

MOTOR UNIT CONTROL OF MOUSE LOCOMOTION

A Dissertation
Presented to
The Academic Faculty

by

Kyle Thomas

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy in the
Wallace H. Coulter Department of Biomedical Engineering

Georgia Institute of Technology
Emory University
December 2025

COPYRIGHT © 2025 BY KYLE THOMAS

MOTOR UNIT CONTROL OF MOUSE LOCOMOTION

Approved by:

Dr. Samuel Sober, Advisor
Department of Biology
Emory University

Dr. Lena Ting
Department of Biomedical Engineering
*Georgia Institute of Technology and
Emory University*

Dr. Megan Carey
Champalimaud Neuroscience Programme
Champalimaud Centre for the Unknown

Dr. Chethan Pandarinath
Department of Biomedical Engineering
*Georgia Institute of Technology and
Emory University*

Dr. Timothy Cope
School of Biological Sciences
Georgia Institute of Technology

Date Approved: August 15, 2025

To the impact we make but don't always recognize.

ACKNOWLEDGEMENTS

I would like to thank my advisor Dr. Sam Sober for his guidance over the course of my PhD. I first joined the lab with very little experience in neuroscience, and you mentored me through learning many difficult skills, concepts, and technologies. You cultivated a lab environment that is collaborative and encouraging, and I'll always remember your excitement at the first motor unit we saw together.

I would like to thank Dr. Megan Carey and Dr. Hugo Marques, who hosted me at Champalimaud early in my PhD. I have enjoyed our collaborations and have learned so much from both of you. I would also like to thank the other members of my committee, Dr. Timothy Cope, Dr. Lena Ting, and Dr. Chethan Pandarinath for the advice you have all shared over the course of many independent and group meetings.

I would like to thank the people in the Sober Lab for countless lessons in both science and life. We came up with the concept of 'data fun', which is very apt given our conversations that range from high-level modern neuroscience to sumo wrestler biomechanics to the spinning egg hypothesis. At the heart of the lab is Dr. Amanda Jacob. You are a pillar in the lab and have been extremely instrumental to our success, so thank you for all that you do. I would also like to thank Rhuna Gibbs for not holding it against me every time I asked you to make 'one last model'. Your energy, eagerness to learn, and willingness to engage in my chaos were more important than you know.

I would like to thank my family for their support and reassurance through my life. Mom and Dad, you guided me towards science before I knew what I was capable of, and your continuous encouragement is a major source of my strength.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	iv
LIST OF TABLES	vii
LIST OF FIGURES	viii
LIST OF SYMBOLS AND ABBREVIATIONS	ix
SUMMARY	x
CHAPTER 1. Introduction	1
1.1 Basis of locomotion	1
1.2 Neural control of locomotion	3
1.3 Methodology to study the neural control of locomotion	5
1.3.1 Techniques for measuring motor units during behavior	5
1.3.2 Mice as a model system	7
1.4 Dissertation overview	9
CHAPTER 2. Literature Review	11
2.1 Movement strategies behind locomotion	11
2.2 Muscles are coordinated to control movement of the body	15
2.2.1 Biomechanical and physiological properties of muscles produce forces on joints	15
2.2.2 Coordinated muscle activity produces locomotion	18
2.3 CNS control of locomotion	22
2.3.1 Central pattern generators (CPGs) produce rhythmic activity through spinal cord circuits	22
2.3.2 Neuromodulation and persistent inward currents shape motor neuron excitability	25
2.3.3 Supraspinal control of locomotion	27
2.4 Motor units provide a high-resolution approach to study motor control	31
2.4.1 Size principle coordination of motor units	31
CHAPTER 3. Motor unit mechanisms of speed control in mouse locomotion	35
3.1 Abstract	35
3.2 Introduction	36
3.3 Methods	38
3.3.1 Surgical implantation	38
3.3.2 Behavioral methods and data collection	39
3.3.3 Electromyography (EMG)	41
3.3.4 Data analysis	44
3.3.5 Joint model of rate and recruitment	44
3.3.6 Confidence intervals in the joint rate-recruitment model	45
3.4 Results	45

3.4.1	Motor units are probabilistically recruited across strides	48
3.4.2	Motor unit firing patterns in the long and lateral heads of the triceps	50
3.4.3	Motor unit mechanisms of speed control	54
3.4.4	Kinematic contributions of motor unit recruitment	57
3.4.5	Motor unit recruitment has population dependence	60
3.5	Discussion	62
3.5.1	Differences in motor unit activity patterns across two elbow extensors	62
3.5.2	Firing rates in mouse locomotion compared to other species	65
3.5.3	Walking speed modulation of firing rate and recruitment	67
CHAPTER 4.	Pairwise coordination of motor units in mouse locomotion	70
4.1	Introduction	70
4.2	Methods	72
4.2.1	Relative firing patterns in pairwise motor units	72
4.2.2	Rate recruitment interaction	72
4.3	Results	73
4.3.1	Motor units have systematic recruitment but not de-recruitment patterns during walking	73
4.4	Motor unit coordination correlations with walking speed and kinematics	76
4.5	Motor unit recruitment may decrease firing rate in currently active units	81
4.6	Discussion	82
4.6.1	Recruitment order has systematic patterns that reflect the behavior	83
4.6.2	De-recruitment patterns demonstrate difference from recruitment order	86
4.6.3	Motor unit firing patterns are functionally coupled during locomotion	88
CHAPTER 5.	Conclusions and future directions	90
5.1	How is motor unit coordination shaped by the task-specific roles of muscles?	90
5.2	How does speed influence motor unit coordination?	94
5.3	Interrogating motor circuits by manipulating motor unit spike patterns	97
REFERENCES		101

LIST OF TABLES

Table 3.1.	Motor units identified per muscle in each experimental mouse. Each thread consisted of 8 electrode contacts used to record bipolar EMG, and numbers in parentheses indicate the number of motor units isolated using the spike sorting algorithm described in Methods. Data from biceps muscle implantation in two mice were not spike sorted and are not included in this report.	39
-------------------	--	----

LIST OF FIGURES

Figure 1.1. Gait cycle in human locomotion on level surface.	2
Figure 1.2. Motor unit firing patterns can vary across the population to produce similar force and total muscle activity.	4
Figure 2.1. Characteristic gait strategies across example species.	13
Figure 2.2. Overview of skeletal muscle.	16
Figure 2.3. Stride-averaged EMG from rat quadriceps muscles across walking speeds.	20
Figure 2.4. Rhythmic generator (RG) circuit with hypothesized connections in producing rhythmic flexor and extensor activity.	24
Figure 3.1. Isolated motor units had consistent waveforms.	43
Figure 3.2. High-resolution muscle recording during mouse locomotion.	47
Figure 3.3. Motor unit spike count distributions.	49
Figure 3.4. Empirical observations of spike count distributions for all units.	50
Figure 3.5. Motor unit firing patterns within and across muscles.	52
Figure 3.6. Motor unit spike patterns evolve differently in the long and lateral heads.	54
Figure 3.7. Motor units alter firing rate and recruitment across walking speeds.	55
Figure 3.8. Altered firing rate and recruitment across walking speed quartiles for all motor units.	56
Figure 3.9. Motor unit recruitment correlates with muscle-specific kinematic differences.	59
Figure 3.10. Motor unit recruitment and kinematic correlations in individual speed quartiles.	60
Figure 3.11. Motor unit recruitment is greater when other motor units within the muscle are recruited.	61
Figure 4.1. Recruitment and de-recruitment latencies across unit pairs.	74
Figure 4.2. Percentages of strides with positive motor unit recruitment and de-recruitment latencies.	75
Figure 4.3. Recruitment and de-recruitment latencies change across locomotor speeds.	77
Figure 4.4. Motor unit recruitment and de-recruitment order influence body kinematics.	80
Figure 4.5. Motor unit instantaneous firing rate (IFR) decreases with second unit recruitment.	82
Figure 4.6. Relation between motor unit amplitude and firing parameters within the stride.	84
Figure 5.1. Comparison of beam walking and treadmill locomotion in the mouse.	94
Figure 5.2. Depiction of Opto-Myomatrix flexible multi-channel electrode arrays with integrated μ LED.	99

LIST OF SYMBOLS AND ABBREVIATIONS

CNS	Central nervous System
EMG	Electromyography
AHP	After-hyperpolarization
SOL	Soleus
MG	Medial gastrocnemius
CPG	Central pattern generation
5-HT	Serotonin
PIC	Persistent inward current
CnF	Cuneiform nucleus
PPN	Pedunculopontine nucleus
MLR	Mesencephalic locomotor region
MRF	Medullary reticular formation
IFR	Instantaneous firing rate
ISI	Inter-spike interval
ChR2	Channelrhodopsin2

SUMMARY

Locomotion relies on neuromuscular circuits that produce rhythmic activity across the limbs and body. Motor neurons within the spinal cord innervate limb muscles, causing them to contract and generate the forces that moves the body. For movements as complex as locomotion, motor units, which consist of a single motor neuron and the muscle fibers it innervates, must be coordinated within and across muscles to not only move, but also to flexibly adjust locomotor rhythm. Although it is well established that motor units modulate the force output in muscles largely through their recruitment and firing rate, it is unclear how these firing patterns, both in individual motor units and in populations of motor units, are coordinated during dynamic movements. Using novel neurotechnology that allows for high-resolution investigation of the neuromuscular system, work presented in this thesis describes the coordination of motor units during locomotion in mice across different speeds. In Chapters 1 and 2, we introduce key concepts regarding the neuromuscular control of locomotion and review literature in the field. In Chapter 3, we identify how the firing rate and recruitment probability of individual motor units correlates with not only movement speed, but also specific kinematic features of each stride. Characterizing these results across different muscles in the mouse forelimb, we highlight how muscles with shared functions may be uniquely controlled by the nervous system. In Chapter 4, we use pair-wise analyses of motor units to determine how populations of motor units are recruited and de-recruited within muscles. Motor units were recruited but not de-recruited in a systematic order, and deviations from this order were correlated with stride-by-stride changes in locomotor behavior. Taken together, these results provide evidence on how the mouse neuromuscular system coordinates robust and flexible locomotor behavior.

CHAPTER 1. INTRODUCTION

1.1 Basis of locomotion

Movement is essential for independent interaction with the world. The movements that a person makes daily, such as walking, waving hello, or speaking, are customarily simple to perform. One can even imagine modifications to these movements. A person may walk slowly if taking a casual stroll or walk quickly if late for an appointment. Skilled athletes can accomplish extraordinary feats, running faster and longer than the average person. Scientists have been intrigued for centuries by how exactly the human body and nervous system accomplish these tasks.

Walking, or more generally locomotion, has been studied extensively, with explorations into the neuromuscular system across species and environmental contexts (Alexander, 2006; Grillner, 1985). Although locomotion itself is widespread across animal species, the exact strategies behind the movement along with the role of the nervous system in generating the movement can vary. From locomotor research, scientists have gained key insights into how different regions of the nervous system enable free and flexible movement.

Locomotion relies on rhythmic, coordinated motion of the body (Grillner, 1975; Grillner & El Manira, 2020). Limbed species, such as humans, mice, and even insects rely on appropriately patterned limb movements to propel the body forward. Strides, typically characterized by a full cycle of the foot touching the ground (stance), lifting off and swinging forward (swing), and then finally touching the ground again, have very stereotyped features (Figure 1.1) (Alexander, 2006; Al-Shuka et al., 2019). For example,

during swing phase, measurable parameters such as the distance the limb swings, the duration the limb is in the air, and the joint angles of the leg are similar on a stride-by-stride basis at a given walking speed (Kadaba et al., 1990; Borghese et al., 1996; Alexander, 2006).

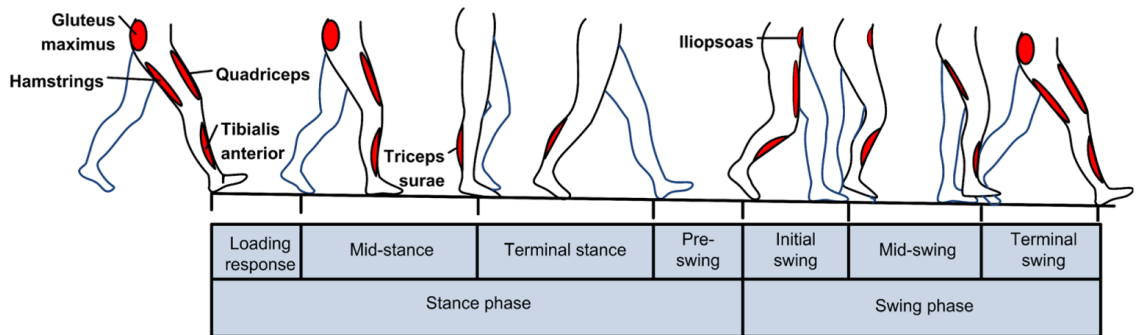


Figure 1.1. Gait cycle in human locomotion on level surface. Muscles are highlighted in red when active during the stride. Adapted from Al-Shuka et al. 2019.

Muscles across the body must be coordinated to produce coherent movement (Ting et al., 2015). Generally, muscle fibers contract to exert force on skeletal joints and generate movement. For example, force produced by the triceps brachii muscle acts to extend the elbow joint. However, muscle force does not have a one-to-one relationship with the output movement. Pushing against a sturdy wall from a stable position will similarly contract the triceps, but there will be no movement of the joint. Additionally, muscles are arranged across the body with various attachments and functions towards different joints, leading to a complex capacity for movement as muscles are engaged simultaneously towards behavior (Latash, 2012). Co-activation of the triceps brachii with the biceps brachii, which flexes the elbow, can again result in no movement at the elbow or even a reduction in the elbow angle. Behaviors as complex as locomotion require coordinated muscle activity, in which

muscles across the body and limbs must rhythmically contract with appropriate timing and strength (Figure 1.1) (Alexander, 2006; Al-Shuka et al., 2019).

1.2 Neural control of locomotion

For coordinating muscle activation during complex behaviors, the central nervous system (CNS) combines movement intentions with both the biomechanical constraints of the body and the environmental context. This results in robust movements that can be adjusted flexibly to meet different demands. Locomotion is continuously shaped by descending signals from the brain, rhythmic circuits within the spinal cord, and peripheral sensory inputs to control the kinematic strategy and speed of movement (Arber & Costa, 2018; Grillner & El Manira, 2020; Kiehn, 2016).

Motor units, which consist of a single motor neuron and all the muscle fibers it innervates, translate CNS inputs into movement (Heckman & Enoka, 2012). Considered the final output of the CNS, each action potential in a motor neuron reliably propagates across its set of muscle fibers, generating force-producing contractions (Heckman & Enoka, 2012). Tens to thousands of motor units may innervate a single muscle depending on the muscle's size (Heckman & Enoka, 2012). The recruitment of each motor unit (De Luca, 1985), rate of its firing activity (De Luca, 1985), and timing of each action potential (Zhurov & Brezina, 2006; Srivastava et al., 2017; Putney et al., 2019) collectively shape the force output in the muscle. Thus, the rhythmic muscle activity behind locomotion comes from motor unit spike patterns across the body, and these spike patterns are shaped by inputs across the nervous system.

Although the CNS can rapidly and flexibly alter locomotor parameters such as speed (Grillner & El Manira, 2020), it remains unknown how these changes are effectively implemented by motor unit spiking patterns. To generate the larger, more rapid forces needed during faster motion, motor units might change their firing rate and/or recruitment in multiple different ways (Figure 1.2). These changes must be coordinated in motor units across the body to produce the correct type of movement. Thus, characterizing how populations of motor units are coordinated during locomotion along with how this coordination changes at different speeds is important towards revealing how the neuromuscular system controls behavior.

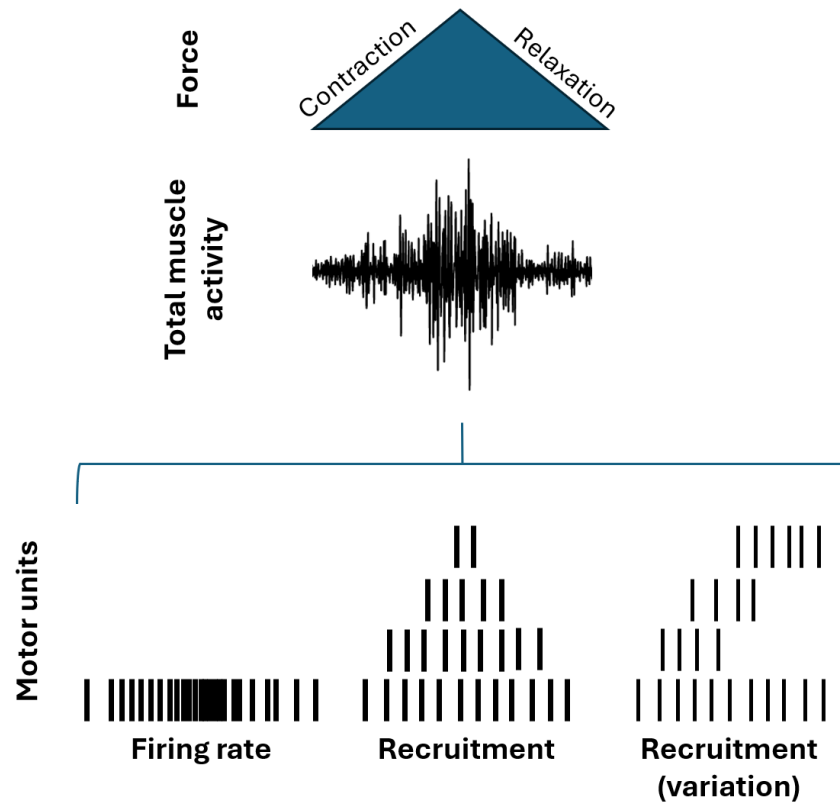


Figure 1.2. Motor unit firing patterns can vary across the population to produce similar force and total muscle activity.

1.3 Methodology to study the neural control of locomotion

1.3.1 Techniques for measuring motor units during behavior

Investigation of motor control at the resolution of motor units offers the potential to best explain how the nervous system controls locomotion. This information would best represent the activity of the CNS and its relation to body movement. However, a major limitation from past studies has been the inability to accurately measure motor units during complex movements in vertebrates. Given their location in the spinal cord, motor neuron cell bodies are impossible to measure during natural movement and behavior. Some investigations attempt these direct measurements but often with experimental preparations that require either spinal fixations or transections that prevent free movement (Hadzipasic et al., 2016).

To overcome these challenges, many techniques rely on less invasive measurement from the muscles to infer activity from motor neuron inputs. Each action potential from the motor neuron reliably innervates a unique set of muscle fibers, and this activity can be measured through electromyography (EMG). Typical EMG recordings do not have the resolution to identify the activity in single fibers but instead make bulk measurement of the entire muscle's electrical activation. In contrast, advanced EMG tools and computational analyses isolate the unique electrical waveforms of each motor unit within this bulk signal.

Traditional methods for measuring the activity of single motor units involve using fine-wire EMG. This technique uses small, wire electrodes that are inserted into a muscle of interest (G. E. Loeb & Gans, 1986; Nelson & Soderberg, 1983). It is difficult to record a population of motor units from a single wire unless the amplitudes and waveforms of

these units are dramatically different at the site of recording. Additionally, experimental subjects tend to have limited mobility and contraction capability because of discomfort caused by the rigidity of the wire, which also limits the muscles able to be studied (Andrew, 1985; Broman et al., 1985; C. K. Thomas et al., 1986; Kidgell et al., 2006). Although several studies across species, including humans (Grimby, 1984) and rats (Eken, 1998; Gorassini et al., 2000), have previously revealed motor unit firing patterns during steady locomotion, it is not yet clear how firing patterns vary as the stepping pattern/rhythm changes.

Newer methods of quantifying motor units involve mathematically identifying their unique waveforms from non-invasive, multi-channel surface recordings (Farina & Enoka, 2023). For example, in motor unit decomposition, the bulk activity from surface recordings is resolved into the constituent motor units that are active simultaneously (De Luca et al., 2006). Through this method, populations of simultaneously active units can be identified over a wide range of contraction sizes and movements including locomotion (Lulic-Kuryllo et al., 2021; Yokoyama et al., 2022). Still, the surface electrodes necessary for this method are not applicable for smaller muscles, smaller species, or deeper muscles not directly near the skin.

Novel Myomatrix electrodes address the limitations of these prior techniques. Myomatrix electrodes include 32 small ($625 - 20,000 \mu\text{m}^2$) recording sites deposited on a polyimide backing (Chung et al., 2023; Zia et al., 2020). The electrodes can be inserted into muscles for either acute or chronic recordings of high-resolution EMG. Unlike fine-wire electrodes, Myomatrix electrodes are flexible and do not impede a subject's range of motion. Additionally, unlike motor unit decomposition, the multiple recording channels

have large signal-to-noise ratio that allows for more direct identification of motor units through spike-sorting, a method which identifies motor units based on their waveforms across channels. Several studies have used modified versions of Kilosort, an open-source spike sorting algorithm originally designed for cortical neurons, to isolate populations of motor units across species (Chung et al., 2023; Pachitariu et al., 2016; K. Thomas et al., 2025). Used by over 100 research labs in more than nine different species, Myomatrix arrays significantly improve neuroscientists' ability to study the neuromuscular system. I contributed directly towards validating this technology, training new users, and translating user needs into design specifications.

1.3.2 Mice as a model system

Mice provide exceptional models to study the neuromuscular control of locomotion. Like humans, mice are altricial and not capable of walking immediately after birth (K. Clarke & Still, 2001; Mistretta et al., 2024). They learn through practiced movements as they gain the movement strategy, strength, and neural coordination. Adult mice successfully navigate their environments in flexible ways, capable of rapid accelerations to increase their speed (Bellardita & Kiehn, 2015). In addition, novel neuroscience tools can be used to perturb the nervous system in mice. Specific neurons within the CNS can be activated or silenced by experimenters, leading to strong conclusions about the role of the neural activity under normal conditions (Machado et al., 2015, 2020; Goñi-Erro et al., 2023; Kirk et al., 2024). Through these methods, cerebellar, brainstem, and spinal cord neurons that regulate body coordination (Machado et al., 2015; Darmohray et al., 2019; Machado et al., 2020) and speed control (Gosgnach et al., 2006; Caggiano et al., 2018; Capelli et al., 2017) have been identified. It is also possible to use

mouse models to study common neuromuscular disorders such as amyotrophic lateral sclerosis (ALS). Experimenters can monitor disease progression both through neural activity patterns and movement to better understand how the disorder might affect human health (Valetdinova et al., 2015; Hadzipasic et al., 2016; Allodi et al., 2021).

When drawing conclusions based on studies in the mouse neuromuscular system, it is important to consider that motor units in mice exhibit different features than other, particularly larger, vertebrate species. For example, average firing rates in mouse limb muscles during locomotion have been shown to exceed 100 Hz (Hadzipasic et al., 2016; K. Thomas et al., 2025). Across different limb muscles, this surpasses the firing rate range of 15-50 Hz observed in cats (Hoffer et al., 1987; Zajac & Young, 1980) and the range of 10-25 Hz in humans (Grimby, 1984). A potential explanation for this contrast is that the cell body size of motor neurons is smaller in mice than the larger species (Manuel et al., 2019). Smaller cell bodies are correlated with lower conductance, meaning that lower stimuli are needed to elicit an action potential (Kernell & Zwaagstra, 1981). Consequently, the conductance of mouse motor neurons is approximately $1/3^{\text{rd}}$ that of cats, making the cell more excitable and enabling more rapid depolarization (Huh et al., 2017; Manuel et al., 2009). Additionally, the after-hyperpolarization (AHP), which denotes the duration after an action potential before a cell can fire again, sets the maximum firing rate of motor neurons. AHPs in mice are particularly interesting because they are shorter than the muscle twitch duration, which is the amount of time that a single action potential elicits force generation in the muscle (Manuel & Heckman, 2011). This means that force generated by consecutive muscle twitches can sum and reach maximum force output before firing at a maximum rate (Manuel et al., 2009; Manuel & Heckman, 2011). This shorter AHP is also

connected to both the lower cell capacitance and shorter membrane time constant, which both influence a neuron's firing response to input stimuli, highlighting further physiological differences across the different species (Powers & Binder, 2001; Manuel et al., 2009, 2019).

The difference in physiology between mice and other species potentially supports their unique behavioral repertoire. During locomotion, mice make more strides per unit time than larger species (Heglund et al., 1974). Additionally, mice often make escape behaviors, which consist of fast, dart-like movements with sudden onset and offset (Kafkafi et al., 2003; Waider et al., 2017). Consequently, mice must be able to more rapidly contract and relax muscles during locomotion than larger species. This rapid force production could rely on the greater excitability in mouse motor neurons (Manuel & Heckman, 2011). Still, open questions remain regarding how motor neurons in mice both receive inputs and drive muscle activity, particularly during dynamic movements such as locomotion.

1.4 Dissertation overview

In this thesis, I present evidence for how motor units in mice are coordinated to achieve robust and flexible locomotor behavior. In the following literature review (Chapter 2), I explore key research on the neuromuscular control of locomotion, expand on differences between mice and other species, and establish the broader impact of my research on the field. Then, using Myomatrix arrays, which I helped create, I quantify how firing patterns in simultaneously recorded motor units change with respect to the kinematic strategy of each stride (Figure 1.2). I approach this through analysis of firing rate and recruitment probability within each motor unit (Chapter 3) and through pairwise analysis

of recruitment patterns between co-active motor units (Chapter 4). Lastly, in Chapter 5, I expand the discussion from the previous chapters to highlight broader interpretability of our conclusions. I also describe future studies that would strengthen our understanding of the neuromuscular control of locomotion.

CHAPTER 2. LITERATURE REVIEW

2.1 Movement strategies behind locomotion

Animals employ different locomotion strategies depending on their body, their environment, and the context of their movement (Dickinson et al., 2000). Locomotion is broadly defined as a body's movement across places. Limbed animals may crawl, walk, run, gallop, etc. as they traverse across the ground (Grillner & El Manira, 2020). Each case consists of rhythmic synchronization across the limbs and body along with stereotyped features on a stride-by-stride basis. Furthermore, each of these movement strategies is shaped by the biomechanical and environmental context (Sober et al., 2018).

Characterizing locomotor patterns in limbed species consists of kinematic and kinetic measurements to identify body movement and the forces behind it respectively. Spatiotemporal parameters of each stride, including joint angles are generally stereotyped within subjects on a stride-by-stride basis (Borghese et al., 1996; Kadaba et al., 1990; Lafortune et al., 1992). Strides are often split into phases by identifying which of the limbs are either on the ground in stance, or in the air in swing (Figure 1.1) (Al-Shuka et al., 2019). During walking in both mice and humans, each foot is in contact with the ground for approximately 60% of the stride (stance phase duration) and each stride involves a period of overlapping stance phases across limbs (double support time) (Borghese et al., 1996; Bellardita & Kiehn, 2015).

Increasing speed, either through faster walking or running, requires a change in the spatiotemporal strategy used during a step. Typically, the stance phase duration and double support time decrease while the length of each stride and leg joint moments (forces that

rotate the joint) increase (Fukuchi et al., 2019; Kirtley et al., 1985). Further increases in speed may require a change in coordinated limb strategy, or gait, which consists of a dramatic alteration in the support patterns and load bearing across limbs (Alexander, 2006; Borghese et al., 1996). During running in bipedal species, feet are not on the ground at the same time and instead spend a portion of the stride in air simultaneously.

Additional limbs allow for more complex patterns across ipsilateral (homolateral), opposite, and diagonal limb pairs, but still result in rhythmic kinematic strategies (Figure 2.1) (Gonçalves et al., 2022). Quadrupeds also have conserved locomotor patterns within species along with additional strategies from the interactions across limbs (Manter, 1938). Key strategies for quadrupeds include walking, trotting, and galloping. Walking consists of sequential and orderly swings of each limb with three or four limbs in stance at a time (Bellardita & Kiehn, 2015). During trotting, diagonal paws move together, landing and lifting in phase with each other and out of phase with the two other limbs. Galloping, like running in humans, includes a portion of the stride with no limbs on the ground and is typically a faster body movement. Hexapods such as insects also control their six limbs rhythmically with similar limb patterns across speeds (Figure 2.1) (Gonçalves et al., 2022).

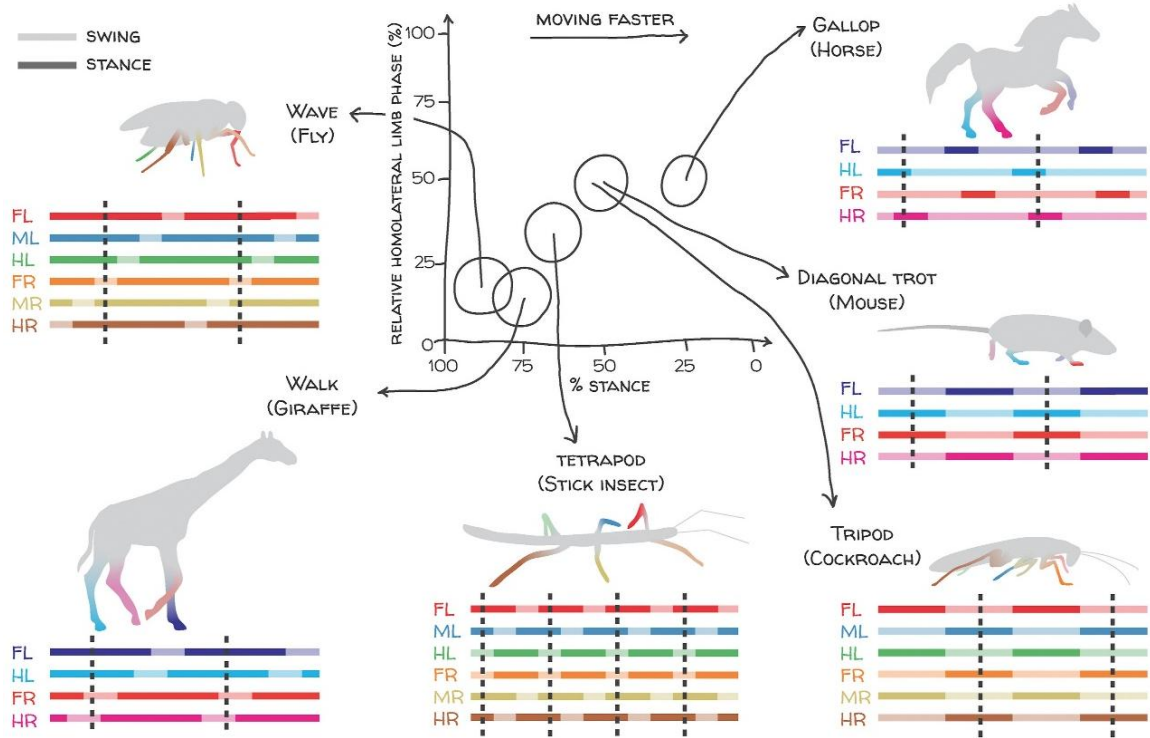


Figure 2.1. Characteristic gait strategies across example species. Each gait can be described by coordinated stance and swing timings across limbs. Adapted from Gonçalves et al. 2022.

Studies across quadrupeds, including mice (Bellardita & Kiehn, 2015; Mendes et al., 2015), cats (Miller et al., 1975a, 1975b; Harnie et al., 2018), and horses (Hoyt & Taylor, 1981; Robilliard et al., 2007) have demonstrated that there is a general preference for different locomotor strategies at different speeds. Transitioning between gaits relates to optimization of the energy consumption needed at faster speeds (Hoyt & Taylor, 1981). Mice, however, may transition between gait strategies less discretely than other quadrupeds (Machado et al., 2015; Gonçalves et al., 2022). They naturally trot over a wide range of both slow and fast speeds instead of solely relying on alternative gait strategies, resulting in more continuous transitions, even on a stride-by-stride basis, between trotting and other strategies (Gonçalves et al., 2022). Speed is naturally constrained by limb length and body

size; although mice can be much slower than the larger quadrupeds, they move with greater stride frequency, taking more strides per unit time (Heglund et al., 1974). Generally, stride frequency scales relative to the body mass and again relates to the energy expenditure (Heglund & Taylor, 1988).

Successful locomotion depends on the ability to accurately interpret different environmental conditions, such as slope or surface, and apply the correct movement strategy towards a given context. Similar to walking on a flat surface, walking up or down a slope consists of stereotyped strides; however, joint angles through the stride differ based on the slope (Lay et al., 2006; Leroux et al., 2002; McIntosh et al., 2006). For example, in humans, the ankle is dorsiflexed (toes pointed up) during the stance phase only on large upward slopes and the ankle moment is also larger in this condition compared to level walking. But, the ankle becomes plantarflexed (toes pointed down) during the swing phase similar to level walking because of further changes in the knee and hip angles (Lay et al., 2006). These stereotyped differences in the relative joint angles and torques could be explained by slope-dependent neural control strategies (Lay et al., 2006; Livingston & Nichols, 2014). Additionally, locomotion on a motorized treadmill as opposed to overground requires different strategies. In humans, stride length changes when running (Riley et al., 2008; Wank et al., 1998) but not necessarily when walking (Alton et al., 1998). For treadmill locomotion in mice, stride length is lower with a greater stride frequency across nearly all speeds (Herbin et al., 2007). Ultimately, each locomotor strategy, across speeds and contexts, relies on coordinated muscle activity to generate the appropriate movement.

2.2 Muscles are coordinated to control movement of the body

Movements result from forces generated by skeletal muscles. These muscles, each with unique physiology and attachments to bone, contract to move the skeletal system. For behaviors as complex as locomotion, muscles across the body must work together, rhythmically controlling the body and limbs. Successful locomotion relies on appropriately patterned sets of activity across muscles given the environmental context and biophysical constraints of the body (Crowninshield & Brand, 1981; Ting & Chiel, 2017).

2.2.1 *Biomechanical and physiological properties of muscles produce forces on joints*

Individual muscles are composed of sets of muscle fibers with heterogeneous physiologies that can be studied through their electrical activity during behavior. Each set of myofibers, or simply fibers, is typically innervated by a single motor neuron in the spinal cord, which are collectively termed the motor unit. The release of acetylcholine at the neuromuscular junction, where the neuron synapses on the fiber, drives an action potential down the length of the fiber. The resulting depolarization propagates across the fiber, releasing internal stores of Ca^{2+} in the myofibril, which in turn initiates sliding of thick and thin filaments within each sarcomere (Figure 2.2) (Ojima, 2019). Assuming adequate concentrations of Ca^{2+} and ATP (adenosine triphosphate, an energy-providing molecule), this sliding will shorten the length of each sarcomere arranged in series along the muscle, serving to contract the muscle and elicit a muscle twitch (Stein, 1980). Fiber contraction properties, such as the time to peak force, depend on Ca^{2+} sensitivity in the filaments and the timescale of Ca^{2+} reuptake (Berchtold et al., 2000). Ultimately, each action potential in the motor neuron reliably leads to depolarization in the muscle fiber and resulting muscle

twitch (Heckman & Enoka, 2012). Summed twitches, both within that fiber and across the muscle, ultimately produce enough force to move the joints and result in movement. Thus, although molecular mechanisms drive force generation, muscle activation, as a reflection of the number of depolarized and contracting fibers, can be more coarsely measured as the total electrical activity in the muscle, recorded through EMG.

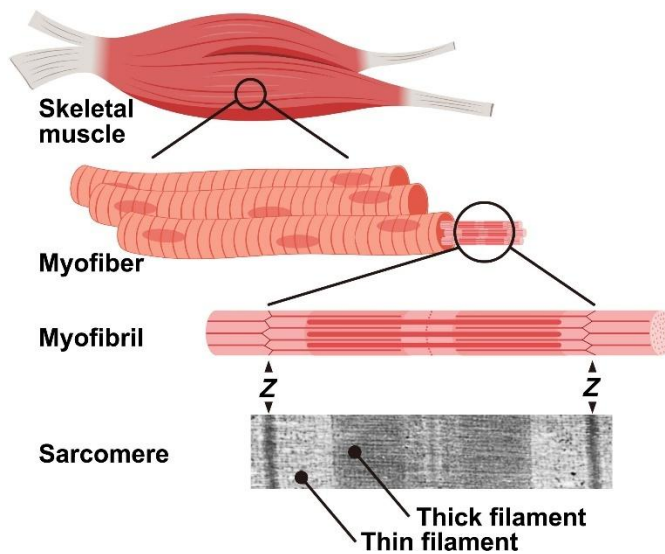


Figure 2.2. Overview of skeletal muscle. Adapted from Ojima 2019.

Activation of different fibers can lead to different force production within a given muscle based on the physiological properties of the fiber. Typically, muscle fibers are grouped into at least three classes with distinct functional outputs and anatomy (Burke et al., 1973; Schiaffino & Reggiani, 2011). Slow twitch fibers produce low forces but with low fatigue, making them useful for posture and slow movement. Fast fatigue resistant fibers produce larger forces but with higher fatigability. Lastly, fast fatigable fibers generate the largest forces but with greater metabolic cost, which limits how long they can remain active. These fiber types can be classified within muscle by profiling myosin, a

protein within the thick filament of the sarcomere (Pette & Staron, 2000; Stein, 1980). Several types of myosin heavy chain (MHC) isoforms exist, and histochemical analysis of their compositions within muscle fibers supported early classification of fiber types (Schiaffino & Reggiani, 2011). Additionally, quantification of metabolic enzymes within each fiber can reveal further distinction of fiber types (Essén et al., 1975; Pette, 1985). Fiber type composition is typically consistent within a single fiber, but hybrid compositions do exist across muscles and species (Augusto et al., 2004; Pette & Staron, 2000). Although muscles fibers exhibit heterogeneous traits, the general agreement between genetic identity and histological measurements supports some level of discrete classification (Schiaffino & Reggiani, 2011).

Generally, each motor neuron reliably innervates a unique set of fibers with consistent physiological and histochemical properties (Burke et al., 1973). Furthermore, properties of the motor neuron correlate with the classification of the muscle fibers it innervates. For example, slow twitch fibers, with their lower force production, are innervated by neurons with smaller cell bodies and slower conduction velocities (Ulfhake & Kellerth, 1982; P. Bawa et al., 1984; Azevedo et al., 2020). Ultimately, the distinction behind slow and fast muscle fibers is also reflected in slow and fast motor units.

The relative composition of fiber types varies across muscles, supporting unique biomechanical roles. For example, the soleus (SOL), which is an ankle plantar flexor, is composed of primarily slow fibers (>70%) (Crow & Kushmerick, 1982; Gollnick et al., 1974), suggesting its role is largely in postural control (Spector et al., 1980). Alternatively, the medial gastrocnemius (MG), which both plantar flexes the ankle and flexes the knee, is composed of a more even distribution of slow and fast fibers (Burkholder et al., 1994;

Edgerton et al., 1975). Consequently, the MG, which is barely active during quiet standing, becomes more active during locomotion as muscle activity and force production scale with the movement speed (Walmsley et al., 1978). The fast twitch fibers in both these and other muscles become most active at fastest speeds (Chanaud et al., 1991).

Muscle attachments to bone further shape the biomechanical role of each muscle (Gans, 1982; Gans & Gaunt, 1991). For example, the SOL and MG share an insertion point on the ankle, contributing to their shared role in ankle plantar flexion (Fukunaga et al., 1996). However, they diverge in their attachments further up the leg as the SOL attaches to the fibula and tibia below the knee while the MG attaches to the femur above the knee (Chow et al., 2000). Crossing two joints, the MG is an example of a biarticular muscle, which provides joint moments across both joints when contracted (Gans, 1982). Monoarticular muscles such as the SOL only act on a single joint. Given all the muscles in the body, their unique fiber-scale physiologies, and their unique biomechanical functions, there are incredibly high degrees of freedom towards how the body generates movement (Latash, 2012).

2.2.2 Coordinated muscle activity produces locomotion

Muscle activity must be properly coordinated across the body to produce locomotion and respond to changing contexts. The diversity and redundancy in muscles across the body provide multiple solutions to produce the same movements (Zajac, 1993; Latash, 2012; G. Loeb, 2021; Ting et al., 2015). Thus, it is striking that electrical activity in the muscle, which generates force-producing contractions, has a very stereotyped pattern as it reflects the muscle's engagement at specific phases of each step (Figure 1.1) (Al-

Shuka et al., 2019). Coordinated activity reflects the neural input to muscles across the body, integrating sensory feedback to produce rhythmic movement. Thus, investigation of muscle patterning through EMG can reveal how neural circuits control movement.

As the context of movement changes, muscles must remain coordinated, although their specific activations may change to meet new constraints. Locomotion at faster speeds requires more rapid changes in the forces that need to be exerted across the body, generally requiring greater muscle activity. Importantly, the relative timing and magnitude of activity for each muscle does not necessarily scale uniformly or evenly with other muscles (Figure 2.3) (Nilsson et al., 1985; Van Hedel et al., 2006; Franz & Kram, 2012; Canu & Falempin, 1998; Alessandro et al., 2020). For example, the tibialis anterior during human walking has lower correlation to itself across walking speeds compared to that of the gastrocnemius (Nilsson et al., 1985; Van Hedel et al., 2006). Gait changes, consisting of altered limb support patterns in addition to speed differences, involve further changes in muscle activity. Still, EMG patterns remain stereotyped on a stride-by-stride basis within individuals (Guidetti et al., 1996; Winter & Yack, 1987).

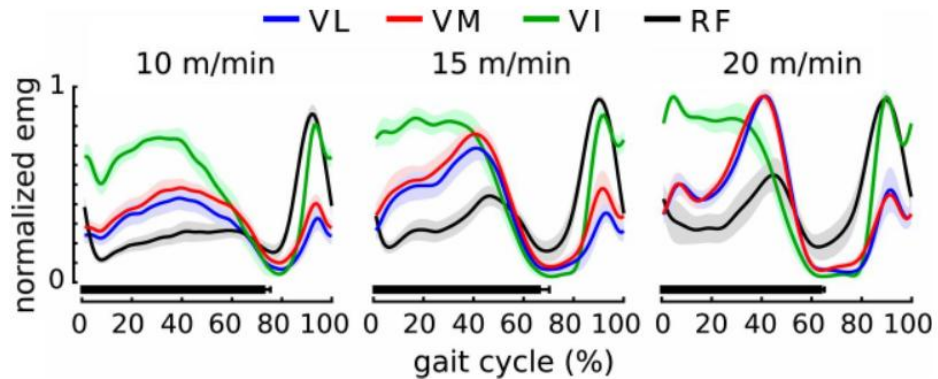


Figure 2.3. Stride-averaged EMG from rat quadriceps muscles [vastus lateralis (VL), vastus medialis (VM), vastus intermedius (VI), rectus femoris (RF)] across walking speeds. Muscle activation, presented as mean \pm SE across strides and animals, has been rectified, filtered, and normalized by its maximum. Black bars denote the average stance phase of each gait cycle. Adapted from Alessandro et al. 2020.

Stereotyped muscle activation patterns can inform about how the nervous system reliably drives movement despite the high degrees of freedom in biomechanical redundancy. Common activation patterns across functionally related muscles, called synergies, may allow for the nervous system to simplify control of the neuromuscular system (D’Avella et al., 2003; Ting et al., 2015). Rather than independently controlling each individual muscle to produce coordinated muscle activity, the nervous system may instead recruit a set of synergies with appropriate timing and weighting. Synergies can be quantified through dimensionality reduction analyses on EMG data from multiple muscles within and across tasks (Steele et al., 2013; Tresch et al., 2006). Typically, most of the variability in the original EMG patterns can be reconstructed from just a few synergies, demonstrating a potential reduction in degrees of freedom from independent muscle control (D’Avella et al., 2003; Ivanenko et al., 2004). Although not a direct measurement of neural inputs, these computationally identified synergies may reflect simplified patterns used by the nervous system.

Rhythmic muscle activity across the body during locomotion further implicates synergies as a potential mechanism for simpler task control. Indeed, locomotor studies in both humans (Chvatal & Ting, 2012; Ivanenko et al., 2004) and mice (Santuz et al., 2019, 2022) have identified synergies in limb muscles that are recruited at specific phases of the stride cycle and robust to changes in context. In human walking, the same synergistic factors can be observed across both walking speeds and subjects (Ivanenko et al., 2004). Locomotion also requires alterations in kinematic strategy and muscle activity on a stride-by-stride basis (obstacle avoidance, stumble correction, etc.) and effective neural control must account for changes at this timescale. To identify how muscles remain coordinated through these conditions, several studies have disrupted normal walking rhythms by introducing perturbations to the limb. For example, shifting the leg position using a moveable platform causes changes in the duration and length of a step (Chvatal & Ting, 2012; You et al., 2001). Despite this, synergies extracted from leg muscles in unperturbed walking can explain the variability introduced by the perturbations (Chvatal & Ting, 2012). Further evidence suggests that synergies in lower limb muscles are present in newborns and become more precise over time as behaviors develop (Hinneken et al., 2023), again suggesting that the underlying circuits driving muscle activity may indeed rely on a simpler control scheme.

Still, caveats exist with this evaluation of synergies that limits the ability to draw conclusions about the neuromuscular system. It is difficult to connect computationally defined synergies with the neural circuits that generate the synergies (Kutch & Valero-Cuevas, 2012; Tresch & Jarc, 2009). Measured synergies may more simply reflect common muscle activation patterns or biomechanical constraints within a given task. Additionally,

experimental selection of muscles, choice of modeling approach, and assumptions on noise within the system influence how well synergies describe muscle activation patterns (Kutch & Valero-Cuevas, 2012; Tresch & Jarc, 2009). For example, past studies have suggested that since trial-by-trial variability in muscle activation patterns does not necessarily affect task success, it may be considered as a feature of neural control rather than noise within it (Kutch et al., 2008; Valero-Cuevas et al., 2009). In this case, synergies do not fully explain the variability in muscle patterning, suggesting that the nervous system is capable of more independent motor control.

2.3 CNS control of locomotion

Locomotor patterns in muscle activity result from CNS circuits that shape the excitability and firing patterns in motor neurons. Successful locomotion demands flexibility across contexts and speeds, and different regions across the nervous system are responsible for executing these changes (Kiehn, 2016; Arber & Costa, 2018). Rhythmic activity within the spinal cord and descending signals from the brain converge on motor pools across the body, where each motor pool consists of the motor neurons that innervate a single muscle (Burke, 1981). The intrinsic physiology of the motor neuron, which affects its excitability, shapes how it responds to these various inputs. Thus, investigation of motor unit coordination to understand the flexibility and robustness behind locomotion relies on deciphering the role of broader regions across the CNS.

2.3.1 Central pattern generators (CPGs) produce rhythmic activity through spinal cord circuits

Several studies have identified that the spinal cord can produce and sustain rhythmic activity without the maintained presence of descending activity (Brown, 1911;

Grillner, 1975; Grillner & El Manira, 2020; Whelan, 1996). The central pattern generator (CPG) describes the spinal cord circuit of interneuron populations capable of generating and shaping motor output without input from the brain (Guertin, 2013; MacKay-Lyons, 2002). This activity results in alternating activation of flexor and extensor motor pools as interneurons drive activity in motor neurons (Burke et al., 2001). However, it is important to note that these studies are typically performed in animal models under ‘fictive locomotion’ in which rhythmic neural activity is inferred as locomotion in the absence of actual movement (Minassian et al., 2017) or in decerebrate preparations in the absence of descending control from the full motor circuit (Bonnot et al., 2002). Thus, several questions have persisted regarding how CPGs operate in complete circuits, which naturally include muscle activation and sensorimotor integration.

Recent advances in neurobiology use molecular genetics to classify and manipulate spinal cord interneurons based on their genetic heterogeneity, establishing a method to investigate CPG neural circuits. Importantly, the spinal cord is structurally and genetically organized, and pre-motor interneurons shape motor neuron activity through excitatory or inhibitory inputs. These interneurons begin as progenitor cells during neural development and gain their circuit identities through the progenitor cells’ expression of specific transcription factors (Goulding, 2009; Jessell, 2000; Kiehn & Butt, 2003; Lewis, 2006). Thus, transcription factors are strongly linked to the resulting anatomical and physiological characteristics of interneurons along with their spatial organization within the spinal cord. Transgenic animal models can be manipulated such that neurons expressing specific transcription factors can be recorded, ablated, activated, or silenced by the experimenter (Luo et al., 2008; A. C. Wilson & Sweeney, 2023). This has allowed for precise

investigation on the contribution of specific cell types to rhythmic motor patterns (Figure 2.4) (Rybak et al., 2015).

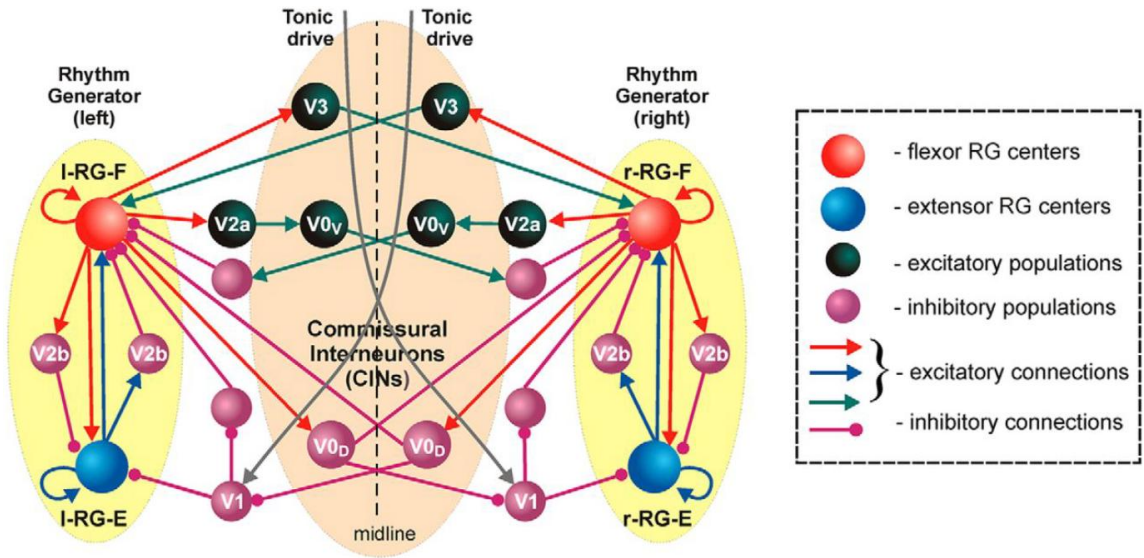


Figure 2.4. Rhythmic generator (RG) circuit with hypothesized connections for V1 and V2 interneurons in producing rhythmic flexor and extensor activity. Adapted from Rybak et al. 2015.

Multiple subclasses of interneurons within the spinal cord have been identified as part of the CPG that play a crucial role in coordinating locomotion and regulating speed. V1 interneurons, identified by their transcription factor Engrailed-1 and their ipsilateral, inhibitory projections on motor neurons, have been shown to be essential for fast locomotor patterning (Brownstone & Bui, 2010; Picton et al., 2025). V1s play a role in alternating flexor-extensor activity in limbed locomotion (Zhang et al., 2014; Britz et al., 2015). In isolated spinal cord preparations, it is possible to combine V1 manipulation with electrophysiological recording from the ventral root, which carries efferent activity from the motor pool as a proxy for muscle activity (Kiehn & Butt, 2003). After inducing locomotor-like activity using excitatory neurotransmitters, several studies have identified

that the absence of V1s correlates with slower rhythmic activity and longer motor neuron bursting within each fictive cycle in both mice (Gosgnach et al., 2006; Falgairolle & O'Donovan, 2019) and zebrafish (Kimura & Higashijima, 2019).

V2a excitatory interneurons, characterized by the transcription factor CHX10, also play a role in locomotor coordination and speed control. In mice, a greater percentage of the total V2a population are recruited at faster speeds (Zhong et al., 2010, 2011). This is potentially important for maintaining left-right limb alternation as mice without V2a interneurons had abnormal gallop-like fictive gaits instead of the typical trot (Crone et al., 2009). Furthermore, in zebrafish, V2a interneurons recruited at faster speeds have stronger connections to the fast motor neuron population that recruit fast-twitch fibers for larger force production (Ampatzis et al., 2014; Song et al., 2018).

2.3.2 Neuromodulation and persistent inward currents shape motor neuron excitability

Neuromodulation affects the excitability of motor neurons and resulting motor output (Jordan et al., 2008; Heckman et al., 2009; Fenstermacher et al., 2024). Motor neurons have both ionotropic and neuromodulatory receptors (Heckman & Enoka, 2012). Ionotropic neurotransmitters, such as excitatory glutamatergic and inhibitory GABAergic inputs, mainly generate post-synaptic potentials by depolarizing or hyperpolarizing the motor neuron. In contrast, neuromodulatory inputs, such as serotonin (5-HT) and norepinephrine (NE), modulates the motor neuron's response to ionotropic inputs (Heckman & Enoka, 2012). These neuromodulatory inputs arise from descending systems related to arousal and the behavioral state of the animal (Alvarez et al., 1998; Heckman et al., 2009; Jacobs & Fornal, 1997). Binding of neuromodulators to receptors on motor

neurons leads to a series of intracellular signals that influence neuron excitability through various measures, including lower thresholds in voltage-gated ion channels and decreased post-spike after-hyperpolarization (see ‘Mouse as a model system’) (Berger et al., 1992; Heckman et al., 2009; Parkis et al., 1995; Rekling et al., 2000).

Neuromodulation is important for setting motor neuron firing patterns during locomotion. Serotonergic neurons in the brainstem that release 5-HT onto motor pools are spontaneously active during walking with activation that scales with the speed of the animal (Jacobs et al., 2002; Rekling et al., 2000; Veasey et al., 1995). Furthermore, optogenetic activation of these neurons in mice, which uses experimentally controlled light to drive depolarization in specific cells, increased movement speed and locomotor bout durations (Fenstermacher et al., 2024). In isolated spinal cord preparations, addition of 5-HT reliably induces locomotion rhythms in motor neurons, and the concentration again correlates with the burst durations (Cazalets et al., 1992; Jordan et al., 2008). Fictive locomotion studies, used to dissect circuit components in fixed conditions, generally rely on neuromodulation to simulate physiological conditions and accurately induce and interpret rhythmic activity (Minassian et al., 2017).

Persistent inward currents (PICs) in motor neurons provide depolarizing currents that are important for generating and sustaining motor neuron firing (Heckman et al., 2005; Huh et al., 2017). Current measurement at the soma has revealed a nonlinear scaling of synaptic input when the cell is artificially held at different voltages (Lee & Heckman, 2000). PICs are responsible for this additional input, amplifying the synaptic current several-fold through voltage-dependent channels in the motor neuron dendrite (Heckman, Johnson, et al., 2008; Lee & Heckman, 2000). PICs largely arise from specific Na^+ and

Ca²⁺ channels located in the dendritic tree that produce long-lasting depolarization (Heckman, Johnson, et al., 2008). Furthermore, the presence of neuromodulators further increases the effect of PICs (Heckman et al., 2005, 2009; Binder et al., 2020; Lee & Heckman, 2000). Importantly, PICs inactivate slowly, generating additional current even after the synaptic input has ended, providing an extended mechanism of excitatory drive (Heckman et al., 2005; Heckman, Johnson, et al., 2008; Lee & Heckman, 1998a).

PICs dramatically influence firing patterns in motor neurons. Prolonged activation of PICs following brief excitatory synaptic input can lead to self-sustained firing in motor neurons, in which the neuron continues firing until inhibited through additional input (Hounsgaard et al., 1988). Similar to having an ‘on’ and ‘off’ switch, this suggests that motor neuron activity may be bistable, in which an excitatory input turns the neuron ‘on’, leading to sustained activity until an inhibitory input turns the neuron ‘off’ (Hounsgaard et al., 1988). This framework potentially simplifies the neural control of these neurons particularly for tonic activity required during posture. Supporting this theory are findings that motor neurons with the longest durations of self-sustained firing tend to have slower conduction velocities, implicating them as slow motor units (Lee & Heckman, 1998b). Still, it is unclear how PICs shape firing patterns during rhythmic behaviors such as locomotion, which seem to rely more on rapid activation and inactivation rather than a multi-second timescale of self-sustained firing.

2.3.3 Supraspinal control of locomotion

The brainstem encompasses several internal regions which interface between the cerebellum, each other, and the spinal cord to influence locomotor speeds and gaits. Recent

advances in the mouse have identified spatially-segregated, cell-type specific circuits that start, modulate, and stop locomotion within the mesencephalic locomotor region (MLR), which contains the cuneiform nucleus (CnF) and pedunculopontine nucleus (PPN) (Gatto & Goulding, 2018; Leiras et al., 2022). Activation of excitatory, glutamatergic neurons within the CnF initiate locomotion. Furthermore, higher frequency stimuli increase the speed of locomotion and can induce walk-, trot-, gallop-, and bound-like gaits (Caggiano et al., 2018). Similarly, activation of excitatory, glutamatergic neurons within the PPN can initiate locomotion, but only of the slower gaits (Caggiano et al., 2018). Inactivation of either of these neuronal populations also reduces the overall movement speed of the animal (Caggiano et al., 2018). The medullary reticular formation (MRF) receives inputs from the MLR and translates them to the spinal cord (Bretzner & Brownstone, 2013; Leiras et al., 2022). Several subregions within the MRF have been identified and similarly to the MLR, manipulations to neural activity have been shown to start and stop locomotion (Bouvier et al., 2015; Capelli et al., 2017) as well as initiate turning behaviors (Cregg et al., 2020; Usseglio et al., 2020), which are important for successfully navigating across contexts.

Given the spinal cord outputs, the above circuits in the MLR and MRF may affect locomotion through activation of CPG rhythmic and pattern generating circuits. However, few studies have identified specific connections in neurons across the regions to support this theory (Leiras et al., 2022). Modeling approaches address this gap through detailed circuits that predict locomotor outputs based on hypothesized functional connections (Danner et al., 2017; Ausborn et al., 2019; Leiras et al., 2022). One compelling theory suggests that brainstem neurons control locomotor speed and gait through separate pathways. In this model, descending, excitatory drive from the MLR sets the rhythm of

limb flexor/extensor pairs (Ausborn et al., 2019). Additionally, interneuron subclasses with commissural (left-right interactions) in the spinal cord receive this information and translate it across the body to set appropriate coordination in limb pairs. This supports the correlation between speed and gait observed kinematically (Bellardita & Kiehn, 2015). Other modeling approaches continue to incorporate regions across the nervous system to better understand the role of specific regions and neurons on locomotor behavior.

Cerebellar circuits calibrate motor outputs for coordinated movement across the body, playing a pivotal role in both normal gait and locomotor adaptation (Morton & Bastian, 2004; Pisotta & Molinari, 2014; Carey, 2024). Damage to the cerebellum in humans, often due to cerebellar stroke or tumor resection, results in ataxic movement. The severity of the ataxia can be clinically measured and correlated to both the location and severity of injury (Earhart & Bastian, 2001; Ilg et al., 2008; Martino et al., 2014). Common features of both bipedal and quadrupedal ataxia are reduced walking speed, shorter stride lengths, and increased stride-to-stride variability in kinematic features. Additionally, humans with cerebellar damage have difficulty producing consistent relationships across joints despite a similar range of motion to healthy controls (Earhart & Bastian, 2001). Instead of the smooth, multi-joint movement characteristic of locomotion, it appears that movement is decomposed into a series of piecewise, single-joint movements (Morton & Bastian, 2007). This implicates the cerebellum in coordinating limb movement, explaining how deficits lead to ataxia.

Investigation in animal models has allowed for more precise recording and manipulation of the cerebellum, identifying the local circuits related to locomotor control. Purkinje cells serve as the sole output of the cerebellar cortex (Herndon, 1963). Purkinje

cells are rhythmically active during locomotion, tuned not only to movement speed (Armstrong & Edgley, 1988), but also broader kinematic features of the movement (Sauerbrei et al., 2015). Induced activity in Purkinje cells during locomotion can slow or stop locomotion (Hoogland et al., 2015), and mice with post-natal degeneration of Purkinje cells exhibit desynchronized movement across front-hind limb pairs (Machado et al., 2015, 2020; Vinueza Veloz et al., 2015). Furthermore, wide oscillations in the movement of the nose and tail highlight the inability of the incomplete neural circuit to predict and compensate for the irregular limb movement, further implicating the cerebellum in whole-body coordination (Machado et al., 2015).

The cerebellum also plays a key role towards locomotor adaptation, which describes the stride-by-stride transition in the learned walking rhythm towards a new spatiotemporal strategy in response to environmental perturbation (Morton & Bastian, 2006). This differs from motor learning, in which a new movement skill is learned *de novo*, for example, walking for the first time (Bastian, 2008). During adaptation, animals acquire a new strategy quickly, often in a few minutes; the strategy then gradually returns to baseline once the perturbation is removed over a period of wash-out. A common method for inducing this adaptation is with a split-belt treadmill. Driving one side of the body at a faster speed produces asymmetries across limbs that become more symmetric over time. Reports in humans (Hoogkamer et al., 2015; Morton & Bastian, 2006), cats (Yanagihara & Kondo, 1996), and mice (Darmohray et al., 2019) demonstrate that this process is cerebellar dependent. Healthy, control mice undergoing split-belt walking adapt both spatial and temporal interlimb coordination across the forelimbs (Darmohray et al., 2019). Mice with Purkinje cell degeneration maintain locomotion on the split-belt treadmill by

adjusting step length and timing in individual limbs, but these asymmetries do not diminish with time, demonstrating no adaptation (Darmohray et al., 2019).

2.4 Motor units provide a high-resolution approach to study motor control

The stereotyped patterns in both locomotor behavior and muscle activity suggest an underlying control scheme that translates movement intent to the muscle fibers that execute the movement. Meanwhile, neural populations in the brain and spinal cord demonstrate various mechanisms and pathways for generating this behavior. There remains a disconnect in how the high degrees of freedom from the nervous system produce smooth, reliable movement. To approach this topic, analysis of motor unit firing patterns is necessary. As the link between the nervous system and muscles, these firing patterns reveal how force develops in the muscle and how muscles may be coordinated across the body. Although difficult to measure during behavior, preliminary investigations into these patterns have revealed much about how the nervous system shapes motor outputs.

2.4.1 Size principle coordination of motor units

The size principle describes a strategy for coordinating motor unit outputs to produce necessary forces (Henneman, 1957; Henneman et al., 1965). Within this core framework, each motor pool, which consists of all motor neurons innervating a muscle, receives a simultaneous common input (De Luca, 1985; Deluca & Erim, 1994; Farina et al., 2014). As that input increases, more units are recruited in an order generally determined by their force output, conduction velocity, and excitability, all of which reflect the cell body size (Burke, 1981; Zajac & Faden, 1985; T. Cope & Pinter, 1995). Cell body size is also strongly linked to motor unit types with small diameter neurons typically being slow

motor units and large diameter neurons being fast, fatigable motor units (McPhedran et al., 1965; Ulfhake & Kellerth, 1982; Wuerker et al., 1965). Thus, the size principle suggests that slow units, which generate less force, should be recruited first. Additionally, motor units that generate more force would only be recruited as the common input increases, potentially optimizing force output while limiting fatigue. Since this model of coordination only requires a single input, it offers a compelling strategy towards how potentially thousands of motor units can be organized efficiently within the nervous system (Farina et al., 2014; Farina & Negro, 2015). Given the anatomical and functional connections, the size principle offers a compelling hypothesis towards how motor units are coordinated.

Decades of research have sought to investigate whether motor unit recruitment order can change systematically along with the implications that this would have for movement. For example, empirical evidence not compatible with the strictest version of the size principle could suggest a more complex capacity of motor control, potentially supporting altered coordination for finely tuned movements (Basmajian, 1963; Marshall et al., 2022). Alternatively, it could more simply reflect an unknown dimension of stochasticity in the system, suggesting that although cell-body size might not be the pivotal factor in determining recruitment order, the basis of the size principle still exists (T. Cope & Pinter, 1995).

Reversals in the recruitment order predicted by the size principle, in which fast motor units are recruited before slow motor units, have been observed across a variety of conditions, including isometric force direction (Desnedt & Gidiaux, 1981; C. K. Thomas et al., 1987; Herrmann & Flanders, 1998), isometric force dynamics (Marshall et al., 2022), and feedback-guided volition (Basmajian, 1963; Harrison & Mortensen, 1962). For

example, motor units in the non-human primate forearm were recruited in reverse order during fast-frequency force productions compared to slow, linear force ramps under isometric conditions (Marshall et al., 2022). However, it is important to note that changing a task condition does not guarantee a reversal to recruitment order (Jones et al., 1994; Feiereisen et al., 1997; P. N. S. Bawa et al., 2014). For example, motor units in the human first dorsal interosseus (index finger abductor) had the same recruitment order across three functional tasks (Jones et al., 1994). Thus, it has remained unclear whether reversals are intentional patterns set by the nervous system for motor control.

Different explanations have been offered to explain reversals based on both anatomical and functional considerations. Motor units within a motor pool receive distinct and non-uniformly shared inputs that influence their activity (Eccles et al., 1961; Clamann et al., 1983; Azevedo et al., 2020; Menelaou et al., 2022). For example, Renshaw cells, which recurrently inhibit the motor pool from motor neuron collaterals, evoke stronger inhibitory post-synaptic potentials in slow motor neurons, suggesting denser connections on slow motor neurons (Eccles et al., 1961; Friedman et al., 1981). Thus, the large inputs that lead to sharp, rapid contractions of the muscle could effectively reduce slow motor unit activity, which may appear as a violation of the size principle (Broman et al., 1985; De Luca, 1985). Similarly, Ia afferent sensory inputs, which innervate motor neurons in response to stretch in the agonist/antagonist muscle, do not uniformly innervate the motor pool (Clamann et al., 1983). Direct stimulation of the sensory nerve (Clamann et al., 1983), muscle stretch (Sokoloff & Cope, 1996), or postural changes (Ter Haar Romeny et al., 1982) can lead to altered recruitment patterns, although it is unclear how this mechanism may affect recruitment order during natural behavior.

Additionally, it is oversimplifying to assume a single input always shapes the activity in the entire motor pool. Instead, task groups, which consist of a subset of motor units with related functions, may provide finer control behind specific, goal-directed behaviors (G. E. Loeb, 1985; Riek & Bawa, 1992; T. C. Cope & Sokoloff, 1999; P. N. S. Bawa et al., 2014). Thus, motor units engaged in different movements may violate recruitment order at the level of the motor pool but not at the scale of the task group. For example, motor units in biarticular muscles can be organized compartmentally across the muscle with unique roles and selective activation for each region (G. E. Loeb, 1985; Chanaud et al., 1991). Similarly, each motor unit may be selectively tuned towards different tasks based on their force production. Motor units may fire with greater recruitment or rate when producing force in different directions, potentially leading to altered recruitment patterns based on the context (Herrmann & Flanders, 1998; Marshall et al., 2022).

Complex behaviors such as locomotion may similarly rely on complex patterns across motor units. Flexible recruitment orders may be advantageous towards adjusting speed and gait patterns, which rely on kinematic and kinetic changes across the whole body. However, few studies have measured motor unit firing patterns under these conditions. Therefore, although much is known about the underlying coordination of motor units, there remains much to learn.

CHAPTER 3. MOTOR UNIT MECHANISMS OF SPEED CONTROL IN MOUSE LOCOMOTION

The following chapter has been adapted from previously published work (K. Thomas et al., 2025):

Thomas, K., Gibbs, R., Marques, H., Carey, M. R., & Sober, S. J. (2025). *Motor unit mechanisms of speed control in mouse locomotion*. <https://doi.org/10.7554/eLife.105829.1>

3.1 Abstract

During locomotion, the coordinated activity of dozens of muscles shapes the kinematic features of each stride, including systematic changes in limb movement across walking speed. Motor units, each of which consists of a single motor neuron and the muscle fibers it innervates, contribute to the total activation of each muscle through their recruitment and firing rate when active. However, it remains unknown how the nervous system controls locomotor speed by changing the firing of individual motor units. To address this, we combined quantitative behavioral analysis of mouse locomotion with single motor unit recordings from the lateral and long heads of the triceps brachii, which drive monoarticular extension of the elbow and biarticular movements of the elbow and shoulder, respectively. In contrast to prior studies employing bulk EMG to examine muscle activity, our recordings revealed the diversity of spike patterning across motor units as well as systematic differences in motor unit activity across muscles and locomotor speeds. First, motor unit activity differed significantly across the lateral and long heads, suggesting differential control of these two closely apposed elbow extensor muscles. Second, we found that individual units were recruited probabilistically during only a subset of strides, showing that bulk EMG signals consistently present in every

stride in fact reflect stochastically varying subsets of individual motor units. Finally, although recruitment probability and firing rate both increased at faster walking speeds, increases in recruitment were proportionally larger than rate changes, and recruitment of individual units accompanied changes in limb kinematics. Together, these results reveal how the firing of individual motor units varies systematically across muscles and walking speeds to produce flexible locomotor behavior.

3.2 Introduction

Skilled behavior depends on the nervous system's precise control of muscle activity. Motor units, which consist of a single motor neuron and all of the muscle fibers it innervates, generate the forces behind movement through their firing patterns. In locomotion, proper neural coordination of motor units within and across muscles allows for the stereotyped yet rapidly adjustable movement used for each step (Akay et al., 2014; Mayer & Akay, 2018; N. P. Schumann et al., 2006). In principle, the total force output of a muscle is modulated by the number of recruited motor units and the firing rate of active units (Enoka & Duchateau, 2017; Heckman & Enoka, 2012), with each newly-recruited unit increasing total muscle force by activating more muscle fibers. The firing rate and inter-spike-interval (ISI) pattern of recruited units then shape force production in concert with the biomechanics of the musculoskeletal system (Sober et al., 2018; Sponberg et al., 2011). Although studies in primates, cats, and zebrafish have shown that both the number of active motor units and motor unit firing rates increase at faster locomotor speeds (Grimby, 1984; Hoffer et al., 1981, 1987; Menelaou & McLean, 2012), the extent to which speed-dependent changes in rate and recruitment vary across muscles and species is unknown.

Mice demonstrate both physiological and biomechanical differences from other vertebrates, potentially leading to unique coordination among their motor units. Compared to cats, for example, mice have highly excitable motor units (Manuel et al., 2019; Manuel & Heckman, 2011) with muscle fibers heavily biased towards fast-twitch fibers (Burkholder et al., 1994; Mathewson et al., 2012), leading to rapid force production. Mice also locomote with greater stride frequency than larger species in order to achieve comparable speeds, requiring faster muscle activation and deactivation (Heglund & Taylor, 1988; Machado et al., 2015). The capability and need for faster force generation during dynamic behavior could implicate motor unit recruitment as a primary mechanism for modulating force output in mice (Manuel & Heckman, 2011; Dideriksen et al., 2020).

To quantify the organization of motor unit firing patterns during locomotion, we recorded mouse motor unit activity from the long head and lateral head of the triceps brachii during treadmill walking at various speeds. Both muscles extend the elbow while the long head also extends, rotates, and abducts the shoulder (Tata Ramalingasetty et al., 2021). Although the triceps are active in every step during quadrupedal locomotion (English, 1978; N. Schumann, 2002; Kirk et al., 2024), it is unknown how individual motor units are coordinated to generate this rhythmic pattern and whether motor pools from closely apposed muscles would exhibit the same coordination. Using Myomatrix electrodes (Chung et al., 2023) to record populations of individual motor units during locomotion, we found that units were recruited probabilistically across strides. When active, units fired in distinct locomotor phases with systematic differences in spike patterns across the long and lateral heads. At faster walking speeds, motor units increased

both their recruitment probabilities and (to a lesser extent) their firing rates. Moreover, motor unit recruitment correlated with variations in limb kinematics both within and across locomotor speeds, with recruitment of long head and lateral head units associated with different changes in limb movement. Overall, our results reveal the systematic changes in motor unit firing that regulate locomotor speed.

3.3 Methods

3.3.1 Surgical implantation

All procedures described below were approved by the Emory University Institutional Animal Care and Use Committee at Emory University (IACUC protocol #201700359). Mice were anesthetized with isoflurane to implant the Myomatrix arrays. Incisions were made in the skin above the skull and above the target muscle. Forceps were used to pull the Myomatrix array through these holes so that the body of the array was entirely subcutaneous, with the Omnetics connector sitting on the skull and the array threads near the muscles. The surface of the skull was lightly scored with a scalpel and dental cement (Metabond Quick Adhesive Cement) was applied generously to fix the Omnetics connector in place and seal the opening. Myomatrix threads were then sutured (8-0 non-absorbable suture from AROSurgical) into the target muscles. Using the four threads of the customizable Myomatrix array (RF-4x8-BHS-5), we implanted a combination of muscles in each mouse, sometimes placing multiple threads within the same muscle. Threads were implanted in the triceps brachii long head and/or the triceps brachii lateral head (Table 3.1) and confirmed through visual inspection. We did not implant in the third (medial) head of the triceps given that it would have required an

additional incision, posing more risk of surgical complications. Some mice also had threads simultaneously implanted in their ipsilateral or contralateral biceps brachii, although due to limited sample size we do not present biceps EMG data in this report. Lastly, 6-0 suture was used to close the incision. Surgeries typically took under three hours and animals were mobile shortly after removal from isoflurane.

Table 3.1. Motor units identified per muscle in each experimental mouse. Each thread consisted of 8 electrode contacts used to record bipolar EMG, and numbers in parentheses indicate the number of motor units isolated using the spike sorting algorithm described in Methods. Data from biceps muscle implantation in two mice were not spike sorted and are not included in this report.

Mouse	Implanted muscle (# motor units identified)
A	2 threads right lateral head, triceps brachii (3) 2 threads right long head, triceps brachii (1)
B	2 threads right lateral head, triceps brachii (0) 2 threads right long head, triceps brachii (3)
C	2 threads right lateral head, triceps brachii (0) 2 threads right long head, triceps brachii (6)
D	2 threads right lateral head, triceps brachii (4) 2 threads right biceps brachii (n/a)
E	2 threads left biceps brachii (n/a) 2 threads right long head, triceps brachii (7)
F	4 threads right lateral head triceps brachii (9)

3.3.2 Behavioral methods and data collection

The treadmill used in this task had a transparent belt and base as described previously (Darmohray et al., 2019; Machado et al., 2015, p. 201). A 45° angled mirror below the base allowed monitoring of side and bottom views from a single camera (FLIR

Grasshopper High Performance USB 3.0 Monochrome Camera) at 330 frames per second. Separate motors controlled the left and right belts, but both were run at the same speed for every experiment. The treadmill, placed within a behavior box, was dark, with the only source of light coming from infrared light.

Experiments were conducted the day following the implant surgery up to five days post-surgery. Data presented in this study came from the first day of recording, in which signal quality tended to be highest (Chung et al., 2023). In each experimental session, mice were first briefly placed under anesthesia using isoflurane to attach a lightweight (1g) digitizing headstage (Intan RHD #C3313 16-Channel Bipolar-Input Recording Headstage) to the Omnetics connector on their skulls. Each recording session lasted approximately 45 minutes in total, and we waited at least 10 minutes after removal from isoflurane to ensure all animals were fully awake before recording began.

For five of six mice, we attempted to record 31 trials - each trial consisted of a single minute continuous running on the treadmill. The first three trials were at 10 cm/s, while the following trials were arranged in seven blocks of four trials each. Each block contained a trial at 12.5 cm/s, 17.5 cm/s, 22.5 cm/s, and 27.5 cm/s in a pseudo-random order presented identically across mice. This pseudo-random order of speeds as opposed to a strict ramping order ensured that we collected data across the full range while reducing potential effects of fatigue. For mice that became uncooperative before completing all trials, we ended the experiment early. Of these five mice, three mice completed all 31 trials, one completed 30, and the last completed 23 trials. For the sixth mouse, we again began with three trials at 10 cm/s, but only increased the speed in 2.5 cm/s increments for either two or three trials each up to a total of 14 trials. Mice were

trained on their given running paradigm and habituated to the treadmill setup twice on the day before surgery. Each trial was initiated using custom Bonsai software (Lopes et al., 2015) and Arduino components to synchronize neural recordings with the camera and motor output.

We used DeepLabCut (Mathis et al., 2018) to track body parts of the mouse during locomotion. We excluded points tracked with less than 90% confidence from DLC and interpolated those points from adjacent high-confidence points. The right elbow angle was estimated using markers from the shoulder, elbow, and wrist. We defined strides using the trough-to-trough minimums of the elbow angle, which occurred approximately 50-100 ms before the footstrike of the right paw. Each stride was also required to contain footstrike and liftoff of the right forepaw during forward movement. From here, we excluded strides with stride durations, stance durations, swing durations, body velocities, or body accelerations outside their respective 95% confidence interval. As a result, we kept about 80% of strides for each animal. Five of six mice had between 2600-3600 total strides, and the remaining mouse, which was run at the slower speed range, had just over 1000 strides included in analyses.

3.3.3 *Electromyography (EMG)*

Bipolar signals from adjacent contact pairs on the Myomatrix array were extracted at 30 kHz (Intan RHD #C3100 Recording Controller) and bandpassed between 300-7500 Hz. Using up to 16 channels of high-resolution EMG from the Myomatrix arrays, motor units were identified using Kilosort 2.5, an open-source multi-channel spike sorting algorithm (Pachitariu et al., 2016). We slightly adjusted the algorithm to better fit the

assumptions behind motor unit activity, including the removal of spatial decay across channels. A full description of adjustments has been previously reported (Chung et al., 2023). A total of 33 units were identified across all animals. Each unit's isolation was verified by confirming that no more than 2% of inter-spike intervals violated a 1 ms refractory limit. Additionally, we manually reviewed cross-correlograms to ensure that each waveform was only reported as a single motor unit. Sorting was high enough quality that no strides were excluded due to inseparable unit activity.

We further validated spike sorting by quantifying the stability of each unit's waveform across time (Figure 3.1). First, we calculated the median waveform of each unit across every trial to capture long-term stability of motor unit waveforms. Additionally, we calculated the median waveform through the stride binned in 50 ms increments using spiking from a single trial. This second metric captures the stability of our spike sorting during the rapid changes in joint angles that occur during the burst of an individual motor unit. In doing so, we calculated each motor unit's waveforms from the single channel in which that unit's amplitude was largest and did not attempt to remove overlapping spikes from other units before measuring the median waveform from the data. We then calculated the correlation between a unit's waveform over either trials or bins in which at least 30 spikes were present. The high correlation of a unit waveform over time, despite potential changes in the electrodes' position relative to muscle geometry over the dynamic task, provides additional confidence in both the stability of our EMG recordings and the accuracy of our spike sorting.

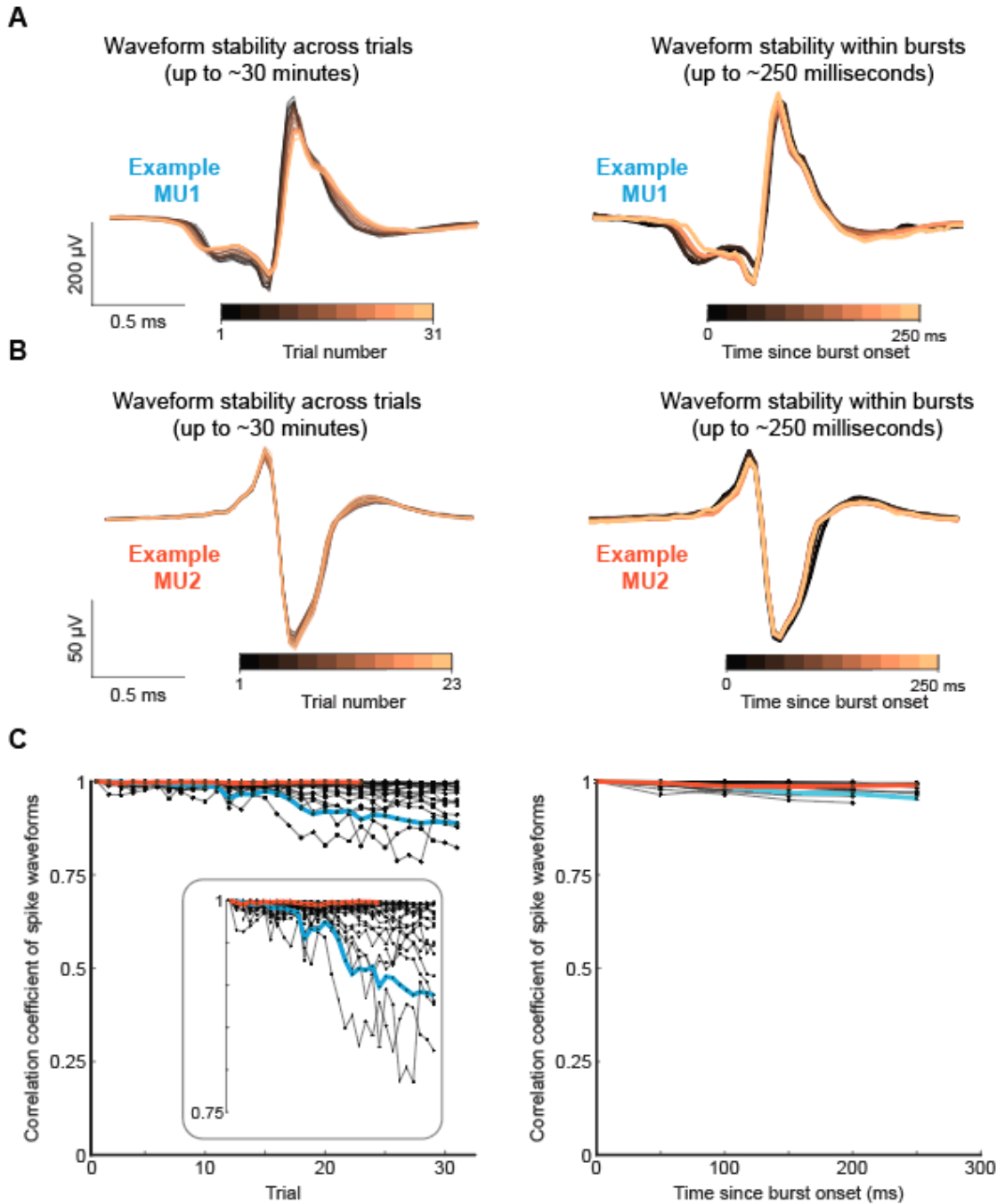


Figure 3.1. Isolated motor units had consistent waveforms. (A,B) Example motor unit waveforms. (Left) Median waveform calculated from a random subset of strides within each trial. (Right) Median waveform calculated from spikes binned in 50 ms increments of the stride. (C) (Left) Auto-correlation of each unit's median waveform between the first trial and subsequent trials. (Right) Auto-correlation of each unit's median waveform between the first 50 ms of its activity and each subsequent 50 ms within the stride.

3.3.4 Data analysis

Continuous firing rates were calculated by convolving raw spike times of a motor unit with a Gaussian kernel with $\sigma = 10$ ms. This continuous result was phase-normalized across strides before calculating the mean continuous rate to identify relevant patterns such as the unit's active duration or peak firing rate. Active duration was measured between the first and last time within a stride that the smoothed curve reached the half-height from a single spike. Overall, this method allowed for quantification of firing rates even when only a single spike was present for a stride.

3.3.5 Joint model of rate and recruitment

We modeled the recruitment probability and firing rate based on empirical data to best characterize firing statistics within the stride. Particularly, this allowed for multiple solutions to explain why a motor unit would not spike within a stride. From the empirical data alone, strides with zero spikes would have been assumed to have no recruitment of a unit. However, to consider both recruitment and rate, it must be possible that a recruited unit can have a firing rate of zero. To quantify the firing statistics that best represent all spiking and non-spiking patterns, we modeled recruitment probability and peak firing rate along the following equations (1,2):

$$P(Y = y) = (1 - p) + p * e^{-\lambda}, \text{ if } y = 0 \quad (1)$$

$$P(Y = y) = p * \frac{e^{-\lambda} \lambda^y}{y!}, \text{ if } y > 0 \quad (2)$$

where y denotes the observed peak firing rate on a given stride, p denotes the probability of recruitment, and λ denotes the expected peak firing rate from a Poisson distribution of outcomes. Thus, an inactive unit on a given stride may be the result of either non-recruitment or recruitment with a stochastically zero firing rate. Peak firing rate was used as opposed to other metrics of firing rate such as the mean or median rate because the peak best reflected changes in the unit activity across strides of varying duration, which was key to drawing conclusions across walking speeds. The above equations were fit by minimizing the negative log-likelihood of the parameters given the data.

3.3.6 *Confidence intervals in the joint rate-recruitment model*

We bootstrapped this model fitting to calculate 95% confidence intervals for the parameters. This was performed for each unit across speed quartiles. To compare changes in parameters at different speeds, we took the difference in bootstrapped parameter estimates from different speed quartiles. The 95% confidence interval was again calculated for these differences. Parameters were statistically significant across speed if this 95% confidence interval did not include zero.

3.4 **Results**

We collected kinematic and high-resolution electromyographic (EMG) data from six mice walking on a transparent treadmill that provided simultaneous lateral and ventral views of the animal (Darmohray et al., 2019). Using DeepLabCut (Mathis et al., 2018) to track body movements during locomotion (Figure 3.2A), we identified the stance phase of the right forelimb, defined as the period between footstrike and liftoff of the right forepaw (Figure 3.2B). Additionally, computing the internal angle of the elbow joint revealed that

the elbow was minimally extended approximately 50 milliseconds before the footstrike (blue squares, Figure 3.2C). Electrode arrays (32-electrode Myomatrix array model RF-4x8-BHS-5) were implanted in forelimb muscles (note that Figure 3.2D shows the EMG signal from only one of the 16 bipolar recording channels), and the resulting data were used to identify the spike times of individual motor units in the triceps brachii long and lateral heads (Table 3.1, Figure 3.2E) as described previously (Chung et al., 2023). To best capture the spike pattern given that some units begin firing prior to footstrike, we defined a stride cycle as the period between minimum elbow angles rather than consecutive footstrikes. Kinematic analysis of locomotor data at different walking speeds revealed systematic variation in the temporal (Figure 3.2F) and spatial (Figure 3.2G, H) components of limb movement, consistent with prior reports (Akay et al., 2006; Bellardita & Kiehn, 2015; Machado et al., 2015; Mendes et al., 2015).

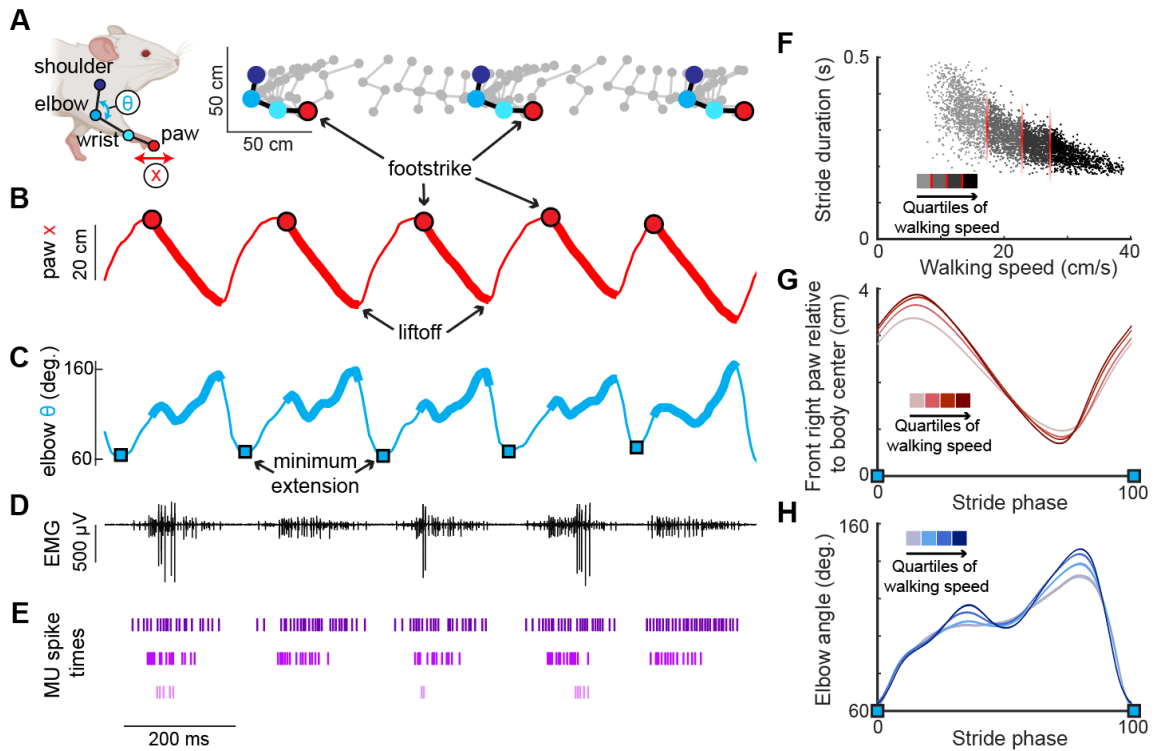


Figure 3.2. High-resolution muscle recording during mouse locomotion. **(A)** (Left) Anatomical landmarks (shoulder, elbow, wrist, paw) and kinematic features (elbow angle θ , paw position x) tracked via high-resolution video during treadmill walking (Darmohray et al., 2019; Mathis et al., 2018). (Right) Position of anatomical landmarks during two stride cycles with limb position captured every 15 ms. **(B)** Position of the right forepaw (x) relative to body center. Thick lines represent the stance phase when the paw is on the ground. **(C)** Interior elbow angle (θ) during locomotion. Troughs of this measure, denoting minimum extension (blue squares), were used to define the spike window for each stride. **(D)** Representative channel of electromyographic (EMG) activity in the long head of the right triceps used to isolate several motor units during walking. **(E)** Three motor units from the long head identified from the above EMG trace (see Methods). Note that units may only be active in a subset of strides. **(F)** Relationship between stride duration and walking speed for all strides in an example mouse. Each dot represents a stride, with shading indicating the speed quartile within which the stride falls (see Methods). **(G,H)** Right forepaw position x (G) and elbow angle (H) within the walking speed quartiles. Note that both (G) and (H) are normalized to total stride duration beginning and ending with elbow minimum extension (blue squares) and show mean (\pm SE).

3.4.1 *Motor units are probabilistically recruited across strides*

Despite the triceps muscles as a whole being reliably activated on every step (English, 1978; N. Schumann, 2002; Kirk et al., 2024), the majority of individual motor units in both the long head and lateral head were active only in a subset of strides during locomotion. Motor units in both muscles exhibited this pattern of probabilistic recruitment, based on empirical observation, but with differing distributions of firing properties across the long and lateral heads (Figure 3.3). For each motor unit, we measured the probability of a unit being recruited as the percentage of strides with at least one spike. Units demonstrated a variety of firing patterns, with some units producing 0 spikes more frequently than any non-zero spike count (Figure 3.3A, B), and units that were less likely to be recruited also had lower average spike counts (Figure 3.3, Figure 3.4). A subset of units, primarily in the long head, were recruited in under 50% of the total strides and with lower spike counts (Figure 3.3C). This distribution of recruitment probabilities might reflect a functionally different subpopulation of units. However, the distribution of recruitment probabilities were not found to be significantly multimodal in either muscle ($p > 0.05$ in both cases, Hartigan's dip test; (Hartigan, 1985)). However, Hartigan's test and similar statistical methods have poor statistical power for the small sample sizes ($n = 17$ and 16 for long and lateral heads, respectively) considered here, so the failure to achieve statistical significance might reflect either the absence of a true difference or a lack of statistical resolution.

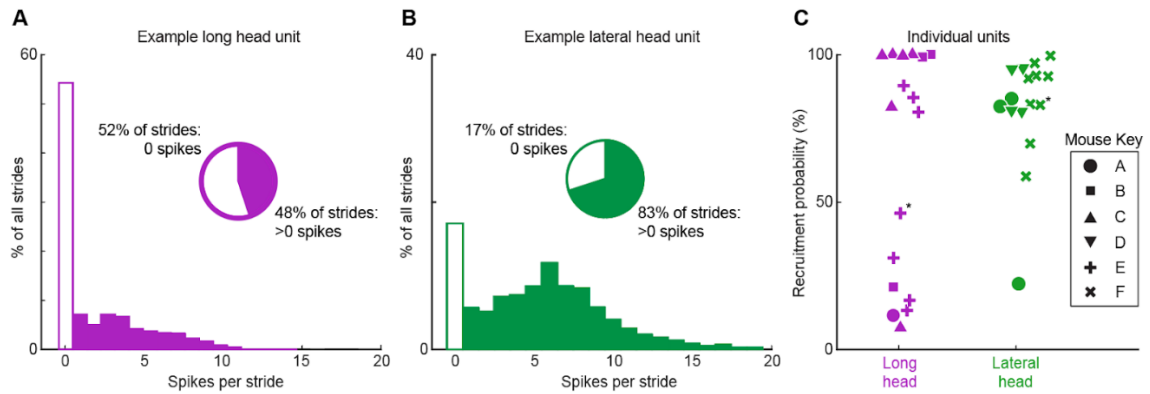


Figure 3.3. Motor unit spike count distributions. **(A)** Example motor unit from the long head of the triceps muscle fired zero spikes on 52% of strides, but on the other 48% of strides fired 1-14 spikes. **(B)** Example motor unit from the triceps lateral head fired zero spikes in 17% of strides but 1-19 spikes on the other 83% of strides. **(C)** Percentage of strides with at least one spike (probability of recruitment) for all recorded motor units in the long (purple) and lateral (green) heads of the triceps. Symbols denote different animals and each point reflects an individual motor unit. Starred points refer to the examples in (A) and (B).

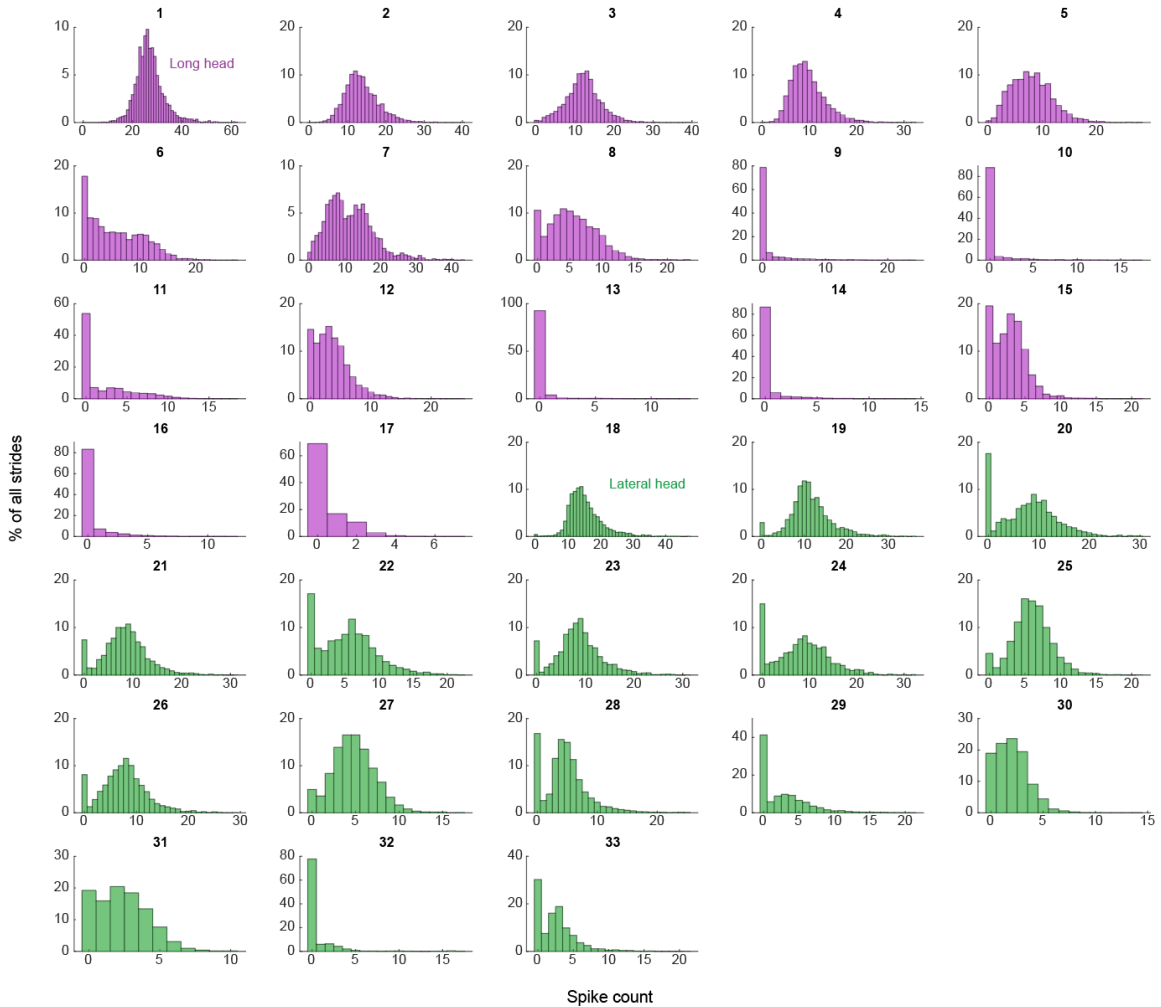


Figure 3.4. Empirical observations of spike count distributions for all units. Units are arranged sequentially to match the descending order presented in Figure 3.5. Units 1-17 are in the long head, while units 18-33 are in the lateral head.

3.4.2 Motor unit firing patterns in the long and lateral heads of the triceps

Motor units within each muscle fired at distinct phases of the stride cycle. Units in the long head typically became active near the time of footstrike, with approximately half of the units reliably recruited prior to footstrike (Figure 3.5A,B). In contrast, units in the lateral head began spiking after the long head was already active and remained active

until just prior to liftoff (Figure 3.5B,C). Furthermore, units in the long head reached their stride-dependent peak rates before the lateral head ($p < 0.01$, two-sample k-s test). These findings demonstrate that despite the synergistic (extensor) function of the long and lateral heads of the triceps at the elbow, there is a consistent, approximately 100 ms delay (Figure 3.5C) between the activation of the two muscles' motor neuron pools. This timing difference suggests distinct patterns of synaptic input onto motor neurons innervating the lateral and long heads. In contrast to the timing differences described above, motor units in the lateral and long heads displayed similar burst durations (Figure 3.5B) and peak firing rates (Figure 3.5D).

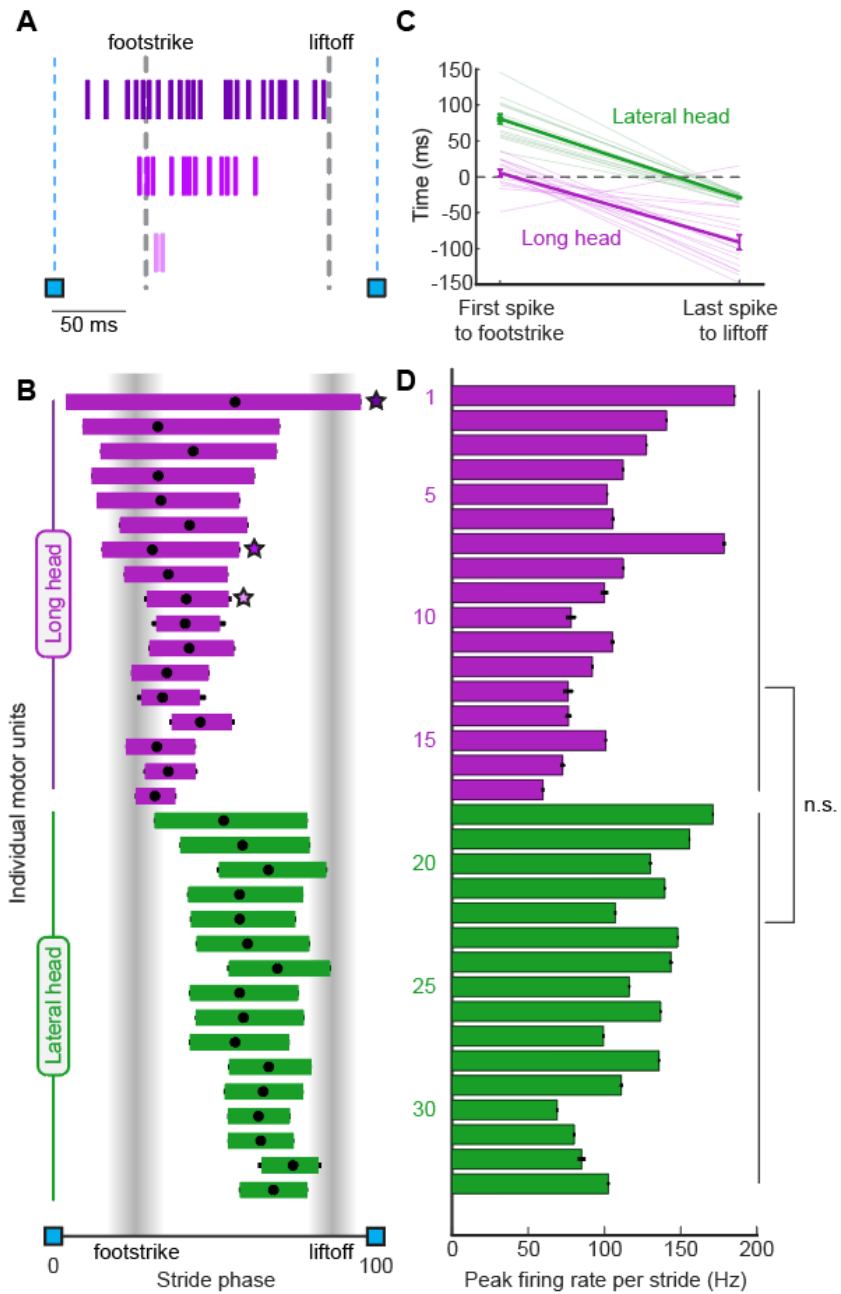


Figure 3.5. Motor unit firing patterns within and across muscles. (A) Example stride with three units from the long head. (B) Mean phase (\pm SE) of motor unit burst activity within each stride duration across all strides. Black dot within each bar shows the mean phase of the unit's peak firing rate. Starred points refer to the examples in A. (C) Left: Mean time (\pm SE) between the first spike of a unit's spike train and the right forepaw footstrike. Positive values denote the spike happening after the footstrike. Right: Mean time (\pm SE) between the last spike of a unit and the liftoff. Light traces denote values for individual motor units while the heavy trace shows the mean and standard error across all units within a muscle. (D) Mean peak firing rate (\pm SE) of each unit. Note that these measurements only include strides in which the given unit was recruited.

The evolution of spike patterns within each stride differed between motor unit populations in the long and lateral heads. In both muscles, motor units with longer burst durations reached higher peak firing rates (Figure 3.6A). However, the slope of this relationship was significantly higher for lateral head units (non-overlapping confidence intervals, Model II regression). We also observed muscle-dependent differences in motor unit patterning when examining the inter-spike intervals (ISIs) between the first three spikes in each stride cycle. Motor units in both muscles began firing with ISIs typically below 12 ms (Figure 3.6B). Furthermore, the second ISI was generally shorter, indicating that firing rate increased throughout the first three spikes fired in the stride cycle. However, the population of motor units in the long head had a larger magnitude in the ratio ISI_1/ISI_2 (Figure 3.6B, $p < 0.01$, two-sample k-s test). Together with the differences in burst timing shown in Figure 3.5B, these results again support the conclusion that the motor pools for the lateral and long heads of the triceps receive distinct patterns of synaptic input, although differences in the intrinsic physiological properties of motor neurons innervating the two muscles might also play an important role.

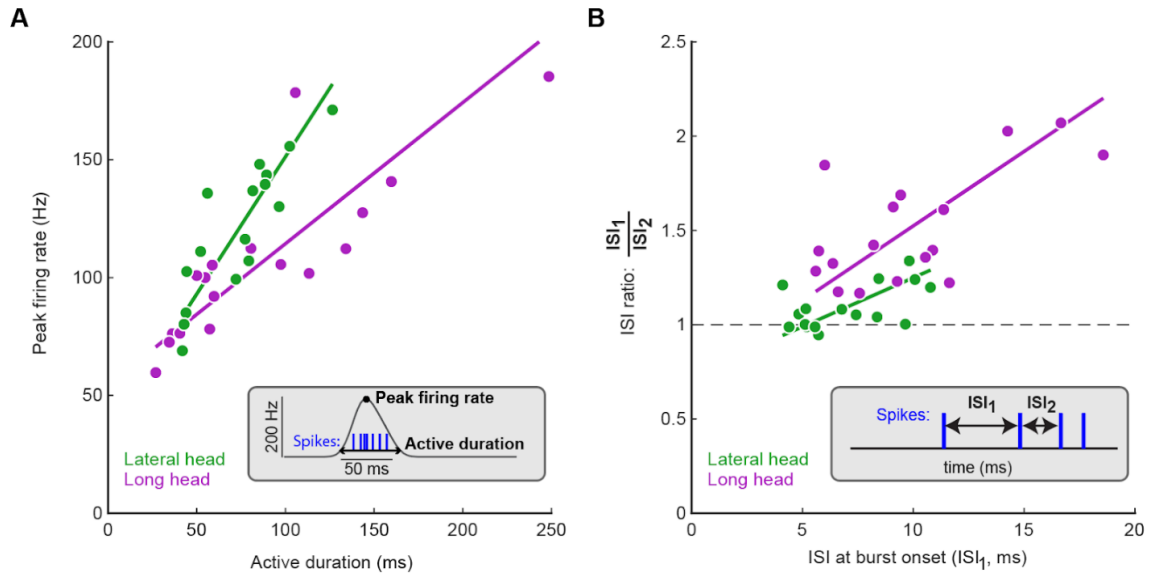


Figure 3.6. Motor unit spike patterns evolve differently in the long and lateral heads. **(A)** Relationships between active duration and peak firing rate across motor pools. Regression slopes were significantly different between the lateral and long heads (non-overlapping confidence intervals, Model II regression). **(B)** Motor unit inter-spike intervals (ISIs) across the first three spikes in motor unit bursts. Each data point shows the mean of the first ISI and the ratio between the first and second ISIs for a single unit. Note that only strides with at least three spikes could be used for the analysis shown in panel (B). Regression slopes were not significantly different between data from the lateral and long heads (overlapping confidence intervals, Model II regression).

3.4.3 Motor unit mechanisms of speed control

Adjusting walking speed requires changes in the firing patterns of individual motor units, which could include speed-dependent changes in units' probability of recruitment and/or changes in firing rate. To investigate the changes in motor unit firing underlying locomotor speed control, we quantified how both recruitment probability and firing rate change across the four quartiles of locomotor speed shown in Figure 3.2F-H. Motor units from the long and lateral heads of the triceps (Figure 3.7A,B, purple and green traces, respectively) displayed significant increases in recruitment probability as

locomotor speeds increased. Figure 3.7C shows each motor unit's difference in recruitment probability between the slowest and fastest locomotor speed quartiles. This increase was statistically significant in 29/33 motor units in our study ($p < 0.05$, rate-recruitment model) when considered individually, and was also significant when the probabilities of all motor units were analyzed as a group ($p < 0.01$, Wilcoxon signed-rank test). Robust increases in recruitment probability across the four speed quartiles were therefore the norm in our dataset (Figure 3.8).

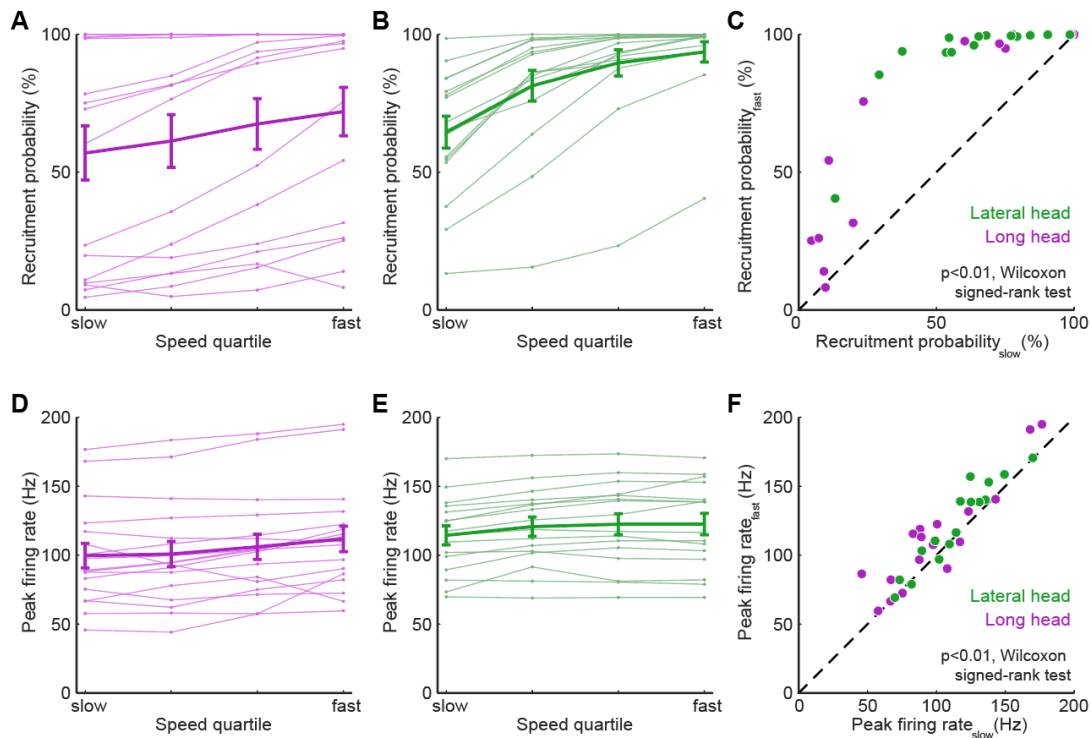


Figure 3.7. Motor units alter firing rate and recruitment across walking speeds. **(A)** Light traces show median of recruitment probability for individual long head motor units while the heavy trace shows mean (\pm SE) across all long head motor units. **(B)** Recruitment probability for lateral head motor units, same plotting conventions as in (A). **(C)** Difference in recruitment probabilities between slowest and fastest speed quartiles for all motor units. **(D)** Light traces show median of peak firing rate for individual long head motor units while the heavy trace shows mean (\pm SE) across all long head motor units. **(E)** Peak firing rates for lateral head motor units, same plotting conventions as in (D). **(F)** Difference in peak firing rates between slowest and fastest speed quartiles for all motor units. Across all motor units, both recruitment probabilities (C) and firing rates (F) were significantly higher at the fastest quartile than at the slowest quartile ($p < 0.01$, Wilcoxon signed-rank tests).

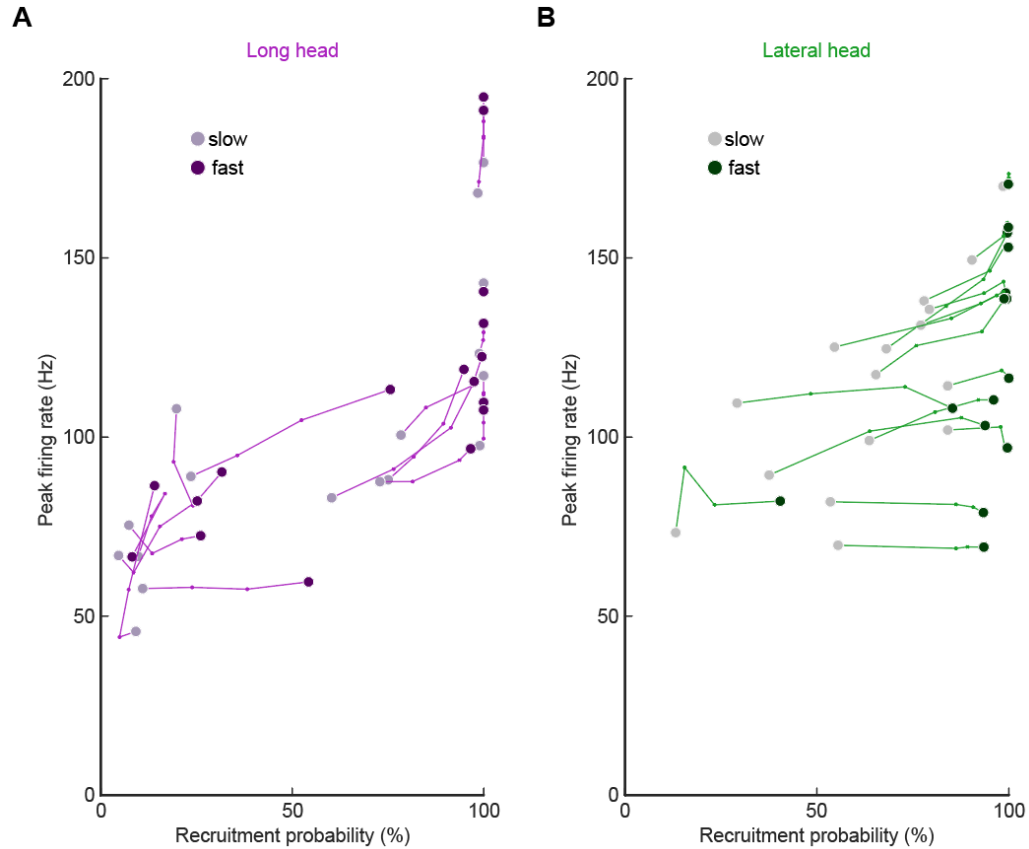


Figure 3.8. Altered firing rate and recruitment across walking speed quartiles for all motor units in the long head (A) and lateral head (B). Each point reflects the median for the model estimate of each unit across the speeds.

Quantitative analysis of motor unit activity also revealed significant speed-dependent changes in firing rate, although these were proportionally smaller than the increases in recruitment probability. Motor units in both the long and lateral heads of the triceps (Figure 3.7D,E, purple and green traces, respectively) often had either marginal increases or no difference in peak firing rate at faster speeds. Across all motor units in our dataset in the slowest and fastest speed quartiles (Figure 3.7F), we observed significant increases in peak firing rate in 20/33 individual motor units in our study ($p < 0.05$, rate-recruitment model), and also a significant speed-dependent increase in peak rate when

considering all motor units together ($p < 0.01$, Wilcoxon signed-rank test). Speed-dependent increases in peak firing rate were therefore also present in our dataset, although in a smaller fraction of motor units than changes in recruitment probability. Furthermore, the mean (\pm SE) magnitude of speed-dependent increases was smaller for spike rates (mean $\text{rate}_{\text{fast}}/\text{rate}_{\text{slow}}$ of $111\% \pm 20\%$ across all motor units) than for recruitment probabilities (mean $p(\text{recruitment})_{\text{fast}}/p(\text{recruitment})_{\text{slow}}$ of $179\% \pm 3\%$ across all motor units). Note that the fraction of motor units with speed-dependent changes in recruitment probability (29/33) and the fraction with speed-dependent changes in peak firing rate (20/33) are not directly comparable, since these fractions reflect different statistical tests with different statistical power. While a direct comparison of rate and recruitment is difficult given their different upper and lower limits, these findings could suggest that while both recruitment and peak rate change across speed quartiles, increased recruitment probability may play a larger role in driving changes in locomotor speed.

3.4.4 *Kinematic contributions of motor unit recruitment*

We next examined whether the probabilistic recruitment of individual motor units in the triceps – an elbow extensor muscle – was correlated with stride-by-stride variations in elbow angle kinematics. To do so, we compared elbow extension ($\Delta\theta$; Figure 3.9A) on strides in which each individual motor unit did or did not fire at least one spike. When kinematic data are combined across all speed quartiles (Figure 3.9B), we found that recruitment of lateral head motor units (green symbols) is associated with greater elbow extension, whereas recruitment of long head units (purple symbols) is correlated with smaller extensions ($p < 0.001$, Wilcoxon signed-rank tests). These correlations might

reflect both an influence of motor unit recruitment on limb kinematics as well as different biomechanical roles for the long and lateral heads.

Since both limb kinematics (Figure 3.2G,H) and recruitment probability (Figure 3.7) are significantly correlated with locomotor speed, the observed correlations between unit recruitment and elbow angle across all speeds (Figure 3.9B) does not necessarily reveal the direct influence of unit firing on limb kinematics. We therefore controlled for speed by repeating the analysis shown in Figure 3.9B for strides within each speed quartile. Strikingly, the correlations between motor unit recruitment and elbow angle persisted in this alternative analysis (Figure 3.9C, Figure 3.10; $p < 0.001$, Wilcoxon signed-rank tests), suggesting that the recruitment of individual motor units in the lateral and long heads might have significant effects on elbow angle in strides of similar speed (see Discussion). We repeated these analyses using the elbow angular velocity rather than just the angle to further identify how firing patterns related to behavior. Motor units in the lateral head had a similar effect with larger velocities correlating with motor unit recruitment across all speeds (Figure 3.9D,E, Figure 3.10; $p < 0.001$).

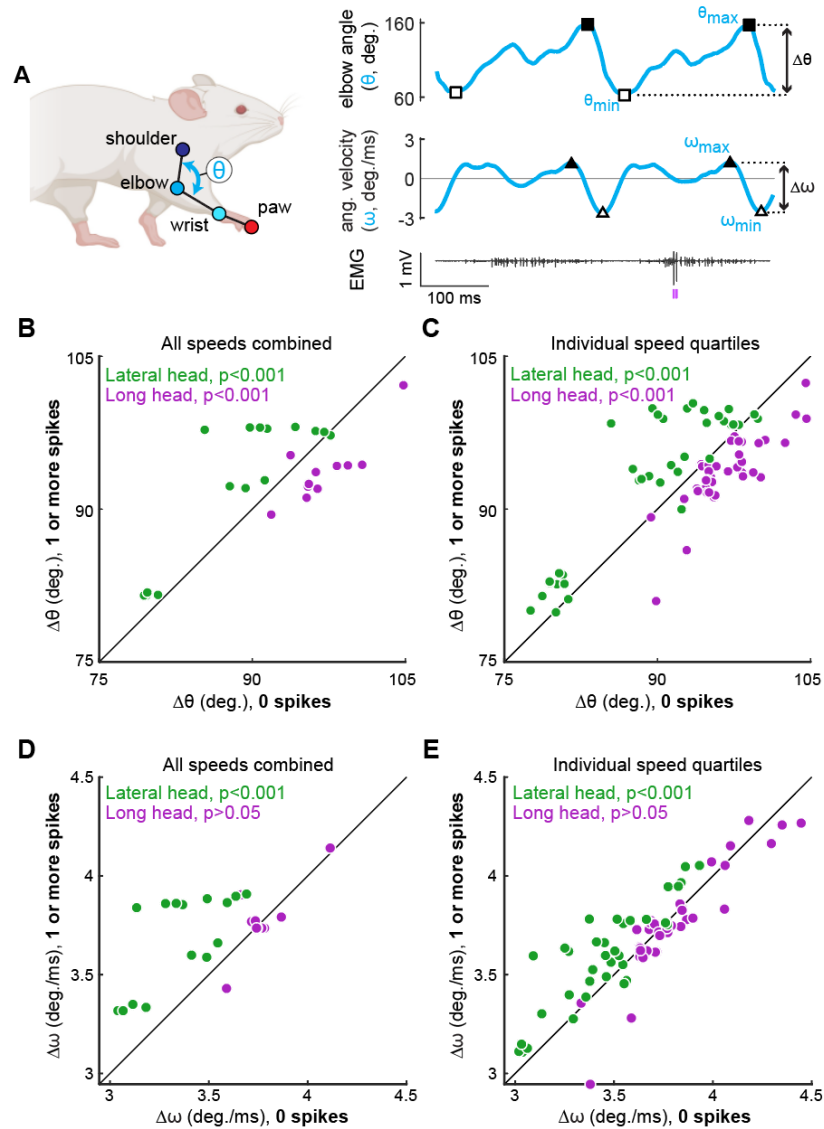


Figure 3.9. Motor unit recruitment correlates with muscle-specific kinematic differences. **(A)** We calculated ranges for elbow angle ($\Delta\theta$) and elbow velocity ($\Delta\omega$) on strides in which each motor unit did or did not fire at least one spike (purple tick marks below EMG trace). **(B,D)** Each point represents the mean $\Delta\theta$ (B) or $\Delta\omega$ (D) observed on strides in which a single motor unit fires zero spikes (horizontal axis) vs. when the motor unit fires at least one spike (vertical axis). Note that in these panels, data for each motor unit were combined across all locomotor speeds. **(C,E)** Same analyses as before, except each motor unit contributes up to four data points, one for each of the four locomotor speed quartiles in which sufficient data were available (at least 30 strides existed in both the spiking and non-spiking conditions within a given quartile). Legends in each panel show statistical significance for a difference in kinematics tested on the motor units within each muscle (Wilcoxon signed-rank tests). Note that most of the muscle-specific differences shown in (C,E) were also present when each of the four quartiles were examined individually for each muscle (Figure 3.10).

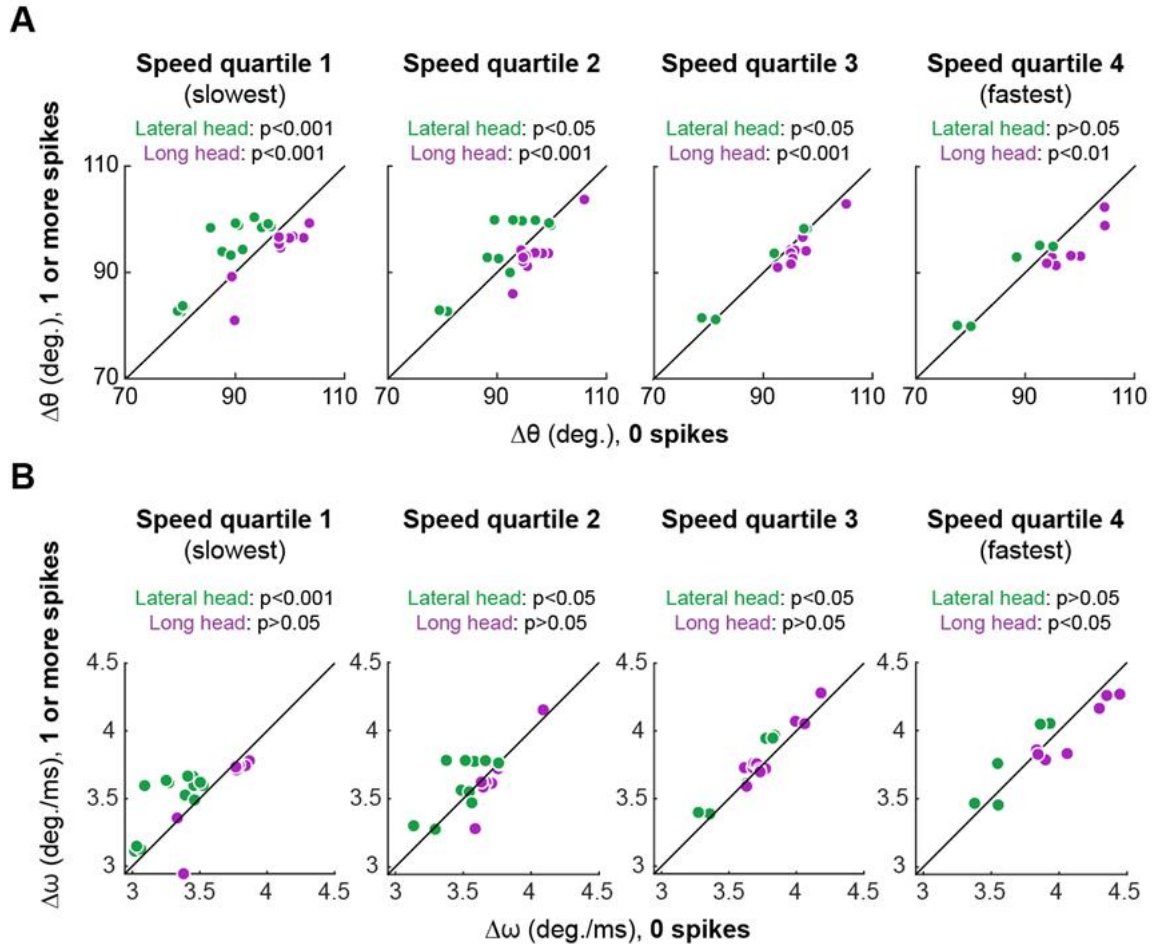


Figure 3.10. Motor unit recruitment and kinematic correlations in individual speed quartiles. Plots show the same analysis as Figure 3.9 for each individual speed quartile of (A) elbow angle ($\Delta\theta$) and (B) elbow velocity ($\Delta\omega$). Speed quartiles 1 and 4 are the slowest and fastest quartiles, respectively, and p-values refer to the results of Wilcoxon signed-rank tests performed separately on data from motor units of the lateral (green) and long (purple) heads of the triceps muscle. Note that most of the muscle-specific differences shown in Figure 3.9 were also present when each of the four quartiles were examined individually for each muscle.

3.4.5 Motor unit recruitment has population dependence

Given that motor units likely have some degree of shared inputs, motor unit firing patterns may not be independent from each other during each stride. For example, two motor units, each with low recruitment probabilities, may still fire during the same set of

strides. To assess the independence of motor unit recruitment across the recorded population, we compared each unit's empirical recruitment probability across all strides to its conditional recruitment probability during strides in which another motor unit from the same muscle was recruited. Doing this for all motor unit pairs revealed that motor units in both muscles were biased towards greater recruitment when additional units were active ($p < 0.001$, Wilcoxon signed-rank test). This highlights that populations of motor units are likely recruited through shared inputs that affect muscle activity by modulating how many units are recruited together.

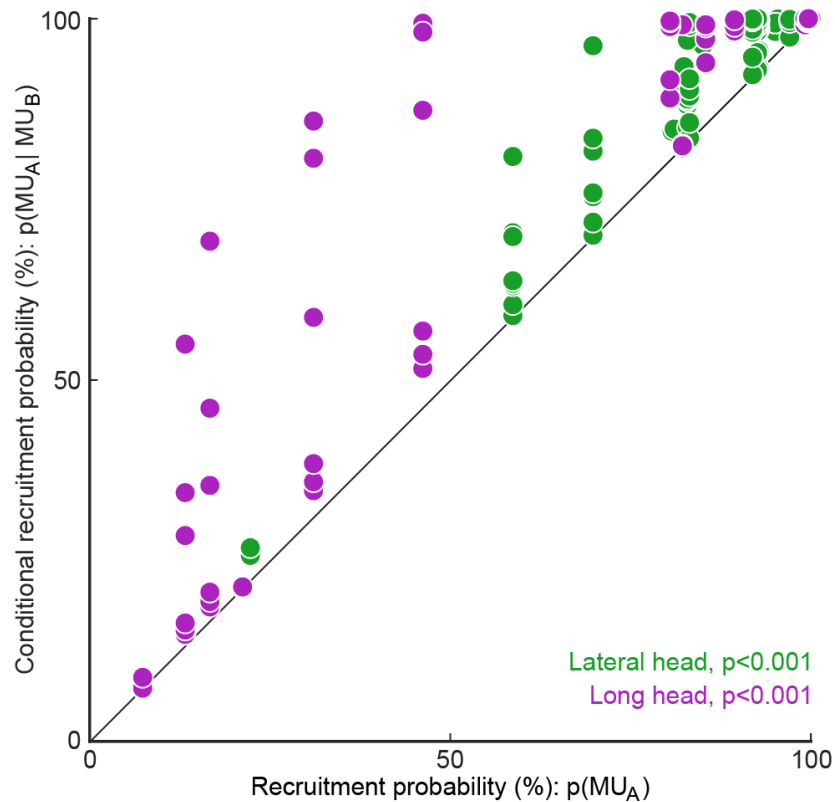


Figure 3.11. Motor unit recruitment is greater when other motor units within the muscle are recruited. Each point reflects a motor unit's empirically measured recruitment probability across all strides compared to strides when an additional motor unit from the same muscle was recruited. Motor units in each muscle had significantly higher recruitment when another unit was recruited in the same stride ($p < 0.001$, Wilcoxon signed-rank tests).

3.5 Discussion

Our data reveal the diversity of motor unit firing patterns in mouse triceps brachii muscles during walking. Motor units were probabilistically recruited on a stride-by-stride basis with peak firing rates between 60-185 Hz when active. Motor units in the long head were recruited before the lateral head and spiking patterns evolved within each stride differently across the two muscles. Motor units in both muscles demonstrated increases in their recruitment probability and firing rate at faster walking speeds. Furthermore, motor unit recruitment was also correlated with differences in limb kinematics for strides of similar speed. As discussed below, firing patterns from motor units in the long and lateral heads likely reflect the functional and anatomical role of these two muscles, highlighting the need for high-resolution quantification of motor unit firing patterns during behavior.

3.5.1 *Differences in motor unit activity patterns across two elbow extensors*

Motor unit spike patterns differed systematically between the long and lateral heads of the triceps brachii. Motor units in the long head were consistently recruited earlier than units in the lateral head (Figure 3.5B,C). The large differences in burst timing and spike patterning across the muscle heads suggest that the motor pools for each muscle receive distinct inputs. However, motor pool-dependent differences in the intrinsic physiological properties of motor units and neuromodulatory inputs across motor pools might also make substantial contributions to the structure of motor unit spike patterns (Martínez-Silva et al., 2018; Miles & Sillar, 2011).

The observed muscle ordering matches past reports in bulk muscle activity in these two muscles across other quadrupedal species (Carroll & Biewener, 2009; Drew et

al., 2008; Livingston & Nichols, 2014; Scholle et al., 2001) and may reflect the biomechanical functions of each muscle. Whereas the lateral head is a monoarticular elbow extensor, the long head is biarticular, both extending the elbow along with extending and rotating the shoulder (Tata Ramalingasetty et al., 2021). Although we did not measure ground reaction forces, prior reports indicate that the vertical ground reaction force on the mouse forepaw reaches two peaks during locomotion (Schmitt et al., 2010). The first peak, which happens soon after the footstrike, has a lower magnitude than the second peak, which occurs closer to liftoff (K. A. Clarke et al., 2001; Schmitt et al., 2010). Studies in both rats (Sarver et al., 2010) and cats (Corbee et al., 2014) have demonstrated that horizontal ground reaction forces in both the medio-lateral and cranio-caudal directions are also greatest soon after footstrike, with more force variability than the vertical reaction force. Since units in the long head are most active following footstrike, this suggests that activity in the long head might be related to stabilizing the limb within each step. Our finding that recruitment of long head motor units (purple symbols, Figure 3.9) accompanied smaller elbow extensions might therefore reflect a more complex biomechanical role for the long head. This interpretation is consistent with past findings that biarticular muscles are power distributors, stabilizing the joint across multiple dimensions, while monoarticular muscles are power generators (Ryan & Gregor, 1992; Van Ingen Schenau et al., 1992, 1994). Conversely, the observed timing of lateral head motor unit activity just prior to liftoff (Figure 3.5B) might therefore reflect the lateral head's role of providing propulsion prior to swing, consistent with our finding that recruitment of motor units in the lateral head is correlated to both larger elbow extension and more rapid changes in angle (Figure 3.9).

Motor units in the lateral and long heads also differed with respect to their recruitment probabilities, with a substantial population of units in the long head (but not the lateral head) with probability of recruitment less than 50% (Figure 3.3C). This difference may reflect different functions of muscle fibers in different subcompartments of biarticular muscles. Prior work has established that different regions within a biarticular muscle can have different contributions across the two joints (Chanaud et al., 1991; English & Weeks, 1987; Watanabe et al., 2021). For example, different regions of the cat biceps femoris are out of phase with each other during walking, with the anterior compartment active during stance as a hip extensor and the posterior compartment active during swing as a knee flexor (Chanaud et al., 1991; English & Weeks, 1987). Additionally, the posterior compartment was only active at faster speeds. In our mouse data, functional compartments within the biarticular long head may thus explain the differently recruited populations for motor units (Figure 3.3C). However, to our knowledge, no studies have investigated anatomical or functional subdivisions across subregions of the triceps long head in the mouse. Nevertheless, the group of less-frequently recruited units might contribute more to forelimb joint stability, which is presumably more variable, whereas the other long head motor units might be recruited in a greater fraction of strides to support the weight of the body. Further examination of the anatomical microstructure of the long head, including precise characterization of the attachment points to the bone (DeWolf et al., 2024; Gilmer et al., 2024), are necessary to answer these questions.

The varied composition of fiber types in the long and lateral heads may also explain the different firing patterns across muscles. Although both muscles are heavily

biased towards the fastest myosin type (type 2B), the long head has a broader composition, including a small percentage of slower isoforms as well (type 1 and 2A) (Mathewson et al., 2012). Type 2B isoforms are related to fast-twitch, fatigable units (FF) while type 1 compose slow-twitch units (S) (Bączyk et al., 2022; Schiaffino & Reggiani, 2011). While we were unable to directly quantify the unit type, the majority of units observed, particularly within the lateral head, are likely FF units given the mouse anatomy. Thus, the probabilistic recruitment may be related to units' susceptibility to fatigue throughout approximately 30 minutes of locomotion (Martínez-Silva et al., 2018). Experimenters randomly changed between slow and fast treadmill speeds every trial, each of which lasted a minute, but fatigue may have still set in within a trial or over the experiment's duration. Motor units that were recruited in nearly every stride with 10 or more spikes per stride (Figure 3.4) could result from units with slower isoforms given their resistance to fatigue. The most prominent example of this came from the single unit in the long head that fired for over 90% of the stride phase in every stride. Still, given the wide range of firing patterns and tendency for units to be recruited more reliably at fast speeds (Figure 3.7), our estimation of unit type differences within each muscle only begins to explain the nuanced details of motor unit function during locomotion.

3.5.2 Firing rates in mouse locomotion compared to other species

The range of firing rates we observed are faster than typically observed across larger species, likely reflecting the unique physiology of mouse motor neurons. Motor units in the lateral and long heads of the triceps exhibited a large and overlapping range of peak firing rates ranging from 50-175 Hz (Figure 3.5D), in agreement with prior reports of motor unit firing rates from mouse forelimb (Kirk et al., 2024) and hindlimb

(Hadzipasic et al., 2016) during locomotion. In rat hindlimb muscles during walking, motor units had mean instantaneous firing rates between 45-109 Hz (Gorassini et al., 2000). Notably, motor units across the tibialis anterior, medial gastrocnemius and lateral gastrocnemius also typically fired rapidly at the beginning of each stride, often with ISIs around 5 ms before settling into a slower, more consistent rate. Since several units in this study only fired once in a large proportion of strides when active, observations of ISIs, while helpful for directly calculating instantaneous firing rates, might also be limiting for an overall interpretation of firing patterns. Still, while we also found short ISIs at the beginning of the stride, these did not seem to precede a consistent rate drop. During cat locomotion, motor units recorded from the toe and hindlimb had firing rates between 15-50 Hz (Hoffer et al., 1987; Zajac & Young, 1980). Human motor units in the short extensors of the toe fire at even lower rates (10-25 Hz) during walking (Grimby, 1984). Compared to these larger species, mice likely reach higher rates through the physiological properties of their motor neurons such as afterhyperpolarization (AHP), which influences how rapidly a neuron returns to baseline voltage after firing a spike. Although AHP durations vary across unit types, AHP durations in mice are approximately two and three times shorter than those in cats and humans respectively (Manuel et al., 2009, 2019; Meehan et al., 2010). Additionally, persistent inward currents (PICs), which amplify excitatory synaptic inputs (Binder et al., 2020; Heckman et al., 2005), might lead to disproportionately large gain in mouse motor neurons compared to other species (Huh et al., 2017; Manuel et al., 2019). Consequently, even mice performing quiet standing have motor unit firing rates reaching up to 68 Hz (Ritter et al., 2014). Our findings (Figure 3.6) highlight that even with the relatively high firing rates

observed in mice, there are still significant changes in firing rate across the spikes within bursts (Figure 3.6B) and across locomotor speeds (Figure 3.7F). Future studies should more carefully examine how these rapidly changing spiking patterns derive from both changing synaptic inputs and intrinsic properties of motor neurons (Berg, 2017; Manuel & Heckman, 2011; Petersen & Berg, 2016).

3.5.3 *Walking speed modulation of firing rate and recruitment*

To investigate the neuromuscular control of locomotor speed, we quantified speed-dependent changes in both motor unit recruitment and firing rate. We found that the majority of units were recruited more often and with larger firing rates at faster speeds (Figure 3.7, Figure 3.8). This result may reflect speed-dependent differences in the common input received by populations of motor neurons with varying spiking thresholds (Henneman et al., 1965). In the case of mouse locomotion, faster speeds might reflect a larger common input, increasing the recruitment probability as more neurons, particularly those that are larger and generate more force, exceed threshold for action potentials (Farina et al. 2014). Importantly, our work only examines a subset of the movement speeds and gait patterns that mice produce. It therefore remains to be determined how rate and recruitment are reshaped as mice increase their speed up to 100 cm/s and alter coordination patterns across their limbs (trotting, bounding, etc.) (Bellardita & Kiehn, 2015; Gonçalves et al., 2022; Herbin et al., 2006, 2007). Since a majority of observed motor units, particularly in the lateral head, were already reliably recruited at the fastest speed quartile (roughly 30-40 cm/s), further speed increases might rely on either more firing rate modulation from these active units or from recruitment of more of the motor pool. Moreover, adjustments to kinematic and kinetic strategy across speeds could result

from more global changes in motor unit coordination. For example, studies in drosophila (Azevedo et al., 2020) and zebrafish (Kishore et al., 2014) have demonstrated preferential recruitment of faster motor unit subtypes during rapid movements. Future studies in mice can therefore examine faster gaits to compare how different species achieve their most rapid forms of locomotion.

Considering the force production of motor units is essential to connect our observations of firing patterns to behavioral outputs. In anesthetized mice, intracellular current injections into individual motor neurons revealed that fast motor units from the triceps surae (gastrocnemius and soleus muscles of the hindlimb) reached near tetanic force at firing rates between 60-80 Hz while slow motor units reached near tetanic forces between 30-40 Hz (Manuel & Heckman, 2011). Furthermore, motor units rapidly reached these rates once active. Despite being recorded from different muscles than the ones we examined, these earlier results are relevant to our findings given that the long and lateral heads of the triceps brachii are (similarly to the gastrocnemius) biased towards fast-twitch muscle fibers (Burkholder et al., 1994; Augusto et al., 2004). Given that motor units recorded in our study had firing rates at or above the aforementioned rates immediately upon recruitment within a stride (Figure 3.6B), it could be that each of the units identified in this study generated near-maximal force whenever active. If units are recruited with near maximal force even at slow walking speeds, generating the additional forces needed for fast walking likely comes from recruitment of additional units. Future studies might answer this question by quantifying the force-production properties of triceps motor units during the rapid changes in the muscle length and shortening velocity that take place during locomotion (Edman, 1979; Gittings et al., 2012; Ting & Chiel, 2017).

Although strong correlations were observed between motor unit recruitment and limb kinematics during locomotion (Figure 3.9, Figure 3.10), it remains unclear whether such correlations actually reflect the causal contributions that those units make to limb movement. To resolve this ambiguity, future studies could perturb the activity in individual motor units during ongoing locomotion and directly test the impact of firing pattern on locomotor movements (Kim et al., 2024; Lu et al., 2024; Srivastava et al., 2015, 2017). The short-latency effects of patterned motor unit stimulation (Srivastava et al., 2017) could then reveal the sensitivity of behavior to changes in muscle spiking or the extent to which the same behaviors can be performed with many different motor commands.

CHAPTER 4. PAIRWISE COORDINATION OF MOTOR UNITS IN MOUSE LOCOMOTION

As demonstrated in the previous chapter, motor unit recruitment in the mouse is correlated with changes in locomotor speed and kinematics. Given that each muscle has tens to hundreds of units that may be recruited, we are left to wonder how the nervous system coordinates recruitment across the population of all motor units within a muscle. In this chapter of unpublished work, I explore this coordination through the relative firing patterns across co-active motor units.

4.1 Introduction

The nervous system must coordinate activity patterns across populations of motor units to accurately produce the muscle force behind complex movements. Current understanding of this organization is that motor units receiving a common input are recruited orderly following the size principle (Henneman et al., 1965). Slow units with smaller cell bodies, as well as lower axonal conduction velocity and lower force output, are recruited before fast units (Henneman et al., 1965; T. Cope & Pinter, 1995; Hodson-Tole & Wakeling, 2009). De-recruitment, however, is not as simply organized since force thresholds may differ at recruitment and de-recruitment (Romaiguère et al., 1993; Gorassini et al., 2002). Inhibitory circuits within the spinal cord are likely responsible for de-recruiting motor units and maintaining rhythmic activity (Conway et al., 1987; Otto Friesen, 1994; Johnson et al., 2017). During mouse locomotion, muscle force across the body must rapidly increase and decrease during each stride. Given that motor unit recruitment may primarily drive force generation in the mouse (Manuel & Heckman, 2011; K. Thomas et al., 2025), it is important to additionally consider de-recruitment patterns at

the population level to understand how the nervous system is controlling dynamic force regulation across the body during behavior. The complexity of locomotion, including speed change and whole-body coordination, may take advantage of flexible activation within the motor pool. However, the relation between coordination patterns and locomotor behavior has yet to be investigated in the intact, freely locomoting mouse.

Further complexity in motor unit populational control may come from functional interactions between motor units. For example, recruitment of a single motor unit can modulate the responses in muscle spindles (Binder & Stuart, 1980) and Renshaw cells (Ross et al., 1975), which each provide inhibitory feedback to the motor pool, potentially influencing motor unit activity. Past studies in the rat (Eken, 1998), cat (Sokoloff & Cope, 1996), and human (Broman et al., 1985; De Luca, 1985) have reported functional coupling between motor units, in which a unit's firing rate abruptly decreases upon another unit's recruitment. These rate-recruitment interactions could enable more complex coordination patterns, allowing a wider range of dynamics in the force production and resulting movement. However, it is unclear which neural circuits are responsible for this mechanism and how it might appear during active behaviors such as locomotion.

To quantify the coordination of motor unit firing patterns during locomotion, we extended our analysis of the data from Chapter 3, focusing now on pairwise relationships in co-active motor units. We found that motor units in both the long and lateral heads of the triceps brachii had strong biases in recruitment order but not de-recruitment order, suggesting differential neural control between muscle contraction and relaxation. Furthermore, reversals in recruitment order correlated with changes in the movement speed. Lastly, motor unit recruitment systematically decreased the activity in previously

active units, highlighting a more complex mechanism behind motor unit coordination during dynamic behaviors.

4.2 Methods

4.2.1 Relative firing patterns in pairwise motor units

We extended our analysis from the dataset collected and described previously in Chapter 3 (see Chapter 3: Methods). To summarize, we collected kinematic and high-resolution electromyographic (EMG) data from six mice during treadmill locomotion between 10-30 cm/s. We identified and validated 33 motor units in the mouse triceps brachii long head (17) and lateral head (16) that were active during the task. Motor unit spike patterns were defined for each motor unit on a stride-to-stride basis as the collective burst of spiking within each stride.

Extended analyses focused on the pairwise relationships in motor unit firing patterns. This scale of measurement allowed close examination of how variations in relative spike timing alter the behavior, enabling broader interpretations of population level control in each motor pool. We quantified the recruitment of each motor unit as the time of its first spike within a stride, and the de-recruitment as the time of its last spike within a stride.

4.2.2 Rate recruitment interaction

We used measurement of the instantaneous firing rate (IFR) to identify how the firing pattern in a motor unit changes upon recruitment of a second unit. For each stride with simultaneously active motor units, we calculated the IFR of the unit that was recruited

first. The IFR at each spike within the burst, except the first, is calculated as the reciprocal of the inter-spike interval (ISI) from the previous two spikes. IFR at the first spike can be considered negligible for the purpose of our analyses. We then identified the time of recruitment for the latter recruited unit and took the difference in IFR for each spike in the first unit relative to this time.

4.3 Results

4.3.1 *Motor units have systematic recruitment but not de-recruitment patterns during walking*

Motor units were recruited in a consistent order during walking. We identified the ‘orderly’ recruitment order by arranging each motor unit sequentially by its average spiking onset within strides (Figure 4.1A). In pairwise comparisons of motor units ordered this way, there was a predictably strong bias towards positive recruitment latencies (Figure 4.1B). This ordering also seemed consistent on a stride-by stride basis (Figure 4.2). Still, reversals from this order occurred, which could be because this pairwise analysis only included strides with both units firing. The initial ordering of units potentially included a different subset of strides given that it was based on a single unit’s activity. The negative recruitment latency could highlight neural circuitry that either drives earlier activation of the second unit or later activation of the first unit when they are co-active.

Interestingly, there was not a strong, systematic bias in the de-recruitment order. 25/39 motor unit pairs in the long head and 22/45 motor unit pairs in the lateral head had positive, mean de-recruitment latencies (long head: $p > 0.05$, lateral head: $p > 0.05$, two-tailed binomial test). Furthermore, the distribution of de-recruitment percentages on a

stride-by stride basis centered about zero, suggesting near random de-recruitment order (Figure 4.2). Still, some units were heavily biased towards a particular order.

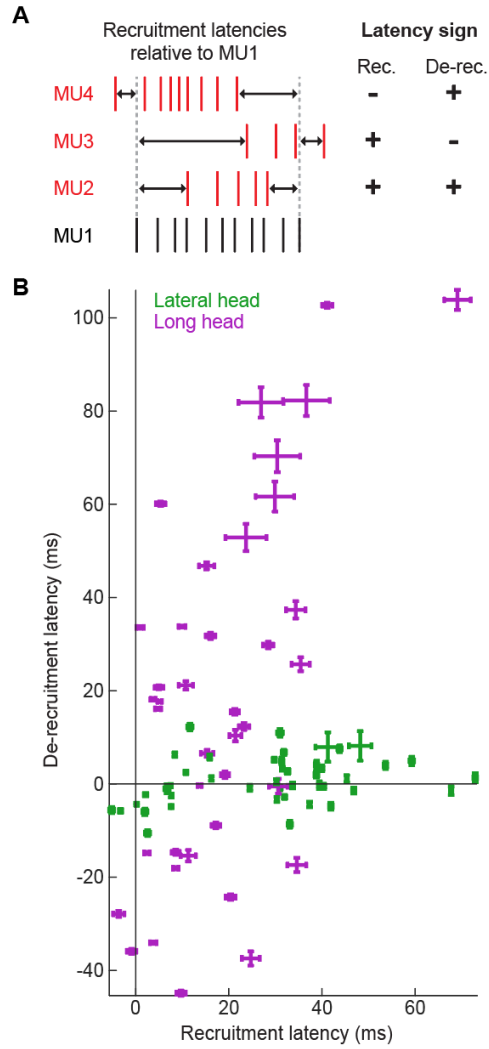


Figure 4.1. Recruitment and de-recruitment latencies across unit pairs. **(A)** Description of how latencies are measured between units to demonstrate spike patterns resulting in either positive or negative latencies. **(B)** Mean latencies with standard error for all simultaneously recorded unit pairs. Units within each pair were ordered by their mean first spike time and both latencies were measured with respect to this ordering.

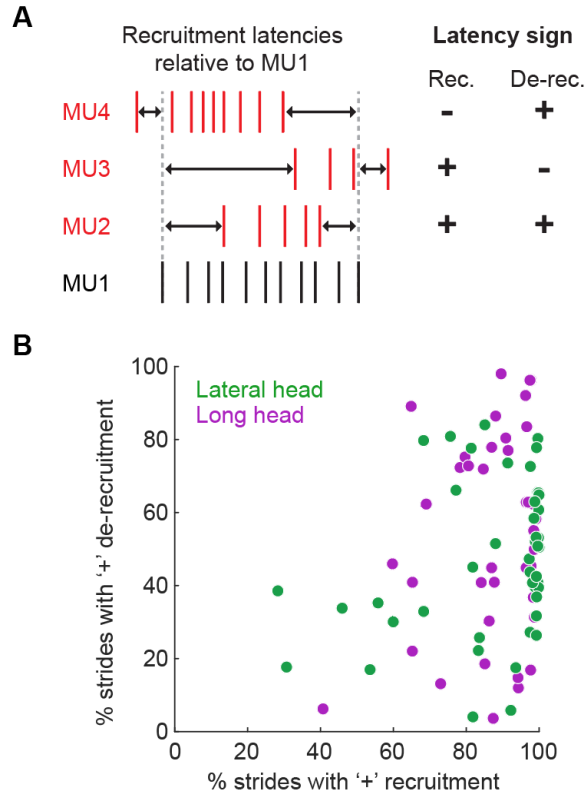


Figure 4.2. Percentages of strides with positive motor unit recruitment and de-recruitment latencies. **(A)** Description of how latencies are measured between units to demonstrate spike patterns resulting in either positive or negative latencies (same as Figure 4.1A). **(B)** Each dot reflects one pair of simultaneously recorded motor units and percentage of strides with positive ('+') recruitment and de-recruitment.

Motor units from the long and lateral heads also displayed muscle-dependent magnitudes in their recruitment and de-recruitment latencies (Figure 4.1B). Motor units in the long head had smaller recruitment latencies than those in the lateral head ($p < 0.01$, two-sample k-s test), which could be important for rapidly producing force to re-distribute weight at footstrike. Conversely, units in the lateral had a different distribution of de-recruitment latencies ($p < 0.01$, two-sample k-s test), likely reflecting the functional role of the lateral head in producing force towards liftoff. This result is particularly striking given the lack of significant pattern in de-recruitment order. Even if de-recruitment is less

important to the behavior for this reason, the difference in trends across muscles and for individual unit pairs suggests some level of control.

4.4 Motor unit coordination correlations with walking speed and kinematics

Faster walking speeds are correlated with greater recruitment of the motor pool (Figure 3.7), but it is unclear how the broader coordination behind population-level motor unit recruitment or de-recruitment would change. At faster speeds, limb muscles more rapidly contract and relax, potentially through altered coordination patterns in the motor pool that support faster force generation, such as reversals to recruitment order.

To investigate the changes in motor unit coordination underlying locomotor speed control, we quantified how relative motor unit firing patterns change at different walking speeds (Figure 4.3). After splitting all strides into quartiles based on the locomotor speed (Figure 3.2D) (K. Thomas et al., 2025), we again calculated the recruitment and de-recruitment latencies for each unit pair within each quartile. Comparing the slowest and fastest quartiles, motor units in the long and lateral heads showed different changes in coordination patterns depending on the muscle. In pairwise comparison of motor units in the long head, the mean de-recruitment latency decreased (Figure 4.3A,C, $p < 0.001$, two-sample k-s test). However, both the recruitment and de-recruitment latency in lateral head units decreased, with a greater magnitude difference in the recruitment latency (Figure 4.3B,C, $p < 0.001$, two-sample k-s test).

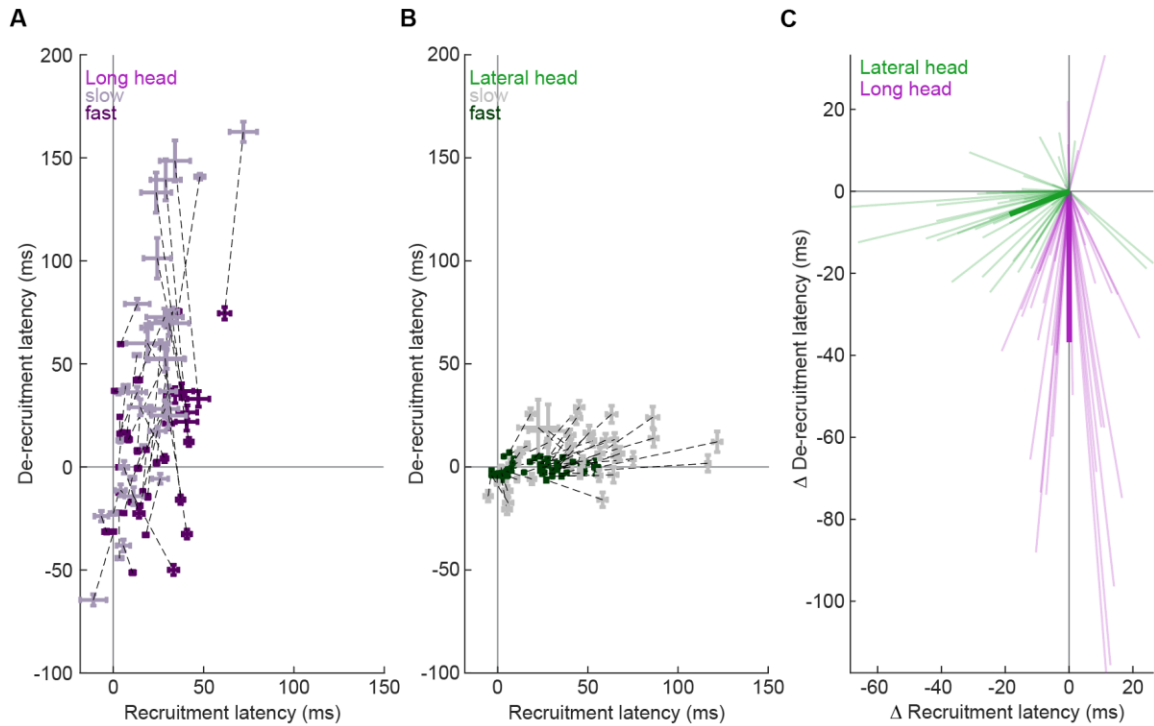


Figure 4.3. Recruitment and de-recruitment latencies change across locomotor speeds. Strides were split into quartiles based on body velocity. Latencies were calculated in the fastest and slowest speeds for motor unit pairs in the (A) long head and (B) lateral head. Each point reflects the mean \pm S.E. of the recruitment and de-recruitment latency in both quartiles and points from the same motor unit pair are connected, showing the difference between the slowest (grayed) and fastest (shaded) strides. (C) Difference in recruitment and de-recruitment latencies for each motor unit pair are represented as vectors offset at the origin. Light traces denote each motor unit pair in either the long or lateral head. Heavy traces denote the mean vector for all pairs within a muscle.

These different adjustments between recruitment and de-recruitment latencies within a muscle highlight further that recruitment and de-recruitment are separately controlled processes. Taken together, these results suggest that the nervous system adjusts speed through muscle-dependent motor unit coordination patterns.

Given the stride-by-stride variability in recruitment and de-recruitment order (Figure 4.2) and the change in pairwise timing across speed (Figure 4.3), we next examined

whether the order motor units were recruited and de-recruited in each stride correlated with systematic differences in movement. We split all strides into groups based on recruitment and de-recruitment order for each motor unit pair. Order was denoted by sign of the latency, agreeing with our earlier definition (Figure 4.1A).

We first identified how body velocity correlated with recruitment and de-recruitment order. Faster body velocities were correlated with positive recruitment latencies in the long head (Figure 4.4B, $p < 0.004$, Wilcoxon signed-rank tests with Bonferroni correction). This correlation was not evident in motor unit pairs in the lateral head. This was unexpected given that the lateral head demonstrated a larger, systematic decrease in recruitment latency across speeds (Figure 4.3B). Additionally, body velocity decreased with positive de-recruitment latencies in both the long and lateral head (Figure 4.4B, $p < 0.004$, Wilcoxon signed-rank tests with Bonferroni correction). These results may reflect that faster movements require more rapid activation of the motor pool, potentially with preference towards motor units that generate more force, leading to reversed recruitment and de-recruitment order within a stride.

In Chapter 3, we identified that motor unit recruitment correlated with changes in elbow extension and elbow angular velocity, suggesting that recruitment in a single motor unit shapes the kinematics behind each stride (Figure 3.9). Still, it is unclear how much a single motor unit can independently move the limb given the need for muscle twitch force to overcome inertial force of the limb. Thus, our previous results might reflect broader motor unit recruitment patterns in the population. Therefore, we next examined whether recruitment and de-recruitment order correlated with stride-by-stride variations in elbow movement. Interestingly, neither recruitment order nor de-recruitment order correlated

with the elbow extension (Figure 4.4C, $p > 0.004$, Wilcoxon signed-rank tests with Bonferroni correction). In the long head, positive recruitment latencies correlated with an increase in the elbow angular velocity while positive de-recruitment latencies correlated with a decrease in the elbow angular velocity (Figure 4.4D, $p < 0.004$, Wilcoxon signed-rank tests with Bonferroni correction). However, these muscle-specific differences were not present when each speed quartile was investigated independently rather than when strides from all speed quartiles were pooled together. Taken together, these results suggest that specific recruitment and de-recruitment patterns may be implemented towards producing certain kinematic features within the stride. Furthermore, these patterns may consist of reversed order for recruitment and de-recruitment.

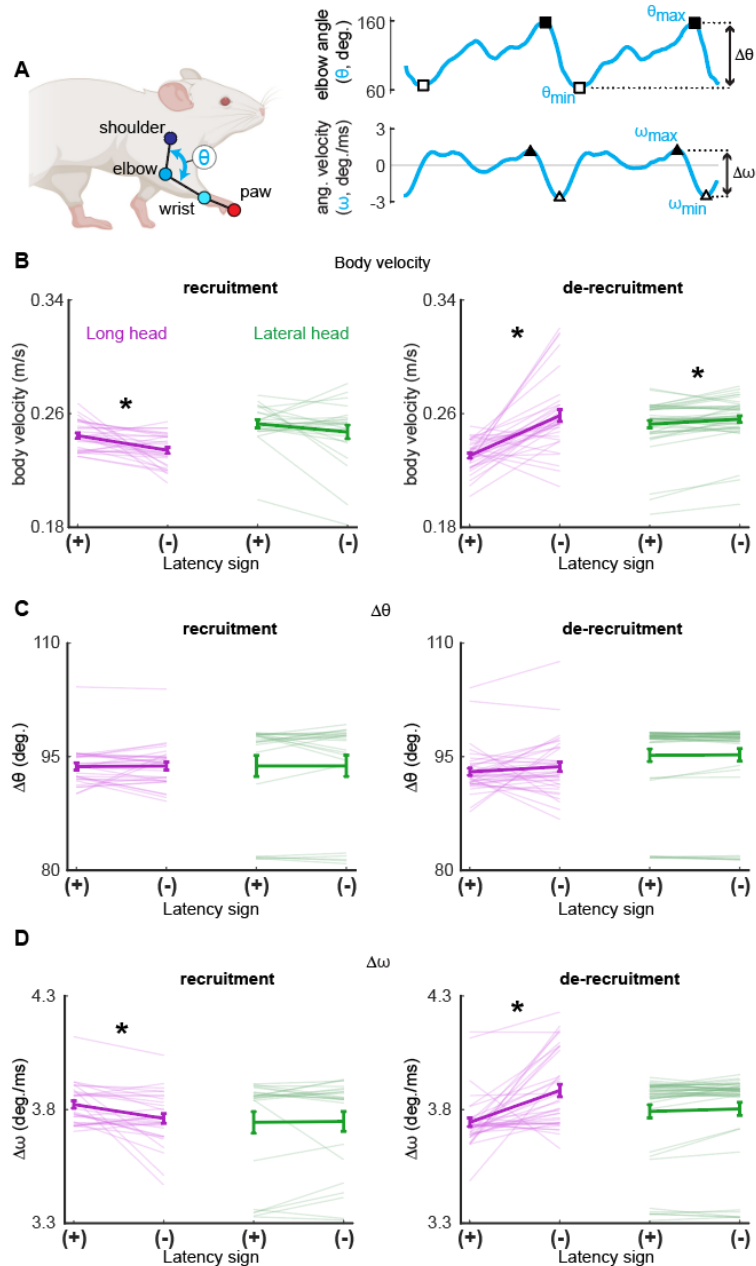


Figure 4.4. Motor unit recruitment and de-recruitment order influence body kinematics. **(A)** We calculated ranges for body velocity, elbow angle ($\Delta\theta$) and elbow velocity ($\Delta\omega$) on strides in which motor unit pairs demonstrated positive or negative latencies in either their relative recruitment or de-recruitment. **(B)** Body velocity for each motor unit pair when strides were split based on the sign of latency in recruitment (left) or de-recruitment (right). Light traces denote values for motor unit pairs while the heavy trace shows the mean (\pm SE) across all unit pairs. Starred traces demonstrate either statistically significant increases or decreases in each parameter ($p < 0.004$, Wilcoxon signed-rank tests with Bonferroni correction). **(C,D)** Same as above for ranges in elbow angle and elbow velocity respectively.

4.5 Motor unit recruitment may decrease firing rate in currently active units

Motor units are functionally coupled through various neural circuits that provide feedback to the homonymous motor pool when a motor unit spikes. It is unclear if and how this feedback alters population-level activity in the motor pool during behavior. Although unable to directly manipulate these circuits within this study, we tested potential functional coupling within the population by measuring a motor unit's change in instantaneous firing rate (IFR) when an additional unit was recruited (Figure 4.5A). Changes in the firing rate, particularly if identified systematically within strides, could help uncover the role of functionally coupling circuits during locomotor behavior.

Motor units in both the long and lateral head had significantly lower firing rates upon recruitment of an additional unit (Figure 4.5B,C, $p < 0.001$, two-sample k-s test). Motor units in the long head demonstrated a larger decrease of about 80 Hz from an apparent steady state while motor units in the lateral head decreased by about 40 Hz. In the lateral head, firing rates seemed to already be decreasing prior to the second unit's recruitment, potentially reflecting the rhythmic input to motor pools that rises and falls independent of motor unit feedback. If this was the sole reason for the decrease in firing rates, we would have expected firing to continually decrease regardless of the second unit's recruitment. Instead, after the recruitment of the second unit, firing rates slightly increased in both the long and lateral heads, supporting our conclusion that the decrease could be attributed to the unit's recruitment.

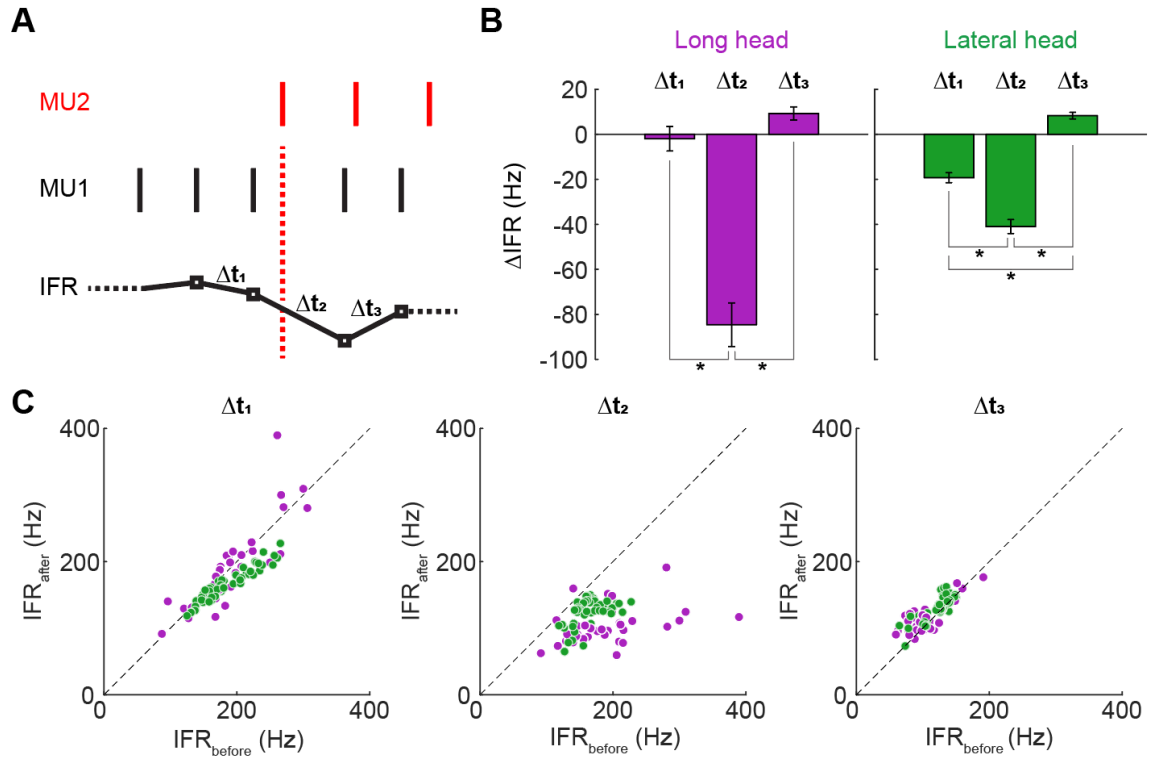


Figure 4.5. Motor unit instantaneous firing rate (IFR) decreases with second unit recruitment. (A) Schematic illustrating the potential interaction between two motor units (MU). IFR is calculated for each spike in MU1 (except the first spike) as the reciprocal of the inter-spike interval (ISI) between the current and previous spikes. Each relevant segment is labeled as Δt for comparing the difference in IFR over time (B) Difference in IFR (ΔIFR) measured for consecutive spikes within MU1 relative to the onset of MU2. The median ΔIFR was first measured across all strides for each motor unit pair at the timepoints depicted in (A). Bars in each figure corresponding to each timepoint denote the mean \pm SE of this measurement across all motor unit pairs. The changes in firing rate across groups were tested for statistical significance (starred points, $p < 0.001$) using the two-sample k-s test. (C) Median IFR calculated at spikes before and after each timepoint. Each point reflects this value for a given motor unit pair.

4.6 Discussion

Our data reveal how motor unit pairs in the triceps brachii muscles are coordinated during walking. Focusing on the first and last spikes (recruitment and de-recruitment respectively) that a motor unit fires in each stride, we identified that motor unit pairs were

recruited in a systematic order that did not necessarily predict the de-recruitment order on a stride-by-stride basis. Furthermore, coordination in recruitment/de-recruitment varied across muscles, highlighting the potential for different neural control strategies used for each muscle. These differences became more apparent across walking speeds, which correlated with unique shifts in the coordination patterns of each muscle. Lastly, there seemed to be functional coupling in the motor pool in which motor unit recruitment may lead to a decrease in firing rate of already active units. As discussed below, interpretations of pairwise motor unit coordination during walking reflect the broader neuromuscular control of the body, allowing us to infer how the nervous system drives locomotor behavior.

4.6.1 Recruitment order has systematic patterns that reflect the behavior

Recruitment order in both the long head and lateral head had systematic patterns. Past reports typically estimate the relative size of the motor unit through its amplitude. It generally follows that the smaller amplitude units are recruited prior to the large amplitude units in accordance with the size principle (Feiereisen et al., 1997; Jones et al., 1994; Zajac & Faden, 1985). Given that we recorded multi-channel EMG data using Myomatrix electrodes, the amplitude, which varies across channels based on proximity to active muscle fibers, does not necessarily reflect the size of the motor unit. Although, some correlations may exist between amplitude and firing patterns (Figure 4.6). To more generally describe recruitment order in a meaningful way, we set an initial order based on the average time a unit fires its first spike within the stride. This enabled us to observe recruitment order based on the onset latency between two units in each stride.

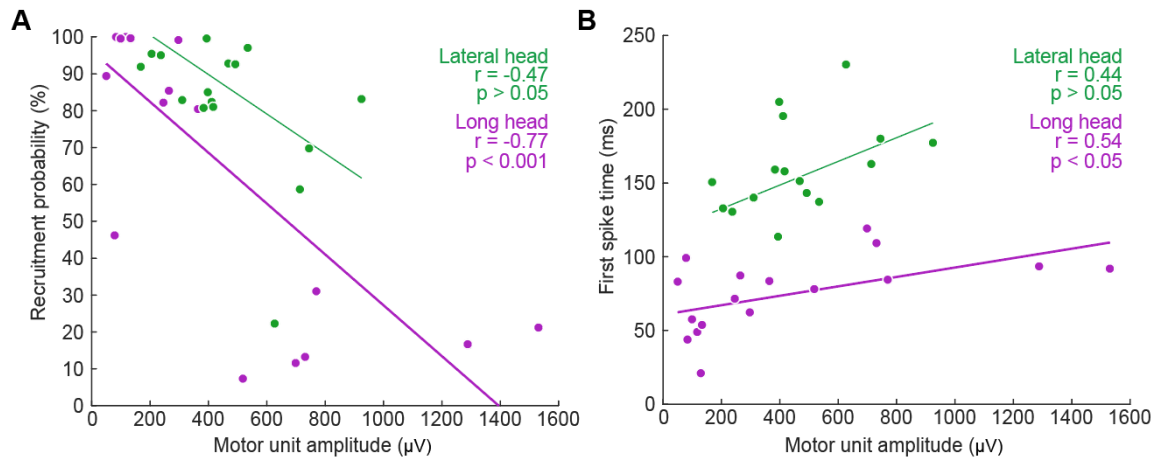


Figure 4.6. Relation between motor unit amplitude and (A) recruitment probability and (B) mean first spike time within the stride. Correlation coefficients were calculated to identify trends in motor units of each muscle.

On a stride-to-stride basis, recruitment order had some variability (Figure 4.1, Figure 4.2). It was still largely biased towards a particular order, but motor unit pairs in individual strides were at times recruited in the opposite order. One compelling explanation is that motor units are recruited within task groups rather than by the entire motor pool, in which each task group consists of a subset of the motor units within and/or across muscles (G. E. Loeb, 1985; Riek & Bawa, 1992; T. C. Cope & Sokoloff, 1999; P. N. S. Bawa et al., 2014). Within each group, motor units would still be recruited in an order that reflects their force production in accordance with the size principle. Still, it is ambiguous how to ascribe motor neurons to a particular task group and how to delineate which task groups might be active at a time. Task groups can perhaps be identified when firing patterns shift based on the behavior. For example, different isometric force directions can elicit a change in apparent recruitment order (Desnedt & Gidoux, 1981; Ter Haar Romeny et al., 1982; C. K. Thomas et al., 1987).

We evaluated the potential that task groups might reflect a change in recruitment patterns by identifying if recruitment order correlated with specific limb and body kinematics. At faster speeds, motor units in the lateral head but not long head were recruited with shorter latencies (Figure 4.3). Still, we did not identify correlations between the order of recruitment in the lateral head and either speed or elbow movements (Figure 4.4). In contrast, when the recruitment order was reversed for the long head, both the body speed and elbow angular velocity were lower (Figure 4.4). These differences in behavior on a stride-by-stride basis could utilize a different strategy in motor unit patterning reflective of task groups. Additionally, task groups might reflect the anatomical and functional heterogeneity of different muscles, supporting our observations across the long and lateral heads. Muscles, particularly biarticular muscles such as the long head, can have subcompartments of muscle fibers that are active towards specific behaviors. For example, in the cat biceps femoris, the anterior component is active during stance as a hip extensor while the posterior component is active during swing as a knee flexor (Chanaud et al., 1991; English & Weeks, 1987). Although no studies have investigated anatomical subdivisions of muscle in either the long or lateral heads of the mouse triceps brachii, it is feasible that the long head has greater anatomical diversity that could be utilized by the nervous system to adjust behavior.

It is difficult to discern from these analyses alone just how flexibly the nervous system may control motor unit recruitment patterns towards behavior. Past studies have similarly identified changes in recruitment patterns based on the intensity of movement (Wakeling, 2004), rate of force development (Marshall et al., 2022), or fatigue in the motor pool (Enoka et al., 1989; Gorassini et al., 2002; Manning et al., 2010). Additional evidence

suggests that cortically mediated circuits may allow higher dimensional control of individual motor units (Marshall et al., 2022). Implications of more independent control are assumed to relate to the force-production needs of different tasks. For example, since faster units produce more force, it makes sense that rapid movements would benefit from recruiting these units quickly. Still, the question remains: can we identify the relevant scale of control for motor unit activation across behaviors? Proper evaluation of this question likely requires simultaneous measurements from nearly all motor units within a motor pool over a wide range of movements. Additionally, rather than artificially selecting individual kinematic parameters, the relevant ‘task’ could be identified through a more continuous representation of whole-body kinematics.

4.6.2 De-recruitment patterns demonstrate difference from recruitment order

Contrary to the recruitment order, de-recruitment did not demonstrate a significant pattern (Figure 4.1, Figure 4.2), suggesting that motor unit de-recruitment is an active process with differences from neural circuits driving recruitment. If a common drive was the only influence towards motor unit firing, it would be expected that the earliest recruited motor units would also be the latest de-recruited (Hodson-Tole & Wakeling, 2009). However, motor unit pairs in both the long and lateral heads had a mix of positive and negative de-recruitment latencies, even on a stride-by-stride basis, suggesting no specific strategy (Figure 4.1, Figure 4.2). Past studies have suggested differential neural control during contractions and relaxations based on the different force thresholds at recruitment and de-recruitment (Romaiguère et al., 1993). Indeed, this may explain reversals in de-recruitment order (Broman et al., 1985; Eken, 1998; Gorassini et al., 2002; Sokoloff &

Cope, 1996). The more variable de-recruitment ordering likely reflects the neural circuits and rhythmic movement.

De-recruitment of active motor neurons during locomotion likely relies on inactivation of persistent inward currents (PICs) rather than just a decrease in common drive input. Excitatory inputs that initially recruit units in each stride activate PICs that raise the intrinsic excitability of the cell (Heckman et al., 2005; Huh et al., 2017). From just brief inputs, PICs initiate self-sustained firing in the motor neuron (Hounsgaard et al., 1988). This leads to hysteresis in which both firing threshold and firing frequency are different at the time of recruitment and de-recruitment (Heckman et al., 2005; Romaguère et al., 1993). The timescale of this sustained activity for both low and high-threshold motor neurons is on the order of seconds although each stride for the mouse happens within 500ms. Thus, it is important that PICs be inactivated to support rapid, rhythmic activity.

Motor unit de-recruitment during locomotion may be mediated by reciprocal inhibition (Johnson et al., 2017). The PIC amplitude scales with inhibitory synaptic input, particularly from 1a reciprocal inhibition (Heckman et al., 2005; Hyngstrom et al., 2008) as the antagonist muscle stretches (Heckman, Hyngstrom, et al., 2008; Hyngstrom et al., 2007). During the stance phase when the long and lateral heads of the triceps are active, the elbow angle gradually increases, stretching the biceps and activating its 1a sensory neurons. The biceps is maximally stretched right before swing, which is when the lateral head is generally de-recruited (Figure 3.5B). Larger reciprocal inhibition at this moment may also explain why motor units in the lateral head were de-recruited with more temporal precision (within 15 ms) as opposed to units in the long head, which are de-recruited partway through stance (Figure 4.1). Furthermore, since the amplitude of inhibition scales

with the speed of the stretch, this may also explain why de-recruitment latencies in both the long and lateral head decreased at faster walking speeds (Fig. 3).

4.6.3 Motor unit firing patterns are functionally coupled during locomotion

To investigate functional coupled spike patterns between motor units, we compared the firing rates of motor units before and after additional units were recruited during a stride. We found that firing rates significantly decreased when the additional unit was recruited (Figure 4.5). This work, which is the first to identify these results both in the intact mouse and during locomotion, agrees with past studies across species (Broman et al., 1985; De Luca, 1985; Eken, 1998; Sokoloff & Cope, 1996). These findings point to potential roles of neural circuits in locomotor behavior that may be important for finely tuning force outputs and providing a smoother force output as more of the population is recruited (Broman et al., 1985; De Luca, 1985).

Spike patterns within the motor pool may be partly organized by anatomical and functional connections across motor units. Recurrent inhibition through Renshaw cells, a class of spinal cord interneurons that receive input from motor neuron axon collaterals and provide inhibition to homonymous motor neurons, may explain our observed decrease in firing rate upon motor unit recruitment (Alvarez & Fyffe, 2007; Renshaw, 1941). Although the role of Renshaw cells during behavior is poorly understood (Alvarez & Fyffe, 2007), past studies in anesthetized and de-cerebrate preparations have found that recurrent inhibition both arises from motor unit spiking (Ross et al., 1975) and modulates the firing patterns of the homonymous motor pool through recurrent inhibitory postsynaptic potentials (RIPSPs) (Noga et al., 1987; Obeidat et al., 2014). Furthermore, slow motor units

have larger RIPSPs (Eccles et al., 1961; Friedman et al., 1981), highlighting that recurrent inhibition is non-uniform across the motor pool. This suggests that as fast motor units are recruited during a behavior, the activity in slower motor units would more sharply decrease, potentially supporting rapid force generation. This well explains the decrease in firing rate we observed upon a second unit's recruitment.

Muscle spindles providing excitatory feedback to the spinal cord may also explain the functional coupling between units. Group Ia muscle fibers excite the homonymous motor pool in response to lengthening of the muscle (stretch reflex) (Conway et al., 1987). Single twitch contractions from motor units, which partly shorten the muscle, have been demonstrated to modulate the responses of Ia fibers, which in turn lowers the net excitation to the motor pool (Binder & Stuart, 1980). This would again explain our results as each newly recruited unit lowers the activity of currently firing units. However, this explanation does not explain how the firing rate in the first recruited unit would decrease while the rate in the second unit continues to increase (De Luca, 1985). Additionally, it is unclear how impactful this effect will be during locomotion, in which the muscle is rhythmically lengthening and shortening to a larger extent.

CHAPTER 5. CONCLUSIONS AND FUTURE DIRECTIONS

The studies presented in this thesis utilize novel Myomatrix technology to demonstrate how motor units are coordinated during locomotion. Taking advantage of high-resolution EMG recordings and kinematic analysis, I provided evidence that motor units are probabilistically recruited on a stride-by-stride basis. Additionally, the recruitment of each unit within the stride seemed to be a key factor in tuning speed along with the other kinematics of each step. Pairwise analysis of motor unit recruitment revealed that populations of motor units were recruited with a systematic order that reflected the kinematics of each stride. However, de-recruitment did not have a similar pattern. These results are important to the fields of neuroscience and neurotechnology, both setting up new questions for basic science research and showcasing Myomatrix electrodes as an ideal tool to answer these questions. The following chapter provides additional discussion on the presented results and posits how future work might build stronger conclusions on the neural control of locomotion.

5.1 How is motor unit coordination shaped by the task-specific roles of muscles?

We identified that motor unit firing patterns were dramatically different across the closely related long and lateral heads of the triceps. For example, motor units in the long head were active earlier in the stance phase than units in the lateral head (Figure 3.5B). Additionally, motor units in the long head seemed to be recruited with immediate faster rates. Compared to motor units in the lateral head, units in the long head had shorter inter-spike-intervals (ISIs) directly upon recruitment (Figure 3.6B) along with shorter latencies in the onset times of simultaneously active units (Figure 4.1B). Since motor unit firing

patterns result from neural inputs, we can use these results to speculate how the nervous system controls muscles to accomplish different movements.

One possible biological basis for different spike patterns across triceps heads is that each muscle receives unique inputs from the nervous system. This differential neural drive may relate to the functional roles for each muscle in the triceps brachii. The triceps brachii includes three muscles – the long, medial, and lateral heads – which have distinct skeletal attachments and joint actions (Tata Ramalingasetty et al., 2021). Across species, studies of motor neurons often consider the whole of the triceps brachii as the relevant functional unit (Qi et al., 2022; Tosolini & Morris, 2012; J. M. Wilson et al., 2015). Our results, which demonstrate the unique firing patterns of the long and lateral heads, suggest that this approach may be limiting. The different roles of each muscle may be related to different neural control of each muscle. One study in the rats triceps brachii did identify that motor neurons supplying each head of the triceps brachii are located in overlapping, but distinct regions of the spinal cord (Lucas-Osma & Collazos-Castro, 2009). Although anatomical location does not provide direct evidence of the inputs into the neurons, this spatial mapping could suggest that the motor pools receive some degree of both shared and independent inputs. This seems to agree with our results as the long and lateral heads were both active during the stance phase but reached their peak firing rates at different times.

We also found that motor units were probabilistically recruited on a stride-by-stride basis (Figure 3.3), potentially reflecting the flexible control of the muscle and limb during movement. The muscles observed in these studies, the triceps brachii long and lateral head, are elbow extensors of the forelimb. The forelimb is important for driving flexible locomotion, contributing to gait strategies (Bellardita & Kiehn, 2015; Machado et al.,

2015), shaping turning behavior (Cregg et al., 2020), and potentially setting the rhythm for novel walking strategies (Darmohray et al., 2019). Several studies suggest that motor units within a muscle may be recruited in a task-dependent manner (G. E. Loeb, 1985; Riek & Bawa, 1992; T. C. Cope & Sokoloff, 1999; P. N. S. Bawa et al., 2014). Small differences in the direction of applied force can correlate with different firing patterns (Desnedt & Gidiaux, 1981; Herrmann & Flanders, 1998; C. K. Thomas et al., 1987). We found that the probabilistic recruitment across the motor pool correlated with the elbow angle and body speed during each stride (Figure 3.9). The recruitment order in pairwise motor units, while mostly consistent, had some variability across strides (Figure 4.2) and correlated with body speed and elbow angular velocity (Figure 4.4). Still, given the various roles of the forelimb in controlling locomotion, it is likely that alternative kinematic and kinetic stride parameters could better predict motor unit firing patterns.

Due to potential differences in motor unit coordination across tasks, it is unclear whether our observations would be consistent for different behaviors. Notably, the forelimb also plays a critical role in non-locomotor behavior, including reaching, feeding, and grooming (Brooks & Dunnett, 2009). Different circuits across the CNS are implicated in specific behaviors, and these circuits converge to motor units in unique ways that may influence firing patterns. For example, chemical inhibition of the lateral rostral medulla in the mouse brainstem leads to poor reaching and handling movements (Ruder et al., 2021). Interestingly, this perturbation had no effect on the mouse's locomotor activity (Ruder et al., 2021). However, this result was based on the total movement of the animal, so it is unclear how motor unit coordination, particularly in spike timing variability (Srivastava et al., 2017; Sober et al., 2018), may have been affected.

To better understand how motor unit firing patterns in muscles across the body coordinate across tasks, future work should investigate motor unit coordination during treadmill locomotion compared to locomotion on a narrow beam. Data from collaboration with Dr. Graziana Gatto has demonstrated that narrow beam walking is correlated with less variability in ankle trajectories, suggesting more precise movement (Figure 5.1) (Gatto & Sober, *in prep.*). Additionally, principal component analysis was used to reduce dimensionality across all observed kinematics, identifying a more systematic difference between beam walking and treadmill locomotion. This difference could be exploited to investigate motor unit coordination across a flexible behavior with different demands in precision. Comparing across the tasks, we might observe that the same motor units are recruited with different likelihoods. Alternatively, we may see that the increased behavioral precision lowers the variance around a unit's recruitment or time of activation.

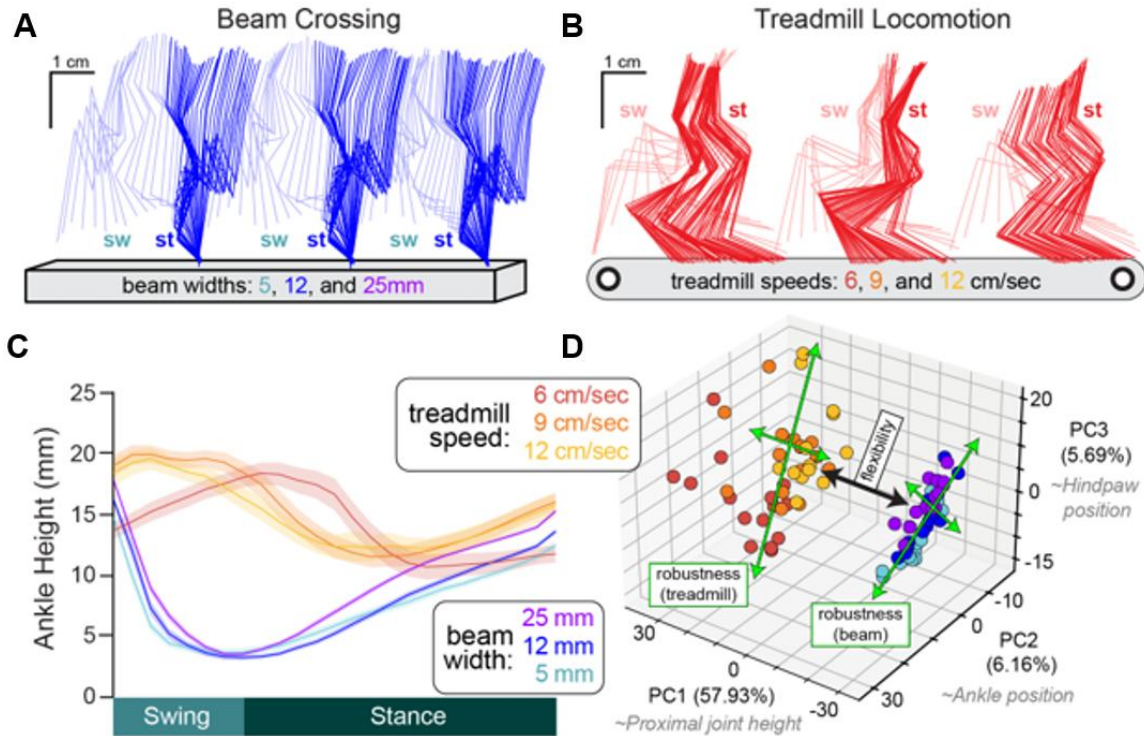


Figure 5.1. Comparison of beam walking and treadmill locomotion in the mouse. Hindlimb trajectory in consecutive (A) beam walking and (B) treadmill locomotion strides with highlighted swing (sw) and stance (st) phases. (C) Ankle height trajectories (mean \pm SE) for different treadmill speeds and beam widths. (D) Principal component analysis of body and limb kinematics across the two behaviors. Each axis reflects a principal component along with the amount of variance it explains and its predominant kinematic feature. Locomotor flexibility reflects the ability to adjust gait strategy while locomotor robustness reflects the ability to maintain a given strategy for each stride. Adapted from Gatto & Sober, in prep.

5.2 How does speed influence motor unit coordination?

Rhythmically active motor units must adjust their firing patterns to enable faster locomotion as limbs move more rapidly and with potentially differently gait strategy. Our results suggested that motor units in the triceps are recruited with greater probability and faster rate as the locomotor speed increases (Figure 3.7). In the presented experiments, mice ran on a treadmill moving between 0.1 and 0.3 m/s. This range of speeds was selected

because mice were able to comfortably and consistently move at these speeds while implanted and tethered with recording equipment. Within our studies, mice mostly trotted or walked but rarely galloped or bounded as these gait strategies are most prevalent at faster speeds up to 1 m/s (Bellardita & Kiehn, 2015). Hierarchically organized neural circuits in the central nervous system are involved in setting the locomotor frequency and relative limb patterns across speeds (Kiehn, 2016; Arber & Costa, 2018; Gatto & Goulding, 2018; Leiras et al., 2022). These circuits converge on motor units, driving and modulating their rhythmic activity to support locomotion across varying contexts and environments. Thus, our observations of greater recruitment and rate reflect these upstream circuits and offer potential explanations on how the nervous system controls speed.

The increase in recruitment probability and firing rate at faster speeds likely reflected the presence of neuromodulators such as serotonin. Serotonin released from the brainstem raises the excitability of motor neurons and enhances persistent inward current responses that lead to sustained firing from the motor neuron (Binder et al., 2020; Heckman et al., 2005, 2009). Serotonergic neurons in the raphe nucleus are active during locomotion and their firing rate scales with locomotor speed over a range of at least 0.1 – 0.5 m/s (Fenstermacher et al., 2024). Therefore, it is likely that our observations of motor unit firing patterns were influenced by serotonin-mediated excitability in the motor neuron. This mechanism may explain how firing rates and recruitment probabilities increased at faster speeds. Reports have also shown that real-time activation of these serotonergic neurons induces locomotor bouts in which the maximum speed exceeds 0.4 m/s (Fenstermacher et al., 2024). Since this exceeds the experimental speeds we observed, it seems that there was still room for greater neuromodulation. Ultimately, the nervous system's capacity to scale

concentrations of neuromodulators such as serotonin is likely a key mechanism to tune motor unit activation patterns across speeds. Future studies that measure motor unit spike patterns while manipulating serotonergic neuron activity could better identify the role of neuromodulation during locomotor behavior.

We also identified that motor unit recruitment correlates with differences in the elbow movement and velocity during the stride (Figure 3.9, Figure 4.4). Although these correlations were mostly independent from movement speed, we did not examine if there was a broader change in gait strategy associated with the different recruitment patterns. Mice can flexibly adjust gaits on a stride-by-stride basis during locomotion given their ability to achieve a wide range of speeds through different strategies (Gonçalves et al., 2022; Machado et al., 2015). Different strategies, particularly those that result in different sets of paws simultaneously on the ground, have the potential to dramatically change motor unit patterning. For example, if just the right forepaw is on the ground, the triceps must generate more force to support more of the body weight than if all the paws are on the ground. For each mouse in our experiments, we split their strides into four quartiles based on the body velocity during the strides. This scheme of normalization made it possible to compare slow against fast strides across all animals regardless of their actual speed (although we still demonstrated that speed ranges in each mouse's quartile were relatively similar). However, this method likely combined changes in both the limb frequency and coordination patterns, making it difficult to more precisely identify how motor unit firing drove the faster speed.

Future studies should more thoroughly characterize movement during each stride through a low-dimensional representation of body and limb kinematics (Gonçalves et al.,

2022; Winner et al., 2023). Our current analyses focused mainly on manually selected parameters such as body speed and elbow angle. While we demonstrated how motor unit spike patterns correlate with these measurements, we also noted that several kinematic parameters correlate with speed, making it difficult to directly identify the behavioral impact of motor unit spiking.

This may be critical for identifying how motor unit coordination supports locomotor flexibility, especially considering that different neural circuits may be responsible for setting both locomotor gait and frequency (Danner et al., 2017; Ausborn et al., 2019). Analysis of motor unit firing patterns across various whole-body representations of limb patterns could reveal how both individual motor units and muscles are coordinated during locomotion. This non-discrete categorization of strides may reveal more of the nuances in motor unit coordination than when strides were categorized by speed. For example, we identified that motor units within a muscle were recruited with consistent latencies but de-recruited without systematic order (Figure 4.1). The need for force generation in an elbow extensor muscle like the triceps brachii depends on which limbs are on the ground supporting the body weight. Variation in motor unit recruitment latencies might reflect the precision of that timing. Furthermore, analysis of motor unit pairs from each limb (e.g. recruitment latency between a motor unit from the triceps and a motor unit from the quadriceps) would highlight potential gait-setting neural circuits that set the rhythmic patterns across the body.

5.3 Interrogating motor circuits by manipulating motor unit spike patterns

Motor unit recruitment elicits a muscle twitch and drives force generation in the muscle, potentially leading to movement of the body (Heckman & Enoka, 2012). More

holistically, we found that recruitment of a unit correlates with an increase in locomotor speed (Figure 3.7), difference in elbow kinematics (Figure 3.9), and a decrease in the firing rates of previously active units (Figure 4.5). Still, a limiting factor in the presented work is the inability to examine the causal relationships between firing patterns and behavior.

Directly perturbing motor unit firing patterns would enable us to make stronger conclusions on how motor unit recruitment influences behavior. For example, in the expiratory muscle of anesthetized songbirds, motor units were reliably stimulated with various spike trains to identify how a millisecond scale shift in spike timing affects respiratory behavior (Srivastava et al., 2017). During mouse locomotion, direct activation or inhibition of motor units would clarify how unit recruitment shapes behavior and firing patterns across units. Furthermore, specific manipulation of slow vs. fast motor units would allow for stronger conclusions on how the physiology of each motor unit influences its functional role.

Novel Myomatrix technology developed in our lab combined with genetic tools in the mouse offers a promising approach for such perturbations. Opto-Myomatrix arrays are a new class of Myomatrix flexible multi-electrode arrays that allow for simultaneous stimulation and recording of muscle fibers during behavior (Kim et al., 2024; Lu et al., 2024). Through optogenetics (Nagel et al., 2003; Fenno et al., 2011), muscle fibers in transgenic mice can be made to express channelrhodopsin2 (ChR2), a light-sensitive ion channel, that depolarizes the cell when activated by blue light (Bruegmann et al., 2015; Magown et al., 2015; Park et al., 2016). In theory, ChR2 expression can also target slow or fast motor unit types specifically based on their unique genetic identifiers (Gundelach et al., 2020). Opto-Myomatrix arrays contain an μ LED interspersed among the electrode

contacts (Figure 5.2) (Lu et al., 2024). The μ LED integrates well with the array, not limiting either the physical flexibility that supports surgical ease or the recording capability of the electrodes. Controlled with millisecond-scale precision, light from the μ LED activates ChR2 to depolarize and contract local muscle fibers. Preliminary experiments using these devices to optogenetically activate mouse jaw muscles expressing ChR2 has demonstrated that the jaw movement scales with the intensity of emitted light (Kim et al., 2024; Lu et al., 2024).

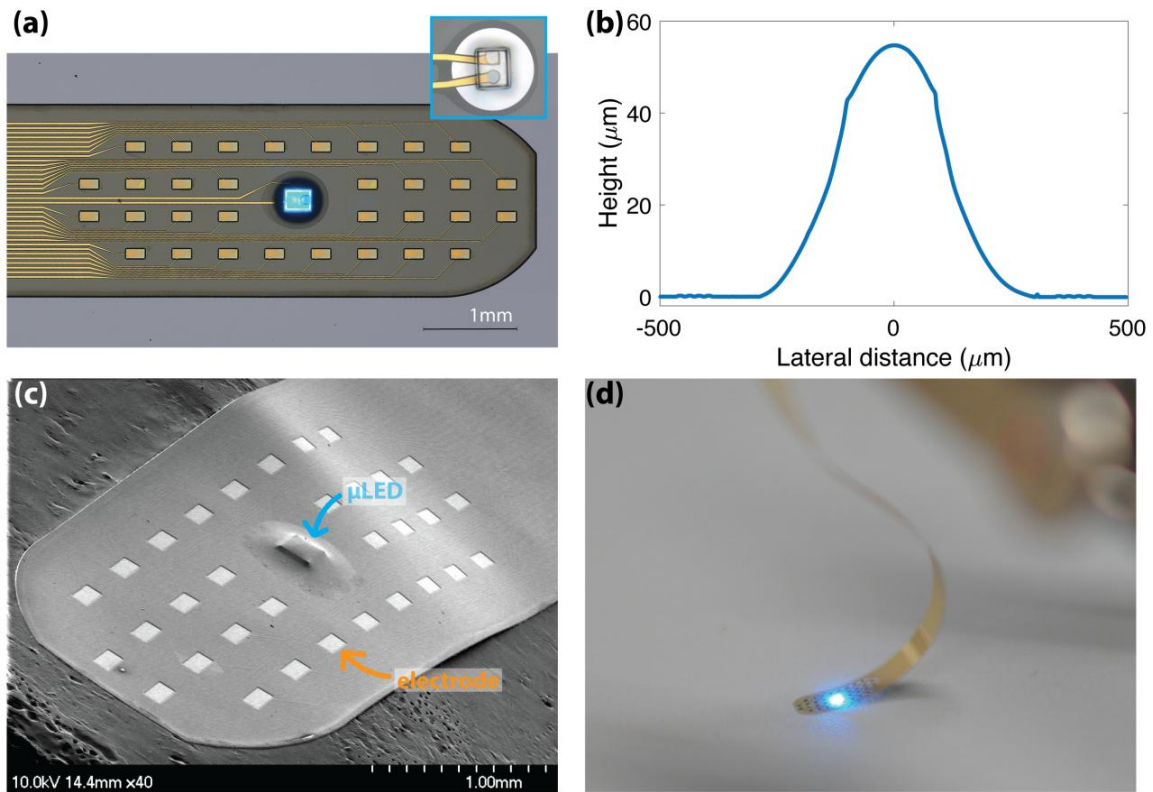


Figure 5.2. Depiction of Opto-Myomatrix flexible multi-channel electrode arrays with integrated μ LED. **(A)** Electrode contacts (gold rectangles) and μ LED (blue square) on Opto-Myomatrix device illustration. The inset shows the μ LED. **(B)** Protrusion of the μ LED from the polyimide array surface. **(C)** Scanning electron microscopy (SEM) image of Opto-Myomatrix and features. **(D)** Opto-Myomatrix arrays are physically flexible, supporting various surgical implementations. Adapted from Lu et al. 2024.

Future experiments could combine real-time kinematic tracking with optogenetic stimulation of the triceps to artificially recruit motor units and determine their impact on behavior. Real-time tracking of the leg angular velocity can be used to identify and predict the stride phase (Quintero et al., 2017). Targeted stimulation of the triceps during stance would increase the recruitment probability of motor units when the muscle is normally active. From here, we could make stronger claims on how body speed and elbow kinematics are tied to motor unit recruitment. Additionally, if we can separate stimulation of slow and fast motor units through optogenetics, we would further identify how motor unit types drive the behaviors given their different force-producing properties. This method would also enable us to specifically activate motor units in the triceps long head or lateral head, allowing us to better determine their roles during behavior. For example, we identified that recruitment in the lateral head was correlated with larger elbow angles during each stride while the long head was correlated with smaller angles (Figure 3.9). Since each muscle is known to extend the elbow, this result may reflect the role of individual motor units in each muscle as the muscle lengthens or shortens through the stance phase. Optogenetic activation of motor units in either muscle throughout the stride cycle would reveal the context dependence for how motor unit recruitment drives behavior.

REFERENCES

- Akay, T., Acharya, H. J., Fouad, K., & Pearson, K. G. (2006). Behavioral and Electromyographic Characterization of Mice Lacking EphA4 Receptors. *Journal of Neurophysiology*, *96*(2), 642–651. <https://doi.org/10.1152/jn.00174.2006>
- Akay, T., Tourtellotte, W. G., Arber, S., & Jessell, T. M. (2014). Degradation of mouse locomotor pattern in the absence of proprioceptive sensory feedback. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(47), 16877–16882. <https://doi.org/10.1073/pnas.1419045111>
- Alessandro, C., Barroso, F. O., Prashara, A., Tentler, D. P., Yeh, H.-Y., & Tresch, M. C. (2020). Coordination amongst quadriceps muscles suggests neural regulation of internal joint stresses, not simplification of task performance. *Proceedings of the National Academy of Sciences*, *117*(14), 8135–8142. <https://doi.org/10.1073/pnas.1916578117>
- Alexander, R. M. (2006). *Principles of animal locomotion*. Princeton University Press.
- Allodi, I., Montañana-Rosell, R., Selvan, R., Löw, P., & Kiehn, O. (2021). Locomotor deficits in a mouse model of ALS are paralleled by loss of V1-interneuron connections onto fast motor neurons. *Nature Communications*, *12*(1), 3251. <https://doi.org/10.1038/s41467-021-23224-7>
- Al-Shuka, H. F. N., Rahman, M. H., Leonhardt, S., Ciobanu, I., & Berteau, M. (2019). Biomechanics, actuation, and multi-level control strategies of power-augmentation lower extremity exoskeletons: An overview. *International Journal of Dynamics and Control*, *7*(4), 1462–1488. <https://doi.org/10.1007/s40435-019-00517-w>
- Alton, F., Baldey, L., Caplan, S., & Morrissey, M. C. (1998). A kinematic comparison of overground and treadmill walking. *Clinical Biomechanics*, *13*(6), 434–440. [https://doi.org/10.1016/S0268-0033\(98\)00012-6](https://doi.org/10.1016/S0268-0033(98)00012-6)
- Alvarez, F. J., & Fyffe, R. E. W. (2007). The continuing case for the Renshaw cell. *The Journal of Physiology*, *584*(1), 31–45. <https://doi.org/10.1113/jphysiol.2007.136200>
- Alvarez, F. J., Pearson, J. C., Harrington, D., Dewey, D., Torbeck, L., & Fyffe, R. E. W. (1998). Distribution of 5-hydroxytryptamine-immunoreactive boutons on alpha-motoneurons in the lumbar spinal cord of adult cats. *The Journal of Comparative Neurology*, *393*(1), 69–83. [https://doi.org/10.1002/\(SICI\)1096-9861\(19980330\)393:1<69::AID-CNE7>3.0.CO;2-O](https://doi.org/10.1002/(SICI)1096-9861(19980330)393:1<69::AID-CNE7>3.0.CO;2-O)
- Ampatzis, K., Song, J., Ausborn, J., & El Manira, A. (2014). Separate Microcircuit Modules of Distinct V2a Interneurons and Motoneurons Control the Speed of Locomotion. *Neuron*, *83*(4), 934–943. <https://doi.org/10.1016/j.neuron.2014.07.018>

- Andrew, P. D. (1985). Motor unit activity under low tensions as muscle changes length. *American Journal of Physical Medicine*, 64(5), 235–254.
- Arber, S., & Costa, R. M. (2018). Connecting neuronal circuits for movement. *Science*, 360(6396), 1403–1404. <https://doi.org/10.1126/science.aat5994>
- Armstrong, D. M., & Edgley, S. A. (1988). Discharges of interpositus and Purkinje cells of the cat cerebellum during locomotion under different conditions. *The Journal of Physiology*, 400(1), 425–445. <https://doi.org/10.1113/jphysiol.1988.sp017130>
- Augusto, V., Padovani, C. R., & Campos, G. E. R. (2004). Skeletal muscle fiber types in C57BL6J mice. *Braz. j. Morphol. Sci*, 89–94.
- Ausborn, J., Shevtsova, N. A., Caggiano, V., Danner, S. M., & Rybak, I. A. (2019). Computational modeling of brainstem circuits controlling locomotor frequency and gait. *eLife*, 8, e43587. <https://doi.org/10.7554/eLife.43587>
- Azevedo, A. W., Dickinson, E. S., Gurung, P., Venkatasubramanian, L., Mann, R. S., & Tuthill, J. C. (2020). A size principle for recruitment of Drosophila leg motor neurons. *eLife*, 9, e56754. <https://doi.org/10.7554/eLife.56754>
- Bączyk, M., Manuel, M., Roselli, F., & Zytnicki, D. (2022). Diversity of Mammalian Motoneurons and Motor Units. In M. J. O’Donovan & M. Falgairolle (Eds.), *Vertebrate Motoneurons* (Vol. 28, pp. 131–150). Springer International Publishing. https://doi.org/10.1007/978-3-031-07167-6_6
- Basmajian, J. V. (1963). Control and Training of Individual Motor Units. *Science*, 141(3579), 440–441. <https://doi.org/10.1126/science.141.3579.440>
- Bastian, A. J. (2008). Understanding sensorimotor adaptation and learning for rehabilitation. *Current Opinion in Neurology*, 129(February), 1–9. <https://doi.org/10.1097/WCO.0b013e328315a293.Understanding>
- Bawa, P., Binder, M. D., Ruenzel, P., & Henneman, E. (1984). Recruitment order of motoneurons in stretch reflexes is highly correlated with their axonal conduction velocity. *Journal of Neurophysiology*, 52(3), 410–420. <https://doi.org/10.1152/jn.1984.52.3.410>
- Bawa, P. N. S., Jones, K. E., & Stein, R. B. (2014). Assessment of size ordered recruitment. *Frontiers in Human Neuroscience*, 8. <https://doi.org/10.3389/fnhum.2014.00532>
- Bellardita, C., & Kiehn, O. (2015). Phenotypic Characterization of Speed-Associated Gait Changes in Mice Reveals Modular Organization of Locomotor Networks. *Current Biology*, 25(11), 1426–1436. <https://doi.org/10.1016/j.cub.2015.04.005>
- Berchtold, M. W., Brinkmeier, H., & Müntener, M. (2000). Calcium Ion in Skeletal Muscle: Its Crucial Role for Muscle Function, Plasticity, and Disease.

- Berg, R. W. (2017). Neuronal Population Activity in Spinal Motor Circuits: Greater Than the Sum of Its Parts. *Frontiers in Neural Circuits*, 11. <https://doi.org/10.3389/fncir.2017.00103>
- Berger, A. J., Bayliss, D. A., & Viana, F. (1992). Modulation of neonatal rat hypoglossal motoneuron excitability by serotonin. *Neuroscience Letters*, 143(1–2), 164–168. [https://doi.org/10.1016/0304-3940\(92\)90257-8](https://doi.org/10.1016/0304-3940(92)90257-8)
- Binder, M. D., Powers, R. K., & Heckman, C. J. (2020). Nonlinear Input-Output Functions of Motoneurons. *Physiology*, 35(1), 31–39. <https://doi.org/10.1152/physiol.00026.2019>
- Binder, M. D., & Stuart, D. G. (1980). Responses of Ia and spindle group II afferents to single motor-unit contractions. *Journal of Neurophysiology*, 43(3), 621–629. <https://doi.org/10.1152/jn.1980.43.3.621>
- Bonnot, A., Whelan, P. J., Mentis, G. Z., & O'Donovan, M. J. (2002). Locomotor-like activity generated by the neonatal mouse spinal cord. *Brain Research Reviews*, 40(1–3), 141–151. [https://doi.org/10.1016/S0165-0173\(02\)00197-2](https://doi.org/10.1016/S0165-0173(02)00197-2)
- Borghese, N. A., Bianchi, L., & Lacquaniti, F. (1996). Kinematic determinants of human locomotion. *The Journal of Physiology*, 494(3), 863–879. <https://doi.org/10.1113/jphysiol.1996.sp021539>
- Bouvier, J., Caggiano, V., Leiras, R., Caldeira, V., Bellardita, C., Balueva, K., Fuchs, A., & Kiehn, O. (2015). Descending Command Neurons in the Brainstem that Halt Locomotion. *Cell*, 163(5), 1191–1203. <https://doi.org/10.1016/j.cell.2015.10.074>
- Bretzner, F., & Brownstone, R. M. (2013). Lhx3-Chx10 Reticulospinal Neurons in Locomotor Circuits. *The Journal of Neuroscience*, 33(37), 14681–14692. <https://doi.org/10.1523/JNEUROSCI.5231-12.2013>
- Britz, O., Zhang, J., Grossmann, K. S., Dyck, J., Kim, J. C., Dymecki, S., Gosgnach, S., & Goulding, M. (2015). A genetically defined asymmetry underlies the inhibitory control of flexor–extensor locomotor movements. *eLife*, 4, e04718. <https://doi.org/10.7554/eLife.04718>
- Broman, H., De Luca, C. J., & Mambrito, B. (1985). Motor unit recruitment and firing rates interaction in the control of human muscles. *Brain Research*, 337(2), 311–319. [https://doi.org/10.1016/0006-8993\(85\)90068-X](https://doi.org/10.1016/0006-8993(85)90068-X)
- Brooks, S. P., & Dunnett, S. B. (2009). Tests to assess motor phenotype in mice: A user's guide. *Nature Reviews Neuroscience*, 10(7), 519–529. <https://doi.org/10.1038/nrn2652>

- Brown, T. G. (1911). The intrinsic factors in the act of progression in the mammal. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character*, 84(572), 308–319. <https://doi.org/10.1098/rspb.1911.0077>
- Brownstone, R. M., & Bui, T. V. (2010). Spinal interneurons providing input to the final common path during locomotion. In *Progress in Brain Research* (Vol. 187, pp. 81–95). Elsevier. <https://doi.org/10.1016/B978-0-444-53613-6.00006-X>
- Bruegmann, T., Van Bremen, T., Vogt, C. C., Send, T., Fleischmann, B. K., & Sasse, P. (2015). Optogenetic control of contractile function in skeletal muscle. *Nature Communications*, 6(1). <https://doi.org/10.1038/ncomms8153>
- Burke, R. E. (1981). Motor Units: Anatomy, Physiology, and Functional Organization. In R. Terjung (Ed.), *Comprehensive Physiology* (1st ed., pp. 345–422). Wiley. <https://doi.org/10.1002/cphy.cp010210>
- Burke, R. E., Degtyarenko, A. M., & Simon, E. S. (2001). Patterns of Locomotor Drive to Motoneurons and Last-Order Interneurons: Clues to the Structure of the CPG. *Journal of Neurophysiology*, 86(1), 447–462. <https://doi.org/10.1152/jn.2001.86.1.447>
- Burke, R. E., Levine, D. N., Tsairis, P., & Zajac, F. E. (1973). Physiological types and histochemical profiles in motor units of the cat gastrocnemius. *The Journal of Physiology*, 234(3), 723–748. <https://doi.org/10.1113/jphysiol.1973.sp010369>
- Burkholder, T. J., Fingado, B., Baron, S., & Lieber, R. L. (1994). Relationship between muscle fiber types and sizes and muscle architectural properties in the mouse hindlimb. *Journal of Morphology*, 221(2), 177–190. <https://doi.org/10.1002/jmor.1052210207>
- Caggiano, V., Leiras, R., Goñi-Erro, H., Masini, D., Bellardita, C., Bouvier, J., Caldeira, V., Fisone, G., & Kiehn, O. (2018). Midbrain circuits that set locomotor speed and gait selection. *Nature*, 553(7689), 455–460. <https://doi.org/10.1038/nature25448>
- Canu, M.-H., & Falempin, M. (1998). Effect of hindlimb unloading on interlimb coordination during treadmill locomotion in the rat. *European Journal of Applied Physiology*, 78(6), 509–515. <https://doi.org/10.1007/s004210050453>
- Capelli, P., Pivetta, C., Soledad Esposito, M., & Arber, S. (2017). Locomotor speed control circuits in the caudal brainstem. *Nature*, 551(7680), 373–377. <https://doi.org/10.1038/nature24064>
- Carey, M. R. (2024). The cerebellum. *Current Biology*, 34(1), R7–R11. <https://doi.org/10.1016/j.cub.2023.11.048>
- Carroll, A. M., & Biewener, A. A. (2009). Mono- versus biarticular muscle function in relation to speed and gait changes: *In vivo* analysis of the goat triceps brachii.

Journal of Experimental Biology, 212(20), 3349–3360.
<https://doi.org/10.1242/jeb.033639>

- Cazalets, J. R., Sqalli-Houssaini, Y., & Clarac, F. (1992). Activation of the central pattern generators for locomotion by serotonin and excitatory amino acids in neonatal rat. *The Journal of Physiology*, 455(1), 187–204. <https://doi.org/10.1113/jphysiol.1992.sp019296>
- Chanaud, C. M., Pratt, C. A., & Loeb, G. E. (1991). Functionally complex muscles of the cat hindlimb: V. The roles of histochemical fiber-type regionalization and mechanical heterogeneity in differential muscle activation. *Experimental Brain Research*, 85(2), 300–313. <https://doi.org/10.1007/BF00229408>
- Chow, R. S., Medri, M. K., Martin, D. C., Leekam, R. N., Agur, A. M., & McKee, N. H. (2000). Sonographic studies of human soleus and gastrocnemius muscle architecture: Gender variability. *European Journal of Applied Physiology*, 82(3), 236–244. <https://doi.org/10.1007/s004210050677>
- Chung, B., Zia, M., Thomas, K. A., Michaels, J. A., Jacob, A., Pack, A., Williams, M. J., Nagapudi, K., Teng, L. H., Arrambide, E., Ouellette, L., Oey, N., Gibbs, R., Anschutz, P., Lu, J., Wu, Y., Kashefi, M., Oya, T., Kersten, R., ... Sober, S. J. (2023). Myomatrix arrays for high-definition muscle recording. *eLife*, 12, RP88551. <https://doi.org/10.7554/eLife.88551>
- Chvatal, S. A., & Ting, L. H. (2012). Voluntary and Reactive Recruitment of Locomotor Muscle Synergies during Perturbed Walking. *The Journal of Neuroscience*, 32(35), 12237–12250. <https://doi.org/10.1523/JNEUROSCI.6344-11.2012>
- Clamann, H. P., Ngai, A. C., Kukulka, C. G., & Goldberg, S. J. (1983). Motor pool organization in monosynaptic reflexes: Responses in three different muscles. *Journal of Neurophysiology*, 50(4), 725–742. <https://doi.org/10.1152/jn.1983.50.4.725>
- Clarke, K. A., Smart, L., & Still, J. (2001). Ground reaction force and spatiotemporal measurements of the gait of the mouse. *Behavior Research Methods, Instruments, & Computers*, 33(3), 422–426. <https://doi.org/10.3758/BF03195396>
- Clarke, K., & Still, J. (2001). Development and consistency of gait in the mouse. *Physiology & Behavior*, 73(1–2), 159–164. [https://doi.org/10.1016/S0031-9384\(01\)00444-9](https://doi.org/10.1016/S0031-9384(01)00444-9)
- Conway, B. A., Hultborn, H., & Kiehn, O. (1987). Proprioceptive input resets central locomotor rhythm in the spinal cat. *Experimental Brain Research*, 68(3). <https://doi.org/10.1007/BF00249807>
- Cope, T. C., & Sokoloff, A. J. (1999). Orderly recruitment among motoneurons supplying different muscles. *Journal of Physiology-Paris*, 93(1–2), 81–85. [https://doi.org/10.1016/S0928-4257\(99\)80138-7](https://doi.org/10.1016/S0928-4257(99)80138-7)

- Cope, T., & Pinter, M. (1995). The Size Principle: Still Working After All These Years. *Physiology*, *10*(6), 280–286. <https://doi.org/10.1152/physiologyonline.1995.10.6.280>
- Corbee, R. J., Maas, H., Doornenbal, A., & Hazewinkel, H. A. W. (2014). Forelimb and hindlimb ground reaction forces of walking cats: Assessment and comparison with walking dogs. *The Veterinary Journal*, *202*(1), 116–127. <https://doi.org/10.1016/j.tvjl.2014.07.001>
- Cregg, J. M., Leiras, R., Montalant, A., Wanken, P., Wickersham, I. R., & Kiehn, O. (2020). Brainstem neurons that command mammalian locomotor asymmetries. *Nature Neuroscience*, *23*(6), 730–740. <https://doi.org/10.1038/s41593-020-0633-7>
- Crone, S. A., Zhong, G., Harris-Warrick, R., & Sharma, K. (2009). In Mice Lacking V2a Interneurons, Gait Depends on Speed of Locomotion. *The Journal of Neuroscience*, *29*(21), 7098–7109. <https://doi.org/10.1523/JNEUROSCI.1206-09.2009>
- Crow, M. T., & Kushmerick, M. J. (1982). Chemical energetics of slow- and fast-twitch muscles of the mouse. *The Journal of General Physiology*, *79*(1), 147–166. <https://doi.org/10.1085/jgp.79.1.147>
- Crowninshield, R. D., & Brand, R. A. (1981). A physiologically based criterion of muscle force prediction in locomotion. *Journal of Biomechanics*, *14*(11), 793–801. [https://doi.org/10.1016/0021-9290\(81\)90035-x](https://doi.org/10.1016/0021-9290(81)90035-x)
- Danner, S. M., Shevtsova, N. A., Frigon, A., & Rybak, I. A. (2017). Computational modeling of spinal circuits controlling limb coordination and gaits in quadrupeds. *eLife*, *6*, e31050. <https://doi.org/10.7554/eLife.31050>
- Darmohray, D. M., Jacobs, J. R., Marques, H. G., & Carey, M. R. (2019). Spatial and Temporal Locomotor Learning in Mouse Article Spatial and Temporal Locomotor Learning in Mouse Cerebellum. *Neuron*, *102*(1), 217–231. <https://doi.org/10.1016/j.neuron.2019.01.038>
- D'Avella, A., Saltiel, P., & Bizzi, E. (2003). Combinations of muscle synergies in the construction of a natural motor behavior. *Nature Neuroscience*, *6*(3), 300–308. <https://doi.org/10.1038/nn1010>
- De Luca, C. J. (1985). Control properties of motor units. *Journal of Experimental Biology*, *115*(1), 125–136. <https://doi.org/10.1242/jeb.115.1.125>
- De Luca, C. J., Adam, A., Wotiz, R., Gilmore, L. D., & Nawab, S. H. (2006). Decomposition of Surface EMG Signals. *Journal of Neurophysiology*, *96*(3), 1646–1657. <https://doi.org/10.1152/jn.00009.2006>
- Deluca, C., & Erim, Z. (1994). Common drive of motor units in regulation of muscle force. *Trends in Neurosciences*, *17*(7), 299–305. [https://doi.org/10.1016/0166-2236\(94\)90064-7](https://doi.org/10.1016/0166-2236(94)90064-7)

- Desnedt, J. E., & Gidoux, E. (1981). Spinal Motoneuron Recruitment in Man: Rank Deordering with Direction But Not with Speed of Voluntary Movement. *Science*, 214(4523), 933–936. <https://doi.org/10.1126/science.7302570>
- DeWolf, T., Schneider, S., Soubiran, P., Roggenbach, A., & Mathis, M. (2024). *Neuro-musculoskeletal modeling reveals muscle-level neural dynamics of adaptive learning in sensorimotor cortex*. <https://doi.org/10.1101/2024.09.11.612513>
- Dickinson, M. H., Farley, C. T., Full, R. J., Koehl, M. A. R., Kram, R., & Lehman, S. (2000). How animals move: An integrative view. *Science*, 288(5463), 100–106. <https://doi.org/10.1126/science.288.5463.100>
- Dideriksen, J. L., Del Vecchio, A., & Farina, D. (2020). Neural and muscular determinants of maximal rate of force development. *Journal of Neurophysiology*, 123(1), 149–157. <https://doi.org/10.1152/jn.00330.2019>
- Drew, T., Kalaska, J., & Krouchev, N. (2008). Muscle synergies during locomotion in the cat: A model for motor cortex control. *The Journal of Physiology*, 586(5), 1239–1245. <https://doi.org/10.1113/jphysiol.2007.146605>
- Earhart, G. M., & Bastian, A. J. (2001). Selection and Coordination of Human Locomotor Forms Following Cerebellar Damage. *Journal of Neurophysiology*, 85(2), 759–769. <https://doi.org/10.1152/jn.2001.85.2.759>
- Eccles, J. C., Eccles, R. M., Iggo, A., & Ito, M. (1961). Distribution of recurrent inhibition among motoneurons. *The Journal of Physiology*, 159(3), 479–499. <https://doi.org/10.1113/jphysiol.1961.sp006822>
- Edgerton, V. R., Smith, J. L., & Simpson, D. R. (1975). Muscle fibre type populations of human leg muscles. *The Histochemical Journal*, 7(3), 259–266. <https://doi.org/10.1007/bf01003594>
- Edman, K. A. (1979). The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres. *The Journal of Physiology*, 291(1), 143–159. <https://doi.org/10.1113/jphysiol.1979.sp012804>
- Eken, T. (1998). Spontaneous Electromyographic Activity in Adult Rat Soleus Muscle. *Journal of Neurophysiology*, 80(1), 365–376. <https://doi.org/10.1152/jn.1998.80.1.365>
- English, A. W. M. (1978). An Electromyographic Analysis of Forelimb Muscles During Overground Stepping in the Cat. *Journal of Experimental Biology*, 76(1), 105–122. <https://doi.org/10.1242/jeb.76.1.105>
- English, A. W. M., & Weeks, O. I. (1987). An anatomical and functional analysis of cat biceps femoris and semitendinosus muscles. *Journal of Morphology*, 191(2), 161–175. <https://doi.org/10.1002/jmor.1051910207>

- Enoka, R. M., & Duchateau, J. (2017). Rate coding and the control of muscle force. *Cold Spring Harbor Perspectives in Medicine*, 7(10), 1–12. <https://doi.org/10.1101/cshperspect.a029702>
- Enoka, R. M., Robinson, G. A., & Kossev, A. R. (1989). Task and fatigue effects on low-threshold motor units in human hand muscle. *Journal of Neurophysiology*, 62(6), 1344–1359. <https://doi.org/10.1152/jn.1989.62.6.1344>
- Essén, B., Jansson, E., Henriksson, J., Taylor, A. W., & Saltin, B. (1975). Metabolic Characteristics of Fibre Types in Human Skeletal Muscle. *Acta Physiologica Scandinavica*, 95(2), 153–165. <https://doi.org/10.1111/j.1748-1716.1975.tb10038.x>
- Falgairolle, M., & O'Donovan, M. J. (2019). V1 interneurons regulate the pattern and frequency of locomotor-like activity in the neonatal mouse spinal cord. *PLOS Biology*, 17(9), e3000447. <https://doi.org/10.1371/journal.pbio.3000447>
- Farina, D., & Enoka, R. M. (2023). Evolution of surface electromyography: From muscle electrophysiology towards neural recording and interfacing. *Journal of Electromyography and Kinesiology*, 71, 102796. <https://doi.org/10.1016/j.jelekin.2023.102796>
- Farina, D., & Negro, F. (2015). Common Synaptic Input to Motor Neurons, Motor Unit Synchronization, and Force Control. *Exercise and Sport Sciences Reviews*, 43(1), 23–33. <https://doi.org/10.1249/JES.0000000000000032>
- Farina, D., Negro, F., & Dideriksen, J. L. (2014). The effective neural drive to muscles is the common synaptic input to motor neurons. *The Journal of Physiology*, 592(16), 3427–3441. <https://doi.org/10.1113/jphysiol.2014.273581>
- Feiereisen, P., Duchateau, J., & Hainaut, K. (1997). Motor unit recruitment order during voluntary and electrically induced contractions in the tibialis anterior. *Experimental Brain Research*, 114(1), 117–123. <https://doi.org/10.1007/pl00005610>
- Fenno, L., Yizhar, O., & Deisseroth, K. (2011). The Development and Application of Optogenetics. *Annual Review of Neuroscience*, 34(1), 389–412. <https://doi.org/10.1146/annurev-neuro-061010-113817>
- Fenstermacher, S. J., Vonasek, A., Gattuso, H., Chaimowitz, C., Dymecki, S. M., Jessell, T. M., & Dasen, J. S. (2024). Potentiation of active locomotor state by spinal-projecting serotonergic neurons. <https://doi.org/10.1101/2024.09.26.615260>
- Franz, J. R., & Kram, R. (2012). The effects of grade and speed on leg muscle activations during walking. *Gait & Posture*, 35(1), 143–147. <https://doi.org/10.1016/j.gaitpost.2011.08.025>

- Friedman, W. A., Sypert, G. W., Munson, J. B., & Fleshman, J. W. (1981). Recurrent inhibition in type-identified motoneurons. *Journal of Neurophysiology*, *46*(6), 1349–1359. <https://doi.org/10.1152/jn.1981.46.6.1349>
- Fukuchi, C. A., Fukuchi, R. K., & Duarte, M. (2019). Effects of walking speed on gait biomechanics in healthy participants: A systematic review and meta-analysis. *Systematic Reviews*, *8*(1), 153. <https://doi.org/10.1186/s13643-019-1063-z>
- Fukunaga, T., Roy, R. R., Shellock, F. G., Hodgson, J. A., & Edgerton, V. R. (1996). Specific tension of human plantar flexors and dorsiflexors. *Journal of Applied Physiology*, *80*(1), 158–165. <https://doi.org/10.1152/jappl.1996.80.1.158>
- Gans, C. (1982). FIBER ARCHITECTURE AND MUSCLE FUNCTION. *Exercise and Sport Sciences Reviews*, *10*, 160–207. <https://doi.org/10.1249/00003677-198201000-00006>
- Gans, C., & Gaunt, A. S. (1991). Muscle architecture in relation to function. *Journal of Biomechanics*, *24*, 53–65. [https://doi.org/10.1016/0021-9290\(91\)90377-Y](https://doi.org/10.1016/0021-9290(91)90377-Y)
- Gatto, G., & Goulding, M. (2018). Locomotion Control: Brainstem Circuits Satisfy the Need for Speed. *Current Biology*, *28*(6), R256–R259. <https://doi.org/10.1016/j.cub.2018.01.068>
- Gilmer, J. I., Coltman, S. K., Cuenu, G., Hutchinson, J. R., Huber, D., Person, A. L., & Al Borno, M. (2024). A novel biomechanical model of the mouse forelimb predicts muscle activity in optimal control simulations of reaching movements. <https://doi.org/10.1101/2024.09.05.611289>
- Gittings, W., Huang, J., & Vandenboom, R. (2012). Tetanic force potentiation of mouse fast muscle is shortening speed dependent. *Journal of Muscle Research and Cell Motility*, *33*(5), 359–368. <https://doi.org/10.1007/s10974-012-9325-6>
- Gollnick, P. D., Sjodin, B., Karlsson, J., Jansson, E., & Saltin, B. (1974). Human soleus muscle: A comparison of fiber composition and enzyme activities with other leg muscles. *Pflugers Archiv European Journal of Physiology*, *348*(3), 247–255. <https://doi.org/10.1007/bf00587415>
- Gonçalves, A. I., Zavatone-Veth, J. A., Carey, M. R., & Clark, D. A. (2022). Parallel locomotor control strategies in mice and flies. *Current Opinion in Neurobiology*, *73*, 102516. <https://doi.org/10.1016/j.conb.2022.01.001>
- Goñi-Erro, H., Selvan, R., Caggiano, V., Leiras, R., & Kiehn, O. (2023). Pedunculopontine Chx10+ neurons control global motor arrest in mice. *Nature Neuroscience*, *26*(9), 1516–1528. <https://doi.org/10.1038/s41593-023-01396-3>
- Gorassini, M., Eken, T., Bennett, D. J., Kiehn, O., & Hultborn, H. (2000). Activity of Hindlimb Motor Units During Locomotion in the Conscious Rat. *Journal of Neurophysiology*, *83*(4), 2002–2011. <https://doi.org/10.1152/jn.2000.83.4.2002>

- Gorassini, M., Yang, J. F., Siu, M., & Bennett, D. J. (2002). Intrinsic Activation of Human Motoneurons: Reduction of Motor Unit Recruitment Thresholds by Repeated Contractions. *Journal of Neurophysiology*, 87(4), 1859–1866. <https://doi.org/10.1152/jn.00025.2001>
- Gosgnach, S., Lanuza, G. M., Butt, S. J. B., Saueressig, H., Zhang, Y., Velasquez, T., Riethmacher, D., Callaway, E. M., Kiehn, O., & Goulding, M. (2006). V1 spinal neurons regulate the speed of vertebrate locomotor outputs. *Nature*, 440(7081), 215–219. <https://doi.org/10.1038/nature04545>
- Goulding, M. (2009). Circuits controlling vertebrate locomotion: Moving in a new direction. *Nature Reviews Neuroscience*, 10(7), 507–518. <https://doi.org/10.1038/nrn2608>
- Grillner, S. (1975). Locomotion in vertebrates: Central mechanisms and reflex interaction. *Physiological Reviews*, 55(2), 247–304. <https://doi.org/10.1152/physrev.1975.55.2.247>
- Grillner, S. (1985). Neurobiological Bases of Rhythmic Motor Acts in Vertebrates. *Science*, 228(4696), 143–149. <https://doi.org/10.1126/science.3975635>
- Grillner, S., & El Manira, A. (2020). Current Principles of Motor Control, with Special Reference to Vertebrate Locomotion. *Physiological Reviews*, 100(1), 271–320. <https://doi.org/10.1152/physrev.00015.2019>
- Grimby, L. (1984). Firing properties of single human motor units during locomotion. *The Journal of Physiology*, 346(1), 195–202. <https://doi.org/10.1113/jphysiol.1984.sp015016>
- Guertin, P. A. (2013). Central pattern generator for locomotion: Anatomical, physiological, and pathophysiological considerations. *Frontiers in Neurology*, 3 FEB(February), 1–15. <https://doi.org/10.3389/fneur.2012.00183>
- Guidetti, L., Rivellini, G., & Figura, F. (1996). EMG patterns during running: Intra- and inter-individual variability. *Journal of Electromyography and Kinesiology*, 6(1), 37–48. [https://doi.org/10.1016/1050-6411\(95\)00015-1](https://doi.org/10.1016/1050-6411(95)00015-1)
- Gundelach, L. A., Hüser, M. A., Beutner, D., Ruther, P., & Bruegmann, T. (2020). Towards the clinical translation of optogenetic skeletal muscle stimulation. *Pflügers Archiv - European Journal of Physiology*, 472(5), 527–545. <https://doi.org/10.1007/s00424-020-02387-0>
- Hadzipasic, M., Ni, W., Nagy, M., Steenrod, N., McGinley, M. J., Kaushal, A., Thomas, E., McCormick, D. A., & Horwich, A. L. (2016). Reduced high-frequency motor neuron firing, EMG fractionation, and gait variability in awake walking ALS mice. *Proceedings of the National Academy of Sciences of the United States of America*, 113(47), E7600–E7609. <https://doi.org/10.1073/pnas.1616832113>

- Harnie, J., Côté-Sarrazin, C., Hurteau, M.-F., Desrochers, E., Doelman, A., Amhis, N., & Frigon, A. (2018). The modulation of locomotor speed is maintained following partial denervation of ankle extensors in spinal cats. *Journal of Neurophysiology*, *120*(3), 1274–1285. <https://doi.org/10.1152/jn.00812.2017>
- Harrison, V. F., & Mortensen, O. A. (1962). Identification and voluntary control of single motor unit activity in the tibialis anterior muscle. *The Anatomical Record*, *144*(2), 109–116. <https://doi.org/10.1002/ar.1091440205>
- Hartigan, P. M. (1985). Algorithm AS 217: Computation of the Dip Statistic to Test for Unimodality. *Applied Statistics*, *34*(3), 320. <https://doi.org/10.2307/2347485>
- Heckman, C. J., & Enoka, R. M. (2012). Motor unit. *Comprehensive Physiology*, *2*(4), 2629–2682. <https://doi.org/10.1002/cphy.c100087>
- Heckman, C. J., Gorassini, M. A., & Bennett, D. J. (2005). Persistent inward currents in motoneuron dendrites: Implications for motor output. *Muscle & Nerve*, *31*(2), 135–156. <https://doi.org/10.1002/mus.20261>
- Heckman, C. J., Hyngstrom, A. S., & Johnson, M. D. (2008). Active properties of motoneurone dendrites: Diffuse descending neuromodulation, focused local inhibition. *The Journal of Physiology*, *586*(5), 1225–1231. <https://doi.org/10.1113/jphysiol.2007.145078>
- Heckman, C. J., Johnson, M., Mottram, C., & Schuster, J. (2008). Persistent Inward Currents in Spinal Motoneurons and Their Influence on Human Motoneuron Firing Patterns. *The Neuroscientist*, *14*(3), 264–275. <https://doi.org/10.1177/1073858408314986>
- Heckman, C. J., Mottram, C., Quinlan, K., Theiss, R., & Schuster, J. (2009). Motoneuron excitability: The importance of neuromodulatory inputs. *Clinical Neurophysiology*, *120*(12), 2040–2054. <https://doi.org/10.1016/j.clinph.2009.08.009>
- Heglund, N. C., & Taylor, C. R. (1988). Speed, Stride Frequency and Energy Cost Per Stride: How Do They Change With Body Size and Gait? *Journal of Experimental Biology*, *138*(1), 301–318. <https://doi.org/10.1242/jeb.138.1.301>
- Heglund, N. C., Taylor, C. R., & McMahon, T. A. (1974). Scaling Stride Frequency and Gait to Animal Size: Mice to Horses. *Science*, *186*(4169), 1112–1113. <https://doi.org/10.1126/science.186.4169.1112>
- Henneman, E. (1957). Relation between Size of Neurons and Their Susceptibility to Discharge. *Science*, *126*(3287), 1345–1347. <https://doi.org/10.1126/science.126.3287.1345>
- Henneman, E., Somjen, G., & Carpenter, D. O. (1965). FUNCTIONAL SIGNIFICANCE OF CELL SIZE IN SPINAL MOTONEURONS. *Journal of Neurophysiology*, *28*(3), 560–580. <https://doi.org/10.1152/jn.1965.28.3.560>

- Herbin, M., Gasc, J.-P., & Renous, S. (2006). How does a mouse increase its velocity? A model for investigation in the control of locomotion. *Comptes Rendus Palevol*, 5(3–4), 531–540. <https://doi.org/10.1016/j.crvp.2005.12.012>
- Herbin, M., Hackert, R., Gasc, J.-P., & Renous, S. (2007). Gait parameters of treadmill versus overground locomotion in mouse. *Behavioural Brain Research*, 181(2), 173–179. <https://doi.org/10.1016/j.bbr.2007.04.001>
- Herndon, R. M. (1963). THE FINE STRUCTURE OF THE PURKINJE CELL. *The Journal of Cell Biology*, 18(1), 167–180. <https://doi.org/10.1083/jcb.18.1.167>
- Herrmann, U., & Flanders, M. (1998). Directional Tuning of Single Motor Units. *The Journal of Neuroscience*, 18(20), 8402–8416. <https://doi.org/10.1523/JNEUROSCI.18-20-08402.1998>
- Hinneken, E., Barbu-Roth, M., Do, M.-C., Berret, B., & Teulier, C. (2023). Generating variability from motor primitives during infant locomotor development. *eLife*, 12, e87463. <https://doi.org/10.7554/eLife.87463>
- Hodson-Tole, E. F., & Wakeling, J. M. (2009). Motor unit recruitment for dynamic tasks: Current understanding and future directions. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, 179(1), 57–66. <https://doi.org/10.1007/s00360-008-0289-1>
- Hoffer, J. A., O'Donovan, M. J., Pratt, C. A., & Loeb, G. E. (1981). Discharge Patterns of Hindlimb Motoneurons During Normal Cat Locomotion. *Science*, 213(4506), 466–467. <https://doi.org/10.1126/science.7244644>
- Hoffer, J. A., Sugano, N., Loeb, G. E., Marks, W. B., O'Donovan, M. J., & Pratt, C. A. (1987). Cat hindlimb motoneurons during locomotion. II. Normal activity patterns. *Journal of Neurophysiology*, 57(2), 530–553. <https://doi.org/10.1152/jn.1987.57.2.530>
- Hoogkamer, W., Bruijn, S. M., Sunaert, S., Swinnen, S. P., Van Calenbergh, F., & Duysens, J. (2015). Adaptation and aftereffects of split-belt walking in cerebellar lesion patients. *Journal of Neurophysiology*, 114(3), 1693–1704. <https://doi.org/10.1152/jn.00936.2014>
- Hoogland, T. M., De Gruijl, J. R., Witter, L., Canto, C. B., & De Zeeuw, C. I. (2015). Role of Synchronous Activation of Cerebellar Purkinje Cell Ensembles in Multi-joint Movement Control. *Current Biology*, 25(9), 1157–1165. <https://doi.org/10.1016/j.cub.2015.03.009>
- Hounsgaard, J., Hultborn, H., Jespersen, B., & Kiehn, O. (1988). Bistability of alpha-motoneurons in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophan. *The Journal of Physiology*, 405(1), 345–367. <https://doi.org/10.1113/jphysiol.1988.sp017336>

- Hoyt, D. F., & Taylor, C. R. (1981). Gait and the energetics of locomotion in horses. *Nature*, 292(5820), 239–240. <https://doi.org/10.1038/292239a0>
- Huh, S., Siripuram, R., Lee, R. H., Turkin, V. V., O’Neill, D., Hamm, T. M., Heckman, C. J., & Manuel, M. (2017). PICs in motoneurons do not scale with the size of the animal: A possible mechanism for faster speed of muscle contraction in smaller species. *Journal of Neurophysiology*, 118(1), 93–102. <https://doi.org/10.1152/jn.00045.2017>
- Hyingstrom, A. S., Johnson, M. D., & Heckman, C. J. (2008). Summation of Excitatory and Inhibitory Synaptic Inputs by Motoneurons With Highly Active Dendrites. *Journal of Neurophysiology*, 99(4), 1643–1652. <https://doi.org/10.1152/jn.01253.2007>
- Hyingstrom, A. S., Johnson, M. D., Miller, J. F., & Heckman, C. J. (2007). Intrinsic electrical properties of spinal motoneurons vary with joint angle. *Nature Neuroscience*, 10(3), 363–369. <https://doi.org/10.1038/nn1852>
- Ilg, W., Giese, M. A., Gizewski, E. R., Schoch, B., & Timmann, D. (2008). The influence of focal cerebellar lesions on the control and adaptation of gait. *Brain*, 131(11), 2913–2927. <https://doi.org/10.1093/brain/awn246>
- Ivanenko, Y. P., Poppele, R. E., & Lacquaniti, F. (2004). Five basic muscle activation patterns account for muscle activity during human locomotion. *Journal of Physiology*, 556(1), 267–282. <https://doi.org/10.1113/jphysiol.2003.057174>
- Jacobs, B. L., & Fornal, C. A. (1997). Serotonin and motor activity. *Current Opinion in Neurobiology*, 7(6), 820–825. [https://doi.org/10.1016/S0959-4388\(97\)80141-9](https://doi.org/10.1016/S0959-4388(97)80141-9)
- Jacobs, B. L., Martín-Cora, F. J., & Fornal, C. A. (2002). Activity of medullary serotonergic neurons in freely moving animals. *Brain Research Reviews*, 40(1–3), 45–52. [https://doi.org/10.1016/S0165-0173\(02\)00187-X](https://doi.org/10.1016/S0165-0173(02)00187-X)
- Jessell, T. M. (2000). Neuronal specification in the spinal cord: Inductive signals and transcriptional codes. *Nature Reviews Genetics*, 1(1), 20–29. <https://doi.org/10.1038/35049541>
- Johnson, M. D., Thompson, C. K., Tysseling, V. M., Powers, R. K., & Heckman, C. J. (2017). The potential for understanding the synaptic organization of human motor commands via the firing patterns of motoneurons. *Journal of Neurophysiology*, 118(1), 520–531. <https://doi.org/10.1152/jn.00018.2017>
- Jones, K. E., Lyons, M., Bawa, P., & Lemon, R. N. (1994). Recruitment order of motoneurons during functional tasks. *Experimental Brain Research*, 100(3), 503–508. <https://doi.org/10.1007/bf02738409>
- Jordan, L. M., Liu, J., Hedlund, P. B., Akay, T., & Pearson, K. G. (2008). Descending command systems for the initiation of locomotion in mammals. *Brain Research Reviews*, 57(1), 183–191. <https://doi.org/10.1016/j.brainresrev.2007.07.019>

- Kadaba, M. P., Ramakrishnan, H. K., & Wootten, M. E. (1990). Measurement of lower extremity kinematics during level walking. *Journal of Orthopaedic Research*, 8(3), 383–392. <https://doi.org/10.1002/jor.1100080310>
- Kafkafi, N., Pagis, M., Lipkind, D., Mayo, C. L., Bemjamini, Y., Golani, I., & Elmer, G. I. (2003). Darting behavior: A quantitative movement pattern designed for discrimination and replicability in mouse locomotor behavior. *Behavioural Brain Research*, 142(1–2), 193–205. [https://doi.org/10.1016/s0166-4328\(03\)00003-2](https://doi.org/10.1016/s0166-4328(03)00003-2)
- Kernell, D., & Zwaagstra, B. (1981). Input conductance, axonal conduction velocity and cell size among hindlimb motoneurons of the cat. *Brain Research*, 204(2), 311–326. [https://doi.org/10.1016/0006-8993\(81\)90591-6](https://doi.org/10.1016/0006-8993(81)90591-6)
- Kidgell, D. J., Sale, M. V., & Semmler, J. G. (2006). Motor unit synchronization measured by cross-correlation is not influenced by short-term strength training of a hand muscle. *Experimental Brain Research*, 175(4), 745–753. <https://doi.org/10.1007/s00221-006-0724-z>
- Kiehn, O. (2016). Decoding the organization of spinal circuits that control locomotion. *Nature Reviews Neuroscience*, 17(4), 224–238. <https://doi.org/10.1038/nrn.2016.9>
- Kiehn, O., & Butt, S. J. B. (2003). Physiological, anatomical and genetic identification of CPG neurons in the developing mammalian spinal cord. *Progress in Neurobiology*, 70(4), 347–361. [https://doi.org/10.1016/S0301-0082\(03\)00091-1](https://doi.org/10.1016/S0301-0082(03)00091-1)
- Kim, J. J., Wyche, I. S., Olson, W., Lu, J., Bakir, M. S., Sober, S. J., & O'Connor, D. H. (2024). *Myo-optogenetics: Optogenetic stimulation and electrical recording in skeletal muscles*. <https://doi.org/10.1101/2024.06.21.600113>
- Kimura, Y., & Higashijima, S. (2019). Regulation of locomotor speed and selection of active sets of neurons by V1 neurons. *Nature Communications*, 10(1), 2268. <https://doi.org/10.1038/s41467-019-09871-x>
- Kirk, E. A., Hope, K. T., Sober, S. J., & Sauerbrei, B. A. (2024). An output-null signature of inertial load in motor cortex. *Nature Communications*, 15(1), 7309. <https://doi.org/10.1038/s41467-024-51750-7>
- Kirtley, C., Whittle, M. W., & Jefferson, R. J. (1985). Influence of walking speed on gait parameters. *Journal of Biomedical Engineering*, 7(4), 282–288. [https://doi.org/10.1016/0141-5425\(85\)90055-X](https://doi.org/10.1016/0141-5425(85)90055-X)
- Kishore, S., Bagnall, M. W., & McLean, D. L. (2014). Systematic Shifts in the Balance of Excitation and Inhibition Coordinate the Activity of Axial Motor Pools at Different Speeds of Locomotion. *The Journal of Neuroscience*, 34(42), 14046–14054. <https://doi.org/10.1523/JNEUROSCI.0514-14.2014>

- Kutch, J. J., Kuo, A. D., Bloch, A. M., & Rymer, W. Z. (2008). Endpoint Force Fluctuations Reveal Flexible Rather Than Synergistic Patterns of Muscle Cooperation. *Journal of Neurophysiology*, *100*(5), 2455–2471. <https://doi.org/10.1152/jn.90274.2008>
- Kutch, J. J., & Valero-Cuevas, F. J. (2012). Challenges and New Approaches to Proving the Existence of Muscle Synergies of Neural Origin. *PLoS Computational Biology*, *8*(5), e1002434. <https://doi.org/10.1371/journal.pcbi.1002434>
- Lafortune, M. A., Cavanagh, P. R., Sommer, H. J., & Kalenak, A. (1992). Three-dimensional kinematics of the human knee during walking. *Journal of Biomechanics*, *25*(4), 347–357. [https://doi.org/10.1016/0021-9290\(92\)90254-X](https://doi.org/10.1016/0021-9290(92)90254-X)
- Latash, M. L. (2012). The bliss (not the problem) of motor abundance (not redundancy). *Experimental Brain Research*, *217*(1), 1–5. <https://doi.org/10.1007/s00221-012-3000-4>
- Lay, A. N., Hass, C. J., & Gregor, R. J. (2006). The effects of sloped surfaces on locomotion: A kinematic and kinetic analysis. *Journal of Biomechanics*, *39*(9), 1621–1628. <https://doi.org/10.1016/j.jbiomech.2005.05.005>
- Lee, R. H., & Heckman, C. J. (1998a). Bistability in Spinal Motoneurons In Vivo: Systematic Variations in Persistent Inward Currents. *Journal of Neurophysiology*, *80*(2), 583–593. <https://doi.org/10.1152/jn.1998.80.2.583>
- Lee, R. H., & Heckman, C. J. (1998b). Bistability in Spinal Motoneurons In Vivo: Systematic Variations in Rhythmic Firing Patterns. *Journal of Neurophysiology*, *80*(2), 572–582. <https://doi.org/10.1152/jn.1998.80.2.572>
- Lee, R. H., & Heckman, C. J. (2000). Adjustable Amplification of Synaptic Input in the Dendrites of Spinal Motoneurons In Vivo. *The Journal of Neuroscience*, *20*(17), 6734–6740. <https://doi.org/10.1523/JNEUROSCI.20-17-06734.2000>
- Leiras, R., Cregg, J. M., & Kiehn, O. (2022). Brainstem Circuits for Locomotion. *Annual Review of Neuroscience*, *45*(1), 63–85. <https://doi.org/10.1146/annurev-neuro-082321-025137>
- Leroux, A., Fung, J., & Barbeau, H. (2002). Postural adaptation to walking on inclined surfaces: I. Normal strategies. *Gait & Posture*, *15*(1), 64–74. [https://doi.org/10.1016/S0966-6362\(01\)00181-3](https://doi.org/10.1016/S0966-6362(01)00181-3)
- Lewis, K. E. (2006). How do genes regulate simple behaviours? Understanding how different neurons in the vertebrate spinal cord are genetically specified. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *361*(1465), 45–66. <https://doi.org/10.1098/rstb.2005.1778>
- Livingston, B. P., & Nichols, T. R. (2014). Effects of Reinnervation of the Triceps Brachii on Joint Kinematics and Electromyographic Patterns of the Feline Forelimb during

- Level and Upslope Walking. *Cells Tissues Organs*, 199(5–6), 405–422. <https://doi.org/10.1159/000371543>
- Loeb, G. (2021). Learning to Use Muscles. *Journal of Human Kinetics*, 76, 9–33. <https://doi.org/10.2478/hukin-2020-0084>
- Loeb, G. E. (1985). Motoneurone task groups: Coping with kinematic heterogeneity. *Journal of Experimental Biology*, 115(1), 137–146. <https://doi.org/10.1242/jeb.115.1.137>
- Loeb, G. E., & Gans, C. (1986). *Electromyography for experimentalists*. University of Chicago Press.
- Lopes, G., Bonacchi, N., Frazão, J., Neto, J. P., Atallah, B. V., Soares, S., Moreira, L., Matias, S., Itskov, P. M., Correia, P. A., Medina, R. E., Calcaterra, L., Dreosti, E., Paton, J. J., & Kampff, A. R. (2015). Bonsai: An event-based framework for processing and controlling data streams. *Frontiers in Neuroinformatics*, 9. <https://doi.org/10.3389/fninf.2015.00007>
- Lu, J., Zia, M., Baig, D. A., Yan, G., Kim, J. J., Nagapudi, K., Anschutz, P., Oh, S., O'Connor, D., Sober, S. J., & Bakir, M. S. (2024). *Opto-Myomatrix: μ LED integrated microelectrode arrays for optogenetic activation and electrical recording in muscle tissue*. <https://doi.org/10.1101/2024.07.01.601601>
- Lucas-Osma, A. M., & Collazos-Castro, J. E. (2009). Compartmentalization in the triceps brachii motoneuron nucleus and its relation to muscle architecture. *Journal of Comparative Neurology*, 516(3), 226–239. <https://doi.org/10.1002/cne.22123>
- Lulic-Kuryllo, T., Thompson, C. K., Jiang, N., Negro, F., & Dickerson, C. R. (2021). Neural control of the healthy pectoralis major from low-to-moderate isometric contractions. *Journal of Neurophysiology*, 126(1), 213–226. <https://doi.org/10.1152/jn.00046.2021>
- Luo, L., Callaway, E. M., & Svoboda, K. (2008). Genetic Dissection of Neural Circuits. *Neuron*, 57(5), 634–660. <https://doi.org/10.1016/j.neuron.2008.01.002>
- Machado, A. S., Darmohray, D. M., Fayad, J., Marques, H. G., & Carey, M. R. (2015). A quantitative framework for whole-body coordination reveals specific deficits in freely walking ataxic mice. *eLife*, 4, e07892. <https://doi.org/10.7554/eLife.07892>
- Machado, A. S., Marques, H. G., Duarte, D. F., Darmohray, D. M., & Carey, M. R. (2020). Shared and specific signatures of locomotor ataxia in mutant mice. *eLife*, 9, e55356. <https://doi.org/10.7554/eLife.55356>
- MacKay-Lyons, M. (2002). Central pattern generation of locomotion: A review of the evidence. *Physical Therapy*, 82(1), 69–83. <https://doi.org/10.1093/ptj/82.1.69>

- Magown, P., Shettar, B., Zhang, Y., & Rafuse, V. F. (2015). Direct optical activation of skeletal muscle fibres efficiently controls muscle contraction and attenuates denervation atrophy. *Nature Communications*, 6(1). <https://doi.org/10.1038/ncomms9506>
- Manning, C. D., Miller, T. A., Burnham, M. L., Murnaghan, C. D., Calancie, B., & Bawa, P. (2010). Recovery of human motoneurons during rotation. *Experimental Brain Research*, 204(1), 139–144. <https://doi.org/10.1007/s00221-010-2295-2>
- Manter, J. T. (1938). Dynamics of Quadrupedal Locomotion. *Journal of Experimental Biology*, 15(4), 522–540.
- Manuel, M., Chardon, M., Tysseling, V., & Heckman, C. J. (2019). Scaling of motor output, from mouse to humans. *Physiology*, 34(1), 5–13. <https://doi.org/10.1152/physiol.00021.2018>
- Manuel, M., & Heckman, C. J. (2011). Adult mouse motor units develop almost all of their force in the subprimary range: A new all-or-none strategy for force recruitment? *Journal of Neuroscience*, 31(42), 15188–15194. <https://doi.org/10.1523/JNEUROSCI.2893-11.2011>
- Manuel, M., Iglesias, C., Donnet, M., Leroy, F., Heckman, C. J., & Zytnicki, D. (2009). Fast Kinetics, High-Frequency Oscillations, and Subprimary Firing Range in Adult Mouse Spinal Motoneurons. *The Journal of Neuroscience*, 29(36), 11246–11256. <https://doi.org/10.1523/JNEUROSCI.3260-09.2009>
- Marshall, N. J., Glaser, J. I., Trautmann, E. M., Amematsro, E. A., Perkins, S. M., Shadlen, M. N., Abbott, L. F., Cunningham, J. P., & Churchland, M. M. (2022). Flexible neural control of motor units. *Nature Neuroscience*, 25(11), 1492–1504. <https://doi.org/10.1038/s41593-022-01165-8>
- Martínez-Silva, M. de L., Imhoff-Manuel, R. D., Sharma, A., Heckman, C. J., Shneider, N. A., Roselli, F., Zytnicki, D., & Manuel, M. (2018). Hypoexcitability precedes denervation in the large fast-contracting motor units in two unrelated mouse models of ALS. *eLife*, 7(2007), 1–26. <https://doi.org/10.7554/eLife.30955>
- Martino, G., Ivanenko, Y. P., Serrao, M., Ranavolo, A., d'Avella, A., Draicchio, F., Conte, C., Casali, C., & Lacquaniti, F. (2014). Locomotor patterns in cerebellar ataxia. *Journal of Neurophysiology*, 112(11), 2810–2821. <https://doi.org/10.1152/jn.00275.2014>
- Mathewson, M. A., Chapman, M. A., Hentzen, E. R., Fridén, J., & Lieber, R. L. (2012). Anatomical, architectural, and biochemical diversity of the murine forelimb muscles. *Journal of Anatomy*, 221(5), 443–451. <https://doi.org/10.1111/j.1469-7580.2012.01559.x>
- Mathis, A., Mamidanna, P., Cury, K. M., Abe, T., Murthy, V. N., Mathis, M. W., & Bethge, M. (2018). DeepLabCut: Markerless pose estimation of user-defined body parts

- with deep learning. *Nature Neuroscience*, 21(9), 1281–1289. <https://doi.org/10.1038/s41593-018-0209-y>
- Mayer, W. P., & Akay, T. (2018). Stumbling corrective reaction elicited by mechanical and electrical stimulation of the saphenous nerve in walking mice. *Journal of Experimental Biology*, 221(13). <https://doi.org/10.1242/jeb.178095>
- McIntosh, A. S., Beatty, K. T., Dwan, L. N., & Vickers, D. R. (2006). Gait dynamics on an inclined walkway. *Journal of Biomechanics*, 39(13), 2491–2502. <https://doi.org/10.1016/j.jbiomech.2005.07.025>
- McPhedran, A. M., Wuerker, R. B., & Henneman, E. (1965). PROPERTIES OF MOTOR UNITS IN A HOMOGENEOUS RED MUSCLE (SOLEUS) OF THE CAT. *Journal of Neurophysiology*, 28(1), 71–84. <https://doi.org/10.1152/jn.1965.28.1.71>
- Meehan, C. F., Sukiasyan, N., Zhang, M., Nielsen, J. B., & Hultborn, H. (2010). Intrinsic Properties of Mouse Lumbar Motoneurons Revealed by Intracellular Recording In Vivo. *Journal of Neurophysiology*, 103(5), 2599–2610. <https://doi.org/10.1152/jn.00668.2009>
- Mendes, C. S., Bartos, I., Márka, Z., Akay, T., Márka, S., & Mann, R. S. (2015). Quantification of gait parameters in freely walking rodents. *BMC Biology*, 13(1), 1–11. <https://doi.org/10.1186/s12915-015-0154-0>
- Menelaou, E., Kishore, S., & McLean, D. L. (2022). Mixed synapses reconcile violations of the size principle in zebrafish spinal cord. *eLife*, 11, e64063. <https://doi.org/10.7554/eLife.64063>
- Menelaou, E., & McLean, D. L. (2012). A Gradient in Endogenous Rhythmicity and Oscillatory Drive Matches Recruitment Order in an Axial Motor Pool. *The Journal of Neuroscience*, 32(32), 10925–10939. <https://doi.org/10.1523/JNEUROSCI.1809-12.2012>
- Miles, G. B., & Sillar, K. T. (2011). Neuromodulation of Vertebrate Locomotor Control Networks. *Physiology*, 26(6), 393–411. <https://doi.org/10.1152/physiol.00013.2011>
- Miller, S., Van Der Burg, J., & Van Der Meché, F. G. A. (1975a). Coordination of movements of the hindlimbs and forelimbs in different forms of locomotion in normal and decerebrate cats. *Brain Research*, 91(2), 217–237. [https://doi.org/10.1016/0006-8993\(75\)90544-2](https://doi.org/10.1016/0006-8993(75)90544-2)
- Miller, S., Van Der Burg, J., & Van Der Meché, F. G. A. (1975b). Locomotion in the cat: Basic programmes of movement. *Brain Research*, 91(2), 239–253. [https://doi.org/10.1016/0006-8993\(75\)90545-4](https://doi.org/10.1016/0006-8993(75)90545-4)
- Minassian, K., Hofstoetter, U. S., Dzeladini, F., Guertin, P. A., & Ijspeert, A. (2017). The Human Central Pattern Generator for Locomotion: Does It Exist and Contribute to

Walking? *The Neuroscientist*, 23(6), 649–663.
<https://doi.org/10.1177/1073858417699790>

- Mistretta, O. C., Wood, R. L., English, A. W., & Alvarez, F. J. (2024). Air-stepping in the neonatal mouse: A powerful tool for analyzing early stages of rhythmic limb movement development. *Journal of Neurophysiology*, 131(2), 321–337. <https://doi.org/10.1152/jn.00227.2023>
- Morton, S. M., & Bastian, A. J. (2004). Cerebellar control of balance and locomotion. *Neuroscientist*, 10(3), 247–259. <https://doi.org/10.1177/1073858404263517>
- Morton, S. M., & Bastian, A. J. (2006). Cerebellar Contributions to Locomotor Adaptations during Splitbelt Treadmill Walking. *Journal of Neuroscience*, 26(36), 9107–9116. <https://doi.org/10.1523/JNEUROSCI.2622-06.2006>
- Morton, S. M., & Bastian, A. J. (2007). Mechanisms of cerebellar gait ataxia. *The Cerebellum*, 6(1), 79. <https://doi.org/10.1080/14734220601187741>
- Nagel, G., Szellas, T., Huhn, W., Kateriya, S., Adeishvili, N., Berthold, P., Ollig, D., Hegemann, P., & Bamberg, E. (2003). Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. *Proceedings of the National Academy of Sciences*, 100(24), 13940–13945. <https://doi.org/10.1073/pnas.1936192100>
- Nelson, R. M., & Soderberg, G. L. (1983). Laser etched bifilar fine wire electrode for skeletal muscle motor unit recording. *Electroencephalography and Clinical Neurophysiology*, 55(2), 238–239. [https://doi.org/10.1016/0013-4694\(83\)90194-3](https://doi.org/10.1016/0013-4694(83)90194-3)
- Nilsson, J., Thorstensson, A., & Halbertsma, J. (1985). Changes in leg movements and muscle activity with speed of locomotion and mode of progression in humans. *Acta Physiologica Scandinavica*, 123(4), 457–475. <https://doi.org/10.1111/j.1748-1716.1985.tb07612.x>
- Noga, B. R., Shefchyk, S. J., Jamal, J., & Jordan, L. M. (1987). The role of Renshaw cells in locomotion: Antagonism of their excitation from motor axon collaterals with intravenous mecamylamine. *Experimental Brain Research*, 66(1), 99–105. <https://doi.org/10.1007/BF00236206>
- Obeidat, A. Z., Nardelli, P., Powers, R. K., & Cope, T. C. (2014). Modulation of motoneuron firing by recurrent inhibition in the adult rat in vivo. *Journal of Neurophysiology*, 112(9), 2302–2315. <https://doi.org/10.1152/jn.00358.2014>
- Ojima, K. (2019). Myosin: Formation and maintenance of thick filaments. *Animal Science Journal*, 90(7), 801–807. <https://doi.org/10.1111/asj.13226>
- Otto Friesen, W. (1994). Reciprocal inhibition: A mechanism underlying oscillatory animal movements. *Neuroscience & Biobehavioral Reviews*, 18(4), 547–553. [https://doi.org/10.1016/0149-7634\(94\)90010-8](https://doi.org/10.1016/0149-7634(94)90010-8)

- Pachitariu, M., Steinmetz, N., Kadir, S., Carandini, M., & Kenneth D., H. (2016). Kilosort: Realtime spike-sorting for extracellular electrophysiology with hundreds of channels. *bioRxiv*, 061481. <https://doi.org/10.1101/061481>
- Park, S., Bandi, A., Lee, C. R., & Margolis, D. J. (2016). Peripheral optogenetic stimulation induces whisker movement and sensory perception in head-fixed mice. *eLife*, 5. <https://doi.org/10.7554/elife.14140>
- Parkis, M. A., Bayliss, D. A., & Berger, A. J. (1995). Actions of norepinephrine on rat hypoglossal motoneurons. *Journal of Neurophysiology*, 74(5), 1911–1919. <https://doi.org/10.1152/jn.1995.74.5.1911>
- Petersen, P. C., & Berg, R. W. (2016). Lognormal firing rate distribution reveals prominent fluctuation-driven regime in spinal motor networks. *eLife*, 5. <https://doi.org/10.7554/elife.18805>
- Pette, D. (1985). Metabolic heterogeneity of muscle fibres. *Journal of Experimental Biology*, 115(1), 179–189. <https://doi.org/10.1242/jeb.115.1.179>
- Pette, D., & Staron, R. S. (2000). Myosin isoforms, muscle fiber types, and transitions. *Microscopy Research and Technique*, 50(6), 500–509. [https://doi.org/10.1002/1097-0029\(20000915\)50:6<500::AID-JEMT7>3.0.CO;2-7](https://doi.org/10.1002/1097-0029(20000915)50:6<500::AID-JEMT7>3.0.CO;2-7)
- Picton, L., Pallucchi, I., Fontanel, P., Bertuzzi, M., Song, J., & El Manira, A. (2025). Circuit Modules for Flexible Locomotion. *Annual Review of Neuroscience*. <https://doi.org/10.1146/annurev-neuro-112723-061241>
- Pisotta, I., & Molinari, M. (2014). Cerebellar contribution to feedforward control of locomotion. *Frontiers in Human Neuroscience*, 8(JUNE), 1–5. <https://doi.org/10.3389/fnhum.2014.00475>
- Powers, R. K., & Binder, M. D. (2001). Input-output functions of mammalian motoneurons. In *Reviews of Physiology, Biochemistry and Pharmacology* (Vol. 143, pp. 137–263). Springer Berlin Heidelberg. <https://doi.org/10.1007/BFb0115594>
- Putney, J., Conn, R., & Sponberg, S. (2019). Precise timing is ubiquitous, consistent, and coordinated across a comprehensive, spike-resolved flight motor program. *Proceedings of the National Academy of Sciences*, 116(52), 26951–26960. <https://doi.org/10.1073/pnas.1907513116>
- Qi, Z., Han, S., Wang, S., Gu, X., Deng, J., Huang, C., & Yin, X. (2022). Visual three-dimensional spatial distribution of motor neurons innervating superficial limb muscles in mice. *Frontiers in Cellular Neuroscience*, 16, 904172. <https://doi.org/10.3389/fncel.2022.904172>

- Quintero, D., Lambert, D. J., Villarreal, D. J., & Gregg, R. D. (2017). Real-Time continuous gait phase and speed estimation from a single sensor. *2017 IEEE Conference on Control Technology and Applications (CCTA)*, 847–852. <https://doi.org/10.1109/ccta.2017.8062565>
- Rekling, J. C., Funk, G. D., Bayliss, D. A., Dong, X.-W., & Feldman, J. L. (2000). Synaptic Control of Motoneuronal Excitability. *Physiological Reviews*, 80(2), 767–852. <https://doi.org/10.1152/physrev.2000.80.2.767>
- Renshaw, B. (1941). INFLUENCE OF DISCHARGE OF MOTONEURONS UPON EXCITATION OF NEIGHBORING MOTONEURONS. *Journal of Neurophysiology*, 4(2), 167–183. <https://doi.org/10.1152/jn.1941.4.2.167>
- Riek, S., & Bawa, P. (1992). Recruitment of motor units in human forearm extensors. *Journal of Neurophysiology*, 68(1), 100–108. <https://doi.org/10.1152/jn.1992.68.1.100>
- Riley, P. O., Dicharry, J., Franz, J., Croce, U. D., Wilder, R. P., & Kerrigan, D. C. (2008). A Kinematics and Kinetic Comparison of Overground and Treadmill Running. *Medicine & Science in Sports & Exercise*, 40(6), 1093–1100. <https://doi.org/10.1249/MSS.0b013e3181677530>
- Ritter, L. K., Tresch, M. C., Heckman, C. J., Manuel, M., & Tysseling, V. M. (2014). *Characterization of motor units in behaving adult mice shows a wide primary range*. 543–551. <https://doi.org/10.1152/jn.00108.2014>
- Robilliard, J. J., Pfau, T., & Wilson, A. M. (2007). Gait characterisation and classification in horses. *Journal of Experimental Biology*, 210(2), 187–197. <https://doi.org/10.1242/jeb.02611>
- Romaiguère, P., Vedel, J.-P., & Pagni, S. (1993). Comparison of fluctuations of motor unit recruitment and de-recruitment thresholds in man. *Experimental Brain Research*, 95(3). <https://doi.org/10.1007/BF00227145>
- Ross, H.-G., Cleveland, S., & Haase, J. (1975). Contribution of single motoneurons to Renshaw cell activity. *Neuroscience Letters*, 1(2), 105–108. [https://doi.org/10.1016/0304-3940\(75\)90053-1](https://doi.org/10.1016/0304-3940(75)90053-1)
- Ruder, L., Schina, R., Kanodia, H., Valencia-Garcia, S., Pivetta, C., & Arber, S. (2021). A functional map for diverse forelimb actions within brainstem circuitry. *Nature*, 590(7846), 445–450. <https://doi.org/10.1038/s41586-020-03080-z>
- Ryan, M. M., & Gregor, R. J. (1992). EMG profiles of lower extremity muscles during cycling at constant workload and cadence. *Journal of Electromyography and Kinesiology*, 2(2), 69–80. [https://doi.org/10.1016/1050-6411\(92\)90018-E](https://doi.org/10.1016/1050-6411(92)90018-E)
- Rybak, I. A., Dougherty, K. J., & Shevtsova, N. A. (2015). Organization of the mammalian locomotor CPG: Review of computational model and circuit architectures based on

- genetically identified spinal interneurons. *eNeuro*, 2(5).
<https://doi.org/10.1523/ENEURO.0069-15.2015>
- Santuz, A., Akay, T., Mayer, W. P., Wells, T. L., Schroll, A., & Arampatzis, A. (2019). Modular organization of murine locomotor pattern in the presence and absence of sensory feedback from muscle spindles. *The Journal of Physiology*, 597(12), 3147–3165. <https://doi.org/10.1113/JP277515>
- Santuz, A., Laflamme, O. D., & Akay, T. (2022). The brain integrates proprioceptive information to ensure robust locomotion. *The Journal of Physiology*, 600(24), 5267–5294. <https://doi.org/10.1113/JP283181>
- Sarver, J. J., Dishowitz, M. I., Kim, S.-Y., & Soslowsky, L. J. (2010). Transient decreases in forelimb gait and ground reaction forces following rotator cuff injury and repair in a rat model. *Journal of Biomechanics*, 43(4), 778–782. <https://doi.org/10.1016/j.jbiomech.2009.10.031>
- Sauerbrei, B. A., Lubenov, E. V., & Siapas, A. G. (2015). Structured Variability in Purkinje Cell Activity during Locomotion. *Neuron*, 87(4), 840–852. <https://doi.org/10.1016/j.neuron.2015.08.003>
- Schiaffino, S., & Reggiani, C. (2011). Fiber Types in Mammalian Skeletal Muscles. *Physiological Reviews*, 91(4), 1447–1531. <https://doi.org/10.1152/physrev.00031.2010>
- Schmitt, D., Zumwalt, A. C., & Hamrick, M. W. (2010). The relationship between bone mechanical properties and ground reaction forces in normal and hypermuscular mice. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, 313A(6), 339–351. <https://doi.org/10.1002/jez.604>
- Scholle, H. Ch., Schumann, N. P., Biedermann, F., Stegeman, D. F., Graßme, R., Roeleveld, K., Schilling, N., & Fischer, M. S. (2001). Spatiotemporal surface EMG characteristics from rat triceps brachii muscle during treadmill locomotion indicate selective recruitment of functionally distinct muscle regions. *Experimental Brain Research*, 138(1), 26–36. <https://doi.org/10.1007/s002210100685>
- Schumann, N. (2002). Multi-channel EMG of the M. triceps brachii in rats during treadmill locomotion. *Clinical Neurophysiology*, 113(7), 1142–1151. [https://doi.org/10.1016/S1388-2457\(02\)00143-8](https://doi.org/10.1016/S1388-2457(02)00143-8)
- Schumann, N. P., Biedermann, F. H. W., Arnold, D., Jinnah, H. A., Grassme, R., Fischer, M. S., & Scholle, H. C. (2006). Treadmill locomotion in normal mice—Step related multi-channel EMG profiles of thigh muscles. *Pathophysiology*, 13(4), 245–255. <https://doi.org/10.1016/j.pathophys.2006.09.002>
- Sober, S. J., Sponberg, S., Nemenman, I., & Ting, L. H. (2018). Millisecond Spike Timing Codes for Motor Control. *Trends in Neurosciences*, 41(10), 644–648. <https://doi.org/10.1016/j.tins.2018.08.010>

- Sokoloff, A. J., & Cope, T. C. (1996). Recruitment of triceps surae motor units in the decerebrate cat. II. Heterogeneity among soleus motor units. *Journal of Neurophysiology*, *75*(5), 2005–2016. <https://doi.org/10.1152/jn.1996.75.5.2005>
- Song, J., Dahlberg, E., & El Manira, A. (2018). V2a interneuron diversity tailors spinal circuit organization to control the vigor of locomotor movements. *Nature Communications*, *9*(1), 3370. <https://doi.org/10.1038/s41467-018-05827-9>
- Spector, S. A., Gardiner, P. F., Zernicke, R. F., Roy, R. R., & Edgerton, V. R. (1980). Muscle architecture and force-velocity characteristics of cat soleus and medial gastrocnemius: Implications for motor control. *Journal of Neurophysiology*, *44*(5), 951–960. <https://doi.org/10.1152/jn.1980.44.5.951>
- Sponberg, S., Spence, A. J., Mullens, C. H., & Full, R. J. (2011). A single muscle's multifunctional control potential of body dynamics for postural control and running. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *366*(1570), 1592–1605. <https://doi.org/10.1098/rstb.2010.0367>
- Srivastava, K. H., Elemans, C. P. H., & Sober, S. J. (2015). Multifunctional and Context-Dependent Control of Vocal Acoustics by Individual Muscles. *The Journal of Neuroscience*, *35*(42), 14183–14194. <https://doi.org/10.1523/JNEUROSCI.3610-14.2015>
- Srivastava, K. H., Holmes, C. M., Vellema, M., Pack, A. R., Elemans, C. P. H., Nemenman, I., & Sober, S. J. (2017). Motor control by precisely timed spike patterns. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(5), 1171–1176. <https://doi.org/10.1073/pnas.1611734114>
- Steele, K. M., Tresch, M. C., & Perreault, E. J. (2013). The number and choice of muscles impact the results of muscle synergy analyses. *Frontiers in Computational Neuroscience*, *7*. <https://doi.org/10.3389/fncom.2013.00105>
- Stein, R. B. (1980). *Nerve and Muscle*. Springer US. <https://doi.org/10.1007/978-1-4684-3797-3>
- Tata Ramalingasetty, S., Danner, S. M., Arreguit, J., Markin, S. N., Rodarie, D., Kathe, C., Courtine, G., Rybak, I. A., & Ijspeert, A. J. (2021). A Whole-Body Musculoskeletal Model of the Mouse. *IEEE Access*, *9*, 163861–163881. <https://doi.org/10.1109/ACCESS.2021.3133078>
- Ter Haar Romeny, B. M., Van Der Gon, J. J. D., & Gielen, C. C. A. M. (1982). Changes in recruitment order of motor units in the human biceps muscle. *Experimental Neurology*, *78*(2), 360–368. [https://doi.org/10.1016/0014-4886\(82\)90054-1](https://doi.org/10.1016/0014-4886(82)90054-1)
- Thomas, C. K., Ross, B. H., & Calancie, B. (1987). Human motor-unit recruitment during isometric contractions and repeated dynamic movements. *Journal of Neurophysiology*, *57*(1), 311–324. <https://doi.org/10.1152/jn.1987.57.1.311>

- Thomas, C. K., Ross, B. H., & Stein, R. B. (1986). Motor-unit recruitment in human first dorsal interosseous muscle for static contractions in three different directions. *Journal of Neurophysiology*, 55(5), 1017–1029. <https://doi.org/10.1152/jn.1986.55.5.1017>
- Thomas, K., Gibbs, R., Marques, H., Carey, M. R., & Sober, S. J. (2025). *Motor unit mechanisms of speed control in mouse locomotion*. <https://doi.org/10.7554/eLife.105829.1>
- Ting, L. H., & Chiel, H. J. (2017). Muscle, Biomechanics, and Implications for Neural Control. In S. L. Hooper & A. Büschges (Eds.), *Neurobiology of Motor Control* (1st ed., pp. 365–416). Wiley. <https://doi.org/10.1002/9781118873397.ch12>
- Ting, L. H., Chiel, H. J., Trumbower, R. D., Allen, J. L., McKay, J. L., Hackney, M. E., & Kesar, T. M. (2015). Neuromechanical principles underlying movement modularity and their implications for rehabilitation. *Neuron*, 86(1), 38–54. <https://doi.org/10.1016/j.neuron.2015.02.042>
- Tosolini, A. P., & Morris, R. (2012). Spatial characterization of the motor neuron columns supplying the rat forelimb. *Neuroscience*, 200, 19–30. <https://doi.org/10.1016/j.neuroscience.2011.10.054>
- Tresch, M. C., Cheung, V. C. K., & d’Avella, A. (2006). Matrix Factorization Algorithms for the Identification of Muscle Synergies: Evaluation on Simulated and Experimental Data Sets. *Journal of Neurophysiology*, 95(4), 2199–2212. <https://doi.org/10.1152/jn.00222.2005>
- Tresch, M. C., & Jarc, A. (2009). The case for and against muscle synergies. *Current Opinion in Neurobiology*, 19(6), 601–607. <https://doi.org/10.1016/j.conb.2009.09.002>
- Ulfhake, B., & Kellerth, J.-O. (1982). Does α -motoneurone size correlate with motor unit type in cat triceps surae? *Brain Research*, 251(2), 201–209. [https://doi.org/10.1016/0006-8993\(82\)90738-7](https://doi.org/10.1016/0006-8993(82)90738-7)
- Usseglio, G., Gatier, E., Heuzé, A., Hérent, C., & Bouvier, J. (2020). Control of Orienting Movements and Locomotion by Projection-Defined Subsets of Brainstem V2a Neurons. *Current Biology*, 30(23), 4665–4681.e6. <https://doi.org/10.1016/j.cub.2020.09.014>
- Valero-Cuevas, F. J., Venkadesan, M., & Todorov, E. (2009). Structured Variability of Muscle Activations Supports the Minimal Intervention Principle of Motor Control. *Journal of Neurophysiology*, 102(1), 59–68. <https://doi.org/10.1152/jn.90324.2008>
- Valetdinova, K. R., Medvedev, S. P., & Zakian, S. M. (2015). Model systems of motor neuron diseases as a platform for studying pathogenic mechanisms and searching for therapeutic agents. *Acta Naturae*, 7(1), 19–36. <https://doi.org/10.32607/20758251-2015-7-1-19-36>

- Van Hedel, H. J. A., Tomatis, L., & Müller, R. (2006). Modulation of leg muscle activity and gait kinematics by walking speed and bodyweight unloading. *Gait & Posture*, 24(1), 35–45. <https://doi.org/10.1016/j.gaitpost.2005.06.015>
- Van Ingen Schenau, G. J., Boots, P. J. M., De Groot, G., Snackers, R. J., & Van Woensel, W. W. L. M. (1992). The constrained control of force and position in multi-joint movements. *Neuroscience*, 46(1), 197–207. [https://doi.org/10.1016/0306-4522\(92\)90019-X](https://doi.org/10.1016/0306-4522(92)90019-X)
- Van Ingen Schenau, G. J., Pratt, C. A., & Macpherson, J. M. (1994). Differential use and control of mono- and biarticular muscles. *Human Movement Science*, 13(3–4), 495–517. [https://doi.org/10.1016/0167-9457\(94\)90051-5](https://doi.org/10.1016/0167-9457(94)90051-5)
- Veasey, S., Fornal, C., Metzler, C., & Jacobs, B. (1995). Response of serotonergic caudal raphe neurons in relation to specific motor activities in freely moving cats. *The Journal of Neuroscience*, 15(7), 5346–5359. <https://doi.org/10.1523/JNEUROSCI.15-07-05346.1995>
- Vinueza Veloz, M. F., Zhou, K., Bosman, L. W. J., Potters, J.-W., Negrello, M., Seepers, R. M., Strydis, C., Koekkoek, S. K. E., & De Zeeuw, C. I. (2015). Cerebellar control of gait and interlimb coordination. *Brain Structure and Function*, 220(6), 3513–3536. <https://doi.org/10.1007/s00429-014-0870-1>
- Waider, J., Popp, S., Lange, M. D., Kern, R., Kolter, J. F., Kobler, J., Donner, N. C., Lowe, K. R., Malzbender, J. H., Brazell, C. J., Arnold, M. R., Aboagye, B., Schmitt-Böhrer, A., Lowry, C. A., Pape, H. C., & Lesch, K. P. (2017). Genetically driven brain serotonin deficiency facilitates panic-like escape behavior in mice. *Translational Psychiatry*, 7(10), e1246–e1246. <https://doi.org/10.1038/tp.2017.209>
- Wakeling, J. M. (2004). Motor units are recruited in a task-dependent fashion during locomotion. *Journal of Experimental Biology*, 207(22), 3883–3890. <https://doi.org/10.1242/jeb.01223>
- Walmsley, B., Hodgson, J. A., & Burke, R. E. (1978). Forces produced by medial gastrocnemius and soleus muscles during locomotion in freely moving cats. *Journal of Neurophysiology*, 41(5), 1203–1216. <https://doi.org/10.1152/jn.1978.41.5.1203>
- Wank, V., Frick, U., & Schmidtbleicher, D. (1998). Kinematics and Electromyography of Lower Limb Muscles in Overground and Treadmill Running. *International Journal of Sports Medicine*, 19(07), 455–461. <https://doi.org/10.1055/s-2007-971944>
- Watanabe, K., Vieira, T. M., Gallina, A., Kouzaki, M., & Moritani, T. (2021). Novel Insights Into Biarticular Muscle Actions Gained From High-Density Electromyogram. *Exercise and Sport Sciences Reviews*, 49(3), 179–187. <https://doi.org/10.1249/JES.0000000000000254>

- Whelan, P. (1996). CONTROL OF LOCOMOTION IN THE DECEREBRATE CAT. *Progress in Neurobiology*, 49(5), 481–515. [https://doi.org/10.1016/0301-0082\(96\)00028-7](https://doi.org/10.1016/0301-0082(96)00028-7)
- Wilson, A. C., & Sweeney, L. B. (2023). Spinal cords: Symphonies of interneurons across species. *Frontiers in Neural Circuits*, 17, 1146449. <https://doi.org/10.3389/fncir.2023.1146449>
- Wilson, J. M., Thompson, C. K., Miller, L. C., & Heckman, C. J. (2015). Intrinsic excitability of human motoneurons in biceps brachii versus triceps brachii. *Journal of Neurophysiology*, 113(10), 3692–3699. <https://doi.org/10.1152/jn.00960.2014>
- Winner, T. S., Rosenberg, M. C., Jain, K., Kesar, T. M., Ting, L. H., & Berman, G. J. (2023). Discovering individual-specific gait signatures from data-driven models of neuromechanical dynamics. *PLOS Computational Biology*, 19(10), e1011556. <https://doi.org/10.1371/journal.pcbi.1011556>
- Winter, D. A., & Yack, H. J. (1987). EMG profiles during normal human walking: Stride-to-stride and inter-subject variability. *Electroencephalography and Clinical Neurophysiology*, 67(5), 402–411. [https://doi.org/10.1016/0013-4694\(87\)90003-4](https://doi.org/10.1016/0013-4694(87)90003-4)
- Wuerker, R. B., McPhedran, A. M., & Henneman, E. (1965). PROPERTIES OF MOTOR UNITS IN A HETEROGENEOUS PALE MUSCLE (M. GASTROCNEMIUS) OF THE CAT. *Journal of Neurophysiology*, 28(1), 85–99. <https://doi.org/10.1152/jn.1965.28.1.85>
- Yanagihara, D., & Kondo, I. (1996). Nitric oxide plays a key role in adaptive control of locomotion in cat. *Proceedings of the National Academy of Sciences*, 93(23), 13292–13297. <https://doi.org/10.1073/pnas.93.23.13292>
- Yokoyama, H., Kaneko, N., Sasaki, A., Saito, A., & Nakazawa, K. (2022). Firing behavior of single motor units of the tibialis anterior in human walking as non-invasively revealed by HDsEMG decomposition. *Journal of Neural Engineering*, 19(6), 066033. <https://doi.org/10.1088/1741-2552/aca71b>
- You, J.-Y., Chou, Y.-L., Lin, C.-J., & Su, F.-C. (2001). Effect of slip on movement of body center of mass relative to base of support. *Clinical Biomechanics*, 16(2), 167–173. [https://doi.org/10.1016/S0268-0033\(00\)00076-0](https://doi.org/10.1016/S0268-0033(00)00076-0)
- Zajac, F. E. (1993). Muscle coordination of movement: A perspective. *Journal of Biomechanics*, 26, 109–124. [https://doi.org/10.1016/0021-9290\(93\)90083-Q](https://doi.org/10.1016/0021-9290(93)90083-Q)
- Zajac, F. E., & Faden, J. S. (1985). Relationship among recruitment order, axonal conduction velocity, and muscle-unit properties of type-identified motor units in cat plantaris muscle. *Journal of Neurophysiology*, 53(5), 1303–1322. <https://doi.org/10.1152/jn.1985.53.5.1303>

- Zajac, F. E., & Young, J. L. (1980). Discharge properties of hindlimb motoneurons in decerebrate cats during locomotion induced by mesencephalic stimulation. *Journal of Neurophysiology*, *43*(5), 1221–1235. <https://doi.org/10.1152/jn.1980.43.5.1221>
- Zhang, J., Lanuza, G. M., Britz, O., Wang, Z., Siembab, V. C., Zhang, Y., Velasquez, T., Alvarez, F. J., Frank, E., & Goulding, M. (2014). V1 and V2b Interneurons Secure the Alternating Flexor-Extensor Motor Activity Mice Require for Limbed Locomotion. *Neuron*, *82*(1), 138–150. <https://doi.org/10.1016/j.neuron.2014.02.013>
- Zhong, G., Droho, S., Crone, S. A., Dietz, S., Kwan, A. C., Webb, W. W., Sharma, K., & Harris-Warrick, R. M. (2010). Electrophysiological Characterization of V2a Interneurons and Their Locomotor-Related Activity in the Neonatal Mouse Spinal Cord. *The Journal of Neuroscience*, *30*(1), 170–182. <https://doi.org/10.1523/JNEUROSCI.4849-09.2010>
- Zhong, G., Sharma, K., & Harris-Warrick, R. M. (2011). Frequency-dependent recruitment of V2a interneurons during fictive locomotion in the mouse spinal cord. *Nature Communications*, *2*(1), 274. <https://doi.org/10.1038/ncomms1276>
- Zhurov, Y., & Brezina, V. (2006). Variability of motor neuron spike timing maintains and shapes contractions of the accessory radula closer muscle of *Aplysia*. *Journal of Neuroscience*, *26*(26), 7056–7070. <https://doi.org/10.1523/JNEUROSCI.5277-05.2006>
- Zia, M., Chung, B., Sober, S., & Bakir, M. S. (2020). Flexible Multielectrode Arrays With 2-D and 3-D Contacts for In Vivo Electromyography Recording. *IEEE Transactions on Components, Packaging and Manufacturing Technology*, *10*(2), 197–202. <https://doi.org/10.1109/TCPMT.2019.2963556>