SCALING OF SENSORIMOTOR CONTROL
IN TERRESTRIAL MAMMALS

by

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Abstract

Terrestrial mammals span a wide range of sizes, with the largest elephant being several million times more massive than the smallest shrew. This huge size range results in small and large animals experiencing very different physical challenges, yet all animals must effectively interact with their environment to survive. In order to sense and respond to stimuli with similar speed and precision, small and large animals may need to control their movement in different ways. To begin to understand whether and how small and large animals coordinate their movement with similar effectiveness despite their different physical challenges, I investigated how size influences the physiological mechanisms underlying sensorimotor control. My general hypothesis was that the sensorimotor systems of larger animals have longer delays and lower precision than those of smaller animals.

To investigate the scaling of delays, I combined my own electrophysiology measurements with data from the literature to determine how total response time and its component delays changed with animal mass. To investigate the scaling of precision, I combined my own histology measurements with data from the literature to determine how nerve fiber number and size distribution changed with animal mass. As part of this, I developed a supervised image analysis method to measure nerve fiber characteristics in scanning electron microscope images.

I found that larger animals have longer absolute delays and more nerve fibers than smaller animals. However, changes to movement times with animal size almost entirely compensate for increases in absolute delays, resulting in similar relative delays for all sizes of animals. Nerve fiber number increases more slowly than animal mass, area, and muscle force, suggesting that larger animals have relatively fewer sensors and motor units than smaller animals. Nerve fiber size distribution becomes more bimodal as animal size increases, ameliorating the potential tradeoff between speed and precision in peripheral nerves. While small and large animals seem to be able to sense and respond to stimuli within the same relative time, large animals may face challenges in situations requiring short absolute delays and high precision, and may need to rely more heavily on predictive methods of control.

Keywords: nerve; muscle; locomotion; delay; precision; size
Dedicated with appreciation to the animals sacrificed for this research.
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Chapter 1

Introduction

Terrestrial mammals span a wide range of sizes, with the largest elephant being several million times more massive than the smallest shrew. As a consequence of their size, large and small animals experience very different physical challenges. If an elephant falls, for example, it risks grave injury, whereas a similar tumble for a shrew would probably be inconsequential [123]. To begin to understand how small and large animals coordinate their movement in the face of differing physical challenges like these, one must first determine how size influences the physiological mechanisms underlying sensorimotor control.

My long-term goal is to determine how size-dependent changes in the nervous system affect the control of movement in large and small animals. By understanding which aspects of the nervous system are preserved and which change with animal size, we can gain insight into the fundamental organization of the nervous system. Although the effect of size on animal structure (e.g. [3,5,18]) and dynamics (e.g. [4,17,72]) has been investigated extensively, the scaling of control has remained relatively unexamined.

The first step in this process, and the overall objective of this thesis, is to determine whether and what structural and functional differences in the sensorimotor system exist in quadrupedal mammals over their size range. My general hypothesis is that the sensorimotor systems of larger animals have longer delays and lower precision than those of smaller animals.
1.1 Sensorimotor system and movement control

Animals must be able to catch prey and escape from predators in order to survive. These crucial activities both require the ability to accurately detect changes in the environment and react appropriately. Effective movement control is therefore critical.

The process of sensing and responding to a stimulus requires interaction between the sensory and motor systems, collectively referred to as the sensorimotor system. Sensory pathways detect changes in the environment and the state of the body, while motor pathways initiate movement by activating appropriate muscles. Control of movement via the sensorimotor system is referred to as sensorimotor control, and delays in sensory and motor pathways are referred to as sensorimotor delays.

There are two distinct mechanisms which may be used, alone or in combination, to control movement [102]. The first mechanism, termed feedback control, senses a stimulus, determines an appropriate response, then initiates that response. For example, detecting a stumble and then initiating a corrective motion can prevent an animal from falling. The second mechanism, termed predictive control, senses the immediate environment, uses this information to predict what stimulus will occur, then initiates a preemptive response. For example, noticing a protruding root and lifting the foot higher can prevent an animal from stumbling.

Feedback control results in efficient and stable motion by allowing quick responses to unexpected disturbances [102]. However, it is effective only when disturbances can be sensed accurately, and when there is little delay between sensing and responding. Inability to accurately sense the location and timing of a stimulus means that feedback control does not have the required information about the body’s state to produce an appropriate response. Long delays mean that by the time the response occurs the body’s state may have changed, making the feedback response irrelevant. When sensing is imprecise and delays are long, feedback control is likely to either make a smaller contribution to overall control or to result in an unstable response – in these situations, predictive control can allow a faster response by giving a ‘head start’ and correcting for sensor imperfections. However, predictive control is extremely sensitive to unexpected or unfamiliar perturbations [102]. In theory, if movement regulation were constrained to one type of control, the frequent occurrence of diverse disturbances in biological systems would make feedback more effective than prediction for short sensorimotor delays [102].


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1.2 Responsiveness and resolution

Sensorimotor control is greatly affected by two factors – the time it takes an animal to sense and respond to stimuli, and the ability of an animal to distinguish between sensory stimuli and generate graded muscle forces [53]. To effectively control movement, an animal must be able to precisely detect the type and magnitude of a stimulus and initiate the correct type and magnitude of response within an appropriate period of time. We refer to the speed at which an animal can sense and respond to a stimulus as responsiveness, and the precision with which it can do this as resolution (Figure 1.1) [131].

![Responsiveness](image1)

**Figure 1.1:** Responsiveness and resolution definitions. (a) Responsiveness refers to the speed at which an animal can sense and respond to stimuli. If all other factors are constant, pathways with larger diameter nerve fibers have higher responsiveness. Resolution refers to the ability of an animal to accurately distinguish stimuli (b) and generate graded muscle forces (c). More nerve fibers innervating a given volume of tissue gives a higher resolution. Modified from [131].

Responsiveness depends on the time between the occurrence of a stimulus and the generation of a response; for example, the time required to detect a stumble and initiate corrective limb movement. This is known as response time, and encompasses many sources of delay in even the simplest and fastest reflexes (Figure 1.2a). As the magnitudes of these delays increase, response time increases and responsiveness decreases. Some delays are larger contributors to responsiveness than others. For example, delays related to sensing stimuli, transferring impulses between nerve fibers, and transferring impulses from nerve fibers...
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to muscle fibers are fairly short, on the order of milliseconds [29, 44, 91, 150, 156]. In contrast, delays related to conducting impulses along nerve fibers, conducting impulses along muscle fibers, and generating force can be relatively long, on the order of tens of milliseconds [32, 131, 152].

Resolution depends on the number of movement-related sensory receptors and motor units that an animal possesses, and encompasses several types of sensors and effectors (Figure 1.2b). As the density of sensory receptors increases, the animal can more precisely determine the location, type, and magnitude of a stimulus, thereby increasing sensory resolution [53, 131]. Similarly, as the number of motor units within a muscle increases the animal can generate more levels of force over its force range, thereby increasing motor resolution [53, 131].

In any animal, a trade-off exists between responsiveness and resolution [131]. Responsiveness is in part determined by nerve conduction delay, which for myelinated nerve fibers is directly correlated with nerve fiber diameter [24, 83, 165]. Therefore, all else being equal, the nerves of an animal with higher responsiveness are likely to contain larger-diameter nerve fibers [131]. Resolution is in part determined by the number of nerve fibers, since each sensory receptor or motor unit is innervated by a given number of nerve fibers [119]. Therefore, all else being equal, the nerves of an animal with higher resolution must contain a larger number of nerve fibers [131]. The total size of a nerve is determined by the number and size of nerve fibers it contains, and is constrained by anatomical and physiological factors such as leg size and energy use [197]. For a given nerve size, an animal can maximize its responsiveness (at the expense of resolution) by having a small number of large axons, maximize its resolution (at the expense of responsiveness) by having a large number of small axons, or reach a compromise by having multiple midsized nerve fibers. Although all animals must compromise in some way, they do not all necessarily have the same combination of nerve fiber number and size but instead may prioritize resolution over responsiveness, or vice versa, depending on their needs.

1.2.1 Dependence of responsiveness and resolution on animal size

Responsiveness may depend strongly on animal size. Specifically, delays associated with nerve conduction, electromechanical processes, and force generation have the potential to substantially increase in larger animals. Nerve conduction delay is partially related to length-related body dimensions, since the time taken for an impulse to be conducted down a nerve
CHAPTER 1. INTRODUCTION

Figure 1.2: Components of responsiveness and resolution. (a) Components of responsiveness. In a simple monosynaptic reflex arc, the total time between stimulus onset and force production (response time) incorporates delays due to sensing of the stimulus (sensing delay), conduction of the resulting nerve impulse along nerve fibers (nerve conduction delay), transfer of the nerve impulse between nerve fibers in the spinal cord (synaptic delay), transfer of the nerve impulse from the motor nerve fiber to muscle fibers (neuromuscular junction delay), conduction of the resulting impulse along muscle fibers and the molecular mechanisms involved in crossbridge formation (electromechanical delay), and muscle force development (force generation delay). (b) Components of resolution. In a large peripheral nerve supplying muscle, joints, and skin, there are movement-related sensory nerve fibers originating from structures detecting hair movement (hair receptors), tissue damage (pain receptors), vibration and pressure (cutaneous/subcutaneous receptors), muscle force (tendon organs), joint angle (joint receptors), and muscle length and velocity (muscle spindles). There are also motor nerve fibers terminating on intrafusal muscle fibers (muscle spindles) and extrafusal muscle fibers (motor units). Part (a) of this figure is modified from [132].
fiber depends both on the distance it must travel and the finite speed of impulse conduction. Similarly, electromechanical delay involves the conduction of impulses along muscle fibers, which, all else being equal, will take more time for longer fibers. Force generation delay reflects the time between force onset and the production of peak force – the maximum velocity of muscle fiber shortening decreases with animal size [160,185], which suggests that it may take larger animals more time to generate peak force.

Resolution may also depend on animal size. The relative number of sensors and effectors in an animal is partially dependent on body dimensions that scale with higher powers of length. For example, comparing two animals that differ in length by a factor of two, the larger animal will have a body volume eight times greater and would thus need eight times as many nerve fibers if the number of nerve fibers per unit volume of tissue (innervation density) were to remain constant [131].

1.2.2 Changes to the responsiveness-resolution trade-off with animal size

Animals face a trade-off between responsiveness and resolution, which may become more acute with increasing body size. Most animals scale approximately with geometric similarity, in which linear body dimensions such as leg length are proportional to other linear dimensions, body areas are proportional to the square of linear dimensions, and body volumes and mass are proportional to the cube of linear dimensions [3]. In the case of nerves, as length-related body dimensions increase nerve fiber conduction velocity must also increase if nerve conduction delay were to remain constant [131]. This can only be arrived at by a concomitant increase in nerve fiber diameter [8,83,165] – to maintain responsiveness, nerve fiber diameter \( d \) must increase in direct proportion to leg length. Similarly, to maintain resolution, the number of nerve fibers may need to increase with animal size – for example, if equal resolution requires innervation density to remain constant, the number \( N \) of nerve fibers would need to increase proportional to mass and with the cube of leg length. In this case, to maintain both responsiveness and resolution, total nerve cross-sectional area \( A \) would need to increase proportional to the fifth power of leg length \( L \) (Figure 1.3):

\[
A \propto Nd^2 \Rightarrow A \propto L^3L^2 \Rightarrow A \propto L^5
\]

If nerves scaled in this way they would quickly reach unsupportable sizes. For example, to have the same absolute responsiveness and resolution as a shrew with a leg length of 3
cm and a sciatic nerve 0.3 mm in diameter, an elephant with a leg length of 3 m would require a sciatic nerve with the impossible diameter of 30 m [131]. To maintain sustainable nerve sizes, animals may need to increasingly choose between responsiveness and resolution as their body sizes increase.

Figure 1.3: Responsiveness and resolution trade-off. Possible nerve structures resulting from increased animal size. The conceptual animal body is shown as a grey cube, with a simplified nerve containing one axon represented by the yellow cylinder. As animal height doubles, geometric scaling predicts that nerve length doubles from \( L \) to \( 2L \), total nerve cross-sectional area increases from \( A \) to \( 4A \), and body tissue volume increases from \( V \) to \( 8V \). This would maintain responsiveness, but would result in decreased resolution if nerve fiber number remains constant. If the large animal were to have nerve fibers of the same cross-sectional area as the small animal and maintain an equal resolution, with each nerve fiber innervating the same tissue volume as in the small animal, it would need 8 nerve fibers and have a total cross-sectional area of \( 8A \). If the large animal were to maintain both responsiveness and resolution, it would need 8 nerve fibers, each with a cross-sectional area of \( 4A \), giving a total nerve cross-sectional area of \( 32A \). Maintaining both responsiveness and resolution over large increases in animal size would require impossibly large increases in the nerve cross-sectional area. Modified from [131].
1.2.3 Ameliorating factors in the responsiveness-resolution trade-off

Animals may not need to maintain constant absolute delays or constant innervation densities. Rather than maintaining constant absolute delays, animals may only need to maintain constant relative delays by increasing sensorimotor speeds at the same rate as other aspects of locomotor dynamics. Many of these characteristic times increase more slowly than linear animal dimensions. For example, an animal may need to respond to a perturbation within the time that its foot is on the ground during locomotion, to avoid falling – both stride period at equivalent speeds and the time required to fall to the ground increase with the square root of linear dimensions [2,72]. Rather than maintaining a constant number of nerve fibers per unit volume, nerve fiber number may need to change in proportion to physical dimensions that increase more slowly than animal mass. For example, the number of cutaneous receptors may need to be proportional to animal surface area, which increases with the two-thirds power of animal mass [119,169], and the number of motor units may need to be proportional to muscle force, which increases with the three-quarters power of animal mass [18]. However, either combination of these responsiveness and resolution possibilities would still result in total nerve cross-sectional area increasing more quickly than predicted by geometric scaling, and would still result in an insupportable increase in nerve diameter over a six order of magnitude increase in size. In addition, because different-sized animals often have to respond to the same stimuli from the external world, it may be important under some circumstances for them to have the same absolute performance in their sensorimotor control. For example, a disease-infected insect may be a threat to both shrews and elephants. If it were to land on either animal, the animal must be able to detect the insect’s position accurately (resolution) and swat the threat away before it gets bitten (responsiveness).

Animals may not need all their nerve fibers to be the same size. While there are many possible solutions to the trade-off between responsiveness and resolution, one way that animals could lessen this trade-off is to have a distribution of nerve fibers with different sizes – the larger faster-conducting fibers would allow fast responses, while the smaller slower-conducting fibers would increase resolution for less time-sensitive pathways. In extreme cases, this could result in two distinct fiber populations, with one population made up of large-diameter fibers, and one population made up of small-diameter fibers.
1.3 Effect of responsiveness and resolution on sensorimotor control

Responsiveness and resolution affect sensorimotor control. If responsiveness and resolution were both high, an animal could precisely sense stimuli and generate fast, accurate responses – feedback control would be very effective, and predictive control would not be necessary. However, if responsiveness or resolution were compromised, feedback control would not be as accurate or fast, and would therefore be less effective. With sufficient impairment of responsiveness or resolution, a response would become too slow or inaccurate to be useful and predictive control could be more effective [131]. If responsiveness were compromised, the animal could predict the timing of the stimulus and initiate its response sooner in order to produce a more timely response. If sensory resolution were compromised, the animal may be able to predict the location of spatial stimuli in order to produce a more accurate response – this would only be possible if sensory resolution could still provide enough information to allow prediction. Under some circumstances, such as when stimuli can be integrated over time, an animal with compromised sensory resolution may also be able to predict the onset of time-varying stimuli; if this is not possible, the animal would experience longer delays and lower responsiveness. If motor resolution were compromised, there would be no way for the animal to compensate for the lower number of force levels its muscles could produce, so predictive as well as feedback control would be affected. Therefore, in some cases, predictive control can be used to compensate for deficits in feedback control caused by decreased responsiveness or resolution.

1.4 Extreme animal size

As the world’s tallest land mammal, the giraffe is a particularly interesting example of extreme morphology. Unlike most land mammals, which are approximately geometrically similar, giraffes have exceptionally long necks, and legs that are over 50% longer than predicted based on their mass (prediction from allometric equations for Bovidae in [3]). The giraffe’s extraordinary height is thought to convey survival advantages, such as enabling it to reach food sources that competitors cannot [34]. However, the giraffe’s extreme height may present challenges for the nervous system to control movement – for example, conducting neural impulses down longer body segments may result in longer delays. This gives giraffes
the potential for a particularly acute trade-off between responsiveness and resolution, and makes them especially suited for understanding compromises in sensorimotor performance. For example, one possible solution to offset increased delays would be for giraffes to have a higher nerve conduction velocity than expected based on measured trends with body mass in other animals. However, if total nerve cross-sectional area were to be maintained, this attempt to maintain responsiveness would occur at the expense of resolution and the giraffe would have fewer nerve fibers than expected compared to measured trends.

1.5 Goals and significance

The need to restrict nerve sizes to physically supportable values may force a trade-off limiting one or both of responsiveness and resolution. An intriguing question is whether responsiveness is prioritized over resolution, resolution over responsiveness, or both are compromised. In this thesis, I seek to determine how total responsiveness and resolution scale with animal size, as well as investigate the scaling of their individual components. I will discuss the component and overall scaling of responsiveness, followed by the component and overall scaling of resolution and nerve size. Finally, I will conclude with some thoughts on how changes in responsiveness and resolution might affect movement control in small and large animals.

This work elucidates a fundamental aspect of nervous system organization. Establishing which aspects of the sensorimotor system are prioritized (and which are not) as the competition for space and resources becomes more intense will give us a better understanding of the body’s underlying structure. Identifying the sources of delays and imprecision in the sensorimotor system, and considering how animals address these challenges, will give us a better understanding of how the body’s control system is designed. My results will help provide insight into animal ecology and behaviour. For example, differences in locomotion between small and large animals affect their foraging techniques, contributing to differences in their use of resources [174] and the tendency of elephants to frequent populated areas where they are more likely encounter hostile humans [195]. Knowing how extreme size affects movement control can help determine whether these behaviours are results of the presence or lack of constraints placed on the sensorimotor system, or if they are due to some other factor. My results will also help in recognizing the basic principles underlying nervous system structure and function, which may help to identify and treat neural disorders in animals and humans. For example, demyelinating polyneuropathies in Charcot-Marie-Tooth disease
and Guillain-Barré syndrome affect the peripheral nerves, causing slower nerve conduction velocities and increased sensorimotor delays [15, 79, 99]. Knowing how nerve conduction delays affect sensorimotor control can assist in pinpointing the best locations and types of intervention, improving the lives of thousands of people with these disorders.

1.6 Publications

I have published portions of this thesis as journal articles. Specifically, Chapter 2 includes content from [131], Chapter 3 includes content from [130], and Chapter 2 and Chapter 3 include content from [132]. Chapter 1 and Chapter 4 include content from all three of my articles.

1.7 Common Methods

The following methods are shared by multiple projects addressing my overall goals.

1.7.1 Power laws

Many anatomical and physiological properties of organisms change in a consistent way with body size. When animals scale with geometric similarity, for example, their linear dimensions such as leg length are proportional to $M^{1/3}$ while their surface areas are proportional to $M^{2/3}$ [3, 169]. This type of exponential representation is referred to as a power law. In general, the power law relating a property $y$ to body mass is given by:

$$y = aM^b$$

(1.1)

where $a$ and $b$ are constants. The coefficient $a$ relates to the magnitude of the response, while the exponent $b$ determines how quickly the property $y$ changes with body mass. If $b = 0$, $y$ does not change with mass. If $b < 0$, $y$ decreases with mass; if $b > 0$, $y$ increases with mass. If $b = 1$, $y$ is directly proportional to mass. If $0 < b < 1$ or $b > 1$, $y$ increases slower or faster than mass, respectively. The relationship between $\log(y)$ and $\log(M)$ is linear, with the log transformed data proportionally related by $b$:

$$\log(y) = b\log(M) + \log(a)$$
Given data on the parameter $y$ for animals of various masses, least-squares linear regression of $\log(y)$ and $\log(M)$ can determine $a$ and $b$.

The exponent $b$ is especially interesting because the way that a parameter changes with mass can have important implications. For example, the metabolic rate per gram of body weight decreases as animal mass increases, meaning that larger animals require relatively fewer calories than small animals to survive [97]. Because we are often particularly interested in examining how a property changes with body mass, power laws are commonly written as a proportionality: $y \propto M^b$.

Throughout my thesis, I use power laws to determine coefficients and scaling exponents when investigating the relationship of specific parameters to mass.

1.7.2 Animal research

Every effort was been made to minimize the number of animals required for this work. However, several experiments had not previously been performed on animals in a sufficiently wide range of sizes to establish a meaningful scaling relationship (in the case of the scaling of nerve conduction velocity), or had never been performed (in the case of the scaling of electromechanical delay, force generation delay, and nerve fiber number). In these cases, I performed the experiments on an appropriate number of animals of the required sizes. The protocols for all the projects I performed were approved by the SFU Animal Care Committee, and all animals used were cared for in compliance with the Canadian Council on Animal Care.
Chapter 2

Responsiveness

2.1 Introduction

It is crucial that an animal be able to quickly sense and respond to a stimulus so that it can avoid injury. We refer to the speed at which an animal can sense and respond to a stimulus as responsiveness \cite{131}. Multiple sources of delay – present even in the simplest neural pathways – contribute to the total time between stimulus onset and response production. We refer to these as component delays, and the total time required as overall responsiveness or response time.

Limbs have inherent mechanical properties and predefined movement patterns which help them respond immediately to some perturbations. For example, it is possible to build simple legged robots that can walk down a smooth shallow slope mechanically, without sensors or actuators. This is known as passive dynamic walking, and relies on the robots’ inertial properties to maintain steady locomotion even in the face of small disturbances \cite{122,154}. Animals cannot rely entirely on passive dynamics, however, in part because they generally encounter more complex environments than smooth shallow slopes; they must therefore have muscles which generate force. The intrinsic properties of muscles may help to correct for perturbations without requiring external neural control – for example, if a disturbance occurs which acts to flex a joint, the resulting stretch in the extensor muscles will generate a passive force opposing the perturbation \cite{89}. If a muscle is active when it is stretched, it will generate an active force opposing the perturbation – this is termed a preflex, and relies on the muscle’s force-velocity relationship to augment the body’s passive resistance to sudden movement \cite{26,193}. During locomotion, animals move their leg
downwards and backwards at the end of swing phase – this is known as **swing-leg retraction**, and automatically adjusts the angle at which the leg strikes the ground so that recovery from a disturbance occurs more quickly [172]. Passive dynamics, preflexes, and swing-leg retraction are some examples of a group of phenomena that help animals respond to small perturbations almost instantaneously. These inherent responses, however, may not always be correct, or the perturbation may require a response which is longer, more complex, or less stereotyped – in these situations higher-level control is required, which necessitates signals travelling to and from the periphery and the central nervous system.

Some impulses travel via the spinal cord to the brain before a response is initiated, while others go through complex polysynaptic reflexes within the spinal cord itself [119]. The simplest and fastest responses, however, are monosynaptic spinal reflex arcs, in which a sensory nerve fiber directly synapses onto the cell body of a motor neuron to generate the appropriate response [7, 80]. Because this represents the minimum feedback response time of an animal via neural pathways, I will use the response time via these monosynaptic reflexes as a measure of responsiveness.

In a simple monosynaptic reflex arc, component delays occur due to sensing of the stimulus (sensing delay), conduction of the resulting impulse along sensory nerve fibers (nerve conduction delay), transfer of the impulse from sensory to motor nerve fibers in the spinal cord (synaptic delay), conduction of the impulse along motor nerve fibers (also nerve conduction delay), transfer of the impulse from motor nerve fibers to muscle fibers (neuromuscular junction delay), conduction of the impulse along muscle fibers and the action of molecular mechanisms involved in crossbridge formation (electromechanical delay), and development of muscle force (force generation delay) (Figure 1.2a). Total response time is the sum of these component delays – as the magnitudes of component delays decrease, response time decreases and overall responsiveness increases. Here, I will consider response time as ending with the generation of force by an animal’s muscles; while limb movement also plays an important role in an animal’s functional response, in this initial investigation into responsiveness I will focus on component delays that depend only on the neuromuscular structures involved in a reflex rather than mechanical properties such as inertia.
2.1.1 Consecutive delays

Time delays depend on the difference in timing between a stimulus (known as an input) and a response (known as an output). Each component delay in a reflex arc has an input (for example, a physical force or a voltage change) and an output (for example, muscle force or a voltage change in an adjacent structure). The conversion of an input to an output can occur abruptly or gradually, leading to two general sources of latency: fixed delays and dynamic processes (Figure 2.1). These terms describe the time course of the output, and do not refer to whether or not the length of the delay can change.

Figure 2.1: Fixed delays and dynamic processes. (a) In a fixed delay, a step input (grey line) results in an instantaneous response (blue line) with a time delay $T_d$. (b) In a dynamic process, a step input (grey line) results in a gradual response (red line) with a time constant $\tau$. At time $\tau$ the dynamic process has reached approximately 63% of its steady state value, at time $2\tau$ it has reached approximately 86%, and at time $3\tau$ it has reached approximately 95%.

Fixed delays happen when an output does not occur until some time $T_d$ after the input, after which it occurs abruptly (Figure 2.1a). Dynamic processes, on the other hand, happen when the output increases gradually (Figure 2.1b). Dynamic processes can be multi-order, with the simplest referred to as first-order systems. The speed of a first-order dynamic process depends on its time constant $\tau$, which represents the amount of time required for

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1The ‘stimulus’ and ‘response’ described here are generic, and are not necessarily the same as the sensory stimuli (for example, a stumble) and motor responses (for example, a limb movement) described in the rest of this thesis. The general concepts, however, are the same.
the response to reach $\sim 63\% \left(1 - \frac{1}{e}\right)$ of its steady state value. The lengths of both fixed delays and dynamic delays can be changed, by changing $T_d$ or $\tau$ respectively.

In a reflex arc, a stimulus initiates an impulse which travels through a chain of component delays. Finding the total response time requires finding the sum of these consecutive delays, which could be difficult since some component delays are fixed delays while others are caused by first-order or multi-order dynamic processes. However, the sensorimotor system thresholds many of the dynamic processes involved in component delays. Thresholding produces an output only when the value of a dynamic process reaches a certain level. This means that, in a monosynaptic reflex arc responding to an isolated stimulus, all the component delays except the last (force generation delay) can be thought of as fixed delays. For instance, the dynamic process of diffusion mediates the transfer of an impulse from one neuron to another at a synapse. The concentration of molecules at the second neuron gradually increases as more molecules diffuse across the synapse – the second neuron does not respond until the number of molecules reaches a threshold level, after which it immediately generates an output. In this example, thresholding converts the dynamic process of diffusion to a fixed delay.

If multiple stimuli occur in sequence, component delays incorporating dynamic processes will be affected. Several rapid inputs will initiate several consecutive dynamic processes, whose magnitudes will sum. This will reduce the time required for the dynamic process to reach the threshold value, thereby reducing the time between the input and response. For example, if a molecule must diffuse across a space to attain a threshold concentration and produce an output, multiple rapid inputs will mean that molecules are released several times in sequence and their concentration will rise more quickly than in the case of a single input – this will result in a shorter delay.

In this thesis, I consider the journey of an isolated stimulus through a monosynaptic reflex arc, which represents the fastest possible response time via neural pathways. Because I consider only a single stimulus and use a threshold to measure force generation delay, I only need to consider fixed delays. Therefore, I will not discuss here the details of adding sequential dynamic processes. In future work considering the effects of repetitive stimuli, dynamic processes must be taken into consideration – this is possible by combining fixed and dynamic component delays in the Laplace domain and examining the resulting transfer function (see [57] for a discussion of transfer functions in fixed delays and dynamic processes).

Total response time is the sum of sequential fixed delays, with the scaling relationship
for each delay depending on the scaling of its respective time delay $T_d$. The power law representing each scaling relationship includes a coefficient $a$, reflecting the magnitude of the delay in a 1 kg animal, and an exponent $b$, reflecting how quickly $T_d$ or $\tau$ changes with animal size (Equation 1.1 on page 11). Adding the scaling relationships for all the component delays determines how response time scales with animal size. A sum of power laws is not necessarily also a power law, and cannot necessarily be approximated by a power law. Therefore, I will use the scaling of component delays to numerically determine how total response time changes with animal size, then figure out what type of equation best describes this relationship.

2.1.2 Component delays

There are six main sources of delay in monosynaptic reflexes, which we refer to as component delays (Figure 1.2a). Some of these delays are short, while others can be relatively long in some animals. As animal size increases, from tiny 5 gram shrews to massive 5,000 kilogram elephants, some component delays have the potential to change whereas others are likely to remain relatively constant.

2.1.2.1 Sensing delay

Each nerve fiber carries information about a single type (modality) of stimulus [89]. The modality transmitted by a nerve fiber depends on modality of the sensory receptor it innervates. While a receptor may respond to several types of stimulus energy, it is usually most sensitive to one type [89]:

- **Photoreceptors** detect electromechanical energy in the form of light.
  e.g. rods and cones in the retina of the eye detect light and colour

- **Thermoreceptors** detect thermal energy in the form of heat or cold.
  e.g. hot and cold receptors in cutaneous and subcutaneous tissue

- **Chemoreceptors** detect chemical energy in the form of the presence of a chemical ligand.
  e.g. free endings in skeletal muscle detect metabolite concentration

- **Mechanoreceptors** detect mechanical energy in the form of physical deformation.
  e.g. corpuscles in cutaneous and subcutaneous tissue detect pressure and change in
Of these receptor groups, I will assume that mechanoreceptors play the greatest role in locomotion, since they detect physical perturbations which threaten stability. I will therefore consider mechanoreceptors exclusively for the remainder of this thesis. I have described the types of mechanoreceptors in more detail in Subsection 3.1.1.

In general, a mechanoreceptor consists of a nerve fiber ending and, in some cases, a specialized end organ surrounding it [113]. Although different types of end organs cause mechanoreceptors to be sensitive to different types of mechanical stimuli, the response of the receptor is generally initiated by stretching of the nerve fiber ending’s cell membrane [95, 113, 143]. Mechanical deformation of the tissue surrounding the receptor opens the stretch-sensitive ion channels in the cell membrane and allows positively charged ions, such as potassium, sodium, or calcium ions, to enter the nerve fiber ending [166]. The influx of positive charge creates local depolarizations at the location of each open ion channel, and spatial summation of these individual depolarizations results in the generation of an action potential in the unmyelinated ending of the nerve fiber [81, 95, 143].

Sensing delay includes the time required for tissue to deform, stretch-sensitive ion channels to open, ions to flow into the nerve fiber, a sufficient amount of depolarization to occur and sum, and an action potential to be generated. The speeds of these processes are likely to be conserved, and the structure of several mechanoreceptor types is fairly consistent across species spanning a range of sizes, from mice to sheep [13, 87, 178]. Taken together, these observations suggest that sensing delay may also be fairly consistent between animal sizes and species.

2.1.2.2 Nerve conduction delay

The time taken for a nerve fiber to conduct an impulse from one point to another depends on two factors: the distance the impulse must travel, and the speed at which the impulse is propagated. In animals, the distance that an impulse must travel depends on body size – for example, larger animals have longer legs, so the impulse must be transmitted further [3, 131]. The speed at which a nerve fiber conducts an impulse depends on its biophysical properties, and several of these properties also depend on size.

Impulses are conducted along nerve fibers by the propagation of a wave of depolarization created by the flow of charge [165]. This wave of depolarization is known as an action
potential, and its speed of propagation depends on the biophysical properties of the nerve fiber [165]. In the absence of an action potential, differences in ion concentrations across the nerve fiber membrane cause an electrical potential difference in which the environment inside the nerve fiber is more negative than the environment outside the fiber [89]. This potential difference is referred to as the membrane potential. During depolarization, the inside of the nerve fiber becomes less negative relative to the outside, making the membrane potential less negative. Once the membrane potential reaches a threshold value, an action potential is generated – voltage-gated ion channels are opened and allow positively charged sodium ions to flow down their electrochemical gradient into the nerve fiber, causing the inside of the fiber to become even less negative and opening more ion channels to allow more sodium ions to flow [89]. This self-perpetuating process does not continue indefinitely, because ion channels become inactivated after only a few milliseconds and because the delayed activation of voltage-gated potassium channels allows an opposing flow of charge [89]. However, the current initiated by the action potential causes neighboring regions of the nerve fiber to depolarize and their voltage-gated ion channels to open, allowing more sodium to diffuse into the nerve fiber and the depolarization process to start again [89]. In this way, the action potential is propagated down the nerve fiber, and the speed at which it travels is termed nerve fiber conduction velocity.

There are two types of nerve fibers, and their different properties affect the speed of action potential propagation: unmyelinated nerve fibers consist of a bare axon, and myelinated nerve fibers consist of an axon surrounded by layers of insulating myelin formed by Schwann cells [89]. In unmyelinated nerve fibers, the action potential is regenerated at each point along the fiber. For small nerve fibers (less than 1 µm in diameter), the high ratio of axon membrane area to axon cross-sectional area in unmyelinated fibers allows action potential propagation to be faster than for myelinated fibers of the same diameter [165]. In large myelinated nerve fibers (greater than 1 µm in diameter), the surrounding myelin increases the effective membrane resistance (radial resistance) relative to the cytoplasmic resistance (axial resistance). This allows more charge to diffuse axially along the fiber, which increases the distance that electrical potential can travel passively – this distance is known as the length constant, and myelinated nerve fibers have greater length constants than unmyelinated fibers of the same diameter. To prevent the action potential from dying out while travelling along the nerve fiber, there are gaps in the myelin, termed nodes of Ranvier, at which it can be actively regenerated. The action potential therefore ‘jumps’
from one gap to another; the combination of a greater length constant and a decreased reliance on active regeneration means that the action potential can be propagated faster [89]. This results in large myelinated nerve fibers conducting nerve impulses more quickly than unmyelinated fibers of the same diameter. In fact, in mammalian peripheral nerves, fibers with diameters less than 1 µm tend to be unmyelinated, fibers with diameters greater than 1 µm tend to be myelinated, and the fastest-conducting nerve fibers are the largest myelinated fibers [47,83]. Since I am interested in the fastest pathways, I will consider myelinated fibers for the remainder of my discussion.

The conduction velocity of a myelinated nerve fiber depends on several factors: the thickness of the myelin surrounding the axon, the distance between nodes of Ranvier (termed internodal distance), and the total diameter of the fiber. In theory, due to biophysical properties of the nerve fiber such as axial resistance, radial resistance, and capacitance of the myelin, there are optimal values for the myelin thickness and internodal distance to maximize the speed of action potential propagation in a nerve fiber of a given size [165]. Measured values for these parameters in cats are almost identical to those predicted based on theory, suggesting that animals have optimized these characteristics of their nerve fibers [8,24,63,83,165]. Theory also predicts that the conduction velocity of myelinated fibers is directly proportional to outer myelin diameter (i.e. nerve fiber diameter); measured values for the relationship between nerve fiber conduction velocity and diameter in cats are in agreement with this [8,24,83,165]. Therefore, since myelin thickness and internodal distance are already optimized, cats must increase the diameter of their nerve fibers to achieve greater speed of impulse transmission. It is likely that, given the long distances that nerve impulses need to travel in large animals, optimal myelin thickness and internodal distance are conserved across different animal sizes. This means that the only way for larger animals to compensate for the longer distances that their nerve impulses must travel is to increase the diameter of their nerve fibers.

2.1.2.3 Synaptic delay

To transfer a nerve impulse between nerve fibers, the signal must cross a small gap between the fibers. This gap is known as a synapse. The arrival of an action potential at the ending of the first nerve fiber opens voltage-gated calcium channels, allowing calcium ions to flow into the cell [38,92]. The increase in intracellular calcium levels triggers the binding of synaptic vesicles with the nerve fiber membrane, allowing the vesicles to release their sequestered
neurotransmitter into the synapse [38]. The neurotransmitter diffuses across the gap, and binds to its specific receptor on the second nerve fiber; in excitatory synapses, such as the synapse involved in the monosynaptic stretch reflex, this binding causes an increase in the permeability of the nerve fiber membrane to cations [89]. The resulting net flow of positive charge into the second nerve fiber causes the membrane potential to become less negative. When the membrane potential reaches the threshold value, an action potential is generated.

Multiple factors can affect the length of synaptic delay, such as the density of vesicles in the presynaptic nerve fiber and the activation history of the synapse, but the main contributor is the time required for calcium channels to open [89]. There is some evidence that the structure of calcium channels is relatively similar across several species of mammals [38, 142], and that the kinetics of at least one type of calcium channel are similar between species of vertebrates [37]. These similarities in the rate-limiting step of synaptic delay suggest that this delay is likely to be relatively constant across animal size, although it may vary slightly if other determinants of synaptic delay are altered.

\[ \text{2.1.2.4 Neuromuscular junction delay} \]

To transfer an electrical impulse between the motor nerve fiber and a muscle fiber, the signal must cross a small gap known as a neuromuscular junction. This is similar to the way in which a signal is passed between nerve fibers at a synapse. The arrival of an action potential at the ending of the motor nerve fiber opens voltage-gated calcium channels, allowing calcium to flow into the cell [38]. The increase in intracellular calcium levels triggers the binding of synaptic vesicles with the nerve fiber membrane, and the release of their sequestered neurotransmitter – acetylcholine – into the neuromuscular junction [40, 78]. Acetylcholine diffuses across the gap, and binds to its receptors on the muscle fiber [78]. The area of the muscle fiber containing acetylcholine receptors is termed the motor end plate. Binding of acetylcholine to its receptor causes an increase in permeability of the muscle cell membrane to sodium and potassium ions, and the resulting net flow of positively charged ions into the muscle cell creates a depolarization called an end plate potential [78]. When the end plate potential reaches a threshold value, an action potential is generated in the muscle fiber [78].

Neuromuscular junctions and synapses are very similar [48, 92]. The length of neuromuscular junction delay is affected by similar factors to those which affect the length of
synaptic delay – for example, the density of vesicles in the presynaptic terminal of the motor nerve fiber, and the activation history of the nerve fiber and muscle fiber – as well as by hormone levels [78]. However, the main contributor to neuromuscular junction delay is the time required to release acetylcholine once the action potential arrives at the presynaptic terminal [78, 91]. This process depends in part on the kinetics of calcium channels, and there is evidence that the general structure of these channels is conserved across species [38]. Therefore, like synaptic delay, neuromuscular junction delay is likely to be relatively constant across animal size. In addition, it is possible that muscle fibers could take more time than nerve fibers to depolarize to the threshold required to generate an action potential, since they have a larger diameter [89] – this could result in neuromuscular junction delay being slightly longer than synaptic delay.

2.1.2.5 Electromechanical delay

Converting an action potential at the motor end plate into muscle force involves several processes. There is no clear definition of electromechanical delay, and different researchers have used different definitions [39, 134, 158]. I will define electromechanical delay as beginning after an action potential is produced at the motor end plate, and ending once muscle force starts being produced – this encompasses action potential propagation and excitation-contraction coupling.

The first step in electromechanical delay is the propagation of the action potential along the muscle fiber surface, and down folds in the cell membrane called T-tubules; this occurs in a similar way as the propagation of an action potential along a nerve fiber [89]. Next, in a process termed excitation-contraction coupling, specialized receptors transmit the depolarization of the T-tubules to a structure inside the muscle fiber called the sarcoplasmic reticulum, which sequesters calcium ions [16, 158]. Activation of the the sarcoplasmic reticulum causes it to release calcium ions into the muscle fiber [158]. The calcium ions travel by diffusion to the myofilaments, and bind to troponin-C, which is a protein located on the actin filaments. The binding of calcium to troponin-C initiates the movement of another protein, tropomyosin, which exposes the myosin binding site on the actin filament [16]. This allows myosin to bind to actin and, once bound, the myosin head changes shape to pull the two filaments past each other [16]. The sliding of the filaments causes the muscle fiber to shorten, thereby generating force.

The processes that determine electromechanical delay may depend on body size. In
particular, impulse conduction along muscle fibers will take more time for longer fibers. Muscle fiber length has been measured to increase substantially with body size in some studies [3], although this finding is certainly not conclusive [60,112]. However, muscle fibers in large animals, such as elephants, are certainly longer than the corresponding whole-muscle length in small animals, such as shrews [5,60,112].

2.1.2.6 Force generation delay

Force generation delay begins at the onset of force production, and ends at peak twitch force. The time required to reach peak force depends on how quickly actin and myosin myofilaments move past each other. This process is referred to as crossbridge cycling, and can only occur when calcium ions are present. During the crossbridge cycle, myosin heads bind to actin and change shape as they release adenosine diphosphate – this shape change pulls the actin and myosin myofilaments past each other, generating force [16]. The binding of adenosine triphosphate to the myosin heads, and its subsequent hydrolysis to adenosine diphosphate, mediates the dissociation of myosin from actin and allows the cycle to repeat. As the myofilaments slide past each other, the muscle shortens and generates force.

Force generation delay depends on muscle fiber type and the amount of slack and stretch in the muscle, which may both change with animal size. Muscle fiber type affects the velocity at which the fiber can contract, with fast fibers contracting more quickly than slow fibers [32,160]. There is some evidence that maximum contractile velocity of muscle fibers may decrease with animal size, particularly in slow fibers [160]. Once muscle fibers contract, and before force output can occur, any slack in the muscle must be taken up by its contractile elements. In addition, muscles contain series and parallel elastic elements, and stretch in these elastic elements delays the production of peak force [16]. Larger animals with longer muscle-tendon units will have more slack and stretch to take up than smaller animals, which may lengthen their force generation delay.

2.1.3 Overall responsiveness

The sum of component delays gives the total response time, which determines overall responsiveness. The magnitude and scaling of the individual component delays therefore determine the way in which response time scales with animal size. Some delays are relatively short and are not expected to change appreciably with animal size (sensing delay, synaptic delay,
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and neuromuscular junction delay), while others can be relatively long and have the potential to increase with animal size (nerve conduction delay, electromechanical delay, and force generation delay). Each of these latter three sources of delay could theoretically increase, decrease, or remain constant with increasing animal size. If the delays increase they will likely dominate the scaling of total response time, causing responsiveness to decrease.

Responsiveness can be expressed in both absolute and relative terms (Subsection 1.2.3). Absolute responsiveness is the absolute speed at which an animal can sense and respond to a stimulus – for example, the number of milliseconds between when an animal starts to stumble and when it moves its limb to recover balance. Relative responsiveness takes into account the size-dependent effects of physical forces by relating absolute responsiveness to a characteristic time – for example, the time required to detect and respond to a stumble expressed as a fraction of the duration of stance phase. Because of the wide variety of situations encountered by animals, both absolute and relative responsiveness are crucial to their survival.

Animals with short response times, and therefore high overall responsiveness, may be able to respond to perturbations effectively using feedback control (Section 1.3; [102]). However, if response times become longer, thereby lowering overall responsiveness, feedback control may be unable to initiate a response within an appropriate amount of time – for example, an animal may be unable to respond to a stumble in time to prevent falling. In addition, if an animal relies too heavily on slow feedback pathways it may experience instability and oscillatory responses as its control system continually attempts to correct for a state which is no longer current; one solution to this problem is for the animal to place less importance on feedback pathways. To avoid slow and unstable reactions, an animal with low responsiveness may be forced to rely more heavily on predictive control to anticipate disturbances in time to initiate a corrective response. Although in many situations predictive control is adequate, it is less likely to allow an effective response to unexpected or unfamiliar perturbations [102]. In unpredictable situations, which feedback control is especially suited for, the decreased reliance on feedback becomes detrimental.

2.1.4 Goals

I sought to determine how component delays and overall responsiveness scale with animal mass, in both absolute and relative terms. Specifically, I considered delays in the hindlimb stretch reflex – this is the simplest and fastest sensorimotor pathway, and its role in regaining
balance after perturbations during locomotion makes it an important aspect of movement control [7,149]. Because the sciatic nerve innervates the hindlimb, and the medial gastrocnemius muscle (innervated by the tibial branch of the sciatic nerve) is a major ankle extensor, I focused my analysis on the sciatic and tibial nerves and medial gastrocnemius muscle. Finally, I used my findings to predict how control methods may change with animal size.

2.2 Methods

Where available, I used literature data to determine component delays. In several cases, where these data were not available, I performed electrophysiology experiments to determine component delays. All electrophysiology procedures were approved by the SFU Animal Care Committee. I performed the shrew experiments with colleagues from the SFU Locomotion Lab, and the giraffe experiments with colleagues from the SFU Locomotion Lab and collaborators from the Danish Cardiovascular Giraffe Research Programme; my colleagues performed the elephant experiments. I analyzed the data from all the experiments along with my colleagues from the SFU Locomotion Lab. While we performed the three sets of electrophysiology experiments using similar methods, the details are slightly different due to the different constraints of experimental locations, the unique challenges inherent to each species, and our evolving experimental techniques.

2.2.1 Shrew electrophysiology

We acquired data from six least shrews (Cryptotis parva) obtained from the Department of Anatomy, Kirksville College of Osteopathic Medicine, MO.

2.2.1.1 Anesthesia

We anesthetized each shrew with 2% isoflurane and monitored their breathing and temperature throughout the experiment, with heating and anesthetic levels adjusted as necessary.

2.2.1.2 Electrophysiology

We acquired motor nerve fiber conduction velocity data by recording surface electromyography (EMG) activity from the medial gastrocnemius while evoking reflexes in this muscle using electrical stimulation of the sciatic nerve at two locations (Figure 2.2a).
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Figure 2.2: Nerve fiber conduction velocity methods. The **top row** shows the nerves and muscles we examined in our experiments in the context of the whole animal. The **middle row** shows our stimulation and recording locations – dark and light blue arrows indicate stimulation locations, black electrodes indicate recording locations. In all cases, we recorded evoked electrical potentials in the medial gastrocnemius muscle. In each experiment, we determined nerve fiber conduction velocity (CV) by dividing the distance $\Delta d$ between stimulation electrodes by the time difference $\Delta t$ of muscle activity onset. The **bottom row** shows examples of evoked electrical potentials that we recorded in the muscle (dark and light blue time-series lines). (a) Shrew CV experiment. We stimulated the sciatic nerve electrically at two points along its length. (b) Giraffe CV experiment. We stimulated the sciatic nerve electrically at two points along its length. We measured the onset time of each giraffe recording as the time at which the evoked electrical potential reached a value of 20% of its maximum (light grey horizontal line). (c) Elephant CV experiment. We stimulated the sciatic nerve electrically (lightning bolt symbol) and the Achilles tendon mechanically (reflex hammer symbol). Modified from [131,132].
We exposed the sciatic and tibial nerves as well as the medial gastrocnemius muscle, and placed the hindquarters of four of the shrews in aluminum pans filled with warmed mineral oil to maintain body temperature and reduce stimulus artifact. We placed two monopolar Ag stimulating electrodes as far apart as possible (5 mm for all shrews) on the surface of the sciatic nerve and two bipolar Pt EMG recording electrodes close together on the surface of the medial gastrocnemius muscle. All electrodes were held in place by three-dimensional electrode manipulators secured to a stereotaxic base (David Kopf Instruments, Tujunga, CA). A Grass S88 stimulator connected to a Grass SIU-5 stimulus isolation unit (Grass Technologies, West Warwick, RI) delivered a train of square wave pulses on the order of 1 V, 0.1 ms duration, and 1 Hz to the sciatic nerve via the proximal stimulating electrode. We used this electrode as the positive pole, and held an identical electrode manually on the surface of nearby tissue for the negative pole. We increased stimulus strength until muscle contractions occurred with 50% of the stimuli, took this voltage as threshold, then increased stimulus strength to two times the threshold value and captured 30 consecutive EMG recordings from the recording electrodes. A Grass 15LT amplifier system with Grass 15A54 amplifiers amplified the signals 500 times and a USB data acquisition device (IOtech, Cleveland, OH) converted them to digital signals before they were collected using Matlab 2007a (The MathWorks, Inc., Natick, MA) on a desktop computer. The stimulation site was then moved to the distal electrode, where the stimulation protocol was repeated to obtain another set of 30 consecutive EMG recordings from the recording electrodes. A Grass 15LT amplifier system with Grass 15A54 amplifiers amplified the signals 500 times and a USB data acquisition device (IOtech, Cleveland, OH) converted them to digital signals before they were collected using Matlab 2007a (The MathWorks, Inc., Natick, MA) on a desktop computer. The stimulation site was then moved to the distal electrode, where the stimulation protocol was repeated to obtain another set of 30 consecutive EMG recordings. We recorded body temperature from the surface of the animal at the time of stimulation. Once all recordings were obtained, we euthanized the shrew with 0.4 cc sodium pentobarbital (240 mg mL$^{-1}$).

For each shrew, we averaged the 30 EMG responses within each trial to reduce noise and produce a single average EMG response. We measured the time between the onset of stimulation artifact and the onset of muscle activity at each of the two stimulation sites, then divided the distance between stimulation sites by the difference in latency between the sites to give nerve fiber conduction velocity (CV). This value was adjusted to 38.5°C using a Q$_{10}$ of 1.6 [145] to find the equivalent CV at shrew body temperature [129]. Specifically, the equation we used for CV adjustment was:

$$CV_2 = CV_1 e^{\ln(1.6) \frac{38.5-T_1}{10}}$$

where $CV_1$ is measured CV, $CV_2$ is temperature-corrected CV, and $T_1$ is the shrew body
temperature at which we measured CV.

2.2.2 Giraffe electrophysiology

We acquired electrophysiology data and sciatic nerve samples from eight male giraffes (*Giraffa camelopardalis*) aged two to four years [132]. Electrophysiology procedures and tissue collection were carried out simultaneously with many other research projects during the 2010 Danish Cardiovascular Giraffe Research Programme expedition to Hammanskraal, South Africa. Due to the nature of these multi-experiment protocols, we performed each procedure on only four of the eight animals. Experiments and procedures were approved by the Danish Animal Ethics Committee, the Animal Ethics Screening Committee at The University of Witwatersrand (Johannesburg), the Animal Use and Care Committee (University of Pretoria, South Africa), and the Simon Fraser University Office of Research Ethics. Permission to euthanize the animals was granted by Gauteng Province, South Africa.

2.2.2.1 Anesthesia

Each animal was anesthetized prior to any invasive procedures. Following overnight fasting, the giraffe was premedicated by remote injection with medetomidine (5.5 µg kg$^{-1}$). Eight minutes later, the giraffe was guided to a chute where it was haltered and blindfolded. An induction dose of etorphine (6.5 µg kg$^{-1}$) and ketamine (0.65 mg kg$^{-1}$) was then administered, and the giraffe was led into an adjacent pen where it became recumbent within 3–7 minutes. A rope connected to the giraffe via a halter and passed through a pulley in the ceiling allowed control of the giraffe’s head to avoid injury during this process. Immediately after the giraffe was recumbent, a cuffed endotracheal tube (ID 20 mm) was inserted through a tracheostomy and ancillary ventilation with oxygen was initiated using a demand valve (Hudson RCI, USA). Breathing was maintained by manually ventilating at a rate of 4 breaths min$^{-1}$. A supplementary dose of ketamine (0.2 mg kg$^{-1}$) was administered intravenously before the giraffe was moved to an adjacent room for the experimental procedures.

In two giraffes, anesthesia was maintained by repeated dosing with etorphine and ketamine based on clinical signs, while in the other six animals anesthesia was maintained by intravenous infusion of alpha-chloralose (15 mg ml$^{-1}$, KVL-pharmacy, Denmark) at 30 mg kg$^{-1}$ h$^{-1}$ decreasing to 20 mg kg$^{-1}$ h$^{-1}$ after 72 min, 15 mg kg$^{-1}$ h$^{-1}$ after 140 min, and
then gradually reducing to 3 mg kg$^{-1}$ h$^{-1}$ over the next 7–8 hours to maintain the animal within the surgical plane. We monitored the giraffe’s electrocardiogram and maintained the giraffe’s heart rate at 30–40 bpm, rectal temperature at 38–39°C, end-expiratory carbon dioxide tension at approximately 40 mmHg, and mean arterial pressure at approximately 150 mmHg using a portable monitor (Mindray PM9000Vet, E-Vet, Denmark) to ensure the giraffe remained stable. In addition, we measured blood gas values every 10 minutes in arterial and venous blood (GEM Premier 3500, Instrumentation Laboratory, Bedford, MA 01730, USA). Values for pH and the partial pressures of carbon dioxide ($pCO_2$) and oxygen ($pO_2$) at the beginning of data collection were $7.2 \pm 0.2$, $42.6 \pm 16.5$ mmHg, and $167 \pm 62$ mmHg, respectively. Toward the end of the procedure, pH, $pCO_2$ and $pO_2$ remained within physiological limits at $7.3 \pm 0.2$, $44 \pm 9$ mmHg, and $249 \pm 153$ mmHg, respectively (mean ± standard deviation). Once all experiments were complete, the giraffe was euthanized with an overdose of pentobarbital.

### 2.2.2.2 Electrophysiology

#### Nerve fiber conduction velocity

We acquired motor nerve fiber conduction velocity data from four giraffes by recording surface EMG activity from the medial gastrocnemius while evoking reflexes in this muscle using electrical stimulation of the sciatic nerve at two locations (Figure 2.2b). At each stimulation site, we inserted a pair of electrodes consisting of two thin insulated wires (0.012 inch (3 mm) diameter, AS 632, Cooner Wire Inc., Chatsworth, CA) with deinsulated (~5 mm) and hooked ends using an epidural needle as a guide (Portex Tuohy, 11 cm long, 1.3 mm outside diameter; Smiths Medical International Ltd., Kent, UK). To insert each electrode, we threaded the wire through the barrel of the needle and, guided by ultrasound (Vivid i, GE Healthcare, curved array 4 MHz probe), we advanced the needle through the overlaying skin and muscle until its tip was adjacent to the nerve. We then retracted the needle, allowing the wire to remain in the tissue. The distances between electrode pairs ranged from 4–8.5 cm, and were measured on images collected by ultrasound. We placed pre-amplified surface EMG recording electrodes (Delsys Inc., Boston, MA) on the skin over the medial gastrocnemius muscle, oriented perpendicular to the muscle fibers. A Grass SD9 stimulator (Grass Technologies, West Warwick, RI) delivered a train of square wave pulses on the order of 10 V, 1 ms duration, and 1 Hz to the sciatic nerve via the proximal pair of stimulating electrodes. We chose a stimulus strength which resulted in
the smallest recognizable EMG signal and collected 11 consecutive EMG recordings at 25 kHz from the recording electrodes. The EMG signals were amplified and filtered by the Delsys system (gain: 1000; bandwidth: 20–450 Hz) and collected on a laptop computer with a data acquisition card. The stimulation site was then moved to the distal pair of stimulating electrodes, where the stimulation protocol was repeated to obtain another set of 11 consecutive EMG recordings.

For each giraffe, we averaged the 11 EMG responses within each trial to reduce noise and produce a single average EMG response. To remove stimulus artifact that obscured muscle activity, we subtracted a modeled stimulus artifact from the average EMG signal. The modeled artifact was based on the band-pass filtering characteristics of the EMG recording hardware [188]. We calculated the onset of muscle activity as the time at which the EMG signal crossed a threshold of 20% of the magnitude of its first peak. This represented muscle activity caused by impulses in the motor nerve fibers stimulated by the stimulating electrodes. We measured the time between the onset of stimulation and the onset of muscle activity at each of the two stimulation sites, then divided the distance between stimulation sites by the difference in latency between the sites to give nerve fiber conduction velocity.

Electromechanical delay and force generation delay
We designed and built a custom device to stimulate muscle fibers and record the timing of the resulting muscle force in four giraffes. The device consisted of two 1 inch (2.54 cm) long hypodermic needles (21G) mounted 1 cm apart on a semi-flexible aluminum plate and inserted into the muscle [198]. Prior to insertion, we threaded two thin insulated wires (0.012 inch (3 mm) diameter, AS 632, Cooner Wire Inc., Chatsworth, CA) with deinsulated (≈5 mm) and hooked ends through the needle barrels – we used these wires as the stimulating electrodes. A single-axis strain gauge (SGD-3/350-LY43, Omega Engineering, Inc., Stamford, CT) mounted to the aluminum plate measured the amount of bend in the plate created when the needles were pushed together during a muscle contraction. We implemented the strain gauge in a standard Wheatstone bridge configuration with a supply voltage of 5 V. A signal conditioner (A2, Vishay Micro-Measurements, Wendell, NC) amplified the strain gauge output by a factor of 1000. Prior to our giraffe experiments, we validated our device by measuring the forces produced in a rat medial gastrocnemius muscle during stimulation through the stimulating electrodes. The resulting force profile had the same shape and force duration as that found in studies that activated the muscle by stimulating the motor
nerve [32,152].

We inserted the needles of our device into the belly of the giraffe medial gastrocnemius muscle at an angle of approximately 35° to the central tendon, following the direction of pennation. A stimulator (SD9, Grass Technologies, West Warwick, RI) delivered a train of square wave pulses on the order of 10 V, 1 ms duration, and 1 Hz to the muscle fibers via the wire stimulating electrodes (Figure 2.3a). We chose the smallest stimulus strength which resulted in clearly identifiable deflections in the force recordings and collected 11 consecutive force profile recordings at 25 kHz on a laptop computer.

Figure 2.3: Electromechanical delay and force generation delay methods. (a) We directly stimulated the medial gastrocnemius muscle of four giraffes, and measured the force profile of the resulting muscle twitch (green time-series line) using a strain gauge. (b) Representative force profile from one giraffe following direct stimulation of the muscle. We determined the onset time of muscle force as the time at which the force signal reached a value of three standard deviations above the baseline noise, calculated over 100 ms before the stimulus (light grey horizontal line). We calculated electromechanical delay as the time between muscle stimulation and muscle force onset, and force generation delay as the time between force onset and the production of peak force. Modified from [132].

For each giraffe, we averaged the 11 force profile recordings within each trial to reduce noise and produce a single average force profile. We defined the onset of force production as the time at which the signal crossed a threshold of three times the standard deviation of the signal occurring in the 100 ms time period before the stimulus. This gave the time required for electromechanical activation. The time between the onset of force production and peak force generation gave the time required for force generation.
2.2.3 Elephant electrophysiology

We acquired sensory nerve fiber conduction velocity data from one Asian elephant (*Elephas maximus*) by recording surface EMG activity from the medial gastrocnemius while evoking reflexes in this muscle using two techniques – electrical stimulation of the tibial nerve in the popliteal fossa and percussion of the Achilles tendon (Figure 2.2c). Throughout the experiment, the elephant was standing and eating under the care of its handlers. To enable us to accurately place stimulating and recording electrodes, we combined results from previous computed tomography scanning and dissection of six Asian elephants [127].

We stimulated the tibial nerve using stimulating electrodes consisting of two thin insulated wires (0.002 inch (0.05 mm) diameter, stainless steel; A-M Systems Inc. Carlsborg, WA), with deinsulated (∼5 mm) ends inserted through the barrel of a hollow catheter needle (15 cm long, 1 mm outside diameter, Milacath, MILA International, Erlanger, KY) and bent to form a hook. We advanced the needle through the skin at the back of the knee to a depth of ∼8 cm before retracting it and allowing the wires to remain in the popliteal fossa. We placed self-adhesive EMG recording electrodes (3M, Red Dot, #2249, Ag/AgCl) on the skin over the medial gastrocnemius muscle. Over a period of 2–3 minutes we delivered 13 stimuli at least 10 seconds apart to the tibial nerve via the stimulating electrodes, at an intensity which evoked a Hoffman reflex in the medial gastrocnemius [128]. We recorded EMG activity at 3 kHz from the recording electrodes, and stored the data on a laptop computer using Noraxon MyoResearch XP programs (Master version 1.04) for subsequent analysis. Next, we used a standard Taylor percussion reflex hammer to percuss the Achilles tendon (n=15) at a strength that was sufficient to evoke a reflexive muscle twitch, and again recorded EMG activity from the recording electrodes. We measured a distance of 0.56 m between the tendon tap and electrical stimulation sites.

We measured the time between the onset of stimulation and the onset of muscle activity at each of the two stimulation sites, and subtracted a total of 2 ms from this to approximate the additional delay for the mechanical stimulus to generate an afferent volley when compared to the electrical stimulus and the additional time required for the unsynchronized Ia action potentials produced by the tendon tap to elicit excitatory post-synaptic action potentials of sufficient amplitude to depolarize motor nerve fibers [29,30,133,150]. Dividing the distance between stimulation sites by the adjusted difference in latency between the sites gave nerve fiber conduction velocity.
CHAPTER 2. RESPONSIVENESS

2.2.4 Systematic reviews

2.2.4.1 Sensing delay and synaptic delay

I searched the literature for sensing delay and synaptic delay values measured for a range of animal masses (Table 2.1, Table 2.2). Specifically, I considered measurements made in vivo and close to body temperature on live adult quadrupedal mammals who were as close to wild type as possible. One study ([111]) did not report the temperature at which their measurements were made, however the delays found in that study were no slower than those measured at body temperature in other studies on the same species so I continued to include them in my analysis.

Some studies did not report the masses of their animals. Where no mass was reported, I estimated the mass from a different publication on the same species by at least one co-author, however in two studies this was not possible. Enough studies reported the mass of their animals that I was able to calculate average masses for all species.

For sensing delay, I searched for measurements of the time between the onset of stretch in the ankle extensors and the onset of action potential generation in the Ia afferent fibers innervating the stretched muscles. These are the fastest-conducting nerve fibers, and are involved in the monosynaptic stretch reflex. Ideally, I would have included only measurements made in the medial gastrocnemius, but due to the scarcity of these data I chose to also include data from other ankle extensors.

For synaptic delay, I searched for measurements made in the lumbosacral spinal cord for synaptic delays between sensory and motor neurons. This is the section of the spinal cord supplying nerve fibers to the sciatic nerve, and is therefore the region where nerve fibers involved in the hindlimb monosynaptic stretch reflex are located [33,70]. I included studies in which nerve fibers were stimulated either electrically or by muscle stretch, and in which delay was measured at single synapses between sensory and motor nerve fibers. Ideally, I would have included only measurements made in nerve fibers in pathways involving the medial gastrocnemius, but due to the scarcity of these data I chose to also include measurements from other lower limb pathways.

In total, I used data from six independent studies reporting delay values for one species each. A total of two species were represented in these studies.
### Chapter 2. Responsiveness

#### Table 2.1: Animal masses and sensing delays. * standard deviation (sd) approximated by range. ** values obtained from figures. Muscles are Ex=ankle extensors, MG=medial gastrocnemius.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Mass (kg)</th>
<th>Mass sd (kg)</th>
<th>Sens. delay (ms)</th>
<th>Sens. delay sd (ms)</th>
<th>Muscle</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat</td>
<td>1</td>
<td>2.75</td>
<td>0.125*</td>
<td>2.1**</td>
<td></td>
<td>Ex</td>
<td>[150]</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.55**</td>
<td>0.057**</td>
<td></td>
<td></td>
<td>MG</td>
<td>[111]</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td><strong>2.75</strong></td>
<td><strong>1.32</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Table 2.2: Animal masses and synaptic delays. * standard deviation (sd) approximated by range. Nerve fibers studied innervate muscles FHL=flexor hallucis longus, MG=medial gastrocnemius. Level indicates the spinal cord segment where recordings occurred.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Mass (kg)</th>
<th>Mass sd (kg)</th>
<th>Syn. delay (ms)</th>
<th>Syn. delay sd (ms)</th>
<th>Muscle</th>
<th>Level</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>4</td>
<td>0.45</td>
<td>0.015*</td>
<td>1.14</td>
<td></td>
<td>FHL</td>
<td>L5</td>
<td>[52, 199]</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td><strong>0.45</strong></td>
<td><strong>1.14</strong></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Cat</td>
<td>5</td>
<td>2.25</td>
<td>0.375*</td>
<td>0.81</td>
<td>0.09</td>
<td>MG</td>
<td>L7-S1</td>
<td>[156]</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>0.375</td>
<td>0.01</td>
<td>0.302</td>
<td>0.01</td>
<td>MG</td>
<td>L6-S1</td>
<td>[136, 137]</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>2.25</strong></td>
<td><strong>0.62</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td><strong>2.25</strong></td>
<td><strong>0.62</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.2.4.2 Nerve conduction delay

I searched the literature for maximum nerve fiber conduction velocity values measured for a wide range of animal masses (Table 2.3). Specifically, I considered measurements made in vivo on live adult quadrupedal mammals who were as close to wild type as possible. I included only measurements for maximum conduction velocity of the whole nerve, and excluded any studies in which conduction velocity of single nerve fibers was found. Measurements were required to be made at body temperature on the sciatic and/or tibial nerves. There was one exception to this – as I was not able to find any conduction values for the sciatic or tibial nerves of the horse, I chose to use the conduction velocity of the forelimb median nerve as it is relatively analogous to the hindlimb tibial nerve.

Some studies did not report masses of their animals – I estimated these masses from the literature.

In total, I used data from 23 independent studies reporting nerve fiber conduction velocity values for only one to three species each. To ensure as accurate a representation of conduction velocity as possible, I included a variety of experimental protocols as well as both motor and sensory values where possible. A one-way ANOVA found that there was no evidence of consistent variation in nerve fiber conduction velocity based on whether it was motor or sensory (p=0.8), therefore I continued to consider both types of conduction velocity in my analysis. In addition, for each species I attempted to obtain values from multiple studies and different researchers. This was possible for all species except the shrew, horse, and elephant.

I averaged the masses and maximum nerve fiber conduction velocity values for each species, then converted each conduction velocity to a conduction delay by predicting leg length from mass. To derive a relationship between animal mass and hindlimb length, I numerically added the mass-length relationships of the femur, tibia, and metatarsals, in meters [3], and used least-squares linear regression of the logarithmically transformed points to determine the exponent and coefficient of the power law relationship:

\[
\text{average hindlimb length} = 0.1649M^{0.3327}
\]

I assumed that the total length over which nerve impulses were conducted was twice the leg length. Implicit in this assumption was that the neglected distance from the femur to the spinal cord and back is compensated for by the included distance from the ground to
the distal leg muscles. Therefore, the minimum nerve conduction delay (in milliseconds) when sensing and responding to a stimulus at the foot was given by twice the leg length (in metres) divided by the maximum nerve fiber conduction velocity (in metres per second) and multiplied by 1000 (to convert from seconds to milliseconds):

$$\text{minimum nerve conduction delay} = \frac{2 \times 0.1649 \times 1000}{\text{nerve conduction velocity}} M^{0.3327}$$

### 2.2.4.3 Neuromuscular junction delay

I searched the literature for neuromuscular junction delay values measured for a range of animal masses (Table 2.4). Specifically, I considered measurements made in vivo and close to body temperature on live adult quadrupedal mammals who were as close to wild type as possible. I included measurements made in hindlimb muscles innervated by branches of the sciatic nerve. Ideally, I would have included only measurements made in the medial gastrocnemius, but due to the scarcity of these data I chose to also include measurements from other muscles crossing the ankle.

Most studies reported the masses of their animals. In one of the two experiments which did not report animal mass, I estimated the mass using a different paper on the same species by the same author; in the other I estimated the mass using the average mass from my nerve fiber conduction velocity systematic review (Table 2.3).

In total, I used data from three independent studies reporting delay values for one species each. A total of three species were represented in these studies.

### 2.2.4.4 Electromechanical delay and force generation delay

I searched the literature for electromechanical delay and force generation delay values measured for a wide range of animal masses (Table 2.5, Table 2.6). Specifically, I considered measurements made on tissue from adult quadrupedal mammals who were as close to wild type as possible. I included measurements from single-twitch non-potentiated responses made in intact medial gastrocnemius muscles at their resting length wherever possible; if this data was not available I also considered averaged measurements from the medial and lateral gastrocnemius where the researchers stated that there was no significant difference between these muscles, and data from large muscles of the hindlimb comprised of
### Table 2.3: Animal masses, maximum nerve fiber conduction velocities (CV), and predicted nerve conduction delays based on leg length (NCD). * standard deviation (sd) approximated by range. ** values obtained from figures. Nerves are Sc=sciatic, T=tibial, Med=median. CV types are M=motor, S=sensory. Modified from [131].

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Mass (kg)</th>
<th>Mass sd (kg)</th>
<th>CV (m/s)</th>
<th>CV sd (m/s)</th>
<th>NCD (ms)</th>
<th>Nerve Type</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shrew</td>
<td>6</td>
<td>0.0057</td>
<td>0.0007</td>
<td>42.1</td>
<td>6.3</td>
<td>1.40</td>
<td>Sc M</td>
<td>[131]</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td><strong>0.0057</strong></td>
<td></td>
<td><strong>42.1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>18</td>
<td>0.0225</td>
<td>0.0013*</td>
<td>41.4**</td>
<td>0.8**</td>
<td>Sc/T M</td>
<td>[75]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>0.0225</td>
<td>0.0013*</td>
<td>58.1**</td>
<td>1.5**</td>
<td>Sc/T S</td>
<td>[75]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.025</td>
<td>0.003*</td>
<td>43.2</td>
<td>0.6</td>
<td>Sc/T M</td>
<td>[94]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>0.0313</td>
<td>0.001*</td>
<td>59.8</td>
<td></td>
<td>Sc</td>
<td>[170]</td>
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</tr>
<tr>
<td>Mean</td>
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<td><strong>0.0253</strong></td>
<td></td>
<td><strong>50.6</strong></td>
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<tr>
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<td>0.43</td>
<td>0.03*</td>
<td>68.7</td>
<td>10.0</td>
<td>Sc M</td>
<td>[35]</td>
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<tr>
<td></td>
<td>12</td>
<td>0.227</td>
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<td>69.4</td>
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<td>Sc</td>
<td>[170]</td>
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<tr>
<td></td>
<td>6</td>
<td>0.325</td>
<td>0.028*</td>
<td>54.7**</td>
<td>2.8**</td>
<td>Sc/T S</td>
<td>[146]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.516</td>
<td>0.026</td>
<td>54.6</td>
<td>2.5</td>
<td>Sc/T M</td>
<td>[173]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.575</td>
<td>0.043*</td>
<td>53.3</td>
<td>2.7</td>
<td>Sc/T M</td>
<td>[184]</td>
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<tr>
<td></td>
<td>20</td>
<td>0.19</td>
<td>0.01*</td>
<td>55.5</td>
<td>2.9</td>
<td>Sc/T M</td>
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</tr>
<tr>
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<td></td>
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<td></td>
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<tr>
<td>Guinea pig</td>
<td>29</td>
<td>0.662</td>
<td>75.2 9.0*</td>
<td>Sc M</td>
<td>[42]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.706</td>
<td>74.5 2.4</td>
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<td>[67]</td>
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<td>Mass sd (kg)</td>
<td>NMJ (ms)</td>
<td>NMJ sd (ms)</td>
<td>Muscle</td>
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<td>Ref.</td>
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<td>1.27</td>
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Table 2.4: Animal masses and neuromuscular junction delays (NMJ). * standard deviation (sd) approximated by \( \frac{\text{range}}{4} \). Muscles are PT=peroneus tertius, G=gastrocnemius. Muscle fiber type is Mix=both fast and slow.

at least 90% fast-twitch fibers (mouse extensor digitorum longus and horse semimembranosus; [14,141,196]). There was one exception to this – as I was not able to find any values for intact horse muscles, I chose to also include measurements made in horse muscle biopsy samples. In all the whole-muscle studies I considered, muscle force was measured using a force transducer attached to the muscle tendon.

Where separate values for slow and fast fibers were given, I used values from the fast fibers; where separate values for slow, fast fatigue-resistant, and fast fatiguable fibers were given, I used values from the fast fatiguable fibers. I chose to select data from faster fibers for two reasons. First, fast fibers have shorter electromechanical and force generation delays [32] so pathways including these fibers are likely to have faster response times. Slow fibers can have delays more than twice that of fast fibers [32], so including both types of fibers would result in a large increase in variability. Second, in my measurements of electromechanical and force generation delay in the giraffe medial gastrocnemius, as well as in measurements recording electrical changes on the surface of the muscle, the activity of the most superficial muscle fibers in the muscle is measured – the superficial portions of the medial gastrocnemius have faster fibers than deeper portions of the muscle [163,164,196], so selecting data from faster fibers where available reduces variability due to muscle fiber type.

For electromechanical delay, I considered measurements made by stimulating either a single nerve fiber or the whole nerve while recording muscle activity. In very small muscles, such as those in the mouse, I also accepted measurements made by directly stimulating the entire muscle. Both whole-nerve stimulation and direct muscle stimulation activate multiple motor units in the muscle; because electromechanical delay is measured as the time from
the onset of electrical activity in the muscle to the onset of force production, the measured delay is likely to be due to the fastest motor units and cannot be longer than the delay in the slowest motor units. In whole-nerve stimulation, the largest nerve fibers are activated first [204], so their associated motor units will contribute to the onset of electrical activity in the muscle. Since these large nerve fibers are associated with the fastest motor units [32], they will also contribute to the onset of force production in the muscle. Similarly, in direct muscle stimulation all motor units will be activated at the same time so the onset of force production in the muscle will be driven by the fastest motor units.

For force generation delay, I considered only measurements made by stimulating a single nerve fiber, because stimulation of the entire muscle nerve has the potential to activate multiple motor units asynchronously [49]. Unlike electromechanical delay, force generation delay is measured from the onset of force production to peak force – any variability in electromechanical delay will affect the onset of force production, and any variability in the time required to generate force will affect the timing of peak force production. Because faster muscle fibers have shorter electromechanical delays and shorter force generation delays [32], if slow and fast motor units are stimulated at the same time the onset of force production will be driven by the fast muscle fibers whereas the time to peak force will be driven by the slower-contracting slow muscle fibers. In this example, measuring the time between force onset and peak force would give a result that is longer than the force generation time of even the slowest muscle fibers. I did, however, accept measurements made by directly stimulating the entire muscle in cases where the muscle contained at least 90% fast-twitch fibers (mouse extensor digitorum longus and horse semimembranosus; [14, 141, 196]), since the similarity in muscle fiber type would reduce the variability in the onset of force production and time to peak force.

Most experiments were performed at body temperature (35–38°C). One experiment (force generation delay in the mouse extensor digitorum longus, [141]) was performed at a temperature outside of this range – for this measurement I adjusted the reported delay to 37°C using a Q_{10} of 2.33, which is the Q_{10} for force generation delay in the mouse extensor digitorum longus [175]. Specifically, the equation I used for delay adjustment was:

\[ t_2 = t_1 e^{\ln(2.33) \frac{37-T_1}{10}} \]

where \( t_1 \) is the measured delay time, \( t_2 \) is the temperature-corrected delay time, and \( T_1 \) is
the temperature at which the delay was measured.

Most studies reported the masses of their animals; in experiments which did not report animal mass, I estimated the mass using a different paper on the same species by the same author.

In total, I used data from seven independent studies reporting delay values for one to two species each. Four of these studies reported electromechanical delay, and six studies reported force generation delay. A total of six species were represented in these studies.

<table>
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<tr>
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<th>Mass sd (kg)</th>
<th>EMD (ms)</th>
<th>EMD sd (ms)</th>
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<td>5.17**</td>
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<td>13.4</td>
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Table 2.5: Animal masses and electromechanical delays (EMD). * standard deviation (sd) approximated by range/4. ** values obtained from figures. Muscles are MG=medial gastrocnemius, LG=lateral gastrocnemius, G=gastrocnemius. Muscle fiber types are F=fast, FF=fast fatiguable, Mix=both fast and slow.

### 2.2.5 Scaling of component delays

For sensing, synaptic, and neuromuscular junction delay, my limited data points did not give evidence for any clear scaling relationship; I therefore assumed constant scaling relationships for these three component delays. I calculated the value of the constant for each scaling relationship by averaging the values of all data points for the respective component delay; this constant became the coefficient in a power law relationship with an exponent of zero.

---

2 After completing this analysis and submitting my thesis, I found additional force generation delay measurements in the rat and cat. Fast fatiguable muscle fibers in the medial gastrocnemii of 4 rats with masses of 0.295±0.023* kg had force generation delays of 11.8±1.6 ms, and fast fatiguable muscle fibers in the medial gastrocnemii of 4 cats with masses of 3.08±0.04* kg had force generation delays of 26.1±5.3 ms (mean±sd, * sd approximated by range/4) [100]. These values are within the variability of the data in Table 2.6, so I do not expect the addition of this information to change my results significantly.
### Table 2.6: Animal masses and force generation delays (FGD).

* standard deviation (sd) approximated by \( \frac{\text{range}}{4} \).  

** values obtained from figures.  

Muscles are MG=medial gastrocnemius, LG=lateral gastrocnemius, EDL=extensor digitorum longus, SM=semimembranosus.  

Muscle fiber types are F=fast, FF=fast fatiguable, Mix=both fast and slow.

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<th>Mass sd (kg)</th>
<th>FGD (ms)</th>
<th>FGD sd (ms)</th>
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</table>

For nerve conduction delay, my data were already expressed as an average for each species; for electromechanical delay and force generation delay, I averaged the values of data points within each species. For each of these three component delays, least-squares linear regression of logarithmically transformed species averages determined the exponent and coefficient of the scaling relationship (Subsection 1.7.1).

#### 2.2.6 Scaling of total delay

To determine how total response time scales with animal size, I numerically added the power law relationships for the six component delays. I created a vector of log-spaced mass points spanning the range of animal masses I studied, from \( 10^{-3} \) to \( 10^4 \) kilograms, then used the power law relationship for each component delay to calculate corresponding vectors of log-spaced component delay points. I added the resulting six component delay vectors together to find a vector of total delay points. Least-squares linear regression of the logarithmically transformed total delay points determined the exponent and coefficient of the power law relationship between animal mass and total response time.

For sensing, synaptic, and neuromuscular junction delay, I assumed constant power law
relationships because my limited data points did not give evidence for any clear scaling relationship (Subsection 2.2.5). While this assumption of constant scaling could affect my results for the scaling of total delay, I expect that the effect would be relatively small since these three delays have relatively small magnitudes compared to nerve conduction, electromechanical, and force generation delay.

### 2.2.7 Scaling of relative delay

My goal was to find the fraction of stance phase needed to sense and respond to a stimulus at the foot using a simple reflex arc. In addition to total response time, I also wanted to find the fraction of stance phase taken up by each component delay. I chose to find these fractions at the trot-gallop transition – a physiologically similar speed for animals of different sizes\(^3\) [72]. I first found a relationship between mass \(M\) (in kilograms) and stride period \(T\) (in seconds) at the trot-gallop transition by inverting the known relationship between mass and stride frequency \(f\) (in minutes\(^{-1}\)), and multiplying by 60 (to convert from minutes to seconds) [72]:

\[
f = 269M^{-0.14} \\
T = \frac{60}{269}M^{0.14} \tag{2.1}
\]

Since stance phase makes up a relatively constant proportion of stride period, with an average stance time fraction (duty factor) for the hindlimb of 0.42 across all animal sizes [17], I multiplied Equation 2.1 by the duty factor to find the relationship between mass and stance duration at the trot-gallop transition, then multiplied by 1,000 (to convert from seconds to milliseconds):

\[
\text{stance duration} = \frac{0.42 \times 60 \times 1,000}{269}M^{0.14} \\
= 93.68M^{0.14} \tag{2.2}
\]

\(^3\)To facilitate comparison of delays between animals, I approximated the locomotor kinematics of elephants as following the same trends as other animals. Compared to other quadrupedal mammals, elephants have different gait transitions and different locomotor patterns at high speeds [84, 86, 155]. Although they do not have a trot-gallop transition in the same sense as other animals, elephants are capable of moving with similar stride frequencies and duty factors to those estimated here [86], suggesting that my approximation is reasonable.
I divided the power law relationship of each delay by Equation 2.2 to determine the fraction of stance time taken up by the delay.

### 2.3 Results

I determined the lengths of six component delays in a variety of species with different body masses, using a combination of my own electrophysiological experiments and systematic reviews of the literature. I defined sensing delay as the time from the application of a stimulus to the generation of an impulse in a sensory nerve fiber, nerve conduction delay as the time required to transmit the nerve impulse along sensory and motor nerve fibers, synaptic delay as the time required for the nerve impulse to be transferred from the sensory nerve fiber to the motor nerve fiber at the synapse in the spinal cord, neuromuscular junction delay as the time required for the impulse to be transferred from the motor nerve fiber to muscle fibers at the neuromuscular junction in the muscle, electromechanical delay as the time required from the beginning of electrical activity in the muscle to the beginning of force generation, and force generation delay as the time required for the muscle to reach peak twitch force (Figure 2.4).

Using a combination of systematic review and my own data, I found that some component delays changed with animal size, while other component delays may remain constant (Table 2.7; Figure 2.5b,e,f). Nerve conduction delay, electromechanical delay, and force generation delay increased with animal size. Of these three increasing delays, nerve conduction delay increased the fastest, in proportion to $M^{0.30}$; electromechanical delay and force generation delay increased more slowly, in proportion to $M^{0.17}$ and $M^{0.19}$, respectively. Data in the literature for sensing delay, synaptic delay and neuromuscular junction delay are sparse, so my results should be considered preliminary; based on these tentative results, the values of these delays appear to be small (Figure 2.5a,c,d). This is consistent with what I expected based on the mechanisms of these delays – the processes of signal transduction within sensors, across synapses, and across the neuromuscular junction all involve fast biophysical processes such as ion diffusion and short-distance signal transduction. Because these mechanisms are likely conserved across different sizes and species of animals, I did not expect these delays to scale strongly with animal size.

Total response time, calculated by fitting a power law relationship to the sum of my six component delay scaling relationships, increased with animal size, in proportion to $M^{0.19}$.
Figure 2.4: Example of component delays superimposed on muscle recordings. The time required between an external stimulus and force production encompasses several sources of delay, here illustrated conceptually by combining my experimental results for nerve conduction delay, electromechanical delay, and force generation delay in the giraffe with my systematic review data for sensing delay, synaptic delay, and neuromuscular junction delay. These delays are considered in the context of the simple monosynaptic reflex pathway initiated by an external stimulus such as a tendon tap, and are superimposed on representative recordings of muscle electrical activity (electromyography; EMG) and force. Sensing, synaptic, and neuromuscular junction delays contribute relatively little (a total of 4%) to the total response time in the giraffe, while electromechanical delay comprises 13% of this time. In contrast, total nerve conduction delay and force generation delay make up the majority of the time required to respond to a stimulus, comprising 38% and 45% of the total response time, respectively.
### Table 2.7: Component delay and total delay power laws and statistics

Coefficient values for sensing, synaptic, and neuromuscular junction delays were determined by averaging all data points for each delay; exponents for these constant delays are zero. Coefficient and exponent values for nerve conduction, electromechanical, and force generation delays were determined by least-squares linear regression of logarithmically transformed data. Coefficient and exponent values for total delay were determined by numerically adding the power law relationships for the six component delays, then performing least-squares linear regression on the logarithmically transformed data. * 95% CI approximated as twice the standard deviation.

<table>
<thead>
<tr>
<th>Delay</th>
<th>Coefficient Value</th>
<th>95% CI</th>
<th>Exponent Value</th>
<th>95% CI</th>
<th>p</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensing</td>
<td>1.324 +/- 2.195*</td>
<td>0</td>
<td>0.301 +/- 0.038</td>
<td>0.000</td>
<td>0.970</td>
<td></td>
</tr>
<tr>
<td>Nerve conduction</td>
<td>5.243 x/÷ 1.178*</td>
<td>0</td>
<td>0.038</td>
<td>0.000</td>
<td>0.966</td>
<td></td>
</tr>
<tr>
<td>Synaptic</td>
<td>0.753 +/- 0.591*</td>
<td>0</td>
<td>0.009 0.927</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuromuscular junction</td>
<td>0.857 +/- 0.723*</td>
<td>0</td>
<td>0.013 0.977</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electromechanical</td>
<td>5.034 x/÷ 1.596*</td>
<td>0</td>
<td>0.099 0.936</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Force generation</td>
<td>18.038 x/÷ 1.526*</td>
<td>0</td>
<td>0.003 0.997</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>33.130 +/- 1.016</td>
<td>0.193</td>
<td>0.000 0.997</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2.5g) – a 5 gram shrew would have a minimum response time of just 12 ms, whereas a 5,000 kilogram elephant would have a minimum response time of over 170 ms. When calculating total response time, I added the power law relationships of three component delays and the assumed constant relationships of the remaining three delays. Because the sum of power law relationships with different exponents does not necessarily result in another power law, I determined the scaling relationship of the total delay by fitting a power law to the sum of component delays. While this approximation is quite accurate, it tended to slightly underestimate total response time in small and large animals by approximately 5% and 8%, respectively, and slightly overestimate total response time in mid-sized animals by about 6%.

The absolute times of component delays were different in small and large animals. My preliminary results for sensing delay, synaptic delay, and neuromuscular junction delay indicate that they are very short – on average, synaptic delay and neuromuscular junction delay had approximately the same magnitude of 0.8 ms and 0.9 ms, respectively, while sensing delay was only slightly longer, at 1.3 ms. The longest delays were force generation delay and electromechanical delay, with force generation delay being about three to four times as long.
Figure 2.5: Component delay and total delay scaling relationships shown individually. Plots show the scaling relationships in Table 2.7 (coloured lines), and the data points used to determine them (filled circles). Each data point in (a), (c), and (d) is from one study; each data point in (b), (e), and (f) is an average of one or more studies. (g) shows the least-squares regression fit of the sum of scaling relationships (a)-(f) (black line), and predicted delays for individual species (open circles) adjusted using the data points in (a)-(f).
as electromechanical delay for animals of all sizes – in a 5 gram shrew, electromechanical delay and force generation delay would be approximately 2 ms and 6 ms, respectively, whereas in a 5,000 kilogram elephant they would be approximately 20 ms and 90 ms, respectively. In small animals, nerve conduction delay was almost equal to the shortest component delays, at about 1 ms, but in large animals it became one of the longest component delays, at about 70 ms (Figure 2.6).

The relative contributions of component delays to total response time were only slightly different in small and large animals. As animal size increased, the constant delays (sensing, synaptic, and neuromuscular junction delay) made up increasingly smaller fractions of total response time, while the contribution of nerve conduction delay increased (Figure 2.7a). The contribution of electromechanical delay and force generation delay to total response time remained a relatively constant fraction of total response time for animals of all sizes.

Relative response time was approximately the same for animals of all sizes, increasing in proportion to $M^{0.05}$ (Figure 2.8). The changes in relative time between small and large animals almost entirely compensated for the increase in total response time with animal size – over a size range of six orders of magnitude, the fraction of stance phase taken up by
Figure 2.7: Relative component delay and total delay scaling. Component delays (coloured areas) expressed as fractions of total response time (a) and as fractions of stance phase at the trot-gallop transition (b). Total response time is the numerical sum of all component delays; the power law determined by least-squares regression of the total response time is $0.4M^{0.19}$, and is shown as a black line in both plots. In each plot, the fraction of an animal’s response time (a) or stance phase (b) required by each component delay is represented by the vertical thickness of the corresponding coloured area at the horizontal location appropriate for the animal’s mass.
total response time only doubled. For example, a 5 gram shrew would require about 1/4 of stance phase to sense and respond to a perturbation at the trot-gallop transition speed, whereas it would take a 5,000 kilogram elephant more than 1/2 the time its foot was on the ground to respond to a similar disturbance. The very similar exponents in the power law relationships for total response time ($\propto M^{0.19}$) and stance phase time ($\propto M^{0.14}$) mean that the fraction of stance phase taken up by response time is approximately constant for all animals sizes; however, the small difference of 0.05 in the exponents of these relationships is still sufficient to double relative response time in large animals compared to small animals.

![Figure 2.8: Relative response time scaling. Total response time increases with animal size, in proportion to $M^{0.19}$ (Figure 2.5g), but this increase is almost entirely compensated for by increases in relative time – stance time at the trot-gallop transition increases in proportion to $M^{0.14}$ (Equation 2.2), resulting in relative response time increasing in proportion to $M^{0.05}$. The fraction of stance phase required to sense and respond to a stimulus only doubles over an increase in animal mass of six orders of magnitude.](image)

2.4 Discussion

I found that several component delays increase with animal size, resulting in longer total response times in larger animals. However, these changes are almost entirely offset by increases in movement time with animal size, resulting in similar relative delays in all sizes of animals.

My preliminary results indicate that synaptic delay and neuromuscular junction delay may have similar lengths, at 0.8 ms and 0.9 ms respectively, while sensing delay is only slightly longer, at 1.3 ms. The similarity in synaptic delay and neuromuscular junction delay...
delay likely reflects similar underlying mechanisms – both these component delays involve the release, diffusion, and binding of a neurotransmitter, and the subsequent generation of an action potential. Sensing delay also involves the generation of an action potential, but mechanical deformation rather than a neurotransmitter stimulates the process. The process of converting this mechanical deformation to an action potential involves taking up slack and stretching the muscle tissue, which is likely to take longer than the release and diffusion of a neurotransmitter across the much smaller synaptic cleft, so it makes sense that sensing delay is slightly longer than synaptic delay and neuromuscular junction delay.

Nerve conduction delay increased in proportion to $M^{0.30}$, which is similar to the $M^{1/3}$ scaling of linear body dimensions. This is because nerve conduction delay depends on the length, for example leg length, that a nerve impulse must travel, as well as the speed at which the impulse travels. Combining my experimental data and systematic review data shows that nerve fiber conduction velocity is almost the same in all animals, less than doubling from 42 m/s to 70 m/s over the six order of magnitude change in body mass from shrew to elephant, and increasing in proportion to $M^{0.04}$ (Table 2.3; [131]). Because nerve fiber conduction velocity is relatively constant regardless of animal size, nerve conduction delay is almost entirely dependent on leg length, and therefore has a similar scaling relationship to that of leg length. In small animals, short conduction distances mean that nerve conduction delay makes up a small fraction of the total response time, but because leg length increases more rapidly than total response time the long conduction distances in large animals mean that nerve conduction delay makes up a larger fraction of the total response time.

Some animals have developed adaptations to lessen nerve conduction delay. For example, the giant axons of squid mediate escape responses, and large-diameter fibers from giant pyramidal cells, known as Betz cells, in the motor cortex of primates play a role in controlling movement of the hands and legs [151, 159]. The high conduction velocities of these fibers decrease nerve conduction delay, allowing responses to occur quickly even in distal appendages.

Electromechanical delay and force generation delay are relatively long compared to other component delays. This is likely due to their slow underlying mechanisms. In the case of electromechanical delay, action potentials must be conducted along muscle fibers to allow the motor unit to contract along its entire length. Muscle fiber conduction velocity is relatively slow, only about 2–3 m/s in rats and 4–6 m/s in cats [49, 104], and is responsible for a large fraction of electromechanical delay [134]. For example, muscle fibers in cats contract
optimally at a length of about 2.6 cm [134] meaning that, if they were innervated in the center, it would take at least 2.2 ms for the resulting action potential to reach the ends of the muscle fiber – this portion of electromechanical delay alone would take almost three times as long as the shortest component delays, to which would be added the time required for excitation-contraction coupling and calcium release. In the case of force generation delay, the time required to move actin and myosin fibers past each other, combined with the necessary physical stretch of series and parallel elastic elements of the muscle, creates a delay in force output. Both electromechanical and force generation delay could be increased by shifting toward faster motor units, but this comes at an energetic cost [22].

My investigations into responsiveness consider animals to be geometrically similar, an assumption that holds for most animals [3]. Geometrically similar animals have similar body proportions – for example, leg lengths of geometrically similar animals are equal to a constant factor multiplied by $M^{1/3}$, and animal surface areas are equal to a constant factor multiplied by $M^{2/3}$ (Subsection 1.2.2). However, some animals deviate from this general geometrically-similar trend. Giraffes, for example, have exceptionally long legs relative to their body size – more than 50% longer than predicted based on their mass (prediction from allometric equations for Bovidae; [3]). Still, giraffes have similar nerve fiber conduction velocities as species with the same mass but which follow the trend of geometric similarity (Table 2.3), giving them the potential for exceptionally long nerve conduction delays. Using my measured values for the nerve fiber conduction velocity and leg length of giraffes – 50.4 m/s and 1.8 m, respectively – I estimate that a giraffe’s nerve conduction delay would be about 71 ms [132]. This is more than 40% greater than the nerve conduction delay predicted based on geometric similarity, and results in a predicted response time almost 20% greater than predicted from the giraffe’s mass and my power law scaling relationship for total response time. In the case of giraffes, their departure from geometric similarity, while presumably providing some advantages, also creates challenges for sensorimotor responsiveness [132].

2.4.1 Limitations

There were several limitations to my study. First, I focused on measurements performed on the medial gastrocnemius muscle, which is located relatively close to the torso, particularly in large animals such as the giraffe. Aware of this limitation, I purposely chose to study this muscle because it is the most distal large muscle in the hindlimb and is widely studied
in other animals, facilitating comparison of our results across species. Also, I focused on only one reflex system: the monosynaptic stretch reflex. There are many other reflexes that are important in the sensorimotor system, such as the monosynaptic reflexes initiated by Golgi tendon organs, however the speed and simplicity of the stretch reflex make this system an ideal place to begin examining component delays. The sensory nerve fibers in the stretch reflex are the fastest conducting nerve fibers [80], and the presence of only one synapse makes it easier to isolate each component delay because each type of delay occurs the minimum number of times.

The delays I measured depend on many assumptions inherent to the measurement process. For example, I considered response time as the time from the start of a perturbation until the time of peak twitch force. While reflexes do cause twitches, in real-world scenarios the movements of animals would be more complex – a stimulus would likely cause more than one action potential, and animals would likely respond to a perturbation with a much greater muscle force composed of the temporal summation of many muscle twitches. A potentially more useful functional measure would be time to peak total muscle force, or time to tetanus. Both these possibilities would require more time than the production of force via a single twitch. However, they are both also affected by many more variables than time to peak twitch force, including the number and types of motor units stimulated and the rate of stimulation. To facilitate comparisons across many different species, in which even homologous muscles may have different numbers of slow and fast twitch fibers, and because time to peak twitch force is correlated with time to peak muscle force and time to tetanus [32], I chose to use time to peak twitch force as the conclusion of response time. The values of other component delays also depend on my definitions of start and end point for each process, but although different definitions of when each component delay began and ended would shift the proportions of component delays it would not alter the length of the total delay.

I did not adjust for evolutionary history when determining the delay scaling relationships. Different degrees of evolutionary relationships between species could affect my results – if closely-related species had similar delays than distantly-related species, my data points could not be considered independent. This would have the potential to change my calculated relationship between animal mass and the length of sensorimotor delays [54]. One widely-used and well-verified method to account for relationships between species before carrying out regression analysis is by using the method of phylogenetically independent contrasts [62].
It uses known phylogenetic relationships to calculate statistically independent weighted
differences between species based on the length of branches in the phylogenetic tree [54,62].
A phylogenetically independent contrasts analysis of a subset of my nerve fiber conduction
velocity data (all species except giraffe; Appendix A) indicated no significant phylogenetic
signal – while this may not be the case for all my scaling relationships, it suggests that the
phylogenetic signal is not strong within the species that I studied. There may still be some
adaptive differences, such as diet, which this analysis did not take into account – I did not
quantify these differences, but, based on visual analysis, whether a species is a herbivore or
carnivore does not seem to correlate with any systematic variation of my data away from
my regression results.

For some component delays, most notably sensing delay, synaptic delay, and neuromuscu-
lar junction delay, I was only able to find a small number of data points. I would ideally
have obtained more data, however due to time constraints and the relative scarcity of these
delay measurements this was not possible. While the number of points I found was not
sufficient to confidently determine the scaling relationship of these delays with animal mass,
the values that I did find suggest that these three component delays are very small. This is
consistent with the main mechanisms mediating each delay – changes in ion channels and
diffusion over a short distance. These processes occur rapidly, leading to relatively short
delays.

2.4.2 Implications for control of movement

Several component delays are longer in larger animals, resulting in big animals having long
absolute response times (Figure 2.5). Large animals may cope with these relatively long
delays by simply moving slowly, explaining at least in part the low maximum speeds of
large mammals [61,86] and providing further evidence for the idea that dinosaurs could
not be both massive and agile [85]. Indeed, differences in movement times between small
and large animals almost entirely compensate for increases in absolute delays, resulting in
similar relative delays for all sizes of animals (Figure 2.8).

Long response times make simple feedback control less effective. By the time sensory
signals are conducted from the periphery to the central nervous system, the information
they contain about the state of the body is no longer current. Similarly, by the time motor
commands are conducted to the periphery, they may be inappropriate for the new body
state. The central nervous system can compensate for these delays using an internal model
CHAPTER 2. RESPONSIVENESS

of the body’s dynamics that takes delayed and incomplete sensory information and predicts the best future motor response [201]. As absolute delays increase in larger animals, they may increasingly depend upon sensorimotor prediction to maintain absolute sensorimotor performance [19,201].

2.5 Future work

Of the three delays contributing most to total response time, I was only able to determine two – nerve conduction delay and force generation delay – in a wide size range of animals. The data I found for the third delay – electromechanical delay – focused on species ranging in size over only three orders of magnitude. Determining the scaling relationship for electromechanical delay would especially benefit from the inclusion of measurements from small animals, such as the shrew and mouse. This would extend the data set over an additional two orders of magnitude, which would provide a much more thorough picture of how electromechanical delay changes with animal size. While I also found limited data for sensing delay, synaptic delay, and neuromuscular junction delay, these component delays were relatively small and their underlying biophysical processes do not indicate that they will change substantially with animal size.

Here, I have considered response time to end at the production of peak twitch force, however an animal must still produce enough muscle force to move its limb or other body part, after which there is an additional delay associated with the movement of that body part. In responses requiring rapid movement, rapid acceleration of a body part must occur in order to quickly reach a high velocity; for a given muscle force generating torque, inertia affects the acceleration that is produced and therefore influences the ability of an animal to move quickly. The effects of inertia are different in different sizes of animals, and would affect the time from the onset of muscle force to the time that the animal’s movement becomes effective. Including the effects of inertia in my response time calculations would illuminate more clearly the functional differences in different sizes of animals.

Once the scaling relationships for component delays are more firmly established, an intriguing avenue for further study is to individually manipulate delays to further investigate their contribution to response time. One possibility is to design experiments manipulating the temperature of various body parts. Lower temperatures are associated with the slowing
of many biological processes, such as nerve fiber conduction velocity and muscle contraction time [145,175], therefore systematically and selectively lowering the temperature at the physical location of each component delay would expose the effects of increasing the respective delay. Another possibility is to create computational models to simulate sensing and response times in different sizes of animals. Using simulations, the magnitudes of various component delays could be changed alone or in combination while keeping all other parameters constant, and the effects on animal movement observed. Simulations would potentially allow a wider range of more precise manipulations than temperature experiments, and could explore possibilities very difficult to achieve experimentally such as setting some component delays to zero. Simulations would also be useful in a clinical setting, where they could investigate the effects of illness or injury on different aspects of sensorimotor pathways.
Chapter 3

Resolution

3.1 Introduction

Regardless of how quickly an animal can respond to a perturbation, its response must be accurate. We refer to the precision at which an animal can sense and respond to a stimulus as \textit{resolution} [131].

Resolution is affected by the density of sensors and effectors throughout the body. In the context of resolution, the term ‘sensor’ refers to a structure which detects a stimulus, and the term ‘effector’ refers to a structure which generates force. Higher sensor and effector densities give an animal a greater potential for sensing and responding precisely [131]. Sensor and effector density is related to sensor and effector number, which in turn is related to the number of nerve fibers contained in peripheral nerves, such as the sciatic nerve.

Each peripheral nerve contains both unmyelinated and myelinated nerve fibers. Unmyelinated fibers are small and conduct impulses relatively slowly, whereas myelinated fibers are larger and conduct impulses relatively quickly due to their size and their insulating layer of myelin (Subsubsection 2.1.2.2). Both unmyelinated and myelinated nerve fibers can be either sensory or motor. Sensory fibers conduct impulses from the periphery of the body toward the central nervous system, whereas motor fibers conduct impulses from the central nervous system to muscles. Each sensory fiber provides information from a single type of sensory receptor, and each motor fiber controls the activation level of a single type of muscle fiber (Figure 1.2; [89]). The more sensory nerve fibers an animal has, the more distinct sensors it can innervate and the more precisely it can sense the location and type of a stimulus. Similarly, the more motor nerve fibers an animal has, the more effectors it can innervate

56
and the more precisely it can generate the force required to respond to a stimulus.

Many types of sensors and effectors contribute to resolution, and there are multiple ways to classify them. Because I am interested in the functional consequences of changes in sensor and effector number, I will use a functional organization, and because I am interested in the fastest pathways, I will discuss only sensors and effectors that are innervated by myelinated nerve fibers. I will classify sensors and sensory nerve fibers based on the type of stimulus they are activated by: touch, force, angle, or length (Figure 1.2b). Correspondingly, I will classify effectors and motor nerve fibers based on their type of functional unit: muscle spindles or motor units (Figure 1.2b).

3.1.1 Components of resolution

3.1.1.1 Sensory resolution components

Myelinated sensory nerve fibers innervate a variety of different sensory receptors detecting light, temperature, chemicals, and mechanical stimuli (Subsubsection 2.1.2.1). Of these receptor groups, I will assume that mechanoreceptors play the greatest role in locomotion, since they detect physical perturbations which threaten stability. The specific type of stimulus detected by a mechanoreceptor can be grouped into four main categories: touch, force, angle, or length (Figure 1.2b). Within each of these categories, there are a wide variety of sensory receptors; rather than a detailed description of each type, I will list the categories of receptors and give examples of the type of stimuli they detect.

- **Touch receptors** detect stimuli applied to the surface of the body. They are located in cutaneous and subcutaneous tissue. Pacinian corpuscles detect vibration, hair receptors detect movement of the body hair, and Merkel discs and Meissner corpuscles detect touch and pressure [76].

- **Force receptors** detect external force applied to muscles. They are located in joint ligaments and tendons, and due to their founder and their location they are called Golgi tendon organs [58]. They are located in parallel with extrafusal muscle fibers.

- **Angle receptors** detect the angles of body joints. They are located in joint capsules. Originally thought to detect intermediate joint angles, they were later determined to be almost solely responsible for sensing extremes of joint flexion and extension;
intermediate joint angles are thought to be sensed by length receptors in nearby muscles [28, 43].

- **Length receptors** detect muscle length. They are composed of fibers called spindle fibers, which are innervated by fast-conducting primary sensory nerve fibers and slower-conducting secondary sensory nerve fibers – these sensory fibers are activated by stretch of the muscle spindles [94]. Spindle fibers are located in series with extrafusal muscle fibers.

### 3.1.1.2 Motor resolution components

Muscle fibers in skeletal muscles can be grouped into two types: intrafusal and extrafusal. Intrafusal muscle fibers are bundled with spindle fibers into muscle spindles, and these bundles are dispersed throughout a muscle. Extrafusal muscle fibers make up the bulk of the muscle. Both types of muscle fibers are capable of contracting and generating force, contributing to motor resolution (Figure 1.2b).

- **Muscle spindles** are length receptors. The intrafusal muscle fibers in muscle spindles contract to provide the spindle fibers with a baseline of stretch, and increase their sensitivity [16]. Motor innervation to muscle spindles is provided by $\gamma$ motor nerve fibers. The number of muscle spindles in a muscle can be determined either by counting the number of $\gamma$ nerve fibers innervating the muscle or by dissecting the muscle to find the spindles.

- **Motor units** are the functional units which generate muscle force. They are composed of several extrafusal fibers which are innervated by a single $\alpha$ motor nerve fiber [16]. Motor units can be fast-fatiguable, fast-fatigue-resistant, or slow, and each of these types of motor unit has a different contraction speed, duration, and resistance to fatigue [32]. The number of motor units in a muscle can be determined either by counting the number of $\alpha$ nerve fibers innervating the muscle or by relating individual motor unit stimulation to force output [41, 120].

### 3.1.2 Scaling of resolution

As body volume increases, the numbers of sensors and effectors may also need to increase if resolution is to remain constant – this would result in an increase in the total number
of nerve fibers in peripheral nerves. It is not clear, however, how the number of nerve fibers should scale with animal size. The numbers of some sensory receptors, such as touch receptors in the skin and subcutaneous tissue, may need to increase in proportion to animal surface area \( (\propto M^{2/3}) \) to allow an animal to sense external stimuli precisely. The number of motor units in the muscles may also need to increase in proportion to area dimensions \( (\propto M^{2/3}) \), because muscle force is proportional to muscle cross-sectional area; alternatively, the number of motor units may need to increase in proportion to muscle force required to support body mass which, due to postural changes, increases in proportion to \( M^{0.74} \) [18].

The number of muscle spindles may need to increase in proportion to animal mass \( (\propto M^1) \) to allow an animal to precisely sense changes throughout its body tissues or, alternatively, in proportion to muscle cross-sectional area \( (\propto M^{2/3}) \) if the placement of muscle spindles in series with motor units reduces the need for spindles along the length of the muscle. If large and small animals have the same number of degrees of freedom – for example, if they have similar numbers of joints and muscles, their limbs move in similar directions, and their muscles produce the same number of discrete force levels – the number of sensors and effectors may not need to increase at all \( (\propto M^0) \). It is unlikely, however, that nerve fiber number would decrease in larger animals as compared to smaller animals. The combination of different scaling relationships for different types of sensory receptors and motor units could therefore result in the total number of nerve fibers scaling with anything from \( M^0 \) to \( M^1 \) or greater.

Scaling of total nerve fiber number provides insight into total resolution, but does not give any information about possible changes in the proportions of sensory receptors and motor units. Since the scaling of sensory receptors and motor units is difficult to determine theoretically, my approach here is to investigate these scaling relationships experimentally and then try to understand their implications. There are many types of receptors that contribute to resolution, and determining how each type changes with animal size is a monumental task. Given my focus on the speed of sensing and responding to perturbations, it is logical to begin with the receptor and effector types involved in the fastest pathway: the monosynaptic spinal reflex arc [7, 80]. In this reflex, muscle spindles are stimulated by muscle stretch, with neural pathways then stimulating motor units in the muscle. I therefore examined the scaling of muscle spindles and motor units as representatives of sensory and motor resolution.

The diameter of an animal’s nerve is determined by the size of nerve fibers it contains,
as well as their number. If nerve fiber number scaled with $M^1$, the increase in nerve fiber number in very large animals relative to very small animals would render their nerve diameters unsupportable if nerve fiber sizes were constant. For example, consider a 5 gram shrew and a 5,000 kilogram elephant. If the shrew had a sciatic nerve 0.3 mm in diameter, and if nerve fiber size as well as the number of nerve fibers per unit mass remained the same, we would expect the elephant’s sciatic nerve to have a diameter of 30 cm – four times the diameter of its femur [3].

The distribution of nerve fiber sizes affects total nerve size. There are numerous possible combinations of nerve fiber size and number for any given nerve size – an animal could maximize its its responsiveness by having a small number of large nerve fibers, maximize its resolution by having a large number of small nerve fibers, or reach a compromise between responsiveness and resolution by having some large and some small nerve fibers. As animal size increases, the trade-off between responsiveness and resolution becomes more acute. One way that large animals could mitigate this trade-off is to change their nerve fiber size distribution. For example, large animals may have more bimodal nerve fiber size distributions, with one distinct population of large fibers to allow fast sensing and response to urgent stimuli, and a second population of small fibers to allow higher resolution for less time-sensitive pathways.

Animals with many sensors and effectors, and therefore high overall resolution, may be able to respond to perturbations effectively using feedback control (Section 1.3; [102]). However, if the number of sensors or effectors decreases, thereby lowering overall resolution, feedback control may be unable to initiate an accurate response – for example, an animal may be unable to swat a small insect successfully, or may swat the insect with too little or too much force. In addition, if an animal relies too heavily on imprecise feedback pathways it may experience instability as its control system receives noisy input. One solution to this problem is for the animal to place less importance on feedback pathways. Although predictive control may be able to adequately compensate for compromised sensory resolution by correcting for sensor imperfections, it may not be able to compensate for lowered motor resolution; predictive control is also less likely to allow an effective response to unexpected or unfamiliar perturbations [102]. In many situations, decreases in resolution have the potential to be detrimental.
3.1.3 Image segmentation

An effective way to analyze nerve fiber number and size is to identify and measure nerve fibers in images of nerve samples.

Image segmentation refers to the process of identifying regions of interest in an image. Many methods to segment nerve fibers in images of nerve cross-sections have been developed, ranging from manual to completely automated, and from approaches which measure a sample of nerve fibers to approaches which measure all fibers within a nerve cross-section [130]. Originally, manual counting and measuring techniques such as manually tracing nerve fibers (e.g. [24]) or cutting out and weighing nerve fiber tracings (e.g. [43]) were used. As technology has advanced, automated and semi-automated techniques have been developed that are faster than manual measurements – for example, the measurement of 1,000 nerve fibers can be reduced from one day to one hour [189]. In some cases, especially in the case of smaller nerves, these more efficient methods allow analysis of all nerve fibers in the nerve cross-section [161,189,200]. However, many approaches require specialized equipment, such proprietary analysis systems (e.g. [82]). Few methods are entirely automated as some form of user input, for example manual addition or deletion of nerve fibers, is generally required to ensure accuracy. In fact, because of the inherent variability present in biological samples, manual confirmation of the results through the process of supervised segmentation is often desirable [11,205].

Most work on nerve segmentation has focused on light microscope images or, less commonly, transmission electron microscope (TEM) images. TEM is more expensive than light microscopy, but is capable of very high-resolution images [25]. Both these methods require samples to be cut into thin slices, which can be very difficult in large-diameter nerves. In addition, TEM imaging is only possible for samples of less than three millimeters in diameter, which would require large-diameter nerves to be divided into several smaller samples. A good alternative to light microscopy and TEM is the use of a scanning electron microscope (SEM). Unlike TEM imaging, which produces images by detecting electrons passed through the sample, SEM imaging produces images by detecting electrons bounced off the surface of the sample. This technique allows thicker sections to be imaged than either of the previous two methods and can therefore be used to effectively image larger-diameter nerves in which thin sections would be difficult to prepare without specialized equipment. It is also capable of imaging sample areas of several centimeters in diameter, eliminating the need to divide
large-diameter nerve samples. While there have been a number of segmentation and analysis methods developed for light microscope and TEM images, to my knowledge there has been no robust method developed to analyze whole-nerve images acquired using an SEM.

Analyzing several sample areas from a nerve, rather than whole-nerve images, to obtain values representative of the whole nerve can introduce bias into the results. Because of their size, larger nerve fibers are more likely to intersect the edge of the sample area and be cut off, making the results biased toward small fibers [106]. As well, the size distribution of nerve fibers can vary in different parts of the nerve, with some areas having a higher percentage of large nerve fibers and other areas having a higher percentage of small nerve fibers [167, 186]. While these problems can be ameliorated by using more and larger sample areas, and appropriate sampling strategies, accuracy and effectiveness could be greatly improved by analyzing all nerve fibers within a nerve or fascicle rather than extrapolating whole-nerve values from a limited number of sample areas [167, 186]. Given that a single nerve can contain many thousands of nerve fibers and that an application can require the analysis of tens or hundreds of nerves [168, 191], it is important to develop methods to obtain the needed measurements efficiently from nerve images.

3.1.4 Goals

I sought to determine how resolution scales with animal mass, and to investigate whether there is any evidence of a tradeoff between responsiveness and resolution in peripheral nerves. Specifically, I considered measurements of resolution from the sciatic nerve and medial gastrocnemius muscle, because the sciatic nerve innervates the majority of tissue in the hindlimb and because the sciatic nerve and medial gastrocnemius muscle contribute to the pathways discussed in Chapter 2. To efficiently measure nerve fiber characteristics in tissue samples, I developed a supervised image analysis method to analyze nerve images. I examined total resolution by determining nerve fiber number in the sciatic nerve, and examined sensory and motor resolution by conducting systematic reviews to determine muscle spindle number and motor unit number in the medial gastrocnemius. To gain insight into the trade-off between responsiveness and resolution, I compared the size distributions of nerve fibers in the sciatic nerves. Finally, I used my findings to predict how control methods may change with animal size.
3.2 Methods

3.2.1 Tissue preparation

3.2.1.1 Tissue acquisition

I obtained sciatic nerve samples from two least shrews (*Cryptotis parva*), one mouse (*Mus musculus*; BALB/cByJ), two rats (*Rattus norvegicus*), one cat (*Felis catus*), two African pygmy goats (*Capra hircus* L.), one horse (*Equus caballus*), four giraffes (*Giraffa camelopardalis*), and one African elephant (*Elephas maximus*; [127]). I acquired the shrew, mouse, rat, and giraffe samples myself, and obtained the cat, goat, horse, and elephant samples from collaborators at the University of Pittsburgh, the Concord Field Station at Harvard University, Cornell University, and the Royal Veterinary College, London, respectively. Depending on the size of the animal, the sections of the nerve ranged from 2 mm to 4 cm long. All tissue collection procedures were approved by the SFU Animal Care Committee.

I perfused one shrew, the mouse, and one rat with 4% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) before the samples were removed; the cat was perfused with 6% paraformaldehyde. Immediately following their removal, nerves from perfused animals were immersed in the same fixative used for perfusion. All other samples were obtained from cadavers as soon after death as possible – the shrew and rat samples were obtained within several minutes, the giraffe samples were obtained within several hours, and the elephant samples were obtained after 24 hours to allow time for the elephant’s herd to mourn. I stored all samples at 4°C until further processing.

Although the elephant specimen was kept at 4°C prior to dissection, unavoidable myelin degradation was still present in many of the elephant nerve fibers as evidenced by the separation of myelin layers (Figure 3.9h, light grey). While this degradation clearly affects the inner diameter of the nerve fibers, its effect on the outer diameter appears to be less severe and, if present at all, would likely increase the apparent size of the nerve fibers making the original elephant nerve fibers smaller than shown here.

3.2.1.2 Histological processing

I processed all the samples in the same manner. First, I cut a thin 1–2 mm thick cross-section from each sample, perpendicular to the direction of the nerve fibers. I washed the sections in 3 changes of 0.1 M phosphate buffer for 10 minutes each, then soaked them in
osmium tetroxide (OsO₄) overnight to stain for lipids and further fix the nerves. I stained the shrew, mouse, and rat sections with 1% OsO₄ in 0.1 M phosphate buffer, and the cat, goat, horse, giraffe, and elephant sections with 2% OsO₄ in 0.1 M phosphate buffer. The next day, I washed the nerves in 2 changes of acetate buffer for 15 minutes each, then dehydrated them in 5 ascending grades of ethanol (20%, 40%, 60%, 80%, two changes of 100%), for 10 minutes each. Afterward, to remove any remaining water, I rinsed them in 2 changes of propylene oxide for 10 minutes each. I immersed the dehydrated nerves overnight in a mixture of propylene oxide and jEmbed 812 embedding compound (Canemco/Marivac Inc., Gore (Lakefield), QC), which allowed the resin to penetrate into all parts of the nerves. The next day, I arranged the nerve slabs in aluminum pans of approximately two inches in diameter (low form aluminum fluted, 42 mL, Fisher Scientific, Ottawa, ON), which were filled with fresh jEmbed resin. To allow any excess propylene oxide to evaporate, I allowed the pans to stand 1–3 hours at room temperature. I then put them on a large Petri dish, and placed them in a 36°C oven overnight. I changed the oven temperature to 48°C the next day, then 60°C overnight for another 24 hours, to polymerize the resin.

I cut each nerve specimen out of the resin disk using a jewel saw. For the shrew, mouse, rat, and cat samples, I placed the plastic block containing the specimen in an ultramicrotome clamp and trimmed it with a glass knife to a depth of 1/4 to 1/3 of the sample length. A glass knife polished the nerve cross-section, and I stained semi-thin cross-sections with Toluidine blue for visualization of structures with a light microscope to ensure that the angle of the cut was satisfactory. I then prepared the nerve block remaining in the ultramicrotome for scanning electron microscopy by securing it to the surface of an scanning electron microscopy (SEM) stub using silicone adhesive. Due to the large size of the goat, horse, giraffe, and elephant samples, I sent the plastic blocks containing the specimens to a local company (Vancouver Petrographics Ltd., Langley, BC) for polishing, then secured them to an SEM stub in a similar manner as the shrew nerve. When the adhesive had dried, I coated all samples with carbon particles, and applied silver paint to connect the carbon-coated samples to the metal stubs. Both these steps prevented charge buildup on the samples, which would have resulted in blurry images.
3.2.2 Image acquisition

3.2.2.1 Fascicle sampling

Due to the large number of fascicles in most of my samples, and realistic time constraints, in most nerves I was not able to analyze each fascicle. In the shrew and mouse nerves, and in one rat nerve, I analyzed all the nerve fibers in the sample; in the remaining nerves I used established systematic random sampling methods to select a subset of fascicles from each sample for further analysis [64]. In nerves where I did not analyze all nerve fibers, I selected between one and seven fascicles representing between 0.8% (in large animals) and 22% (in small animals) of the total fibers within the nerve. The variability between fascicles in each sampled nerve was not large, therefore even small sampling fractions gave very reasonable estimates of the characteristics of the nerve. In the future, I would like to obtained even better estimates by collecting data from a greater percentage of fibers within the samples from large animals.

3.2.2.2 Fascicle imaging

A Bausch & Lomb 2100 Nanolab scanning electron microscope (SEM) imaged the embedded nerves at approximately 1,500x magnification. The microscope scanned at 10–12 kV using the backscatter detector, a spot size of between 3 and 7, and an image quality setting of ‘low 6’ to ‘high 6’; the settings were chosen to give the clearest image, and were sometimes different between samples due to sample variation and the status of the microscope’s filament. Prior to nerve imaging, I confirmed the accuracy of the microscope scalebar with a standard test sample of known size. Nerves had total diameters of approximately 0.3–20 mm, with most fascicle diameters ranging from approximately 0.3 mm to approximately 1 mm. Due to instrument limitations, I usually could not acquire one single image of an entire fascicle cross-section but instead scanned through the cross-section to obtain a set of overlapping sub-images with identical size and resolution. The amount of overlap on each side of each sub-image was approximately 12–20% of the sub-image size to allow sufficient overlap to align adjacent images and allow cropping.

3.2.2.3 Image stitching

SEM imaging produced a set of sub-images for each nerve or fascicle cross-section which fit together in an overlapping grid-like fashion, with the approximate position of each sub-image
known. The size of a typical sub-image was 1024×954 pixels. I developed an algorithm, implemented in Matlab (Matlab 2007a, The MathWorks, Inc., Natick, MA), to automatically stitch these sub-images together and give mosaics showing the whole nerve or fascicle.

My program first cropped the edges of the sub-images to remove any edge distortion. It then used normalized cross-correlation to align the sub-images and stitch them together into a single image (Figure 3.1). While other studies have reported more sophisticated image stitching methods [183,192], I found that cross-correlation was simple, fast, and accurate for my images. To accomplish this, my program compared each sub-image to an overlapping section of its neighboring sub-image. This overlapping section was termed the template, and typically had a width of 50 pixels and a length of 80% of the relevant sub-image dimension. The template was translated over the entire area of its neighboring sub-image, termed the target, with the position of the target remaining fixed. A normalized cross-correlation index describing the similarity of pixel values between the overlapping regions of the template and the target was calculated at each translation [68,109]. I considered only translations, because throughout the imaging process the sample was not rotated or moved vertically.

The optimal position of the template with respect to the target was the position at which the normalized cross-correlation index was maximal. My program used this optimal position to stitch the sub-images together so that there was the highest similarity possible between the overlapping regions. Mathematically, if $I_0$ and $I_1$ are the template and target, respectively, the normalized cross-correlation of these images as a function of their relative horizontal position $u$ and vertical position $v$ was given by:

$$\text{normalized cross-correlation}_{(u,v)} = \frac{\sum_{x,y}(I_0(x-u,y-v)-\bar{I}_0(u,v))(I_1(x,y)-\bar{I}_1(u,v))}{\sqrt{\sum_{x,y}(I_0(x-u,y-v)-\bar{I}_0(u,v))^2 \sum_{x,y}(I_1(x,y)-\bar{I}_1(u,v))^2}}$$

Here, $\bar{I}_0(u,v)$ and $\bar{I}_1(u,v)$ are the mean pixel values of the template and target, respectively, over the overlap region and are expressed as a function of displacement position. The algorithm first aligned all sub-images in each row, then aligned the rows with each other to create the final image.
Figure 3.1: Stitching of sub-images for one rat fascicle. Overlapping scanning electron microscope (SEM) images are taken sequentially to cover the entire fascicle (left), then stitched together using cross-correlation into one image of the entire fascicle (right). All images are shown at the same scale. Modified from [130].
3.2.3 Supervised image analysis

A combination of automatic and manual techniques, referred to as supervised image analysis, identified and labelled nerve fibers in the stitched fascicle images. Prior to analysis, I manually cropped each stitched image to remove extraneous surrounding information. I analyzed each fascicle individually to enable slight adjustments of segmentation parameters such as the greyscale threshold. To analyze the images more efficiently, I developed a supervised image analysis algorithm in collaboration with researchers from the SFU Medical Image Analysis Lab; the steps of our algorithm are illustrated in Figure 3.4, and were published in [130]. We implemented our algorithm in Matlab (Matlab 2007a, The MathWorks, Inc., Natick, MA). While the individual operations used by our algorithm are well-established, our algorithm combines these operations in a unique way.

My SEM images of nerve cross-sections show nerve fibers consisting of dark axons surrounded by a lighter ring of myelin, all on a grey background. We used these intensity characteristics to automatically and separately identify axons and myelin using a series of image manipulations known as morphological operations.

3.2.3.1 Definition of morphological operations

Morphological operations transform images by iteratively carrying out set operations between the image $I$ and a smaller geometric shape (such as a circle or square) known as a structuring element $se$ (Figure 3.2a,c). Combinations of morphological operations are frequently used to identify particles in various types of applications, but comparatively few studies have focused on using these concepts to analyze nerve images [82,121,161,187,191,192].

Commonly used set operations include the union $\cup$ of two images, which retains only light areas present in both images, the intersection $\cap$ of two images, which retains light areas present in either image, and the complement $I^c$ of an image $I$, which reverses the intensities of light and dark areas (Figure 3.2b). The two most basic morphological operations are dilation, which expands light areas ($I \oplus se$), and erosion, which shrinks light areas ($I \ominus se$) (Figure 3.2d,e). Performing dilation and erosion in sequence gives two other important morphological operations: opening, which removes small light areas by performing erosion followed by dilation ($(I \ominus se) \oplus se$), and closing, which removes small dark areas by performing dilation followed by erosion ($(I \oplus se) \ominus se$) (Figure 3.2f,g).

A specific class of morphological operations, known as morphological reconstructions,
Figure 3.2: Basic morphological operations. A digital image $I$ is made of pixels (a). Set operations compare two images: the union $\cup$ retains only light areas present in both images, and the intersection $\cap$ retains light areas present in either image. The complement $I^c$ of $I$ reverses the intensity of each pixel (b). Morphological operations perform set operations with a structuring element $se$ (c). Dilation $\oplus$ expands light areas (d); erosion $\ominus$ shrinks light areas (e). Opening performs an erosion followed by a dilation, removing thin light areas (f); closing performs a dilation followed by an erosion, removing thin dark areas (g). Sequentially dilating or eroding a marker image and comparing the result to a mask image with a union or intersection reconstructs elements of the mask indicated by the marker (h, i); coloured lines from red to blue indicate image boundaries after each union or intersection.
transforms images using sequences of morphological and set operations. One image, denoted
the marker, is dilated or eroded before being compared to the second image, denoted the
mask. In morphological reconstruction by dilation, the marker is dilated and only light areas
which are present in both the dilated marker image and the mask are retained \((\text{marker} \oplus \text{se}) \cap \text{mask}\); the resulting image is used as the new marker, and the process is repeated until
no further change in the marker image occurs (Figure 3.2h). In morphological reconstruction
by erosion, the marker is eroded and all light areas in the eroded marker image as well as the
mask are retained \((\text{marker} \ominus \text{se}) \cup \text{mask}\); the resulting image is used as the new marker, and
the process is repeated until no further change in the marker image occurs (Figure 3.2i). The
structuring element for these procedures defines connectivity – the central element denotes
a representative pixel of the image and the surrounding elements denote the surrounding
pixels. In binary images, these eight elements can each have a value of either 1, meaning
the respective pixel is considered connected to the center pixel, or 0, meaning the respective
pixel is not considered connected to the center pixel.

3.2.3.2 Noise removal

Due to the system noise of the SEM, we observed scattered grey-level variations throughout
the images. This noise was likely caused by a combination of factors including biological and
chemical variability in the tissue, slight changes in electron emission and detection produced
by electrical interference and thermal activity, and small differences in detector sensitivity.
The noise decreased the clarity of the boundary between each axon and its surrounding
myelin and reduced the uniformity of pixel values within these areas, effects detrimental for
accurate segmentation.

To improve accuracy, we used a series of combined morphological operations and mor-
phological reconstructions to de-noise the stitched image before segmentation (Figure 3.3;
Figure 3.4a; [190]). Our algorithm performed one morphological reconstruction by dilation
using an eroded version of the original image as the marker and the original image as the
mask. It then dilated the resulting image and performed a second morphological recon-
struction by dilation, using the complement of the dilated image as the marker and the
complement of the first reconstruction as the mask. The complement of the result was the
denoised image. The overall result of this de-noising process was to remove small localized
areas of higher or lower intensity (noise) and instead bring the intensities of these areas
closer to that of the surrounding image. Image de-noising based on morphological reconstruction has the advantage of reducing noise while preserving edges in the image. This is unlike mask-based filtering methods, such as the commonly-used Gaussian filter, which rely on convolution and can potentially smooth object edges making subsequent segmentation more difficult.

Figure 3.3: Noise removal. The original scanning electron microscope image (top left) contained small localized areas of higher or lower intensity, referred to as noise. To remove the noise, first the original image is eroded (a). Next, a morphological reconstruction by dilation is performed, using the eroded image as the marker and the original image as the mask (b). The resulting image is dilated (c), and the reconstructed image as well as the dilated image are complemented (d, e). A second morphological reconstruction by dilation is performed, using the complement of the dilated image as the marker and the complement of the first reconstructed image as the mask (f). The complement of the second reconstruction is the final de-noised image (g; bottom right). All structuring elements used for noise removal are squares with a width of two pixels.

3.2.3.3 Axon identification

After noise removal, our algorithm identified and labeled potential axons in the de-noised image. SEM images of nerve cross-sections show axons as areas of dark grey (low intensity
**Figure 3.4:** Axon and myelin segmentation. The original greyscale image is first de-noised (a, Figure 3.3), then thresholded to convert it to black and white (b). Any remaining small white areas are removed, and the complement is taken (c). Three criteria are used to determine which white connected components are potential axons: first, they must be within a certain size range (d); second, they must have a perimeter to area ratio below a given threshold (e); and third, they must have a low average grey level (f). White connected components meeting these criteria are labeled as potential axons, coloured to enable visualization, and overlaid on the original image for verification by the user (g). Through a graphical user interface, the user is then able to manually remove areas which are not axons (h) and manually add axons which have been missed (i). Using these final axon labels, the algorithm scans radially outwards from the center of each axon to find the surrounding myelin (j). Finally, the labeled area of each axon is combined with its respective myelin area to give labeled nerve fibers (k). Modified from [130].
value) pixels and the surrounding myelin as lighter (high intensity value) pixels. The background area between the myelinated nerve fibers has a grey-level value between that of the axons and myelin. Our method set all grey-level values below a user-defined threshold to black and all grey-level values above this threshold to white, thus converting the greyscale image into a black and white image – known as a binary image – showing potential axons as black areas on a white background (Figure 3.4b). The algorithm removed all white areas too small to represent myelin, as determined by a user-defined threshold. It then inverted the image by taking its complement, so that the potential axons were shown as white areas (known as connected components) on a black background (Figure 3.4c). With this method, our algorithm could identify the majority of axons. However, because the grey-level values of the axons and background can be similar, some background areas were mislabeled as potential axons. To reduce the mislabeling of connected components, we used prior knowledge of axon properties to add the following inclusion/exclusion rules:

1. **Number of pixels in a connected component**
   Myelinated axons have a finite range of possible sizes – a connected component with size outside this user-defined range is not an axon, and is therefore designated as part of the background (Figure 3.4d).

2. **Shape of a connected component**
   Axons generally have approximately round shapes, while background areas can have much more convoluted shapes. We measured the degree of roundness using the parameter $R = p/A$, where $p$ is the number of pixels on the perimeter of a connected component and $A$ is the total area in pixels of that component. With a user-defined upper limit of $R$ for axons above a given size, components with shapes closer to round have smaller $R$ values and are designated as axons, whereas components with more convoluted shapes have larger $R$ values and are designated as part of the background (Figure 3.4e). Examples of axons with different sizes and relative $R$ values are shown in Figure 3.5.

3. **Average grey-level value of a connected component**
   Axons are generally shown as areas of dark grey (low intensity value) pixels in the SEM image, whereas background sections are generally shown as areas of lighter grey (higher intensity value) pixels. To refine the initial axon identification, which relied only on the grey-level values of individual pixels, we found the average grey-level value
of each connected component and designated all components with an average value greater than a user-defined threshold as part of the background and all components with average grey-levels below this threshold as axons (Figure 3.4f).

![Figure 3.5: Examples of various axon shapes detected automatically with our algorithm.](image)

We used axon perimeter to area ratio ($R = p/A$) as a metric of shape, with axons having more convoluted shapes also having a higher ratio of edge pixels to total pixels. Here, axon perimeter to area ratio increases from bottom to top, whereas axon size increases from left to right. Pixels on the perimeter of the identified axon are coloured red. Modified from [130].

### 3.2.3.4 Myelin identification

Once the axons were identified, we used this information to identify the surrounding myelin (Figure 3.4j; [130]). We developed an algorithm which started from the geometric center of each identified axon and scanned for changes in image intensity along lines radiating outward. Myelin labeling along each scan line began when the radial outward scan line exited the labeled axon area, and terminated when a change in pixel grey-level from high to low, indicating the outer boundary of myelin, was detected. The length of the scanning line, defined by the user, was constrained to be within physiological limits to prevent labeling.
the myelin of other nerve fibers in the case of two myelin areas touching. Upon completion of one scan line, the angle of the scan was incremented and the scan repeated until all 360° of myelin surrounding one axon were scanned. Once this procedure was performed on all axons, morphological closing with a small structuring element was used to smooth and fill in any small gaps in the labeled myelin. A conceptually similar procedure has been previously proposed in the context of cardiac wall segmentation in ultrasound images, denoted as the star algorithm [203].

3.2.3.5 User interface and manual correction

To increase the usability of our method and visualize the computed results, I integrated our algorithms into a user interface which included the ability to input parameters, display the image, and manually correct segmentation results by adding or deleting labeled axons and myelin. Input parameters, along with their typical values for segmentation of a rat nerve, are shown in Table 3.1. Once the segmentation algorithms were complete, the unique identifying labels corresponding to each automatically segmented axon and myelin area were converted to unique colours, and visually overlaid on the original image (Figure 3.4g,j). This visualization step was critical to allow the user to evaluate the effectiveness of the chosen parameters and manually correct axon labels. I developed manual correction tools for the deletion and addition of axons – based on prior knowledge of axon appearance, the user manually deleted axons by identifying mislabeled areas (Figure 3.4h), and manually added axons by tracing their outline with similar accuracy to the algorithm (Figure 3.4i). I trained two colleagues to use my manual correction methods, and compared the results for several images that all three of us analyzed – there were no systematic errors in nerve fiber number or size. We therefore all contributed to correcting the inevitable errors in automatic segmentation before finalizing data for statistical analysis.

3.2.3.6 Output

Upon completion, our method had quantified the location and size of all myelinated axons and their corresponding myelin sheaths. I used this information to calculate the size of each myelinated nerve fiber as the diameter of a circle with equivalent area to the sum of the corresponding axon and myelin areas (Figure 3.4k). I chose equivalent circle diameter as a measure of nerve fiber size because, in addition to being the most accurate method
<table>
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<td>pixels</td>
</tr>
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</table>

Table 3.1: Supervised image analysis input parameters. Values were obtained during the segmentation of one fascicle from a rat sciatic nerve. Modified from [130].

[90], diameter is directly correlated with physiological properties of nerve fibers such as conduction velocity [63, 83, 165]. Since diameter is a commonly-used size measure [51, 90] this also allowed me to compare our measurements to those found in other studies. I used the output from our algorithm to calculate the total number of myelinated nerve fibers and create fiber size distributions. For nerves where we did not analyze all fascicles, I estimated the total number of nerve fibers by calculating the number of nerve fibers for the measured fascicles, dividing this value by the area of the measured fascicles, and then multiplying the result by the total area of all fascicles in the nerve. I averaged the number of nerve fibers over all animals within each species to give representative data for the species.

In addition to nerve fiber number and size distribution, our method can also output other characteristics such as nerve fiber shape and myelin thickness. A summary of preliminary statistics describing fascicle number and area, as well as total nerve fiber area, fiber diameter metrics, myelin thickness, g-ratio, and fiber shape in the images we analyzed is given in Appendix B.

### 3.2.3.7 Performance

To test the performance of our methods, we examined the supervised analysis of one average-sized fascicle, approximately 0.5 mm in diameter, from one rat sciatic nerve [130]. We measured the time required for each step of our image analysis method, and counted the
number of misidentified axons (false positives) and unidentified axons (false negatives) given by our segmentation algorithm. We compared the results obtained using our supervised method with those obtained using a completely manual method in which we manually traced the outline of each nerve fiber using a stylus and graphics tablet (Wacom Graphire 4, Wacom Co., Ltd, Saitama, Japan). We implemented both supervised and manual methods in MATLAB (MathWorks, Natick MA) and performed all analyses on a PC with a 3.40GHz Pentium D processor and 2GB of RAM. A stylus and graphics tablet helped us to quickly and accurately add and delete axons.

3.2.4 Systematic reviews

3.2.4.1 Nerve fiber number

I searched the literature for the number of nerve fibers in the sciatic nerve measured for a range of animal masses (Table 3.2). Specifically, I considered measurements made in tissue obtained from mature quadrupedal mammals who were as close to wild type as possible. I included only counts of total nerve fiber number in the sciatic nerve, and excluded estimates based on the number of nerve fibers in the branches of the sciatic nerve.

One study ([138]) did not report masses of their animals – I estimated these masses using the age of the animals studied and growth charts from an animal supplier website (The Jackson Laboratory; http://jaxmice.jax.org).

In total, I used data from three independent studies reporting nerve fiber numbers for one to five species each.

3.2.4.2 Muscle spindle number and motor unit number

I searched the literature for muscle spindle numbers and motor unit numbers measured for a range of animal masses (Table 3.3, Table 3.4). Specifically, I considered measurements made in vivo or in tissue obtained from mature quadrupedal mammals who were as close to wild type as possible. I included counts of total spindle and motor unit number in the medial gastrocnemius muscle. Muscle spindles have been studied extensively by Banks, but he does not report spindle number in the medial gastrocnemius ([12, 13]).

I included estimates for muscle spindle numbers and motor unit numbers based on studies which reported the total number of gamma or alpha motor nerve fibers innervating the medial gastrocnemius. To convert the number of gamma nerve fibers to the number of
Table 3.2: Animal masses and numbers of nerve fibers in the sciatic nerve (Fibers), with their standard deviations (sd) where available. ‡ unpublished data obtained using my supervised image analysis method. [130,132] published data obtained using my supervised image analysis method. All other values are from the literature.
muscle spindles in cats, I used a conversion factor of 2.4 gamma fibers per muscle spindle, as given in [23] for the cat medial gastrocnemius. I was unable to find any studies directly reporting the ratio of gamma fibers to the number of muscle spindles in the rat medial gastrocnemius, however I was able to find this information for the rat soleus. I therefore calculated a conversion factor for the rat medial gastrocnemius by combining data from multiple studies and assuming that rats and cats had similar relative magnitudes of gamma fiber–muscle spindle ratios in their medial gastrocnemius and soleus muscles: the rat soleus has 2.75 gamma fibers per spindle [6], and the cat soleus and medial gastrocnemius have 2.1 and 2.4 gamma fibers per spindle, respectively [23], so I estimated that a rat medial gastrocnemius has $2.75 \times \frac{2.4}{2.1} = 3.14$ gamma fibers per muscle spindle. To convert the number of alpha nerve fibers to the number of motor units, I used a conversion factor of 1 alpha nerve fiber per motor unit for all species – this value is considered to be applicable to most animals [31].

Some studies did not report masses of their animals – I estimated these masses using the age of the animals studied and growth charts for the appropriate species. These charts were available from animal supplier websites, including Charles River Laboratories (http://www.criver.com), Harlan Laboratories (http://www.harlan.com), and The Jackson Laboratory (http://jaxmice.jax.org).

In total, I used data from 10 independent studies reporting muscle spindle and/or motor unit numbers for one species each. To ensure as accurate a representation of muscle spindle and motor unit number as possible, I included a variety of experimental protocols, including anatomical observations and electrophysiological measurements. In addition, for each species I obtained values from multiple studies and different researchers.¹

¹After completing this analysis and submitting my thesis, I found additional muscle spindle measurements and corrected motor unit number measurements in the rat. The values reported in [41] were found by the authors to be an underestimate of true motor unit number; they have since found that the medial gastrocnemii of 8 male rats with masses of $0.474 \pm 0.031$ kg have $28.7 \pm 6.6$ gamma fibers and $65.8 \pm 7.3$ alpha fibers, and the medial gastrocnemii of 8 female rats with masses of $0.251 \pm 0.044$ kg have $27.6 \pm 3.9$ gamma fibers and $56.1 \pm 7.6$ alpha fibers [126]. Using the conversion factors in Subsubsection 3.2.4.2, this equates to $9.1 \pm 2.1$ muscle spindles and $65.8 \pm 7.3$ motor units in male rats, and $8.8 \pm 1.2$ muscle spindles and $56.1 \pm 7.6$ motor units in female rats. (mean±sd). These muscle spindle numbers are slightly lower than the data in Table 3.3, and these motor unit numbers are within the variability of the data in Table 3.4; I therefore expect that the addition of this information would slightly decrease my reported scaling of muscle spindle number, but would not change my reported scaling of motor unit number significantly.
### Table 3.3: Animal masses and numbers of muscle spindles (Spind).

- * standard deviation (sd) approximated by \( \frac{\text{range}}{4} \).
- † standard error. Muscles are MG=medial gastrocnemius. Measurement types are Anat=counted anatomically, Gamma=estimated using number of gamma motor neurons, Avg=average of multiple studies.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Mass (kg)</th>
<th>Mass sd (kg)</th>
<th>Spind</th>
<th>Spind sd</th>
<th>Muscle</th>
<th>Type</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>6</td>
<td>0.0125</td>
<td></td>
<td>10.2</td>
<td>0.3†</td>
<td>MG</td>
<td>Anat</td>
<td>[101]</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.0230</td>
<td></td>
<td>9.6</td>
<td>0.2</td>
<td>MG</td>
<td>Anat</td>
<td>[202]</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td><strong>0.0178</strong></td>
<td></td>
<td><strong>9.9</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>4</td>
<td>0.300</td>
<td></td>
<td>17.5</td>
<td>2.5</td>
<td>MG</td>
<td>Gamma</td>
<td>[148]</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.455</td>
<td>0.0225*</td>
<td>12.6</td>
<td>1.1</td>
<td>MG</td>
<td>Gamma</td>
<td>[70]</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td><strong>0.378</strong></td>
<td></td>
<td><strong>15.0</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>2</td>
<td>2.5</td>
<td>0.3</td>
<td>36.9</td>
<td>3.2</td>
<td>MG</td>
<td>Gamma</td>
<td>[23]</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td><strong>2.5</strong></td>
<td><strong>54.1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3.4: Animal masses and motor unit (MU) numbers.

- * standard deviation (sd) approximated by \( \frac{\text{range}}{4} \).
- ** values obtained from figures.
- † standard error. Muscles are MG=medial gastrocnemius. Measurement types are Force=estimated using force measurements, Alpha=estimated using number of alpha motor neurons.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>Mass (kg)</th>
<th>Mass sd (kg)</th>
<th>MU</th>
<th>MU sd</th>
<th>Muscle</th>
<th>Type</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>1</td>
<td>0.022</td>
<td></td>
<td>95.5**</td>
<td>8.8**</td>
<td>MG</td>
<td>Force</td>
<td>[71]</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.025</td>
<td></td>
<td>105</td>
<td></td>
<td>MG</td>
<td>Force</td>
<td>[96]</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td><strong>0.0235</strong></td>
<td></td>
<td><strong>100.3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>4</td>
<td>0.300</td>
<td></td>
<td>77</td>
<td>8</td>
<td>MG</td>
<td>Alpha</td>
<td>[148]</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.455</td>
<td>0.0225*</td>
<td>97.5</td>
<td>4.2</td>
<td>MG</td>
<td>Alpha</td>
<td>[70]</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.4966</td>
<td>0.045</td>
<td>57.2</td>
<td>4.0†</td>
<td>MG</td>
<td>Force</td>
<td>[41] (males)</td>
</tr>
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<td>6</td>
<td>0.2583</td>
<td>0.0248</td>
<td>52.1</td>
<td>3.3†</td>
<td>MG</td>
<td>Force</td>
<td>[41] (females)</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>0.453</td>
<td>0.052</td>
<td>93</td>
<td>28</td>
<td>MG</td>
<td>Force</td>
<td>[50]</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td><strong>0.3926</strong></td>
<td></td>
<td><strong>75.4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>2</td>
<td>2.5</td>
<td>0.3</td>
<td>280</td>
<td></td>
<td>MG</td>
<td>Alpha</td>
<td>[23]</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td><strong>2.5</strong></td>
<td><strong>270</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

...
3.2.5 Scaling of nerve fiber number, muscle spindle number, and motor unit number

For nerve fiber number, I combined my systematic review results with my own histology measurements of nerve fiber number, and averaged the values of data points within each species; for muscle spindle number and motor unit number, I averaged the values of my systematic review results within each species. For each of these three relationships, least-squares linear regression of logarithmically transformed species averages determined the exponent and coefficient of the scaling relationship (Subsection 1.7.1).

3.2.6 Nerve fiber size distribution

For each of the samples I analyzed using our supervised image analysis method, I calculated the size of each nerve fiber as the diameter of a circle with equivalent area (Subsubsection 3.2.3.6). For each species, I constructed a nerve fiber size distribution by combining nerve fiber diameters from all the fascicles analyzed. To account for the different total numbers of nerve fibers I measured in each species and make it easier to compare the distributions of nerve fiber sizes, I normalized each size distribution by expressing the number of identified fibers of each size as a percentage of the total number of fibers analyzed in the species.

3.3 Results

3.3.1 Supervised image analysis

I validated our supervised image analysis method using one representative fascicle from a rat sciatic nerve. To cover the entire fascicle, I collected 45 SEM images and stitched them together using my normalized cross-correlation algorithm (Figure 3.6a). Of the 2,010 axons ultimately identified in the fascicle (Figure 3.6b), our automated axon segmentation algorithm correctly identified 84.3%, leaving 15.7% of axons unidentified – these false negatives were added using my manual correction tools. Of the 1,963 potential axons initially identified by our automated axon segmentation algorithm, 13.4% were in fact background areas – these false positives were also deleted using my manual correction tools. Ultimately, our supervised method and the completely manual method identified 2,010 axons and 2,025 axons, respectively – a 0.5% difference.
Figure 3.6: Example of supervised image analysis results from one rat fascicle. Following sub-image stitching (Figure 3.1), I manually cropped the fascicle image to remove extraneous surrounding information; the resulting greyscale image (a) was the input for our algorithm. Our algorithm automatically identified the axons in the original image, after which we removed misidentified axons and added missing axons using our manual correction tools. Overlaying the final axon labels on the original image enabled visual evaluation of image analysis (b). Our algorithm then automatically identified the myelin surrounding each axon; overlaying the resulting myelin labels on the original image again allowed visual evaluation (c). Finally, we combined each labeled axon with its respective labeled myelin sheath to obtain labeled nerve fibers, and overlaid the nerve fiber labels on the original image (d). Modified from [130].
After all axons had been identified, our automated myelin segmentation algorithm identified myelin surrounding 98.5% of the axons (Figure 3.6c). The identified myelin was not always accurate, however. In cases where cracks appeared in the myelin, the algorithm ceased labeling at the crack. In cases where the boundary between myelin and background was indistinct, or where two myelin sheaths touched, the algorithm often continued labeling past the outer myelin border. Myelin surrounding 1.5% of axons was not identified. The majority of these axons had convoluted shapes in which the geometric center did not fall within the labeled axon area. Combining the labelled myelin areas with their respective labelled axons gave labelled nerve fibers (Figure 3.6d).

Because the number of axons in an image can vary, we found it useful to express the speed of our algorithm steps on a per-axon basis in addition to reporting the overall speed of our method. Performance metrics for each stage of analysis are given in Table 3.5. Automatic identification of each potential axon by our algorithm took approximately 4 milliseconds per axon, while manual deletion of one axon took approximately 3.2 seconds and manual addition took just over 7.4 seconds per axon. After axon identification, automatic myelin identification took approximately 22 milliseconds per axon. The total time required to fully process the sample image was just under 1 hour, including manual correction. Stitching the sub-images together comprised 0.3% of this time, manually cropping the fascicle comprised 5.9%, automatic segmentation comprised 0.2%, and manual deletion and addition comprised 24.6% and 67.6% respectively. Identification of myelin comprised the remaining 1.3% of image analysis time. Our supervised image analysis method was approximately 7.8 times faster than completely manual segmentation of the entire fascicle, reducing the time required for the analysis of all nerve fibers in the fascicle from over 7 hours to under 1 hour. After automatic stitching of sub-images and cropping, we estimate that the time needed to analyze an entire rat sciatic nerve sample (approximately 4.5 times the area of the fascicle that we analyzed) would be approximately 4.5 times as long as the analysis of one fascicle, taking roughly 4 hours using our supervised image analysis method as compared to almost 32 hours using manual segmentation methods.

3.3.2 Scaling of nerve fiber number, muscle spindle number, and motor unit number

Using a combination of my own histology data and systematic review data, I found that nerve fiber number in the sciatic nerve increased with animal size in proportion to $M^{0.35}$. 
| Time to automatically stitch | 12 | 00:00:12 |
| Time to manually crop | 206 | 00:03:26 |
| Time to automatically segment axons | 8 | 00:00:08 |
| Time to manually delete axons | 856 | 00:14:16 |
| Time to manually add axons | 2,351 | 00:39:11 |
| Time to automatically segment myelin | 44 | 00:00:44 |
| Total processing time, one fascicle (supervised method) | 3,477 | 00:57:57 |
| Total fiber segmentation time, one fascicle (supervised method) | 3,259 | 00:54:19 |
| Total fiber segmentation time, one fascicle (manual method) | 25,445 | 07:04:05 |
| Predicted fiber segmentation time, whole nerve (supervised method) | 14,666 | 04:04:26 |
| Predicted fiber segmentation time, whole nerve (manual method) | 114,503 | 31:48:23 |

| # axons |
| Initially identified | 1,963 |
| Correctly identified | 1,694 |
| False positives | 269 |
| False negatives | 316 |
| Total in fascicle (supervised method) | 2,010 |
| Total in fascicle (manual method) | 2,025 |
| Predicted total in whole nerve (supervised method) | 9,045 |
| Predicted total in whole nerve (manual method) | 9,113 |

**Table 3.5:** Performance characteristics of our supervised image analysis method. Values were obtained during the analysis of one fascicle from a rat sciatic nerve. Modified from [130].
CHAPTER 3. RESOLUTION

(Chapter 3.6; Figure 3.7a). This relationship predicts that a 5 gram shrew would have just over 2,000 nerve fibers in its sciatic nerve, while a 5,000 kilogram elephant would have almost 280,000 nerve fibers – an increase of more than 140 times over a size difference of six orders of magnitude. However, expressed with respect to body weight ($M^1$), relative nerve fiber number decreases with animal size in proportion to $M^{-0.64}$, predicting a relative decrease in nerve fiber number from the shrew to the elephant of almost 7,500 times, from more than 400 fibers per gram of body tissue in a 5 gram shrew to just over 0.05 fibers per gram in a 5,000 kilogram elephant. Expressed with respect to animal surface area ($M^{2/3}$), relative nerve fiber number decreases in proportion to $M^{-0.31}$, and expressed with respect to muscle force ($M^{0.74}$; [18]), relative nerve fiber number decreases in proportion to $M^{-0.38}$. Expressed with respect to degrees of freedom ($M^0$), relative nerve fiber number increases in proportion to $M^{0.35}$.

<table>
<thead>
<tr>
<th>Number</th>
<th>Coefficient</th>
<th>Exponent</th>
<th>Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value</td>
<td>95% CI</td>
<td>Value</td>
</tr>
<tr>
<td>Nerve fibers</td>
<td>13.587</td>
<td>$\times$</td>
<td>1.24</td>
</tr>
<tr>
<td>Muscle spindles</td>
<td>31.14</td>
<td>$\times$</td>
<td>90.59</td>
</tr>
<tr>
<td>Motor units</td>
<td>160.33</td>
<td>$\times$</td>
<td>532.79</td>
</tr>
</tbody>
</table>

**Table 3.6:** Nerve fiber number, muscle spindle number, and motor unit number power laws and statistics. Coefficient and exponent values were determined by least-squares linear regression of logarithmically transformed data. Only the nerve fiber number power law is statistically significant; due to the limited range of data for muscle spindle number and motor unit number, these two relationships should be considered preliminary.

My systematic review suggests that muscle spindle number and motor unit number in the medial gastrocnemius muscle increase with animal size, in proportion to $M^{0.32}$ and $M^{0.17}$, respectively (Table 3.6; Figure 3.7b,c); however, these relationships are not statistically significant and are based on data from a limited range of animal sizes, so they should be considered as preliminary results only. The three species for which I was able to find data all had more motor units than muscle spindles – mice had approximately 10 times as many motor units as muscle spindles, and both rats and cats had approximately 5 times as many motor units as muscle spindles (Table 3.3; Table 3.4; Figure 3.8). I estimate that the medial gastrocnemius makes up approximately 9% of the muscle mass innervated by nerve fibers in the sciatic nerve (assuming that the medial gastrocnemius is 50% of the mass of
Figure 3.7: Nerve fiber number, muscle spindle number, and motor unit number scaling relationships shown individually. Plots show the scaling relationships in Table 3.6 (black and coloured lines), and the data points used to determine them (filled circles). Where information from more than one study per species was used, the data point is an average of values from the studies. Nerve fiber number is in the sciatic nerve; muscle spindle number and motor unit number are in the medial gastrocnemius muscle, which comprises approximately 9% of the muscle mass innervated by the sciatic nerve and its branches [9]. Due to the limited range of data for muscle spindle number and motor unit number, these two relationships should be considered preliminary.
the gastrocnemius muscle; [9]) – based on this assumption and my scaling relationships in Table 3.6, the percentage of nerve fibers in the sciatic nerve that are Ia afferents and alpha motor nerve fibers decreases from about 3% and 35%, respectively, in a 5 gram shrew to about 2% and 3%, respectively, in a 5,000 kilogram elephant.

![Diagram of nerve fiber number, muscle spindle number, and motor unit number scaling relationships superimposed. Solid lines show the scaling relationships in Figure 3.7; vertical dashed lines indicate the masses of each species in Figure 3.7(a) – from left to right, shrew, mouse, rat, guinea pig, cat, dog, goat, horse, giraffe, and elephant. Nerve fiber number is in the sciatic nerve; muscle spindle number and motor unit number are in the medial gastrocnemius muscle, which comprises approximately 9% of the muscle mass innervated by the sciatic nerve and its branches [9]. Due to the limited range of data for muscle spindle number and motor unit number, these two relationships should be considered preliminary.]

3.3.3 Nerve fiber size distribution

I measured the equivalent circle diameters of 36,442 nerve fibers in sciatic nerve samples from a total of eight species, and used these measurements to construct nerve fiber size
distributions for each species. Images showing a portion of a typical nerve cross-section in each species are shown in Figure 3.9, alongside the total nerve fiber size distribution for the respective species. Both minimum and maximum nerve fiber size remained relatively constant in all species – the smallest fibers in all animals were about 1 µm, and the largest fibers in big animals were less than twice the diameter of those in small animals (23 µm vs. 13 µm) despite an increase in body mass by almost six orders of magnitude. Qualitatively, larger animals tended to have more bimodal fiber size distributions than small animals, and the heights of the distribution peaks became increasingly different with animal size. Among animals with bimodal nerve fiber size distributions, as animal size increased the small-fiber peak became increasingly higher than that of the large-fiber peak – the small-fiber peak in the 4.4 kg cat was slightly lower than the large-fiber peak, whereas in the 2,500 kg elephant the small-fiber peak was almost 3.5 times higher than the large-fiber peak.

3.4 Discussion

3.4.1 Supervised image analysis

Our supervised image analysis method is efficient. With a 7.8-fold increase in speed over traditional manual segmentation methods and the ability to identify both axon and myelin characteristics in every nerve fiber in the nerve, our supervised method markedly reduces the time and effort needed to analyze SEM images of nerve cross sections. The quality control tools help users quickly identify and correct any errors made by the algorithm, as well as visually evaluate the accuracy of the results. Due to our algorithm’s ability to stitch together multiple SEM images into one larger image showing a whole nerve or fascicle cross-section, our method allows us to analyze an entire nerve or fascicle at one time. This eliminates the need to sample only a few regions of the nerve, thereby reducing sampling error and producing more accurate data. While a time savings of over 27 hours for small nerves (containing on the order of $10^4$ nerve fibers) is quite substantial, we anticipate that the time savings for very large nerves (containing on the order of $10^6$ nerve fibers; [131]) will be even more impressive, potentially saving months of work. Although the development of this algorithm was time-consuming, we expect that its continued use by us and other research groups will result in a net cost savings.

One of the major advantages of our algorithm is its simplicity and ease of implementation. Methods of segmentation previously developed to analyze nerve images often use
Figure 3.9: Scanning electron microscope images and nerve fiber size distributions. For each species, part of a fascicle cross-section is shown alongside total nerve fiber size distribution. The number of nerve fibers of each size is expressed as a percentage of the total number of fibers measured in the species; the mean number of fibers in the sciatic nerve of each species (from Table 3.2) is noted on its respective size distribution. Dark grey bars on horizontal axes span minimum to 95th percentile diameters. Scale bars (white) are 20 µm.
complex algorithms involving zonal graphs [161], clustering [36], and bit-plane analysis [82].
In contrast, our algorithm uses a unique sequence of basic morphological operators and
reconstructions, making it highly customizable, computationally efficient, and easy to un-
derstand and execute in a wide range of software programs. Additionally, the input pa-
rameters for the automated portions of our algorithm are based on physiological properties,
making them intuitive and our method easy to learn. These factors allow almost anyone
to employ our supervised image analysis method without the investment of a large amount
of time and money. Our method provides a straightforward workflow that most clinicians
and researchers can use. While our method has a slightly longer analysis time than some
others [82,161], its use of SEM imaging and simplicity of implementation make it a worthy
addition to existing methods of nerve segmentation.

Our supervised image analysis method produces results similar to those of other re-
searchers. Based on the analysis of a typical rat fascicle, our method predicts total nerve
fiber numbers within the range of those found in other studies [138,168,170]. Although I
was not able to find previously published histograms of sciatic nerve fiber sizes for most of
the species described in Figure 3.9, rat fiber size distributions found by other researchers
have shapes that are similar to mine [168]. Although my measurements of rat nerve fiber
diameter are larger on average than those in other studies [82,168] I attribute this to the
nature of my samples rather than to any error in our algorithm. One of the rat fascicles I
analyzed included several regions of large elongated nerve fibers, and because our method
identifies and measures every nerve fiber in the nerve rather than several representative ar-
eas these outliers will tend to skew the distribution toward larger nerve fiber sizes. In fact,
I view this as a strength of our method – rather than extrapolating from a limited sample
set, as in most other studies, all nerve fibers in the sample, including those with noncircular
or irregular shapes are identified.

3.4.2 Scaling of nerve fiber number, muscle spindle number, and motor
unit number

Nerve fiber number increased in proportion to \( M^{0.35} \), which is not significantly different
from changes in animal linear dimensions [3]. It is not clear what the underlying reason
for my observed scaling of nerve fiber number is, in part because total nerve fiber number
reflects the sum of fibers from many different sensors and effectors. It is probable that the
numbers of different types of sensors and effectors would need to change in different ways
with animal mass to maintain resolution (Subsection 3.1.2) – for example, the number of muscle spindles may need to increase in proportion to animal volume ($\propto M^1$), the number of motor units may need to increase in proportion to muscle force ($\propto M^{0.74}$), the number of touch receptors may need to increase in proportion to animal surface area ($\propto M^{2/3}$), and the number of joint receptors, may not need to increase at all ($\propto M^0$). The sum of these relationships could result in total nerve fiber number increasing at an intermediate rate if resolution were maintained. However, because my observed increase in nerve fiber number with animal size is slower than three of these four theoretical possibilities, another, perhaps more likely, possibility is that resolution decreases as animal size increases. Having relatively fewer nerve fibers would mean that larger animals were not able to innervate as many distinct sensorimotor structures with respect to their body size. If all other factors were equal, the resulting relative decrease in sensory receptor and motor unit number would result in larger animals being less able to precisely sense and respond to stimuli compared to smaller animals. This could force larger animals to develop compensatory mechanisms.

To determine whether larger animals do indeed face challenges to resolution, we need to determine how the components of resolution change with animal size.

To investigate the contribution to total resolution of one type of sensor (muscle spindle) and one type of effector (motor unit), I systematically searched the literature for data on muscle spindle number and motor unit number in the medial gastrocnemius. Because of the limited data available, my results for both these measures of resolution should be considered preliminary. My systematic review suggests that muscle spindle number may increase in proportion to $M^{0.32}$ and motor unit number may increase in proportion to $M^{0.17}$. My observed increase in muscle spindle number is slower than would be expected for animal areas ($M^{2/3}$), suggesting that the number of spindles per unit area of muscle decreases as animal size increases. My observed increase in motor unit number is slower than the increase in muscle force ($M^{0.74}$; [18]), suggesting that individual motor units produce more force in larger animals than in smaller animals. These preliminary results could indicate that neither muscle spindle number nor motor unit number increase sufficiently to maintain resolution – larger animals may be less able to precisely sense muscle length changes and less able to produce smoothly varying muscle forces relative to smaller animals. My tentative scaling relationships for muscle spindle number and motor unit number also suggest that muscle spindle number may increase at close to the same rate as total nerve fiber number, while motor unit number may increase at a slower rate. If motor unit number does scale in this
way, the decrease in motor unit number relative to total nerve fiber number could reflect a prioritizing of sensory components of resolution.

My preliminary results for the scaling of muscle spindle number are consistent with other studies. Muscle spindle number in the combination of medial and lateral gastrocnemii of humans, combined with several other mammals spanning a 14 kilogram size range, has been shown to increase in proportion to muscle mass raised to the power of 0.23 [12]. Because extensor muscle mass and body mass increase in almost direct proportion to each other [5], this suggests that muscle spindle number in the gastrocnemii also increases with body mass raised to the power of 0.23. This exponent is within the confidence interval predicted by my preliminary data. On average, in animals ranging in size from mouse to human, muscle spindle number in muscles other than the medial gastrocnemius increases in proportion to linear dimensions [12]; this scaling relationship is very close to my preliminary findings in the medial gastrocnemius. Banks speculates that, given that muscle spindles sense muscle length, there may be a relationship between the scaling of a sensor and the dimension that it provides information about [12]. While this is an interesting idea, more measurements need to be made for different sensors in animals spanning a wider size range before it can be considered a general relationship.

Muscle spindles may not be the only sensor that increases more slowly than mass. Dissections of tissue from several species of animals suggests that some cutaneous touch sensors become less dense in the skin of larger animals [178] – this would decrease an animal’s ability to precisely sense the location of stimuli, such as insects, on the surface of its body. Like mechanoreceptors, the number of photoreceptors may also increase more slowly than mass – the number of myelinated fibers in the optic nerves of animals ranging in size from the rat to sheep increase approximately with $M^{0.33}$ (based on fiber numbers from [27] and estimates of animal masses), which is almost identical to my preliminary results for muscle spindle number.

3.4.3 Nerve fiber size distribution

The range of nerve fiber sizes was relatively constant across all species. Two measurements of nerve fiber range – minimum diameter and 95th percentile diameter – are particularly interesting in the context of sensorimotor control. Across all animals, minimum nerve fiber diameter was approximately 1 µm, and 95th percentile diameter was about 7–16 µm (Figure 3.9). My observed average minimum nerve fiber diameter is consistent with theoretical
predictions that the different biophysical characteristics of unmyelinated and myelinated nerve fibers will result in myelinated fibers only having higher conduction velocities than unmyelinated fibers if they have diameters larger than 1 $\mu$m – nerve fibers with diameters less than 1 $\mu$m have higher conduction velocities if they are unmyelinated [165]. My observed range of 95th percentile fiber diameters is consistent with the range of conduction velocities found in my electrophysiology experiments and systematic review (Table 2.3). I focused on maximum nerve fiber conduction velocities, which depend upon the size of the largest nerve fibers stimulated – because there are only a few very large fibers in each nerve, and because nerve stimulation does not always activate 100% of the fibers in a nerve, the 95th percentile fiber diameter is likely the best measure of the largest fibers stimulated. The conduction velocities of these large nerve fibers increase by 5.7–6.0 m/s per $\mu$m of fiber diameter [8, 83]; when combined with my measurements of 95th percentile nerve fiber diameters, this gives a range of predicted nerve fiber conduction velocities of 40–96 m/s, which is very close to the range of 41–101 m/s in Table 2.3. The maximum nerve fiber size in the shrew was lower than the maximum fiber size in other animals; excluding the shrew from the range of 95th percentile nerve fiber diameters results in an even more constant 95th percentile fiber diameter of about 11–16 $\mu$m, with no qualitative dependence on animal size. Figure B.1 and Table B.2 in Appendix B report more detailed data describing the nerve fiber size distributions in Figure 3.9.

In addition to the shrew’s small maximum nerve fiber diameter, the peak of its nerve fiber size distribution was located at a small diameter – about 3 $\mu$m. A large fraction of the myelinated fibers in the shrew, therefore, have diameters only slightly greater than the 1 $\mu$m division between unmyelinated and myelinated fibers [165]. At nerve fiber diameters below 1 $\mu$m, unmyelinated fibers conduct more quickly than myelinated fibers (Subsubsection 2.1.2.2; [165]). This, in combination with the short distances that shrews need to conduct their nerve impulses, might make it beneficial for shrews to have a larger proportion of unmyelinated nerve fibers relative to larger animals. The increase in resolution gained by having more, slightly slower-conducting, unmyelinated fibers may outweigh the benefits of having fewer, but slightly faster-conducting, myelinated fibers. However, the number of myelinated nerve fibers I measured in the shrew sciatic nerve is consistent with that expected based on my measurements in other species Figure 3.7, suggesting that any increase which may occur in the number of unmyelinated nerve fibers in shrews does not decrease the number of myelinated nerve fibers.
Many of the species I studied had nerve fiber size distributions that were clearly bimodal, similar to those found in the peripheral nerves of rats, rabbits, and cats [25,59,63,186]. While this bimodal nerve fiber distribution was true across whole nerves as well as within most fascicles, I found some fascicles that exhibited a unimodal distribution with a single peak at the lower end of the size range. The reasons for this variation in distribution type between fascicles are unclear, but may reflect somatotopic organization, with nerve fibers in fascicles running to different parts of the body having different size distributions. For example, it is intriguing to speculate that the fascicles with distinctly bimodal distributions innervate distal tissue, dedicating the large and fast nerve fibers to govern behaviours that require high responsiveness (analogous to the giant neurons that coordinate escape responses in invertebrates and fish) and dedicating the small nerve fibers to preserving high resolution. However, since it is not yet known to what degree somatotopic organization occurs in more proximal peripheral nerves such as the sciatic [177], this possibility remains uncertain.

As animal size increased, the nerve fiber size distribution became more bimodal and the relative peak heights changed, with the small-fiber peak becoming higher than the large-fiber peak. This shift toward more bimodal nerve fiber size distributions in larger animals may help compensate for or prevent a trade-off between responsiveness and resolution, which has the potential to become more acute as animal size increases. Limits to the total size of a nerve constrain the size and number of nerve fibers it can contain – large animals may reserve some space in their nerves for large fast-conducting nerve fibers to be used for pathways requiring high responsiveness, and fill the remaining space with smaller slower-conducting fibers to offset decreased resolution.

3.4.4 Limitations

Although our supervised image analysis method is an improvement over existing methods, it is not without limitations. Our axon segmentation algorithm is very efficient and automatically identifies the majority of axons, but there are still quite a few false positives (background areas identified as axons) and false negatives (missed axons). This underscores the need for supervision of segmentation – the inherent variability in biological samples means that manual input, rather than a completely automated method, is required to get accurate results. Accurate tuning of parameters helps to reduce the total number of false positives and false negatives. It is important to note that the time required to re-analyze the image after each iterative adjustment is only about eight seconds, making parameter
tuning a rapid process.

Like most automated image segmentation methods, the performance of our method depends heavily on image quality. Due to variability in tissue characteristics, small but unavoidable differences in nerve processing between animals, and sample height differences in the SEM itself, images acquired from each nerve are slightly different. Differences in contrast between axons, myelin, and background are the most common, and differences in noise level are also present. This variation requires manual adjustment of parameters for each image to achieve the highest accuracy, however each adjustment can be made very quickly due to our algorithm’s speed. A potentially productive future area of research is the development of an automated method to optimize parameter values using training data, for example a small manually-segmented section of the nerve image.

Some nerve fibers appeared blurry and difficult to identify or to distinguish from one another, particularly in samples of large nerves. I attribute this to minor degradation caused during the time taken for fixative to penetrate our large samples, as well as limits to the imaging resolution of our samples with our microscope. This was not a large effect – I estimate that we may have missed identifying approximately 3% of myelinated nerve fibers due to this blurring.

Our myelin segmentation algorithm detects the majority of myelin in ideal samples and non-blurry images. However, it tends to underestimate myelin thickness in areas with cracks or blurring. In the first case, the algorithms stop labelling myelin at the onset of the crack, underestimating the myelin area; in the second case, the lack of a clear boundary between adjacent myelin sheaths means that the algorithms underestimate some myelin thicknesses and overestimate others. More sophisticated methods such as active contours [56] may help solve these problems and improve accuracy by growing a continuous smooth line outward from the axon perimeter and using criteria based on smoothness and thickness to detect when the outer myelin edge is reached.

My imaging methods only allow the detection of myelinated nerve fibers – unmyelinated nerve fibers are not visible. These nerve fibers generally have much smaller diameters than myelinated nerve fibers, and conduct impulses more slowly [165]. Therefore, I chose to focus on the analysis of larger, faster-conducting myelinated fibers as response speed is important for effective movement control.

I restricted my analysis to the sciatic nerve, and one type of sensor and effector in one muscle. Other systems also contribute to resolution – for example, the body is innervated
by many peripheral nerves, and many different sensors can provide information to increase sensing and response precision. However, a logical place to begin investigating resolution is in a pathway important to movement control – the fastest pathway is the monosynaptic stretch reflex, and the hindlimb is important for animal locomotion. I therefore chose to examine the nerve fibers, sensors, and effectors that make up this pathway. In addition, the sciatic nerve innervates a large portion of the hindlimb and reflects a relatively large fraction of the sensors and effectors in an animal; the number of nerve fibers in this nerve therefore provides a good indication of overall sensorimotor resolution.

Although my nerve fiber number and size distribution results are compelling, they are based on the analysis of relatively few nerve fibers from only one to four animals per species. In some nerve samples, I measured fewer than 1% of nerve fibers. Analyzing more fibers would decrease uncertainty in my estimates of nerve fiber number and nerve fiber size distribution in different species. However, because the scaling relationship between nerve fiber number and animal mass is already very strong, I do not expect that obtaining larger samples would change the relationship significantly. Similarly, most nerve fiber size distributions of fascicles in the same nerve had generally similar shapes and size ranges, so I do not expect that sampling more fascicles will change the trends in nerve fiber size distribution that I observed. I did find some fascicles with unimodal rather than bimodal nerve fiber size distributions, which could misrepresent the true nerve fiber size distribution if my sample comprised a non-representative fraction of unimodal fascicles relative to their prevalence in the nerve. In this case, obtaining more samples would give a more accurate result.

For both muscle spindle number and motor unit number, I was only able to find a small number of data points in animals ranging in size over only two orders of magnitude. I would ideally have obtained more data in animals spanning a wider size range, however my systematic review, as well as discussion with experts in the field, indicate that these data likely do not exist. While the number of points I found was not sufficient to determine the scaling relationship of these structures with animal mass, the values that I did find suggest that neither muscle spindle number or motor unit number increase in direct proportion to animal mass. This may indicate that larger animals have both relatively fewer sensors and relatively fewer effectors, at least in the stretch reflex.

As in Chapter 2, I did not adjust for evolutionary history when determining my nerve fiber number, muscle spindle number, and motor unit number scaling relationships. Differences in the degree to which species are phylogenetically related could affect my results, as
described in Subsection 2.4.1. However, a phylogenetically independent contrasts analysis of a subset of my nerve conduction velocity data, collected from similar species as my nerve fiber number data, indicated no significant phylogenetic signal (Appendix A), suggesting that the phylogenetic signal is not strong within the species that I studied.

3.4.5 Implications for control of movement

Nerve fiber number in the sciatic nerve increases slower than body mass, body surface area, and muscle force (Subsection 3.3.2). This results in larger animals having relatively fewer nerve fibers, and therefore fewer total sensory receptors and motor units, than smaller animals. While it is not clear whether this affects all or only some components of resolution, the decreased innervation density may present challenges for large animals to sense and respond to stimuli precisely. All else being equal, having fewer sensors and effectors results in lower resolution – if this is the case for large animals, one possibility is that they may compensate for their loss of precision by relying on internal models of their body dynamics and their environment to integrate information from multiple sensors [201]. As innervation density decreases in larger animals, they may depend more heavily upon prediction to maintain sensorimotor performance.

Larger animals may have developed more extensive lower-level techniques and higher-level models to enhance precision within the constraints of fewer sensors. Pathways of this type have been studied in humans and monkeys. For example, inhibitory networks of nerve fibers from cutaneous receptors help the sensory system combine information from receptors to distinguish between multiple stimuli [89]. Similarly, parallel pathways of ganglion cells in the retina help the visual system to enhance image features [89]. By using similar types of sensory integration, large animals could increase their sensory precision despite a relative lack of increase in sensors. Sensory precision can also be enhanced using internal models. For example, information from muscle spindles is the main method by which the body senses joint angle, despite muscle spindles not directly detecting angle [28, 43]. In this case, the body has an internal model relating the length of certain muscles to nearby joint angles. This allows the body to use one sensor for multiple purposes, effectively increasing resolution without increasing the number of sensors.

It is less clear what mechanisms larger animals may use to increase motor precision within the constraints of fewer motor units. One possibility is to rely on different levels of activation of motor units to generate varying levels of force per motor unit – this is possible
in particular for slow motor units, which modulate their force output in response to the firing rate of their associated motor nerve fiber [31].

Taken together, my observed scaling of nerve fiber number and size distribution suggest that total nerve area may increase slower than predicted by geometric similarity. Calculated as the sum of individual nerve fiber areas, total nerve area depends on nerve fiber number and nerve fiber size. My histology results show that nerve fiber size remains relatively constant (Figure 3.9), which suggests that total nerve area increases in direct proportion to nerve fiber number $M^{0.35}$ (Figure 3.7a). Geometric similarity, however, predicts that areas will increase in proportion to $M^{2/3}$ – this is much faster than the increase in total nerve area predicted by my results, meaning that large animals may have sufficient space to increase the size of their nerves. In this case, large animals could increase responsiveness by having larger nerve fibers, and/or increase resolution by having more nerve fibers. The fact that this does not occur could indicate that responsiveness and resolution are not restricted by nerve fiber size and nerve fiber number. Alternatively, or additionally, nerve fiber size and number may be constrained by factors other than the need for fast, precise sensing and response – for example, there may be limitations to cell size that make it unfavourable to have nerve fibers above a certain diameter [55]. Constraints such as this would set limits on the sensorimotor system that could affect which methods of sensorimotor control are used by animals, and how those methods of control change with animal size.

3.5 Future work

In addition to my presented data on total nerve fiber size, other characteristics such as nerve fiber shape, spatial distribution of nerve fiber sizes throughout the fascicle and nerve, and the axon/myelin ratio are readily available for output from our supervised image analysis method (for some examples, see Appendix B). This capability gives our method the potential to be useful for a wide range of applications including studies of nerve structure, function, and response to injury; in fact, it is already being used by research groups in Toronto and Berlin, and may soon be used by a group in London. To enable the use of our algorithm by even more researchers, I would like to improve its user interface and its robustness to different image types. Increasing the efficiency of my own and of other researchers’ work would be useful for both clinical and comparative studies, and may greatly enhance the understanding of nervous system organization and function.
Our myelin segmentation method based on radial scanning was less robust than we would like, and it frequently over- or under-labelled the myelin even with changes to its segmentation parameters. We recently developed a new segmentation method, which uses a quantity termed *pixel energy* to label the entire nerve fiber. Like our radial scanning method, the pixel energy method uses the segmented axons as a starting point for myelin identification. Our algorithm uses greyscale values of pixels just outside the axons to determine likely greyscale values for the myelin. For each axon, individual pixels within a given radius of the axon are given an energy value based on their greyscale value and distance from the axon. The Fast Marching algorithm [147, 171] uses the energy values of all pixels surrounding the axon to determine the likelihood that each individual pixel belongs to the axon’s surrounding myelin; this creates an image of myelin likelihood. After all the areas surrounding all the axons have been analyzed, the labelled axons are used to enforce local minima in the myelin likelihood image; the Watershed transform [124] then separates distinct nerve fibers. The resulting image of separated likelihood values is thresholded to give a labelled image of nerve fibers, which can be used in subsequent analysis. This new pixel energy algorithm performs better than our original radial scanning algorithm, and requires less manual correction; it is also robust with respect to axon shape. While we have not yet quantified its performance, it appears to be faster and easier to use than our original method.

One particularly interesting application of our supervised image analysis method is the investigation of somatotopic organization (Subsection 3.4.3). While most fascicles I analyzed had bimodal nerve fiber size distributions, with one population of small fibers and one population of large fibers, a few fascicles had unimodal nerve fiber size distributions, with a single population of small fibers. One possible reason for the two types of nerve fiber size distributions we observed is that fascicles with bimodal distribution innervate more distal parts of the body, with their large-diameter fiber population conducting nerve impulses quickly and reducing nerve conduction delay in pathways with long conduction distances. The speed of our supervised image analysis method would make it relatively easy to analyze multiple samples from different levels of sciatic nerve branches – this information could be used investigate how fiber number and size distribution change between nerves innervating more proximal parts of the body and nerves innervating more distal parts of the body. The results of this investigation would provide more insight into the organization of the nervous system, particularly in distal regions of the body which have the potential for long nerve
conduction delays.

My scaling relationships for muscle spindle and motor unit number scaling relationships are based on data from only three species each, representing only a two order of magnitude difference in mass. This lack of data prevents me from drawing any firm conclusions about how muscle spindle and motor unit number scale. Measuring muscle spindle number and motor unit number in additional species, particularly large animals such as the horse and elephant, would give a much better picture of how these values change with animal mass. In addition, expanding my analysis to include the scaling of other sensor types would result in a more complete picture of how sensory pathways are affected by animal size.

While nerve fiber size distributions can be compared between species by measuring parameters such as the locations and heights of peaks, a more rigorous comparison would help to elucidate more subtle differences. Fitting normal distributions to each of the peaks and comparing their means and standard deviations is one possibility [25], but it requires making assumptions about the number of peaks and the appropriateness of normal distributions for the data. Although the number of peaks can appear very obvious, it is often a more qualitative decision. Comparing nerve fiber size distributions would be more objective and less prone to error if a statistically sound method to compare size distributions with multiple peaks, and to determine how many distinct peaks are present, were developed.

Computational models are an intriguing avenue for further investigation of nerve fiber size distribution. Using simulations, the numbers and sizes of nerve fibers could be optimized for different constraints – for example, for a given nerve size, number of nerve fibers, and fiber size bounds, simulations could determine the optimal fiber size distribution to achieve the greatest number of large fibers. These simulations could be used to test the effects of various constraints on nerve fiber characteristics, and gain insight into some of the principles underlying the responsiveness-resolution trade-off.
Chapter 4

Conclusion

While increasing mass has advantages, my results demonstrate that it also brings challenges. I found that bigger animals have longer absolute delays than smaller animals, slightly longer relative delays, and relatively fewer sensors and effectors – this indicates that, while some aspects of responsiveness are maintained, neither responsiveness nor resolution is entirely preserved in large animals. These findings support my hypothesis. Response time in larger animals is much longer than that of smaller animals in terms of absolute time, but only twice as long when adjusted for differences in relative time between large and small animals. Big animals also have fewer total sensory receptors and motor units relative to their body mass, their body areas, and their muscle force than small animals which, all else being equal, would result in a decreased ability to precisely sense and respond to stimuli.

Large animals exist and thrive, so they must have developed mechanisms to overcome challenges to responsiveness and resolution. Rather than being at an overall disadvantage in the wild, bigger animals may need to use additional mechanisms such as prediction of their environment and body state to effectively sense stimuli and control their movement [201]. There are several possible methods by which large animals could compensate for the constraints placed upon their sensorimotor system, including integration of sensory information using lower-level neural circuitry and prediction of motor outputs using higher-level internal models of body dynamics. Internal models combine sensed information about the body’s state with knowledge of body dynamics – acquired through prior experience – to predict the consequences of movement [93, 201].
4.1 Potential implications of changes to movement control

The possibility that larger animals, such as elephants, have developed better internal models would likely result in these animals having more complex neural circuitry in their central nervous systems than smaller animals, such as shrews. Internal models require computational resources in order to integrate sensorimotor information and store learned body dynamics, which means that a greater reliance on internal models will likely be associated with an increase in neuron number [162]. The number of neurons in the brains of rodents and insectivores increases in proportion to $M^{0.5} - M^{0.7}$ [74] – almost twice as quickly as I found that nerve fiber number increases in peripheral nerves. While this relative increase in the number of neurons in the central nervous system compared to the peripheral nervous system is likely due to many factors, the related increase in central processing capability may partially reflect an increased predictive capacity of large animals. The brain structures which perform most movement planning and sensorimotor integration are the cerebral cortex and cerebellum [77, 93], and neurons in both these structures represent an increasing fraction of brain neurons in larger animals [74]. This relative increase in the number of neurons in the cortex and cerebellum may relate to increased learning and cognitive abilities with animal size [74, 108], although the difficulty in comparing cognition between species with very different abilities and ecological niches, and the difficulty of defining what determines cognitive ability or intelligence, mean that this is currently only speculation [45, 74, 88, 162]. The cerebellum has traditionally been considered as being primarily involved in motor control, however more recently it has been shown that the cerebellum also contributes to non-motor functions such as learning and language [108]. It is intriguing to speculate that the increase in number of cerebellar neurons, although perhaps originally developed to address longer sensorimotor delays and lower sensorimotor precision by improving internal models of the body, may have been co-opted by animals for other purposes – although constraints to responsiveness and resolution may create challenges for sensorimotor control in animals, the resulting solutions may have contributed to the evolution of higher brain functions.
4.2 Future work

4.2.1 Larger data set

While I was able to determine how several components of responsiveness and resolution change with animal size, there were three important aspects for which I could not find sufficient data to confidently determine a scaling relationship: information on electromechanical delay, muscle spindle number, and motor unit number was sparse, particularly in very small and very big animals. My preliminary results suggest that these components are large contributors to sensorimotor control, contributing to the potential for big animals to have longer delays and lower precision than small animals. Electromechanical delay is one of the three component delays that appear to increase total response time the most – it has a relatively large magnitude in all animals, and the data that I was able to find indicated that it may increase in larger animals. Muscle spindles sense muscle length and stretch, and initiate monosynaptic stretch reflexes as well as providing information about an animal’s body position; motor units allow one muscle to generate varying levels of force. Both these abilities are crucial in controlling body position and in detecting and responding to perturbations, and the data that I was able to find suggest that larger animals may have relatively fewer muscle spindles and motor units compared to small animals. While my research was not able to draw any firm conclusions about how electromechanical delay, muscle spindle number, and motor unit number change with animal size, it does suggest that these components are important contributors to sensorimotor control and highlights the need for more work to be done determining these values in animals with extreme sizes.

4.2.2 Computer simulations

My empirical approach allows me to determine what animals do, but does not necessarily allow me to test ideas that don’t appear in nature. For example, I determined how nerve conduction delay, total nerve fiber number, and nerve fiber size distribution change with animal size, but this information does not necessarily tell us what aspects of sensorimotor control, if any, are being optimized. Computer simulations can provide more insight into how the components of sensorimotor control affect movement, as well as insight into the trade-off between responsiveness and resolution, by allowing systematic variation of some control aspects and observing how other aspects are affected.
CHAPTER 4. CONCLUSION

Computational simulations of locomotion are widely used to investigate the mechanics and control of locomotion. These range from simple inverted pendulum models \cite{103} to complex models involving multiple muscles and degrees of freedom. Models incorporating the component delays involved in responsiveness and having the ability to change sensory and motor resolution could investigate the contributions of each of these factors to movement control. Within a model, individual components could be changed, and their effect on body movement could be assessed. For example, nerve conduction delay could be increased, while the resolution and the remaining component delays could stay the same. Or, error could be introduced into the model’s sensed information, and its effect observed. These and similar experiments could give information into which components of responsiveness and resolution are crucial for movement control, and which are less important. As animal size increases, the sensorimotor system may prioritize crucial components at the expense of less critical ones. Combining modelling results with observed changes would give a much deeper insight into the mechanisms underlying sensorimotor control.

One possibility for modelling that I find especially interesting is the trade-off between nerve fiber number and nerve fiber size in peripheral nerves. For a given nerve size animals need to balance the benefits and costs of having relatively few large nerve fibers, and therefore high responsiveness but low resolution, against the benefits and costs of having relatively more small fibers and therefore low responsiveness but high resolution (Section 1.2, page 4). The process of packing nerve fibers into nerves adds additional complexity to the trade-off between responsiveness and resolution. Nerve fibers are approximately circular in cross-section, and closely spaced similarly-sized circular items cannot pack together without leaving gaps between them – these gaps could be filled with smaller fibers, and the remaining gaps filled with even smaller fibers, and so on. These smaller fibers can be added without any increase in nerve cross-sectional area. With all of these contributing factors, it is not clear what the optimal solution is to offset the seemingly necessary decreases in nerve fiber size and number. My preliminary results on the scaling of nerve fiber size distributions suggest that larger animals have a more bimodal size distribution, supporting the idea that an increasingly acute trade-off between nerve fiber number and size results in two distinct fiber populations (Subsection 3.4.3). However, this does not give any insight into whether this is the optimal solution, or whether responsiveness or resolution (or both or neither) is being prioritized. It is next to impossible to manipulate nerve fiber size distribution in controlled experiments, so simulations are an excellent candidate to investigate the effect of
different nerve fiber size combinations on sensorimotor control. Simulations of nerve fiber packing could test various restrictions to nerve fiber size and number, and determine the optimal fiber size distribution to maximize responsiveness and resolution. This procedure could be used in combination with computational simulations of movement to investigate how anatomical and biophysical constraints to nerve properties affect sensorimotor control.

4.3 Summary

As animal size increases, sensorimotor delays increase while the relative number of nerve fibers decreases. Changes in movement time almost entirely compensate for the increase in absolute delays, resulting in similar relative delays for all sizes of animals. Despite maintenance of relative responsiveness across animal sizes, and no evidence that nerve size is constrained, larger animals still have longer absolute delays and lower innervation densities relative to smaller animals – as animal size increases, both absolute responsiveness and resolution decrease. Larger animals therefore face challenges in sensing and responding to stimuli as quickly and precisely as small animals, and may compensate by moving more slowly and relying more heavily on predictive mechanisms to control their movement.
References


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Appendix A

Phylogenetically independent contrasts analysis

To test whether the phylogenetic relationships of the mammals in our study might influence our conclusions regarding the scaling of nerve fiber conduction velocity (CV) with body mass, John Hutchinson from the Royal Veterinary College in London, England, applied phylogenetically-independent contrasts (PIC) analysis [54, 62, 65, 144] to our data set. Logarithmically-transformed values of mean body mass and conduction velocity for each species were entered into a taxon-character data matrix in Mesquite 2.6 [115] and two phylogenetic trees were constructed (Figure A.1), here termed Trees 1 and 2. Tree 1 followed the recent “Afrotheria” hypothesis placing elephants outside other placentals in our analysis and shrews as the sister group to carnivores and ungulates [10, 20]; Tree 2 followed traditional hypotheses placing elephants closer to (other) ungulates and shrews outside other placentals [110, 139, 140].

We conducted only simple analyses of PIC, setting the tree branch lengths to 1 (a “speciation” model of phylogeny) because divergence times for mammals remain highly controversial [20], our sample of 11 species was limited compared with total mammalian diversity, and our initial ordinary least squares (OLS) scaling results indicated very little or no allometry.

Using the PDAP:PDTREE module of Mesquite 2.6 [125], we first conducted diagnostic tests for correlations between the resulting standardized PIC values and their standard deviations, normal distribution of these data, and visual inspection of outliers. For conduction...
APPENDIX A. PHYLOGENETICALLY INDEPENDENT CONTRASTS

velocity, Tree 1 had a significant correlation (OLS slope=-0.136; $R^2=0.400$; Pearson Product Moment Correlation=0.633; 2-tailed t-test: df 8; $F=5.34$; p=0.0496); Tree 2 did not (OLS slope=-0.0450; $R^2=0.0295$; Pearson Product Moment Correlation=-0.172; 2-tailed t-test: df 8; $F=0.243$; p=0.635). Both trees had adequately standardized contrasts, but shrews and elephants (owing to their relatively small and large body masses respectively) were obvious outliers so their associated nodes were as well.

We then proceeded to examine the PIC values for log(body mass) vs. log(CV), with the y-axis intercept set at 0. Tree 1 again had a significant correlation and slight positive allometry (OLS slope=0.0488; $R^2=0.502$; Pearson Product Moment Correlation=0.708; $F_{1,9}=9.07$; p=0.0147); Tree 2 implied similar allometry but it did not achieve a significant relationship (OLS slope =0.0363; $R^2=0.247$; Pearson Product Moment Correlation=0.497; $F_{1,9}=2.95$; p=0.120). Major axis and reduced major axis regressions gave only trivially different results.

We inferred from these analyses that the phylogenetic signal in our data was minimal but deserved an additional test. Using REGRESSIONv2 [107], we compared our original OLS regressions (i.e. assuming a single, inclusive polytomy at the root node or “star phylogeny”), PIC-based generalized least squares (GLS) with arbitrary designation of branch lengths (all branches = 1), and GLS with branch length transformations imposed by an Ornstein-Uhlenbeck evolutionary model [21]. Akaike Information Criterion (AIC) values for the OLS regressions were lowest in all analyses, indicating that there was no significant phylogenetic signal in our data [107, 180]; OLS regressions performed superior to GLS (AIC values for Trees 1 and 2 = -23.0 and -21.0 for OLS regression vs. -21.0 to -17.5 for GLS analyses).
These analyses robustly support the OLS results presented in the main text. Finally, we checked in our AIC analysis if our elephant outlier had a significantly different conduction velocity value than other taxa; it did not (p=0.44).
Appendix B

Nerve, fiber, and fascicle geometry measurements

Our supervised segmentation method is capable of calculating many size and shape characteristics of nerve fibers. Table 3.2 shows nerve fiber numbers for the sciatic nerves of several species of animals, eight of which have been calculated using information from our method. Figure 3.9 shows total nerve fiber size distributions for each of the species we studied, all of which have been calculated using information from our method. In addition to nerve fiber number and size distribution, our method can also output many other descriptive parameters of nerve fibers. By combining nerve fiber areas with fascicle areas, which we measured separately by manually tracing fascicles in low-magnification nerve images, we can also see how nerve fiber characteristics relate to whole-nerve size. Table B.1 and Table B.2 show some preliminary statistics describing fascicle number and fascicle area, as well as total nerve fiber area, nerve fiber diameter metrics, myelin thickness, g-ratio, and nerve fiber shape in the images we analyzed. This information was calculated from the same images used to obtain the measurements in Table 3.2 and Figure 3.9, and the numbers, masses, and species of animals are the same as those reported in Table 3.2. Figure B.1 shows several of the statistics describing nerve fiber diameter, taken from Table B.2, plotted with animal mass, taken from Table 3.2.
Table B.1: Total number and area measurements for nerves, nerve fibers (Fib), and fascicles (Fasc). The number of animals of each species studied (N) is the same as reported in Table 3.2. Average measurements are calculated over all nerves analyzed. Nerve area (Nerve A) is calculated as the sum of fascicle areas, and does not include any space between fascicles. The average number of fascicles contained in a nerve (Fasc/nerve) is reported. Aggregate measurements are calculated by combining data from all fascicles analyzed (Fasc) and all fibers analyzed (Fib). Fiber area (Fib A) is the sum of all nerve fiber areas measured. Fascicle area (Fasc A) is the sum of the areas of all fascicles measured, and was measured on whole-nerve images rather than segmented images – measured areas in segmented images were consistently lower than areas reported here. Therefore, although the trend in the ratio of fiber area to fascicle area (Fib:fasc A) reported here is likely correct, each individual ratio value is probably an underestimate. All counts of discrete objects, as well as areas, are rounded to the nearest whole number. All ratios are given to two decimal places. Animal masses and average nerve fibers number are reported in Table 3.2. Values reported as X ± Y are means ± standard deviations.
<table>
<thead>
<tr>
<th>Species</th>
<th>Fiber equivalent diameter (µm)</th>
<th>Myelin thickness</th>
<th>G ratio</th>
<th>major:minor</th>
<th>p:C</th>
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<td>(µm)</td>
<td></td>
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</tr>
<tr>
<td>Shrew</td>
<td>1.3  2.5  3.8  4.2  6.9  13.4</td>
<td>0.9 ± 0.2</td>
<td>0.56 ± 0.08</td>
<td>1.44 ± 0.32</td>
<td>1.17 ± 0.14</td>
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<tr>
<td>Mouse</td>
<td>0.9  3.3  6.7  6.9  11.1  19.5</td>
<td>1.4 ± 0.3</td>
<td>0.58 ± 0.09</td>
<td>1.45 ± 0.34</td>
<td>1.17 ± 0.19</td>
</tr>
<tr>
<td>Rat</td>
<td>0.4  2.9  7.5  7.8  13.3  18.6</td>
<td>1.6 ± 0.6</td>
<td>0.57 ± 0.08</td>
<td>1.53 ± 0.39</td>
<td>1.24 ± 0.24</td>
</tr>
<tr>
<td>Cat</td>
<td>1.4  3.0  10.0 10.1 16.3  18.4</td>
<td>2.1 ± 0.9</td>
<td>0.55 ± 0.10</td>
<td>1.25 ± 0.22</td>
<td>1.05 ± 0.15</td>
</tr>
<tr>
<td>Goat</td>
<td>2.4  4.8  9.0  9.4  15.6  21.0</td>
<td>2.1 ± 0.6</td>
<td>0.53 ± 0.10</td>
<td>1.37 ± 0.26</td>
<td>1.11 ± 0.14</td>
</tr>
<tr>
<td>Horse</td>
<td>0.4  4.4  9.6  10.3 16.5  20.0</td>
<td>2.5 ± 0.8</td>
<td>0.51 ± 0.08</td>
<td>1.25 ± 0.18</td>
<td>1.07 ± 0.21</td>
</tr>
<tr>
<td>Giraffe</td>
<td>0.3  2.9  6.7  7.7  14.2  23.0</td>
<td>2.1 ± 1.0</td>
<td>0.43 ± 0.11</td>
<td>2.02 ± 0.87</td>
<td>1.20 ± 0.18</td>
</tr>
<tr>
<td>Elephant</td>
<td>0.3  2.3  4.6  6.3  13.4  21.6</td>
<td></td>
<td>1.79 ± 0.60</td>
<td>1.18 ± 0.14</td>
<td></td>
</tr>
</tbody>
</table>

Table B.2: Nerve fiber geometry measurements; this table continues Table B.1. Minimum (Min), 5th percentile (5%ile), median (Med), mean, 95th percentile (95%ile), and maximum (Max) fiber diameters are calculated over all nerve fibers analyzed, where fiber equivalent diameter is the diameter of a circle with equivalent area. Myelin thickness (My thick) is calculated by subtracting axon diameter from nerve fiber diameter and dividing by two. G ratio is calculated for each fiber as (fiber diameter - 2(myelin thickness))/fiber diameter, and is the ratio of axon diameter to total nerve fiber diameter. Fiber major and minor axes are the major and minor axes, respectively, of an ellipse with the same normalized second central moments as the nerve fiber cross-section; the mean ratio of major axis length to minor axis length (major:minor) is reported here. The ratio of nerve fiber perimeter to the circumference of a circle with equivalent area (p:C) is calculated by measuring the number of pixels on the perimeter of the fiber, converting this to µm, and dividing by \(2\sqrt{\pi A}\), where \(A\) is the area of the nerve fiber in µm. It was not possible to distinguish between axon and myelin areas in the elephant samples, therefore myelin thickness and G ratio could not be calculated. All lengths are given to one decimal place, and all ratios are given to two decimal places. Animal masses and average nerve fiber numbers are reported in Table 3.2. Values reported as X ± Y are means ± standard deviations.
Figure B.1: Ranges and medians of nerve fiber size distributions. For each of the species nerve fiber size distributions in Figure 3.9, the 5th percentile diameter (grey filled circles) gives a measure of the smallest nerve fibers, the median diameter (black open circles) gives a measure of the midrange nerve fibers, and the 95th percentile diameter (black filled circles) gives a measure of the largest nerve fibers. Animal masses are reported in Table 3.2, and nerve fiber diameter measurements are reported in Table B.2.