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Therapeutic

Cosmetic Medicine

Exploratory Analysis of Upper Facial Muscle Interplay During Emotional Expressions: Magnetic Resonance Imaging (MRI) Insights From Young, Caucasian, Toxin-naïve Individuals

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Abstract

Background: Understanding the interplay of muscle activity in the upper face is crucial because it can significantly impact the effectiveness and safety of aesthetic treatments. Traditional injection algorithms typically focus on the general 2-dimensional and 3-dimensional anatomy of muscles, often neglecting the areas where muscles exert the greatest force during facial expressions.

Objectives: The goal of this study was to analyze the location of greatest morphological change in the upper facial muscles including the procerus muscle (PM), corrugator supercilii muscle (CSM), orbicularis oculi muscle (OOM), and frontalis muscle (FM) during various facial expressions.

Methods: A total of 34 healthy young individuals (17 females, 17 males), with a mean age of 23.6 ± 2.4 years [range 20-30], were examined with magnetic resonance imaging (MRI) to assess the length, thickness, and width of the upper facial muscles (PM, CSM, OOM, and FM) for 5 different facial expressions: repose, anger, joy, surprise, and sadness.

Results: Facial muscle thickness is a key indicator of activity during expressions such as anger, joy, surprise, and sadness. During anger, the PM and CSM decreased in length and width but increased in thickness, whereas the FM passively contracted to stabilize the expression. The OOM showed increased thickness in its medial, inferior, lateral, and superior portions during various expressions, with specific regions activating differently depending on the expression, such as the medial and lateral parts during surprise and the inferior and lateral parts during joy. The medial third of the CSM was the most active region during contraction.

Conclusions: Upper facial muscles—as either agonists or antagonists—act together during facial expressions to stabilize facial expressions, emphasizing the need to assess both groups in neuromodulator treatments. The medial third of the corrugator supercilii shows the most significant MRI changes, making it the primary target for injections.

Level of Evidence: 4 (Therapeutic)

The annual statistics of The Aesthetic Society reveal that the most frequently performed aesthetic surgical procedure in 2023 was liposuction, with 394,527 procedures performed in the US. In comparison, the American Society of Plastic Surgeons reported a total number of 9,480,949 neuromodulator injections during that same period, reflecting the popularity and ease of minimally invasive procedures.[1,2] The glabella and the forehead are the most frequently requested facial regions for neuromodulator injections, targeting the procerus

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muscle (PM), corrugator supercilii muscle (CSM), orbicularis oculi muscle (OOM), and the frontalis muscle (FM).[3,4]

Upper facial anatomy and neuromodulator treatment algorithms are closely related to each other, because the knowledge of where a muscle is located and what its function is determine injection points and a successful treatment outcome. However, most upper facial injection algorithms are based on the 2-dimensional and 3-dimensional location of a specific muscle, without regard for where a muscle might have its greatest activity or where a facial muscle might exert its greatest force. Knowing where a facial muscle has its highest activity may help injectors more selectively target facial muscles, achieving the most optimal aesthetic outcome and reducing adverse events.

Previous studies reported on the distribution of neuromuscular junctions within facial muscles and reported that such junctions were found in close proximity to the motor nerve entry but also located eccentrically, with a high variation between the investigated facial muscles.[5-8] The clinical application of such basic science studies is unfortunately limited because no clear guidance can be obtained about whether the location of the neuromuscular junction corresponds to the area of greatest activity of a muscle. Magnetic resonance imaging (MRI) on the contrary can be utilized to identify the location of the muscle's greatest activity by measuring differences between a muscle's morphology at rest and during contraction.[9-11] It can be assumed that, if a muscle's greatest change during contraction occurs in its medial aspect but not in its lateral aspect, injections should focus on the medial and not the lateral aspect. Targeting the medial aspect of the muscle would administer the product specifically where its greatest contraction occurs, resulting most likely in more effective treatments and ultimately superior aesthetic outcomes. On the contrary, targeting the lateral aspect of such a muscle would administer neuromodulator product in an area of a muscle that is most likely involved in the respective contraction to a lesser degree due to the minimal change the muscle displays.

Therefore, the objective of this study was to identify for the muscles of the upper face (the PM, CSM, OOM, and FM) where the greatest morphologic change occurred during various facial expressions, such as anger, joy, surprise, and sadness, by utilizing MRI. It is hoped that the results of this investigation will increase understanding of the movement of upper facial muscles and provide guidance that increases precision during neuromodulator treatments.

METHODS

Study Population

In this MRI-based observational exploratory study we examined the glabellar muscles in young, healthy, Caucasian, Polish volunteers who had not undergone any previous neuromodulator treatments. This study was conducted between March 2022 and July 2024, and parts of this study have been previously published by Rams et al.[12] Ethical approval for the study was granted by the Jagiellonian University Ethics Committee in Krakow, Poland, under registration number 1072.6120.209.2022. All participants provided written informed consent, agreeing to participate in the study and allowing the use of their demographic and imaging data.

No specific inclusion criteria were imposed, ensuring a broad range of community-based MRI data sets. Exclusion criteria included individuals with contraindications for MRI (such as metal implants) or those with a history of facial injuries or aesthetic procedures that might affect the visibility of the upper facial muscles during MRI.

Facial Expressions

Before entering the MRI machine, study participants were instructed to perform 4 specific basic facial expressions—anger, joy, surprise, and sadness—based on a facial animation chart. To ensure consistency and reproducibility, these expressions were practiced in front of a mirror or with their cell phones.

Once this preparation was completed, participants were asked to enter the MRI machine in a supine position with a relaxed facial expression. After the initial MRI scan was completed (at rest), participants were asked to perform the first facial expression (anger) and maintain it during the subsequent scan. A total of 5 scans were conducted, 1 at baseline (repose) and the remainder for each of the 4 facial expressions (anger, joy, surprise, and sadness). The MRI scan for each facial expression lasted 4 minutes, followed by a 15-second relaxation period before transitioning to the next expression and scan. This resulted in a total scanning duration of 21 minutes per participant (5 facial expressions plus 4 relaxation periods).

MRI Sequence and Analysis

MRI data were collected with a 1.5T Siemens Sola MR System (Siemens, Erlangen, Germany) equipped with a 20-channel coil. A customized T1 MPRAGE sequence was employed with the following parameters: TR = 2340 ms, TE = 5.1 ms, TI = 1180 ms, FA = 8°, FOV = 240 × 240 mm, slice thickness = 0.9 mm, and 288 axial slices. Participants were instructed to maintain a relaxed facial expression throughout the scanning process to accurately capture the muscles at rest.

The resulting medical images were retrospectively analyzed for the glabellar region of interest. After verifying the quality of the MRI images, DICOM data sets were analyzed by 2 radiologists with expertise in head and neck imaging. They utilized a standardized radiology workstation (syngo.via software, Siemens, Erlangen, Germany) and the multiplanar mode for image reconstruction in all 3 planes. Each measurement was repeated twice, with both sides of the muscles being analyzed separately. The following muscle measurements were performed as outlined in a previous protocol as detailed below, accompanied by a detailed graphic representation illustrating the evaluation of MRI scans to enhance clarity and understanding.[12]

Procerus Muscle

Length

Maximum vertical linear distance from the muscle's origin at the most inferior point on the nasal bone to its insertion at the eyebrow level in the median plane.

Width

Maximum horizontal linear distance measured at the midpoint between the nasion and the glabella.

Thickness

Maximum anteroposterior dimension measured at the midpoint between the nasion and the glabella.

Corrugator Supercilii Muscle

Length

Maximum vertical linear distance from the muscle's medial origin to its lateral insertion near the midpupillary line.

Width (Medial Third, Middle Third, Lateral Third)

Horizontal linear distance from the inferior to the superior border of the muscle.

Thickness (Medial Third, Middle Third, Lateral Third)

Sagittal linear distance from the most anterior to the most posterior surface of the muscle. (Figure 1)

Orbicularis Oculi Muscle

Surface Area

The best-fit area determined by approximating the elliptical shape formed by the muscle's boundary points.

Height

Maximum vertical linear distance from the lower border to the upper border of the muscle at the vertical midpupillary line.

Width

Maximum horizontal linear distance from the medial to the lateral border of the muscle at the horizontal midpupillary line.

Thickness (Medial, Inferior, Lateral, Superior)

Distance between the muscle's surface and undersurface.

Frontalis Muscle

Length

Maximum vertical distance from the upper margin of the eyebrows to the transition into the galea aponeurotica at the vertical midpupillary line.

Width

Maximum horizontal distance between the most lateral borders of the muscle

Thickness

Average anteroposterior distance measured 1.5 cm above the bony supraciliary arch in the midpupillary line.

Statistical Analysis

An independent sample *t* test was run to identify differences between genders for age, BMI, and all investigated muscle parameters. Differences during the 4 performed facial expressions in reference to baseline muscle parameters (facial expression at repose) were calculated as changes in percentage. Values below 100% indicated a decrease, and values above 100% indicated an increase in the respective measurement. One-sample *t* tests were run to compare whether the change in percentage was statistically significantly different from the baseline value. A *P* value of .05 indicated statistical significance. Absolute muscle morphological parameters are presented in Tables 1-4 for each individual muscle (expressed in millimeters, unless otherwise noted). Relative changes in percentage, along with SD, are provided in the "Results" section, stratified by facial expression, with the percentage (SD) convention.

RESULTS

General Description

In total 34 healthy young individuals of Caucasian Polish descent were investigated (17 females and 17 males), with a mean age of 23.6 (2.4) years [range: 20-30] and a mean body mass index (BMI) of 22.8 (2.3) kg/m^2 [range: 18.6-27.8]. Female participants were of older age and lower BMI when compared to male study participants, both with $P < .001$. PM length, CSM medial width, CSM thickness, OOM surface area, FM thickness, and FM width displayed significantly higher values in males when compared to female study participants, with $P \leq .05$. The exact values of the measured parameters, stratified by facial expressions can be found in Tables 1-4.

Angry Facial Expression

When compared to the baseline morphology of the respective glabellar muscles, it was identified that PM length decreased to 86.1% (10.1) with $P < .001$, width decreased to 86.7% (10.9) with $P < .001$, and thickness increased to 165.7% (55.3) with $P < .001$.

CSM length decreased to 84.1% (9.6) with $P < .001$, and CSM thickness increased in its medial third to 127.5% (33.2) with $P < .001$, in its middle third to 131.6% (24.1) with $P < .001$, and in its lateral third to 131.5% (24.1) with $P < .001$. CSM width changed in its medial/middle/lateral third to 106.0% (6.2) with $P = .002$; 103.1% (16.5) with $P = .122$; and 97.2% (12.9) with $P = .075$, respectively.

OOM surface area decreased to 97.4% (17.0) with $P = .209$, height decreased to 99.0% (11.6) with $P = .494$, and width decreased to 98.1% (10.2) with $P = .117$. OOM thickness increased in its medial/inferior/lateral/superior portions with 121.5% (22.1) with $P < .001$; 133.0 (27.3) with $P < .001$; 116.1% (22.6) with $P < .001$; and 130.2% (33.5) with $P < .001$, respectively.

FM length increased to 105.4% (9.1) with $P < .001$, width decreased to 98.8% (5.7) with $P = .086$, and thickness increased to 111.0% (19.5), $P < .001$.

Joyful Facial Expression

PM length/width/thickness changed to 100.5% (15.3) with $P = .773$; 112.3% (37.6) with $P = .111$; and 97.2% (14.3) with $P = .009$, respectively.

CSM length decreased to 99.6% (11.6) with $P = .786$, and CSM thickness medial/middle/lateral increased to 104.0% (23.5) with $P = .169$; 101.9% (21.0) with $P = .455$; and 105.3% (20.1) with $P = .035$, respectively. CSM width changed in its medial/middle/lateral third to 109.0% (15.4) with $P = .635$; 97.5% (14.8) with $P = .162$; and 100.8% (15.7) with $P = .658$, respectively.

OOM surface area decreased to 97.8% (17.5) with $P = .312$, height decreased to 98.4% (12.1) with $P = .274$, and width decreased to 99.0% (8.5) with $P = .316$. OOM thickness increased in its medial/inferior/lateral/superior portions with 117.3% (30.4) with $P < .001$; 123.5 (29.6) with $P < .001$; 125.1% (23.6) with $P < .001$; and 114.4% (24.5) with $P < .001$, respectively.

FM length increased to 101.3% (9.2) with $P = .243$, width increased to 100.7% (5.4) with $P = .303$, and thickness increased to 109.9% (19.7), $P < .001$.

Surprised Facial Expression

PM length/width/thickness changed to 108.6% (9.6) with $P < .001$; 95.5% (12.1) with $P = .003$; and 95.6% (30.2) with $P = .234$, respectively.

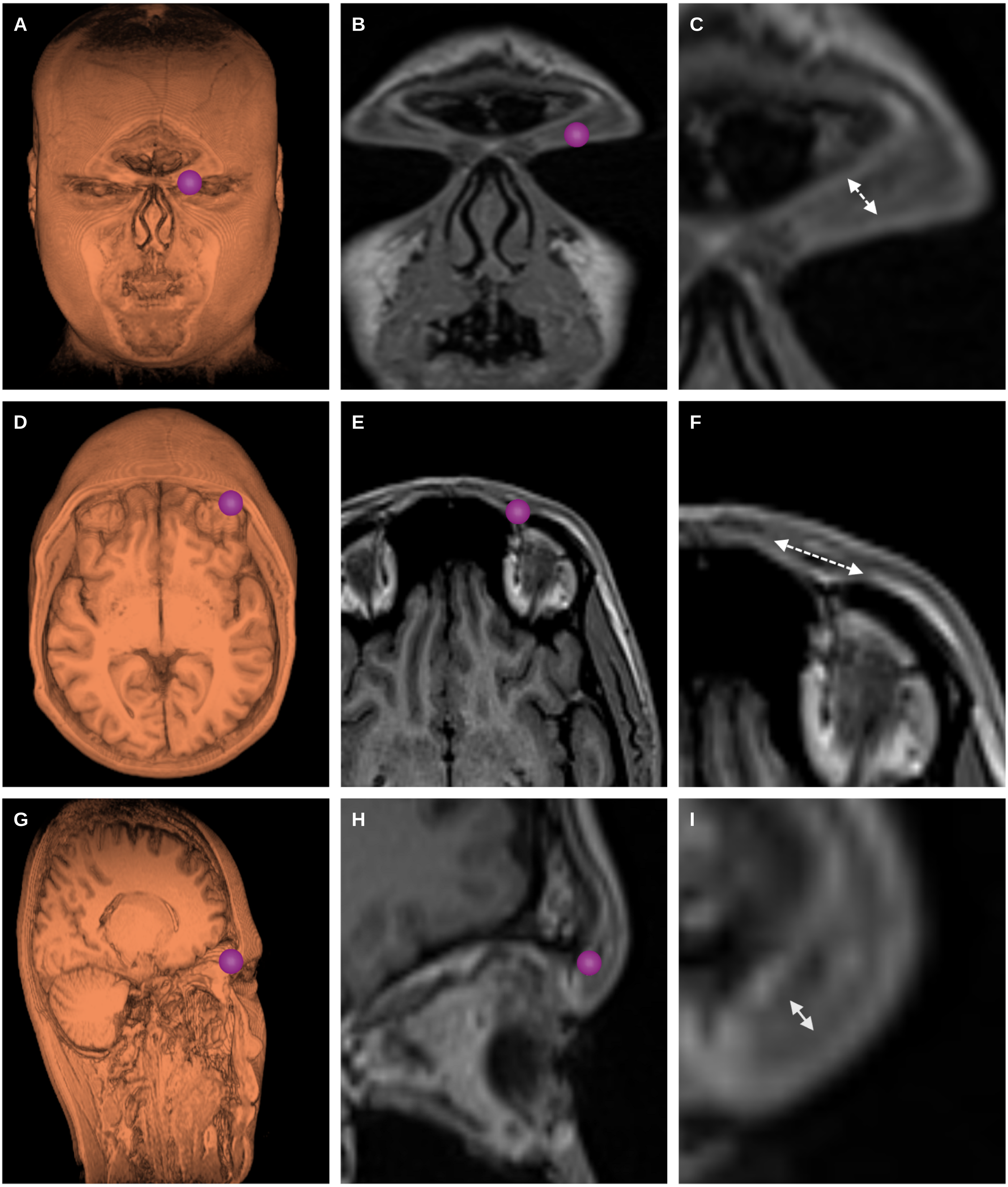

Figure 1. Multiplanar measurements exemplified for the corrugator supercilii muscle. In the coronal view (A and B), the muscle's width (C) was measured. In the axial view (D and E), the muscle's length (F) was measured. In the sagittal view (G and H), the muscle's thickness (I) was measured.

Table 1. Absolute Measurements of Procerus Muscle for Each Facial Expression

	Procerus muscle (PM)		
	Length mm	Thickness mm	Width mm
Repose	25.90 (3.65) [19.81-35.85]	0.99 (0.29) [0.51-1.95]	27.72 (4.32) [20.25-39.10]
Anger	22.21 (3.53)** [16.20-28.75]	1.60 (0.56)** [0.66-2.81]	23.87 (4.13)** [18.60-35.41]
Joy	25.87 (4.51) [19.50-36.95]	1.09 (0.40)* [0.55-2.14]	26.67 (4.30) [18.19-40.03]
Surprise	27.94 (3.28)** [21.38-36.57]	0.92 (0.29) [0.47-1.73]	26.39 (4.84)* [16.94-36.74]
Sadness	24.03 (2.31)** [20.19-28.79]	1.34 (0.42)** [0.78-2.58]	26.12 (4.48)* [17.82-35.57]

Values are provided as mean with standard deviation and the respective data range [minimum-maximum]. Asterisks indicate statistical significance when compared to a repose facial expression, with a single asterisk (*) denoting $P \leq .05$ and 2 asterisks (**) denoting $P \leq .001$.

Table 2. Absolute Measurements of Corrugator Supercilii Muscle for Each Facial Expression

	Corrugator supercilli muscle (CSM)						
	Length mm	Width (medial) mm	Width (middle) mm	Width (lateral) mm	Thickness (medial) mm	Thickness (middle) mm	Thickness (lateral) mm
Repose	24.25 (3.62) [17.29-32.79]	8.39 (1.06) [5.95-10.50]	8.79 (1.05) [6.44-11.80]	8.24 (1.08) [6.09-10.59]	0.88 (0.21) [0.41-1.35]	0.91 (0.23) [0.56-1.65]	0.86 (0.20) [0.52-1.45]
Anger	20.27 (3.18)** [15.68-29.58]	8.83 (1.31)* [6.46-12.47]	8.99 (1.40) [6.57-12.39]	7.93 (0.92) [6.07-9.94]	1.08 (0.22)** [0.45-1.59]	1.17 (0.26)** [0.66-1.72]	1.10 (0.23)** [0.77-1.85]
Joy	24.04 (3.89) [16.46-34.59]	8.39 (1.22) [6.40-11.80]	8.49 (1.22) [6.47-11.66]	8.23 (1.22) [6.31-10.78]	0.89 (0.18) [0.57-1.36]	0.91 (0.23) [0.50-1.73]	0.89 (0.21)* [0.49-1.51]
Surprise	25.33 (4.20)* [17.39-33.95]	8.58 (1.35) [6.44-11.61]	8.68 (1.28) [6.22-11.89]	8.29 (1.27) [6.09-12.00]	0.80 (0.20)* [0.46-1.55]	0.86 (0.25) [0.48-1.94]	0.79 (0.18)* [0.36-1.27]
Sadness	21.73 (3.62)** [15.92-28.81]	8.39 (1.25) [6.48-11.41]	8.70 (1.28) [6.31-13.81]	8.07 (1.03) [6.16-10.50]	1.09 (0.26)** [0.65-1.94]	1.16 (0.34)** [0.71-2.12]	1.06 (0.24)** [0.63-1.77]

Values are provided as mean with standard deviation and the respective data range [minimum-maximum]. Asterisks indicate statistical significance when compared to a repose facial expression, with a single asterisk (*) denoting $P \leq .05$ and 2 asterisks (**) denoting $P \leq .001$.

Table 3. Absolute Measurements of Orbicularis Oculi Muscle for Each Facial Expression

	Orbicularis oculi muscle (OOM)						
	Area mm^2	Height mm	Width mm	Thickness (inferior) mm	Thickness (superior) mm	Thickness (medial) mm	Thickness (lateral) mm
Repose	3136.62 (339.50) [2444.80-3668.42]	59.30 (4.67) [50.32-69.79]	67.33 (4.87) [57.83-78.00]	0.99 (0.23) [0.60-1.56]	0.93 (0.19) [0.65-1.40]	0.83 (0.16) [0.52-1.18]	0.92 (0.15) [0.67-1.28]
Anger	3010.92 (348.02) [2129.86-3858.45]	58.40 (5.45) [43.36-68.15]	65.61 (3.99) [58.11-73.39]	1.29 (0.28)** [0.61-2.15]	1.18 (0.28)** [0.63-2.24]	1.00 (0.20)** [0.68-1.48]	1.05 (0.17)** [0.74-1.60]
Joy	3028.13 (406.55) [2223.16-4372.67]	57.95 (5.25) [47.24-73.79]	66.37 (4.45) [57.67-76.13]	1.18 (0.23)** [0.70-1.92]	1.04 (0.18)** [0.52-1.37]	0.94 (0.18)** [0.53-1.31]	1.14 (0.18)** [0.83-1.65]
Surprise	3071.23 (362.79) [2114.98-3765.99]	58.89 (5.64) [46.88-69.11]	66.30 (3.02) [55.95-73.75]	0.98 (0.23) [0.61-1.72]	0.91 (0.19) [0.42-1.34]	0.86 (0.15)* [0.54-1.18]	0.99 (0.13)* [0.61-1.35]
Sadness	3063.46 (328.72) [2360.85-3950.38]	58.57 (4.24) [49.30-70.36]	66.49 (3.54) [57.94-74.94]	1.14 (0.21)** [0.75-1.67]	1.02 (0.21)** [0.66-1.59]	0.96 (0.16)** [0.63-1.29]	1.10 (0.16)** [0.85-1.63]

Values are provided as mean with standard deviation and the respective data range [minimum-maximum]. Asterisks indicate statistical significance when compared to a repose facial expression, with a single asterisk (*) denoting $P \leq .05$ and 2 asterisks (**) denoting $P \leq .001$.

CSM length changed to 105.2% (14.4) with $P = .004$, and CSM thickness medial/middle/lateral decreased to 93.6% (24.7) with $P = .036$; 96.1% (22.5) with $P = .158$; and 93.9% (19.8) with $P = .013$, respectively. CSM width changed in its medial/middle/lateral third to 103.0% (15.0) with $P = .102$; 99.4% (13.6) with $P = .700$; and 101.4% (15.6) with $P = .470$, respectively.

OOM surface area decreased to 99.3% (17.8) with $P = .750$, height increased to 100.1% (13.8) with $P = .959$, and width decreased to 99.0% (9.1) with $P = .382$. OOM thickness changed in its medial/inferior/lateral/superior portions with 106.0% (22.7) with $P = .017$; 103.1 (26.1) with $P = .164$; 108.6% (18.8) with $P < .001$; and 99.6% (21.5) with $P = .887$, respectively.

FM length decreased to 95.5% (12.1) with $P = .003$, width increased to 101.4% (4.8) with $P = .020$, and thickness increased to 126.9% (26.6) with $P < .001$.

Sad Facial Expression

PM length/width/thickness changed to 93.9% (11.4) with $P < .001$; 94.8% (13.6) with $P = .003$; and 141.6% (42.1) with $P < .001$, respectively.

CSM length changed to 90.4% (13.7) with $P < .001$, and CSM thickness medial/middle/lateral decreased to 129.6% (44.4) with $P < .001$; 132.4% (45.7) with $P < .001$; and 127.5% (38.6) with $P < .001$,

Table 4. Absolute Measurements of Frontalis Muscle for Each Facial Expression

	Frontalis muscle (FM)		
	Length mm	Thickness mm	Width mm
Repose	59.90 (12.18) [39.84-87.82]	1.02 (0.21) [0.66-1.58]	139.13 (10.91) [122.17-174.75]
Anger	62.93 (12.95)** [38.75-93.27]	1.11 (0.18)** [0.79-1.51]	137.24 (10.76) [119.66-170.38]
Joy	60.25 (11.28) [38.92-85.29]	1.10 (0.21)** [0.73-1.88]	139.98 (12.43) [122.67-185.81]
Surprise	56.47 (9.82)* [35.65-79.14]	1.27 (0.26)** [0.72-1.91]	140.90 (10.85)* [125.40-178.68]
Sadness	60.28 (11.00) [35.36-83.61]	1.11 (0.22)** [0.64-1.51]	138.80 (9.36) [124.18-168.70]

Values are provided as mean with standard deviation and the respective data range [minimum-maximum]. Asterisks indicate statistical significance when compared to a repose facial expression, with a single asterisk (*) denoting $P \leq .05$ and 2 asterisks (**) denoting $P \leq .001$.

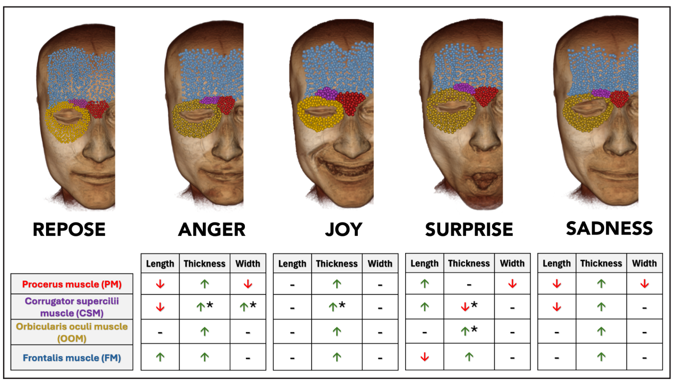

Figure 2. Three-dimensional hemifacial models showing the investigated facial expressions (repose, anger, joy, surprise, sadness) with overlaid borders of the glabellar muscles, illustrating muscle interactions. The table presents only statistically significant parametric changes associated with each expression ($P < .05$). An asterisk (*) denotes partial involvement of the muscle in the respective expression. (Procerus muscle (PM)—red, corrugator supercilii muscle (CSM)—purple, orbicularis oculi muscle (OOM)—yellow, frontalis muscle (OOM)—blue).

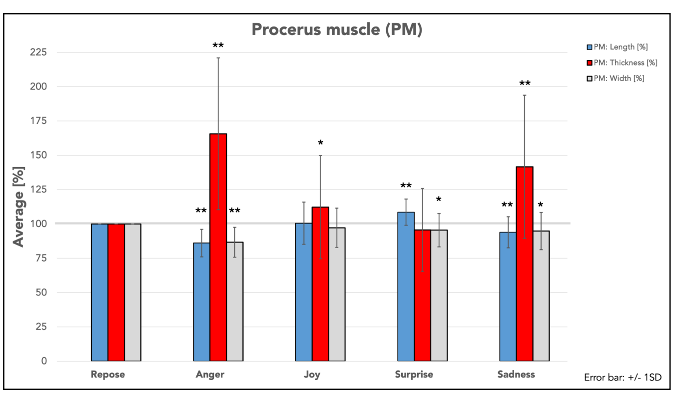

Figure 4. Paired bar graphs showing the average muscle measurements of the procerus muscle (PM) for facial expressions of repose, anger, joy, surprise, and sadness.

respectively. CSM width changed in its medial/middle/lateral third to 101.1% (16.9) with $P = .598$; 99.7% (14.1) with $P = .842$; and 99.0% (15.0) with $P = .599$, respectively.

OOM surface area decreased to 99.0% (16.7) with $P = .637$, height increased to 99.4% (10.9) with $P = .655$, and width decreased to 99.3% (9.6) with $P = .557$. OOM thickness changed in its medial/inferior/lateral/superior portions to 118.2% (19.4) with $P < .001$; 119.0 (23.9)

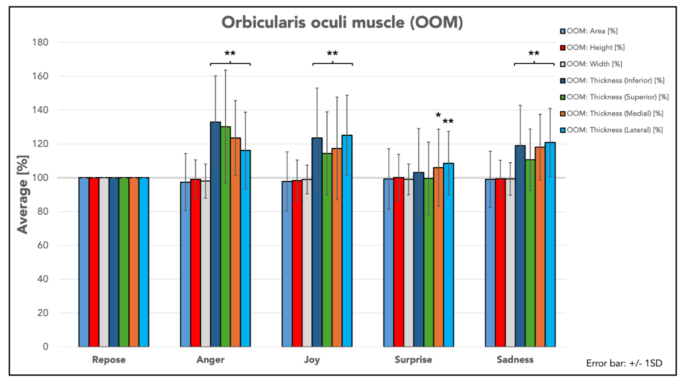

Figure 3. Paired bar graphs showing the average muscle measurements of the orbicularis oculi muscle (OOM) for facial expressions of repose, anger, joy, surprise, and sadness.

with $P < .001$; 120.9% (20.2) with $P < .001$; and 110.6% (18.2) with $P < .001$, respectively.

FM length increased to 101.8% (12.7) with $P = .247$, width decreased to 99.9% (4.5) with $P = .909$, and thickness increased to 109.9% (19.6), with $P < .001$.

Muscle measurements for each facial expression are summarized in Tables 1-4 and Figures 2-5.

DISCUSSION

This study was initiated to identify morphologic changes as identified by MR imaging of upper facial muscles during facial expressions of anger, joy, surprise, and sadness. The underlying idea for this study was recently published by Rams et al, who identified in a similar MRI-based study that the increase in thickness of a facial muscle was more reflective of its innate activity than other measured parameters.[12] The authors based their conclusion on a previous publication by Frank et al, who found that a reduction in frontalis muscle activity by 44.3% was sufficient to achieve a wrinkle severity grade of 0, and a reduction by 80.7% was sufficient to eliminate frontal skin movements.[13,14] These findings indicated that a facial muscle can be active, but its activity might not be observed on the skin surface in the form of skin movements or skin rhytids. However, to identify whether a muscle is active or not, measurements of its thickness can be conducted to reflect its activity: increase in thickness can be regarded as a positive sign, whereas no increase in thickness can indicate that the respective facial muscle is not active. The present study utilized this knowledge and conducted MRI-based measurements of facial muscles while the contraction of facial muscles was

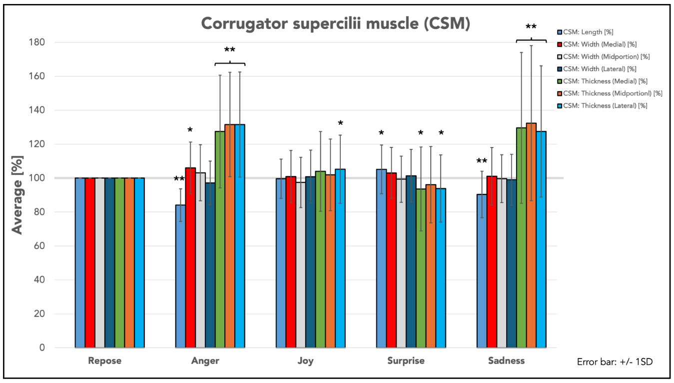

Figure 5. Paired bar graphs showing the average muscle measurements of the corrugator supercilii muscle (CSM) for facial expressions of repose, anger, joy, surprise, and sadness.

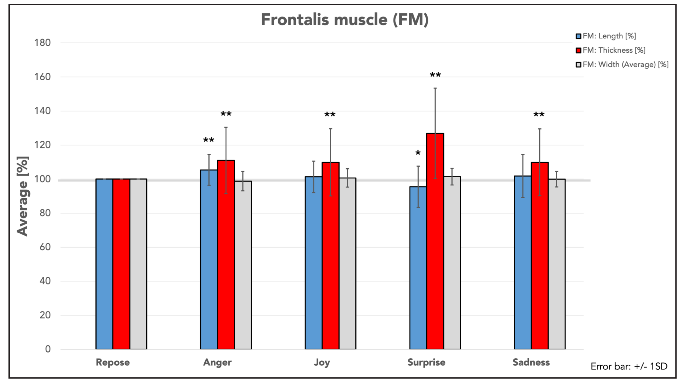

Figure 6. Paired bar graphs showing the average muscle measurements of the frontalis muscle (FM) for facial expressions of repose, anger, joy, surprise, and sadness.

maintained during the MR scanning process; this was achieved by asking study participants to exert a specific facial expression (anger, joy, surprise, sadness) while inside the MR machine.

The validity of the conducted MR measurements is reflected in the obtained results, which are both confirmatory and self-explanatory. During the facial expression of anger, the PM decreased in length and width (both $P < .001$) and increased in thickness ($P < .001$) when compared to the facial expression in repose. This reflects an isotonic muscle contraction pattern, characterized by reduced dimensions and increased thickness. On the contrary, during the facial expression of surprise, the length of the PM increased ($P < .001$), whereas the width ($P = .003$) and the thickness decreased; this behavior corresponds to a passive stretching phenomenon of the PM during the motion of eyebrow elevation, which is a component of the surprised facial expression.

The results of this study provided additional confirmatory knowledge of facial muscle behavior during animation: no facial expression is the result of a single muscle movement but always a result of multiple muscles acting together in synchronicity. We found that during an expression of anger, the PM, CSM, OOM, and FM were active at the same time. For the PM, OOM, and CSM this was understandable, because these muscles were needed to move the eyebrows medial and downward. However, the MR measurements revealed that the thickness of the FM had a statistically significant increase ($P < .001$), indicating that this muscle was active as well, despite being an eyebrow elevator, such that its contraction would oppose the movements exerted by the PM, OOM, and CSM. This behavior most likely indicates that despite being an antagonist (an eyebrow elevator) of the specific facial movement seen during the expression of anger (eyebrow depression), the FM is contracting at the same time to moderate the downward movement, similar to a muscular sling in which all involved muscles are active and contracting. This type of coactivation is most likely the reflection of the need to precisely position the eyebrows, which requires active agonists and antagonists at the same time to establish a stable muscle contraction equilibrium. It follows that if the FM was not active the PM, OOM, and CSM would have depressed the eyebrow too much and the position of the eyebrow would be too low; repositioning would rely solely on the retraction capability of the frontal soft tissues to reposition the eyebrows, which would be slower and less precise. If, however, the FM was active at the same time, a precise and fast repositioning of the eyebrows could occur, allowing the facial expression to change quickly and accurately.

A similar behavior was observed during the facial expression of joy: the thickness of the PM ($P = .009$), CSM ($P = .035$), OOM ($P < .001$), and FM ($P < .001$) increased at the same time. Despite smiling predominantly occurring in the lower face (the perioral region), the upper facial muscles all acted together. When looking in greater detail at the magnitude of changes, it was revealed that the greatest change was observed for the lateral (125%) and the inferior (124%) OOM muscle fibers; this corresponds to the coactivation of the OOM during Duchenne-type smiling, which results clinically in the formation of lateral canthal lines (crow's feet) during this type of smiling.

Another example of simultaneous coactivation of agonists and antagonists was observed between the FM and OOM during the facial expression of surprise. This specific facial expression included active eyebrow elevation, which resulted in an increase in thickness of the FM, but at the same time in an increase in thickness of the OOM in its medial vertical ($P = .017$) and lateral vertical fibers ($P < .001$), but not in its horizontally oriented superior ($P = .887$) or inferior ($P = .164$) muscle fibers. This regionally specific analysis allowed us to additionally identify that the OOM can activate different parts, depending on what facial expression needs to be actively executed or passively modulated. These findings are of clinical relevance because they show that different parts of facial muscles (here the OOM) can selectively support facial expressions with their different components; a finding that is in line with the histologic findings of Happak et al in 1997.[8] Clinically, it is known that the height of the lateral eyebrow can be increased by administering neuromodulators to the lateral OOM, which is known to act as a depressor of the lateral eyebrow.[4,15-17] By targeting the lateral OOM, the muscular sling between the OOM and the FM is unbalanced and more influence is assigned to the FM as the sole elevator of the eyebrow; this allows the eyebrow to be repositioned in a more cranial location. This indicates that an eyebrow elevation effect is possible just by targeting the eyebrow depressors (lateral: OOM, central: CSM, medial: PM) due to their coactivation during eyebrow elevation. To estimate the magnitude of this effect it would be advisable to palpate the respective muscles (OOM, CSM, PM) or compress them against the underlying bone during forced eyebrow elevation to feel whether there is any muscular activity of these depressors present. If no muscular activity is present, a small elevation effect may be expected; whereas a larger elevation effect may be expected if strong contractile activity is palpated. The depressor function would be blocked during neuromodulator treatment, and the eyebrow could be elevated by just targeting the depressors.

Another important clinical finding of this study was the behavior of the CSM during an angry facial expression: length decreased ($P < .001$), thickness in all thirds (medial, middle, and lateral) increased (all $P < .001$), but width increased in the medial third ($P = .002$) and not in the middle ($P = .122$) or the lateral third ($P = .075$). These findings indicate that the medial third is the dominant location of CSM activity during its contraction and should therefore be the primary neuromodulator target for glabellar rhytid treatments. A precise injection of the CSM should suffice in most cases to block its muscular activity. This finding was clinically verified with the introduction of the 3-point glabella injection technique as published by Cotofana et al in 2021.[18]

Despite its clinically relevant findings, this study was not free of limitations. First, the study sample consisted of young (age range: 20-30 years) and healthy individuals, raising questions about the expansion of the obtained results to older study populations. Given the age-related physiological changes in muscle tone and elasticity that occur in some facial muscles, further research is needed to understand how these changes impact facial muscle activity and treatment outcomes. Second, the population investigated consisted of Polish Caucasians; it is recommended that other ethnicities are studied, reflecting the heterogenous patient clientele healthcare providers see on a daily basis. Third, although our study design made an effort to standardize facial expressions across participants through preliminary training, the inherently individual nature of emotional expression poses a challenge to consistency and reproducibility. Achieving perfect reproducibility of facial expressions is difficult, because emotional intensity and expression execution vary between individuals. Finally, the slice thickness of the MR scan was 0.9 mm. This is the current standard in most radiology centers but introduces a certain degree of inaccuracy when it comes to the small and thin muscles of facial expression. The results need to be understood in this context. Of note, this study received no funding and was conducted on a voluntary basis.

CONCLUSIONS

The results of this MRI-based study revealed that upper facial muscles act together during the investigated facial expressions, independent of their action as agonists or antagonists in exerting specific facial movements. Coactivation allows understanding of the interplay between eyebrow elevators and depressors, a crucial factor during neuromodulator treatments. Clinical examinations should focus on both muscle groups when estimating the resulting clinical effect. The corrugator supercilii muscle displayed its greatest change in MRI morphology in its medial third (and not its middle or lateral portions) which predisposes this muscle part to be the major target during neuromodulator injections. Yet this study was limited by its focus on a young, healthy, and ethnically homogenous population as well as by challenges in the reproducibility of emotional expressions and the inherent limitations of imaging techniques. Future studies should explore these factors in broader populations and also investigate the impact of age-related changes on treatment outcomes.

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