

Fibre types of human suboccipital muscles

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SUMMARY

Understanding the functional role of the cervical muscles is important for the effective diagnosis and treatment of cervical disorders. The suboccipital muscles are targets for treatment in whiplash and chronic headache, although their function remains unclear. There are no data on suboccipital muscle fiber type composition to facilitate an understanding of their function. Suboccipital muscles (n=95; rectus capitis posterior major, rectus capitis posterior minor, obliquus capitis superior, obliquus capitis inferior) were dissected bilaterally from 12 cadavers (6 male; mean age 81 years). Immunohistochemistry was used to identify type I/II muscle fibers. Fibers were counted using stereology (random systematic sampling) and data analyzed (descriptive statistics, ANOVA, paired and independent t-tests) to examine differences between muscles, sex and laterality ($p < 0.05$). Mean [SD] type I fiber proportion overall was 62.3% [10.9]; rectus capitis posterior minor had the smallest proportion of type I fibers (58.8% [9.5]), obliquus capitis inferior the largest (69.2% [10.5]). There were no significant differences overall between muscles or sides. There was a significant difference between sexes overall when data from the four muscles were pooled ($p = 0.027$), but no difference when muscles were compared separately. Individual suboccipital muscles showed similar type I/II fiber type proportions, suggesting homogenous function for muscles in this group. Fiber type composition indicated high levels of both postural and phasic activity. Conservative management of cervical disorders involving the suboccipital muscles

(e.g. exercise therapy) should consider the homogenous function of this muscle group, and include rehabilitation promoting both postural and phasic function.

Key words: Suboccipital muscles – Fiber type – Muscle function – Dissection – Immunohistochemistry

INTRODUCTION

Cervical spine pathologies involving chronic pain are estimated to affect 67% of the population during their lifetime, contributing to increased healthcare costs and decreased work productivity (Falla, 2004). Effective management and treatment of cervical pathologies is therefore of great financial and social importance, and a detailed understanding of the function and morphology of cervical spine structures is consequently required in order to effectively deliver targeted management and rehabilitation.

The suboccipital muscles (SOM) are a group of four muscles located posteriorly at the top of the cervical spine between the occiput, C1 and C2 (Fig. 1). They have been identified as playing a role in cervical pain, and are therefore a target for rehabilitation and intervention in some cervical spine disorders. They are of interest clinically (McPartland and Brodeur, 1999) as they have been identified as undergoing altered motor activity post whiplash (Bexander and Hodges, 2012), are said to develop myofascial trigger points (De las Penas et al., 2006), and contribute to both cervicogenic headache (Alix and Bates, 1999) and chronic tension type headache (De las Penas et al., 2007). They are also important for some surgical procedures of the upper cervical spine (Heros,

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1986). Despite the potential clinical significance of the SOM, there is little morphological data available to further our understanding of these muscles' function or to provide evidence supporting current rehabilitation strategies involving these muscles.

The SOM, rectus capitis posterior major (RCPMa), rectus capitis posterior minor (RCPMi), obliquus capitis superior (OCS) and obliquus capitis inferior (OCI) are said to be active during head extension (RCPMa, RCPMi, OCI) (Standring, 2008), ipsilateral head and neck rotation (RCPMa, RCPMi, OCI), and ipsilateral lateral flexion (OCS) (Standring, 2008; Moore et al., 2014). The OCI is also said to show an increase in electrical activity during contralateral rotation (OCI) (Bexander et al., 2005). Studies using electromyography have shown that activity in the RCPMi increases during voluntary head retraction while it becomes less active in a neutral posture (Hallgren et al., 2014), and the relationship between OCI and oculomotor activity has been shown to be altered in whiplash patients (Bexander and Hodges, 2012). They are innervated by the first branch of the cervical dorsal ramus (Bogduk, 1982), and their function is generally considered to be adjustment and stabilization of the skull, or in postural control (Standring, 2008).

There are no fiber type data from humans available to assist interpretation of SOM function. Previous investigations into the fiber type of the SOM in rhesus monkeys (Richmond et al., 1999, 2001) has demonstrated marked non-uniformity of fiber type distribution in all four muscles. The moderate to low proportions of type I fibres across all SOM muscles (RCPMa 54%, RCPMi 23%, OCS 33%, OCI 45%) indicate that in this primate model phasic activity is high and is the predominant activity in three of the four muscles (Richmond et al., 2001).

Some information is available on muscle spindle density and the general morphology of the SOM. Muscle spindle density of the SOM has been examined in a limited number of studies (Cooper and Daniel, 1963; Peck et al., 1984), with results indicating high levels of spindle density suggestive of a highly proprioceptive function. Spindles from the SOM have also been shown to have muscle-related differences in morphology, indicating potential specialization of function within cervical muscles (Liu et al., 2003). A myodural bridge forming a connection between the dura and the RCPMi (Hack et al., 1995; Scali et al., 2011), RCPMa (Scali et al., 2011), and OCI (Pontell et al., 2013) has been identified, suggesting a mechanical link between these muscles and the generation of some headache-types (Scali et al., 2011).

Fiber type analysis, where different fiber types are identified to determine proportions of extrafusal fibers throughout a muscle, provides data that contribute to a clearer understanding of muscles' roles and function (Johnson et al., 1973; Uhlig et al., 1995; Boyd-Clark et al., 2001; Cornwall et al.,

2011). Given the lack of data on SOM fibre type, it is unclear whether these muscles should i) be considered a functional 'group' that operates in concert, ii) be thought of as separate muscles with different individual functions, or iii) whether their actual function is 'postural' as described in anatomy texts (Standring, 2008; Moore et al., 2014). Such data would enable improved planning of conservative treatment and rehabilitation, contribute information relevant to biomechanical modelling and electromyography studies, and further elucidate the function of the SOM. This study therefore aimed to examine the fiber type distribution of the SOM to provide such information.

MATERIAL AND METHODS

Human cadaveric tissue was accessed through the Department of Anatomy, University of Otago, Dunedin, New Zealand. All tissue was obtained from donated cadavers, in accordance with the Human Tissue Act (2008), and utilized in line with local institutional ethical requirements and the Declaration of Helsinki. The upper cervical spines of six male and six female cadavers (embalmed with an alcohol-based mix) were dissected; the RCPMa, RCPMi, OCS and OCI muscles were identified, then removed (bilaterally) and placed in individual, labelled containers containing neutral buffered formalin prior to processing. Male cadavers had a mean age 79.2 years (SD 6.9, range 67-85), and females had a mean age of 82.8 years (SD 8.6, range 69-93), with an overall mean age of 81 years (SD 7.7) (Table 1).

Immunohistochemistry

Muscles were removed from containers and a

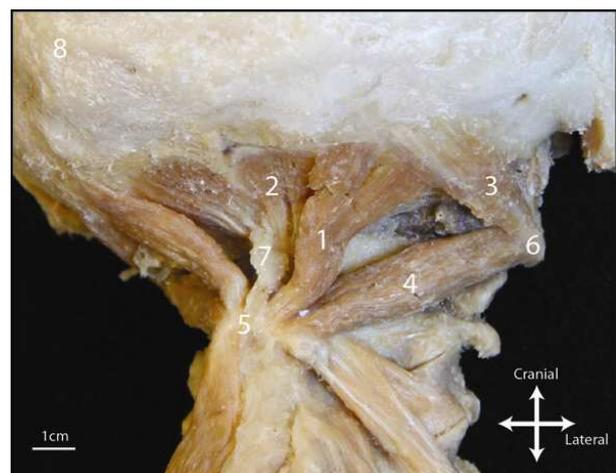


Fig. 1. Individual suboccipital muscles (right side) identified on a plastinated specimen. The suboccipital muscles viewed posterolaterally from the right: 1. Rectus capitis posterior major; 2. Rectus capitis posterior minor; 3. Obliquus capitis superior; 4. Obliquus capitis inferior; 5. Spinous process of C2; 6. Transverse process of C1; 7. Posterior tubercle of C1; 8. External occipital protuberance.

Table 1. Information on cadaver sex and age

Specimen number	Sex	Age
1	M	85
2	M	76
3	F	85
4	M	67
5	F	69
6	M	85
7	M	83
8	F	93
9	M	79
10	F	89
11	F	84
12	F	77

M: male; F: female.

transverse tissue block approximately 4mm wide removed from the midpoint of each muscle. This tissue block was paraffin embedded before 5µm sections were cut and mounted on slides. Tissue was then processed using a protocol adapted from Cornwall et al. (2011). Briefly, tissue was run through a series of graded xylene and ethanol baths prior to undergoing antigen retrieval via microwave at 95°C in citrate buffer (6.0 pH) for 22 minutes. Tissue sections were incubated with anti-type I (NOQ7.1.1A, mouse monoclonal antibody; courtesy of Dr Robin Fitzsimons, dilution 1:100) and anti-type II (MY32, mouse monoclonal antibody, identifies all type II fibers and sub-groups, Sigma, MO, dilution 1:200) antibodies overnight before being incubated with secondary antibody (EDL, undiluted, Dako Envision Link System) for 30 minutes at room temperature. Slides were the dehydrated through graded ethanol and xylene baths before having coverslips applied and being stored for examination (Fig. 2).

Stereology

Random systematic sampling of whole sections was performed to estimate the proportion of fiber type numbers. An Aperio Scanscope CS2 (Leica Biosystems, Auckland, New Zealand) scanned slides at x20 magnification; resulting images were montaged using Adobe Illustrator (Adobe Systems, San Jose, CA). Montaged images had a numbered grid superimposed to allow random systematic sampling of at least 7% of the area of each section to provide a similar or higher fiber count than other work examining fiber type proportions (Peck et al., 1984).

Statistical analysis

Count data were entered into a Microsoft Excel spreadsheet (Microsoft Corporation, Los Alamos, WA), then STATA v12.1 (StataCorp, College Sta-

tion, TX) statistical software was used to analyse data and generate descriptive statistics. A one-way ANOVA with *post hoc* Sidak test was used to compare fiber type proportions between muscle types (e.g. between RCPMa and RCPMi) from data for each individual muscle pooled from both sides of each cadaver (left and right side data pooled), and between cadavers (data for all four muscles pooled for each cadaver). A paired t-test was used to compare the proportion of fibers between sides (left versus right), and independent t-tests were used to examine potential differences in fiber type proportion between the sexes (overall, and for each individual muscle, with data from left and right sides pooled) and differences in age of cadavers between the sexes. Significance was $p < 0.05$ for all analyses.

RESULTS

In total 95 muscle sections were successfully processed. One muscle block was destroyed during processing (OCS from the right side of one female cadaver). Therefore, fiber data were generated from 24 RCPMa, 24 RCPMi, 23 OCS and 24 OCI muscles.

Overall, the total numbers of fibers counted was 196,269 (2066 / slide) including 121,442 type I (1278 / slide) and 74,827 type II (788 / slide) fibers with an average of 24.2 points systematically and randomly sampled from each of the 95 muscle sections. The proportion of type I fibers is presented with the proportion of type II, being the inverse of the percentage of type I (e.g. 60% type I indi-

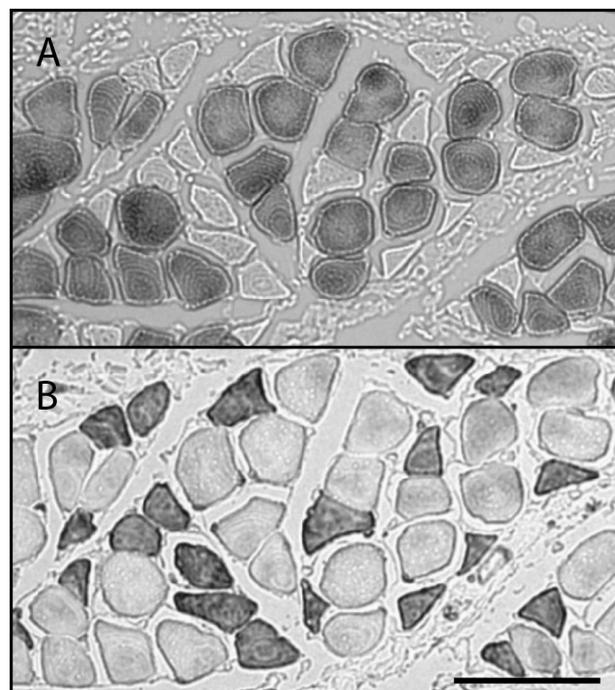


Fig. 2. Photomicrograph of a section of obliquus capitis inferior processed using immunohistochemistry to identify (A) type I and (B) type II muscle fibers. Scale bar, 50 micrometers.

cates 40% type II). Standard deviations are presented in square brackets following means.

Descriptive statistics

The mean [SD] type I fiber type proportion for all SOM was 62.3% [10.9]; data for individual muscles are presented in Table 2 and Figure 3. OCI had the largest proportion of type I fibers (69.2% [10.5]) while RCPMi had the smallest (58.8% [9.5]). Fiber type proportions for type I fibers ranged from 40.9% (one male RCPMa) through to 92.7% (one female OCI). Between sexes the overall means were 58.9% [8.9] for males and 65.8% [11.7] for females.

Significance testing

There was no significant difference between type I fiber proportions for any SOM, although there was a trend towards significance (p=0.08). There was no significant difference in the type I fiber proportions between the left and right side. A significant difference was observed in the proportion of type I fibers between cadavers (p<0.0001), with type I fiber proportions from one cadaver (female, 93 years) being significantly different to six others. There was a significant difference in type I fiber proportions between males and females (p=0.027) with all data pooled, yet there was no significant difference between any of the muscles when each were individually compared by sex. There was no significant difference in age between the two sexes (p=0.43).

DISCUSSION

This study is the first to provide data on SOM fiber type, with results indicating that these muscles have similar fiber type composition to each other (e.g. are likely homogenous in activity) and are not exclusively postural in function. Findings indicate that SOM contributed to a greater amount of postural than phasic activity during daily activity in our elderly sample, and should be considered a 'group' rather than individual muscles with exclusively different functions, in this age group at least.

Descriptive data

Fiber type proportions in this study provide infor-

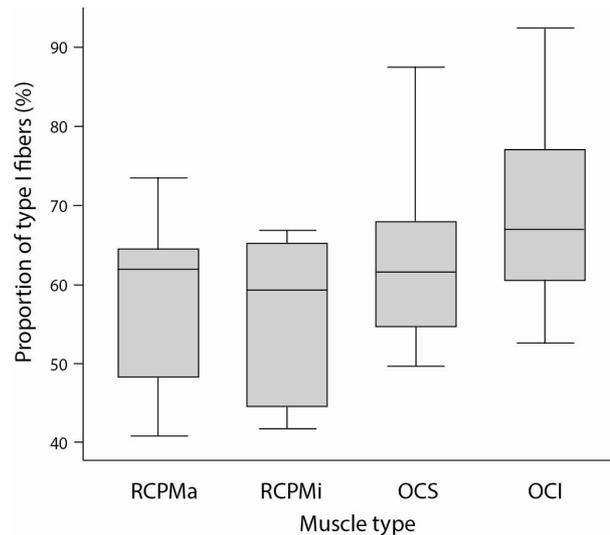


Fig. 3. Box plot of type I fiber proportions for individual suboccipital muscles. RCPMa: rectus capitis posterior major; RCPMi: rectus capitis posterior minor; OCS: obliquus capitis superior; OCI: obliquus capitis inferior.

mation about the basic function of the SOM in an elderly population. The average fiber type of the SOM was 62.3%, with a range of average values across the four muscles of 58.8% (RCPMi) to 69.2% (OCI) (Fig. 3). This indicates that while all SOM are predominantly comprised of type I fibers, they are not highly postural muscles like the soleus with 87.7% type I fibers (Johnson et al., 1973). The SOM are similar, and in some cases lower in type I fiber proportion, than muscles that are traditionally considered 'phasic' such as biceps femoris (66.9% type I fibers) (Johnson et al., 1973), though they are higher in type I fiber proportion than triceps brachii (32.6%) and orbicularis oculi (15.4%), muscles which have an extremely low type I fiber proportion and are highly phasic. It is possible the type I proportion of these muscles is lower in a younger population, given that sarcopenia increases the proportion of type I fibers in the elderly, meaning in a younger population these muscles may be more phasic than postural. Based on our findings, textbooks (Standring, 2008; Moore et al., 2014) could be revisited to further clarify their descriptions of SOM function as not exclusively pos-

Table 2. Mean, standard deviation (SD) and range observed for proportions of type I fibers identified in 95 suboccipital muscles from 12 cadavers (six male, six female).

Muscle	Proportion of type I fibers (%)			
	Mean	SD	Minimum	Maximum
RCPMa	60.1	8.9	40.9	73.6
RCPMi	58.8	9.5	41.7	66.9
OCS	61.3	12.3	49.7	87.6
OCI	69.2	10.5	52.9	92.7

RCPMa: rectus capitis posterior major; RCPMi: rectus capitis posterior minor; OCS: obliquus capitis superior; OCI: obliquus capitis inferior.

tural.

The results for SOM type I fiber proportions were similar to those reported for spinal muscles longus colli of 52.8% (Johnson et al., 1973) and 69.1% (Uhlrig et al., 1995), although lower than the cervical multifidus (77%) (Boyd-Clark et al., 2001) or thoracolumbar transversospinal muscles (89%) (Uhlrig et al., 1995). Findings indicate that the SOM may play a dual role by contributing to both postural and phasic movements in the upper cervical spine, perhaps like the anterior cervical muscle longus colli, given their similar fiber type proportion values. Data also suggest OCI has a larger fiber type range and higher mean and quartiles than all the other muscles (Fig. 3); further data are required in order to see whether this trend is apparent in all age groups and not just the elderly group sampled.

Significance test outcomes

No significant variation was seen amongst the SOM, indicating a similarity in function. However, there was a trend towards significance ($p=0.08$) that may suggest a potential relationship. Further data are required to determine whether this trend is consistent among different age groups and populations, and a larger sample size may be required to assist our understanding of fiber type composition.

There was a difference in the overall SOM fiber type composition between cadavers, with one cadaver being significantly different to half the total sample ($p<0.0001$). No other differences were noted between any cadavers, suggesting that there is the potential for muscle fiber type proportions for varied fiber type proportions in elderly SOM. It is important that clinicians and scientists are aware that these differences exist when planning and undertaking research involving functional assessment of the SOM in an elderly population.

A significant difference in type I fiber proportions was observed between males and females when all data were pooled ($p=0.027$), indicating that there may be functional differences in the SOM between elderly males and females. This difference was no longer apparent when each muscle was individually compared by sex, suggesting that overall SOM function may be different between males and females in regards to how these muscles function as a group. Clinicians and investigators should be made aware that such sex differences may exist in SOM function when examining these muscles in clinical practice.

Limitations

This study only used elderly cadavers, and therefore the results may not be generalizable to younger populations; muscle sections from the cervical spine of younger populations are exceptionally difficult to procure, given the average age of body donors is usually greater than sixty years

(Cornwall et al., 2012). Any future studies of fiber type in these muscles that aimed to utilize biopsy samples as an alternative methodology is unlikely due to their size and difficult access, meaning that these data are a useful platform on suboccipital fiber type from which additional data on potential sarcopenic change will likely prove useful, should data from younger donors ever become available. In addition, medical notes were not available, and therefore it is unclear whether any had pre-existing cervical pathologies that may have affected data from the cervical spine muscles. We are also unable to report on pre-death activity levels of the donated cadavers, which may also affect data. The number of cadavers sampled was limited, yet despite this the sample size for each muscle is similar to other work examining spinal muscle function (Boyd-Clark et al., 2002) and morphology. The fiber counts performed are higher than previously published investigations assessing and quantifying fiber type in humans (Johnson et al., 1973; Uhlrig et al., 1995; Boyd-Clark et al., 2001), therefore providing robust data from those cadavers examined. Ethanol-embalmed cadavers were used and it is possible muscle cells did undergo some shrinkage as a result (Brown et al., 2002). However, any such morphometrical alterations would not change cell numbers and therefore it is unlikely fiber counts were affected.

Conclusions

This study provides the first data on fiber types of the SOM. Findings of our investigation indicate that these posterior, upper cervical spine muscles are a functionally homogenous group that are likely responsible for mostly postural but also phasic movements of the upper cervical spine in elderly populations. Some significant differences between fiber type proportions were noted between sex overall, but not when muscles were individually compared, and RCPMa proportions were different for side though these differences were not apparent when all muscle data were pooled. Further work examining SOM fiber types is necessary in younger populations to develop knowledge of SOM function and morphology across different age groups, and to examine whether fiber-type differences across sex and laterality exist in different populations.

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