Topographical Characteristics of Motor Units of the Lower Facial Musculature Revealed by Means of High-Density Surface EMG

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Lapatki, Bernd G., Robert Oostenveld, Johannes P. Van Dijk, Irmtrud E. Jonas, Machiel J. Zwarts, and Dick F. Stegeman. Topographical characteristics of motor units of the lower facial musculature revealed by means of high-density surface EMG. J Neurophysiol 95: 342–354, 2006. First published July 6, 2005; doi:10.1152/jn.00265.2005. The objective of this study was to systematically characterize motor units (MUs) of the musculature of the lower face. MU endplate positions and principal muscle fiber orientations relative to facial landmarks were identified. This was done by the analysis of motor unit action potentials (MUAPs) in the surface electromyogram. Thirteen specially trained, healthy subjects performed selective contractions of the depressor anguli oris, depressor labii inferioris, mentalis, and orbicularis oris inferior muscles. Signals were recorded using recently developed, 0.3-mm thin and flexible high-density surface electromyography (sEMG) grids (120 channels). For each subject and each muscle and for different low contraction levels, representative MUAPs (“MU fingerprints”) were extracted from the raw sEMG data according to their spatiotemporal amplitude characteristics. We then topographically characterized the lower facial MUs’ endplate zones and main muscle fiber orientations on the individual faces of the subjects. These topographical MU parameters were spatially warped to correct for the different sizes and shapes of the faces of individual subjects. This electrophysiological study revealed a distribution of the lower facial MU endplates in more or less restricted, distinct clusters on the muscle often with eccentric locations. The results add substantially to the basic neurophysiological and anatomical knowledge of the complex facial muscle system. They can also be used to establish objective guidelines for placement of conventional (surface or needle) EMG electrodes as well as for clinical investigations on neuromuscular diseases affecting the facial musculature. The localized endplate positions may also indicate optimal locations for botulinum toxin injection in the face.

Introduction

The facial musculature is a three-dimensional (3-D) assembly of multiple small muscle bundles and sheetlike muscle fiber arrays (Fig. 1). The independently controlled subcomponents of this complex muscle system are of great importance for the functioning of the orofacial sense organs and in the mediation of emotional and affective states (mimic expression). Characteristic anatomical features of the facial muscles are 1) non-tendinous attachments to soft tissue (in some muscles including origins), i.e., to facial skin or other muscle tissue; 2) interdigitation and overlap in relatively small areas (especially in the lower facial area); and 3) high inter- and intraindividual variability in location and morphology (Blair 1986; Braun and Elze 1954; Kennedy and Abbs 1979; Naim 1975; Salmon 1995). Facial movements are mediated by an integrated network that includes neural systems mediating both voluntary and emotional drive from the cortex and the facial nucleus (Hopf et al. 1992; Morecroft et al. 2001).

Despite the great functional importance and the unique morphological and neural characteristics of the facial musculature, “there is no doubt that very little research of any sort has been done on the muscles innervated by the facial nerve” (May and Schaitkin 2000). This is especially true for systematic electrophysiological investigations at a single motor unit (MU) level. In the few electromyographic (EMG) studies on facial MU characteristics, firing behavior (Blair 1988; Kamen and De Luca 1992), recruitment patterns, and reflex responses (Lansing et al. 1991; Mateika et al. 1998; McClean 1991; McClean and Smith 1982; Smith et al. 1981; Valls-Sole et al. 1992; Wohlet 1996b) were examined mostly with needle electrodes (the standard technique for electrophysiological studies at the MU level). Until now, other basic properties of facial MUs, such as their spatial spread and orientation, and the location of the endplate zones, were not specified (except a rough determination of MU territories in the human lip musculature; Goffman and Smith 1994). High-density surface electromyography (sEMG; Blok et al. 2002; Farina et al. 2000; Huppertz et al. 1997; Masuda and Sadoyama 1988; Prutchi 1995; Wood et al. 2001; Yamada et al. 1987) is the obvious method of choice to fill that gap of knowledge. However, this technique could not be applied to the facial musculature because of technical limitations and lack of appropriate signal processing tools, combined with the methodological demands for EMG studies in the facial area (Cole et al. 1983; Lapatki et al. 2003). The latter are related to 1) uneven skin contours in the face requiring highly flexible electrode arrays; 2) skin distortions during facial muscle contractions and flow of saliva, both increasing the risk of sensor attachment loss; and 3) general problems in attaching an electrode array to the face. These problems have been solved by the development of thin and highly flexible multielectrode grids together with a suitable
skin attachment technique for this type of sensor (Lapatki et al. 2004). This study was undertaken to systematically determine topographical MU characteristics (i.e., their location, spatial orientation, and endplate zone) of the facial musculature by means of high-density sEMG, and to compare the results to basic anatomical knowledge and data obtained in histochemical studies. It is expected that the results will significantly contribute to our basic knowledge on human (neuro-)physiology, and also enable informed guidelines to be derived for the placement of conventional (surface or needle) EMG electrodes. In this article, results of measurements in the lower face are presented. In a subsequent paper we will publish the results from the mid- and upper face.

METHODS

Subjects

The study group consisted of 13 subjects (six men and seven women, mean age 27.2 yr, range 21–43 yr) without known neurological or general health disorders. Nine of the subjects were trumpeters (seven music students and two professionals), who were expected to have good facial motor control and to be motivated to improve on that. In the run-up to the measurements, a special training program was designed and implemented to improve the ability of the participants in performing selective contractions (i.e., isolated activations of the individual facial muscles). The Ethics Commission of the University Medical Centre Nijmegen (The Netherlands) approved the protocol of the study.

Sensors for high-density sEMG measurements

The recently developed multielectrode sEMG grids (Digiraster Tetzner GmbH, Stuttgart, Germany) applied in this study were manufactured using flexprint techniques. They consist of regularly arranged, chlorided silver electrodes 2 mm in diameter with an inter-electrode distance (IED) of 4 mm (center to center) in both directions. This IED value allowed a sufficient spatial sampling for the facial musculature. These electrode grids (Fig. 2A) were attached to the skin using double-sided adhesive tape (1522 medical double-coated tape, 3M, St. Paul, MN) that had been prepared for this application by creating regular perforation patterns.

The elaborated skin attachment procedure yields firm sensor fixation without requiring external fixations. High signal quality was achieved as a result of the relatively low electrode-to-skin impedances (Lapatki et al. 2004).

Data acquisition

Electrical connections to the amplifiers were made with the help of a headset equipped with flexible arms and connectors for the electrodes (Fig. 2B). Data were acquired “monopolarly,” i.e., referred to a remote reference electrode (material: Ag/AgCl, diameter: 4 mm) attached on the dorsum nasi. A second reference electrode provided a common mode signal (CMS). In addition, a driven right leg (DRL) electrode was attached at the forehead (Metting van Rijn et al. 1990).

The system used for data acquisition (Mark-6, BioSemi, Amsterdam, The Netherlands) has an input impedance >100 MΩ and a common mode rejection ratio (CMRR) >120 dB. Signals were band-pass filtered (3.2–400 Hz; high-pass: first-order Bessel; low-pass: fourth-order Bessel) and synchronously sampled at 2,000 Hz with a resolution of 0.5 μV/bit over a range of ±16 mV (16 bits). The acquisition software allows the experiments to be controlled by on-line inspection of the raw monopolar or bipolar data of selected
electrode rows or columns (Blok et al. 2002). Because contractions of facial muscles at a constant level are difficult to perform, we implemented an sEMG amplitude feedback tool in the acquisition software that visually displayed the normalized root mean square (RMS) value of selected bipolar signals (and so the activity level of the underlying muscle) to the subject by the PC monitor. The RMS was calculated for data windows of 500 ms; normalization was made to a maximal reference contraction of the muscle.

**Setup for the measurements and electrode positioning**

We examined the subjects’ lower facial musculature unilaterally either on the right (seven subjects) or the left (six subjects) side of the face. Two grids of 6 × 10 electrodes were positioned side by side, but vertically displaced by one electrode row according to criteria explained in the legend to Fig. 3.

**Recording procedure**

After electrically connecting the grids, subjects were again instructed and trained in the performance of the experimental tasks now controlled by on-line inspection of selected signal amplitudes (i.e., the visual amplitude feedback derived above). When the selective motor control in the lower face was considered to be sufficient and the subjects were accustomed to the recording conditions, data (120 channels) were acquired while the depressor anguli oris (DAO), the depressor labii inferioris (DLI), the mentalis (MEN), or the orbicularis oris inferior (OOI) muscle was selectively activated at different levels. The facial poses corresponding to the selective contractions of these four lower facial muscles are shown in Fig. 4. In each muscle we performed two recordings per activity level, each lasting about 20 s. The first recordings were made at the activity level at which clear MU firings could be determined in the bipolar signals over the area of the corresponding muscle; this level corresponded most often with 2–3% of maximal voluntary contraction (MVC). Then, the subjects were asked to perform contractions at 5, 10, 15, 20, 25, and 30% of MVC so that in total 14 × 20 s of data were recorded per subject and muscle.

**Data analysis**

Data were analyzed off-line using algorithms programmed in Matlab, Version 6.5 (The MathWorks, Natick, MA). After extracting MUAPs from all recorded data (decomposition) we selected for each subject and muscle a data file containing a representative set of MUAPs; the physiological parameters of interest, the MU endplate positions and muscle fiber orientations, were then extracted from these MUAPs; finally, to allow the comparison and combination of the individual results, the extracted topographical MU parameters were normalized.

**Decomposition of the sEMG pattern**

MU action potentials were extracted from the first 16 s of the recorded data on the basis of the specific topographical profiles and amplitude characteristics of the peaks. The decomposition procedure was based on a previously described method (Kleine et al. 2000) and primarily consisted of the following three components: 1) peak detection in three selected bipolar signals, 2) classification of the data around the detected peaks according to their differential spatiotemporal amplitude characteristics, and 3) averaging broader data windows around the classified peaks, for each peak group, and for all recording channels. Most of these steps were largely automated.

Peak detection was performed in three bipolar signals recorded in distinct locations along the fibers of the contracted muscle (indicated as an example by the three small gray rectangles in the left grid of Fig. 3). Thus we took into account that facial MUs can lie in distinct areas of the muscle. The algorithm guaranteed that peaks within one channel occurring <5 ms apart were eliminated from the determined list of peak-occurrence times, and that peaks resulting from a specific MU firing, detected in several channels, were considered only once.

Peak classification was based on the Ward’s clustering algorithm (Everitt et al. 2001) using “Euclidean distances” between the spatio-temporal amplitude characteristics of 16 ms of data around the detected peaks. One important issue in this classification is the consideration of the differential amplitude topographies of the peaks. Preliminary observation of data from the facial muscles revealed that the amplitude topographies of facial MUAPs could be significantly distinguished in both the dimensions perpendicular and parallel to the muscle fiber direction. As a consequence, we selected an extended set of input channels for the peak classification procedure that represented distinct topographical regions of the corresponding muscle in both dimensions (indicated by the three rows of four bipolar signals illustrated in Fig. 3). The time shift between peaks from distinct channels, resulting from signal propagation, was accounted for by determining the peak latency (using a cross-correlation technique) and aligning the signals accordingly. The stopping criterion of the hierarchical clustering algorithm requires that the number of clusters be predefined. We chose this number to be approximately four times the number of active MUs expected. The latter number was estimated from the length of the evaluated data segment, the number of detected peaks, and the average firing rates of facial MUs (Blair 1988; Kamen and De Luca 1992). In this manner we ensured that there was a low probability that peaks with significantly distinct amplitude topographies were classified as belonging to the same cluster.
phies (i.e., peaks belonging to distinct MUs) were classified into one group.

After performing spike-triggered averaging of the peaks for each classified peak group and recording channel (here we used 80 ms of data around the classified peaks to include the whole width of the MUAP), we obtained a set of low-noise MUAP templates for each activity level and repetition, which could be regarded as representative “fingerprints” of the MUs that contributed to the contraction. From this data set, MUAPs averaged from a small number of peaks were removed.

Selection of a representative MUAP template set

All decomposed data were inspected to select one set of MUAP templates (belonging to a certain recording level and repetition, respectively) per subject and muscle that best represented the distinct MUAP topographies observed in the template sets obtained from all recording levels and repetitions. To facilitate the differentiation of the MUAPs’ distinct amplitude topographies, we scanned the template sets in their bipolar montage (see also Fig. 6). Topographical MU parameters were determined for only the selected MUAP template sets. The corresponding methods used the MUAP data in a monopolar montage.

Determination of innervation zone location and muscle fiber orientation

The location of the innervation zone and the main muscle fiber orientation of a MU correspond to the location where the MUAP starts and in which direction it propagates. From the spatial and temporal information contained in an averaged monopolar MUAP, which can be illustrated either as a template or as a time sequence of amplitude maps (see Fig. 5, A or D), both of these parameters can be derived. Our method included the following steps: 1) two-dimensional interpolation of the monopolar MUAP data; 2) determination of the initial phase of the MUAP (MUAP initiation); 3) localization of the standing maximal amplitude area in the initial MUAP phase (and endplate zone, respectively) on the electrode grid; 4) determination of the latencies (in both directions of signal propagation) when the propagation terminated or became diffuse (MUAP termination); and 5) localization of the maximal amplitude area on the electrode grid at the terminal MUAP latency (in both directions of propagation) and subsequent calculation of the main muscle fiber orientations.

The interpolation was performed in two stages using a bicubic method. First, we calculated the monopolar data of the missing electrode column in between the two attached electrode grids (see Fig. 3). Then, we interpolated the complete template data set in both dimensions eight times between adjacent electrodes. In this manner we increased the topographical resolution of the localization of the MUAP initiation and termination to a value of 0.5 mm (instead of the IED value of 4 mm).

The method for determining the initial phase of the MUAP was based on the fact that the main peak of the monopolar MUAP appears first in the grid area around the endplates. Another important aspect is that the monopolar amplitude values in this area are usually high compared with the amplitudes in other grid areas and thus contribute significantly to the average of the absolute monopolar MUAP signals over the whole grid (called “single time series”; see Fig. 5C). For these reasons, the MUAP initiation can be determined by means of a predefined amplitude threshold in the single time series. We used a threshold of 40% of the maximum level: this value was on the one hand usually above the average noise and smaller peaks in the single time series and, on the other hand, below the average value of the signals around the endplate (so that their leading edge was detected). It is important to note that not the real start of the
MUAP but a slightly longer latency was determined by our algorithm. However, because there is no apparent signal propagation in the initial MUAP stage, this had only a negligible influence on the accuracy of localizing the endplate zone.

The MUAP initiation was topographically characterized by searching for the (interpolated) electrode grid location of maximal amplitude in the corresponding interpolated monopolar amplitude map (Fig. 5D, latency 19). To reduce the influence of inhomogeneities in the area of maximal MUAP amplitude, the algorithm did not localize the single maximum amplitude value, but a maximal amplitude area (see black frame in Fig. 5D, latency 19).

The two latencies indicating the terminal stage of signal propagation away from the endplate zone in both (opposite) directions were determined by observing the complete amplitude map sequences of the corresponding MUAP. The main criteria for detecting the terminal MUAP stage included finding either a drastic reduction in amplitude [i.e., a decrease of about 50% within one IED (Fig. 5D, upper fiber direction)], a beginning diffuse displacement of the propagating high-amplitude zone (Fig. 5D, lower fiber direction), or a stationary amplitude maximum.

The position on the electrode grid corresponding to MUAP termination was localized in the same way as described for MUAP initiation, i.e., we also determined the position of the zone of maximal amplitude in the interpolated monopolar amplitude maps of the corresponding latencies (as shown by the black frames in Fig. 5D, latencies 23.5 and 24.5). The orientation of the muscle fibers could then be characterized as two vectors pointing from the endplate position to the (two) positions where the MUAP termination was localized.

Accurate MUAP endplate location and muscle fiber orientation were verified by a final, careful observation of the complete amplitude map sequence (or “MUAP movie”) with the superimposed results. If necessary, determination of these parameters was repeated with adapted threshold values and latencies, respectively.

**Normalization**

The electrode grids were positioned according to the criteria specified in the legend of Fig. 3. The distances from the electrode grids to the facial midline, to the corner of the mouth, as well as to the median and lateral lower mandibular border were quantitatively determined after the sEMG recordings directly on the subject’s face. Using the known interrelations between these specified facial landmarks and the electrodes it was possible to define the endplate positions and muscle fiber orientations (previously determined in electrode coordinates) in an individual facial coordinate system. Separately for each subject, we then applied a spatial scaling of the data to account for the different sizes and shapes of the subjects’ faces; the individual facial dimensions (characterized by the center and corner of the mouth as well as the lower mandibular border at the facial midline and at the mediolateral level of the corner of the mouth) were warped so that they agreed with the average facial dimensions of the study group. The (constant) horizontal scaling factor for this warp was determined by the relation between the individual and average distance between the corner and the center of the mouth. In the vertical dimension, separate scaling factors were calculated at the facial midline and at the corner of the mouth. The vertical scaling at other mediolateral levels varied linearly between these two scaling factors. After applying this
spatial normalization, we could combine the data of all subjects in one figure and perform interindividual comparisons. Because no systematical differences between endplate positions and fiber orientations determined on the right and left face were found in any muscle, we finally performed a “mirror-image transformation” of the parameters obtained from measurements on the left facial side to facilitate interindividual comparison.

RESULTS

Figure 6 shows two monopolar (Fig. 6, A and B) and two bipolar (Fig. 6, C and D) MUAP templates decomposed from data recorded during a 5% (MVC) contraction of the DAO muscle. Only the signals derived from the lateral electrode grid (gray area in central graphic) are shown because the signal amplitudes of the medial electrode grid were negligible for this muscle. Endplate positions and muscle fiber orientations are represented by gray dots and lines, respectively.

The correct localization of the endplate (our method used the monopolar MUAPs) can be verified in the bipolar templates (Fig. 6, C and D), in which the endplate position is indicated by low signal amplitude (in between signals of high amplitude) or reversal of signal polarity. The MUAP with higher mono- and
bipolar amplitudes (Fig. 6, A and C) represents the majority of the templates decomposed from this recording and has its endplates in the craniocaudal (i.e., vertical) center of the grid. The graphic in the middle of Fig. 6 shows that this location roughly corresponds with the craniocaudal center of the muscle. The MUAP presented on the right side (in monopolar and bipolar montage) has a smaller-amplitude territory and its endplate zone has a more cranial and medial location.

Figure 7 shows the endplate zones and DAO muscle fiber orientations of two representative subjects (Fig. 7, A and B) and those parameters for all subjects included in our study (Fig. 7, C and D). The data illustrated in Fig. 7A were derived from the MUAP template set from which two examples were presented in Fig. 6. The occurrence of a dominant endplate zone could be observed in 12 of the 13 subjects (one subject could not voluntarily contract the DAO muscle at all). In nine of these 12 subjects, a second endplate cluster (or single additional endplates) was found either in the cranial–medial direction (in eight subjects; see examples in Fig. 7, A and B), or in the caudal–lateral direction (in one subject). With the exception of one subject, the MUs of this additional cluster had a smaller electrical size (i.e., the MUAPs had lower-amplitude magnitudes) and smaller territories (indicated by the somewhat shorter muscle orientation lines).

Figure 7C showing the data of all subjects proves that most DAO endplates are concentrated in a band-shaped area (running across the muscle in the mediolateral direction) which is located slightly inferior to half the distance between the modiolus and the mandibular’s lower border. The dispersed endplates above this “band” were those of the additional cluster as mentioned earlier. The course of the muscle fibers was obviously different from subject to subject (Fig. 7D), varying from almost straight to rather curved courses (the latter is indicated by increased angulations of the superior and inferior muscle orientation lines). To demonstrate the effect of the normalization procedure, endplate locations and fiber orientations of all subjects are also presented in this muscle in absolute facial coordinates (Fig. 7, E and F). Comparisons of Fig. 7C with E and Fig. 7D with F illustrate the reduction of the interindividual variability after correction for the individual sizes and shapes of the subject’s faces. In the following figures only normalized data are shown.
The data from two representative subjects from the DLI muscle (Fig. 8, A and B) demonstrate the occurrence of MU endplates only in the lower portion of the muscle, typically in a lateral and medial cluster. The fibers above the endplates are mainly orientated toward the medial portion of the respective lower lip quadrant. Fiber orientation in the caudal portion of the DLI muscle could not be determined for most decomposed MUAPs because of the short distance from the endplates to the origin.

Figure 8C proves that eccentric locations of DLI MU endplates (near the origin of this muscle) were a typical finding. The clear separation of the endplate locations in medial and lateral clusters in most of the individual data are only vaguely visible in the combined data of all subjects (obviously arising from interindividual variability). DLI fiber orientation as specified above was a common finding in all subjects. Moreover, in some MUAPs, signal propagation could be traced across the lower mandibular border.

Figure 9, A and B exemplifies that MEN MU endplates could be found in both superior and inferior muscle portions, with a predominance of the superior location in most of the subjects. Typically, the superior endplates were concentrated in a relatively delimited area; a few inferior endplates were more widely distributed in the muscle's caudal portion (see as an example Fig. 9A).

Figure 9C, showing the combined data of all subjects, indicates that the main concentration of MEN MU endplates lies approximately between 25 and 50% of the distance from the inferior border of the lower lip vermilion to the median inferior border of the mandibula. MEN muscle fiber orientation could be determined only inferiorly to the endplates because of the very low amplitude values in the higher portions of the muscle. Inferior fiber orientation was predominantly vertical, with a relatively large variability (Fig. 9, B and D).

Figure 10, A and B, which shows individual results from the OOI muscle, exemplifies the distribution of endplates on the whole area of the muscle and the presence of horizontal and short skew OOI fibers, as well as stationary (i.e., nonpropagating) MUAPs.

Although in the individual data from the OOI we could not find clearly delimited endplate clusters, there was a tendency in 11 subjects for muscle innervation in the mediolateral center, i.e., at a location about halfway between the corner of the mouth and the facial midline (Fig. 10C). Additional endplates were found in the medial OOI portion (in 10 subjects) and/or in an area medial and inferior to the corner of the mouth (in four subjects). One subject showed endplates only near the facial midline and near the modiolus, i.e., the typical occurrence of endplates in the center of the lower lip quadrant was missing. Figure 10D indicates the dominant OOI fiber orientation in parallel to the lower vermilion border, although short skew OOI fibers could also be found in most of the subjects.

DISCUSSION

Sensor technique and data analysis

High signal quality was achieved with the multielectrode sEMG grids especially developed for this study (Lapatki et al. 2004). Because of the high mechanical flexibility of the new sensors, good electrical connections of the single electrodes could be achieved even in subjects with very uneven contours in the recording area, such as those attributed to a deep mentolabial sulcus. Moreover, the flexible electrode grids could, to a large extent, follow the skin movements resulting from the lower facial muscle contractions. As a result the sensors did not significantly hinder the performance of the experimental tasks.

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Two-dimensional topographical information about the electrical activity of a muscle allows the extraction of single MU potentials from the sEMG interference pattern (Masuda et al. 1985; Rau and Disselhorst-Klug 1997; Stegeman et al. 2004). Because we applied a largely automated decomposition method and we did not consider the information about the firing moments of the MUs, the signal decomposition in our study was inherently incomplete. The risk that peaks of MUs with distinct amplitude profiles could have been grouped together and averaged respectively was largely minimized by classifying the detected peaks into a much greater number of groups than the number of expected active MUs. Thus the only

FIG. 9. Endplate locations and muscle fiber orientations of the motor units (MUs) of the MEN muscle. A and B: representative results from 2 subjects. C: combined, normalized endplate positions of all subjects with schematic representations (gray ovals) of the muscle’s origin (apical region of lower lateral incisors) and insertion (in the soft tissue mass of the chin). D: normalized MEN fiber orientations of all subjects, showing the relatively high inter- and intrainsdividual variability in this parameter.

FIG. 10. MU endplate locations and muscle fiber orientations in the OOI muscle. A and B: individual results from 2 subjects exemplifying the relatively dispersed endplate distribution in this muscle and the presence of both horizontal and skew OOI fibers. C: combined, normalized endplate locations of all subjects indicating a high density of endplates in the mediolateral center of the lower lip quadrant. D: combined, normalized muscle fiber orientations of all subjects showing the predominance of the horizontal OOI fibers.
possible compromises for our results were (1) either the averaging of more or less identical MUAP peaks belonging to distinct, but electrophysiologically (and probably anatomically) similar MUs, or (2) the subdivision of the peaks belonging to a single MU into two (or more) different peak groups. In both cases, the consequences for the determined endplate zone distributions and muscle fiber orientations were only of minor significance with regard to the mapping of these topographical MU parameters and the interpretation of the results with respect to anatomical features.

Besides histological and histochemical techniques (Aquilonius et al. 1984; Christensen 1959; Desmedt 1958; Snobl et al. 1998), high-density sEMG techniques (Hilfiker and Meyer 1984; Masuda and Sadoyama 1988; Masuda et al. 1983) and EMG techniques with several inserted invasive electrodes (Lateva et al. 2002) have already proven in previous studies to be suitable to determine the locations of the MU endplates and their distribution on the muscle. The underlying hypothesis that the MUAP amplitude waveforms are closely related to aspects of MU (and muscle fiber) morphology is well studied and an important issue in electrodagnostic medicine (Dumitru 2000; Lateva and McGill 2001). In the present study, we focused on two main aspects of MU topography: the endplate zone location and the main muscle fiber orientation. With respect to the latter parameter, it is important to note that we determined the muscle fiber orientation in the main (central) area of each MU and did not always trace the propagating signal to the end of the muscle fibers (e.g., in the case of diffuse terminal propagation). The only possibility to localize fiber ends with an electrophysiological technique would rely on the detection of “end effects” in the MUAP (Dumitru 2000; Dumitru and King 1999; Stegeman et al. 1997), which were in most instances not visible in our averaged facial MUAPs. End effects may not be visible from these data because of relatively short muscle fiber lengths (i.e., the end effects would be covered by the main peak of the MUAP) and aspects of facial muscle architecture (i.e., the diffuse interdigitation and interweaving of muscles at their origins and insertions; see Fig. 1), which reduce the “sharpness” of the end effect.

A general problem in the interpretation of sEMG data in anatomical respects is connected with the differential movement of the muscle tissue and the electrodes during the contraction. This problem can be especially relevant in recordings with multielectrode sEMG grids that reduce the mobility of the skin in the relatively large recording area and therefore may impede the skin in following the contracting muscle tissue. In our study, the MUAP template sets selected for further evaluation were decomposed from contractions between 5 and 15% of MVC. In an exemplary analysis in the DAO muscle (contraction of this muscle is accompanied by relatively large skin movements) in three subjects and three MUs per subject, we estimated an average relative movement between the electrode grid and the underlying muscle when the contraction level was increased from 5 to 15% MVC of about 1 mm. This value indicates the possible, relatively small bias of our results arising from this effect. Accurate determination of topographical MU characteristics requires that the recording area completely spans the skin area above the examined muscles. With the 128 available recording channels and an IED of 4 mm, the recording area of the two attached electrode grids was about 20 cm² in size. Because we optimally adapted the shape of this area to the average anatomical configuration of the underlying facial musculature, it was rare that the MU territory occurred at the border or even out of the recording area. An exception in this respect were the OOI MUs; in this muscle, endplate locations above the upper border of the medial electrode grid were unavoidable because sEMG grids cannot be placed on the lip vermilion where the density of the OOI fibers is the highest (Blair 1986). To minimize this “vertical” bias in our results as far as possible we positioned the sensors directly at the lower lip’s vermilion border.

Discussion of results in an anatomical and physiological context

Topographical facial MU parameters (i.e., endplate positions and muscle fiber orientations) were imaged and quantitatively determined in relation to facial landmarks. The characteristic spatial profile allowed the decomposed MUAPs to be classified as belonging to certain facial muscle subcomponents. Thus it can be concluded that the complex facial muscle architecture could be successfully mapped with the applied techniques. Discussion of our results is possible in the context of both macro- and microanatomical knowledge.

In general, we found the facial muscle positions (estimated from the mono- and bipolar amplitude profiles and endplate locations) and fiber orientations in good agreement with descriptions and drawings (see, as an example, Fig. 1) in anatomical textbooks (Braus and Elze 1954; Nairn 1975; Salmons 1995; Sobotta 2000), as well as quantitative macroanatomical data (Kennedy and Abbs 1979). In anatomical literature, facial muscle architecture has often been described as having considerable interindividual variability compared with that of other musculature. An interesting finding in our study was that interindividual variability, mainly that of the MU endplate positions, could be significantly reduced by the spatial scaling of the data on the basis of the individual and average facial dimensions (see Fig. 7). Based on this finding, it is possible that a great part of the variation in facial muscle anatomy discussed in the literature is attributed to complex interindividual variations in facial size and shape.

Detailed histological studies on the myoneuronal junctions revealed that normal adult mammalian skeletal muscles are innervated by a single motoneuron (i.e., consist of fibers with a single MU endplate in the middle) and that the MU endplates are usually arranged to form a narrow band crossing the muscle’s central region, described as the “motor band” (Aquilonius et al. 1984; Christensen 1959). This dogma of classical anatomy and physiology has been revised in several respects. There is growing histological and electrophysiological evidence that some mammalian muscles—including the human laryngeal muscles (Perie et al. 1997; Rossi 1990)—may contain fibers with more than one endplate (Lateva et al. 2002, 2003). Further studies with sophisticated electrophysiological techniques indicate that a variety of architectural organizations of MUs, including the presence of single and multiple endplate zones in central and noncentral locations (Lateva and McGill 2001), are present in normal human limb muscles. Such differences from the classical arrangement of MU endplates in one motor zone or band could also be observed in human facial muscles. Histochemical studies revealed that myoneural junctions are distributed on the facial muscles in round or oval-
shaped clusters (Happak et al. 1997). Histological examinations provided evidence that most investigated facial muscles show a small number of clusters corresponding to the number of innervating terminal motor nerve branches. Exceptions were the orbicularis oris, orbicularis occuli, and buccinator muscles, in which MU endplates were found to be spread over the whole muscle resulting in a greater number of small motor zones. Other characteristic findings in these studies were a predominance of one MU endplate cluster (in most of the examined muscles) and eccentric positions of the MU endplates on the corresponding muscle fiber bundles. These distinctive characteristics of facial MU topography could also be observed in our electromyologic study.

In the DAO muscle, which is innervated by two nerve branches from the lateral margin (Happak et al. 1994, 1997), MU endplates were typically identified in two clusters (Fig. 7). The finding in a few subjects of DAO endplates in only a single cluster might be attributable to either an absence of a second endplate cluster (as a sign of interindividual variability) or, more likely, peaks of the smaller MUs belonging to an additional cluster were “covered” by the peaks of the larger MUs (Fig. 6) and were therefore not detected by our peak-detection algorithm.

The DLI, which is innervated from several motor nerve branches of the marginal mandibular branch, showed extreme eccentric endplate locations near its lower border, with a distribution of endplates in the mediolateral dimension in two (or in some subjects three) clusters (Fig. 8). Position of the MU endplates near the origin of this muscle at the lower mandibular border might be connected with the phylogenetic migration of the fibers from the neck to the area near the corners of the mouth and the interconnection with the platysma, respectively (Braus and Elze 1954). The predominance of either lateral or medial endplate location in the DLI muscle varied from subject to subject. Most DLI MUAPs had amplitude territories that spanned the region of the lower lip. This confirms histological findings of serial sagittal sections of human lower lip in which groups of vertical and skew muscle fibers with an origin inferior to the lower lip (i.e., in the central DLI muscle area) were found in between the horizontal OOI fibers (Blair 1986; Blair and Smith 1986). An interesting additional finding during our experiments was that some subjects were able to contract the DLI muscle either in its lateral or medial region. This ability reflects the endplate zone distribution on this muscle and can be interpreted as compartmentalization, i.e., within-muscle differences of anatomical and physiological parameters that partition the motoneuron pool of a single muscle into independently controlled subsets (Binder and Stuart 1980). The possibility of such heterogenic contraction was also observed in masticatory muscles (Blanksma and van Eijden 1995; Phanachet et al. 2001), which—similar to the facial muscles—stem from the branchial arches.

In the MEN muscle, a predominant MU endplate cluster was usually found in a location slightly lateral to the facial midline (vertically) between a quarter and half the distance from the lower lip vermilion’s inferior border to the lower bony margin of the mandible. One or two additional, often less clearly delimited endplate zones were often located in the muscle’s inferior portion. These results cannot be compared with other data because, to our knowledge, no histological study has been carried out on the MEN muscle. The finding of relatively dispersed MEN endplate locations in the combined data of all subjects might be connected with the high interindividual variability in soft-tissue thickness and contour in the lower lip and chin area (Kennedy and Abbs 1979) because the measured sEMG signals are a “projection” onto the surface of the electrode grids (which follows the uneven skin contours; see Fig. 2A). The difficulties in determination of the superior MEN muscle fiber orientation and the low amplitudes of the MEN MUAPs above the endplates, respectively, are explained by the three-dimensional fiber course and the filtering properties of the soft tissues: the MEN muscle has its origin on the anterior border of the alveolar process of the mandible and is therefore covered by the thick soft-tissue mass of the chin and the lower lip in its superior part.

The orbicularis oris muscle consists of four (upper/lower/right/left) quadrants, each of them anatomically subdivided in a marginal portion located within the lip vermilion, and a peripheral portion around the lips. The marginal and peripheral subcomponents of the investigated inferior orbicularis oris muscle (OOI) could not be separately examined in our study because of the impossibility of positioning sEMG electrode grids directly over the lip vermilion and the incapability of most subjects (even trained trumpeters) to contract the subcomponents separately. Anatomical and histological studies have shown that the OOI is innervated from its lateral side by two terminal nerve branches and has MU endplates spread over the muscle in multiple clusters. In agreement with these data from former studies, we observed dispersed MU endplate locations in the investigated inferior part of this muscle in most of the subjects, with a main concentration of endplates, however, in the center of the right or left lower orbicularis oris quadrants (i.e., approximately half the distance between the corner of the mouth and the facial midline). We were surprised to find MUs with only relatively small territories (when compared with the whole muscle’s length) because in the anatomical literature horizontal OOI fibers are described to run from the modiolus to the facial midline without interruption (Braus and Elze 1954). This issue requires further electrophysiological and histological investigations. On the other hand, the small territories and distinct muscle fiber orientations might reflect the muscle’s complicated fiber architecture. As already mentioned in the context of overlapping electrical territories of OOI and DLI MUs, serial sagittal sections of human lower lip revealed the presence of fiber groups with distinct orientation (i.e., horizontal, tangential, and vertical fibers) in the region “traditionally described as OOI.” The relative masses of these fiber groups showed considerable variation as a function of medial–lateral and superior–inferior position (Blair 1986). Electrophysiological studies suggest that these distinct fiber groups in the OOI belong to MUs that are independently controlled and have differential mechanical functions (Abbs et al. 1984; McClean and Smith 1982). From all these electrophysiological and anatomical/histological data it can be concluded that MU architecture of the muscle tissue in the area of the lower lip is highly developed and very complex. Dispersed location of horizontal OOI MUs along the lips as well as the presence of intrinsic nonhorizontal OOI MUs and DLI MUs in this area endow the lips with the ability of highly differential variation of tension and 3-D shape. Such fine and differential motor control is required such as in production of speech sounds, drinking, mastication, and other oral functions.
An additional, frequent finding in averaged OO1 MUAPs was the presence of two widely (i.e., ≤10 mm) separated, propagating zones of high amplitude. Possible explanations for this phenomenon are either branching of the motor nerve in its distal part (Happak et al. 1994, 1997) or synchronization of the firings of distinct MUs. Synchronization of facial MUs has been described in previous studies (Folkins et al. 1988; Wohlert 1996a) and seems to be one of the components of differential neural regulation of lip muscle activity on a premotoneuron level depending on the motor task (Wohlert and Goffman 1994).

To conclude, in this electrophysiological study, the position and distribution of MU endplates and principal muscle fiber orientations of facial muscles have been mapped and quantitatively determined. Such fundamental data add substantially to the basic neurophysiologic and anatomical knowledge of the complex facial muscle system. The results obtained can also be regarded as a basis for improving both the accuracy and the reproducibility of conventional facial sEMG measurements. Facial sEMG has proved to be a valuable, noninvasive tool in many research and clinical applications in psychophysiology (Jancke et al. 1996; Lundqvist 1995; Root and Stephens 2003; Sloan et al. 2002), speech physiology and pathophysiology (Kelly et al. 1995; Leanderson et al. 1971; Raark and Moore 1997; Wohlert 1996a,b; Wohlert and Hammen 2000), as well as in general dentistry and orthodontics (Lapatki et al. 2002; Tosello et al. 1998, 1999; Yamaguchi et al. 2000). Electrode placement in these studies has been oriented only toward the estimated positions and fiber directions of the examined muscles, with the intention in most of the studies to position the sensors on the center or “belly” of the examined muscle. Our results prove that this electrode position is suboptimal at least in some muscles, such as in the DAO muscle, because of the central location of most endplates (Fig. 7), which means that relevant factors for electrode placement (Freriks et al. 1999) had to be ignored in previous studies because of the lack of quantitative data on these variables. In our study, these gaps in fundamental anatomical and neurophysiological knowledge have been bridged to a large extent and, consequently, foundations have been laid for objective guidelines for facial EMG electrode placements. The presented results may also be relevant from a clinical perspective. The information about MU topography obtained in a group of healthy individuals is useful as normal data in the observation of regeneration and reinervation of MUs after peripheral nerve injuries or muscle transplantation, and in the study of the topographical aspects and the characteristic alterations on the MU level of neuromuscular diseases affecting the facial musculature [e.g., facioscapulohumeral dystrophy (FSHD) and Möbius syndrome]. Finally, maxillofacial surgical reconstruction of facial musculature (e.g., in patients with cleft lip or tumors in the face) and therapeutic interventions with botulinum toxin (with respect to the definition of optimal locations for injections) may also benefit from this fundamental data on the facial musculature and myoneural junctions in healthy individuals.

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