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Neuromuscular partitioning of the gastrocnemius based on intramuscular nerve distribution patterns: implications for injections

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SUMMARY

Spasticity of the gastrocnemius is commonly treated with botulinum toxin injections; however, the optimal injection sites within each head have not been evaluated in relation to neuromuscular partitions. The purpose of the present study was to (1) document the intramuscular innervation patterns of the medial and lateral heads of gastrocnemius using 3 dimensional modeling; (2) determine if the medial and lateral heads of gastrocnemius are neuromuscularly partitioned; and (3) propose botulinum toxin injection strategies based on these findings. In this cadaveric study (n=24) the extramuscular and intramuscular innervation was serially dissected followed by digitization and 3D reconstruction and/or photography of the innervation pattern throughout the muscle volume. Intramuscular innervation patterns were defined to determine if the heads of gastrocnemius were neuromuscularly partitioned and based on these findings approaches for botulinum toxin injections were proposed. In all specimens except one, both heads of the gastrocnemius received independent innervation from three discrete nerve branches. Therefore, each head had three neuromuscular partitions defined by location as superior, inferomedial and inferolateral. In one specimen, the lateral head also received nerve branches via the soleus that innervated the inferolateral partition distally. Functionally, independent activation of the neuromuscular partitions of the gastrocnemius may result in differential contribution of the partitions to knee flexion and ankle plantarflexion. To capture all partitions, four injection sites into each belly were proposed. Future clinical studies are needed to determine if there is improved spasticity reduction by targeting neuromuscular partitions.

Key words: Gastrocnemius muscle – Tibial nerve – Injections – Muscle spasticity – Botulinum toxin

INTRODUCTION

In stroke, cerebral palsy and other upper motor neuron conditions, ankle plantarflexion spasticity can cause significant functional impairment (Crosbie et al., 2012; Hsu et al., 2003; Sosnoff et al., 2011). The medial (MG) and lateral (LG) heads of the gastrocnemius muscle are commonly injected with botulinum toxin A (BoNT-A) in the focal management of ankle plantarflexion tone. However, the recommended location of injection sites for MG and LG has varied in the literature (Childers et al., 1996; Im et al., 2014; Sätilä et al., 2005, 2008). Improvements in ankle plantarflexor spasticity

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were not shown to differ significantly between techniques that targeted the neuromuscular junction zone or direct muscle belly injection.

In a more recent study, Elwischger et al. (2014) suggested that to optimize biceps brachii BoNT-A injection, the orientation of the muscle fibers need to be considered and recommended injection perpendicular to the direction of the fibers, with additional injections along the length of the fiber bundles (Elwischger et al., 2014). In both healthy and spastic biceps brachii the BoNT-A bolus was found to diffuse between muscle fiber bundles as a thin longitudinal layer. To understand diffusion patterns along muscle fiber bundles, a detailed knowledge of muscle architecture is required. It has been suggested that targeting injections at the fiber bundle level may enhance clinical outcomes. However, strategies for injection of MG and LG, based on neuromuscular architecture, have not been explored (Elwischger et al., 2014; Warden et al., 2014).

A portion of a muscle that has distinct fiber bundle architecture and receives independent intramuscular innervation is described as a neuromuscular partition (English et al., 1993). Electromyographic (EMG) studies have provided evidence for neuromuscular partitioning of MG and LG (Table 1). For example, Wolf et al. (1993, 1998) demonstrated differential activation in the distal lateral region of LG compared to the proximal lateral and medial regions during leg tasks. The significance of differential activation and the functional role of each partition are not well understood.

In the three anatomical studies that examined the neuromuscular partitioning of MG and/or LG the results were conflicting (Table 1). Two studies,

Table 1. Summary of previous studies investigating the neuromuscular partitioning of MG and LG

Study	Muscle Head	n	Findings		Neural Partitioning
Wolf et al., 1993	LG	20 EMG	Activity: 8 leg tasks 3 heads tested		Yes
Wolf et al., 1998	LG	5 EMG	Activity: body perturbations 3 heads tested as in Wolf et al., 1993		Yes - Further investigation suggested
Segal et al., 1991	LG	6 CS	1°: 1	2°: 2	No
Wolf et al., 1997	MG	8 CS	1°: 1 3°: 3-10	2°: 2 4°: 0-8	No
Sheverdin et al., 2009	LG & MG	18 CS	1°: 2-3 2°, 3°, 4°: X		Yes

Abbreviations: CS, embalmed cadaveric specimens; EMG, electromyography; LG, lateral head of gastrocnemius; MG, medial head of gastrocnemius; X, not reported; 1°-4°, first to fourth order nerve branches.

Table 2. Summary of previous studies investigating the location of extramuscular nerve entry points of MG and LG

Study	n	Extramuscular nerves		
		Number of entry points		Location of entry point(s)
		LG	MG	
Bryce, 1923	1	2-3	2-3	Proximal 1/5 muscle belly MG/LG
Kim et al., 2002	LG: 25 MG: 26	1 (20CS) 2 (3CS) 3+ (2CS)	1 (18CS) 2 (5CS) 3+ (3CS)	Mean distance from intercondylar line (% of lower leg length): MG: 1st: 4.1 ± 3.0cm (11.6 ± 8.5%) 2nd: 3.9 ± 2.2cm (10.7 ± 6.1%) LG: 1st: 4.0 ± 1.5cm (10.7 ± 3.8%) 2nd: 4.0 ± 0.7cm (11.4 ± 2.0%) Lower leg length: intercondylar line to intermalleolar line
Parratte et al., 2002	36	1	1	X
Kim et al., 2005	8	1-2 (7CS) 3+ (1CS)	1-2 (7CS) 3+ (1CS)	X
Sheverdin et al., 2009	18	3-8	3-8	Calf length: knee crease to intermalleolar line Superior 30% of calf length

Abbreviations: LG, lateral head of gastrocnemius; MG, medial head of gastrocnemius; CS, embalmed cadaveric specimens; X, not reported.

Segal et al. (1991) (LG) and Wolf and Kim (1997) (MG), found no evidence of partitioning. However, Sheverdin et al. (2009) reported the presence of 4 neuromuscular partitions, 1 proximal and 3 distal, in both MG and LG based on intramuscular nerve distribution patterns. These studies were descriptive and used schematic diagrams and photographs to report findings.

Since neuromuscular partitioning is based on innervation pattern, extramuscular innervation also needs to be considered. Previous studies examining the number and location of extramuscular nerve entry points are summarized in Table 2. Results were variable with the number of reported nerve entry points ranging from 1 to 8 located within the proximal third of the muscle belly.

Recent studies conducted in our laboratory have shown that digitization of intramuscular nerve distribution with subsequent 3D modeling provides comprehensive data sets that can be used to fully document intramuscular nerve distribution throughout the entire muscle volume (Fattah et al., 2013; Loh et al., 2003; Warden et al., 2014). Digitization captures the intramuscular innervation within the muscle volume as in situ, and the 3D models provide a fully manipulable reconstruction of the specimen that can be analyzed to determine the presence of neuromuscular partitions and nerve distribution relative to the orientation of the fiber bundles.

More detailed knowledge of the intramuscular innervation of MG and LG could assist in developing new strategies for distributing the dose of BoNT-A. Therefore, the objectives of this study were to: (1) document the intramuscular innervation patterns of MG and LG using digitization and 3D modeling; (2) determine the presence of neuromuscular partitions and nerve distribution relative to the orientation of the fiber bundles in MG and LG; and (3) propose BoNT-A injection strategies based on these findings.

MATERIALS AND METHODS

Twenty-four formalin embalmed cadaveric specimens with an average age of 77.7±11.2 years (13M/11F) were included in this study. In 20 specimens (11M/9F), the intramuscular innervation pattern was exposed using microdissection and documented with illustrations and photographs. Four additional specimens (2M/2F) that were representative of each of the distinct innervation patterns were serially dissected and digitized for 3D modeling. Exclusion criteria included evidence of musculoskeletal deformities, pathologies, surgery and/or trauma. Ethics approval was obtained from the University of Toronto Health Sciences Research Ethics Board.

To prepare the specimens, MG and LG were exposed by removal of overlying soft tissues. The tibial nerve was then identified and traced from the bifurcation of the sciatic nerve, and all branches innervating MG and LG were located.

In all specimens, the number of branches entering MG and LG was recorded. The locations of the most proximal and most distal nerve entry points were measured from the intercondylar line and then quantified as a percentage of muscle belly length. The length of the muscle belly was defined as the distance from the most proximal to the most distal attachments of the fiber bundles.

Each nerve branch entering MG and LG was serially dissected intramuscularly, in short segments, throughout the muscle volume until no longer visible with a dissection microscope. At each level of serial dissection, the nerve distribution pattern was illustrated and photographed. Patterns of innervation were identified according to the distribution of the nerve branches within MG and LG. Muscular regions receiving independent innervation were identified.

Specimens representative of each variation of intramuscular innervation pattern were digitized (n=4). Following exposure of MG and LG, the knee and ankle joints were stabilized in neutral position using metal plates. Three screws were placed into bony landmarks to serve as reference markers for reconstruction of the digitized data. The extramuscular branches of the tibial nerve to MG and LG were identified and digitized to their entry points into the muscle belly. Subsequently, each nerve was traced intramuscularly and sequentially exposed in short segments by removing overlying fiber bundles. As the nerves were exposed, they were sequentially digitized using a MicroScribe™ G2X Digitizer (Immersion Corp, 30 Rio Robles, San Jose, CA 95134). This process was continued until each intramuscular branch was no longer visi-

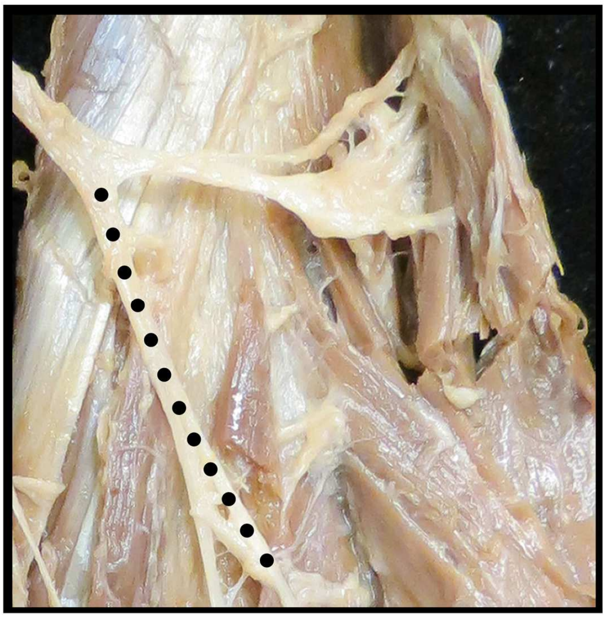

Fig. 1. Digitization of nerves. Points along each nerve were digitized at 3-5 mm intervals (black dots).

ble under a dissection microscope (Fig. 1). To enable volumetric reconstruction of MG and LG, the muscle bellies were digitized prior to and throughout the nerve dissection process.

The digitized data were imported into Autodesk Maya 2011 (Autodesk, Inc, 111 McInnis Pkwy, San Rafael, CA 94903), a 3D modeling and animation software, enhanced with plug-ins developed in our laboratory. A fully manipulable, 3D digital model of the nerve distribution within the muscle volume as in situ was generated for each specimen. These models were used to quantify the location of the entry points of the extramuscular nerve branches and to determine nerve distribution patterns relative to the orientation of the fiber bundles in MG and LG.

RESULTS

A database of nerve entry points and intramuscular distribution patterns in 24 specimens was compiled and analyzed. The 3D models allowed for visualization of the intramuscular innervation pattern relative to the muscle volume as in situ. Both MG and LG were found to be neuromuscularly partitioned based on intramuscular innervation patterns.

Medial Head of Gastrocnemius

One primary branch from the tibial nerve was found to innervate MG in all specimens by dividing into proximal and distal branches extramuscularly in 87.5% of specimens (n=21/24) and intramuscularly in 12.5% of specimens (n=3/24). Specimens where the primary branch divided intramuscularly (n=3/24), 1 nerve entry point was found (Fig. 2). However, if the primary branch divided extramuscularly, there were 2 to 4 nerve entry points: 2 in 25% (6/24) of specimens, 3 in 41.7% (10/24), and 4 in 20.8% (5/24).

The average distance from the intercondylar line to the proximal and distal nerve entry points was 4.2 ± 1.7 cm and 5.9 ± 1.9 cm, respectively (Table 3). All extramuscular nerve entry points were located between 10-35% of muscle belly length.

Two intramuscular innervation patterns were identified (Fig. 3). In both patterns, the proximal branch and its divisions were distributed to the superior quarter of the muscle belly, while the inferior three-quarters of the muscle belly were innervated by the distal branch, which further divided into medial and lateral branches (Fig. 3). In the most common pattern (Pattern 1), occurring in 70.6% of specimens (Fig. 3A):

- The lateral branch supplied the lateral part and entire distal end of the belly
- The medial branch supplied the superomedial part of the belly

In the less frequently found innervation pattern (Pattern 2; 29.4% of specimens), the areas inner-

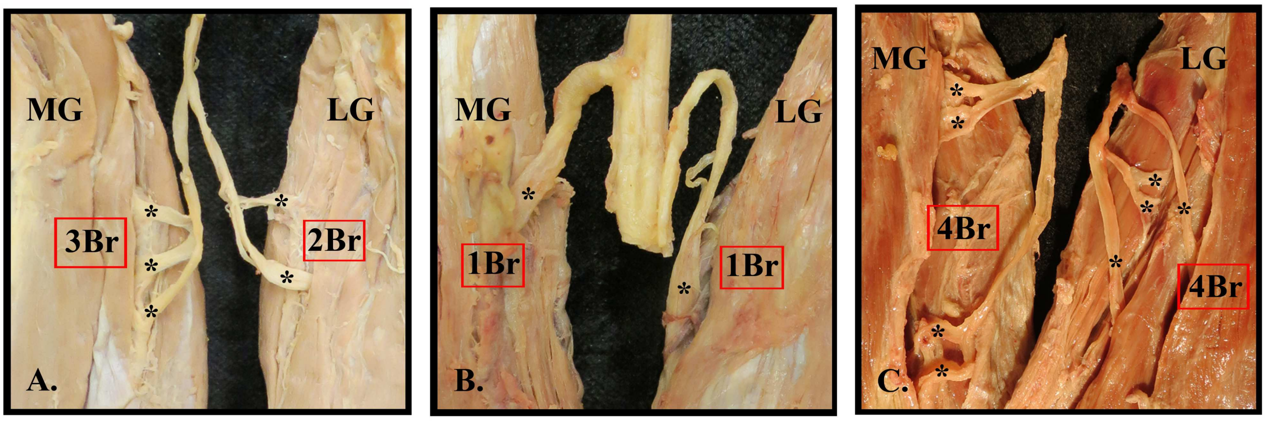

Fig. 2. Patterns of MG and LG nerve entry points. (A) MG with 3 entry points and LG with 2 entry points. **(B)** MG and LG with 1 entry point each. **(C)** MG and LG with 4 entry points each. Nerve entry points (*); extramuscular nerve branches (Br).

Table 3. Summary of findings: extramuscular nerve entry points into MG and LG

	MG		LG	
	Proximal entry point	**Distal entry point**	**Proximal entry point**	**Distal entry point**
Average % of muscle belly length (range)	18.4 ± 5.1% (9.9 – 28.6%)	26.1 ± 4.8% (20.0 – 34.8%)	19.1 ± 5.7% (6.6 - 28.8%)	26.6 ± 6.9% (9.6 – 38.0%)
Average distance from intercondylar line (range)	4.2 ± 1.7cm (1.5 – 8.5cm)	5.9 ± 1.9cm (3.1 – 10.1cm)	3.9 ± 1.3cm (1.1 – 6.7cm)	5.3 ± 1.5cm (1.6 – 8.5cm)

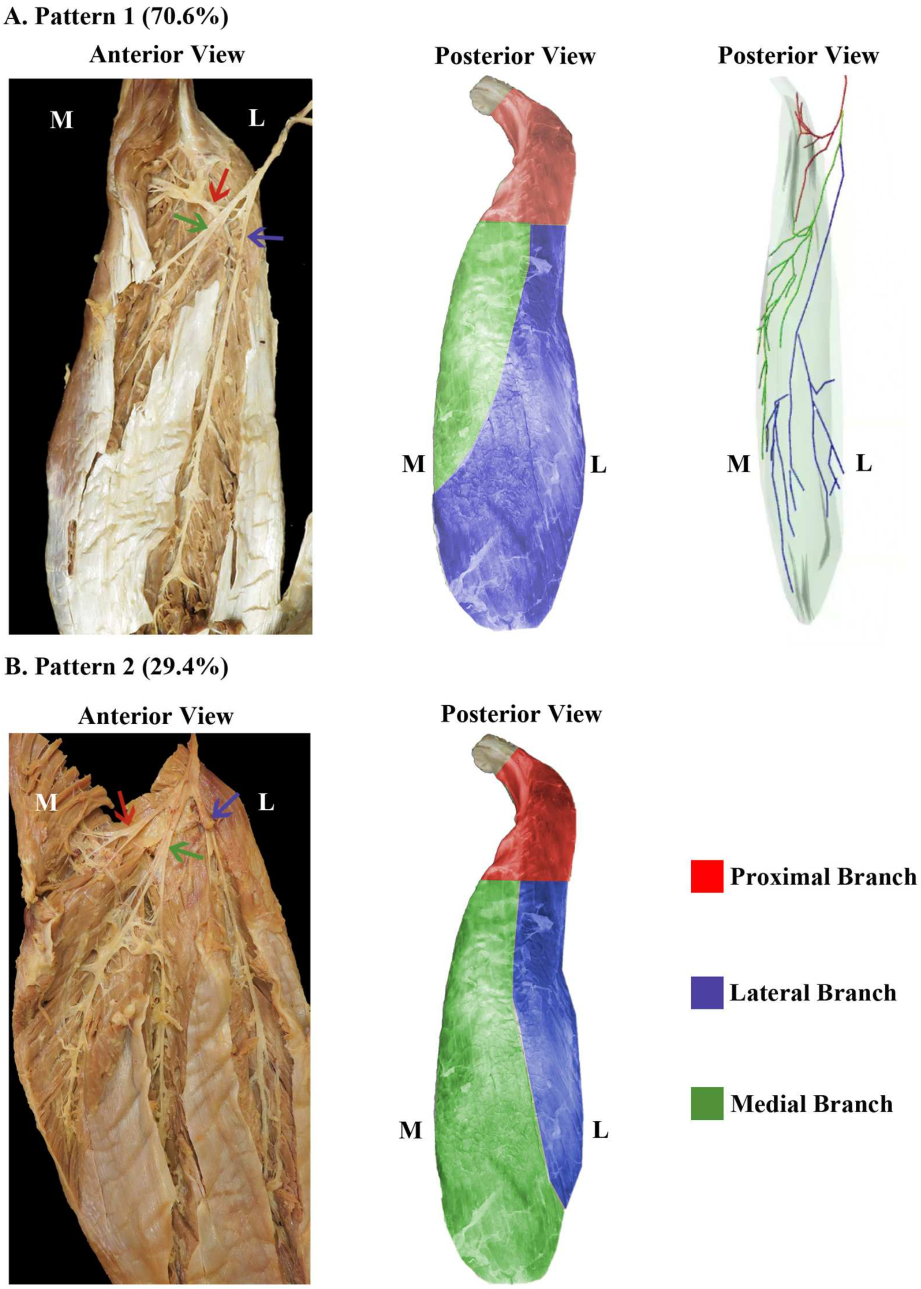

Fig. 3. Intramuscular innervation patterns of MG. (A) Pattern 1. Photograph (left), neuromuscular partitions (center), and 3D model (right). **(B)** Pattern 2. Photograph (left) and neuromuscular partitions (right). Lateral (L); Medial (M).

vated by the medial and lateral branches were reversed (Fig. 3B):

- The lateral branch supplied the superolateral part of the belly
- The medial branch supplied the medial part and entire distal end of the belly

The superior quarter of MG had a large tendon medially, and obliquely oriented fiber bundles laterally, whereas the inferior quarter in contrast had vertically oriented fiber bundles. Fiber bundles in the central portion of the muscle were obliquely oriented both medially and laterally.

In summary, MG is most commonly divided into 3 neuromuscular partitions:

- Superior partition, supplied by the proximal branch;
- The inferomedial partition, supplied mainly by the medial branch; and
- The inferolateral partition, supplied mainly by the lateral branch.

Lateral Head of Gastrocnemius

One primary branch from the tibial nerve was found to innervate LG by dividing into proximal and distal branches extramuscularly in 91.7% of specimens (n=22/24) and intramuscularly in

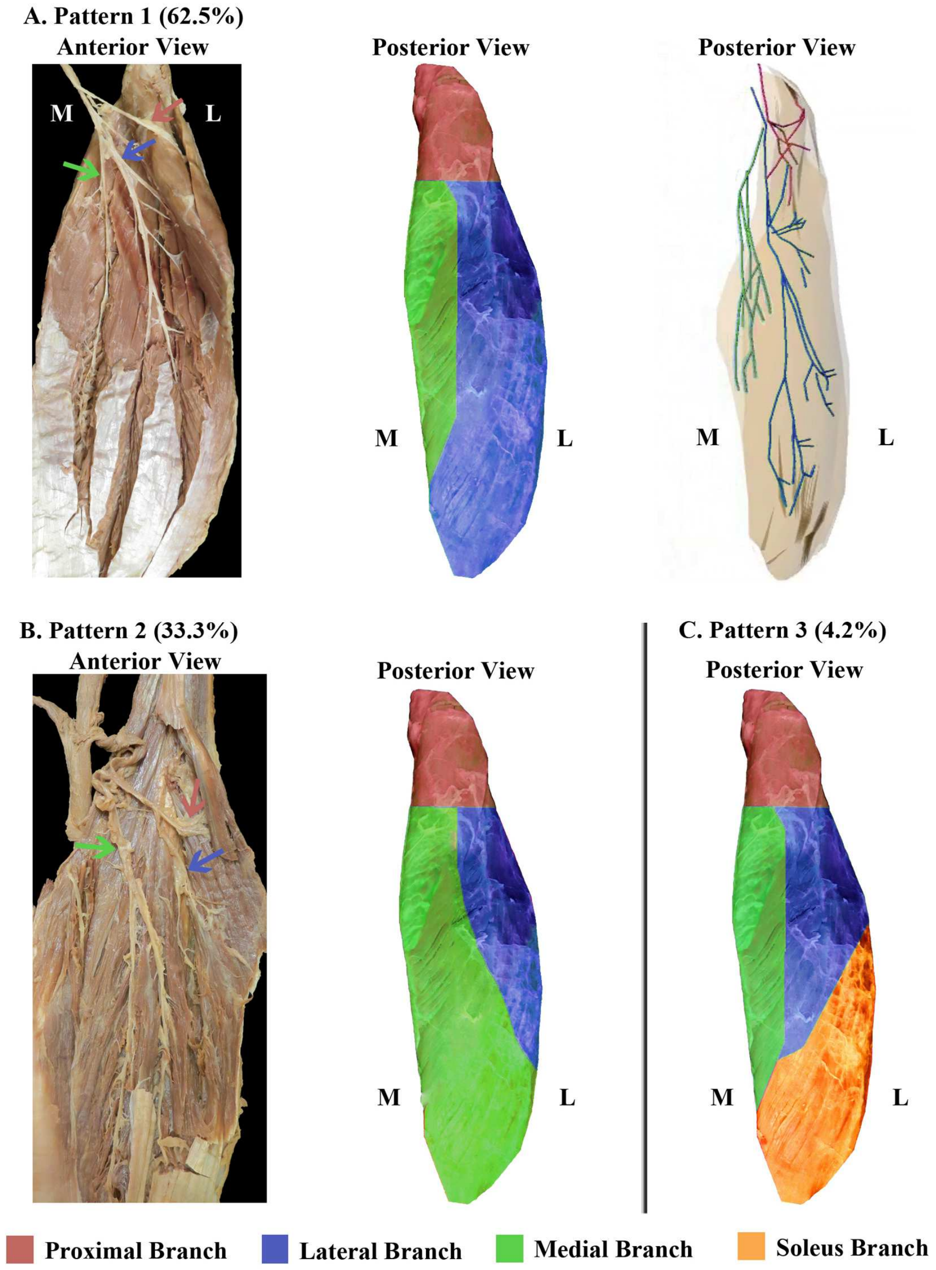

Fig. 4. Intramuscular innervation patterns of LG. (A) Pattern 1. Photograph (left), neuromuscular partitions (center), and 3D model (right). **(B)** Pattern 2. Photograph (left) and neuromuscular partitions (right). **(C)** Pattern 3. Neuromuscular partitions. Lateral (L); Medial (M).

8.3% of specimens (n=2/24). In specimens where the primary branch divided intramuscularly (n=2/24), 1 nerve entry point was found. However, if the primary branch divided extramuscularly, there were 2 to 5 nerve entry points: 2 in 50% (12/24) of specimens, 3 in 12.5% (3/24), 4 in 20.8% (5/24), and 5 in 8.3% (2/24).

The average distance from the intercondylar line to the proximal and distal nerve entry points was 3.9 ± 1.3 cm and 5.3 ± 1.5 cm, respectively (Table 1). All extramuscular nerve entry points were located between 6.6% and 38.0% of the muscle belly length. In 1 specimen, 2 additional extramuscular nerve entry points were found from branches originating from the soleus muscle. These 2 branches entered the inferolateral aspect of the muscle belly and continued intramuscularly.

The intramuscular innervation pattern of LG resembled that of MG, but in 1 specimen, 4 partitions were found. Three intramuscular innervation patterns were identified (Fig. 4A-C).

1. In 62.5% (15/24) of specimens, the muscle belly was supplied as follows:

- Proximal branch: superior quarter
- Medial and lateral branches: inferior three-quarters
  - Medial branch: superomedial part
  - Lateral branch: lateral part and entire distal end

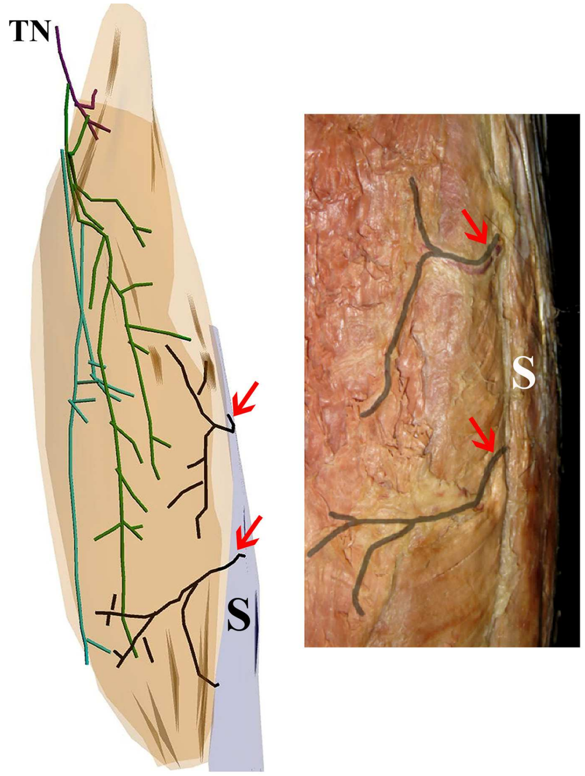

Fig. 5. Innervation of LG by branches of soleus, posterior view. 3D model (left) and photograph (right). Marginal soleus (S); tibial nerve (TN); nerve branches from soleus (red arrows).

2. In 33.3% (8/24) of specimens, the muscle belly was supplied as follows:

- Proximal branch: superior quarter
- Medial and lateral branches: inferior three-quarters
  - Medial branch: medial part and entire distal end
  - Lateral branch: superolateral part

3. In 4.2% (1/24) of specimens, the muscle belly was supplied as follows:

- Proximal branch: superior quarter
- Medial, lateral and soleal branches: inferior three-quarters
  - Medial branch: superomedial part
  - Lateral branch: superolateral part
  - Branches from soleus: entire distal end

The superior quarter of LG had a large tendon laterally and obliquely oriented fiber bundles medially. In the remaining three quarters of the muscle belly the fiber bundles of the inferior and lateral aspect were vertically oriented and the fiber bundles of the medial aspect obliquely oriented.

Similar to MG, LG is most commonly divided into 3 neuromuscular partitions:

- The superior partition, supplied by the proximal branch;
- The inferomedial partition, supplied mainly by the medial branch; and
- The inferolateral partition, supplied mainly by the lateral branch.

In the specimen with additional branches from soleus, there was a fourth partition (Figs. 4C and 5).

DISCUSSION

In all specimens except one, both heads of the gastrocnemius received independent innervation from three discrete nerve branches and thus were divided into superior, inferomedial and inferolateral partitions. One specimen had four partitions due to the additional innervation received via soleus.

Previous cadaveric studies reported nerve entry points and intramuscular innervation patterns utilizing illustrations and Sihler's staining technique (Frohse and Frankel, 1908; Segal et al., 1991; Sheverdin et al., 2009; Wolf and Kim, 1997). In these studies, fiber bundles were excised to expose the intramuscular innervation, which was recorded photographically and therefore could not be volumetrically reconstructed. In contrast, the current study provided manipulable, 3D models of detailed intramuscular innervation patterns as in situ, which is unique in that it enables viewing of the intramuscular innervation relative to the muscle volume.

The number of extramuscular nerve entry points into MG and LG found in the current study was consistent with previous studies. In previous studies the number of entry points for MG and LG ranged from 1-8 (Bryce, 1923; Kim et al., 2002; Kim et al., 2005; Parratte et al., 2002; Sheverdin et al., 2009). In the current study, 1-4 entry points were found in MG and 1-5 in LG all entering in the proximal third of the muscle belly.

The results of previous cadaveric studies that examined the intramuscular innervation patterns and neuromuscular partitions of MG and LG are inconclusive. The intramuscular innervation pattern of MG and LG described by Frohse and Frankel (1908) is consistent with innervation pattern 2 identified in the current study, with 1 superior partition and 2 inferior partitions. Only one previous study was found that identified neuromuscular partitions within both MG and LG based on intramuscular innervation patterns (Sheverdin et al., 2009). In this study, Sheverdin et al. (2009) described 4 neuromuscular partitions in MG and LG based on the presence of 4 primary intramuscular nerve branches, 1 in the "head" of the muscle and 3 in the "belly". In the current study, 2 inferior partitions were found, rather than the 3 reported by Sheverdin et al. (2009).

Partitioning of MG and LG into superior and inferior partitions suggests that there may be differences in the contributions of these partitions to knee and ankle movement. The superior partitions of both heads were found to cross the posterior aspect of the knee joint, suggesting a greater contribution to knee flexion, whereas the inferomedial and inferolateral partitions may play a greater role in ankle plantarflexion. Concurrent activation of the soleus and the distal part of LG may also be possible if LG is innervated via soleus. Using the data from the current study, it is feasible to design more comprehensive EMG studies to investigate differential dynamic activation of MG and LG.

Studies that have evaluated BoNT-A injections at the areas of greatest NMJ concentration in gastrocnemius have not demonstrated a significant advantage over other injection strategies (Im et al., 2014). Two BoNT-A injection techniques for the gastrocnemius include a single injection of MG and LG in the distal muscle belly either 8 to 10 cm inferior to the knee crease (Jost, 2008) or at the level of the proximal third of the fibula (Fheodoroff et al., 2008). Neither of these techniques would be likely to capture the superior partition and may not capture both inferior partitions. Based on the results of the current study, neuromuscular partitions may provide an alternate BoNT-A injection strategy. To capture all of the partitions, taking into account the variations in innervation pattern, four injection sites into each muscle belly are proposed (Fig. 6):

- One injection into the superior quadrant of the belly
- Two injections medially and laterally at the midpoint of the belly
- One injection into the inferior quadrant of the belly

Ultrasound or EMG guidance would be required to ensure that the needle is placed within the gastrocnemius.

In conclusion, based on the results of this study, MG and LG consist of three neuromuscular partitions each having distinct intramuscular innervation. Functionally, the superior, inferomedial and inferolateral partitions of MG and LG may contribute differently to knee flexion and ankle plantarflexion. Currently, dosing of BoNT-A into gastrocnemius can range from 30-100 units (Fheodoroff et al., 2008; Jost, 2008), but the optimal dosing and concentration has not been determined. Targeting injections in the neuromuscular partitions may allow for the use of a lower overall dosage with a higher concentration. Further clinical studies are needed to determine the functional and dose dependent outcomes of BoNT-A injection of the neuromuscular partitions of MG and LG.

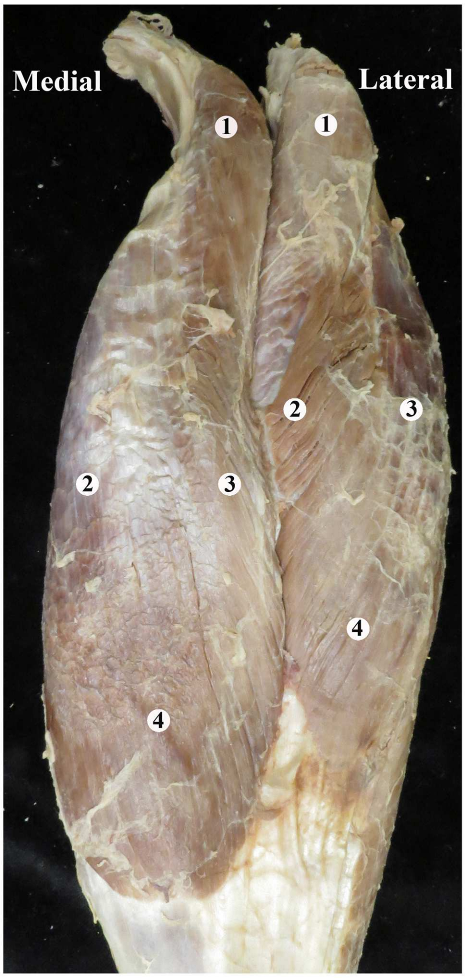

Fig. 6. Proposed Botulinum toxin injection sites for MG and LG. Numbers indicate the four injection sites in each head. 1, superior partition; 2, inferomedial partition; 3, inferolateral partition; 4, remainder of distal belly.

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Dissemination History

Preliminary results of this study were presented at the American Association of Clinical Anatomists Annual Meeting, July 9-13, 2013, Denver, CO, USA.

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