 1230 ISIs in 15 MU samples in the older group are represented. B) Muscle shortening contraction during unconstrained-velocity movement, between the young (n = 8) and older group (n = 13). In total 403 ISIs from 15 MU samples in the young and 425 ISIs from 15 MU samples in the older group are represented. Depicted p values were from likelihood ratio test comparisons as described in the results. C and D) Stratified by age group and participant.

During constrained-velocity movement (i.e., isokinetic), the difference in firing rates between age groups was also investigated across three different movement velocities at 25, 50 and 75% normalized to the maximum elbow extension velocity (table 3.1). For comparison between age groups, the firing rate variance attributed to each fixed movement velocity was accounted for as a fixed-effect. In this condition (Figure 3.4), the age-related effect on firing rate was not significantly different between age groups.
(negative log-likelihood, reduced model = -991.0, full model = -990.5; $X^2 = 0.99; p = 0.32$), and bootstrap simulation supported that the probability of a difference between models was low ($X^2$ mean ± standard deviation, 1.0 ± 0.2). Therefore, during muscle shortening contractions with unconstrained- and constrained-velocity, differences in firing rates from single MU recordings in the anconeus muscle were not statistically different between age groups during elbow extension limb movement.

Figure 3.4 Empirical cumulative distribution plots depict summary firing rates measured from the anconeus muscle during constrained-velocity limb movements.

Muscle shortening contraction during constrained-velocity movement (i.e., isokinetic). There was no significant age-related effect of firing rate between age groups for each movement velocity based on the LRT model comparisons. Each movement occurred at 25, 50 and 75% of the maximum movement velocity ($V_{max}$) for each participant. **A-C** In total 1670 ISIs from 15 MU samples in the young ($n = 8$), and **D-F** 1530 ISIs from 15 MU samples in the old ($n = 13$) are represented. The rounded ensemble median (1st-3rd interquartile range) of averaged firing rates (in Hz) per participant at 25, 50 and 75% of
Vmax was: A) 24 (22-25), B) 31 (29-32) and C) 32 (28-34) Hz. D) 21 (16-27), E) 28 (23-34) and F) 28 (23-33), respectively.

**Comparisons as a function of contraction time course**

As a function of contraction time course, firing rates for each MU train were compared between age groups during 1) firing recruitment threshold that occurred prior to movement, and 2) the firing rate change (i.e., increase or decrease) was estimated as a function of contraction time course during movement.

The firing recruitment threshold for each MU train (i.e., the first recorded MU potential) was aligned to time zero in Figure 3.5. In comparing isometric and shortening contraction with unconstrained-velocity, the firing recruitment thresholds occurred prior to a large effect of elbow extension limb position displacement (median <1 deg elbow extension), with position change occurring ~0.2 s after the first observed MU potential (Figure 3.5, panels H and J). In isometric contraction, the firing rate of the first ISI was 2.9-fold greater in the young as compared to the old (median, young = 35.9 Hz, old = 12.4 Hz, Wilcoxon rank sum test, p < 0.001). Furthermore, the related torque at firing recruitment threshold was significantly greater (Wilcoxon rank sum test, p < 0.01) in the older group (median, min-max: 57, 18-80% of MVC) as compared to the young (27, 13-81% of MVC). During unconstrained-velocity shortening movement, the firing rate during muscle shortening contractions was 1.6-fold greater in the young as compared to the old (median, young = 44.7 Hz, old = 27.2 Hz, p = 0.002). Additionally, the related torque at firing recruitment threshold was significantly greater (p < 0.01) in the older group (36, 12-79% of MVC) as compared to the young (18, 7-53% of MVC). The firing rate of the first ISI for each MU train in the anconeus muscle and elbow extension torque was negatively correlated (r mean range -0.61 to -0.31, p < 0.05), across both age groups and conditions, and in agreement with prior reports during isometric contraction (Kamen et al. 1995; De Luca and Hostage 2010). Therefore, firing recruitment threshold at contraction onset occurred largely in an isometric contraction, as all contractions essentially start in an isometric state prior to shortening (Figure 3.5, panels H and J), and
in agreement with lower firing rates in the older group during isometric contraction as compared to the young (Figure 3.3, panel A).

**Figure 3.5** Representative data and summary relationships of MU firing rate activity from the anconeus muscle and elbow joint kinematics during isometric and unconstrained-velocity movements.
Individual MU samples (30 total) were followed between conditions for each age group with the first detected MU potential (i.e., firing recruitment threshold) aligned with time-zero. A-B) Representative data from a younger (33 years old) and older participant (79 years old). From top to bottom: waveform overlays of the same followed MU train between conditions, with 306 spikes in the young and 111 spikes in the older participant, respectively; firing graph in Hz; 1 kHz high pass filtered intramuscular EMG; unfiltered intramuscular EMG recorded from the anconeus muscle; bi-polar surface EMG recorded from the long head of the triceps brachii muscle; elbow extension torque; velocity; and position. C-F) Line-joined scatterplots of firing rate throughout contraction time course for MU trains during each contraction (black lines), with LOESS fit in red, with a data span of 50%. G-J) Linked vertically with the above row, each line represents the LOESS fits of each kinematic parameter dependent on contraction time course, with grey shading representing the 95% confidence interval. Each parameter is normalized in relative percentage to the maximum for each participant, except for firing rate in Hz. G) Isometric contraction in the young, I) and older group. H) Muscle shortening contraction during unconstrained-velocity movement in the young J) and older group.

As a function of contraction time course, firing rates for each MU train were associated with elbow extension kinematic parameters (Figure 3.5). During isometric contraction (Figure 3.5, panels C, E, G and I), firing rates remained relatively constant for many seconds throughout contraction time course, as elbow extension torque was increased and sustained. In contrast, during unconstrained-velocity movement (Figure 3.5, panels D, F, H and J), firing rates decreased as kinematic parameters of elbow extension torque, velocity and position peaked in successive phases. The observable difference in firing rates between age groups during-movement (quantified in the next paragraph) was that the averaged firing rates decreased to a greater extent in the young as compared to the older group, especially within the first 0.2 s of contraction time course (Figure 3.5, panels D and F). The averaged torque of elbow extension increased within the first 0.2 s and the RTD decreased (appendix A). In the young, firing rates associated to RTD during unconstrained-velocity movement was 1.5-fold greater in the young (mean r = 0.48, 95% CI 0.38-0.55) as compared to the older group (mean r = 0.31, 95% CI 0.22-0.40, Z-score -4.2, p < 0.001), indicating a stronger relationship between contractile responses to firing rates inputs in the young. The RMS amplitude of bi-polar surface EMG recorded from the long head of the triceps brachii was aligned to the time coordinate of each MU firing
throughout each contraction (examples in Figure 3.5, panels A and B). Although variability of the surface EMG amplitude was related to anconeus MU firing rates (Pearson’s covariance = 0.35), surface EMG amplitude was not correlated with elbow extension RTD for either age group (mean r = 0.03, p = n.s.). Instead, surface EMG amplitude was weakly correlated with torque in the young (mean r = 0.19, 95% CI 0.08-0.27) and older group (mean r = 0.11, 95% CI 0.03-0.20), but was not significantly different between age groups.

The estimated firing rate change (i.e., increase or decrease) as a function of contraction time course for each MU train was quantified as the slope of the simple linear regression (table 3.2). Therefore, the firing rate change was used to compare the magnitude of firing rate increase or decrease throughout contraction. Contraction time course was used as the independent variable because time invariantly increased across conditions, as compared to kinematic parameters of elbow extension (Figure 3.5, panels H and J). In isometric contraction, there was no difference of the firing rate change with contraction time course between age groups (Wilcoxon rank sum test, p = 0.22). In contrast, during unconstrained-velocity movement, firing rates decreased with contraction time course for all MU trains in the young and decreased significantly more than the older group (Wilcoxon rank sum test, p = 0.009). In the older group, the slope of the firing rate change during unconstrained-velocity movement was attenuated by a 3-fold difference (median, young -41, old -12.5) as compared to the young (table 3.2). This difference of the firing rate change between age groups was attributed to lower firing rates at recruitment thresholds (Figure 3.5) and that one-third of MU trains (table 3.2) from older participants had firing rates that increased with contraction time course, as compared with the young. The difference of firing rate change with contraction time course between age groups did not occur during constrained-velocity movement (i.e., isokinetic), showing that when movement velocity was controlled (Figure 3.6), firing rates similarly decreased with contraction time course across MU samples (table 3.2). In both age groups the slope of the firing rate decrease during constrained-velocity movement was negatively correlated to the 2- and 3-fold difference in movement velocity (Spearman’s rank, rho = -0.36, p < 0.001), indicating that faster limb movements were related to a greater MU firing rate decrease (i.e., negative slope).
Table 3.2 Average firing rate change as function of contraction time course.

<table>
<thead>
<tr>
<th>Age</th>
<th>Condition</th>
<th>Velocity range</th>
<th>Linear slope coefficient</th>
<th>µSSE</th>
<th>Number of contractions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>Isometric</td>
<td></td>
<td>+1.5 (-1.0, 3.9)</td>
<td>47.6</td>
<td>26</td>
</tr>
<tr>
<td>O</td>
<td></td>
<td></td>
<td>-0.3 (-1.3, 1.4)</td>
<td>11.8</td>
<td>30</td>
</tr>
<tr>
<td>Y</td>
<td>Unconstrained-velocity</td>
<td>Vmax</td>
<td>179-214</td>
<td>-41.0 (-82.3, -14.3)</td>
<td>79.2</td>
</tr>
<tr>
<td>O</td>
<td></td>
<td></td>
<td>159-205</td>
<td>-12.5 (-32.8, 5.9) *</td>
<td>74.1</td>
</tr>
<tr>
<td>Y</td>
<td>Constrained-velocity</td>
<td>25%</td>
<td>45-53</td>
<td>-3.8 (-9.2, -0.8)</td>
<td>23.4</td>
</tr>
<tr>
<td>O</td>
<td></td>
<td></td>
<td>40-51</td>
<td>-0.3 (-7.0, 2.1)</td>
<td>29.6</td>
</tr>
<tr>
<td>Y</td>
<td></td>
<td></td>
<td>90-107</td>
<td>-16.7 (-25.9, -10.5)</td>
<td>36.5</td>
</tr>
<tr>
<td>O</td>
<td></td>
<td></td>
<td>80-103</td>
<td>-12.2 (-21.5, -1.6)</td>
<td>45.4</td>
</tr>
<tr>
<td>Y</td>
<td></td>
<td></td>
<td>134-161</td>
<td>-37.6 (-37.6, -13.7)</td>
<td>37.9</td>
</tr>
<tr>
<td>O</td>
<td></td>
<td></td>
<td>119-154</td>
<td>-35.4 (-35.4, -7.4)</td>
<td>71.2</td>
</tr>
</tbody>
</table>

The ensemble median (1st, 3rd interquartile range) are reported from the distribution of MU trains subset by condition for the young (Y) and older (O) age groups. The velocity range (deg • s⁻¹) shows the peak or constant voluntary elbow extension movement velocity for each condition across participants. Characteristics of the firing rate change during contraction was quantified as the slope of the simple linear regression (in Hz) as a function of contraction time course (in seconds) calculated for each MU train. Linear regression was adjusted based on the averaged firing rate variance explained by elbow extension torque, that was variable across conditions. The ensemble median of the averaged sum of squared error (µSSE) was calculated from residuals of the linear regression. The number of contractions refers to the total number of MU firing rate trains that were quantified for each age group and condition, with each MU sample representing 1-3 slope coefficients. In both age groups, the firing rate slope negative during muscle shortening contraction as compared to the isometric contraction (Wilcoxon signed rank, p ≤ 0.001). Significance (*) between age groups within the same condition was tested using the Wilcoxon rank sum test, p < 0.01.
Figure 3.6 Summary MU firing rate activity from the anconeus muscle and elbow joint kinematics during constrained-velocity movement (i.e., isokinetic).

Individual MU samples (30 total) were followed between conditions for each age group with the first detected MU potential (i.e., firing recruitment threshold) aligned with time-zero. The constant movement velocity (% of Vmax) for each participant was normalized to the maximum velocity (Vmax) achieved during unconstrained-velocity movement. A) Representative data from a younger participant (27 years old). From top to bottom: firing graph of the same MU potential waveform; selected waveforms (in blue); 1 kHz high pass filtered intramuscular EMG; unfiltered intramuscular EMG recorded form the anconeus muscle; bi-polar surface EMG from the long head of the triceps brachii muscle;
elbow extension torque; velocity; and position. **B-D)** For each movement velocity (25, 50 and 75% of Vmax), MU trains from the young and older groups were combined, the x-axis (time) was normalized to the maximum contraction time of each steady-state velocity, and each line represents the LOESS fits (span 0.5 s) of firing rates and each kinematic parameter dependent on contraction time course, with grey shading representing the 95% confidence interval. Each parameter is normalized in relative percentage to the maximum for each participant, except for firing rate in Hz.

### 3.4 Discussion

Unlike isometric contractions, very old adults did not have significantly lower MU firing rates compared to younger adults during voluntary elbow extension movements. This finding was contrary to our hypothesis and suggested that neural drive to the anconeus muscle during maximal voluntary efforts is not reduced during muscle shortening contractions, but is relatively increased, despite contractions being ~33% weaker and ~10% slower in the very old compared with the young. As movement is time-dependent, firing rates were further compared as a function of contraction time course across MU samples. At firing recruitment threshold, the associated torque was greater and firing rates were lower in the older group compared to the young, indicative of an age-related change of MU function. However, throughout shortening contractions that occurred with unconstrained-velocity, firing rates were greater than isometric contractions in both age groups, but as a function of time, the firing rate decrease was 3-fold greater in the young compared to the old. Whereas, during constrained-velocity movements (i.e., isokinetic contractions), the firing rate decrease was not different between age groups, despite the older group moving at slower isokinetic speeds. Overall, these findings indicate that neural drive to the agonist muscle, measured as MU firing rates, are facilitated during movements in very old adults, or become minimally decreased.

**Relating motor unit activity to limb contractile outputs**

Muscle twitch responses in older adults have slowed contractile responses (Allman and Rice 2004; Vandervoort and McComas 1986), explained by muscle fibre atrophy (Lexell et al. 1988), reduced calcium ion sensitivity (Straight et al. 2018), and overall altered
contractile mechanics (Gries et al. 2019; Łochoński et al. 2008). In sustained isometric contractions, lower firing rates in the older group occurred with lower voluntary elbow extension torque. However, during elbow extension movement, reduced elbow extension torque may not be dependent on neural drive to the same extent as during isometric contractions (Kwon et al. 2020) because MU firing rates were not different between age groups. During elbow extension movements, the relationship between MU firing rates and elbow extension RTD was used as a proxy, similar to the relationship of contractile speed-matching in reduced preparations (Eccles et al. 1958; Gardiner 1993; Hammarbarg and Kellerth 1975; Kernell 1979). In relating firing rates to RTD (rate of torque development), the older group had a weaker correlation as compared to the young, supporting reduced muscle contractile capacity of the elbow extensors (Allman and Rice 2004; Barry et al. 2005; Gilmore et al. 2017; Gries et al. 2019; Larsson and Edström 1986; Lexell et al. 1988; Straight et al. 2018). Therefore, a reduction of muscle contractile responsiveness from the survived MU pool (Campbell et al. 1973; Gilmore et al. 2016; Lexell et al. 1988; Ling et al. 2009; Łochoński et al. 2008; Piasecki et al. 2016; Tomlinson and Irving 1977) may limit voluntary strength despite being provided frequency (Łochoński et al. 2010) and MU firing rate inputs that are similar to the young during-movements. Indeed, the relationship between firing rates and RTD would be especially important at contraction onset (i.e., isometric contraction that is pre-movement) to affect the absolute velocity of the limb apparatus (Partridge 1966). Expectedly, this relationship at the single MU level would be stronger in comparisons of the principal agonist muscle – the triceps brachii.

**Firing rates decreased less during-movement in the older group**

Few investigations have discriminated single MU activity during movements that are unconstrained (Cowling et al. 2016; Desmedt and Godaux 1979; Garland et al. 1996; Gydikov et al. 1986; Harwood et al. 2011, 2012; Harwood and Rice 2014). The anconeus muscle was utilized due to the clear intramuscular EMG recordings during elbow extension movements, attributed to the smaller absolute changes of fascicle length and pennation angle as compared with larger limb muscles (Nelson et al. 2016; Stevens et al. 2014). In young adults, the anconeus muscle is estimated to have fewer than 100 MUs
(Stevens et al. 2013), and in very old adults undergoes MU loss (~40% lower MU number estimates as compared to the young) with diminished compensatory remodeling (Gilmore et al. 2017), contributing to the well isolated MU potentials observed in the older group. In some muscles of very old adults, including the anconeus (Gilmore et al. 2017), the process of collateral reinnervation following MU loss may cease to become an effective compensatory mechanism, resulting in a muscle with smaller surviving MUs (Aare et al. 2016; Gilmore et al. 2017; Lexell et al. 1988). The current group of very old adults very likely had MU loss of the anconeus muscle, because six of the thirteen older participants were also part of a previous investigation that quantified electrophysiological characteristics of MU quantity and quality (Gilmore et al. 2017). However, despite these age-related alterations, differences in firing rates throughout elbow extension movements (i.e., larger effect of muscle shortening) were not likely explained by reduced neural drive to the muscle at the MU level, and was contrary to isometric contractions and broader literature of isometric contractions in other limb muscles (Connelly et al. 1999; Dalton et al. 2010; Erim et al. 1999; Kamen et al. 1995; Kamen and Knight 2004; Kirk et al. 2018, 2019, 2021b). In support of our findings, single MU recordings of low threshold MUs in the diaphragm muscle in young, middle-age and older adults during quiet breathing showed that despite MU waveforms having electrophysiological characteristics of remodelling and collateral reinnervation (i.e., larger MU potential area), the averaged firing rates (14-15 Hz) were not different between young and older participants (Nguyen et al. 2019). Instead, age-related differences of firing rates were found when compared as a function of contraction time course, with the older group having delayed firing onset during inspiration, associated with muscle shortening phase of diaphragm contraction (Nguyen et al. 2019).

During unconstrained-velocity movement, the firing rate decrease (i.e., negative slope) throughout muscle shortening contraction was attenuated in the older group. In contrast, during constrained-velocity movement, there was no statistical difference in the firing rate decrease between age groups. As all contractions were required to be of MVC efforts, observed differences during movements, and between age groups may be explained by the integration of afferent synaptic inputs (Christou and Enoka 2011; Pascoe et al. 2013; Pruszynski et al. 2011; Weiler et al. 2019; Wolpe et al. 2016), especially as
Peripheral nerves in the upper limb are comprised of ~90% afferent axons (Gesslbauer et al. 2017). For example, during sustained isometric contractions, firing rates recorded from the anterior leg are lower during afferent nerve block (Macefield et al. 1993), therefore, lower firing rates in the older group during isometric contraction may be due to reduced afferent drive to the MU pool. Similarly, increased afferent feedback during movement may explain the increase of MU firing rates observed in both age groups. From the muscle spindle system (Macefield and Knellwolf 2018; Windhorst 2007), differences in excitatory afferent feedback between conditions related to elbow extension acceleration (appendices B and C) may explain how firing rates decrease during unconstrained-velocity movements (Blum et al. 2017; Day et al. 2017; Partridge 1967; Weiler et al. 2019). Because Ia monosynaptic feedback are lower in older adults (Kallio et al. 2010) and are slower conducting in experiments of older cats (Chase et al. 1985; Morales et al. 1987), reduced or altered excitatory drive from the muscle spindle system may further explain how MU firing rates started lower and decreased less in older adults, as compared to the young.

At contraction onset, isometric contractions have a component of muscle shortening despite a lack of change in joint angle (Griffiths 1991). However, when continued throughout a range of motion, overall muscle shortening is significantly greater than during a sustained isometric contraction (Nelson et al. 2016; Stevens et al. 2014). Unlike prior reports (Garland et al. 1996; Partridge 1966), single MU firing rates here were not closely matched to elbow joint acceleration during unconstrained-velocity movements, perhaps because the effects of electromechanical delay (Buchthal and Rosenfalck 1960) and limb momentum made the comparison more complex. Our data showed MU firing rates were significantly lower in older adults during sustained isometric contractions. From this, it is reasonable to expect that with slower muscle shortening movements at heavier loads, the isometric state (i.e., pre-movement) would be prolonged, resulting in greater differences of MU firing rates between age groups until the moment of inertia is overcome (Partridge 1966). Beyond the modest 20% normalized torque resistance used here and with prior studies of low-loaded movements in younger adults (Garland et al. 1996; Harwood et al. 2011; Harwood and Rice 2014; Ivanova et al. 1997), heavier loaded movements would be slower with larger muscle torques, and presumably have greater
effects of the length-tension relationship (Brown and Loeb 2000a; Partridge and Benton 1981), potentially impacting the complexity of the MU firing rate requirement.

Are voluntary movements less inhibited in older adults?

During sustained isometric contractions, MU firing responses are expected to be closely related to descending synaptic inputs (Fuglevand et al. 1993; Heckman and Binder 1991; Monster and Chan 1977), with the input-output relationship between synaptic inputs and firing rate outputs altered by persistent inward currents (Binder et al. 2020) and extrinsic feedback to the MU pool (Fuglevand et al. 2015; Revill and Fuglevand 2017). In the exploration between age groups only during isometric contraction, lower firing rates could be reasonably explained by degradations of synaptic inputs (Maxwell et al. 2018; Rowe et al. 2006), altered intrinsic excitability, and prolonged extrinsic monosynaptic reflex inputs to the lower motor neuron (Chase et al. 1985; Morales et al. 1987). As a function of contraction time course, we did not observe MU firing rates to have clear exponential decreases (Granit et al. 1963b; Kernell 1965a; Miles et al. 2005) during isometric contractions or elbow extension movements, and possibly explained by differences in synaptic drive to, or conductance properties of anconeus motor neurons (Harwood et al. 2011). Furthermore, in comparison to lower limb muscles, corticospinal projections to the upper limb are more direct (Brouwer and Ashby 1990, 1992), further complicating the comparison of MU firing rate adaptation (i.e., exponential decrease with time) across muscles based on estimates of the strength of synaptic inputs in human participants. During elbow extension movements (i.e., greater effects of muscle shortening), MU firing rates were higher than during isometric contractions in both age groups and the age-related decreases of elbow extension strength or movement velocity were not clearly explained by differences of neural drive at the single MU level. Because firing rates were measured from direct recordings of MU EMG activity during MVC efforts, but had different age-related effects dependent on the condition, this indicated that different intrinsic and extrinsic controls of the input-output relationships at the motor neuron likely occurred (Heckman and Binder 1991; Revill and Fuglevand 2017), that were relatively facilitated, or minimally decreased in the aged system during movement.
As a function of contraction time course, firing rates decreased during muscle shortening contractions, but to a lesser extent in the older group. This observation likely resulted from large excitatory inputs at the lower motor neuron at pre-movement initiation as firing rates generally were highest, and facilitated from corticospinal descending inputs (Chen et al. 1998; Devanne et al. 1997; Lawrence and Kuypers 1968a) that were becoming intrinsically amplified at the motor neuron (Granit et al. 1963a; Heckman 1994; Heckman and Binder 1991; Lee et al. 2003a). After this, firing rates largely decreased throughout contraction time course, and was likely attributed to more than only a reduction of descending synaptic drive, or firing rate adaption (Kernell 1965a; Miles et al. 2005), as extrinsic feedback from antagonist reciprocal inhibition during a small joint rotation will decrease intrinsic currents (Hyngstrom et al. 2007). However, the potency of this extrinsic affect remains unknown in the system of aged adults. In the older group, lower firing rates at contraction onset would have influenced the immediate catch-like response of the muscle (Binder-Macleod and Lee 1996; Christie and Kamen 2006), however, this did not explain why firing rates decreased less in the older group (table 3.2). A more likely explanation as to how firing rates decreased less in the older group during movement is based on sensorimotor deficit from the periphery (Chase et al. 1985; Dunn et al. 2015; Goble et al. 2009; Klever et al. 2019; Maxwell et al. 2018; Morales et al. 1987; Vaughan et al. 2017), requiring greater descending synaptic inputs to the survived MU pool during movements (Wolpe et al. 2016). There is support for this idea from small finger and hand movement in older adults that demonstrated sensory deficit with structural and functional changes of the pre-supplemental motor area (Wolpe et al. 2016). Altogether, lower firing rates at MU recruitment threshold may correspond to structural and intrinsic decrement of the MU pool in response to initial descending synaptic inputs, but during-movement, neural drive to the muscle in very old adults was like the young adults during maximal voluntary efforts.
3.5 Limitations

Although we had no direct way to assess whether the MU pool was fully recruited in each age group, contractions were required to be maximal efforts (Desmedt and Godaux 1977, 1979; Harwood and Rice 2012), as the anconeus muscle becomes fully recruited during submaximal contraction intensities >50% of MVC in younger adults (Harwood et al. 2013; Harwood and Rice 2012; Stevens et al. 2013). Likewise, we were not able to rank single MU samples by an electrophysiological threshold other than elbow extension torque or the presumed size-ordered relationship. The anconeus was used as a model muscle (Le Bozec and Maton 1982, 1987; Davidson and Rice 2010; Gilmore et al. 2017; Harwood et al. 2011; Harwood and Rice 2012; Miguel-Andres et al. 2017; Stevens et al. 2014, 2013), sharing the same main innervation (i.e., radial nerve) as the triceps brachii, and acts as both a stabilizer and weak elbow joint extensor during contraction (Le Bozec and Maton 1987; Coriolano et al. 2009; Miguel-Andres et al. 2017; Stevens et al. 2014). We did not characterize agonist-antagonist muscle activation (Hallett et al. 1975) between age groups, but antagonist and joint stabilizer muscles likely have importance in explaining firing rate variability during movements (Gribble et al. 2003). As a measure of firing rate change throughout contraction, simple linear regression was limited by the number of firing rates for each MU train that was dependent on both the absolute frequency and movement velocity, without adjustments for nonlinear components other than torque. Similarly, calculation of the likelihood-ratio test statistic relied on mixed-effects regression modelling that weighted firing rates equally between age groups. Bipolar surface EMG was recorded from the long head of the triceps brachii and was related weakly to kinematic torque during elbow extension movements. Surface EMG amplitude was positively related to anconeus MU firing rates through calculation of the joint probability distribution covariance. This indicated that an increase of anconeus MU activity corresponded to an increase of triceps brachii MU activity, however, because surface EMG represents more than just firing rates (Enoka and Duchateau 2015) the use of surface EMG as a measure of neural drive was limited in combination with direct MU recordings.
General limitations were in the cross-generational comparisons between age groups, as we could not account for genetic and lifestyle influences which likely impact neuromuscular function. To control for age-dependent effects based on biological sex and physical activity, only males were tested and recruited from an active healthy population of older adults (Hunter 2016), however, systematic comparisons of MU activity during movement between the sexes is a necessary next step. Furthermore, frail adults with severe sarcopenia (Gilmore et al. 2016) were not investigated, but with impaired limb movements, neural drive to the muscle is likely altered and different from healthy aged adults.

3.6 Conclusions

Voluntary elbow extension movements were weaker and slower in the older group but occurred within a firing rate distribution like the young, providing evidence that firing rates are dependent on the contraction condition and less dependent on the effects of ageing. As only males were tested, these findings have yet to be confirmed in the female sex. Between conditions, MU firing rates for each participant were greater during elbow extension movements compared to sustained isometric contractions in both age groups. Between age groups, firing rates were lower during isometric contraction in the older group, but during elbow extension movements requiring active muscle shortening to assist in joint rotation, firing rates were similar. This indicates that despite voluntary weakness and in a muscle that undergoes significant MU loss (Gilmore et al. 2017), neural drive to the agonist muscle can be relatively facilitated or diminished to a lesser extent in very old adults during these kinds of joint-movements. This finding was supported when comparing firing rates as a function of contraction time course during muscle shortening contractions, as firing rates were significantly lower in the older group at contraction onset (i.e., pre-movement isometric contraction), but throughout contraction duration when movement velocity was unconstrained, the firing rate decrease was 3-fold lower in the older group as compared to the young. These results highlight that spinal motor neuron outputs during elbow extension movements are more complex
and facilitated as compared to sustained isometric contractions. When integrated with MU loss and voluntary weakness in very old adults, neural drive to the muscle at the single MU level was not the limiting factor.

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Chapter 4

4. The relationship of agonist muscle single motor unit firing rates and elbow extension limb movement kinematics

A version of this chapter has been published in Experimental Brain Research (Kirk and Rice 2021) and is presented here with permission (license number: 5118781193907).

This study explored the relationship between single motor unit (MU) firing rates (firing rates) and limb movement velocity during voluntary shortening contractions when accounting for the effects of time course variability between different kinematic comparisons. Single MU trains recorded by intramuscular electromyography in agonist muscles of the anconeus (n = 15 participants) and lateral head of the triceps brachii (n = 6) were measured during each voluntary shortening contraction. Elbow extension movements consisted of a targeted velocity occurring along the sagittal plane at 25, 50, 75 and 100% of maximum velocity. To account for the effect of differences in contraction time course between parameters, analysis of each MU potential was time-locked throughout the shortening muscle contraction and linked with separated kinematic parameters of the elbow joint. Across targeted movement velocities, instantaneous firing rates were significantly correlated with elbow extension rate of torque development \((r = 0.45)\) and torque \((r = 0.40)\), but firing rates were not correlated with velocity \((r = 0.03, p = n.s.)\). Instead, firing rates had a weak indirect relationship with limb movement velocity and position assessed through multiple correlation of the stepwise kinematic progression. Results show that voluntary descending synaptic inputs correspond to a more direct relationship between agonist muscle firing rates and torque during shortening contractions, but not velocity. Instead, firing rates were indirectly correlated to preparing the magnitude of imminent movement velocity of the lagging limb through torque.

4.1 Introduction

The rate of limb movement is ultimately determined by the shortening contraction speed of the agonist muscle (Edman 2014). Under voluntary control, tension in the muscle, and
its corresponding force, are mediated by motor unit (MU) recruitment and firing rate modulation that are first controlled by descending synaptic inputs (Devanne et al. 1997; Fuglevand et al. 1993; Heckman and Binder 1991). Yet, understanding how firing rates correspond to movement kinematics is largely based on inference from isometric contraction.

From the few comparisons in human tibialis anterior, soleus, hand and upper limb muscles (Desmedt and Godaux 1979; Garland et al. 1996; Gydkov et al. 1986; Harwood et al. 2011; Harwood and Rice 2012, 2014; Howell et al. 1995; Kallio et al. 2013; Oliveira and Nergo 2021), averaged firing rates are higher during muscle shortening as compared to isometric contraction, and are positively related to movement speed (Desmedt and Godaux 1979; Garland et al. 1996; Gydkov et al. 1986; Harwood et al. 2011; Harwood and Rice 2014). Whereas, in extra-ocular muscles in non-human primates firing rates are directly related to eye movement velocity with proportional changes throughout the contraction duration (Van Gisbergen et al. 1981; Robinson 1970). This work has been used to inform theory on goal directed arm movements (Harris and Wolpert 1998). However, in understanding relationships between firing rates and limb kinematics, the effect of differences in contraction lag (i.e., time shifted or electromechanical delay) between individual kinematic parameters are not well integrated; despite the importance of maximal work and contraction duration being described in some of the first analyses of fast movements (Hill 1922). Recently, this has been supported from experiments investigating the cortical (Churchland et al. 2012; Churchland and Shenoy 2007a) and spinal (Gydkov et al. 1986; Weiler et al. 2019) control of muscle activity and movement (Flash and Hogan 1985; Hallett et al. 1975; Harris and Wolpert 1998) framing neuromuscular control as changing time-evolving process throughout volition. The importance of understanding which kinematic parameters are directly or indirectly related to firing rates throughout contraction duration would provide a clearer understanding of the underlying timing of descending or sensory synaptic inputs that predict and shape movement. Therefore, in building on relatively few prior MU studies during voluntary limb movements (Garland et al. 1996; Harwood et al. 2011; Harwood and Rice 2014), our aim was to compare the strength of relationships
between firing rates and separated kinematic parameters when accounting for an effect of contraction lag.

Unlike eye movement (Van Gisbergen et al. 1981; Robinson 1970), limb movement has relatively larger intrinsic force lag in response to firing rate inputs during the onset of isometric contractions (Baldissera et al. 1998; Partridge 1965) or load-moving shortening contractions (Brown et al. 1999; Buchthal and Rosenfalck 1960; Partridge 1966), with force lag attributed to the electromechanical delay (EMD) across muscle fibres and tendon structures in transferring intrinsic muscle tension (Balnave and Allen 1996; Ebashi and Endo 1968; Ford et al. 1977; Herzog et al. 2012; Kjaer 2004). During limb movement, EMD, which represents the time between measured electromyography activity and a kinematic movement response, is greater during muscle shortening as compared to isometric or lengthening contraction (Cavanagh and Komi 1979; Norman and Komi 1979), and is attributed to differences in muscle stiffness, because frequency-dependent catch-like responses of human muscle can be more enhanced during shortening contractions (Binder-Macleod and Lee 1996). Therefore, during shortening contractions, the relationship of firing rates to kinematic parameters with minimal contraction lag are expected to be more directly predictive of each other (Van Gisbergen et al. 1981; Goldberg et al. 1998; Robinson 1970), as compared to relationships with greater lag, for example firing rates with limb movement velocity.

From this, firing rates would be expected to have a weaker direct relationship with limb movement velocity as compared with torque, however, there is little evidence to support this at the single MU level during whole muscle shortening contractions. The analysis was designed to account for the effect of differences in contraction lag between correlations of firing rates and measured kinematic comparisons. This was accomplished by instantaneously relating each firing rate interval from single MU train recordings throughout agonist muscle shortening contractions to separated kinematic parameters at the same time coordinate (figure 4.1, panels A and B). We hypothesized a stronger relationship will occur between firing rates and elbow extension torque as compared with either velocity or position.
Figure 4.1 Relationship of agonist muscle single MU firing activity with measured limb kinematics during voluntary elbow extension movement.

Intramuscular EMG of the lateral head (LH) of the triceps brachii muscle with a MU train identified and followed within each targeted velocity movement at A) V25% and B) V100% of maximum velocity during a MVC (i.e., Vmax). C) Cartoon depiction of the targeted velocity and elbow extension during 120 deg range of motion (ROM). For intramuscular sites, the location of the anconeus (A) and LH of the triceps brachii muscles are depicted. D) Summary raw data for V25 and V100 targeted velocity conditions from an adult male participant (age 23 years old). Kinematic parameters were linked to each firing rate interval throughout the common contraction time course. The MU potential (MUP) waveforms for MU1 and MU2 identified in panels A and B, are overlayed for each targeted movement velocity, respectively. As an example, the blue arrow denotes the relationship between the firing rate, torque, velocity, and position tracings linked to the first firing rate interval for MU1 at the same time coordinate, with 12.7 Hz, 17.2 Nm, 0.6 deg • s⁻¹, and 0.2 deg, respectively. This was done for each
measured firing rate interval across MU trains, and these relationships from the composite of MU trains are summarized in figures 4.2-3.

4.2 Methods

Participants. Fifteen adults (11 males and 4 females, 20-34 years old) free of neurological or orthopedic illness consented and participated in 34 experimental sessions. These experiments were approved by the local University’s research ethics board and conform to the latest revision of the Declaration of Helsinki (appendices R and S). Each participant gave their informed written consent.

Experimental set-up. Seated upright in a dynamometer (Humac Norm, Computer Sports Medicine Inc., MA, USA) the upper limb, shoulders and hip were secured. The left elbow was aligned to the axis of rotation and the range of motion (ROM) occurred along the sagittal plane with the forearm in the neutral or semi-pronated position. Signal acquisition of calibrated torque, angular velocity, and position from the dynamometer during elbow extensions were analog-digital converted (power-1401, Cambridge Electronic Design, Cambridge, UK) and each sampled at 1 kHz (spike2, version 7, Cambridge Electronic Design).

Intramuscular electromyography (EMG). Sterilized intramuscular electrodes (102 µm diameter insulated steel wire, California Fine Wire company, USA) were inserted into the mid-point and distal aspect of the anconeus and distal aspect of the lateral head of the triceps brachii using a 27-gauge hypodermic needle. Intramuscular EMG in the anconeus was recorded from a bi-polar configuration and grounded over the clavicle with a 2.5x2.5 cm surface electrode (Harwood et al. 2011; Harwood and Rice 2012, 2014). A monopolar intramuscular electrode configuration was used for the lateral head of the triceps brachii with a reference and ground surface electrode placed on the acromion process and deltoid muscle, respectively. Through pilot testing, a monopolar configuration resulted in clearer MU train recordings in the triceps brachii (figure 4.1, panels A, B and D), likely due to anatomical features that differ from the anconeus (Stevens et al. 2014) in which a bipolar electrode configuration provided better recordings (Harwood et al. 2011; Harwood and
Rice 2012, 2014). Each EMG channel was differentially hardware amplified (x100-500), band pass filtered (0.01-10 kHz, Neurolog, Digitimer, UK) and sampled at 25 kHz.

**Elbow extension movement.** Real-time feedback of elbow joint velocity on a computer monitor was provided (figure 4.1). Elbow extension movement was commonly loaded with 20% of isometric MVC torque normalized for each participant, with the dynamometer in the unconstrained-velocity (i.e., isotonic-like) configuration. This low-load baseline of elbow joint torque acted as common tension against each movement and was used to normalize contraction initiation across movement speeds and participants. After familiarization and practice, each participant exerted elbow extension MVC limb movements with peak velocity defined as their maximum movement velocity (Vmax), and consisted multiple (6-9) attempts. The range of motion for each movement was 120 deg, starting at 40 and extended to 160 deg along the sagittal plane. Four velocity ranges (0-25, 25-50, 50-75 and 75-100% of Vmax) were calculated relative to each participant’s Vmax that were used during targeted velocity movements (figure 4.1, panel A). The velocity for each targeted movement was self-initiated and in a self-defined order. Each set consisted of the four different targeted movement velocities and sets were repeated 6-12 times for each participant. To minimize any effects of fatigue, rest periods of 3-5 minutes were provided between contraction sets. On-line examination of the quality of the intramuscular EMG signals helped to determine when sufficient representative recordings were captured for off-line analysis for each condition and participant. This often-required repositioning and reinsertion of electrodes in a different region of the muscle belly between contraction sets.

**Data analysis.** Intramuscular EMG signals were high pass filtered (IIR, second order, 1 kHz), and individual MU potential waveforms were sorted using the wavemark algorithm requiring manually inspection and correction based on template-matching (spike2 software, version 7.20). For inclusion, each distinct waveform, attributed to a single MU train was required to have consistent, and repeatable waveform shape within each contraction (Harwood et al. 2011; Harwood and Rice 2012, 2014). Furthermore, the amplitude of a distinct MU waveform slowly changed across successive MU potentials (figure 4.1, panels A and B), however, the shape remained relatively constant (see
overlays, figure 4.1, panel D). Within each contraction, inter-spike-interval (ISI) time in seconds was calculated from pairs of successive MU potentials and the frequency was calculated. Across different contractions, electrode displacement resulted in different and new MU potential waveforms being recorded, therefore MU trains were analyzed as unpaired between each contraction.

Alignment of firing rate intervals to measured elbow joint kinematics. For each contraction, MU potentials measured within each MU train were locked to the corresponding time coordinate of the contraction time course in spike2 software. These coordinates were then used to link the measured kinematic parameters of elbow extension that occurred at the same time points (see blue arrow, figure 4.1, panel D). As the sampling rate was 1 kHz for each kinematic parameter, MU potentials were linked with calibrated torque, angular velocity, and position displacement of the elbow joint with a resolution of 1 ms. In addition, the rate of torque development (RTD), angular acceleration, and instantaneous power (product of torque and velocity) were calculated relative to the time point of the first MU potential at firing recruitment threshold for each MU train. Data from Spike2 were exported, with statistical analysis and figures written in R (version 3.6).

Principal component analysis (PCA). The aggregated dataset comprised both elbow joint kinematic (RTD, torque, acceleration, velocity, position) and non-kinematic parameters (participant, muscle, participant’s sex, testing session, targeted velocity, and MU train). Parameters were scaled to a standard normal distribution and centered prior to PCA using the package ‘ggfortify’ (Tang et al. 2016).

Mixed effects linear regression Additive linear regression from the packages ‘lme4’ (Bates et al. 2015) and ‘lmerTest’ (Kuznetsova et al. 2017; Luke 2017) was used to estimate the statistical dependence of firing rates from the aggregated dataset. To minimize the effect of leverage on linear regression by the few high frequency firing rates that are physiologically relevant (i.e., doublets), firing rates were transformed by the natural logarithm. The model was fit with fixed effects that included elbow joint kinematic parameters, participants’ sex, and muscle (anconeus or triceps brachii), and
random intercept effects that were non-kinematic including the participant (Tenan et al. 2014), MU train and the targeted velocity.

**Correlation.** Between firing rates and kinematic parameters, probability distribution estimates of Pearson’s correlations were calculated using bootstrap simulation from the package ‘boot’ (Canty and Ripley 2019; Davison and Hinkley 1997). As distributions were unimodal, we used bootstrapping as a nonparametric approach with data normalized per participant (Efron 1979; Wagstaff et al. 2009). Furthermore, bootstrap resampling was stratified by participant and consisted of $1 \cdot 10^5$ iterations per probability distribution estimate (figure 4.3). When subset by participant, correlations were stratified by targeted velocity. Statistical significance was determined by the range of bootstrapped Pearson’s r coefficients not crossing zero. To quantify indirect relationships between firing rates and kinematic parameters, multiple correlation was calculated based on the product of statistically significant stepwise correlations throughout contraction time course.

### 4.3 Results

During voluntary elbow extensions at four different targeted movement velocities along a fixed plane, 856 MU trains were measured from intramuscular EMG recordings during separate agonist muscle shortening contractions (table 4.1). The effect of contraction lag between targeted limb movement comparisons was accounted for by instantaneously linking each firing rate interval to limb kinematic parameters (see figure 4.1, panel D). From PCA of the aggregated dataset, the proportion of cumulative variance explained from included parameters was >90% by the eighth principal component rotation (appendix D). With the explained variance in principal components one (27%) and two (19%), attributed to the largest eigenvectors of elbow extension torque and velocity, respectively.

From the aggregated dataset, the response of firing rates analyzed by linear regression (adjusted r-squared 63%), resulted in kinematic parameters of elbow extension RTD (t value 24.3, p < 0.001) and torque (t value 16.4, p < 0.001) having the strongest estimated
statistical difference to effect firing rates, as compared to acceleration (t value -6.2, p < 0.001), velocity (t value -12.8, p < 0.001) and position (t value -5.4, p < 0.001). From non-kinematic parameters, the difference between the anconeus and triceps brachii muscles had no significant effect on firing rates (t value -0.14, p = 0.88), but a sex-related effect (t value -4.0, p = 0.002) indicated that firing rates were ~8 Hz higher in females as compared to males based on the slope constant.

**Table 4.1** Dataset summary of motor unit trains during voluntary elbow extension movements.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Anconeus</th>
<th>Triceps brachii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isometric MVC (Nm)</td>
<td>65 ± 18</td>
<td>(37-90)</td>
</tr>
<tr>
<td>Movement MVC (deg • s⁻¹)</td>
<td>200 ± 28</td>
<td>(150-263)</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participants (n)</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Sex (male: female)</td>
<td>11: 4</td>
<td>2: 4</td>
</tr>
<tr>
<td>Total MU trains (#)</td>
<td>628</td>
<td>228</td>
</tr>
<tr>
<td>MU trains (#) / participant</td>
<td>42 ± 19</td>
<td>38 ± 14</td>
</tr>
<tr>
<td>MU trains (#) / targeted velocity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-25% Vmax</td>
<td>166</td>
<td>72</td>
</tr>
<tr>
<td>25-50</td>
<td>153</td>
<td>69</td>
</tr>
<tr>
<td>50-75</td>
<td>128</td>
<td>26</td>
</tr>
<tr>
<td>75-100</td>
<td>181</td>
<td>61</td>
</tr>
<tr>
<td>Median firing rate (Hz) / targeted velocity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-25% Vmax</td>
<td>15 (12-18)</td>
<td>17 (13-21)</td>
</tr>
<tr>
<td>25-50</td>
<td>16 (12-21)</td>
<td>20 (15-26)</td>
</tr>
<tr>
<td>50-75</td>
<td>20 (14-26)</td>
<td>24 (18-35)</td>
</tr>
<tr>
<td>75-100</td>
<td>26 (19-36)</td>
<td>31 (23-42)</td>
</tr>
</tbody>
</table>

The aggregated dataset comprised 11,022 firing rate intervals from 856 MU trains that were measured from 15 participants. Single MU recordings were in two agonist muscles during voluntary shortening contraction, that resulted in elbow extension movement. Values are mean ± standard deviation, and for firing rates the median (1st - 3rd inter-
quartile range). Successful recordings of MU trains occurred in 15 participants for the anconeus and 6 participants for the lateral head of the triceps brachii muscle.

**Figure 4.2** Representative from a female participant (21 years old).

Scatterplots depict the relationship between firing rates from agonist muscle shortening contraction in the anconeus (A-F) and lateral head of the triceps brachii muscles (G-L) with elbow extension kinematic parameters. Each dot represents an instantaneous
association of a firing rate interval from single MU EMG activity across measured elbow joint kinematic parameters, with the effect of contraction time course accounted for, with 497 firing rate intervals in the anconeus and 790 firing rate intervals in the triceps brachii. Elbow extension torque, velocity, and position are relative to the participant’s maximum. Elbow extension rate RTD is depicted in the log scale. Histograms representing summary frequency of firing rate intervals for each muscle with the gray vertical as the median statistic for the anconeus (median 22 Hz) and triceps brachii (median 21 Hz). For scatterplots, the mean± standard deviation of correlation values (r) were calculated from the simulated probability distribution of bootstrap correlations stratified by targeted velocity.

The relationship between firing rates and limb kinematic parameters across targeted velocities was explored through bootstrap correlation (figures 4.2 and 4.3) in the anconeus (panels A-F) and lateral head of the triceps brachii muscles (panels G-L). In both muscles, the strength of correlations was similar for the same associations, with the variability and shifts of data points having similar patterns between the different targeted movement velocities (25, 50, 75 and 100% of Vmax). Correlations are depicted in a female participant (figure 4.2) and from the aggregated dataset of all participants (figure 4.3).

The relationship between firing rates with elbow extension RTD and torque was significantly correlated (figures 4.2 and 4.3, panels B, C, H and I), however, the relationship between firing rates and elbow extension velocity was not significantly correlated (figure 4.3, panels D and J). Through correlation, this indicated that firing rates were more directly related to torque parameters but were less directly related to elbow extension velocity. Instead, firing rates were indirectly related to elbow extension velocity through a stepwise ordering of correlations between firing rates and torque (figures 4.2 and 4.3, panels C and I), and torque with velocity (figures 4.2 and 4.3, panels E and K).

From raw data tracings during limb movement (figure 4.1, panel D), the stepwise ordering is observed from the succession of peak values throughout contraction time course from firing rates to torque to velocity and to position, which were all significantly
correlated when compared in a stepwise ordering (figures 4.2 and 3). However, if firing rates were compared to a non-ordered kinematic parameter, such as between firing rates and elbow extension velocity (that was confounded by torque), the correlation was non-significant (figure 4.3, panels D and J). Therefore, it was unlikely that firing rates were directly related to limb velocity or position, but that higher movement velocity was attributed to higher preceding magnitudes of elbow extension torque (i.e., acceleration) and firing rates (figures 4.2 and 4.3, panels E and K). However, at the single MU level, this indirect relationship between firing rates and limb velocity, quantified through multiple correlation between firing rate to torque and torque to velocity, had a low predictive value in both the anconeus and triceps brachii muscles (r-squared = ~0.17).
Figure 4.3 Aggregated data from all participants.

Scatterplots depict the relationship between firing rates from agonist muscle shortening contraction in the anconeus (A-F) and lateral head of the triceps brachii muscles (G-L) with elbow extension kinematic parameters. Each dot represents an instantaneous association of a firing rate interval from single MU EMG activity across measured elbow joint kinematic parameters, with the effect of contraction time course accounted for, with
7,640 firing rate intervals in the anconeus and 3,382 firing rate intervals in the triceps brachii. Elbow extension torque, velocity, and position are in percentages relative to each participant’s maximum. Elbow extension RTD is depicted in the log scale. For the number of MU trains for each targeted velocity condition refer to table 4.1. Histograms representing summary frequency of firing rate intervals for each muscle with the gray vertical as the median statistic for the anconeus (median 17 Hz) and triceps brachii (median 19 Hz). For each scatterplot, the mean correlation value (r) was calculated from the simulated probability distribution of bootstrap correlations stratified by participant, and the standard deviation of the bootstrap distribution was between r = 0.01-0.02, see appendix E for estimate values and ranges.

4.4 Discussion

In this study, firing rates measured from recordings of single MU trains during agonist muscles shortening contraction, were instantaneously related to elbow extension kinematics throughout movement. Each voluntary effort was self-initiated (Chen et al. 1998), and graded by contraction intensity through different targeted velocity movements (Harwood et al. 2011). To account for the effects of contraction time course across associations, each firing rate interval was time-locked and instantaneously linked to separated kinematic parameters with a time resolution of 1 ms, that was well below the biological delay attributed to EMD during shortening contraction elbow extension movements that is 26±11 ms (Norman and Komi 1979). From the aggregated dataset that included both non-kinematic (i.e., participant, sex, etc.) and kinematic parameters, our results show that RTD and torque were most likely to have a significant effect on firing rates, as compared to acceleration, velocity, and position. Through correlation (figures 4.2-3), firing rates were directly related to elbow extension torque, but not directly related to elbow extension velocity. Instead, through multiple correlation between stepwise relationships of the kinematic progression (figure 4.3), firing rates were indirectly related to elbow extension velocity (r-squared = 0.17) and position (r-squared = 0.04-0.08), however these relationships had relatively lower predictive values.

At the single MU level during voluntary shortening contraction, the weak indirect relationship between firing rates and limb movement velocity (figure 4.3), suggests that
limb velocity is predicted but never directly controlled by firing rates, and this observation is important to consider in experiments that relate the timing of MU firings to movement speeds. Kinematic parameters of the limb lag throughout contraction time course (Baldissera et al. 1998; Binder-Macleod and Lee 1996; Cavanagh and Komi 1979; Norman and Komi 1979; Partridge 1966). Such lag was evident in the disassociation of firing rate to movement velocity (figure 4.3, panels D and J) suggesting that voluntary limb movement relies on the coordination of synaptic inputs between cortical (Churchland et al. 2012; Churchland and Shenoy 2007a) and sensory sources (Weiler et al. 2019) that can predict and correct MU activity throughout an effect of contraction lag.

In naturalistic movements that have unconstrained-velocities, the velocity tracings are bell-shaped (Garland et al. 1996; Hallett et al. 1975; Harris and Wolpert 1998; Harwood et al. 2011), and a general principle exists across anatomical structures, as both eye and limb movement velocity tracings have been explained by a single model (Van Gisbergen et al. 1981; Harris and Wolpert 1998). However, in limb movement we show that resulted movement velocity occurs from an indirect relationship to firing rates (figure 4.3), with the underlying relation between movement speed and firing rates. From this we can infer that anatomical characteristics can determine the tension and consequent torques in a muscle. From MU recordings of extra-ocular muscles in non-human primates, firing rates are directly related to eye movement velocity and are described to function as a 1:1 ratio (Robinson 1970), because tension was instantaneous and occurred without a significant effect of lag (Van Gisbergen et al. 1981; Robinson 1970). From single extra-ocular MU recordings, the time-delay between a stimulated input and force response is ~5 ms (Goldberg et al. 1998), with eye displacement controlled by few MUs that generate minimal forces under a high input frequency range (Goldberg et al. 1998). In contrast, elbow extension movement operated within a lower firing rate range (table 4.2), and had no instantaneous relationship between firing rates and elbow extension velocity (figures 4.2-3), and this difference was attributed to contraction lag, due to the limb moment of inertia and the torque measuring device requiring the generation of relatively larger forces. For example, during limb movement, kinematic parameters of torque, velocity, and position relative to firing rates, lag throughout contraction time course (figure 4.1, panel D), and in agreement with a reduced preparation (Partridge 1966), with the limb
expectedly gaining momentum throughout contraction duration. Likewise, effects of gravitational forces also would likely modify the required state of MU activity (Virji-Babul, et al., 1994). As compared to limb movement, minimal contraction lag (i.e., EMD) may not significantly impact motor control in smaller and lighter anatomical arrangements that have lower relative mass (i.e., eyes, fingers, toes, etc.), and this may implicate how voluntary descending (Economo et al. 2018; Lawrence and Kuypers 1968a) or sensory feedback controls (Weiler et al. 2019) are anatomically dependent. An extremity with lower relative mass would have a closer relationship between force and acceleration (based on Newton’s second law, force = mass • acceleration) to be controlled by motor unit inputs, like during eye movements (Goldberg et al. 1998; Robinson 1970) or presumably during finger movements. For example, fingers are evolved to have low mass that can perform dexterous interaction with the environment, therefore, if the fingers have less contraction lag, firing rates would be expected to have a more direct relationship to finger movement velocity, and may explain how finger movement velocity is correlated to specific representations in sensory-motor cortex (Ejaz et al. 2015). By comparison, an extremity with higher relative mass (i.e., upper limb), would have a greater disparity between force and acceleration during faster movements (figure 4.3) and may rely more on corrective short-latency sensory feedback to aid in movement precision (Weiler et al. 2019).

During shortening contractions that result in limb movement (figure 4.3), current results of an indirect relationship between firing rates and movement velocity are in some agreement with other reports during isometric contraction that infer ballistic movement speed (Baudry and Duchateau 2021; Desmedt and Godaux 1979; Del Vecchio et al. 2019). However, this remains a tenuous comparison between isometric and shortening contraction, as differences are found across cortical motor neurons (Churchland et al. 2012; Churchland and Shenoy 2007a), lower motor neuron intrinsic excitability (Hyngstrom et al. 2007), averaged MU firing rates (Desmedt and Godaux 1979; Garland et al. 1996; Gydikov et al. 1986; Harwood et al. 2011; Harwood and Rice 2012, 2014; Howell et al. 1995; Kallio et al. 2013), and with length-dependent and altered calcium sensitivity at the muscle fibre (Balnave and Allen 1996; Edman 2014; Ford et al. 1977). During shortening contraction, the patterns in figure 4.3 were representative of each
participant (figure 4.2) in both the anconeus and lateral head of the triceps brachii muscle. Although quantifying power has been used widely to describe muscle work during movement (Hill 1922), we found it was less informative when related to instantaneous firing rates during limb movement (appendix F, panel A), as firing rates were correlated to torque, but not velocity (figure 4.3). Furthermore, additional associations were calculated that did not follow an ordered kinematic progression, and to highlight some firing rate relationships that were spurious and weakly correlated to kinematic parameters that had greater contraction lag (appendix F).

In humans, we have no direct way to quantify descending synaptic inputs to the MU pool, but similar to other reports in humans (Desmedt and Godaux 1979; Garland et al. 1996; Gydikov et al. 1986; Harwood et al. 2011, 2012; Harwood and Rice 2014) firing rate increases dependent on targeted movement velocity (table 4.1) were representative of increasing gradations of likely descending excitatory drive to the MU pool (Harwood et al. 2011; Lawrence and Kuypers 1968a). From reduced preparations in the cat, another factor that may have explained the disassociation between firing rates and limb movement velocity during elbow joint rotation, was that the intrinsic state of lower motor neuron excitability. This likely would have been lower during movement through antagonist muscle stretch reflex inhibition (Hyngstrom et al. 2007; Johnson and Heckman 2014), and has only been observed during decerebrate conditions.

The firing rate ranges in this muscle group (table 4.1 and figure 4.3) were in agreement with prior observations (Dalton et al. 2010; Harwood et al. 2011, 2012, 2013; Harwood and Rice 2014; Del Valle and Thomas 2005). However, based on linear regression, we found that higher firing rates measured in the triceps brachii were also explained by a sex-related effect, and this requires further investigation, because during isometric contraction there is no reported difference in firing rates between sexes in the first dorsal interosseous muscle (Parra et al. 2020). Despite similar firing rate responses to voluntary contraction between muscles (figures 4.2-3), the anconeus additionally acts to stabilize the elbow joint (Le Bozec and Maton 1982, 1987; Coriolano et al. 2009; Miguel-Andres et al. 2017) but undergoes relative fascicle length changes like the triceps brachii (Stevens et al. 2014), which is the primary agonist muscle (Le Bozec and Maton 1987;
Harwood et al. 2013). From ramp isometric contractions, MUs in both the anconeus and triceps brachii muscles have firing rate increases, but differ in MU recruitment thresholds (Harwood et al. 2013). Although the effect of the muscle on firing rates was non-significant from linear regression, it remains important to consider how anatomical differences within muscle synergists may affect the relationship between firing rates and movement velocity. For example, as the anconeus muscle stabilizes the elbow joint and is active just prior to the triceps brachii during voluntary elbow extension (Le Bozec et al. 1980; Le Bozec and Maton 1987; Harwood et al. 2013; Maton et al. 1980), this would likely contribute towards a small EMD increase (on the order of ms) in the anconeus as compared to the triceps brachii muscles.

4.5 Limitations

We were limited in our ability to classify recorded MU EMG activity by MU type or size, and we did not measure the effect of ordered MU recruitment within participants. However, faster movement speeds would be expected to have compressed timing of MU recruitment thresholds towards contraction onset (Desmedt and Godaux 1979; Harwood and Rice 2012). We relied on a relatively small sample of MU trains per participants to what are likely active during shortening contraction at high movement velocity, and sampled a larger proportion of the MU pool in the anconeus than the triceps brachii, as MU number estimates in the anconeus are approximately four-fold lower compared to other upper limb muscles tested (Boe et al. 2004, 2006; Stevens et al. 2013). Across targeted velocity movements, MU trains were likely resampled within participants during lower contraction intensities (Rich et al. 1998), and therefore we relied on stratifying MU data per participant (Tenan et al. 2014) and statistically accounted for the participant and re-sampled MU trains using linear regression and bootstrapping. Because bootstrap correlation can be used as a nonparametric approach (Efron 1979; Wagstaff et al. 2009), we did not adjust for nonlinear components in scatterplot relationships (figure 4.2-3), but instead used a higher number of bootstrap iterations to increase confidence in the resulted correlation and error estimates (appendix E). Relating elbow joint kinematics as a proxy
to contractile mechanics at the level of the muscle provides only indirect estimates. Because at the muscle fibre and sarcomere levels, there are force-velocity, force-length and length-dependent differences during shortening contractions in response to injected current inputs (Balnave and Allen 1996; Brown et al. 1999; Brown and Loeb 2000a; Edman 1979, 2014; Ford et al. 1977). Furthermore, as movement occurred with a 20% of MVC torque load, this may have contributed to greater contraction lag between firing rate and velocity that would be minimized during lower loads (i.e., faster movements). However, as the torque load was relatively low and easily overcome by participants, this was unlikely to change the main conclusions. More general limitations were in only studying a stereotypic movement behaviour that occurred along a single joint and plane of movement, however, velocity tracings were comparable to goal-direct arm movements in other reports (Desmedt and Godaux 1979; Garland et al. 1996; Hallett et al. 1975; Harris and Wolpert 1998). Likewise, we did not measure single MU EMG activity in antagonist muscles (Hallett et al. 1975) or in other supporting muscles that stabilize the upper limb apart from the elbow, but are likely important during more complex movement tasks (Weiler et al. 2019).

4.6 Conclusions

In summary, during limb movement, agonist muscle firing rates were more directly related to the kinematic parameter of torque, but not velocity or position due to contraction lag. Instead, firing rates were indirectly related to velocity and position through a stepwise ordering of the kinematic progression (multiple correlation of firing rate to torque to velocity and to position). In highlighting the importance of contraction lag between firing rates and kinematic steps, this increased buffering time in the limb may serve as a beneficial component between cortical and sensory neural computations, as reducing noise variance (Harris and Wolpert 1998) or permitting redundant variance (Todorov 2004; Todorov and Jordan 2002) are likely represented at the single MU level. Understanding how characterized patterns (figure 4.3) may be different during more complex movement behaviour with comparisons between muscles that actively shorten
(agonist) versus actively and passively lengthen (antagonist) are important next steps to understand how voluntary neuromuscular controls coordinate muscle tension and force throughout limb movements.

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Chapter 5

Agonist muscle motor unit firing rate trajectories underlying stereotype voluntary elbow extension contractions in humans

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During voluntary contraction, the timing of motor unit (MU) firings controls the state of muscle contraction. The pattern of MU firings as a function of contraction time course is defined here as the firing rate trajectory. To date, there remains minimal experimental evidence on how the MU firing rate trajectory corresponds to differences of contraction states, that can remain static or become load-moving. Our purpose was to explore changes of agonist muscle MU firing rates throughout voluntary elbow extension contractions that were isometric or moving. From intramuscular single MU electromyogram recordings in the anconeus (n = 17) and triceps brachii (n = 6) of adult participants, two experiments were performed. Experiment one comprised self-initiated voluntary contractions at a range of contraction intensities up to maximum. Whereas experiment two required voluntary contractions that occurred in response to reaction cue stimuli, with similar contraction torque-time profiles. During voluntary contractions, the firing rate trajectory of each MU train was estimated through linear regression. We observed that firing rates decreased during limb movements, and that the steepness, but not the direction of the trajectory was contraction intensity related. During reaction-cued voluntary contractions, the same MU sample was followed across contractions and averaged. We found that the summary firing rate distribution was not different between isometric or limb movement conditions, but that the timing of firings relative to the go cue occurred sooner during limb movements. These results suggest that the MU firing rate trajectory is largely determined from descending synaptic inputs that are task specific, but the timing can be further modified, and was quickened in the movement-related state.
5.1 Introduction

Voluntary movements are controlled ultimately by skeletal muscle force that contracts in response to neural inputs from the spinal cord or brainstem. There are considerable efforts to probe the characteristics of neuronal firing rates in the motor cortex that control and modify movement (Ajiboye et al. 2017; Churchland et al. 2012; Churchland and Shenoy 2007a; Russo et al. 2018; Sergio et al. 2005), however, these neurons are not directly connected to the muscle. Moreover, during voluntary movements, there remains minimal experimental evidence characterizing the final neural inputs to the muscle. In human participants, firings from lower motor neurons can be assessed by recording the innervated muscle fibre activity of the motor unit (MU). During sustained isometric contractions under different contraction intensities (Bigland and Lippold 1954a; Desmedt and Godaux 1978; Kanosue et al. 1979; Monster and Chan 1977; Oya et al. 2009), voluntary neural drive to the muscle has been explored broadly. However, during ‘real-life’ voluntary movements, the underlying MU firing rate trajectory is unknown, despite higher averaged firing rates observed during faster movements (Denny Brown and Denny-Brown 1949; Grimby 1984; Gydikov et al. 1986; Harwood et al. 2011; Ivanova et al. 1997). From Chapter 4, we found that during elbow extension movements, firing rates were decreased as the limb was displaced. Because MU firing rates were most closely related to torque that determines movement, understanding of the MU firing rate pattern as a function of contraction time course is necessary to understand how movement is controlled by the brain and spinal cord. Therefore, quantifying the MU firing rate trajectory in relation to movement states could provide understanding towards how neural inputs encode movements across contraction intensities.

At contraction onset the agonist muscles shortens without joint angle changes (Cavanagh and Komi 1979; Griffiths 1991; Partridge 1965), but when the moment of inertia is overcome, muscle shortening continues as the contraction becomes load-moving (Brown et al. 1999; Brown and Loeb 2000a; Buchthal and Rosenfalck 1960; Demieville and Partridge 1980; Edman 1979, 2014; MacIntosh et al. 2008; Partridge 1966). This suggests that voluntary limb movements may require more complex MU firing rate control as compared to sustained isometric contractions, as the limb mass and extrinsic forces are
integrated throughout the shortening contraction. Unlike motor neurons in the cortex, motor neurons in the spinal cord reside in anatomically defined columns that innervate a single skeletal muscle (Watson et al. 2009). This suggests, based on fixed organization (Rothwell 2012), that MU firing rate trajectories may require ‘flexible’ modification by descending synaptic inputs and related interneurons to suit the task.

During movements of different voluntary contraction intensities, the influence of the corticospinal tract in humans (Devanne et al. 1997; Nicolas et al. 2001; Nielsen and Petersen 1994), that is largest among primates (Watson et al. 2009), has been minimally characterized with regard to the MU firing rate trajectory as a function of contraction time course. Likewise, the corresponding descending synaptic input state should be importantly considered; because inputs from self-initiated movements have greater pre-movement corticospinal excitability (Chen et al. 1998), whereas sub-cortical inputs studied by startle reflexes have faster movement onsets (Rothwell 2006; Valls-Solé et al. 1999). These studies suggested that characteristics of MU firing rate trajectories would vary dependent on the contribution from different states of the underlying descending synaptic input (Binder et al. 2002; Nicolas et al. 2001; Nudo and Masterton 1988; Schieber and Rivlis 2005, 2007). Furthermore, this is evidenced from the resilience of gross voluntary limb movements with bilateral pyramidotomy (Lawrence and Kuypers 1968a, 1968b) and some forms of somatosensory impairment (Taub 1976). Altogether, motor planning of the voluntary contraction, that can occur with or without a controlled external stimulus would be hypothesized to result in different MU firing rate trajectories in addition to expected differences that are necessary to control isometric contractions and limb movements.

Movements, unlike sustained isometric contractions, are time-dependent (Lupton and Hill 1923), involving different time-frequency relationships between the motor cortex, spinal cord and muscle (Crammond and Kalaska 1996; Oya et al. 2020; Shalit et al. 2012). Therefore, and likely, theories regarding the MU firing rate trajectory are also required to be time-dependent during movement, as compared to sustained isometric contractions that are statistically modelled to be stationary (Clamann 1969). From limb movement speeds that occur in daily life, kinematic parameters are oscillatory and phase shifted in
time (Flash and Hogan 1985; Hallett et al. 1975; Harris and Wolpert 1998; Ivanova et al. 1997), suggesting that the underlying MU firing rate trajectories that controls the resultant kinematic outputs are also non-stationary and changing (Partridge 1966). Therefore, our purpose was to measure single MU firing rates during self-initiated (i.e., without a visual cue), and reaction-cued voluntary contractions, comparing directional characteristics of the MU firing rate trajectories between sustained isometric contractions and limb movements. During self-initiated contractions, we hypothesized that MU firing rate trajectories during limb movements would have greater time-dependent changes when compared to sustained isometric contractions. Whereas during reaction-cued contractions, that involved the same pre-contraction set up and go-cue stimulus, MU firing rate trajectories would not be different between isometric contractions and limb movements.

Experimentally, single MU activity was recorded using intramuscular electromyography in the anconeus and triceps brachii muscles of young adult participants during voluntary elbow extension contractions. In two paradigms, MU firing rate trajectories were explored as a function of contraction time course. In experiment one, firing rate trajectories were compared between isometric contractions and elbow extension movements that were self-initiated at a range of contraction intensities (peak torque 25-100% of maximum). A second experiment (experiment 2) was required to control motor planning prior to voluntary contractions that were randomized between isometric contractions and limb movements that had similar torque-time profiles. In experiment 2, firing rate trajectories were compared between isometric contractions and elbow extension movements in response to reaction-cued stimuli. The go-cue stimulus was randomized with a delay onset and included a sham control with the participant blinded to the contraction condition that was either isometric (dynamometer resistance was >100% of MVC torque) or permitted to move (dynamometer resistance was equal to 7% of MVC torque). This enabled a standard preparation and initial execution of voluntary contractions to compare differences of the underlying MU firing rate trajectory between isometric and movement conditions. In figure 5.1 (also see appendix R), the experimental set-up and raw data from a single MU train recording is depicted.
Figure 5.1 Voluntary contraction experimental set-up.

A) Elbow extension contractions along the sagittal plane occurred with visual feedback of torque or velocity. The external load (i.e., passive resistance) was adjusted and known to the participant during experiment one, and unknown to the participant during experiment two. Intramuscular fine-wires recorded single MU EMG activity in the anconeus (A) and lateral head (LH) of the triceps brachii muscles. B) From experiment one, raw data of a single MU train recording in the anconeus muscle during elbow extension movement is depicted.

5.2 Methods

Participants. In agreement with the latest revision of the declaration of Helsinki, the local University’s research ethics board approved all procedures. Seventeen adults (4 females and 13 males, 20-34 years old) free of neurological, metabolic, or orthopedic illness provided written consent, and were each involved in 2-4 experimental sessions on
separate days. Experiment one involved fifteen participants, and experiment two involved only five participants.

**Experimental set-up.** Participants were seated on a dynamometer (Humac Norm, Computer Sports Medicine Inc., MA, USA) with the upper limb, shoulders and hip secured. The left elbow was aligned to the axis of rotation and the range of motion occurred along the sagittal plane with the forearm in the neutral or semi-pronated position. Signal acquisition (Power-1401, Cambridge Electronic Design, UK) of calibrated torque, angular velocity, and position were each sampled at 1 kHz (Power-1401 and Spike2 software, Cambridge Electronic Design).

**Electromyography (EMG).** Pairs of surface EMG electrodes (1.25×2.5 cm) were placed over the anconeus, triceps brachii and biceps brachii muscles in monopolar configurations. For each muscle and muscle group, the active electrode was placed midway on the muscle belly and was referenced on the distal tendon, respectively. A common ground electrode (2.5x2.5 cm) was placed over the deltoid muscle at the posterior spine of the scapula. Surface EMG was amplified (x100, Neurolog, Digitimer, UK) and sampled at 5 kHz (Power-1401 and Spike2 software). Intramuscular EMG of the anconeus and lateral head of the triceps brachii muscles recorded single MU EMG following existing protocols (Harwood et al., 2011, 2012; Harwood & Rice, 2014; Kirk & Rice, 2021). In the anconeus muscle, two separate channels recorded intramuscular EMG activity, with each channel in a bi-polar configuration having ~1 cm inter-electrode distance. For experiment 1, the lateral head of the triceps brachii muscle was investigated using a monopolar configuration (Kirk & Rice, 2021). The active electrode was inserted deep into the distal muscle belly, with the reference and ground electrodes placed on the acromion and deltoid muscle, respectively. Each intramuscular EMG channel was amplified (x100-500), band pass filtered (0.01-10 kHz) and sampled at 25 kHz.

**Experiment 1. Self initiated voluntary contractions.**

An account of MU firing rates in relation to elbow extension limb kinematics explored through correlation analysis has been published from only the limb movement data for
experiment one (Kirk & Rice, 2021). Here, we analyse the MU firing rate trajectory as a function of contraction time course.

**Task.** During contractions, visual feedback of torque and velocity were provided to each participant (figure 1). After familiarization and practice, each participant made self-initiated contractions of the elbow extensors with 1-3 min of rest between each effort to mitigate effects of fatigue. During isometric contractions the elbow joint was fixed at 90 deg. Isometric maximal voluntary contractions (MVC) were required to be as fast and strong as possible (i.e., a rapid contraction), with each MVC lasting ~5 s in duration and consisted of 2-4 attempts across participants with at least 3 min of rest between attempts. Submaximal torque ramps were ~15 s in duration (~6 s torque rise, 1-3 s at the plateau and ~6 s of lowering on the descending phase) and the torque range (up to 25-70% of isometric MVC) was dependent on intramuscular EMG signal quality to identify single MU trains. During elbow extension movements, the upper limb was loaded equal to 20% of the isometric MVC torque for each participant, with movements of an unconstrained-velocity. During movements, the range of motion was 120 deg, starting from 140 deg of flexion and extended along the sagittal plane (with 0 deg flexion having the elbow fully extended). Relative to the maximum movement velocity (Vmax) under the 20% load for each participant, four velocity ranges were calculated in 25% velocity increments that were used by the participant to target different limb movement velocities.

**Data analysis.** Surface EMG was digitally filtered (infinite impulse response (IIR), second order, 20-500 Hz), full-wave rectified and averaged with a time constant of 0.01 s. Intramuscular EMG was high pass filtered (IIR, second order, 1 kHz) to identify local field potentials of MU fibres (Stashuk, 1999). For each MU train, the muscle fibre action potentials were sorted based on waveform characteristics using Spik2 software (Cambridge Electronics Design) that required manual inspection and correction based on template-matching (Harwood et al., 2011; Harwood & Rice, 2012, 2014; Cowling et al., 2016; Kirk & Rice, 2021). For inclusion, waveforms attributed to a single MU train were required to have consistent shape with gradual developing changes of amplitude that were dependent on elbow extension position change and inter-spike-interval (ISI) times were calculated. Kinematic parameters of limb torque, angular velocity and position were
measured from resting baseline, and normalized to the maximum MVC or Vmax for each participant. Elbow extension kinematics were linked to each measured MU potential at a resolution of 1 ms based on the minimum sampling rates (1 kHz). For each parameter, locally weighted scatterplot smoothing (LOESS) (Cleveland et al., 1992) was used to estimate the ensemble trajectories of MU firing rates and limb kinematics. LOESS averages a percentage of neighbouring data points (defined as the span) using a tri-cubic weighting function. All statistical analyses and data figures were written using R software (version 4.1), with the alpha set to 0.05.

**Modelling the firing rate trajectory as linear or non-linear.** To compare the firing rate trajectory of each MU train, firing rates (in Hz) were fit as competing functions of contraction time course (in seconds). For each MU train, the hypothesis of firing rate trajectory non-linearity was tested in a similar way as Fuglevand et al., (Fuglevand et al., 2015) comparing calculated residuals of the linear fit (null, equation 1) against the non-linear fit (alternative, equation 2). Sum of squared errors (SSE) were calculated from residuals of each fit and were compared between models using the F-test statistic. In some instances, the half-life constant ratio was violated (i.e., Tau >1) when firing rates increased throughout contraction time, therefore the MU was determined to be linear (i.e., the null) with a positive slope.

\[
\text{firing rate}(x) = mx + b
\]  

[1] Equation 1. Firing rate response modelled as a linear function of time \(x\), fitted with ordinary least squares. The y-intercept \(b\) was the extrapolated firing rate at contraction time zero (i.e., the firing recruitment threshold).

\[
\text{firing rate}(x) = \text{firing rate}_0 \left( \frac{1}{2} \right)^{\frac{x}{\tau}}
\]  

[2] Equation 2. Firing rate response modeled as a half-life function of time \(x\). For each MU train, the half-life constant \(\tau\) was calculated as a ratio from dividing the final by the initial firing rate (firing rate\(_0\)) and was required to be <1. Because there was firing rate
variability, the ratio between the initial and final firing rate values used in calculation of the half-life constant (Tau) were averaged between the first 0-30%, and last 70-100% of sequential firings for each MU train, respectively. Tau was used as a scaling factor to normalize the magnitude of the firing rate difference per half-life fit. In *ex vivo* motor neuron recordings in response to constant step excitatory currents, half life exponentials have been observed and modelled (Kernell, 1965; Spielmann *et al.*, 1993; Gorman *et al.*, 2005; Miles *et al.*, 2005; Button *et al.*, 2007) and are purported to also occur in human MU recordings (Desmedt, 1980).

*Estimate of the firing rate trajectory using linear regression.* For each MU train, firing rates were fit using a simple linear regression as a function of contraction time course. Each MU train was required to have ≥4 inter-spike intervals, and the linear slope was calculated in three steps. First, firing rates were transformed by the natural logarithm (i.e., lognormal, log Hz) to minimize effects of leverage on linear models by skewness of the firing rate distribution (Petersen & Berg, 2016; Linden & Berg, 2021). Secondly, the firing rate variance explained by normalized elbow extension torque, that is correlated with firing rates during elbow extension movements (Kirk & Rice, 2021) was calculated as residuals from simple linear regression in response to torque. Finally, simple linear regression of firing rates (in log Hz) as a function of contraction time course (in seconds) was calculated for each MU train and was adjusted based on the averaged variance explained by elbow extension torque (from step two). The p-value for the linear slope coefficient for each MU firing trajectory was determined using the F-test. For each MU train, a metric of normality was assigned to the distribution of residuals using the Shapiro-Wilk test.

*Likelihood ratio test.* From the dataset of linear slope coefficients, mixed-effect additive linear regression using the packages ‘lme4’ (Bates *et al.*, 2015) and ‘lmer’ (Kuznetsova *et al.*, 2017) were used to determine if the firing rate linear slope coefficient was different between the two conditions of isometric contraction and elbow extension movement. Comparisons were made using the likelihood ratio test between maximal likelihood estimation of regression models. Each model included fixed effects of the normalized torque at MU firing recruitment threshold and the muscle (anconeus or triceps brachii),
and intercept random effects of participant (Tenan et al., 2014). Only the full regression
model comprised the additional factor of the contraction condition (isometric or
movement). Likelihood ratio test statistics were calculated for the entire MU dataset. In
addition, the same test was performed on MU trains filtered by normality (i.e., the
Shapiro-Wilk test, p > 0.05) and from significant time-dependent directionality of the
MU firing trajectory (i.e., linear slope F-test, p < 0.05). Case bootstrapping was used to
estimate error of the likelihood ratio test statistic for each approach.

Although MU trains were recorded across different contraction intensities (up to 100% of
MVC) and from separate intramuscular EMG channels, muscles, days and participants,
MU sample duplication was likely. Therefore, to estimate the variance from the effect of
MU duplication on the maximal likelihood estimation of the full mixed-effects linear
model, we graded the odds of duplication events using Monte Carlo simulation. From the
empirical dataset, separate simulations of high (25%), moderate (12.5%), and rare
(6.25%) MU duplication was investigated and was added as an intercept random effect in
a fully crossed subject and item factor design.

Experiment 2. Reaction-cued voluntary contractions.

Task. In the same experimental arrangement as described for experiment 1, single MU
trains recorded from the anconeus muscle of five participants (one female and four
males) were followed across voluntary isometric and elbow extension movements in
response to visual cues. Isometric contractions occurred with the elbow joint fixed at 100
deg flexion, while elbow extension movements were permitted between 100-20 deg of
extension (0 deg flexion is full elbow extension). During elbow extension movements,
the upper limb was minimally loaded for each participant, with movements having
unconstrained velocity. Only elbow extension torque feedback was provided, and each
voluntary contraction occurred in response to reaction timed cues that consisted of a ‘get-
ready’ cue (yellow light), followed 2 s later by a preparatory ‘ready’ cue (red light) that
required the participant to perform a weak isometric contraction at 5% of MVC torque.
After this, a final ‘go’ cue (red or green light) instructed the participant to rest (sham, red
light) or go (green light), requiring the participant to quickly reach 50% of MVC torque as fast as possible. The delay between the preparatory ‘ready’ and ‘go’ cue was randomized between 0.6-1 s across trials to avoid anticipation by the participant. Both the participant and investigator were blinded to the randomized final cue (70% green, 30% red), and the condition of isometric contraction or elbow extension movement was randomized and unknown to the participant.

The contraction condition was dependent on the passive load of the dynamometer arm that was increased beyond the capacity of the participant during isometric contractions or was decreased to 7% of MVC torque, enabling elbow extension movements. This was chosen because each contraction started with a sustained isometric contraction at 5% of MVC torque that did not overcome the passive resistance for either condition (7% and >100% of MVC, respectively), and therefore had similar initial starting conditions prior to the visual ‘go’ cue. Prior to each contraction, the low (7% of MVC torque) or high (>100% of MVC torque) load was randomly determined on the dynamometer by the investigator using method with the participant blinded. After familiarization and practice, each participant completed >20 contractions per condition, with 10 s rest between voluntary contractions. After every 20 contraction cycles, ~5 min breaks occurred to mitigate effects of fatigue. During the experiment, the computer monitor was positioned ~1 m away at eye level, with the LED (brightness 1250 mcd) controlled through the analog-digital Spike2 software interface.

Data analysis. The same MU was reliably tracked by waveform template matching in the same participant across separate voluntary contractions, with MU firing rates measured after the go cue. For each condition of isometric contraction and elbow extension movement, MU firing rates measured across contraction replicas were grouped and a fixed kernel bandwidth was determined for estimating the time dependent firing rate trajectory per MU (Shimazaki & Shinomoto, 2010). The two-sided Kolmogorov-Smirnov test was used to determine if the timing or frequency of MU firings were different between isometric contraction and movement conditions for each MU, and a measure of the distance error was determined using case bootstrapping (Praestgaard, 1995). This method in comparing the cumulative distribution of firing rates is routinely used in
comparing firing rate distributions of cortical neurons and is similarly applied here to firing rate distributions of motor units, i.e., motor neurons of the spinal cord. The data and computer code described can be accessed upon request to the corresponding authors.

5.3 Results

Experiment 1. Self-initiated voluntary contractions.

From voluntary self-initiated contractions, trains of single MU firings in the anconeus and lateral head of the triceps brachii muscle were analyzed in relation to contraction time course and elbow extension kinematics during sustained isometric and elbow extension movements (table 5.1). In the triceps brachii muscle, intramuscular EMG recordings yielded fewer identified MU trains as compared to the anconeus. Because elbow extension movement velocities were non-ordered across participants and of different contraction intensities, this resulted in considerable changes of agonist muscle activity and electrode displacement between contractions. Therefore, MU trains were analyzed as unpaired between contractions, and firing rate trajectories were adjusted statistically. In relation to separate kinematic parameters of elbow extension, the first detected firing of each MU train, an estimate of the recruitment threshold across MU trains (appendix G), was aligned at time zero for summary comparisons (figures 5.2 and 5.3).
### Table 5.1 Summary dataset of MU trains in experiment one.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Isometric MVC (Torque (Nm))</th>
<th>65 ± 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Movement MVC</td>
<td>Velocity (deg • s⁻¹)</td>
<td>200 ± 28</td>
</tr>
<tr>
<td>Muscle</td>
<td>Anconeus</td>
<td></td>
</tr>
<tr>
<td>Participants (n)</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Sex (f/m)</td>
<td>4/11</td>
<td>4/2</td>
</tr>
<tr>
<td>MU trains / condition</td>
<td>Isometric ramp</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>Isometric MVC</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Extension movement</td>
<td>628</td>
</tr>
<tr>
<td>MU trains / participant</td>
<td>Isometric ramp</td>
<td>6 ± 3</td>
</tr>
<tr>
<td></td>
<td>Isometric MVC</td>
<td>3 ± 1</td>
</tr>
<tr>
<td></td>
<td>Extension movement</td>
<td>42 ± 19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38 ± 14</td>
</tr>
</tbody>
</table>

Values are total counts or mean ± standard deviation, rounded in whole numbers. Each MU train was unpaired between contractions. Isometric contractions were loaded beyond the torque wielding capacity of the participant, and elbow extension movements were loaded at 20% of isometric MVC torque. Recordings occurred in the lateral head of the triceps brachii muscle.

In human experiments of the intact system, voluntarily targeting different torque or velocity gradations normalized to the maximal voluntary contraction (MVC) are used to modify the underlying background of descending synaptic inputs to the agonist muscle MU pool (Devanne et al. 1997; Fuglevand et al. 2015; Harwood et al. 2011), analogous to ramp or step-like injected currents in reduced preparations. During self-initiated voluntary contractions, efforts were made to measure MU firing rates during single trial elbow extension contractions that can occur in daily life, such that isometric contractions were prolonged and sustained, or that elbow extension movements occurred under relatively small loads of torque resistance (% of MVC torque). During sustained isometric contractions with increasing elbow extension torque over several seconds (peak torque ranged from 25-70% of MVC), firing rates slowly increased from MU recruitment
and remained largely stable across contraction time course (figure 5.2, panels A, C and E), suggesting that some MU firing rate trajectories had saturated (Fuglevand et al. 2015) or that firing rates were slowly increasing. During sustained isometric MVCs, the torque was rapidly increased with correspondingly higher firing rates at MU recruitment threshold and throughout the contraction as compared to submaximal ramps (figure 5.2, panels B, D and F).

Figure 5.2 Voluntary self-initiated isometric contractions at targeted elbow extension torque ramps and MVCs.
A and B) Representative recordings, showing surface EMG, intramuscular EMG of the anconeus muscle; elbow extension torque, firing rates and the MU potential waveform overlays that were high-pass filtered. Note the saturated firing rate trajectory during the increasing phase of the torque ramp (A), and the higher frequency interval at recruitment threshold during MVC (B). C and D) Scatterplots depict summary firing rates across MU trains recorded from the anconeus (n = 15) and lateral head of the triceps brachii (n = 6) muscles dependent on contraction time course, with each MU train line joined. The firing recruitment threshold for each MU train is aligned to time zero. In total, data from 131 MU trains are included during ramps (C) and 48 MU trains during MVCs (D). The ensemble smoothed curve shows the LOESS fit with a span of 25% (in red). E and F) Depicted as separate LOESS fits with a span of 50% for summary elbow extension RTD and torque (in percentage of each participant’s voluntary maximum) and are vertically linked in time with panels C and D, respectively. Grey shading represents the 95% confidence interval. In the appendices J-M, the same summary patterns are observed when MU trains are grouped for each muscle.

In contrast to sustained isometric contractions, firing rates during elbow extension movements had relatively larger changes, with firing rates observed to decrease throughout the contraction time course from MU recruitment (figure 5.3). Across MU trains (figure 5.3, panels B and C), and targeted limb movement velocities (figure 5.3), firing rates decrease occurred as the limb transitioned across different kinematic phases of elbow extension torque, velocity, and position. Unlike isometric contractions, elbow extension movements required displacement of the joint, and 1 deg rotation occurred at the median contraction time of 0.2 s (interquartile range 1st and 3rd, 0.11 and 0.44 s, respectively) relative to recruitment threshold across MU trains (figure 5.3). Although movements had a range of motion that was 120 deg, MU activity in this experimental set-up rarely occurred throughout the entirety of elbow joint displacement, as shown from the alignment of kinematic parameters to MU firing rates (figure 5.3, panels C). This was likely due to a combination of effects from decreasing descending inputs to the MU pool that were measured as decreasing MU firing rates (figure 5.3, panels B). That was further explained by limb momentum from increasing velocity in this task (positive torque phase and gravity).
Figure 5.3 Voluntary self-initiated limb movements at targeted elbow extension velocities.
For each contraction, MU firing rates were measured from the first burst of activity related to the positive phase of movement. For each participant, the elbow extensors had a passive load of 20% torque relative to isometric MVC. A) Representative recordings for each movement velocity, showing elbow joint position, velocity, torque, surface EMG, intramuscular EMG of the anconeus muscle, firing rates from a followed MU sample across contractions based on waveform matching, and the MU potential waveform overlay that was high-pass filtered. The red arrow denotes one degree of elbow joint position displacement relative to baseline in both the position and firing rate channels. B) Scatterplots depict summary firing rates across MU trains recorded in the anconeus (n = 15) and lateral head of the triceps brachii (n = 6) muscles dependent on contraction time course, with each MU train line joined. The firing recruitment threshold for each MU train is aligned to time zero. For each participant, the targeted velocities in 25% bins (V25-100), were normalized to the maximum movement velocity (Vmax). In total, the number of MU trains per targeted velocity were: 238, 222, 154 and 242, respectively. The smoothed curve shows the LOESS fit (in red). C) Depicted as separate LOESS fits for summary elbow extension parameters, and are linked vertically in time with B, respectively. Grey shading for each LOESS fit represents the 95% confidence interval. Torque and velocity were normalized to the voluntary maximum for each participant. In appendices J-M, MU trains are grouped for each muscle.

Quantifying an estimate of the MU train firing rate trajectory

From intramuscular recordings (figure 5.3, panel A), a relatively small change in limb position to extend the elbow joint (red arrow denotes a 1 deg displacement from baseline) corresponded to the contraction time of the firing rate linear decrease during a slower targeted movement (figure 5.3, V25, peak velocity 50 deg • s\(^{-1}\)). In relatively faster movements (figure 5.3, V50, V75 and V100), firing rates were also were observed to decrease with a linear component (figure 5.3, panel B). To quantify an estimate of the firing rate trajectory that could reasonably explain the variance in data points for each MU train, firing rates were separately modelled as a function of contraction time course by simple half-life decay and linear regression. The fit of each model, and corresponding SSE output was then statistically compared (table 5.2), with the linear model representing the null hypothesis for each MU train.
Overall, firing rate trajectories of single MU trains were modelled to be linear (~90%) when tested systematically across both sustained isometric contractions and elbow extension movements of different contraction intensities and time durations. This indicated that an estimate of the firing rate trajectory could reasonably be compared based on characteristics of the linear slope during elbow extension movements (table 5.2). During isometric contractions, the firing rate trajectory was increasing in 73% of MU trains (130 out of 179; table 5.2), and therefore, these MU trains were not determined to have a half-life decay or negative direction characteristic.

**Table 5.2** Comparing the firing rate trajectory for each MU train as half-life or linear.

<table>
<thead>
<tr>
<th>Condition</th>
<th>MU trains (total)</th>
<th>Half-life decay (% significant)</th>
<th>Linear slope coefficient, median (1\textsuperscript{st}, 3\textsuperscript{rd} IQR)</th>
<th>Averaged SSE, ensemble median</th>
<th>Averaged firing rate (Hz), ensemble median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isometric</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increasing ramp</td>
<td>131</td>
<td>6.1</td>
<td>1.3 (-3.0, 5.8)</td>
<td>592.8</td>
<td>13.1</td>
</tr>
<tr>
<td>MVC</td>
<td>48</td>
<td>6.2</td>
<td>0.7 (-0.04, 1.4)</td>
<td>299.8</td>
<td>25.8</td>
</tr>
<tr>
<td>Movement</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-25</td>
<td>238</td>
<td>10.1</td>
<td>-2.3 (-7.1, 1.0)</td>
<td>194.8</td>
<td>15.0</td>
</tr>
<tr>
<td>25-50</td>
<td>222</td>
<td>9.5</td>
<td>-7.9 (-19.7, 0.1)</td>
<td>118.2</td>
<td>16.8</td>
</tr>
<tr>
<td>50-75</td>
<td>154</td>
<td>7.8</td>
<td>-14.4 (-39.2, -2.2)</td>
<td>198.3</td>
<td>20.8</td>
</tr>
<tr>
<td>75-100</td>
<td>242</td>
<td>10.5</td>
<td>-20.0 (-53.2, -2.6)</td>
<td>306.1</td>
<td>27.3</td>
</tr>
</tbody>
</table>

As a function of contraction time course (in seconds), firing rate responses (in Hz) of each MU train were fit as a half-life decay and simple linear regression. Values represent the summary data of linear regression slope coefficients for each MU train. For each condition, the percentage represents the relative number of MU trains significantly better fit by half-life decay. Ramp isometric contractions only included the increasing torque phase. Linear slope coefficients were not different between isometric contraction conditions (Wilcoxon rank sum test, p = 0.4).
Comparing characteristics of linear firing rate trajectory estimates

For each MU train, characteristics of the firing rate trajectory were estimated using the linear regression slope (i.e., the direction and steepness) and were compared between sustained isometric contractions and elbow extension movements. To minimize effects of outliers, firing rates were transformed by the natural logarithm, and adjusted based on the averaged variance explained by elbow extension torque (figure 5.4, panels A-C). In sustained isometric contractions, the elbow joint was fixed, therefore, both submaximal and MVCs were combined into a single isometric condition (figure 5.4, panel F). In contrast, elbow extension movements were also compared across movement velocities (figure 5.4, panel G).
Figure 5.4 Linear estimate of the MU firing rate trajectory during self-initiated voluntary contractions.

For each MU train, the linear slope coefficient was calculated as the firing rate response (log Hz) to contraction time (seconds) that was adjusted by the averaged variance explained by elbow extension torque. Only MU firing rate trajectories with a normal distribution of residuals (i.e., Shapiro-Wilk confirmed the null) were included. In total, 762 MU trains were measured across conditions in the anconeus (n = 15) and 273 MU trains were measured in the lateral head of the triceps brachii (n = 6) muscles. A) Example of the observed firing rate trajectory for a single MU train during elbow
extension movement at 100% of Vmax. The elbow extension torque at firing recruitment threshold was 27% of MVC torque. B) Average firing rate variance explained by normalized elbow extension torque for the MU train in panel A. Variance was estimated by simple linear regression. C) Estimated firing rate trajectory for the MU train in panel A. The simple linear regression (grey line) was adjusted by the average variance explained by normalized torque (panel B) and fit as function of contraction time; Shapiro-Wilk, p = 0.3; F-test, p = 0.08. D and E) Summary comparisons of the linear slope coefficient direction, grouped by the voluntary contraction condition. Coefficients were filtered by significance (p < 0.05) that resulted in 42 and 222 MU trains being included, during the isometric and movement conditions, respectively. D) Between bar-plots, a chi-square goodness of fit test indicated that the observed ratios of the slope coefficient direction were statistically different (asterisk, df = 2, p < 0.01) between isometric and elbow extension movements. E) During elbow extension movements, slope coefficients were grouped by targeted movement velocity. In total, the number of MU trains per targeted velocity were: 54, 62, 46 and 60, respectively. F and G) Volcano plots of the linear slope coefficient and statistical significance among MU trains (e.g., panel C). For inclusion, each MU train was required to have residuals of the simple linear regression that were not significantly skewed, assessed by the Shapiro-Wilk test. For the x-axis, the slope coefficient is in units of log Hz • s⁻¹.

From the entire dataset including 1031 MU trains, the slope coefficient represented an estimate of the average MU firing rate trajectory as a function of contraction time course. We found that slope coefficients of MU trains were statistically different between sustained isometric contractions and limb movements across participants (null negative log-likelihood -2590.5, alternative negative log-likelihood –2586.5, $X^2$ = 8.0; p = 0.004). This result was confirmed with case bootstrapping (simulated $X^2$: mean = 9.4, standard deviation = 5.8). To test how MU duplication events influenced the variance of the slope coefficient in the mixed effects linear regression model, we simulated different odds of the random effect of MU duplication on the measured dataset related to high (25%), moderate (12.5%) and rare (6.25%) probability of a single MU being recorded across contractions in the same participant. From this, ~4% of the slope coefficient variance was attributed to high probability of MU duplication. By comparison, the variance attributed to the participant, that was adjusted for, was ~40%.
Next, from the dataset of measured firing rate trajectories, only 264 MU trains were accepted for further analysis based on statistical filtering of the slope coefficient (figure 5.4, panel F). This approach confirmed that included MU trains for comparisons had significant firing rate directionality as a function of contraction time course. This included 42 (23%) during isometric contractions and 222 (29%) during elbow extension movements from the total number of measured MU trains for each condition (table 5.1). From the filtered dataset (figure 5.4, panels D-E), the directions of firing rate trajectories were statistically different between isometric contraction and elbow extension movements during self-initiated voluntary contractions (figure 5.5, panel A). The firing rate trajectory was positive (i.e., increasing) for 88.1% of MU trains during isometric contractions, as compared to elbow extension movements that were negative (i.e., decreasing) for 88.3% of MU trains (figure 5.4, panel D).

With MU trains grouped by the targeted movement velocity (figure 5.4, panels E and G), the relationship between the steepness of the linear slope coefficient and targeted movement velocity was negatively correlated (Spearman’s rank, rho = -0.43, p < 0.001), illustrating that greater contraction intensity and elbow extension position change was associated with a greater steepness of the firing rate trajectory decrease. This relationship was also observed during elbow extension movements because the linear slope coefficient was negatively correlated with the torque of firing recruitment threshold (figure 5.5, panel C). In experiments on human participants, the limb torque at firing recruitment threshold is used to estimate size-ordered relationships between the MU train and a characteristic of the firing rate trajectory (Monster and Chan 1977; Oya et al. 2009). This further indicated that the firing rate trajectory of higher torque threshold MU trains had a greater decrease during faster elbow extension movements.
Figure 5.5 Comparisons of summary MU firing rate trajectories that passed the statistical filter.

A) Boxplots depicting linear slope coefficients grouped by isometric (42 MU trains, n = 15) or elbow extension movement (222 MU trains, n = 15). From MU trains that passed the statistical filter (p < 0.05), a statistical difference between conditions was determined by the likelihood ratio test between mixed-effects linear regression models (null negative log-likelihood –485.9, alternative negative log-likelihood -470, \(X^2 = 32\); p < 0.001), and confirmed with case bootstrapping (simulated \(X^2\): mean = 33.2, standard deviation = 9.3). Models were adjusted based on fixed-effects of the muscle, torque at firing recruitment threshold, mean elbow extension velocity, and from random-effects of the participant. B) Relationship between the linear slope coefficient and normalized torque at firing recruitment threshold during isometric contraction (Pearson’s \(r = 0.16\), 95% confidence interval = -0.15 to 0.45, \(p = 0.3\), \(df = 40\)). C) During elbow extension movements (Pearson’s \(r = -0.35\), 95% confidence interval = -0.46 to -0.23, \(p < 0.001\), \(df = 220\)).
Experiment 2. Reaction-cued voluntary contractions.

From voluntary contractions in response to a visual preparatory and go cues, single MU trains recorded in the anconeus muscle were followed across contractions (per condition, mean 17, maximum 26) of five participants during isometric contractions and elbow extension movements. In this paradigm (figure 5.6, panel B), each participant was provided the same timing of visual cues, enabling the ~3 s of preparation prior to each voluntary contraction to be similar across participants and contraction conditions. The final visual cue had a randomized delay onset (0.6-1 s) to control against the participant synchronizing the voluntary contraction to the final visual stimulus, that was also randomized between a go or sham stimulus. The contraction condition consisted of either an isometric or load-moving contraction that was also randomized and unknown to the participant. To facilitate this, the ready cue that occurred prior to the final visual stimulus, required the participant to perform a voluntary isometric contraction equal to 5% of MVC torque. To set the contraction condition, that was blinded to the participant, the load of the dynamometer was changed to permit or disable movement, therefore, movement was load-dependent. To permit elbow extension movement, the loaded resistance of the dynamometer arm was set to 7% of MVC torque, while during the isometric contraction, the load exceeded >100% of MVC torque. For each reaction-cued voluntary contraction, that occurred from the final visual cue, the participant was instructed to reach 50% of MVC torque as fast as possible.
Figure 5.6 Single MU firing rate trajectories of the anconeus muscle during reaction-cued voluntary elbow extension contractions.

A) Recorded from a female participant, data of a single MU sample is depicted. Relative to the time of the go cue (at time zero), contraction replicas are depicted (from top to bottom) as torque overlays; velocity overlays; raster of firing times with each indexed row representing a separate contraction in order throughout the experiment; firing rates were smoothed with a fixed kernel bandwidth, see appendix Q (Shimazaki and Shinomoto 2010) for the isometric (MU6, 0.0524 s) and movement (MU6, 0.0509 s) contraction conditions; underlying density plot from the optimized kernel bandwidth. B) Schematic of the reaction-cued experimental design. C) Smoothed firing rate trajectories for each MU sample from the summary of voluntary contraction replicas as a function of
contraction time. Firing rates for each MU sample and contraction condition were smoothed with a fixed bandwidth kernel regression. See appendices N-P for detailed information on each MU sample. Differences between isometric and movement conditions were determined using the Kolmogorov-Smirnov test with significance set at $p \leq 0.05$, * denotes a difference between firing rate distributions, and † denotes a difference between the time of firings relative to the go cue (time zero), see appendices H and I for precise values.

In relation to movement kinematics, MU firing rates across all samples occurred prior to and with elbow extension torque, with peak firing rates preceding peak elbow extension velocity. In comparing firing rates between isometric contractions and elbow extension movements at the level of single MU activity, we found that 40% of MU samples (4 out of 10, n = 5) had altered firing rates when the elbow joint was permitted to move (figure 5.6, panels A and C). We also found that 60% of MU samples had different timing of firings relative to the go cue during the elbow extension movement, as compared to the isometric condition. In these MUs, firing rates occurred sooner and within a shorter (i.e., compressed) window of contraction time course than those recorded during isometric contractions. This was an unexpected finding because participants were planning and initially executing the same motor task and were blinded to the contraction condition with appropriate randomized controls. In the MU samples that showed significant differences between isometric and movement conditions, median firing rates were higher (MU samples: 3, 4 and 7; figure 5.6) and the proportion of firings relative to the go cue stimulus occurred sooner during movements (MU samples: 4, 6-8 and 10 figure 5.6), apart from one MU sample that had both lower median firing rates and longer duration of MU firings (MU sample 2, figure 5.6). Through correlation analysis, these differences of the firing rate distribution or timing of firing relative to the go cue were not explained by different relationships of elbow extension torque, velocity, or position across MU samples.

Summary analysis of MU samples showed that a difference of the firing rate distribution between isometric or movement conditions was less likely (figure 5.7, panel A). Through mixed-effects linear regression analysis, the minimal difference between firing rate
distributions was supported from larger variance attributed to the participant (residual variance 55, standard deviation 7) as compared to across contraction replicas (residual variance 7, standard deviation 3). Like experiment one, a similar approach was used to quantify characteristics of the firing rate trajectory for each MU sample (see figure 5.4), but with the addition of adjusting for the random effect of multiple contraction replicas. Based on a linear estimate of the MU firing rate trajectory, we found no difference in the direction of linear slope coefficients (median -0.45, range -1.83 to 1.7 log Hz • s⁻¹, 20 linear slope coefficients, 10 MU samples, 2 conditions) between isometric and movement conditions (likelihood ratio test, $X^2 = 0.06, p = 0.8$). This further supported that the underlying MU firing rate trajectory was minimally different between isometric contractions and elbow extension movements during the reaction-cued experiment, when voluntary contractions were similarly prepared, cued and carried through.

Unlike summary comparison of the MU firing rate distribution or trajectory, the timing of MU firings relative to the go cue stimulus occurred sooner during elbow extension movements (figure 5.7, panel B), and had a median time difference of ~27 ms between the conditions of isometric contractions and elbow extension movements. Although the firing rate trajectories delivered to the muscle were not markedly different between conditions when provided similar preparation and cueing, the timing of the MU firing rate trajectory occurred sooner during elbow extension movement. This outcome suggests that in the movement condition, the timing of MU firings was modified; but the MU firing rate trajectory, analyzed using linear regression and with comparisons of the firing rate distribution, was not different between movement and isometric contractions. Therefore, in experiment 2, the MU firing rate trajectory was dependent on the voluntary contraction task (i.e., descending synaptic inputs), but the timing of the trajectory, relative to the go-cue occurred sooner and was ‘quickened’ in the movement-related state.
A statistical difference between isometric and movement conditions was assessed using the Kolmogorov-Smirnov test, with the firing rates and firing times relative to the go cue combined from all MU samples and contraction replicas. A) Summary firing rates were not different between conditions (D = 0.04, p = 0.21; bootstrap simulation of D, mean = 0.06, standard deviation = 0.01). B) Summary firings relative to the time of the go cue occurred at different time points between conditions (D = 0.11, p < 0.001; bootstrap simulation of D, mean = 0.12, standard deviation = 0.02). The median difference in time of the cumulative probability distribution (see inlay) was 26.8 ms. C) Example of one MU sample (MU6) followed between isometric and movement conditions, separated by ~10 s of rest. Depicted intramuscular EMG channels were high pass filtered.

5.4 Discussion

During relatively fast elbow extension contractions in adult participants, we tracked single MU firing rates in two voluntary contraction paradigms. In experiment one we found that the direction of single MU firing rate trajectories was different between
sustained isometric contractions and movements during self-initiated voluntary contractions. This demonstrated that neural inputs to the muscle controlling joint torque rely on directional characteristics of the firing rate trajectory that are task specific, and less dependent on gradations of voluntary inputs. This was supported from changes of the steepness, but not the direction of the MU firing rate trajectory in relation to contraction intensity throughout limb movements, in-line with our hypothesis of greater firing rate changes as compared to sustained isometric contractions. In experiment two, voluntary contractions occurred in response to reaction-cued stimuli with the contraction condition that was either isometric or moving, unknown to the participant until voluntary torque was produced. Although having similar gross preparatory activity to self-initiated voluntary contractions (Ibáñez et al. 2020), reaction cueing enabled the preparation and initial execution of voluntary contraction to be better controlled. Presumably, experiment two had greater similarity of descending synaptic inputs to the MU pool between contraction conditions. Because the contraction intensity range was only up to 50% of maximum voluntary torque and movements occurred throughout a relatively smaller range of motion as compared to experiment one, we were able to confidently follow the same MU sample across repeated contractions in both isometric and elbow extension movements. Across firing rate trajectories, the absolute firing rate and timing of firings relative to the go cue were different in 40 and 60% of MU samples, respectively. This demonstrated that with limb movement, as compared to isometric contractions, the underlying neural drive to the muscle can be altered in the movement-related state. Summary comparisons provided support that the timing of firings relative to the go cue occurred sooner during elbow extension movements, further suggesting that movement-related state, that likely requires greater afferent feedback could alter the timing (Edin et al. 1998; Ivanova et al. 1997; Oya et al. 2020; Ribot-Ciscar et al. 2009), but not markedly the distribution of the MU firing rate trajectory during stereotyped limb movement. However, the contribution of movement-related afferent pathways that modify the MU firing rate trajectory, likely attributed to fast conducting Ia and Ib feedback, requires further investigation. Overall, during voluntary contractions, our results suggest that the direction of the single MU firing rate trajectory is determined from descending synaptic inputs that are task specific, and the steepness of the MU firing rate trajectory is
influenced by the contraction intensity. However, during movement as compared to the isometric contraction in experiment two, we found that the timing of firings could occur sooner and within a shorter contraction duration relative to the contraction go-cue. This provided evidence at the single MU level, that the movement-related state can modify the timing of the MU firing rate trajectory, but that characteristics of the trajectory (i.e., direction and steepness) were unchanged when the same torque target was provided across voluntary contractions.

During voluntary contractions with unconstrained movement velocity, few studies have followed single MU activity during moderate or high contraction intensities (Cowling et al. 2016; Desmedt and Godaux 1979; Garland et al. 1996; Gydkov et al. 1986; Harwood et al. 2011; Harwood and Rice 2012, 2014; Ivanova et al. 1997). More commonly, MU activity has been investigated during low intensity contractions (Nguyen et al. 2019), and during constrained-velocity (i.e., isokinetic) movements that are relatively slow (Bigland and Lippold 1954b; Howell et al. 1995; Kallio et al. 2013, 2014; Kossev and Christova 1998; Oliveira and Nergo 2021; Pasquet et al. 2006; Tax et al. 1990; Theeuwen et al. 1994). Regardless of the experimental approach, these experiments agree that average MU firing rates of the agonist muscle are increased during active muscle shortening as compared to sustained isometric contractions of similar intensity (Harwood et al. 2011; Howell et al. 1995; Ivanova et al. 1997; Kallio et al. 2013, 2014; Kossev and Christova 1998; Nguyen et al. 2019; Oliveira and Nergo 2021; Pasquet et al. 2006; Tax et al. 1990; Theeuwen et al. 1994), suggesting that movements require summary increased excitatory inputs to the MU pool. During self-initiated voluntary contractions, we found that the directionality of the MU firing rate trajectory was related to the task, as sustained isometric contractions generally had increasing firing rates with lower variability of the trajectory steepness. During isometric contractions, this was likely related to stability of the spinal motor neuron intrinsic state that amplifies synaptic inputs (Bennett et al. 1998; Fuglevand et al. 2015; Revill and Fuglevand 2011), while this may not be the case during limb movements (Johnson and Heckman 2010; Oya et al. 2020), as we observed the direction of firing rate trajectories to be decreasing. This contrast between conditions suggested that descending synaptic inputs to and the intrinsic state of the MU pool are critical in determining characteristics (i.e., direction and steepness) of the resulted MU
firing rate trajectory, with the steepness of the trajectory more strongly modified during movements. This was supported from the steepness, but not the directionality of the firing rate trajectory being dependent on voluntary contraction intensity during self-initiated limb movements (in experiment one). During limb movement, this suggested that descending synaptic inputs to the MU pool (Binder et al. 2002; Devanne et al. 1997; Evarts 1968; Heckman 1994; Hyngstrom et al. 2008; Lawrence and Kuypers 1968a, 1968b; Schieber and Rivlis 2007; Valls-Solé et al. 1999) were decreased uniformly (Binder et al. 2002; Fuglevand et al. 1993), or commonly inhibited throughout contraction (Hyngstrom et al. 2007; Johnson and Heckman 2010). Although minimal comparative studies have been made relating MU firing rate changes during voluntary movements in vivo, muscle groups with similar function likely have similar firing rate control (Kirk and Rice 2021), but expectedly would be stratified based on the task (Ryan and Kalmer 2019; Ivanova et al. 1997), anatomical mechanics (Loeb et al. 2002) and innervation (Tadros et al. 2016).

We found that firing rate trajectories of MU trains measured on a per contraction basis (i.e., statistically unpaired) were better modelled as a linear function of contraction time as compared to a half-life decay. This contrasts firing rate trajectories described during rapid (i.e., ‘ballistic’) voluntary isometric contractions of the tibialis anterior, soleus and first dorsal interosseous muscles that are purported to resemble a half-life decay (Desmedt 1980; Desmedt and Godaux 1978; Del Vecchio et al. 2019) but were not statistically tested against a more parsimonious linear fit (Fuglevand et al. 2015). From direct recordings of single MU firing rates during limb movements, the firing rate trajectory was often observed to have a strong linear component (figure 5.3, panel A), and is supported from similar linearity of spinal motor neuron firing rate trajectories in vivo during locomotion in the intact cat (Hoffer et al. 1987b, 1987a; Hoffer and Loeb 1980). Likewise, mechanisms that determine the linear component of the firing rate trajectory during movement may be attributed to movement-related feedback inhibition from the antagonist muscle spindle system (Hyngstrom et al. 2007; Johnson and Heckman 2010), with extrinsic inhibition shown to linearize the input-output relationship at the lower motor neuron (Harvey et al. 2005; Hounsgaard et al. 1988; Kuo et al. 2003; Revill and Fuglevand 2017). As we only studied elbow extension movements along a
single plane (that was assisted by gravitational force) and were stereotyped from a motor control perspective (Hallett et al. 1975), it remains likely that more complex movements that integrate many planes of movement, would require more complex firing rate trajectories at the single MU level. Presumably, these would also be influenced from different contributions across descending tracts (e.g., corticospinal, rubrospinal, reticulospinal, etc.), and pre-motor neurons related to task specificity (Buford and Davidson 2004; Lawrence and Kuypers 1968a, 1968b; Nicolas et al. 2001; Rothwell 2006; Whishaw et al. 1998).

In contrast to experiment one, the directionality of the firing rate trajectory was not different between isometric or movement contractions in experiment two, that only involved voluntary contractions in response to the same provided preparatory and reaction-cued stimuli (figure 5.7, panel B). These findings are in agreement with a similar experimental approach in the triceps brachii muscles that found MU firings per contraction were similar between conditions, but occurred less often during the late phase of isometric contractions as compared to movements (Ivanova et al. 1997). Although the time of preparation and anticipation is shown to reduce firing rate variability in muscles of the antebrachium during submaximal isometric voluntary contractions (Duclos et al. 2008), the utility of the reaction paradigm was to enable commonly prepared and executed voluntary contractions prior to the feedback effects of muscle length changes and limb rotation (Day et al. 2017; Merton 1953; Vallbo 1971). The approach of experiment two had some similarity to paradigms of more complex upper limb reaches recording cortical motor neuron (Churchland et al. 2012; Churchland and Shenoy 2007a) and medial pontomedullary reticular formation (i.e., reticulospinal system) neuronal activity in non-human primates (Buford and Davidson 2004). Unlike upper motor neurons, that can have firing rates peak throughout any point of the movement cycle (Churchland and Shenoy 2007a), the output of lower motor neurons, measured as MU firing rates of the anconeus corresponded to elbow extension torque and occurred prior to peak limb torque and velocity. Furthermore, as the ‘feedforward’ aspect provided to the MU pool was similar between contraction conditions in experiment two, differences of the firing rate trajectory in the movement-related state were more reasonably explained.
from the influence of movement dependent afferent feedback from the limb and periphery.

Summary analysis showed that the timing of MU firing rates relative to the go cue occurred sooner, or in other words, compressed, during limb movements as compared to isometric contractions, with a median time difference of ~27 ms. As the timing of MU firings rates were shifted closer to contraction onset, muscle state changes during movements may have resulted in greater short latency excitatory Ia/Ib afferent activity (Vallbo 1971), that modified and ‘quickened’ the already occurring MU firing rate trajectory, and can be interpreted as possible evidence supporting the ‘forward sensory model’ (Dimitriou and Edin 2010). We speculate that an oscillatory stretch of the agonist muscle could theoretically initiate short latency excitatory feedback to the active MU pool explaining the time-shifted MU firing trajectories. For example, this could be caused by faster conducting larger MUs (orderly recruited after slower conducting and smaller MUs) that would first depolarize type II muscle fibres based on slight differences of conduction velocity (Desmedt 1980), and would apply negative force to slower type I muscle fibres (Edman 2014). This would presumably result in slower type I muscle fibres being passively stretched and resulting in excitatory muscle spindle (e.g., Ia) stretch responses of the agonist muscle (Reinking et al. 1975), however, despite some evidence for the role of Ia excitatory inputs at contraction onset (Meunier and Pierrot-deseilligny 1989) and Ib excitatory (oligosynaptic) feedback (Houk and Henneman 1967; Jami 1992; Pratt 1995), we have no data in humans or the elbow extensors to support this hypothesis. Indeed, in the upper limb, short latency afferent feedback to the spinal cord (Weiler et al. 2019) and primary motor cortex (Pruszynski et al. 2011) can modify voluntary EMG activity. However, feedback modulation from the agonist muscle spindle system has unknown effects onto single MU firing rate trajectories during voluntary movements, despite importance to drive higher MU firing rates during isometric contraction (Macefield et al. 1993). Appreciably, afferent firings of the agonist muscle had minimal importance during peak muscle shortening velocity (Al-Falahe et al. 1991; Burke et al. 1978; Vallbo 1971) but was graded by voluntary contraction intensity (Vallbo 1974), whereas the afferent feedback from the antagonist muscle would be length dependent (Al-Falahe et al. 1991; Al-falahe et al. 1990; Day et al. 2017). Alternatively, during
voluntary movements, increased afferent drive from joint or cutaneous afferents, like in the hand (McNulty and Macefield 2001) can provide short latency excitation to the MU pool, and correspondingly may also occur in a relative sense but in the elbow joint.

Relationships between controlled ‘neural’ inputs (e.g., electrical, and chemical) to the muscle, and mechanical responses during movements have been more extensively studied in reduced *ex vivo* preparations, providing important clues to understand the functional importance of the underlying MU firing rate trajectory observed *in vivo*. At the muscle, contractile forces of load-moving skeletal muscle involves time-dependent lag relative to the neural inputs, therefore, accounting for effects of contraction time are important when describing movement control (Brown et al. 1999; Buchthal and Rosenfalck 1960; Cavanagh and Komi 1979; Churchland and Shenoy 2007a; Edman 1979; Partridge 1965, 1966). Although the limb starts in an isometric state, contraction onset involves intrinsic muscle shortening (Griffiths 1991), and compensation (Partridge 1967) that has load-dependent duration (Edman 2014; Partridge 1966). In experiment two, isometric contractions and movements based on the go cue had ~0.6-1 s of isometric contraction time at 5% of MVC torque to set a baseline voluntary preparatory state that would have also reduced the myotendinous slack and delay (Cavanagh and Komi 1979). During upper limb movements, agonist muscles have greater contractile shortening throughout a range of motion (Nelson et al. 2016; Stevens et al. 2014), and the implications of a decreasing firing rate trajectory would therefore coincide with time-dependent changes of the length-tension and force-velocity relationships (Brown et al. 1999; Brown and Loeb 2000a; Demieville and Partridge 1980; Edman 2014; Partridge 1965, 1966). Importantly, the highest MU firing rates occurred at the earlier part of contraction, likely accompanied by greater muscle shortening velocity due to activation of actin binding and cross bridge formation (Edman and Josephson 2007; Huxley 1957; Josephson and Edman 1998). Furthermore, the decreasing firing rate trajectory during larger voluntary efforts likely occurred with increasing sensitivity of type II muscle fibres to recurrent depolarization, as post-activation potentiation especially during faster speed movements (i.e., faster shortening velocity) would alter muscle mechanical outputs relative to the ‘neural’ inputs (Balnave and Allen 1996; Brown and Loeb 1999, 2000a; Buchthal and Rosenfalck 1960; Kristensen et al. 2018; MacIntosh et al. 2008; Moore and Stull 1984).
5.5 Limitations

General limitations were that we did not directly assess corticospinal (or other spinal tract) excitability, but instead relied on self-initiated and reaction-cued voluntary contractions (Chen et al. 1998; Jenkins et al. 2000) graded by relative contraction intensity (Devanne et al. 1997). Experiment one was limited in following the same MU sample across entire experiments based on MU potential waveform matching because switching between isometric and limb movements, and across different contraction intensities resulted in significant displacement between muscle and intramuscular electrode. We simulated an effect of MU duplication on our dataset, finding minimal effect on MU firing rate trajectory variance, however, this true unknown variance remains a possible confound. Furthermore, across different contraction intensities, expected differences in the number of recruited MUs (Desmedt and Godaux 1979; Harwood and Rice 2012; Oliveira and Nergo 2021) further modified MU waveform shapes between contractions. Therefore, in experiment one, MU trains were statistically unpaired between contractions and grouped for each participant (Tenan et al. 2014). A major assumption from recorded single MU activity was that it is representative of the larger MU pool and across other agonist muscles, and current findings remain to be validated with population-based analyses, and with stratification based on classification of MU type. Other limitations were in relating single MU activity to elbow joint kinematics, because the anconeus functions as both an elbow joint stabilizer and extensor (Le Bozec and Maton 1982; Coriolano et al. 2009; Miguel-Andres et al. 2017; Stevens et al. 2014), whereas the triceps brachii is the primary agonist (Harwood et al. 2013). However, both agonist muscles had similarities in how MU firing rates changed throughout contraction time course for each condition (appendices J-M). In addition, movements did not have specified target in space, but instead relied on reaching a set torque or velocity, therefore the voluntary control of deceleration (Garland et al. 1996; Ivanova et al. 1997; Sergio et al. 2005) was likely less prominent. Likewise, the antagonist MU firing rate trajectories were not investigated, but are necessary during targeted limb movements (Flash and Hogan 1985; Sanes and Jennings 1984; Sergio et al.
2005), and expectedly, would correspond to torques profiles that stabilize the joint and slow limb movement velocity (Ivanova et al. 1997).

5.6 Conclusions

The implications of these results are in understanding how the balance of descending and afferent inputs shape firing rate trajectories of the MU pool, as a different pattern of the underlying MU firing rate trajectory was observed between sustained isometric and limb movements during self-initiated contractions. This finding is supported from different time-frequency relationships across the brain, spinal cord and muscle that are different between hand movement and sustained isometric contractions in non-human primates (Oya et al. 2020). This insight was further supported in the reaction-cued experiment, as the summary timing of MU firings occurred sooner during voluntary limb movements, suggesting time-dependent neural control of muscle can be modified within the movement-related state, despite contractions being similarly prepared and initiated. Our findings highlight that task dependent descending synaptic inputs determine the direction of the MU firing rate trajectory, but the steepness is modified based on voluntary intensity. Explained another way, the direction and the steepness of the MU firing rate trajectory can ‘encode’ the task (i.e., isometric vs. limb movement) and voluntary contraction intensity, respectively. Likewise, that role of the movement-related state, that likely involved greater afferent feedback (Ellaway et al. 2015), was shown to alter the timing but not the general characteristics (i.e., linear slope and firing rate distribution) of the firing rate trajectory, making the control of agonist muscle contraction adaptable and within a compressed time duration during voluntary contractions.

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Chapter 6

6 Thesis conclusions

6.1 Thesis summary

This thesis explored the characteristics of neural drive at the single MU level underlying the voluntary control of isometric and limb movements in agonist muscles of human participants. Firing rates measured from single MUs were compared across several skeletal muscles during sustained isometric contractions in both young and older adults. In an experimental contrast, during elbow extension limb movements, that comprised greater effects of agonist muscle shortening (Nelson et al. 2016; Stevens et al. 2014), comparisons between age groups and movement speeds occurred primarily in the anconeus muscle and to a lesser extent in the triceps brachii muscle. Altogether, these data provide information as to the magnitude of different MU firing rate ranges inherent to muscles, along with how changes in MU firing rates enable simple limb movements at various contraction intensities up to maximum voluntary efforts. In a broader context, these findings can be used to inform theory and future experiments regarding voluntary mammalian movement, electrical control of skeletal muscle, brain-machine interfaces, and perhaps even in treatments to support successful ageing. For example, if we know the pattern of muscle activation during movement (see chapters 3-5) we can use the pattern to stimulate skeletal muscle contraction to control movement velocity.

In chapter 2, the range of MU firing rates was measured and compared across 12 different muscles that included a hand and several limb muscles. Findings showed that different muscles operate within different firing rate ranges during isometric contraction, that become more similar (i.e., overlapping) during ageing, but have greater dissimilarity in response to voluntary contraction intensity. This study further standardized an averaged comparison from a significant proportion of the present published literature on single MU firing rates at different contraction intensities up to MVC and enabled depiction of a ‘firing rate landscape’ to be built upon in future work.
In chapter 3, differences in MU firing rates recorded from the anconeus muscle were explored and compared in young and very old adults during voluntary elbow extension contractions. During conditions of both isometric efforts and limb movements, neural drive to the muscle was limited and lower in the older group in isometric contractions, but during limb movement (i.e., larger effect of muscle shortening contractions) the neural drive represented at single MU firing rates was not considerably different between age groups, despite the older group being weaker and slower during maximal voluntary contraction efforts as compared to the young. These data indicated that age-related effects are not limited by the neural drive frequency of the survived MU pool, but more likely attributed to other factors related to MU loss and contractile deficit at the muscle. This finding challenges many pre-conceived ideas regarding age-related decline of voluntary neural drive, as single MU firing rates, a form of information transfer to the agonist muscle, are not decreased with age when the limb is moving.

Unlike sustained isometric contractions, limb movement (i.e., motion), is measured as a time-dependent construct, requiring an acceleration, a velocity and ultimately a change of position. Using this framework, chapter 4 compared single MU firing rate activity with the aim to make comparisons to limb kinematic parameters, and through association analysis the effects of contraction time course were minimized. This was achieved by instantaneously relating MU firings recorded from the anconeus and lateral head of the triceps brachii muscles with separated kinematic parameters throughout each elbow extension contraction, with the strength of associations quantified through correlation analysis. Altogether, firing rates were directly related to limb torque, but not velocity or position. Although a straightforward finding, these results provided support that all the complex computations of the brain and spinal cord, are essentially reduced to a neural drive component (i.e., MU firing rates) that produce a direct torque (i.e., force) effect, with future velocity and position changes dependent on this interaction across movement speeds.

Finally, in chapter 5, MU firing rates recorded from the anconeus and lateral head of the triceps brachii muscles were analyzed as a function of contraction time course. In two experimental paradigms, the firing rate trajectory was compared between voluntary
elbow extension contractions that were isometric and limb moving. It was observed that firing rates decreased during limb movements, and that the steepness, but not the direction of the trajectory was voluntary contraction intensity dependent. During reaction-cued voluntary contractions, the same MU sample was followed across contraction replicas and averaged. The summary firing rate distribution was not different between isometric or limb movement conditions, but that the timing of firings relative to the go cue occurred sooner during limb movements. These results suggested that the MU firing rate trajectory was determined from descending synaptic inputs that are task and contraction intensity specific, but the timing of the trajectory at the single MU level can be shifted based on the state of the voluntary contraction that is permitted to involve joint displacement.

In summary, results from my studies show that neuronal firing rates that drive voluntary muscle contraction are muscle dependent and task dependent, and to a lesser extent, age dependent. A key objective of this thesis was to understand and compare the underlying neural activity of the agonist musculature during voluntary isometric and shortening contractions. The main findings from these studies were that firing rate ranges have up to 3-fold differences across muscles during isometric contraction, with the absolute firing rate range becoming lower and more similar with an age-related effect. However, during shortening contractions that coexist in limb movement, firing rates did not decrease with an age-related effect. In exploring single MU firing rate activity in the anconeus and triceps brachii muscle in young adults, firing rates in both muscles were directly associated with limb kinematic torque, but not velocity or position change. This finding supported that single MU activity of the anconeus muscle corresponded to the larger elbow extensor muscles that are primary agonists during elbow extension. Moreover, during elbow extension movements, the firing rate trajectory was time-dependent, was largely linear and decreasing, and was time-shifted dependent on the movement-related state. These findings provided evidence that neural drive to the agonist muscle during movement is steadily decreased but the timing can be slightly modified. The decreasing MU firing rate trajectory was likely explained by increasing extrinsic inhibition and decreasing descending synaptic drive, as the magnitude of the trajectory decrease (i.e., steepness of the slope) was associated with voluntary intensity (i.e., movement speed).
Altogether, from direct intramuscular recordings of agonist muscle MU activity, these results provide a further characterization of the neural drive underlying voluntary contractions between states of isometric tasks and stereotyped limb movements.

6.2 Thesis limitations

The physiological study of human participants occurred in all studies, therefore, experimental designs required necessary limitations based on ethical considerations, and for each experiment are considered in each chapter. In single MU EMG recordings of awake participants, a main consideration throughout the thesis is that we were likely sampling alpha motor neuron outputs to extrafusal muscle fibres, but in rare circumstances we could have also recorded single MU activity from beta- or gamma-motor neurons, a form of voluntary fusimotor drive (Edin et al. 1998). In all chapters, apart from having direct evidence related to the firing rate and recruitment threshold characteristics of motor unit activity in relation to limb kinematic outputs, many other factors, such as the state of afferent feedback, spinal and supra-spinal activity, and electrophysiological dynamics of the lower motor neuron were cited from the more extensive literature in single cell, and vertebrate experimental models (e.g., birds, turtles, rodents, cats, and non-human primate). The general assumptions therefore relate to the conserved evolutionary processes, that do have inter-species differences (Manuel et al. 2018) or were interpreted with known contrasts of anatomical of functional differences (Manuel and Heckman 2011). Likewise, in humans, a large amount of work comes from experimental designs that occur post-mortem or during voluntary contraction tasks that rarely occur in daily life (e.g., very slow isokinetic movements; or very rapid contractions that remain isometric… pain!), making the functional implications harder to interpret. However, a unique strength of this thesis, was a central focus throughout the four studies in exploring MU activity during sustained isometric contractions and movement behaviours that occur daily, involving a considerable range of voluntary contraction intensities, movement speeds and range of motion. Although, limb movements were indeed stereotypical in a motor control sense as only one plane of movement was
investigated (chapters 3-5). Likewise, as many of the experiments were association-based and sought to characterize the state of MU activity during simplified movement, it was important to first study participants that were deemed healthy and exercised on a regular basis (Booth et al. 2017), with future comparisons to participants that are limited by means of health status or functional ability.

6.3 Future labours

Regarding the neural control of movement, there remains considerable work and questions yet to be addressed at the MU level in both reduced and integrated (i.e., human) preparations. All the experiments in this thesis were done to record electrophysiological signatures from single MU firings, as these types of direct recordings during movements can track single MU activity throughout length changing contractions. However, understanding the relationships to other active MUs within the same muscle (or other synergist muscles, or antagonists) during voluntary movement, that simultaneously occur at the level of spinal circuitry (Petersen and Berg 2016) or the muscle (Demieville and Partridge 1980), are in early days. Throughout the last hundred years, *Herculean* scientific advancements related to voluntary movement neuroscience have come from a few very influential scientists (Burke 2007; Reid and Sherrington 1890), whereas the majority of breakthroughs have come from gradual advancements to characterize the intricate workings of brain-to-spinal-to-muscle relationships based on applications of new experimental tools. Of note, the amount of data a researcher can acquire in the last number of decades has increased exponentially, despite the biological state of the studied organisms being relatively unchanged. As it appears, the engineering of better tools and devices will continue, but what remains essential is that the quality of such data at both large and small scales be integrated, as useful theory developed from understanding large data should be able to explain the workings at smaller scales. At the scale of the single lower motor neuron and therefore what we observed as MU activity, these ideas are currently playing out, as new and powerful scaling tools can enable easier access to collect larger data sets that may indeed revolutionize our understanding of voluntary
movement control. However, like all technologies there are distinct known and unknown limitations that should be appreciated, hopefully tempering opinions that every experiment worth funding needs to disrupt the status quo.

Conceptually, MU outputs during voluntary movement represent only one side of the equation, with complex computations of neuron-to-neuron exchanges in series, parallel, mixing, etc., represented on the other side. In broader contexts, there is considerable interest to characterize population-based spinal and MU activity during limb movements (Glaser and Holobar 2019; Linden and Berg 2021; Petersen and Berg 2016; Zia et al. 2020), and building upon dynamical system frameworks in the cortex is a logical first analysis approach (Churchland et al. 2012; Russo et al. 2018, 2020; Vyas et al. 2020). Furthermore, if theory regarding firing rate dimensionality of the cortex can be applied to the MU population, the dynamical systems framework may be more simplified having fewer ‘dimensions’ as main outputs of lower motor neuron firing rates are related to muscle tension (e.g., chapter 4). Therefore, it may be redundant to require recordings of the complete MU population in order to make accurate predictions related to movements based on firing rate characteristics (Gao et al. 2017). In other contexts, comparisons of MU firing rate trajectories between agonist and antagonist muscle groups during movements are logical next steps, as antagonist and supporting postural muscles are necessary (Garland et al. 1996; Hallett et al. 1975; Ivanova et al. 1997; Merton 1953; Sanes and Jennings 1984; Sergio et al. 2005). Furthermore, as afferent feedback are required (beyond the first millisecond or so) for time-dependent movement correction, regulation, prediction and planning beyond the ‘feedforward’ initiation (Al-Falahe et al. 1991; Al-falahe et al. 1990; Dimitriou and Edin 2010; Matthews 1964; Merton 1953; Partridge 1967; Pruszynski et al. 2011; Vallbo 1971; Weiler et al. 2019), the integration of additional extrinsic inputs apart from the efferent pathways are necessary to inform how voluntary movements are carried through (Capaday and Stein 1987; Houk and Henneman 1967; Jami 1992; Meunier and Pierrot-deseilligny 1989; Pratt 1995). Likewise, at the muscle level, the mixing and synchronization of forces, from different MU samples (Edman 2014) and anatomically dependent effects of the shared myofibrillar matrix (Willingham et al. 2020) is another area of open possibility, as firing rates transduced into twitch-like and fused forces across MUs are nonlinear (Brown and Loeb
1999, 2000b; Edman 2014) and chaotic (Demieville and Partridge 1980). In comparison to sustained voluntary isometric contractions, studies outside this thesis exploring muscle shortening or lengthening contractions have minimally characterized MU firing rate activity as a function of contraction time course, with few exceptions (Grimby 1984; Gydikov et al. 1986; Oliveira and Nergo 2021), despite the application of load-moving characteristics of mammalian movement being time- and space-dependent. Future work regarding MU control of voluntary muscle contraction should continually focus on characterizations as a function of time, and with comparisons between altered and relevant physiological states; for example, during ageing (e.g., chapters 2 and 3), disease (e.g., disuse), different tasks and voluntary intensities (e.g., chapters 4 and 5), and movement complexity (e.g., synergist, spinal tract contributions, etc.).

In summary, voluntary mammalian movement is produced through a series of biochemical and electrical steps that ultimately become initiated in supraspinal tissues, are transferred throughout the spinal cord, and result in muscle fibre contractile forces at the skeletal muscle tendon. Although the lower motor neuron has provided some of the first and best studied models of neuronal activity under voluntary control due to the relative ease of single MU recordings (Adrian and Bronk 1929; Wachholder 1928), our understanding comes largely from isometric contractions under low voluntary intensities. In human participants, future work is required to probe how muscle contraction and complex movements are controlled at the scale of each MU sub-type and what contributions of efferent and afferent inputs to the MU and MU pool are redundant or critical in driving voluntary efforts.
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Appendices

Chapter 3

Appendix A: Chapter 3. Rate of torque development and time graphs.

Relationship between elbow extension rate of torque development (RTD) and the first 400 ms of contraction time course for each contraction, stratified by age group. A) Isometric contraction. B) Unconstrained-velocity movement. Correlation values represent the mean based on bootstrapping.
Appendix B: Chapter 3. Firing rate and limb acceleration time graphs of the older group.

Line-joined scatterplots for acceleration and FR as a function of time stratified by each contraction (black lines) from 15 MU samples in the older group. The LOESS fit (span 0.5 s) was coloured red. Data for acceleration (panels A-D) and FR (panels E-H) are linked to each MU potential, with the first MU potential for each MU train aligned to time-zero. Unconstrained-velocity movements were baseline loaded with 20% of the maximum torque per participant. Constrained-velocity movements were normalized based on the maximum movement velocity (Vmax).
Appendix C: Chapter 3. Firing rate and limb acceleration time graphs of the young group.

Line-joined scatterplots for acceleration and FR as a function of time stratified by each contraction (black lines) from 15 MU samples in the young group. The LOESS fit (span 0.5 s) was coloured red. Data for acceleration (panels A-D) and FR (panels E-H) are linked to each MU potential, with the first MU potential for each MU train aligned to time-zero. Unconstrained-velocity movements were baseline loaded with 20% of the maximum torque per participant. Constrained-velocity movements were normalized based on the maximum movement velocity (Vmax).
Chapter 4

Appendix D: Chapter 4. Principal component analysis of the aggregated dataset.

<table>
<thead>
<tr>
<th>Principal component rotation</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalue</td>
<td>4.0</td>
<td>2.8</td>
<td>2.3</td>
</tr>
<tr>
<td>Proportion (%)</td>
<td>27</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>Cumulative (%)</td>
<td>27</td>
<td>46</td>
<td>61</td>
</tr>
</tbody>
</table>

| Eigenvector                |      |      |      |
|-----------------            |      |      |      |
| Participant       | 0.13 | -0.17 | -0.42 |
| Muscle           | 0.17 | -0.13 | -0.36 |
| Sex              | -0.20| 0.05  | 0.40  |
| Session          | 0.16 | -0.22 | -0.13 |
| Targeted velocity condition | -0.38 | -0.10 | -0.16 |
| MU train         | 0.20 | -0.28 | -0.35 |
| Firing rate      | -0.18| 0.04  | -0.35 |
| Torque           | -0.40| -0.17 | 0.04  |
| RTD              | -0.27| 0.10  | -0.27 |
| Acceleration     | -0.31| -0.21 | -0.14 |
| Velocity         | -0.26| -0.44 | 0.10  |
| Power            | -0.33| -0.38 | 0.10  |
| Position         | 0.16 | -0.42 | 0.06  |

The summary dataset comprised 11,025 firing rate intervals measured from 856 MU trains across 15 participants, in two muscles during one of four targeted velocity elbow extension movements. In total, 12 principal component rotations were required to attain a cumulative proportion of 99% explained variance.
**Appendix E: Chatper 4. Pearson’s correlation estimates.**

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Associated parameters</th>
<th>Mean ± SD</th>
<th>Minimum, maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anconeus</td>
<td>Firing rate (Hz)</td>
<td>0.44 ± 0.02 *</td>
<td>0.37, 0.50</td>
</tr>
<tr>
<td></td>
<td>RTD (Nm/s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Firing rate (Hz)</td>
<td>0.42 ± 0.01 *</td>
<td>0.38, 0.46</td>
</tr>
<tr>
<td></td>
<td>RTD log(Nm/s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Firing rate (Hz)</td>
<td>0.36 ± 0.02 *</td>
<td>0.30, 0.42</td>
</tr>
<tr>
<td></td>
<td>Torque (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Firing rate (Hz)</td>
<td>0.03 ± 0.01</td>
<td>-0.02, 0.08</td>
</tr>
<tr>
<td></td>
<td>Velocity (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triceps brachii</td>
<td>Torque (%)</td>
<td>0.48 ± 0.01 *</td>
<td>0.44, 0.52</td>
</tr>
<tr>
<td></td>
<td>Velocity (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Position (%)</td>
<td>0.48 ± 0.01 *</td>
<td>0.44, 0.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triceps</td>
<td>Firing rate (Hz)</td>
<td>0.45 ± 0.02 *</td>
<td>0.34, 0.53</td>
</tr>
<tr>
<td>brachii</td>
<td>RTD (Nm/s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Firing rate (Hz)</td>
<td>0.42 ± 0.02 *</td>
<td>0.36, 0.48</td>
</tr>
<tr>
<td></td>
<td>RTD log(Nm/s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Firing rate (Hz)</td>
<td>0.43 ± 0.02 *</td>
<td>0.33, 0.50</td>
</tr>
<tr>
<td></td>
<td>Torque (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Firing rate (Hz)</td>
<td>0.02 ± 0.02</td>
<td>-0.04, 0.09</td>
</tr>
<tr>
<td></td>
<td>Velocity (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Torque (%)</td>
<td>0.40 ± 0.02 *</td>
<td>0.33, 0.48</td>
</tr>
<tr>
<td></td>
<td>Velocity (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Position (%)</td>
<td>0.24 ± 0.01 *</td>
<td>0.18, 0.29</td>
</tr>
</tbody>
</table>

From the aggregated dataset in figure 3, Pearson’s correlations (r) were calculated by bootstrap simulation, with the mean ± standard deviation (SD) and the range calculated from the simulated probability distribution of bootstrap correlations stratified by participant. All MU trains and targeted movement velocities in table 4.1, were grouped for each muscle. Values in percentage (%) were normalized to each participant’s maximum value during elbow extension movement. Statistical significance (*) was determined by the range of Pearson’s r coefficients not crossing zero.
Appendix F: Chapter 4. Associations of indirect relationships between MU firing rate and limb movement kinematics.

Aggregated data from all participants with MU trains combined from both the anconeus and lateral head of the triceps brachii muscles. Here we depict correlations from combined and non-ordered stepwise relationships of kinematic parameters throughout contraction time course. Although associations were significantly weakly correlated, panels A-C were confounded by different effects, and included A) firing rates were not related to limb velocity, B) confounded by torque and velocity, C) confounded by velocity. D) the relationship between elbow extension power and position was weakly correlated and was not confounded. For scatterplots, the mean± standard deviation of correlation values (r) were calculated from the simulated probability distribution of bootstrap correlations stratified by participant.
**Appendix G:** Chapter 5. Torque and firing rate at motor unit firing recruitment threshold, from experiment one.

<table>
<thead>
<tr>
<th>Contraction condition</th>
<th>Contraction intensity</th>
<th>Torque (% of MVC)</th>
<th>Median firing rates (Hz)</th>
<th>Pearson’s correlation</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isometric</td>
<td>MVC</td>
<td>59.7 (14.7, 94.9)</td>
<td>23.3 (5.3, 49.3)</td>
<td>-0.35</td>
<td>-0.56, 0.07</td>
</tr>
<tr>
<td></td>
<td>Ramp</td>
<td>24.5 (10.9, 55.1)</td>
<td>9.6 (2.1, 60.1)</td>
<td>0.24 *</td>
<td>0.09, 0.37</td>
</tr>
<tr>
<td>Limb movement</td>
<td>V25</td>
<td>33.9 (13.4, 75.4)</td>
<td>14.1 (4.6, 115.2)</td>
<td>0.08</td>
<td>-0.06, 0.20</td>
</tr>
<tr>
<td></td>
<td>V50</td>
<td>38.5 (11.7, 76.0)</td>
<td>17.5 (2.3, 124.4)</td>
<td>-0.01</td>
<td>-0.10, 0.07</td>
</tr>
<tr>
<td></td>
<td>V75</td>
<td>44.2 (10.6, 82.1)</td>
<td>21.0 (4.7, 102.0)</td>
<td>0.01</td>
<td>-0.12, 0.14</td>
</tr>
<tr>
<td></td>
<td>V100</td>
<td>49.4 (12.3, 96.3)</td>
<td>27.6 (5.4, 257.7)</td>
<td>-0.003</td>
<td>-0.11, 0.10</td>
</tr>
</tbody>
</table>

Values are median (min, max). For each condition, summary values of elbow extension torque and the frequency of the first inter-spike-interval (ISI) from MU trains recorded in the anconeus or lateral triceps brachii muscle. The number of MU trains used for each condition can be found in table 5.1. Statistically significance (*) of Pearson’s correlations were determined from the 95% confidence interval (CI) not spanning zero.
### Appendix H: Chapter 5. Comparing firing rates between isometric and movement conditions for each MU, from experiment two.

<table>
<thead>
<tr>
<th>Participant</th>
<th>MU number</th>
<th>#firings: isometric /movement</th>
<th>Isometric median firing rate (Hz)</th>
<th>Movement median firing rate (Hz)</th>
<th>p value</th>
<th>D</th>
<th>D bootstrapped mean ± standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>111/97</td>
<td>22.7</td>
<td>22.7</td>
<td>0.13</td>
<td>0.16</td>
<td>0.18 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>126/284</td>
<td>20.4</td>
<td>16.7</td>
<td>&lt;0.001</td>
<td>0.23</td>
<td>0.25 ± 0.04</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>68/69</td>
<td>34.5</td>
<td>47.6</td>
<td>0.03</td>
<td>0.24</td>
<td>0.28 ± 0.07</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>91/84</td>
<td>32.2</td>
<td>50.0</td>
<td>0.002</td>
<td>0.28</td>
<td>0.31 ± 0.06</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>162/117</td>
<td>23.8</td>
<td>24.4</td>
<td>0.96</td>
<td>0.06</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>185/126</td>
<td>21.3</td>
<td>23.8</td>
<td>0.10</td>
<td>0.14</td>
<td>0.16 ± 0.04</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>76/76</td>
<td>20.4</td>
<td>28.6</td>
<td>0.006</td>
<td>0.28</td>
<td>0.28 ± 0.07</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>133/123</td>
<td>21.0</td>
<td>24.4</td>
<td>0.45</td>
<td>0.11</td>
<td>0.15 ± 0.05</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>93/122</td>
<td>21.3</td>
<td>26.3</td>
<td>0.14</td>
<td>0.16</td>
<td>0.15 ± 0.05</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>84/88</td>
<td>23.2</td>
<td>26.7</td>
<td>0.83</td>
<td>0.09</td>
<td>0.16 ± 0.05</td>
</tr>
</tbody>
</table>

Statistical comparisons for each MU sample were made using a two-sided Kolmogorov-Smirnov test. The distance value (D) between cumulative probability distributions is in decimals, with the value of 1 equal to 100%. Bootstrapping of $1 \cdot 10^3$ iterations calculated a simulated probability distribution estimate of the D statistic for each test.
Appendix I: Chapter 5. Comparing the timing of firings relative to the go stimulus cue between isometric and movement conditions for each MU, from experiment two.

<table>
<thead>
<tr>
<th>Participant</th>
<th>MU number</th>
<th>#firings isometric /movement</th>
<th>Isometric median firing time (s)</th>
<th>Movement median firing time (s)</th>
<th>p value</th>
<th>D</th>
<th>D bootstrapped mean ± standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>117/107</td>
<td>0.383</td>
<td>0.396</td>
<td>0.87</td>
<td>0.08</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td>139/307</td>
<td>0.420</td>
<td>0.527</td>
<td>&lt;0.001</td>
<td>0.28</td>
<td>0.30 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>71/70</td>
<td>0.346</td>
<td>0.316</td>
<td>0.20</td>
<td>0.18</td>
<td>0.23 ± 0.06</td>
</tr>
<tr>
<td>4</td>
<td>94/90</td>
<td>0.374</td>
<td>0.328</td>
<td>0.03</td>
<td>0.22</td>
<td>0.24 ± 0.06</td>
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<tr>
<td>3</td>
<td>5</td>
<td>163/122</td>
<td>0.460</td>
<td>0.428</td>
<td>0.07</td>
<td>0.15</td>
<td>0.18 ± 0.04</td>
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<tr>
<td>6</td>
<td>190/135</td>
<td>0.498</td>
<td>0.441</td>
<td>0.001</td>
<td>0.22</td>
<td>0.24 ± 0.05</td>
<td></td>
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<tr>
<td>4</td>
<td>7</td>
<td>76/80</td>
<td>0.382</td>
<td>0.312</td>
<td>0.001</td>
<td>0.31</td>
<td>0.35 ± 0.07</td>
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<tr>
<td>8</td>
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<td>0.354</td>
<td>0.272</td>
<td>&lt;0.001</td>
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<td>0.36 ± 0.05</td>
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<tr>
<td>5</td>
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<td>0.367</td>
<td>0.345</td>
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<td>0.16</td>
<td>0.19 ± 0.05</td>
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<tr>
<td>10</td>
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<td>0.389</td>
<td>0.351</td>
<td>0.04</td>
<td>0.21</td>
<td>0.25 ± 0.06</td>
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Statistical comparisons for each MU sample were made using a two-sided Kolmogorov-Smirnov test. The distance value (D) between cumulative probability distributions is in decimals, with the value of 1 equal to 100%. Bootstrapping of $1 \cdot 10^3$ iterations calculated a simulated probability distribution estimate of the D statistic for each test.
Appendix J: Chapter 5. Anconeus MU firing rate and time graphs.

Voluntary self-initiated elbow extension contractions. Summary activity from single MU recordings in the anconeus muscle (n = 15), depicting firing rates as a function of contraction time course, with each MU train line joined. The smoothed curve was LOESS fit (in red), with a span of 25%. The 95% confidence interval of the LOESS fit is shaded in grey. Firing recruitment threshold for each MU train is aligned to time zero.
**Appendix K**: Chapter 5. Elbow extension kinematic and time graphs.

Voluntary self-initiated elbow extension contractions. Measures of limb kinematics were linked to the timing of each MU firing rate in corresponding panels of figure S1. Summary limb kinematics are depicted as a function of contraction time course (n = 15), with each parameter shown as a separate LOESS fit, with a span of 50%. Blue denotes RTD (Nm • s⁻¹), Black is torque (normalized % of MVC torque), green is velocity (normalized % of Vmax velocity) and purple is position (deg). The 95% confidence interval of each LOESS fit is shaded in grey.
Appendix L: Chapter 5. Lateral head of the triceps brachii MU firing rate and time graphs.

Voluntary self-initiated elbow extension contractions. Summary activity from single MU recordings in the lateral head of the triceps brachii muscle (n = 6), depicting firing rates as a function of contraction time course, with each MU train line joined. The smoothed curve was LOESS fit (in red), with a span of 25%. The 95% confidence interval of the LOESS fit is shaded in grey. Firing recruitment threshold for each MU train is aligned to time zero.
Appendix M: Chapter 5. Elbow extension kinematic and time graphs.

Voluntary self-initiated elbow extension contractions. Measures of limb kinematics were linked to the timing of each MU firing rate in corresponding panels of figure S3. Summary limb kinematics are depicted as a function of contraction time course (n = 6), with each parameter shown as a separate LOESS fit, with a span of 50%. Blue denotes RTD (Nm • s^{-1}), Black is torque (normalized % of MVC torque), green is velocity (normalized % of Vmax velocity) and purple is position (deg). The 95% confidence interval of each LOESS fit is shaded in grey.
Appendix N: Chapter 5. Summary of MU samples 1-4 in experiment two.

Single MU firing rate activity of the anconeus muscle during reaction-cued voluntary elbow extension contractions. Black denotes isometric contractions and blue denotes movements. Each column of rows depicts a different MU sample (MU1-10). Relative to the time of the go cue (at time zero), contraction replicas are depicted (from top to bottom) as torque overlays; velocity overlays; raster of firing times with each indexed row representing a separate contraction in order throughout the experiment; firing rates smoothed with a fixed bandwidth of the kernel regression that was optimized (Shimazaki and Shinomoto 2010) for the isometric and movement contraction conditions; underlying density plot from the optimized kernel bandwidth.
Appendix O: Chapter 5. Summary of MU samples 5-7 in experiment two.

Single MU firing rate activity of the anconeus muscle during reaction-cued voluntary elbow extension contractions. Black denotes isometric contractions and blue denotes movements. Each column of rows depicts a different MU sample (MU1-10). Relative to the time of the go cue (at time zero), contraction replicas are depicted (from top to bottom) as torque overlays; velocity overlays; raster of firing times with each indexed row representing a separate contraction in order throughout the experiment; firing rates smoothed with a fixed bandwidth of the kernel regression that was optimized (Shimazaki and Shinomoto 2010) for the isometric and movement contraction conditions; underlying density plot from the optimized kernel bandwidth.
Appendix P: Chapter 5. Summary of MU samples 8-10 in experiment two.

Single MU firing rate activity of the anconeus muscle during reaction-cued voluntary elbow extension contractions. Black denotes isometric contractions and blue denotes movements. Each column of rows depicts a different MU sample (MU1-10). Relative to the time of the go cue (at time zero), contraction replicas are depicted (from top to bottom) as torque overlays; velocity overlays; raster of firing times with each indexed row representing a separate contraction in order throughout the experiment; firing rates smoothed with a fixed bandwidth of the kernel regression that was optimized (Shimazaki and Shinomoto 2010) for the isometric and movement contraction conditions; underlying density plot from the optimized kernel bandwidth.
Appendix Q: Chapter 5, experiment 2. Calculating the fixed kernel bandwidths.

To estimate the MU firing rate trajectory for isometric and movement contraction conditions, firing rates across contraction replicas were smoothed with a fixed kernel bandwidth (Shimazaki and Shinomoto 2010). The relationship depicts the cost and size of the fixed kernel bandwidth in seconds, with the most negative point on the y-axis denoting the optimal cost. Using this approach, it’s likely that the bandwidth is more bias towards lower values but is the appropriate choice given that some MUs were only followed <20 contractions (Shimazaki and Shinomoto 2010).
Appendix R: Experimental set-up used in chapters 3-5.

Voluntary elbow extension contractions occurred in a dynamometer with floating intramuscular wire EMG connections. A) Posterior view of the elbow joint. B) Floating intramuscular wire and EMG setup to the pre-amplifier. Depicted is the set-up for two intramuscular recording channels of the anconeus muscle. C) Another view of the experimental set-up, during experiment two from Chapter 5.
Appendix S: Research ethics initial approval notice.
Appendix T: Research ethics continued approval.
Curriculum Vitae

Eric Andrew Kirk
May 10, 1991
Citizenship: Canadian

Degrees earned:
1. MSc. Kinesiology integrated biosciences. Supervisor: Dr. Charles L. Rice. The University of Western Ontario.
2. BSc. Biology honours specialization in genetics. Supervisors: Dr. Shiva M. Singh and Dr. Charles L. Rice. The University of Western Ontario.

Graduate scholarships and funding:
1. Society for Neuroscience 2021 Trainee Professional Development Award (TPDA).
2. Natural sciences and engineering research council of Canada (NSERC) – Michael Smith foreign study supplement. Collaboration with the lab of Dr. C.J. Heckman at Northwestern University, Chicago, USA. Funded for 2021.
4. Ontario graduate scholarship (OGS). Funded for 2016-2017 (MSc), and 2018 (first year PhD studies).

Academic service:
1. Member of the full health sciences research ethics board (HSREB) for human experimentation at the University of Western Ontario. From 2018-present.
2. Reviewed for scientific journals as the primary reviewer with support from Dr. Rice (PhD supervisor), including Scientific Reports (2021), the Journal of Clinical Medicine (2020) and Applied Physiology, Nutrition, and Metabolism (2019).
3. In 2017, led successful change in the school of Kinesiology policy with the associate dean of health sciences (Dr. R. Martin) to enable graduate students awarded external scholarships to participate as graduate teaching assistants.

Peer reviewed publications:

Other peer reviewed contributions:


Relevant presentations:
1. Lecture on neuromuscular electrical stimulation techniques, January 26, 2021. York University, Kin 3675 3.0 techniques in muscle physiology, Dr. A. Cheng.
4. Presenter at the department of physical medicine and rehabilitation research day, April 15, 2019, Parkwood Institute - main auditorium. Title: Insights from motor unit firing rates of 13 muscles.
6. Lecture on neuromuscular system ageing, October 18, 2018, KIN 4430F (85 students).
7. Age-related differences in motor unit discharge rates of the upper trapezius (2017) Exercise neuroscience group, University of Guelph, Canada (Presentation).

Graduate teaching assistantships:

1. 2020: Exercise physiology (2230).