An exploratory study on the presence of sensory nerves in the caudal part of the trapezius

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SUMMARY

Intramuscular onabotulinumtoxinA (BTX) injections and nerve decompression surgery both aim to release compressed sensory nerves and are used in the treatment of migraine/headaches. Although for many BTX injection sites, sensory nerves and their potential entrapment sites have been established, for the trapezius this information is incomplete. A macro- and microscopic cadaveric study was performed in which the suprascapular part of the trapezius was explored for the presence of piercing or penetrating nerves and whether these nerves contained sensory nerve fibers. One side of six human cadavers were dissected to reveal trapezius-associated nerves in its suprascapular part. Schematic overview drawings were made of nerves either piercing or penetrating the trapezius in this region. Piercing or penetrating cervical nerves were resected and microscopically studied for the presence of sensory nerve fibers.

For this suprascapular region, correlating potential entrapment sites of the supraclavicular nerves were detected. In all specimens, plexus-like connections between the accessory nerve and branches of the cervical plexus, varying between CII-CV, were observed to innervate the trapezius at locations that showed overlap with BTX injection sites. Moreover, all these nerves contained sensory nerve fibers as confirmed by immunohistochemical staining with the sensory nerve marker CGRP. The presence of potential entrapment sites for supraclavicular nerves and other cervical nerve branches might explain why BTX injection in the suprascapular part of the trapezius show therapeutic effectivity in the treatment of migraine/headaches.

Key words: Cervical plexus – Supraclavicular (scapular) nerves – Entrapment – Migraine headache – Trapezius – OnabotulinumtoxinA – BTX

ABBREVIATIONS

CGRP	Calcitonine gene-related peptide
HE	Hematoxylin eosin
TBS	Tris buffered saline
BTX	OnabotulinumtoxinA

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INTRODUCTION

Migraine headache is a debilitating clinical condition which is associated with a high socioeconomic burden (Cady and Dodick, 1999; Stovner et al., 2018). Migraine headache affects both sexes and all age groups, although migraine headaches mostly occur in young adult and middle-aged women (Stovner et al., 2018). Migraine headaches can be divided into two subgroups; 1) episodic migraine, which is characterized by patients suffering from headaches during 14 days or fewer a month, and 2) chronic migraine in which patients have headaches during at least 15 days a month (Schwedt, 2014). Each year, 2.5% of episodic migraine patients turn into chronic patients (Schwedt, 2014). Frequent use of abortive migraine drugs has been associated with this disease progression (Schwedt, 2014). Due to the high disease burden caused by migraine, and because preventive and abortive medication is ineffective or poorly tolerated in a relatively large group of patients, alternative therapies are desired. Migraines are classically considered to represent a central phenomenon; however, various peripheral nerves have been identified as extracranial trigger sites (Guyuron et al., 2000, 2005; Behmand et al., 2003; Mosser et al., 2004; Austad, 2005; Totonchi et al., 2005; Blumenfeld et al., 2010, 2017; Gfrerer and Guyuron, 2017; Muehlberger, 2018; de Ru et al., 2019). Both intramuscular onabolinumtoxinA (BTX) injections causing paralysis of the muscles that are probably strangling the nerves, and decompression surgery were proven to diminish headache burden (Janis et al., 2014). Therapeutic BTX injections result in a significant reduction of up to 9 headache days as described in the PREEMT study (Diener et al., 2010). Furthermore, more recent studies confirm these findings and observe reduction in the use of abortive migraine drugs (Aicua-Rapun et al., 2016; Bilgiç et al., 2021). For most commonly used BTX injection sites – the temporalis region, the frontal region, the occipital region, and cervical paraspinal muscles – the involved sensory nerves and their potential entrapment sites in the pericranial musculature have been identified (Guyuron et al., 2000, 2005; Behmand et al., 2003; Mosser et al., 2004; Austad, 2005; Totonchi et al., 2005; Blumenfeld et al., 2010, 2017; Gfrerer and Guyuron, 2017; Muehlberger, 2018; de Ru et al., 2019; Yi et al., 2021). Especially, the anatomy and possible nerve entrapment sites of the occipital nerves in the upper part of the trapezius were studied extensively (Mosser et al., 2004; Austad, 2005). However, information on potential entrapment sites in the suprascapular part of the trapezius, a region described as additional injection site by Blumenfeld et al., is incomplete. So far, three suprascapular BTX injections sites were described: 1) in the center between the acromion and the necklace line, 2) between the first injection site and the acromion and 3) between the necklace line and the first injection site (Blumenfeld et al., 2017). When reviewing literature on trapezius-associated nerves in this region, potential nerve entrapment sites have been previously observed. These nerve entrapment sites involved the medial, intermediate and lateral supraclavicular nerves, which pierced the muscle or the clavicle (Macalister, 1871; Le Double, 1897; Jelev and Surchev, 2007). Furthermore, some studies hypothesized the existence of a supraclavicular entrapment syndrome, resulting in anterior shoulder girdle pain (Gelberman et al., 1975; Douchamps et al., 2012). How these entrapment sites correlate to the earlier mentioned injection sites is unclear. Therefore, the objective of this study was to confirm the previous observations and to explore the additional injection sites for the presence of sensory nerves piercing or innervating the suprascapular part of the trapezius. The presence, origin, and course of trapezius piercing and innervating nerves were determined by means of cadaveric dissections. If these piercing or penetrating nerves represented other nerves than the supraclavicular nerves (and hence it was unknown if these contained sensory nerve fibers), samples of these nerves were collected and subsequently microscopically studied for the presence of sensory nerve fibers.

MATERIALS AND METHODS

Six human cadavers were used for this study; five males and one female with an age varying between 64-94 years. These bodies entered

the Department of Anatomy of the University Medical Center Utrecht through a body donation program. Written informed consent was obtained during life allowing the use of these bodies for educational and research purposes. No signs of head and/or neck surgery were observed. Whole body preservation was accomplished by arterial perfusion with 3% formaldehyde. Dissections were performed on the right side of the head and neck area only. Skin, platysma and subcutaneous adipose tissue were removed, revealing the sternocleidomastoid, trapezius, and trapezius-associated nerves which were represented by branches of the supraclavicular nerves, the accessory nerve and direct branches of the cervical plexus. Both the sternocleidomastoid and trapezius were dissected and lifted, allowing a better view on relevant neural structures. Small blood vessels were removed if they disturbed the view. Each location where nerves pierced or penetrated the trapezius was documented by both photographs and schematic anatomical drawings.

If these nerves represented nerves other than supraclavicular nerves, they were resected and preserved in 70% ethanol until they were further processed for microscopy (the location of the samples is indicated in Fig. 1). Quantification of the relative contribution of sensory nerve fibers was performed on tissue sections of one level of each nerve only. All samples were placed in increasing percentages of ethanol and xylene, whereafter they were embedded in paraffin and cut into 5 µm thick sections on a microtome (Leica 2050 Super Cut, Nussloch, Germany). Transverse sections of nerves were collected on glass slides, air-dried and subsequently heat fixed for two hours on a slide drying table of 60°C (Medax, 14801, Kiel, Germany). Sections of each sample were routinely stained with hematoxylin/eosin (HE) to provide a general tissue overview and to identify nerves. An adjacent section of each nerve was incubated with antibodies raised against calcitonin gene-related peptide (CGRP) to determine the presence of sensory nerve fibers in these nerves.

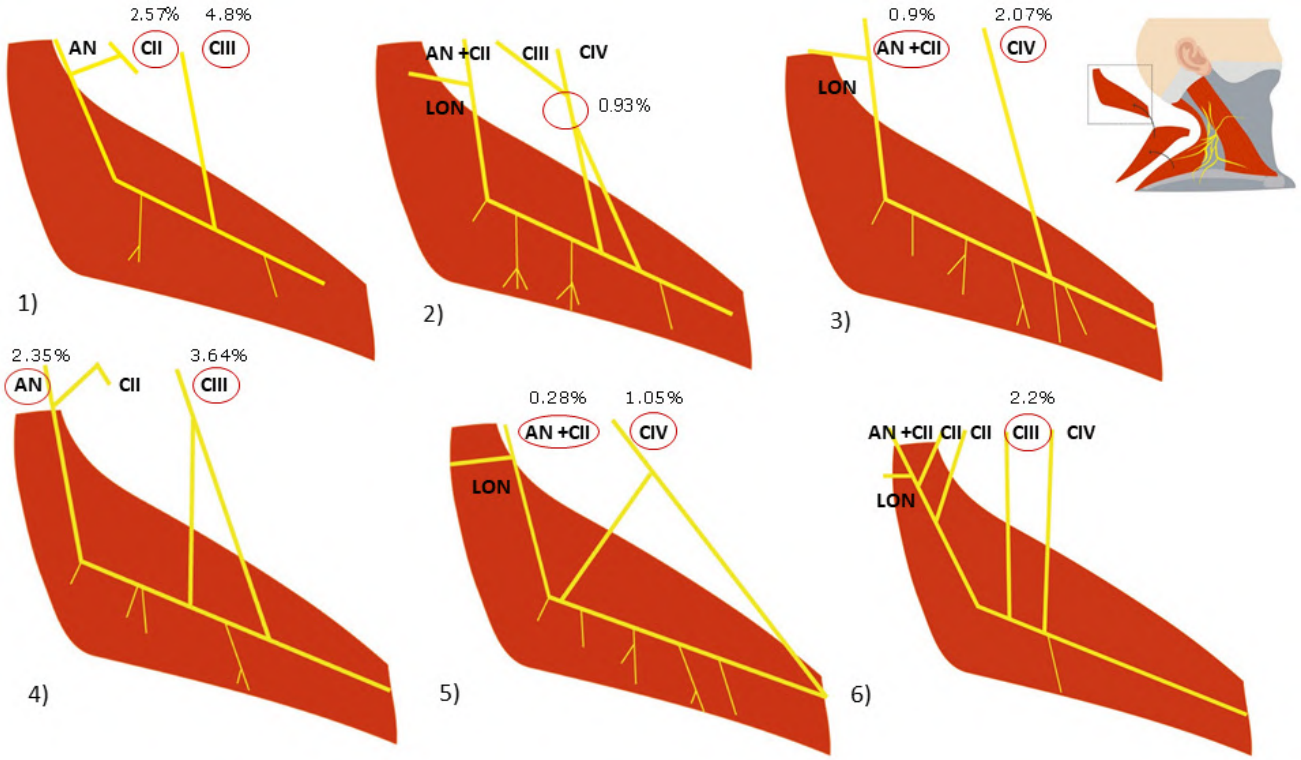

Fig. 1.- Schematic overview of the accessory nerve (AN) forming an accessory-cervical plexus with different branches (CII-CIV) in all six cadavers. In some figures the lesser occipital nerve (LON) is displayed to clarify when AN and CII are already merged. The numbered branches enter the trapezius. In this schematic overview the trapezius has been detached from the clavicle and turned open laterally to reveal a view of the deep surface of the muscle. The muscle is still attached at the level of the acromioclavicular joint. The samples of nerves, which were taken for histochemical analysis are indicated by a red circle. The relative contribution of sensory nerve fibers are expressed as area%.

Immunohistochemistry

Sections were dewaxed in xylene and rehydrated in graded alcohols, followed by 20 min of antigen retrieval in citrate buffer (pH6.0) at 95°C. After washing in Tris-buffered saline (TBS) with 0.05% Tween20 (TBS-T), sections were pre-incubated with 5% Normal Human Serum in TBS for 10 min, followed by incubation with mouse anti-CGRP (Sigma C7133, Saint Louis, USA) 1:1500 overnight at 4°C) in TBS with 3% bovine serum albumin (BSA). Sections were washed with TBS-T several times and incubated for 30 minutes at room temperature with Brightvision Poly-AP Goat-anti-Mouse (ImmunoLogic, Duiven, the Netherlands). After washing with TBS, sections were incubated with liquid permanent red (DAKO, Glostrup, Denmark) for ten minutes. Tissue sections were then washed with distilled water and counterstained with hematoxylin, airdried at 60°C for 90 minutes and cover-slipped using Entellan (Merck, Darmstadt, Germany). Negative controls were obtained by incubation of one additional section with TBS-3%BSA without primary antibodies. Vagus-nerve sections were included as a positive control.

Microscopic imaging

All samples were studied by brightfield (HE) and fluorescent microscopy (CGRP). Stitched overview images (also known as tile scans) of CGRP stained slides were captured with a 10x objective using fluorescent microsocopy. Image acquisition was performed using a DM6 microscope with a motorized scanning stage, a I3 fluorescent filter, a DFC7000 T camera and LASX software (all from Leica, Nussloch, Germany).

Image analysis

Tile scans of CGRP stained nerve sections were analyzed for their relative amount of sensory fibers with respect to the total area of a nerve using Fiji (Schindelin et al., 2012). The outer lining of the neural tissue (thereby excluding the epineurium) was manually selected in each slide and its total area was determined (Fig. 2). A threshold was set allowing to select all CGRP-immune reactive (IR) tissue inside these areas. The pixel area of CGRP-IR nerve tissue was then calculated for each nerve and its amount was then expressed as area% with respect to the total tissue area of the nerve. If a nerve appeared to be composed of multiple smaller nerves, each small nerve was analyzed independently and the results were added up.

RESULTS

All cadavers showed various nerves piercing and/or innervating the trapezius. These represented supraclavicular nerves or branches of the cervical plexus that were joined by branches of the accessory nerve, respectively.

Macroscopic observations

Supraclavicular nerves

In two out of six specimens, three branches of both the lateral and intermediate supraclavicular nerves pierced the trapezius via separate foramina (Fig. 3B, C, and Fig. 4). These foramina correspond with the first two BTX injection sites described by Blumenfeld et al. (2017). Fig. 3A shows the location of BTX injection sites 1-3. All branches originated from ventral rami of the third or fourth cervical spinal nerve. Furthermore, in one of these two specimens the medial supraclavicular nerve pierced through a tendinous arch of the trapezius (Fig. 3B) at a location which might correspond with the third injection site.

Cervical plexus branches and branches of the accessory nerve

No isolated cervical plexus branches or branches of the accessory nerve entered the trapezius in the studied region. In all six specimens several connections between the branches of the cervical plexus (CII-CV) and branches of the accessory nerve were formed prior to entering the trapezius. Each specimen showed an early connection between the accessory nerve and CII. Furthermore, three out of six showed a connection between the accessory nerve and CIII. Lastly four out of six showed connections between the accessory nerve and CIV (Fig. 1). All of them entered the trapezius in the middle portion between the necklace line and the acromion, locations which correspond with the first two injection sites. Unfortunately, some nerves of the sixth dissected cadaver were not preserved due to frailty.

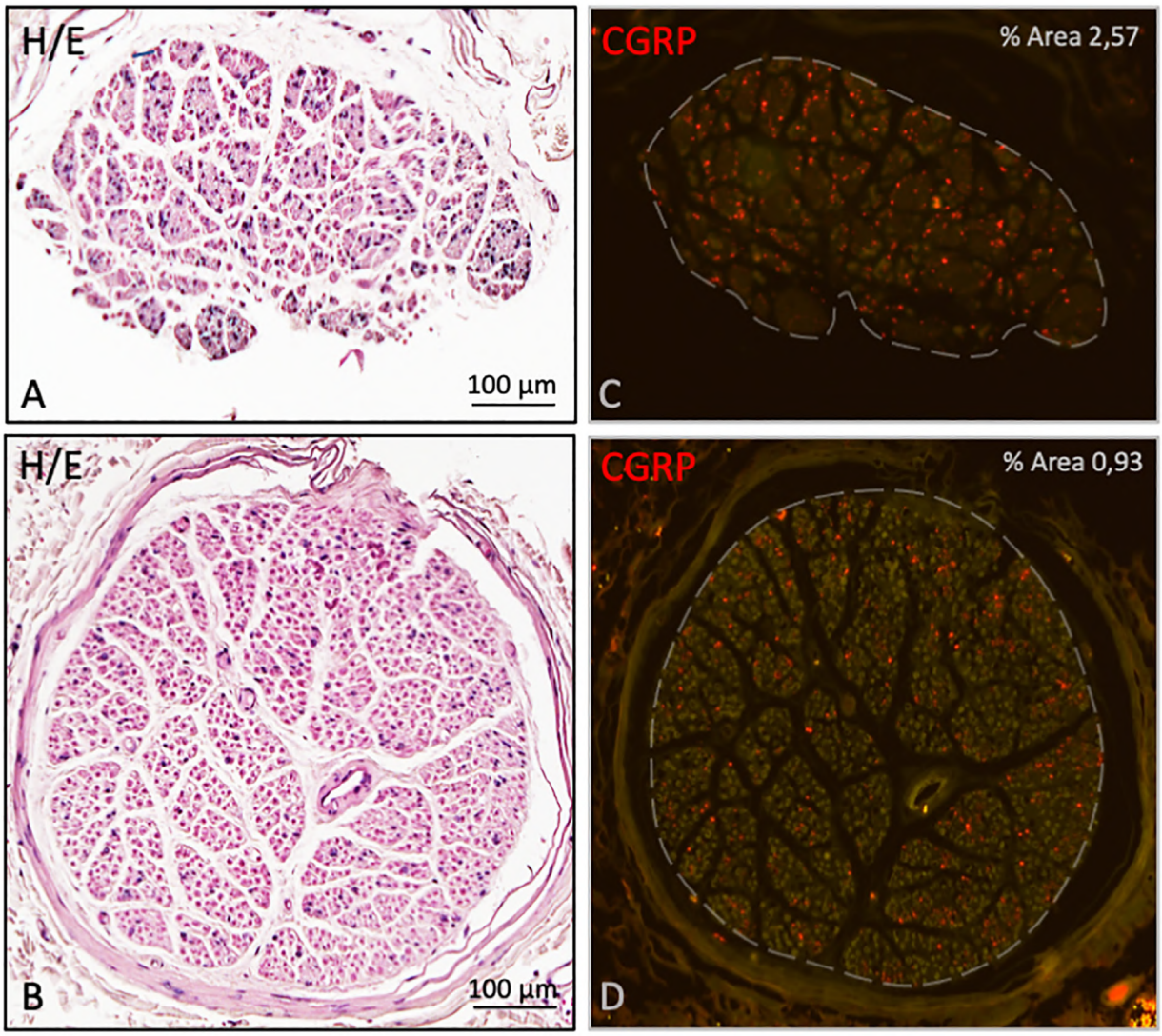

Fig. 2.- Microscopic images of nerves with relative high and low amount of sensory nerve fibers. **A:** Nerve CII of cadaver 1 has a relative high density of senosory nerve fibers. **B:** Nerve CIII/CIV of cadaver 2 has a relative low densitiy of sensory nerve fibers. **C, D:** Grey dotted lines: selection of neural tissue in which the density of sensory nerve fibers is determined.

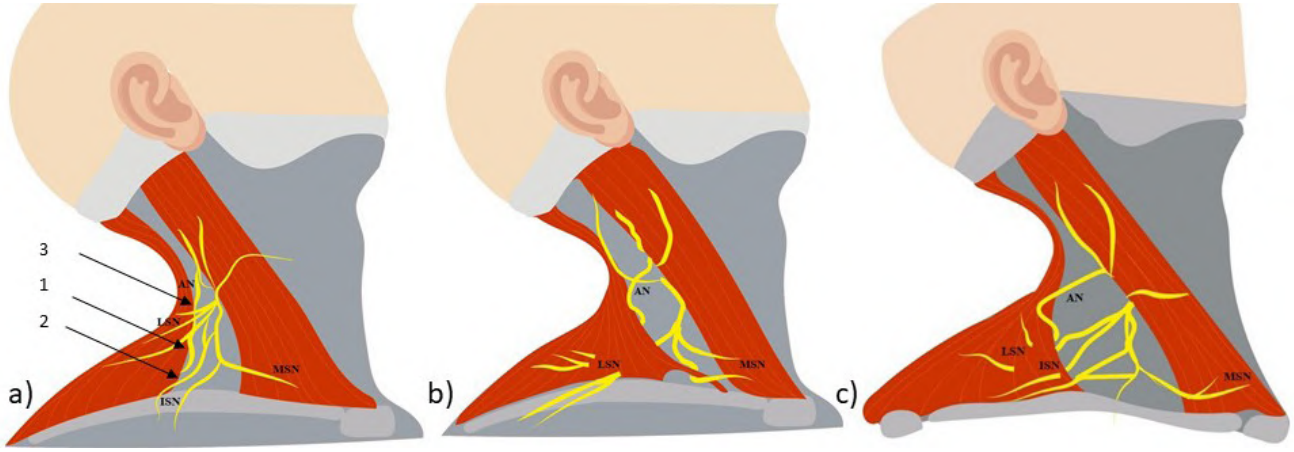

Fig. 3.- Drawing of supraclavicular nerves (lateral (LSN),intermediate (ISN), medial(MSN)), accessory nerve (AN), great auricular nerve, transverse nerve and lesser occipital nerve. **a)** Cadaver 5- male 75 years. Without any potential entrapment sites of the supraclavicular nerves. Thee arrows indicate the location where the BTX injection are commonly placed. **b)** Cadaver 2- male 64 years with potential entrapment sites of the lateral and medial branches of the supraclavicular nerves. **c)** Cadaver 4- male 72 years, with similar entrapment site of the intermediate and lateral branches of the supraclavicular nerve.

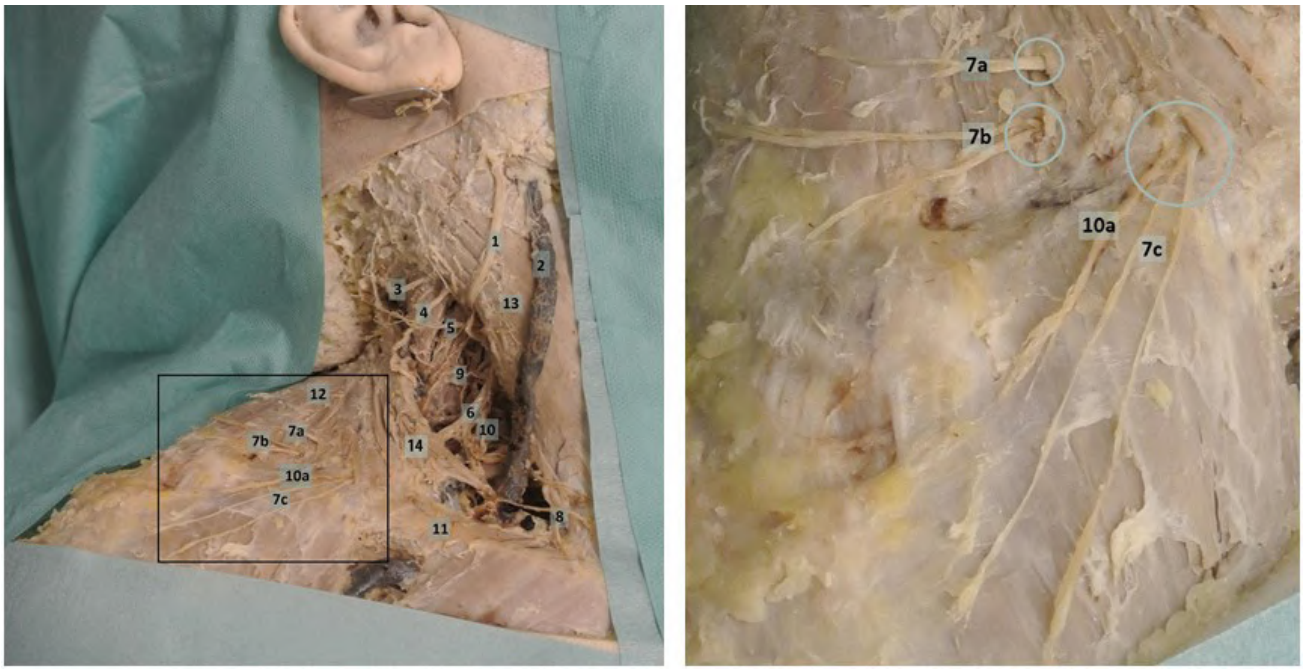

Fig. 4.- Left: Photographic overview of the posterior triangle of the neck in cadaver 2: 1. Great auricular nerve, 2. External jugular vein, 3.Lesser occipital nerve, 4. Accessory nerve, 5. Lesser occipital nerve, 6. CIV, 7.a,b,c Lateral supraclavicular nerve, 8. Medial supraclavicular nerve, 9.CIV, 10. Superficial branch of the transverse cervical artery, 10a Superficial branch of the transverse cervical artery, 11. Clavicle, 12. Trapezius, 13. Sternocleidomastoid, 14.Tendinous arch. **Right:** Enlargement of region of interest.

Microscopic observations

The neural identity of all sampled nerves was confirmed in HE-stained slides as they all represented nerve specific morphological characteristics (Fig. 1). Some nerves were composed of various fascicles within one surrounding epineurium (Fig. 1), whereas other nerves were composed of multiple discrete fascicles, each with their own epineurium. All nerves contained sensory nerve fibers but their number was highly variable (Table 1). The median amount of sensory nerve fibers was 2.34% with an interquartile range of 0.91-2.84.

Quantitative microscopic data on the presence of CGRP-IR nerve fibers in non-supraclavicular nerves are expressed in Table 1. For each nerve the % area of CGPR-IR nerve fibers with respect to the total area of neural tissue is listed. If a nerve was composed of multiple smaller nerve branches, each smaller branch was analyzed separately and the data was pooled and then the %area was calculated.

DISCUSSION

This study shows that in two out of six cases, lateral and intermediate supraclavicular nerves pierced the trapezius. Additionally, in one of these two cadavers, the medial supraclavicular nerve pierced through a tendinous arch formed by the trapezius. These potential entrapment locations of the lateral, intermediate and medial supraclavicular nerves correspond with the second, first and third additional BTX injection site (Blumenfeld et al., 2017), respectively. Since supraclavicular nerves contain sensory fibres (Drake et al., 2014), entrapment of these nerves by the surrounding muscle tissue might result in a pain trigger. This might explain some of the effectivity of BTX injections at these locations; the muscle relaxes and the nerve is no longer entrapped. The occurrence of supraclavicular nerves piercing muscles has been described previously (Macalister, 1871; Le Double, 1897; Jelev and Surchev, 2007). In these studies, nerves piercing the trapezius were mainly observed in conjunction with anatomical variations. These variations comprised a broader attachment of the trapezius to the clavicle which forces the supraclavicular nerves to pierce through it, or a tendinous arch formed over the supraclavicular nerves (Macalister, 1871; Le Double, 1897; Jelev and Surchev, 2007). Jelev and Surchev (2007) solely found one nerve piercing the lateral

part of the trapezius without any anatomical anomalies in one cadaver. Both previous and current findings show that supraclavicular nerve entrapment sites are rare, and, if present, they are mostly located in the medial or more lateral part of the trapezius, close to the acromion and above the clavicle. It is interesting to note that branches of the plexus-like formation consisting of cervical nerve branches and the accessory nerve were found entering the trapezius in all six cadavers. Immunohistochemical analysis confirmed that the cervical nerve branches of these plexus contained sensory nerve fibers, more specifically nociceptive fibers, since CGRP is known for staining nociceptive nerves (Patil et al., 2018). The presence of sensory nerve fibers in cervical branches has been described previously in both animals (Zhao et al., 2006) and humans (Tubbs et al., 2011). Both articles consider the fibers to be proprioceptive, although both studies did not use a specific proprioceptive staining. Based on the immunostaining used in the current study, we hypothesize that these nerves are potentially nociceptive and might detect pain within the trapezius region. Consequently, the muscle paralysis caused by BTX injections might result in less compression of these nerves. In this way, additional migraine/headache trigger reduction due to BTX injections in this region becomes plausible. The relief of migraine headaches after the release of extracranial sensory nerves might be explained by a theory on convergence mechanism (Piovesan et al., 2003). This theory suggests that pain registered by a CI or CII branch can excite the caudal part of the spinal trigeminal nucleus via the lateral cervical nucleus (Piovesan et al., 2003). This could explain why pain registered in the neck (by CI or CII branches) can be perceived as referred pain in areas normally innervated by the trigeminal nerve (Piovesan et al., 2003).

Table 1. Study measurements, including gender, age, length (*head till toe), width (**left acromion till right acromion).

Cadaver	Nerve	% Area CGRP-IR tissue	# nerve branches	Area neural tissue	Area CGRP-IR tissue
1	CII	2.57	1	84260	2169
	CIII	4.8	1	247575	10189
			2	114133	7171
2	CIII/CIV	0.93	1	244570	2268
3	AN/CII	0.9	1	22235	167
			2	45793	346
			3	160183	1734
			4	18367	282
			5	11345	16
	CIV	2.07	1	177382	3022
			2	54396	1776
4	AN	2.35	1	43954	969
			2	196799	3841
			3	89505	2762
			4	32712	975
	CIII	3.64	1	130363	4791
			2	88768	3194
5	AN/CII	0.28	1	61749	172
			2	142682	541
			3	8836	18
			4	49724	12
	CIV	1.05	1	98059	1034
6	CIII	2.2	1	130671	2880

Recommendations

As this study on a relatively small group of specimens was just an exploratory study, more anatomical dissection is needed in this region of interest. Moreover, clinical studies using BTX injections in patients who specifically point out the trapezius as additional trigger site should be performed to further evaluate its efficacy. Furthermore, next to evaluation of subjective symptom improvement, advanced imaging techniques might be used to objectify the degree of nerve compression. For example, using functional magnetic resonance imaging (fMRI) in patients before and after administrating BTX could be of great interest.

Limitations

The low number of included study specimens might be considered a limitation. However, even with this low number, this study clearly underlines previous observations on the heterogeneity of trapezius-piercing nerves. Another limitation was caused by the fragility of some small nerve branches, which resulted in less sampling. Despite these limitations, this exploratory study is a first step in identifying possible treatment options for pain triggers in the trapezius and provides support for further studies on the functional efficacy of trapezius BTX injections.

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AUTHORS'CONTRIBUTIONS

IC designed the study, performed the dissections and histological examination, interpretated the data, designed the figures and wrote the manuscript.

CC supervised the project, performed the histological examination, and helped writing the manuscript.

JR initiated, supervised the project and helped writing the manuscript.

RB initiated, supervised the project and helped writing the manuscript.

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