RESEARCH ARTICLE

Sensorimotor responsiveness and resolution in the giraffe

Heather L. More[1,*,†], Shawn M. O'Connor[1,*], Emil Brøndum[2], Tobias Wang[3], Mads F. Bertelsen[4],
Carsten Grøndahl[4], Karin Kastberg[5], Arne Hørlyck[5], Jonas Funder[6] and J. Maxwell Donelan[1]

[1]Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Canada, [2]Department of Biomedicine,
Aarhus University, Denmark, [3]Zoophysiology, Department of Biosciences, Aarhus University, Denmark, [4]Center for Zoo and
Wild Animal Health, Copenhagen Zoo, Frederiksberg, Denmark, [5]Department of Diagnostic Imaging, Aarhus University Hospital,
Skejby, Denmark and [6]Department of CardioThoracic Surgery, Aarhus University Hospital, Skejby, Denmark

*These authors contributed equally to this work
†Author for correspondence (hmore@sfu.ca)

SUMMARY

The ability of an animal to detect and respond to changes in the environment is crucial to its survival. However, two elements of sensorimotor control – the time required to respond to a stimulus (responsiveness) and the precision of stimulus detection and response production (resolution) – are inherently limited by a competition for space in peripheral nerves and muscles. These limitations only become more acute as animal size increases. In this paper, we investigated whether the physiology of giraffes has found unique solutions for maintaining sensorimotor performance in order to compensate for their extreme size. To examine responsiveness, we quantified three major sources of delay: nerve conduction delay, muscle electromechanical delay and force generation delay. To examine resolution, we quantified the number and size distribution of nerve fibers in the sciatic nerve. Rather than possessing a particularly unique sensorimotor system, we found that our measurements in giraffes were broadly comparable to size-dependent trends seen across other terrestrial mammals. Consequently, both giraffes and other large animals must contend with greater sensorimotor delays and lower innervation density in comparison to smaller animals. Because of their unconventional leg length, giraffes may experience even longer delays compared with other animals of the same mass when sensing distal stimuli. While there are certainly advantages to being tall, there appear to be challenges as well – our results suggest that giraffes are less able to precisely and accurately sense and respond to stimuli using feedback alone, particularly when moving quickly.

INTRODUCTION

As the world's tallest land mammal, the giraffe is a particularly interesting example of extreme morphology. The giraffe's height, due to its exceptionally long neck and legs, is thought to convey survival advantages, such as enabling it to reach food sources that competitors cannot (Cameron and du Toit, 2007). However, the survival of an animal also depends on its ability to detect changes in the environment and react appropriately, as when responding to a stumble or uneven terrain. A giraffe's extreme height, although helpful for feeding, may present challenges for the nervous system to control movement – for example, delays associated with conducting neural impulses down longer body segments. Here we study the neurophysiology of the giraffe hindlimb in terms of two characteristics of sensorimotor performance that we have previously termed 'responsiveness' and 'resolution' (More et al., 2010). These refer to how quickly and precisely, respectively, an animal can sense and respond to a stimulus. These two elements of sensorimotor control compete for space in peripheral nerves, and this competition only becomes more extreme as animal size increases. In this paper, we sought to understand how the physiology of giraffes – the land mammals with the longest legs (Holdrege, 2005) – manages these particularly challenging size-related sensorimotor constraints. The few previous studies of the giraffe nervous system have focused on central nervous system morphology (Badlangana et al., 2007a; Badlangana et al., 2007b), the gross morphology of the peripheral nervous system (Heinze, 1964) and the morphology of the recurrent laryngeal nerve (Harrison, 1981). This study is the first to examine the histology of giraffe hindlimb peripheral nerves and their functional characteristics. It adds to our earlier work on the scaling of nerve conduction delay (More et al., 2010) and investigates two additional delays, as well as resolution.

Responsiveness incorporates a number of neuromuscular delays. In the simplest and fastest reflexes, such as the monosynaptic stretch reflex, delays occur as a result of sensing the stimulus (sensing delay), conducting the resulting nerve impulse along sensory nerve fibers (nerve conduction delay), transferring the nerve impulse across synapses in the spinal cord (synaptic delay), conducting the impulse along motor nerve fibers (also conduction delay), transferring the nerve impulse from the motor axon to muscle fibers (neuromuscular junction delay), conducting the resulting impulse along muscle fibers and activating molecular mechanisms involved in crossbridge formation (electromechanical delay), and generating muscle force (force generation delay) (Fig. 1). The sum of these delays determines the total time required between stimulus onset and response production, known as response time – for example, the time required to detect a stumble and initiate corrective limb movement.
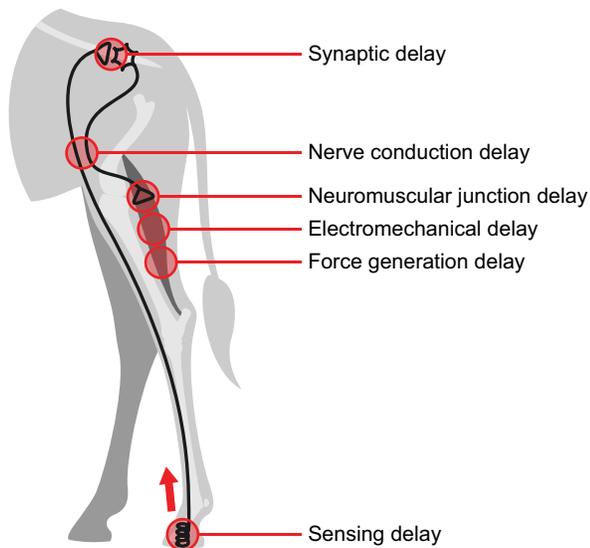
Fig. 1. Sources of sensorimotor delay. In a simple monosynaptic reflex arc, the total time between stimulus onset and force production (response time) incorporates delays due to sensing the stimulus (sensing delay), conducting the resulting nerve impulse along nerve fibers (nerve conduction delay), transferring the nerve impulse between axons in the spinal cord (synaptic delay), transferring the nerve impulse from the motor axon to muscle fibers (neuromuscular junction delay), conducting the resulting impulse along muscle fibers and activating the molecular mechanisms involved in crossbridge formation (electromechanical delay), and generating muscle force (force generation delay).

Some delays affect responsiveness more than others. For example, sensing delay, synaptic delay and neuromuscular junction delay are fairly short, on the order of milliseconds (Renshaw, 1940; Katz and Miledi, 1965; Prochazka et al., 1976; Datyner and Gage, 1980; Burke et al., 1983), whereas nerve conduction delay, electromechanical delay and force generation delay can be relatively long, on the order of tens of milliseconds (Burke et al., 1973; Raikova et al., 2007; More et al., 2010).

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, as the time taken for an impulse to be conducted down a nerve fiber depends both on the distance it must travel and the finite speed of impulse conduction. The processes that determine electromechanical delay may also depend on body size. One such process is impulse conduction along muscle fibers, which, all else being equal, will take more time for longer fibers. Although the scaling relationship between muscle fiber length and body size has not been well characterized, muscle fibers in large animals, such as giraffes, are certainly longer than the corresponding whole-muscle length in small animals, such as rats (Alexander et al., 1981; Gans et al., 1989; Loeb and Richmond, 1994). Force generation delay reflects the time between force onset and the production of peak force; it is known that the maximum velocity of muscle fiber shortening decreases with animal size (Rome et al., 1990; Toniolo et al., 2004), suggesting that it will take larger animals more time to generate peak force.

Resolution depends on the density of nerve and muscle fibers throughout the body. For a given body size, if an animal has more sensory nerve fibers it can more precisely sense the location of a stimulus; similarly, if an animal has more motor units it can generate finer gradations of muscle force within the same force range (Enoka, 1995; More et al., 2010). Therefore, like responsiveness, resolution is partially dependent on body dimensions. However, rather than directly depending on length, resolution is dependent on body dimensions that scale with higher powers of length, such as body volume. 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 will thus need eight times as many nerve fibers if each fiber is to innervate the same amount of tissue (More et al., 2010).

Animals face a trade-off between responsiveness and resolution, which becomes more acute with increasing body size. In the case of nerves, as length-related body dimensions increase, nerve conduction velocity must also increase if nerve conduction delay is to remain constant (More et al., 2010). A myelinated nerve fiber's conduction velocity is proportional to its diameter (Hursh, 1939; Rushton, 1951), so an increase in conduction velocity can only be arrived at by a concomitant increase in nerve fiber diameter. However, as body volume increases, the number of nerve fibers must also increase if resolution is to remain constant. If absolute responsiveness and resolution were maintained in very large animals as compared with small animals, the combination of more and larger diameter nerve fibers would render their nerve diameters insupportable: in our previous paper we calculated that if an elephant had the same absolute responsiveness and resolution as a shrew, its sciatic nerve would have a diameter of 30 m (More et al., 2010).

It is possible that, rather than maintaining constant absolute delays, sensorimotor speeds may only need to maintain constant relative delays by increasing at the same rate as other aspects of locomotor dynamics. Many of these characteristic aspects increase more slowly than linear animal dimensions; for example, stride period at equivalent speeds, as well as the time required to fall to the ground, increases with the square-root of linear dimensions (Heglund et al., 1974; Alexander, 2002). It is also possible that nerve fiber number may only need to increase at the same rate as animal surface area, such as in the case of cutaneous receptors (Matthews, 1972). However, even if these two scenarios occurred, total nerve cross-sectional area would still need to increase proportional to the cube of leg length. This is faster than predicted by geometric scaling and would still result in an insupportable increase in nerve diameter over an increase in size of six orders of magnitude.

The need to restrict nerve sizes to physically supportable values forces a trade-off that may limit one or both of responsiveness and resolution. Indeed, we have found in previous work that there is no notable increase in nerve conduction velocity as animal size increases, resulting in increased conduction delays in larger animals (More et al., 2010). Similarly, when we combine data from other studies (Boyd and Davey, 1968; Schnepp et al., 1971; Hashizume et al., 1988), they collectively suggest that increases in the number of nerve fibers and motor units with animal size are not sufficient to maintain resolution. This does not imply that large animals are disadvantaged in the wild, but rather suggests that they may need to use additional mechanisms such as prediction of their environment and body state to more effectively sense stimuli and control their movement (Wolpert and Ghahramani, 2000).

Here, we examined neuromuscular delays and nerve fiber number in the giraffe hindlimb as representative measurements of responsiveness and resolution in the giraffe sensorimotor system. The giraffe is especially suited for understanding compromises in sensorimotor performance – as one of the largest land mammals, it

Table 1. Physical dimensions and summary results from electrophysiology experiments and sciatic nerve sampling in eight giraffes

Subject	Mass (kg)	Height (m)	Leg length (m)	Age (months)	Nerve conduction velocity (m s^{-1})	Force onset (ms)	Force onset to peak force (ms)	Total no. fascicles	Analyzed no. nerve fibers	Estimated no. nerve fibers in sciatic nerve
1	527	—	—	33	—	12.6	44.8	—	—	—
2	445	3.3	—	33	50.0	14.5	43.6	190	3466	104,600
3	420	3.4	—	24	—	14.6	60.5	—	—	—
4	654	3.9	2.0	48	—	—	—	172	4716	96,820
5	456	3.5	1.7	36	30.4	—	—	304	3335	110,696
6	475	3.6	1.8	42	43.4	—	—	—	—	—
7	603	3.8	1.8	51	—	11.8	34.9	—	—	—
8	490	3.4	1.7	42	77.8	—	—	244	4042	114,670
Mean ± s.d.	509±82	3.6±0.2	1.8±0.1	39±9	50.4±20.0	13.4±1.4	45.9±10.7	228±59	3890±631	106,697±7778

Leg length is the sum of femur, tibia and metatarsal lengths.

has the potential for a particularly acute trade-off between responsiveness and resolution. These compromises may be even more extreme for the giraffe in the case of sensing stimuli at the foot because the giraffe has legs that are over 50% longer than predicted based on its mass [prediction from allometric equations for Bovidae (Alexander, 1979)]. For example, if a giraffe had the same number and size of nerve fibers as an average-shaped animal of the same mass, the giraffe's nerve conduction delay from the foot would be 50% greater while its relative nerve fiber number would remain the same. We sought to learn whether giraffes have patterns of neuromuscular delays and nerve fiber number similar to those of other animals, or whether they have developed a unique solution to this trade-off. 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 with measured trends. To understand responsiveness, we measured three major sources of delay (nerve conduction delay, electromechanical delay and force generation delay) using a combination of electrical stimulation, muscle activity recording and force recording. To understand resolution, we measured the number and size distribution of axons in the sciatic nerve. We then compared these values with those of other animals to understand how the giraffe's extreme size and unique limb proportions impact its sensorimotor control.

MATERIALS AND METHODS
We acquired electrophysiology data and sciatic nerve samples from eight male giraffes [*Giraffa camelopardalis* (Linnaeus 1758)] aged 2–4 years (Table 1). 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 of the 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.

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 min. 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 (20 mm internal diameter) was inserted through a tracheostomy and ancillary ventilation with oxygen was initiated using a demand valve (Burtons Medical Equipment, Marden, Kent, UK). Breathing was maintained through manual ventilation 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 α-chloralose (15 mg ml^{-1}, KVL-pharmacy, Frederiksberg, 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 h to maintain the animal within the surgical plane. We monitored the giraffe's electrocardiogram and maintained the giraffe's heart rate at 30–40 beats min^{-1}, rectal temperature at 38–39°C, end-expiratory carbon dioxide tension at ~40 mmHg and mean arterial pressure at ~150 mmHg using a portable monitor (Mindray PM9000Vet, E-Vet, Haderslev, Denmark) to ensure the giraffe remained stable. In addition, we measured blood gas values every 10 min in arterial and venous blood (GEM Premier 3500, Instrumentation Laboratory, Bedford, MA, USA). Values for pH and the partial pressures of carbon dioxide (P_{CO_2}) and oxygen (P_{O_2}) at the beginning of data collection were 7.2±0.2, 42.6±16.5 and 167±62 mmHg, respectively. Towards the end of the procedure, pH, P_{CO_2} and P_{O_2} remained within physiological limits at 7.3±0.2, 44±9 and 249±153 mmHg, respectively (means ± s.d.). Once all experiments were complete, the giraffe was euthanized with an overdose of pentobarbital.

Electrophysiology
Motor nerve conduction velocity
We acquired motor nerve conduction velocity data from four giraffes by electrically stimulating the sciatic nerve at two locations along its length. At each stimulation site, we inserted a pair of electrodes consisting of two thin insulated wires (0.012 inch diameter, AS 632, Cooner Wire, Chatsworth, CA, USA) with de-insulated (~5 mm) and hooked ends using an epidural needle as a guide (Portex Tuohy,

A

Muscle activity

10 mV

10 ms

$$\text{Conduction velocity} = \frac{\Delta d}{\Delta t}$$

B

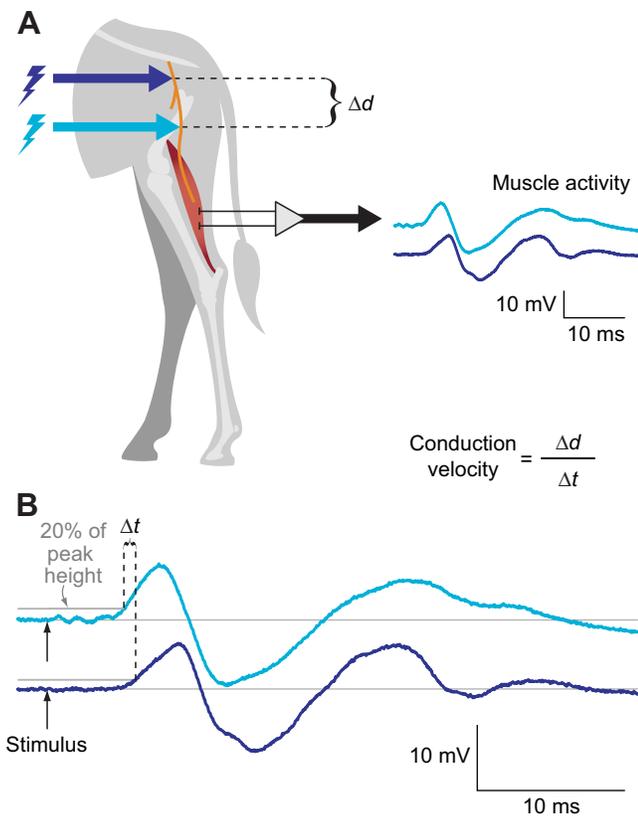20% of peak height Δt

Stimulus

10 mV

10 ms

Fig. 2. Nerve conduction velocity measurements. (A) We stimulated the sciatic nerve at two points along its length, and measured the resulting activity in the medial gastrocnemius muscle using electromyography (EMG). We measured the onset time of each recording as the time at which the EMG signal reached a value of 20% of its maximum, then determined the difference in onset times between the two recordings. Finally, we calculated motor nerve conduction velocity by dividing the measured distance between stimulating electrodes by the difference in response onset times. (B) Representative EMG recordings from one giraffe following stimulation of the sciatic nerve at proximal and distal locations.

11 cm long, 1.3 mm outside diameter; Smiths Medical International, 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, Haifa, Israel), 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 to 8.5 cm, and were measured on images collected by ultrasound. A stimulator (SD9, Grass Technologies, West Warwick, RI, USA) 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 (Fig. 2A). We chose a stimulus strength that resulted in the smallest recognizable electromyography (EMG) signal and collected 11 consecutive EMG recordings at 25 kHz from the medial gastrocnemius muscle using pre-amplified surface EMG electrodes (Delsys, Boston, MA, USA) placed on the skin over the muscle and oriented perpendicular to the muscle fibers. 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 switched 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 artifacts 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 (Tracey and Krishnamachari, 2006). 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 axons 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 yield motor conduction velocity.

Muscle activation and force generation

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 2.54-cm-long hypodermic needles (21 G) mounted 1 cm apart on a semi-flexible aluminum plate and inserted into the muscle (Wardle et al., 1989). Prior to insertion, we threaded two thin insulated wires (0.03 cm diameter, AS 632, Cooner Wire) with de-insulated (~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, Stamford, CT, USA) 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, USA) 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 (Burke et al., 1973; Raikova et al., 2007).

We inserted the needles of our device into the belly of the giraffe medial gastrocnemius muscle at an angle of ~35 deg to the central tendon, following the direction of pennation. A stimulator (SD9, Grass Technologies) 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 (Fig. 3A). We chose the smallest stimulus strength that resulted in clearly identifiable deflections in the force recordings and collected 11 consecutive force profile recordings at 25 kHz on a laptop computer.

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 point where 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. Similarly, we measured the time between the onset of force production and peak force generation to give the time required for force generation.

Histology

After euthanasia, we collected one sciatic nerve sample from the left hindlimb of four giraffes for estimation of axon number and size distribution. Immediately after each sample was removed, we immersed it in 4% paraformaldehyde and 1% glutaraldehyde in 0.1 mol l^{-1} phosphate buffer and refrigerated it until further
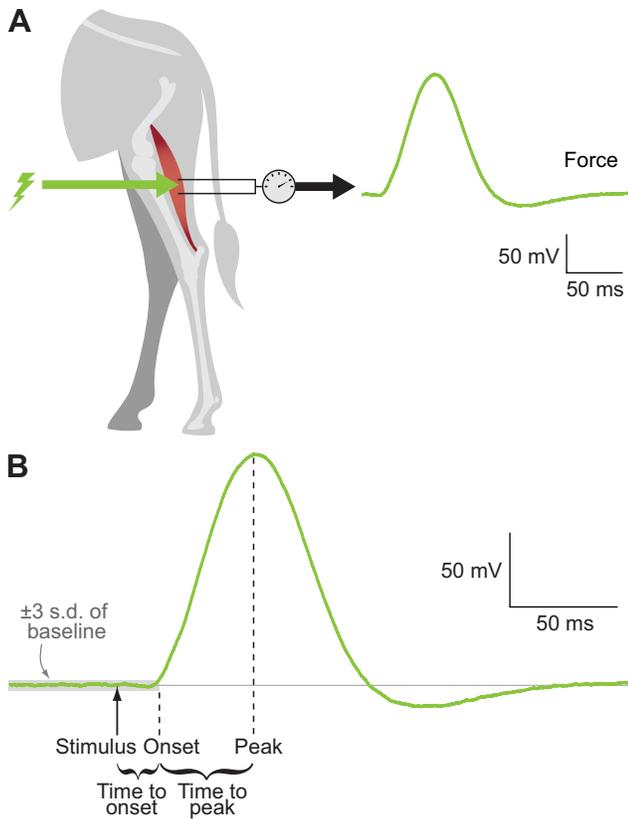
Fig. 3. Muscle force measurements. (A) We directly stimulated the medial gastrocnemius muscle and measured the force profile of the resulting muscle twitch using a force transducer inserted into the muscle between the stimulation electrodes. We determined the onset time of muscle force as the time at which the force signal reached a value of three standard deviations above the baseline noise, calculated over 100 ms before the stimulus. Finally, we calculated electromechanical delay as the time between muscle stimulation and force onset, and force generation delay as the time between force onset and the production of peak muscle force. (B) Representative force recording from one giraffe following direct stimulation of the muscle.
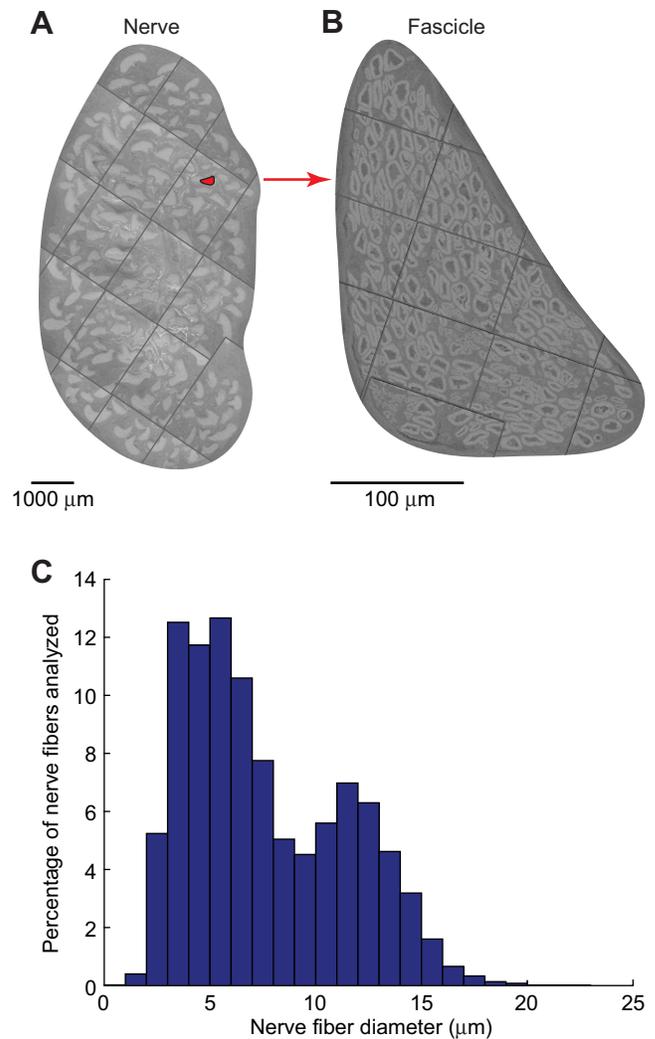

Fig. 4. Nerve fiber number and size measurements. (A) Giraffe sciatic nerve samples were imaged using a scanning electron microscope. Many small images were combined to show the entire nerve cross-section; a representative nerve is shown here, with individual images separated by dark grey lines. (B) We randomly selected seven fascicles for detailed analysis, and obtained high-resolution images of each one by combining many smaller images; a representative fascicle, highlighted in red in A, is shown here, with individual images separated by dark grey lines. On each fascicle image, we measured the size of each nerve fiber and expressed it as the diameter of a circle with equivalent area. In total, we analyzed 28 fascicles from four sciatic nerve samples. (C) A histogram showing the sizes of all 15,559 fibers measured.

processing. We stained the fixed nerves with 2% osmium tetroxide, and then dehydrated them in ascending grades of ethanol. We embedded the dehydrated nerves in plastic, polished transverse sections of each nerve and secured the nerves to stubs that were then coated with carbon in preparation for scanning electron microscopy. A scanning electron microscope (Strata DualBeam 235, FEI Company, Hillsboro, OR, USA) imaged the nerves at a magnification of ~×50 using a secondary electron detector. It was necessary for us to take multiple images to cover the entire cross-section of one nerve; the images were stitched together using a custom-written MATLAB program (R 2007a, The MathWorks, Natick, MA, USA) to give mosaics showing the whole nerve (More et al., 2011) (Fig. 4A).

Because of the large number of fascicles in the nerves, we were not able to analyze each fascicle in detail. We therefore used established random sampling methods to select a subset of fascicles from each nerve for further analysis (Geuna et al., 2000). In order to obtain a sufficient sample of the total fibers within a nerve (Bronson et al., 1978; Mayhew and Sharma, 1984; Larsen, 1998) we selected seven fascicles from each nerve. The scanning electron microscope imaged each selected fascicle at a magnification of

~×1500 using a backscatter detector, and the resulting images were stitched together into mosaics showing one fascicle each (Fig. 4B).

Custom-written and previously validated MATLAB software (More et al., 2011) automatically identified each myelinated axon in the fascicle mosaics. After we manually corrected any misidentified axons, the software automatically identified the myelin surrounding each axon and combined it with the axon area to determine the area of the nerve fiber. We expressed nerve fiber area as the diameter of a circle with equivalent area (Karnes et al., 1977). Finally, we estimated the total number of nerve fibers for each nerve by calculating the number of nerve fibers in the seven 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. We averaged the number of nerve fibers over all four giraffes to give representative data for the animals used in our study.

RESULTS

We quantified three major sources of sensorimotor delay related to responsiveness – nerve conduction delay, electromechanical delay and force generation delay – and estimated the numbers and sizes of axons in the sciatic nerve as a measure of resolution. A summary of our results is given in Table 1. Unless otherwise noted, all results reported in the text are given as means ± s.d.

We measured motor nerve conduction velocity in four giraffes by electrically stimulating the sciatic nerve at two points and recording the resulting muscle activity in the medial gastrocnemius muscle. Fig. 2B shows representative EMG recordings from one animal. The average nerve conduction velocity over all four giraffes was $50.4 \pm 20.0 \, \text{m s}^{-1}$. While the most direct estimates of nerve conduction velocity are arrived at by comparing evoked potentials from two stimulating sites, estimates are also possible using only one stimulation site and measuring the nerve path length from stimulation site to recording electrodes relative to the time from stimulation to evoked potential. Considering estimates of conduction velocity using the latter method allowed us to estimate nerve conduction velocity including two additional giraffes where we were only able to successfully stimulate the nerve at one site. Using single-site estimates gave very comparable nerve conduction velocities of $52.0 \pm 14.0 \, \text{m s}^{-1}$, based on our observed EMG onset times of ~6 ms and taking into account neuromuscular junction delay.

We measured electromechanical and force generation delays in four animals by electrically stimulating the medial gastrocnemius muscle directly and recording the force produced between the stimulation sites. Fig. 3B shows a representative force recording from one animal, as well as the two delays that we measured. On average, electromechanical delay as determined by the time from stimulus to the onset of force production was $13.4 \pm 1.4 \, \text{ms}$, and force generation delay as determined by the time from force onset to peak force was $45.9 \pm 10.7 \, \text{ms}$.

We measured a total of 15,559 nerve fibers from 28 fascicles in the sciatic nerves of four giraffes. Images showing a typical nerve and fascicle are shown in Fig. 4A and 4B, respectively. On average, we analyzed 3890 ± 631 fibers per nerve. A histogram showing the sizes of all fibers measured is shown in Fig. 4C, and demonstrates a markedly bimodal distribution with peaks at 5 and 12 μm. The smallest and largest fibers had diameters of 0.3 and 23 μm, respectively. The bimodal size distribution was often strikingly apparent within individual fascicles, with clear populations of large and small nerve fibers but very few with mid-range sizes. Approximately one-third of the fascicles had unimodal and positively skewed nerve fiber size distributions, with peaks at 4–5 μm. While the number of fascicles per nerve varied (228 ± 59 fascicles per nerve) the size of the fascicles was even more variable, with each fascicle containing between 125 and 1250 nerve fibers (mean=556 ± 305 nerve fibers). However, taking fascicle area into account and extrapolating our sampled fiber counts to whole-nerve values resulted in a relatively consistent estimate of $106,697 \pm 7778$ nerve fibers in the sciatic nerve.

DISCUSSION

Our results show that the nerve conduction velocity of the giraffe is similar to that of other mammals with similar mass. Based on previous comparisons of nerve conduction velocities across a range of animal sizes, an animal of the giraffe's mass is expected to have a maximum nerve conduction velocity of $83 \pm 13 \, \text{m s}^{-1}$ (mean ± s.e.m.) (More et al., 2010). This expected value is within the variability of our measured data ($50.4 \pm 20.0 \, \text{m s}^{-1}$). One can also roughly estimate maximum nerve conduction velocity based on established correlations with nerve fiber diameter. In myelinated fibers, conduction velocity is approximately $6 \, \text{m s}^{-1}$ faster per micrometer gained in fiber diameter (Hursh, 1939). Averaging the 95th percentile fiber diameter for each of the four animals in which fiber diameter were measured yields a mean of $14.2 \pm 0.9 \, \mu\text{m}$, and thus an estimated maximum nerve conduction velocity of $85 \, \text{m s}^{-1}$ – a value higher than our measured conduction velocities, but almost the same as that predicted based on trends seen in other animals. We suspect the difference between our measured and estimated values is due to testing only the conduction velocity of nerve fibres supplying the medial gastrocnemius, as well as the large size of the giraffe sciatic nerve resulting in only a subpopulation of the nerve's axons being depolarized past their threshold for generating action potentials. Irrespective of whether the best estimate of conduction velocity is determined from our electrophysiology or histology measurements, the giraffe's conduction velocity, like that of other large animals, is not sufficiently high to maintain nerve conduction delay relative to smaller animals. For example, in order to have the same absolute delays as a rat, which has an average nerve conduction velocity of $59.4 \, \text{m s}^{-1}$ (More et al., 2010), the giraffe would require a nerve conduction velocity of $650 \, \text{m s}^{-1}$ and therefore fiber diameters that were almost eight times the largest we measured (based on the 95th percentile diameter). Even if nerve conduction delays scaled with movement time, to have the same relative delay as a rat, the giraffe would still require a nerve conduction velocity of $200 \, \text{m s}^{-1}$, and nerve fiber diameters that were almost 2.5 times the largest we measured.

Our measured nerve conduction velocity is from motor rather than sensory nerve fibers. It is possible to measure sensory nerve conduction velocity by electrically stimulating sensory nerve fibers, which in turn activate motor nerve fibers and cause muscle contraction – this is termed the Hoffmann reflex (H-reflex) (Misiaszek, 2003). Although we tried to make these measurements in eight giraffes, we were largely unsuccessful. This could have been due to a number of reasons, including lack of background muscle activity in the anesthetized animals or reflex suppression due to anesthetic. Fortunately, we were able to generate H-reflexes in one giraffe. In this animal, we evoked H-reflexes from each of the stimulation electrodes and divided the distance between the stimulation sites by the difference in onset timing of the H-wave to yield sensory conduction velocity (Misiaszek, 2003). From these measures, our best estimate of sensory conduction velocity was $56.3 \, \text{m s}^{-1}$, which is very close to the giraffe's motor conduction velocity. This estimation may suggest that sensory conduction velocity is not appreciably larger than motor conduction velocity in the giraffe, a conclusion that would be consistent with the similarity of sensory and motor conduction velocities measured over several species (Stanley, 1981; More et al., 2010).

The most massive giraffes and elephants have leg lengths of over 2 m – the longest of any living animal (Holdrege, 2005; Hutchinson, 2006). Consequently, among the longest nerve fibers in terrestrial mammals are those originating from the most distal sensors in giraffe legs, such as cutaneous receptors in the feet. We estimate a total conduction distance of 2.3 m for these sensory fibers based on our leg length measurements (1.8 m; Table 1) and our estimated nerve length between the femoral head and spinal cord (0.5 m). Using our measured motor conduction velocity as an approximation of sensory conduction velocity, we estimate that the conduction delay between
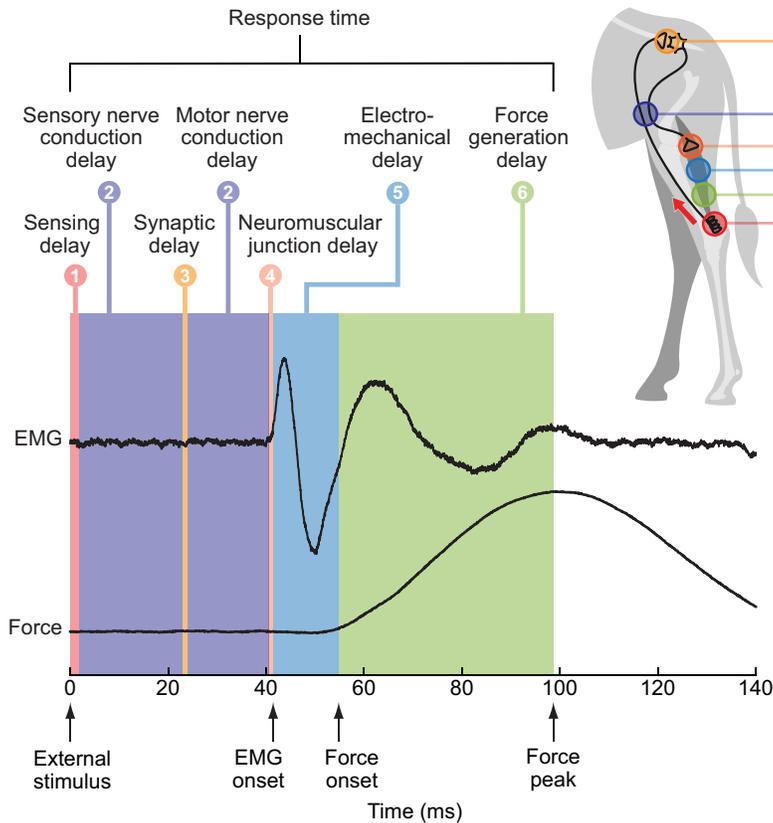
Fig. 5. Components of response time. The time required between an external stimulus and force production encompasses several sources of delay, here illustrated conceptually by combining our experimental results with data from the literature. We estimated values for sensing, synaptic and neuromuscular junction delays, and combined them with our measured values for conduction, electromechanical and force generation delays in the giraffe. 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 electromyography (EMG) activity and force. Sensing, synaptic and neuromuscular junction delays contribute relatively little (a total of 4%) to the total response time in a large animal such as the giraffe, whereas 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.

the foot and the spinal cord would be 46 ms. Higher conduction velocities, such as our estimated maximum nerve conduction velocity of 85 m s^{-1}, would reduce this delay, whereas longer leg lengths, as would typically be found in giraffes older than those we tested, would increase the delay. Our estimated conduction delay, derived entirely from our measurements, lies in the middle of these two extremes. Because giraffes have relatively long legs and similar nerve conduction velocities compared with other animals of similar mass, conduction delay appears to be longer in giraffes than in any other terrestrial mammal.

Our results also indicate that electromechanical delay and force generation delay are longer in giraffes than in smaller animals. Though the shape of the force response curve in the giraffe was very similar to the shape observed in the rat during our force measurement pilot experiments, as well as those reported in other studies (Burke et al., 1973; Raikova et al., 2007), the measured delays were longer. We measured an electromechanical delay that is approximately three times the length of the 4.5 ms delay observed in the rat. Although the scaling relationship for electromechanical delay is not yet known, this finding suggests that it may be longer in larger animals. Furthermore, the measured force generation delay is approximately 46% larger than the 31.5 ms delay observed in the rat, suggesting that force generation delay also increases with animal size. This is consistent with measured decreases in muscle fiber shortening velocity with increasing animal size (Rome et al., 1990; Toniolo et al., 2004). These size comparisons should be taken as tentative until these measurements are made using consistent methodology in animals spanning a wide size range.

Our measured sensorimotor delays allow us to estimate how quickly a giraffe can respond to changes in its environment, such as uneven terrain. For example, if we consider a discrete external stimulus such as a tendon tap on the medial gastrocnemius tendon,

which elicits a simple reflex arc, we estimate that the giraffe would have a total response time of ~100 ms. A total of ~4% of this time would be taken up by sensing, synaptic and neuromuscular junction delays, while 16% would comprise motor nerve conduction delay, 22% would comprise sensory nerve conduction delay, 13% would comprise electromechanical delay and the final ~45% would comprise force generation delay (Fig. 5). This time delay is not insignificant as it is almost half of the ~210 ms stance phase of the giraffe's galloping gait, calculated from their average observed stride frequency and average duty factor (Alexander et al., 1977). Given the very long necks of giraffes, it is interesting to estimate how long it will take for a signal to reach the brain. To do so, we assumed that the conduction velocity for corticospinal tract neurons is similar to what we have measured for peripheral nerve conduction velocity – an assumption that appears to hold for humans and some other animals (Elger et al., 1977; Janzen et al., 1977; Boyd et al., 1986; Koh and Eyre, 1988). In the giraffes studied, the distance from the lumbar spinal cord to brain was 1.8 m, equating to a conduction delay of ~58 ms between stimulation of the tendon receptors and arrival of the resulting signal at the brain. Total conduction delay from the tendon receptors to the brain (a distance of ~2.9 m in the giraffes studied) and back to the muscle (a distance of ~2.6 m in the giraffes studied) would take 109 ms, which is over half of the stance phase during galloping. Because other sources of sensorimotor delay would only serve to increase response time, this suggests that giraffes must rely on spinal reflexes, rather than long-loop or other brain-mediated reflexes, to respond to stimuli within the time their foot is on the ground.

While the scaling relationship for nerve fiber number is not yet known, our estimate of the total number of nerve fibers in the giraffe's sciatic nerve is comparable with trends seen across other

mammals. One study reports that myelinated nerve fibers in five mammalian species increase from approximately 4000 to 23,500 over the 250-fold increase in mass from mouse to dog (Schnepp et al., 1971). If giraffes followed this trend, we would expect them to have just over 100,000 nerve fibers – a number fairly close to what we estimate in this study. Still, the giraffe and other large animals seem constrained to have a low resolution relative to smaller animals because these measured increases in nerve fiber number with body size do not scale with higher powers of body lengths, such as body surface area or volume. In order for the giraffe to have the same number of nerve fibers per unit mass as a smaller animal, such as a rat (More et al., 2011), it would need to have over 5.6 million myelinated nerve fibers in its sciatic nerve – over 50 times more than what we observed. If the scaling of nerve fiber number was relative to body surface area rather than mass, the number of myelinated nerve fibers required by the giraffe would be over 0.65 million, which is still over six times more than we observed. The clearly bimodal size distribution of myelinated nerve fibers in the giraffe nerve is strikingly similar to that found in nerves of humans and other animals (Gasser and Grundfest, 1939; Bronson et al., 1978; Torch et al., 1989). While this bimodal nerve fiber distribution is true across the whole nerve as well as within most fascicles, some fascicles exhibit 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 behaviors 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, as it is not yet known to what degree somatotopic organization occurs in more proximal peripheral nerves such as the sciatic nerve (Stewart, 2003), this possibility remains uncertain.

There were several limitations to this study in addition to the previously mentioned effects of anesthesia. First, we performed all muscle measurements on the medial gastrocnemius muscle, which is located relatively close to the giraffe's torso. Aware of this limitation, we 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. Second, when measuring images of nerve fibers, we found that small fibers were often blurry and difficult to identify or distinguish from one another. We attribute this to minor degradation caused during the time taken for fixative to penetrate our large samples, as well as to limits of the imaging resolution of our samples with our microscope. This was not a large effect – we estimate that we may have missed identifying ~3% of myelinated nerve fibers because of this blurring.

In summary, we found that nerve conduction velocity, electromechanical delay, force generation delay and nerve fiber number in giraffes are broadly comparable to those of other animals based on measured trends with size. In comparison to smaller animals, both giraffes and other large animals must contend not only with relatively long sensorimotor delays, but also with more limited sensorimotor resolution. Because of their unconventional leg length, giraffes may experience even longer delays than other animals of the same mass when sensing distal cutaneous stimuli. Although increasing mass or height must certainly have its advantages, our results demonstrate that both these changes bring challenges.

Giraffes are less able to precisely and accurately sense and respond to stimuli using feedback alone, particularly when moving quickly. This suggests that giraffes may require additional compensatory mechanisms, such as sensorimotor prediction, for more effective sensorimotor control.

REFERENCES

Alexander, R. M. (2002). Stability and manoeuvrability of terrestrial vertebrates. *Integr. Comp. Biol.* **42**, 158-164.

Alexander, R. M., Langman, V. A. and Jayes, A. S. (1977). Fast locomotion of some African ungulates. *J. Zool.* **183**, 291-300.

Alexander, R. M., Jayes, A. S., Maloiy, G. M. O. and Wathuta, E. M. (1979). Allometry of the limb bones of mammals from shrews (*Sorex*) to elephant (*Loxodonta*). *J. Zool.* **189**, 305-314.

Alexander, R. M., Jayes, A. S., Maloiy, G. M. O. and Wathuta, E. M. (1981). Allometry of the leg muscles of mammals. *J. Zool.* **194**, 539-552.

Badlangana, N. L., Bhagwandin, A., Fuxe, K. and Manger, P. R. (2007a). Distribution and morphology of putative catecholaminergic and serotonergic neurons in the medulla oblongata of a sub-adult giraffe, *Giraffa camelopardalis. J. Chem. Neuroanat.* **34**, 69-79.

Badlangana, N. L., Bhagwandin, A., Fuxe, K. and Manger, P. R. (2007b). Observations on the giraffe central nervous system related to the corticospinal tract, motor cortex and spinal cord: what difference does a long neck make? *Neuroscience* **148**, 522-534.

Boyd, I. A. and Davey, M. R. (1968). *Composition of Peripheral Nerves.* Edinburgh: E and S Livingstone.

Boyd, S. G., Rothwell, J. C., Cowan, J. M., Webb, P. J., Morley, T., Asselman, P. and Marsden, C. D. (1986). A method of monitoring function in corticospinal pathways during scoliosis surgery with a note on motor conduction velocities. *J. Neurol. Neurosurg. Psychiatry* **49**, 251-257.

Bronson, R. T., Bishop, Y. and Hedley-Whyte, E. T. (1978). A contribution to the electron microscopic morphometric analysis of peripheral nerve. *J. Comp. Neurol.* **178**, 177-185.

Burke, R. E., Levine, D. N., Tsairis, P. and Zajac, F. E., III (1973). Physiological types and histochemical profiles in motor units of the cat gastrocnemius. *J. Physiol.* **234**, 723-748.

Burke, D., Gandevia, S. C. and McKeon, B. (1983). The afferent volleys responsible for spinal proprioceptive reflexes in man. *J. Physiol.* **339**, 535-552.

Cameron, E. Z. and du Toit, J. T. (2007). Winning by a neck: tall giraffes avoid competing with shorter browsers. *Am. Nat.* **169**, 130-135.

Datyner, N. B. and Gage, P. W. (1980). Phasic secretion of acetylcholine at a mammalian neuromuscular junction. *J. Physiol.* **303**, 299-314.

Elger, C. E., Speckmann, E. J., Caspers, H. and Janzen, R. W. (1977). Cortico-spinal connections in the rat. I. Monosynaptic and polysynaptic responses of cervical motoneurons to epicortical stimulation. *Exp. Brain Res.* **28**, 385-404.

Enoka, R. M. (1995). Morphological features and activation patterns of motor units. *J. Clin. Neurophysiol.* **12**, 538-559.

Gans, C., Loeb, G. E. and Vree, F. D. (1989). Architecture and consequent physiological properties of the semitendinosus muscle in domestic goats. *J. Morphol.* **199**, 287-297.

Gasser, H. S. and Grundfest, H. (1939). Axon diameters in relation to the spike dimensions and the conduction velocity in mammalian A fibers. *Am. J. Physiol.* **127**, 393-414.

Geuna, S., Tos, P., Battiston, B. and Guglielmone, R. (2000). Verification of the two-dimensional disector, a method for the unbiased estimation of density and number of myelinated nerve fibers in peripheral nerves. *Ann. Anat.* **182**, 23-34.

Harrison, D. F. N. (1981). Fibre size frequency in the recurrent laryngeal nerves of man and giraffe. *Acta Otolaryngol.* **91**, 383-389.

Hashizume, K., Kanda, K. and Burke, R. E. (1988). Medial gastrocnemius motor nucleus in the rat: age-related changes in the number and size of motoneurons. *J. Comp. Neurol.* **269**, 425-430.

Heglund, N. C., Taylor, C. R. and McMahon, T. A. (1974). Scaling stride frequency and gait to animal size: mice to horses. *Science* **186**, 1112-1113.

Heinze, W. (1964). Die Muskulatur der Hintergliedmaße von *Giraffa camelopardalis angoliensis* mit einigen Hinweisen zur Blutgefäß- und Nervenversorgung. *Anat. Anz.* **115**, 476-496.

Holdrege, C. (2005). *The Giraffe's Long Neck: From Evolutionary Fable to Whole Organism*. New York: The Nature Institute.

Hursh, J. B. (1939). Conduction velocity and diameter of nerve fibers. *Am. J. Physiol.* **127**, 131-139.

Hutchinson, J. R., Schwerda, D., Famini, D. J., Dale, R. H. I., Fischer, M. S. and Kram, R. (2006). The locomotor kinematics of Asian and African elephants: changes with speed and size. *J. Exp. Biol.* **209**, 3812-3827.

Janzen, R. W., Speckmann, E. J., Caspers, H. and Elger, C. E. (1977). Cortico-spinal connections in the rat. II. Oligosynaptic and polysynaptic responses of lumbar motoneurons to epicortical stimulation. *Exp. Brain Res.* **28**, 405-420.

Karnes, J., Robb, R., O'Brien, P. C., Lambert, E. H. and Dyck, P. J. (1977). Computerized image recognition for morphometry of nerve attribute of shape of sampled transverse sections of myelinated fibers which best estimates their average diameter. *J. Neurol. Sci.* **34**, 43-51.

Katz, B. and Miledi, R. (1965). The measurement of synaptic delay, and the time course of acetylcholine release at the neuromuscular junction. *Proc. R. Soc. Lond. B* **161**, 483-495.

Koh, T. H. and Eyre, J. A. (1988). Maturation of corticospinal tracts assessed by electromagnetic stimulation of the motor cortex. *Arch. Dis. Child.* **63**, 1347-1352.

Larsen, J. O. (1998). Stereology of nerve cross sections. *J. Neurosci. Methods* **85**, 107-118.

Loeb, G. E. and Richmond, F. J. R. (1994). Architectural features of multiarticular muscles. *Hum. Mov. Sci.* **13**, 545-556.

Matthews, P. (1972). *Mammalian Muscle Receptors and Their Central Actions*. London: Edward Arnold Ltd.

Mayhew, T. M. and Sharma, A. K. (1984). Sampling schemes for estimating nerve fibre size. I. Methods for nerve trunks of mixed fascicularity. *J. Anat.* **139**, 45-58.

Misiaszek, J. E. (2003). The H-reflex as a tool in neurophysiology: its limitations and uses in understanding nervous system function. *Muscle Nerve* **28**, 144-160.

More, H. L., Hutchinson, J. R., Collins, D. F., Weber, D. J., Aung, S. K. and Donelan, J. M. (2010). Scaling of sensorimotor control in terrestrial mammals. *Proc. Biol. Sci.* **277**, 3563-3568.

More, H. L., Chen, J., Gibson, E., Donelan, J. M. and Beg, M. F. (2011). A semi-automated method for identifying and measuring myelinated nerve fibers in scanning electron microscope images. *J. Neurosci. Methods* **201**, 149-158.

Prochazka, A., Westerman, R. A. and Ziccone, S. P. (1976). Discharges of single hindlimb afferents in the freely moving cat. *J. Neurophysiol.* **39**, 1090-1104.

Raikova, R., Krutki, P., Aladjov, H. and Celichowski, J. (2007). Variability of the twitch parameters of the rat medial gastrocnemius motor units – experimental and modeling study. *Comput. Biol. Med.* **37**, 1572-1581.

Renshaw, B. (1940). Activity in the simplest spinal reflex pathways. *J. Neurophysiol.* **3**, 373-387.

Rome, L. C., Sosnicki, A. A. and Goble, D. O. (1990). Maximum velocity of shortening of three fibre types from horse soleus muscle: implications for scaling with body size. *J. Physiol.* **431**, 173-185.

Rushton, W. A. (1951). A theory of the effects of fibre size in medullated nerve. *J. Physiol.* **115**, 101-122.

Schnepp, G., Schnepp, P. and Spaan, G. (1971). Analysis of peripheral nerve fibres in animals of different body size. I. Total fibre count, fibre size, and nerve conduction velocity. *Z. Zellforsch. Mikrosk. Anat.* **119**, 77-98.

Stanley, E. F. (1981). Sensory and motor nerve conduction velocities and the latency of the H reflex during growth of the rat. *Exp. Neurol.* **71**, 497-506.

Stewart, J. D. (2003). Peripheral nerve fascicles: anatomy and clinical relevance. *Muscle Nerve* **28**, 525-541.

Toniolo, L., Patruno, M., Maccatrozzo, L., Pellegrino, M. A., Canepari, M., Rossi, R., D'Antona, G., Bottinelli, R., Reggiani, C. and Mascarello, F. (2004). Fast fibres in a large animal: fibre types, contractile properties and myosin expression in pig skeletal muscles. *J. Exp. Biol.* **207**, 1875-1886.

Torch, S., Usson, Y. and Saxod, R. (1989). Automated morphometric study of human peripheral nerves by image analysis. *Pathol. Res. Pract.* **185**, 567-571.

Tracey, B. H. and Krishnamachari, S. (2006). Automated removal of stimulus artifact in nerve conduction studies. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* **1**, 6360-6363.

Wardle, C. S., Videler, J. J., Arimoto, T., Franco, J. M. and He, P. (1989). The muscle twitch and the maximum swimming speed of giant bluefin tuna, *Thunnus thynnus* L. *J. Fish Biol.* **35**, 129-137.

Wolpert, D. M. and Ghahramani, Z. (2000). Computational principles of movement neuroscience. *Nat. Neurosci.* **3**, 1212-1217.