Location and 3D Reconstruction of Motoneurons Innervating Gastrocnemius Medialis and Tibialis Anterior in the Rat

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Introduction & Objectives

Incomplete spinal cord injury (SCI) can alter locomotor output, not just due to interruption in supraspinal-spinal interaction, but also to alterations in spinal circuitry below the level of injury arising as a result of activity-dependent plasticity.

Spinal motoneurons are the "final common pathway" of motor control—ultimately determining locomotor output—and are known to undergo significant morphological and electrophysiological changes following SCI (1,3). The ultimate effects of these changes on locomotion are poorly understood.

Also, computational simulations of motoneurons and related locomotor spinal circuitry that can account for the changes in locomotor output seen after SCI could be a useful tool in exploring the activity-dependent plasticity of the spinal cord. Realistic computational simulations of spinal circuitry will require quantitative information as to the number and morphology of spinal motoneurons and spinal motoneuron pools with and without SCI but detailed distribution of motoneuronal pools and quantitative properties of the morphology is only partially available (1,2,3).

Objective of this study: To characterize the number, location, and morphology of spinal motoneurons of an ankle flexor (tibialis anterior), ankle extensor (gastrocnemius medialis), hip flexor (iliacus), and hip extensor (biceps femoris) in the uninjured rat.

Long term objective: to better understand the role of morphological changes in activity-dependent alterations of locomotor function after SCI (i.e. understand how form affects function)

Methods

Spinal motoneurons were retrogradely labeled via injection of fluorescein-conjugated cholera toxin β subunit (CTβ) 0.1% aqueous solution into the selected muscles: Alexafluor 594 "red" for **iliacus (IL)** and **tibialis anterior (TA)**, Alexafluor 488 "green" for **biceps femoris (BF)** and **gastrocnemius medialis (GM).**

Animals: Female adult (250-300g) Long Evans rats (n=5) anesthetized with 2% isoflurane gas.

CTβ Injection Procedure: Muscles were surgically exposed and their motor points determined with a neurostimulator. 5 μL of CTβ solution was injected into the motor point slowly over 5 minutes, and the needle was left in place for 5 minutes following the injection. Cyanoacrylate was used to seal the injection site and the incisions were sutured closed.

Spinal Cord Harvesting: 72 to 84 hours after CTβ injection, under deep anesthesia (40 mg/kg sodium pentobarbitol) animals underwent transcardiac perfusion with 0.1 M phosphate-buffered saline, followed by 4% paraformaldehyde (PFA). The spinal cords were removed and post-fixed in PFA for 24 hours, then transferred to a 30% sucrose solution for 24 to 48 hours. The cords were embedded and frozen for sectioning.

Histology: Each cord was transversely cut in 40 μm sections using a cryostat. The sections were coverslipped using an anti-fade material. The sections were examined using a fluorescent microscope and reconstructions of individual motoneurons and the motoneuron pools were completed using Neurolucida[TM] software.

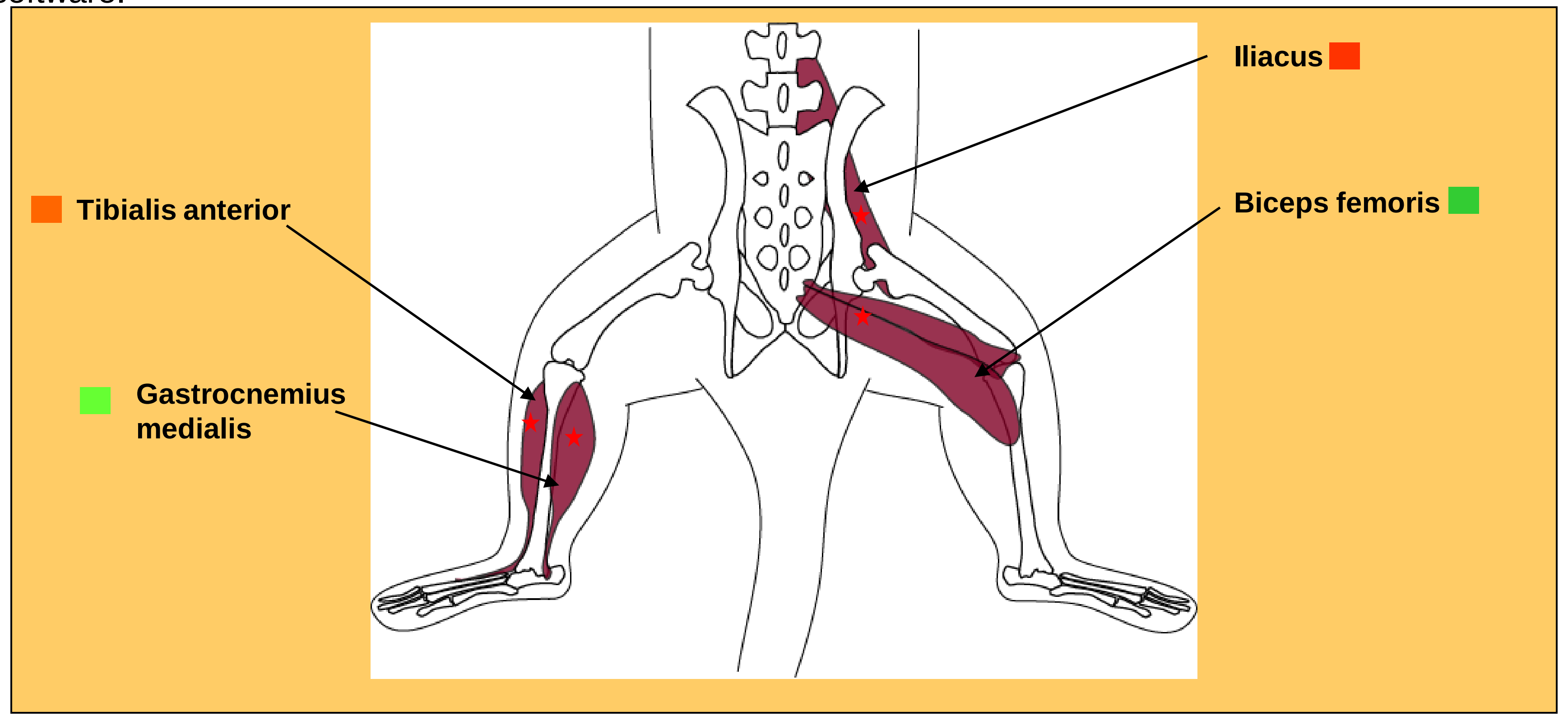
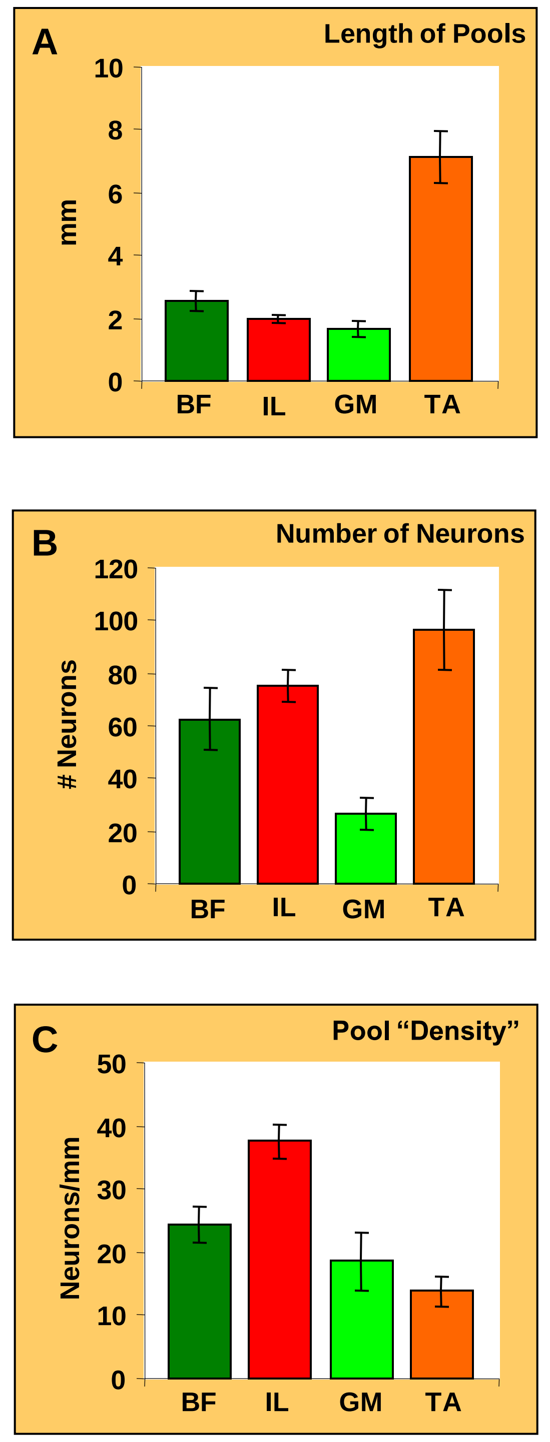
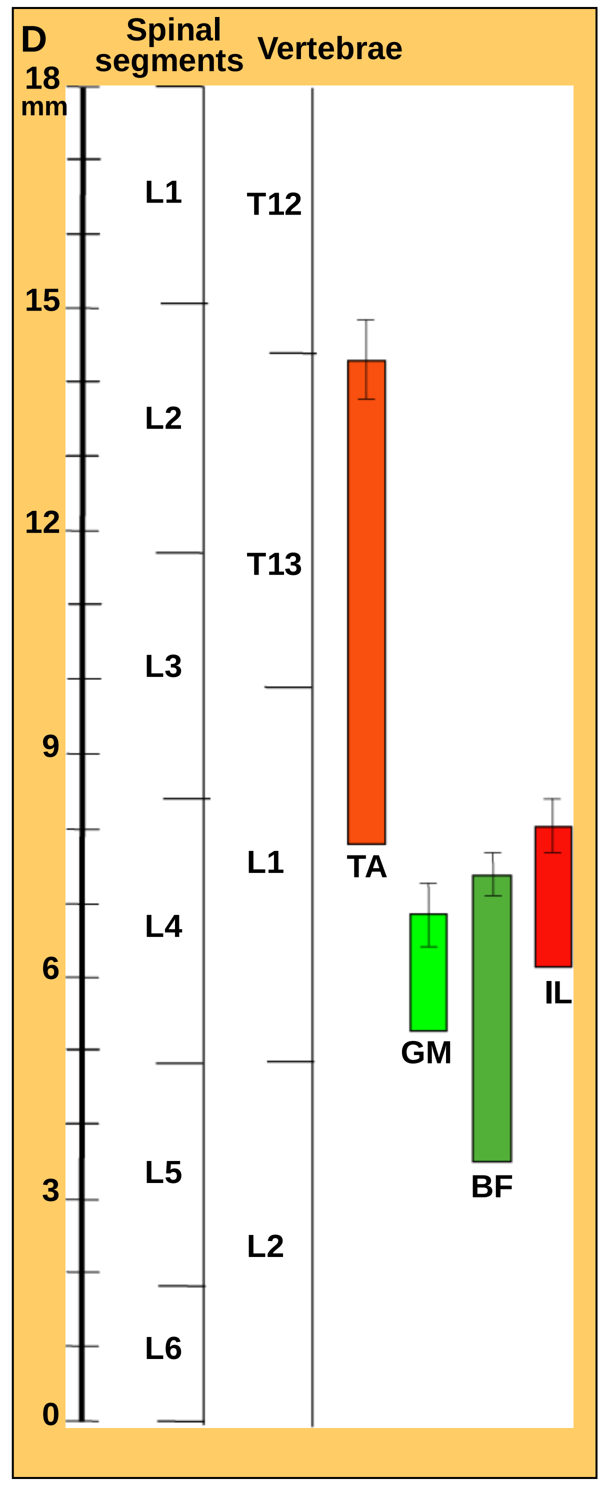


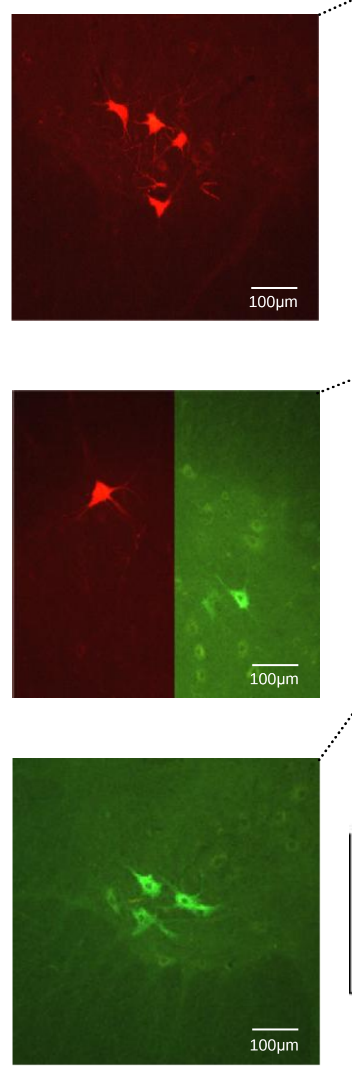
Figure 1: Target muscles. Alexafluor 594 "red" Alexafluor 488 "green" ★ Motor point location
Left site - Tibialis anterior and gastrocnemius medialis. Right site - Iliacus and biceps femoris.

Results

Motoneuron Pools







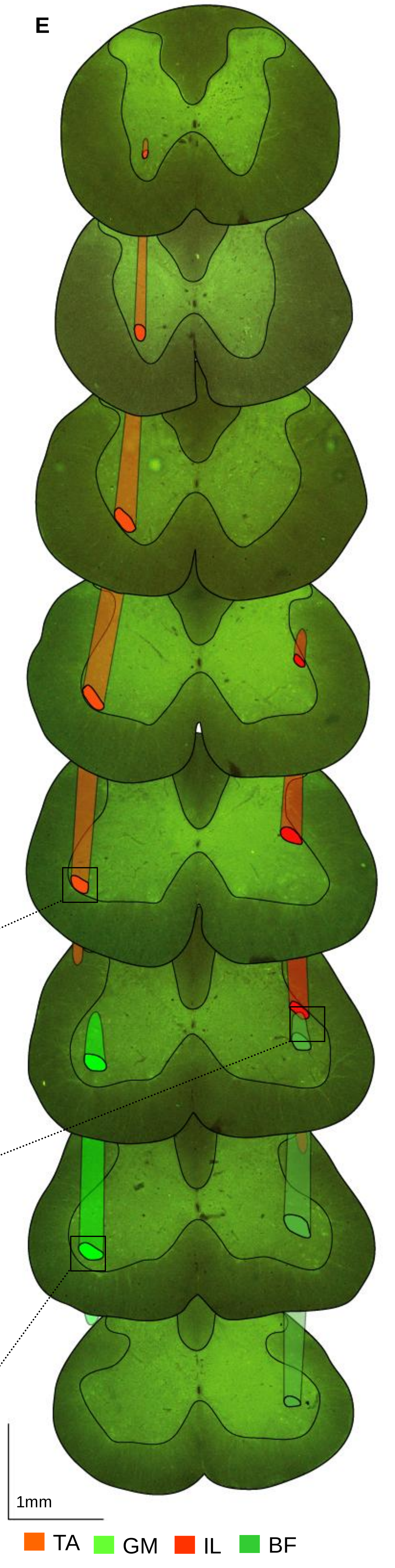


Figure 2. Properties of the Motoneuron Pools: Bars are standard error, data for A – D is from is from n=5 animals, data for E is from n=1 animal. Innervations for four muscles

A. Length of Pools: Mean length of the motoneuron pool in the spinal cord for each muscle in millimeters. Calculated as the distance from the most rostral to the most caudal labelled neurons.

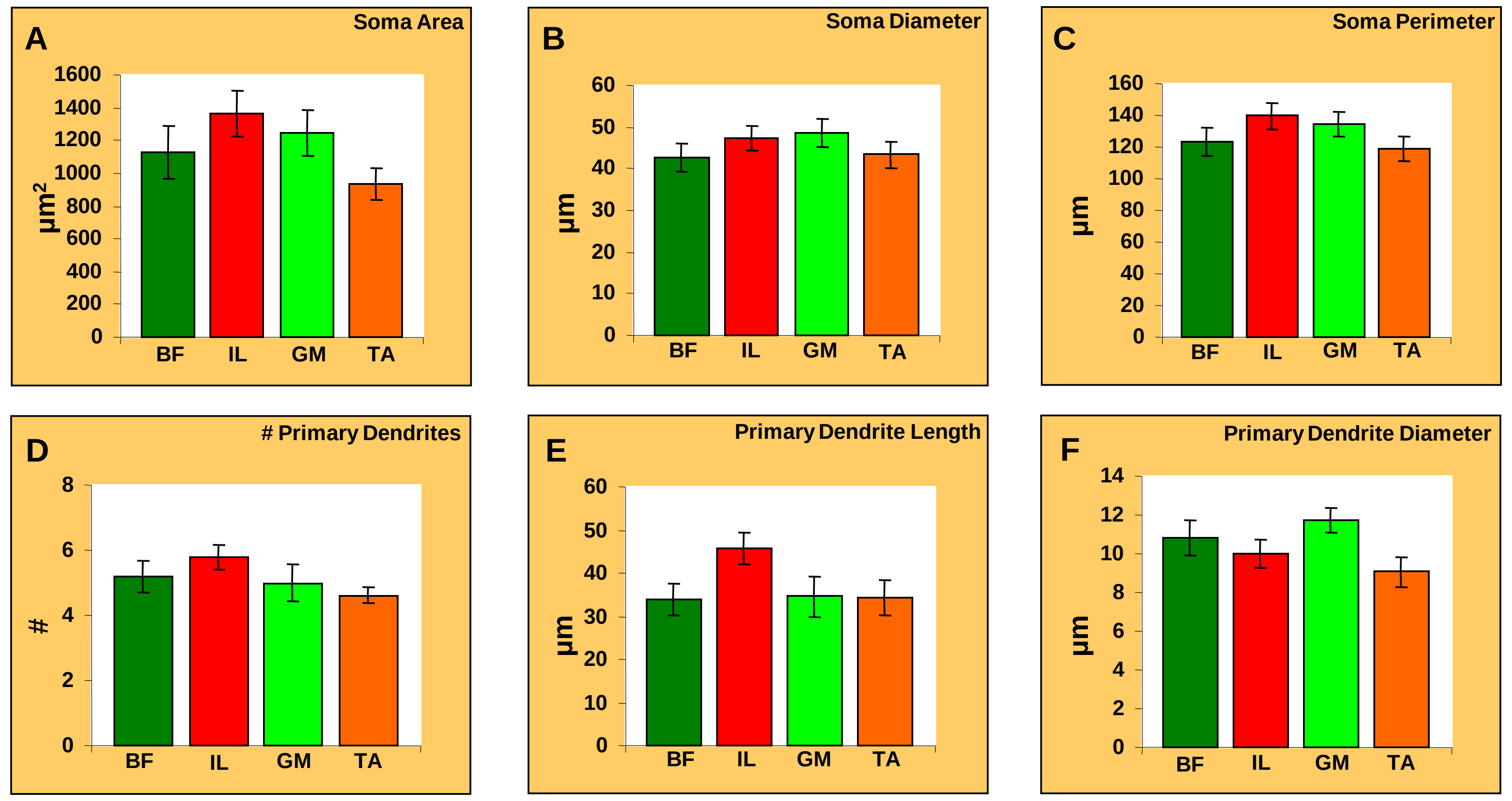
B. Number of Neurons: Mean number of neurons labelled for each muscle.

C. Pool "Density": Number of neurons per unit length in each motoneuron pool. Calculated as the number of neurons in each pool divided by the length of the pool.

D. Pool Location: Rostro-caudal location (only) of motoneuron pools within the spinal cord for each muscle.

E. Pool Location: Serial sections showing rostro-caudal and medio-lateral location of motoneuron pools for each muscle in one animal. Insets show actual picture of labelled neurons.

Motoneuron Morphology



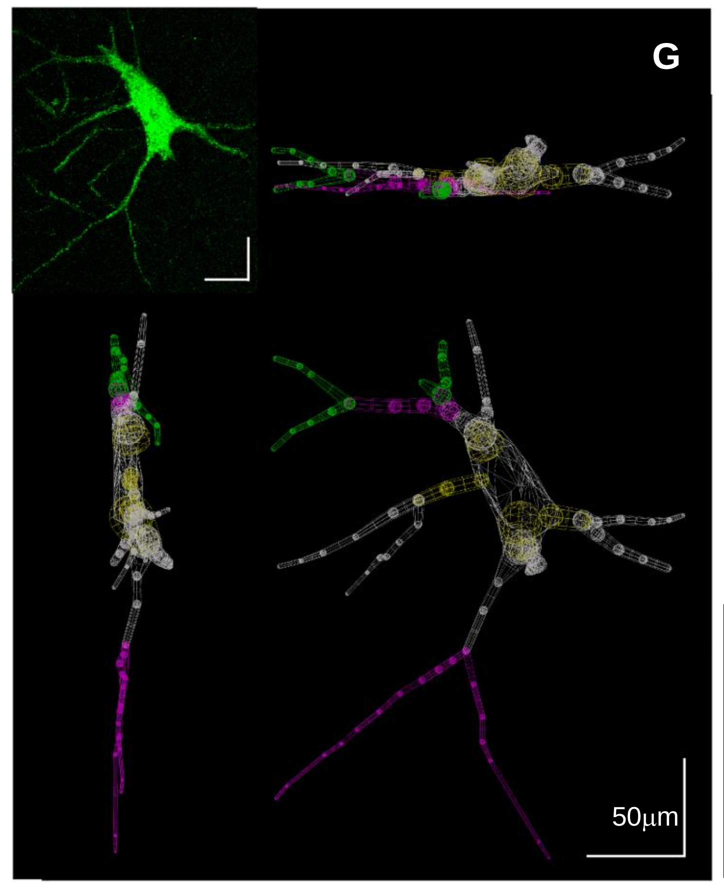


Figure 3. Motoneuron Morphology: Bars are standard error, data for A – F: n=1 animal, p=5 motoneurons, 2D measurements

A. Soma Area (μm²)

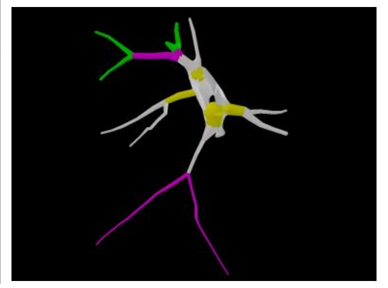
B. Soma Diameter: maximum diameter of the soma (μm)

C. Number of Primary Dendrites

D. Soma Perimeter (μm)

E. Primary Dendrite Length: mean length of the primary dendrites measured from soma to first branch point (μm)

F. Primary Dendrite Thickness: mean value of the maximum thickness of primary dendrites (μm).



G. Three dimensional reconstruction of BF motoneuron from confocal image. Top left image is 2D projection of confocal image stack.

Conclusions

The data presented here characterize the number, location, and morphology of spinal motoneurons and their pools for an ankle flexor (tibialis anterior), ankle extensor (gastrocnemius medialis), hip flexor (iliacus), and hip extensor (biceps femoris) in the uninjured rat.

These data will form a baseline for comparison with spinal cord injured rats and will be used in future computational models of spinal cord electrophysiology in relation to spinal cord injury.

References

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