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Tissue motion pattern of ventral neck muscles investigated by tissue velocity ultrasonography imaging

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Abstract

We designed this experimental study to investigate tissue motions and thus infer the recruitment pattern of the ventral neck muscles (sternocleidomastoid (SCM), longus capitis (Lca), and longus colli (Lco)) at the C4–C5 level in healthy volunteers during isometric manual resistance of the head in flexion in a seated position. This exercise is used in the physiotherapeutic treatment of neck pain and is assumed to activate the deep ventral muscles, but the assumption has not been clearly evaluated. Neck flexors of 16 healthy volunteers (mean age 24 years, SD 3.7) were measured using ultrasonography with strain and strain rate (SR) tissue velocity imaging (TVI) during isometric contraction of flexor muscles. TVI involves using Doppler imaging to study tissue dynamics. All three muscles showed a deformation compared to rest. Except for the initial contraction phase, Lco exhibited a lower strain than Lca and SCM but was the only muscle with a significant change in SR between the phases. When the beginning of the contraction phase was analysed, Lco was the first to be deformed among most volunteers, followed by Lca and then SCM. The exercise investigated seems to be useful as a “stabilizing” exercise for Lco. Our suggestion is that in further research, Lco and Lca should be investigated as separate muscles. TVI could be used to study tissue motions and thus serve as an indicator of muscle patterning between the neck flexors, with the possibility of separating Lco and Lca.

Keywords: Neck muscles, Ultrasonography, Coordination, Recruitment pattern
Introduction

The neck muscles predominantly (about 80%) provide the mechanical stability of the cervical spine (Panjabi et al. 1998). Conley et al. (1995) reported the main flexors of the neck to be the deep muscles, longus colli (Lco) and longus capitis (Lca), and the superficial sternocleidomastoid (SCM). Several authors (Falla et al. 2004a, b; 2006; 2007a; Johnston et al. 2008; Jull et al. 2004a; O’Leary 2007) have described an association of disturbed performance of the neuromuscular coordination among neck flexors and chronic neck pain in people.

Activation of the deep ventral neck muscles or coordination between the deep and surface flexor muscles in different tasks or situations has been studied with surface electromyography (EMG) (Falla et al. 2004a, 2006, 2007a; Jull et al. 2004a; O’Leary et al. 2007). Functional (T2-weighted) magnetic resonance imaging (MRI) (Cagnie et al. 2008; Conley et al. 1995), computerized tomography (CT) (Mayoux-Benhamou et al. 1994), and ultrasonography (US) (Cagnie et al. 2009; Jesus et al. 2008) have been used to study differences in signal activity or tissue deformations. With EMG, deep ventral neck muscles have been investigated invasively by surface electrodes inserted via the nose and fixed to the posterior mucosa of the oropharynx, which “indirectly” captures signals of interest from Lco and Lca, with some risk of cross-talk from other muscles. US is non-invasive, less expensive than CT and MRI, and offers the possibility of capturing functional movements in real time.

Traditional US (using the grey-scale technique), which was used in earlier studies of ventral neck muscles (Cagnie et al. 2009; Jesus et al. 2008), provides information about anatomical landmarks, giving data about the structure of the tissue, fascicle length, and pennation angle (Blazevich et al. 2006; Chino et al. 2008; Fukunaga et al. 1997; Kawakami et al. 2006; Narici
1999; Oda et al. 2007). Muscle deformation in ventral neck muscles (Cagnie et al. 2009; Jesus et al. 2008) has been studied during measurement of the change in cross-sectional area (CSA)/muscle thickness during rest and contraction.

US in conjunction with tissue velocity imaging (TVI) makes it possible to identify active and passive muscular movements (Brodin 2004; Grubb et al. 1995; Mannion et al. 2008; Peolsson et al. 2008; Pulkowski et al. 2008). According to Mannion et al. (2008), TVI could be used to study muscular feed-forward activity, and according to Pulkowski et al. (2008), to study the onset of muscle activation. TVI could also be used to differentiate muscle dynamics between patients with muscular disorder and healthy controls (Peolsson et al. 2008). TVI has not, according to our knowledge, been used to study activation patterns of ventral neck muscles.

Strong evidence suggests the effectiveness of physical exercise in the rehabilitation of patients with neck disorders (Kay et al. 2005), but a consensus is lacking regarding how to optimise the exercise. A specific exercise of the deep ventral neck flexors (Jull et al. 2004b) is reported to reduce pain in patients with neck pain (Falla et al. 2006). Physiotherapists (PTs) use manual isometric resistance of the head in flexion in the seated position in the treatment of patients with neck pain, which is assumed to activate the deep ventral neck muscles; however, the truth of this assumption remains to be established. Thus, more knowledge is required about the activation patterns in the ventral neck muscles in the clinical setting.

The purpose of this study was to use US with strain and SR TVI techniques to describe and investigate tissue motions within each muscle (SCM, Lca, and Lco). The goal was to identify the recruitment pattern of the ventral neck muscles at the C4–C5 level in healthy volunteers during manual resistance of the head in flexion in the seated position.
Methods

Participants

The inclusion criterion for this study was a healthy neck. Exclusion criteria were pain >10 mm (Croft et al. 1998) on the Visual Analogue Scale (VAS) (Scott and Huskisson 1976) or discomfort (>20%) (Fairbank et al. 1980) on the Neck Disability Index (NDI) (Vernon and Mior 1991) from the neck during the last 3 months, repeatable neck discomfort, using any kind of medical treatment for neck discomfort, trauma to the neck, severe back pain, generalized myalgia, or the presence of rheumatologic or neurological diseases.

All participants were university student volunteers (responding to a group mailing) with the following characteristics: 11 women and 5 men, mean age 24 years (SD 3.7, range 19–33). They were without neck discomfort; pain on VAS averaged 1 mm (SD 2.1, range 0–8) and the NDI average was 0.8% (SD 1.2, range 0–4). They had a high level of physical activity (mean 3.5, SD 0.6) on a 4-point scale (Peolsson et al. 2007). All participants, except one woman, were right handed.

Identification of the ventral neck muscles

US was performed on the right side at the C4 segmental level. C4 was identified by palpation by an experienced PT and marked with a pen. Apart from manual palpation to identify the segmental level, the bifurcation of arteria vertebralis, commonly at the C4 level (as seen with the US), verifies measurements at the segmental level of interest. The C4 level was also noted on US by way of the horizontal projection plane of the PT’s marker and the fork of the spinosis (Fig. 1). The probe was rotated around its axis 90 degrees from the transversal (axial) to longitudinal (sagittal) projection (Fig. 1) to provide an optimal image plane for post-process analysis. All registrations were taken in a longitudinal projection. Pulkovski et al.
(2008) reported a good reliability (intra-class correlation coefficient (ICC), 0.67–0.99) for onset of muscle activation using TVI and a good correlation for onset of muscle activation using TVI and EMG (r=0.78–0.80). The procedure used in the present study has also proved to have good reliability for ventral neck muscles (ICC, 0.80–0.99) (Table 1) (Peolsson A, unpublished data). The C4 segmental level was chosen to optimise the image field for studying tissue motions and thus infer the coordination between the three muscles in the mid-cervical spine. At the C4 level, we captured different parts of the Lco, Lca, and SCM. If the registrations had been performed above the C4 level, we would have been unable to capture the SCM; projections below C5 make it difficult to get clear pictures because of shadows.

![Image of anatomical landmarks](image)

Fig. 1 Anatomical landmarks of ventral neck muscles, from the superficial towards the deep muscle layer: sternocleidomastoid (SCM), longus capitis (Lca), and longus colli (Lco) at the C4–C5 level

Test procedure

The experimental set-up was designed to be similar to the clinical setting. Volunteers took a seated position on a stool in an erect posture, with the feet flat on the floor and resting their arms on their legs. They were instructed to perform a small ventral flexion in the upper neck. The PT held her right hand on the participant’s forehead and instructed the participant to do a
sub-maximal isometric head flexion for about 8 s, as PTs do in neck rehabilitation (Fig. 2). The PT gently increased the resistance in the beginning of the procedure and gradually decreased it towards the end. Before all measurements, participants were informed about the procedure and practiced it once.

Fig. 2 Test position of the submaximal head flexion during manual resistance
Equipment used

To measure deformation variables, an Ultrasound Vivid 7 Dimension system (GE Healthcare, Horten, Norway) was used together with a 14-MHz linear transducer and research software. The US images were analysed post-process using an external software package (EchoPac, GE Healthcare, Horten, Norway) for offline analysis.

TVI

TVI offers two fundamental calculating parameters: tissue movement and tissue deformation (D’Hooge et al. 2000; Heimdal et al. 1998; Mirsky et al. 1973; Stoylen 2001). The difference between the two is whether a velocity alteration is present during the movement. If a constant force affects a tissue, the velocity parameter will remain constant, while an accelerating or decelerating force affecting the tissue causes a tissue deformation. Consequently, the tissue response will be either compression or elongation.

In the musculoskeletal case, several anatomical muscles often lie close to each other, separated by a fascia. To study the regional deformation of each separate muscle layer and thus to indicate the corresponding coordination pattern, regional velocities are required. Strain rate (SR) (D’Hooge et al. 2000; Heimdal et al. 1998) is such a tool. With this tool, the image lines are divided at a specified measuring distance, offset by, for example, 5 mm, generating a calculation grid (Fig. 3A). Within each and every grid, cell velocities are calculated (Fig. 3A). The implication of this procedure is that tissue velocities are measured independently within local regions. As a consequence, different muscle layers will be covered by different cells. Regional SRs are calculated within each and every grid cell (Fig. 3B). When compared, the relative differences between the cell velocities can be investigated, and the result can be transformed into colour-coded, superimposed images.
Strain is described as the momentary tissue deformation expressed in percent compared to rest, i.e., the change in muscle tissue length (Mirsky et al. 1973). Negative strain thus signifies compression, and positive indicates elongation. In the colour-coded images, the degree of blue represents the degree of compression/contraction, green and yellow indicate more passive tissue segments because of low regional velocities, and the degree of red represents elongation/relaxation.

Fig. 3  a The principle of global velocity (TVI). Velocities were calculated along the vertical image lines. b The principle of regional velocities (strain rate). The image lines were divided according to a chosen offset (6 mm), resulting in a measuring grid map. Strain rate were calculated within each and every cell
The final result is generation of a regional tissue deformation map, presented frame by frame using these four colours (Fig. 3). The map also adds information about changes in the degree of deformation (strain) and deformation rate (SR) as part of muscle deformation in real time. Thus, the colour-coded images present qualitative information about tissue motions as an indicator for inter-muscular coordination patterns during neck movements.

Quantification of tissue velocities from velocity images

To investigate tissue movements as an indicator of the coordination of the neck muscles quantitatively, regions of interest (ROIs) are often manually placed within the images. A ROI is a shape that can be drawn and placed arbitrarily. In this study, an ellipsis was chosen that captured the centre part of the Lco, Lca, and SCM. The ROI was placed in the first frame of the loop and then followed frame by frame in the video sequence. Within each ROI, average SR and strain values were calculated frame by frame. As a result, the relationships between the individual ROIs were delineated and are presented as one curve per ROI, as a function of individual neck muscle deformation progression in real time (Fig. 4). Three variables were calculated from the quantitative curves: RMS for SR and mean and maximal values for strain.

Definition of tissue motions as an indicator of activation order

To analyse tissue motions as an indicator of the order of muscle activation, three ROIs were inserted into the LCa, LCo, and SCM, respectively, in the US grey-scale loop and coloured individually (Fig. 4A). The ROIs followed the same tissue region during the whole registration of the functional movement in real time. Fig. 4A contains the grey-scale US loop (video). Figure 4B shows the corresponding colour-coded overlaid transparency video, which contains the tissue velocity data (SR). The changes in the tissue velocity data were captured in
real time and thus alter as a function of tissue alteration in the muscles as the movement progresses. The tissue velocity alterations are presented as shifting colours and represent the characteristic of the tissue movement correlated with the progress of the neck movement.

Fig. 4 Quantification of inter-muscle tissue motion patterns in the neck. a The image shows three regions of interest (ROIs) inserted into the separate muscles, sternocleidomastoid (SCM), longus capitis (Lca), and longus colli (Lco). b The image presents the calculated curves, each colour curve (presented in the online version/shades of grey in the printed version) showing the resulting deformation rate of the separate muscles during exercise (strain rate). c The right part of the image shows the corresponding result according to the degree of deformation rate (strain rate).

Fig. 4C presents the quantitative result of the strain rate registrations during the neck movement, where the coloured curves correspond to the mean value of strain rate within the colour-coded muscle regions in 4A and 4B. The vertical red line is the time reference. It can be used to investigate the correspondence between the colour shifts in 4B and the time point in the resulting curve. The curves represent the mean values of the calculated strain rates within the ROIs as a function of time (ms). As an example, Fig. 4B images a specific time point and shows that the tissue within Lca (blue coded ROI) is coloured deep blue,
representing higher strain rates (positive velocities represent contraction) while Lco (yellow coded ROI) is coloured yellow/orange, representing low velocities and low activity. This representation can also be seen in Fig. 4C where the blue ROI curve shows a larger strain rate while the yellow alternates around the zero baseline (close to zero). The slopes of the curves thus represent the magnitude of an increase (positive) or decrease (negative) in the tissue velocity and the relationship between the tissue velocities in regard to the specific muscle regions (LCa, LCo, or SCM). As a result, the curves can be used to decide the order of deformation rate based on the initiation, direction, and magnitude of each muscle segment. The vertical time line in Fig. 4C thus shows the deformation rate relationship among LCa, LCo, and SCM. In this particular individual, LCa was contracted before LCo while SCM was not contracted simultaneously.

Definition of the three phases

Three phases were identified based on SR curves during the isometric resistance exercise. The first phase was derived from tissue motions indicating the activation of the neck muscles as a consequence of maintaining the head position during the initial manual resistance compared to the neck muscles at rest. This phase is indicated by a rapid increase in activity in the SR curve and is labelled ‘the initial contraction phase’. The second phase refers to the maintenance of the muscle tension during the external hand-held resistance. The SR curve is characterized by a flat, low activity level, indicating that the deformation has reached a ‘steady-state level’. This phase, called ‘the plateau phase’, is indicated by a horizontal SR curve. The third phase is characterized by a letting-go of the external hand-held resistance and thus is labelled ‘the relaxation phase’, as indicated by an inverted SR curve compared to the initial contraction phase.
Statistical analysis

For descriptive statistics, median and interquartile range were used. For paired tests (of patterns in the same sequence of movement of the same muscle), Friedman’s test (three-group analysis) and the Wilcoxon signed-rank test (two-group analysis) were used. For unpaired tests (between the muscles), the Kruskal-Wallis (three-group analysis) and Mann-Whitney U tests (two-group analysis) were used. A $P$ value of $\leq 0.05$ was considered to be statistically significant.
Results

Muscle deformation and deformation rate

Muscles did not differ with regard to SR either in the total sequence or in the phases (Table 2). Except for maximum strain in the relaxation phase, Lco had lower mean and maximum strain values in the total sequence as well as in the plateau and relaxation phases compared with Lca and SCM ($P = 0.0006–0.05$) (Table 2). We identified no significant differences between Lca and SCM in either of the phases or during the total sequence (Table 2).

Differences in the three phases

Lco had a higher SR in both the contraction and relaxation phases compared to the plateau phase ($P = 0.003–0.03$) (Table 3). Except for mean strain of Lco and maximum strain for Lca, which did not differ among the phases, the strain was higher in the plateau phase than in the contraction phase ($P = 0.0005–0.02$) (Table 3). For Lca, the mean strain in the plateau phase was also higher than in the relaxation phase ($P = 0.03$) (Table 3); otherwise, there were no differences between plateau phase and relaxation phase (Table 3). In the relaxation phase, mean strain for SCM and maximum strain for Lco were higher than in the contraction phase ($P = 0.008–0.02$) (Table 3).

Tissue motions as an indication of the order of muscle recruitment

When tissue motions were analysed as an indicator of the most common order of muscular recruitment according to the initiation of the contraction phase, Lco was the first muscle to be deformed in most volunteers prior to Lca (Table 4). Lco and SCM differed significantly ($P = 0.03$), and Lco and Lca showed a trend to a difference ($P = 0.09$) (Table 4). SCM was the last muscle to be deformed in most volunteers (but non-significant vs. Lca, $P = 0.68$) (Table 4).
Discussion

Summary of results

All three muscles exhibited a change in deformation during the ventral isometric contraction compared to rest. Despite great inter-individual variation and a small population, some patterns and differences among muscles emerged. They did not differ significantly with regard to SR either in the total sequence or in the phases. However, when the start of the contraction phase was analysed, Lco was first to be deformed among most volunteers followed by Lca and then SCM. Compared to Lca and SCM, and except for the contraction phase, Lco had the lowest strain but was the only muscle with a significant change in SR between the phases. One possible explanation for these results is that even if the exercise was isometric, the PT applied and stopped the manual resistance gently, causing a small movement in the start and end phases that elicited an Lco reaction. Such a conclusion could be interpreted as confirmation of, in particular, Lco as a sensitive stabilizer in the cervical spine (Boyd-Clark et al. 2002; Falla et al. 2007; Mayoux-Benhamou et al. 1994). Another explanation for the results could be different biomechanical parameters, such as the length of each muscle, the locations of origins and insertions, and/or the location where the force was applied relative to the muscles; however, these parameters could not be measured with the methodology used here.

Different tissue motion patterns of the three muscles

Previous authors (Conley et al. 1995; Falla et al. 2004a, c; 2006; 2007a; Jesus et al. 2008; Jull et al. 2004a; O’Leary et al. 2007) often referred to Lco and Lca as “deep ventral neck muscles” and analysed the two together as one entity. In the present paper, the synergists Lco and Lca emerge as having different tissue motions, indicating different activation patterns. This finding verifies the results of Cagnie et al. (2008), who concluded that it is necessary to
distinguish between the two. The primary function of Lca is as a craniocervical flexor (Cagnie et al. 2008). Lco, on the other hand, has been reported to have a high rate of muscle spindle distribution (Boyd-Clark et al. 2002) and primarily serves a stabilizing and postural function with a more important role for the cervical curvature than Lca (Falla et al. 2007b; Mayoux-Benhamou et al. 1994).

Today there is no consensus about whether the deep flexor muscles or the SCM have the higher activity during cervical flexion (Conley et al. 1995; Falla et al. 2006; Jesus et al. 2008; Vasavada 1998). This lack of agreement could be the result of different experimental set-ups with different study designs, purposes, participant positions, and exercises. In the present study, using a sitting position and an exercise that differed from those previously examined, Lco had lower strain than the other muscles, but the differences between Lca and SCM were not significant.

Present results compared with earlier studies
No one has, to our knowledge, used US with TVI to study tissue motions as an indicator of ventral neck muscle function. Jesus et al. (2008) and Cagnie et al. (2009) have presented data using US, albeit with the grey-scale technique. Jesus et al. (2008) studied changes in CSA between rest and activity in the supine position and found a greater thickness in the later phases than in the starting phase, both for the deep muscles and for SCM. Cagnie et al. (2009) measured CSA of Lco by US and the “gold standard” MRI in the supine position and found a moderate validity (ICC 0.68–0.71) of US; in addition, they found with both techniques an increase in CSA in Lco during activity compared with rest. In the present study of tissue dynamics, a higher deformation in the later phases was found in Lca and SCM. The deformation of Lco was mostly constant during the phases, possibly because of its postural
function (Falla et al. 2007b; Mayoux-Benhamou et al. 1994) in an upright position. An explanation for the differences among the studies could be the different experimental set-ups with the different US methods used and the different study purposes. All measurements were done on healthy young adults. Jesus et al. (2008) did not differentiate between Lco and Lca, and Cagnie et al. (2009) studied Lco only. Jesus et al. (2008) and Cagnie et al. (2009) performed their measurements with volunteers in the supine position; in the present study, volunteers were in a functional upright position.

Cagnie et al. (2009) discuss the difficulties associated with boundaries of Lco that are not always distinct when measuring CSA of Lco with grey-scale US. They suggest that it would have been helpful to watch the muscle contraction before taking measurements at rest. In the present study using US with TVI, tissue dynamics during the contraction were studied but not CSA. It is possible, however, that in the present study, a falsely low SR and strain of Lco were captured because when the ROI of Lco is placed over two vertebral levels (C4 and C5), the bony structure will be a part of the ROI. To avoid the bony structures, an alternative analysis of several smaller ROIs summed together was done, but the outcome did not change; thus, we infer that the results remain trustworthy.

Representativeness of the population
Young adults only were included in the present study, which could be a drawback in terms of how representative the results are. Using MRI, Elliot et al. (2007) found a weak correlation between age and relative CSA of neck extensor muscles at the level of C5. Rankin et al. (2005) presented reference values of neck extensor muscle size and found muscle size to be altered by gender but not by age. One can presume that this association is true also for the
neck flexors, and we believe that our population can be considered representative as an indicator of muscle function in healthy individuals.

Only speculation is possible about whether or not the tissue motion patterns would have been altered in patients with neck disorders compared to healthy participants, as a result of lowered tonic deep muscle activity and higher activity in superficial torque-produced muscles (Falla 2004a, 2006, 2007a; Johnston et al. 2008; Jull et al. 2004a). Future studies involving patients with neck disorders would be required to address this question.

Manual resistance

To imitate the clinical rehabilitation technique used by PTs, a submaximal non-standardized resistance was used in the present study. In patients with severe neck pain, trying one repetition maximum in isometric extension could be contraindicated because of pain, fear of movement, and high segmental load (Choi et al. 2000). The results of the study could have been different if a different load, i.e., maximal resistance, had been applied. SCM especially, with its great moment-generating capacity (Vasavada 1998), could have behaved differently. The results could also have been altered if measurements had been done at a different segmental level. Additionally, use of a strain-gauge to control for potential differences in resistance might have been advantageous.

TVI: strain and SR

SR is a parameter that is arguably related to the rate of contraction while strain can be viewed as a description of the consequence expressed in terms of muscle expansion. On the one hand, the colourization of these parameters gives visually qualitative information about the progression of the specific muscle deformations as well as the time-related inter-muscular
coordination pattern during exercise. On the other hand, the curves give quantitative graphical information about these items. The benefit of strain and SR, therefore, was that they identified information about regional tissue deformation relative to nearby tissues. A drawback was that all deformations were angle dependent; i.e., tissue velocities were measured in an axial direction to the probe and not along the fascicles. Taking for perspective the principle of muscle volume incompressibility, which states that if a muscle is shortened (contracted) in one dimension, the muscle volume has to expand in the other two dimensions, the measurements carry relative information that could not be used as absolute values. Another drawback is that TVI provides an indirect measure of tissue movement patterning of the ventral neck muscles. “Activation” and “recruitment pattern” are neurophysiological terms and imply that electrical parameters were monitored (i.e., EMG). In this study, TVI provided a method to assess tissue motion patterns within various muscles, which is argued to be an indicator of tissue contraction patterning. However, because of the lack of possibility for direct and non-invasive registrations with surface EMG of deep ventral neck muscles, TVI seems to be useful.

As with all medical-imaging work, the question of quality needs to be considered concerning image resolution and adequate anatomic imaging. This consideration is also necessary when performing the time-consuming post-process analysis of TVI. Yet, we suggest that the benefits of this methodology outweigh such costs. US is a safe, non-invasive, and reliable technique, inexpensive compared to MRI, and has the great benefit of making it possible to investigate muscle tissue response in real time during functional movements.
Conclusion

All three muscles showed a deformation during the submaximal ventral isometric contraction. Except for the contraction phase, Lco exhibited a lower deformation than Lca and SCM and was the only muscle with a significant change in deformation rate between the phases. When the beginning of the contraction phase was analysed, Lco was the first to be deformed among most volunteers, followed by Lca and then SCM. The exercise investigated here seems to be useful as a “stabilizing” exercise for Lco. Our suggestion is that Lco and Lca in further research should be investigated as separate muscles. US with TVI was sensitive enough to identify Lco and Lca as separate muscles and to study tissue motions as an indicator of coordination between the neck flexors.
**Ethical standards**

All procedures were conducted according to the Declaration of Helsinki. The experiments complied with the current laws of Sweden, and the study was approved by the Ethics Committee at the Faculty of Health Sciences, Linköping University.

**Conflict of interest**

The authors declare that they have no conflict of interest.
References


**Table 1**) Reliability data from two measurements (two weeks apart) in 10 of the 16 study participants (age 23 years; SD 2.7, range 20–27)\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>SR, RMS</th>
<th>Strain mean</th>
<th>Strain max</th>
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<tbody>
<tr>
<td>LCO(^b)</td>
<td>0.98</td>
<td>0.84</td>
<td>0.84</td>
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<tr>
<td>LCA</td>
<td>0.89</td>
<td>0.84</td>
<td>0.83</td>
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<tr>
<td>SCM</td>
<td>0.99</td>
<td>0.80</td>
<td>0.90</td>
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</tbody>
</table>

\(^a\)Intra-class correlation coefficient (ICC), two-way random absolute agreement, single measure was used to obtain the ICC values of the total sequence. ICC values of the root mean square (RMS) values of strain rate (SR) and mean and maximal (max) strain are presented for each of the three muscles.

\(^b\)Lco = longus colli; Lca = longus capitis; SCM = sternocleidomastoid.
Table 2 Strain and strain rate (SR) of ventral neck muscles, longus colli (Lco), longus capitis (Lca), and sternocleidomastoid (SCM) in 16 healthy volunteers

<table>
<thead>
<tr>
<th></th>
<th>Lco</th>
<th>Lca</th>
<th>SCM</th>
<th>Lco-Lca</th>
<th>Lco-SCM</th>
<th>Lca-SCM</th>
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<tr>
<td><strong>Total sequence</strong></td>
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<td><strong>SR</strong></td>
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<tr>
<td>RMS</td>
<td>0.22</td>
<td>0.17</td>
<td>0.20</td>
<td>0.53</td>
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<tr>
<td><strong>Strain</strong></td>
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<tr>
<td>Mean</td>
<td>0.28</td>
<td>6.82</td>
<td>14.32</td>
<td>0.003</td>
<td>0.02</td>
<td>0.002</td>
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<tr>
<td>Max</td>
<td>3.66</td>
<td>18.46</td>
<td>34.59</td>
<td>0.002</td>
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<tr>
<td><strong>Contraction phase</strong></td>
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<tr>
<td><strong>SR</strong></td>
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<tr>
<td>RMS</td>
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<td>0.14</td>
<td>0.18</td>
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<tr>
<td>Mean</td>
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*Median and interquartile range for measurements in SR, mean strain, and maximal (Max) strain. Significance level (P) from Kruskal-Wallis test (K-W) is presented; if P < 0.05, results from further analyses with Mann-Whitney U tests are also presented.*
Table 3 Significance test between contraction phase (cp), plateau phase (pp), and relaxation phase (rp), respectively, for longus colli (Lco), longus capitis (Lca), and sternocleidomastoid (SCM) in 16 healthy volunteers

<table>
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<tr>
<th></th>
<th>Friedman</th>
<th>cp-pp</th>
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<tr>
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<td>0.03 (cp ↑)</td>
<td>0.08</td>
<td>0.003 (rp ↑)</td>
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<td>Lca</td>
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<tr>
<td>SCM</td>
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Strain

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<tr>
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<tr>
<td>Lca</td>
<td>0.006</td>
<td>0.003 (pp ↑)</td>
<td>0.08</td>
<td>0.03 (pp ↑)</td>
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<tr>
<td>SCM</td>
<td>0.001</td>
<td>0.0005 (pp ↑)</td>
<td>0.02 (rp ↑)</td>
<td>0.10</td>
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</tbody>
</table>

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<tr>
<td>Lco</td>
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<td>0.02 (pp ↑)</td>
<td>0.008 (rp ↑)</td>
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<tr>
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<tr>
<td>SCM</td>
<td>0.05</td>
<td>0.02 (pp ↑)</td>
<td>0.06</td>
<td>0.49</td>
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*If Friedman’s test was significant (P < 0.05), further analyses were done using the Wilcoxon signed-rank test (descriptive data available in Table 2.) ↑ = higher; i.e., rp↑ indicates that relaxation phase/end phase was the highest.*
**Table 4** The most common order of tissue deformations as an indicator of activation in the ventral neck muscles (longus colli (Lco), longus capitis (Lca), and sternocleidomastoid (SCM)) in 16 healthy volunteers at the start of the contraction phase during ventral isometric contraction against manual resistance (1= the first muscle to be deformed, 3= latest muscle to be deformed)³

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<tr>
<th>Test Person</th>
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<th>SR Lca</th>
<th>SR SCM</th>
<th>P value Lco-Lca</th>
<th>P value Lco-SCM</th>
<th>P value Lca-SCM</th>
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</tbody>
</table>

Most common activation order

³Significance level, P, from Mann-Whitney U test results is presented. Video sequences (obtained by Doppler-based tissue velocity imaging) were visually analysed with respect to regional tissue velocity alternations correlated to the progress of the movement (see also Figure 4).