OASIS Progress Report

Digestion

Digestion 2004;69:123–130
DOI: 10.1159/000077878

Published online: April 14, 2004

Innervation Zones of the External Anal Sphincter in Healthy Male and Female Subjects

Preliminary Results

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Key Words
External anal sphincter · Surface EMG · Motor unit · Electrode arrays · Innervation zone

Abstract
Objectives: The objective of this work was to investigate the distribution of the innervation zones of the motor units that make up the external anal sphincter (EAS) in healthy males and females. ***Methods:*** A cylindrical probe carrying a circumferential array of 16 electrodes was used to detect the generation, propagation and extinction of individual motor unit action potentials (MUAPs) at 1, 2, and 3 cm depth from the orifice of the anal canal during maximal voluntary contractions of the EAS. Fifteen healthy males and 37 healthy nulliparous females were investigated. ***Results:*** IZs could be detected in all males and in 34 out of 37 females. In the males, the IZs are scattered in the right and left hemisphincter at each of the three levels and their distribution is not affected by depth. In the females, the distribution is also concentrated in the right and left hemisphincter at depth 1 cm but is more uniform at depth 2 cm and more concentrated in the dorsal and ventral regions at depth 3 cm. ANOVA shows a statistically significant dependence of the IZ distribution on depth only in females and not in males. ***Conclusions:*** It is concluded that (a) IZs of the EAS can indeed be detected with a circumferential array placed at different depths along the anal canal; (b) large individual variability is observed, and (c) IZs show similar distribution at the three depth levels in males and different distributions in females.

Introduction

Non-invasive recording of surface-EMG (S-EMG) from pelvic floor sphincter muscles usually adopts either longitudinal or ring-shaped pairs of electrodes or perianal electrodes [1–5] for assessment of latencies and velocities of signal transmission along the neural pathway to the sphincter muscles [6]. In biofeedback training, S-EMG is used for within-subject control of overall improvement of S-EMG amplitude during therapy. Occasionally, side-separated recording of S-EMG by two pairs of electrodes has been performed to study laterality of sphincter innervation [7]. In all these cases, detection, recognition and clas-

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0012–2823/04/0692–0123$21.00/0

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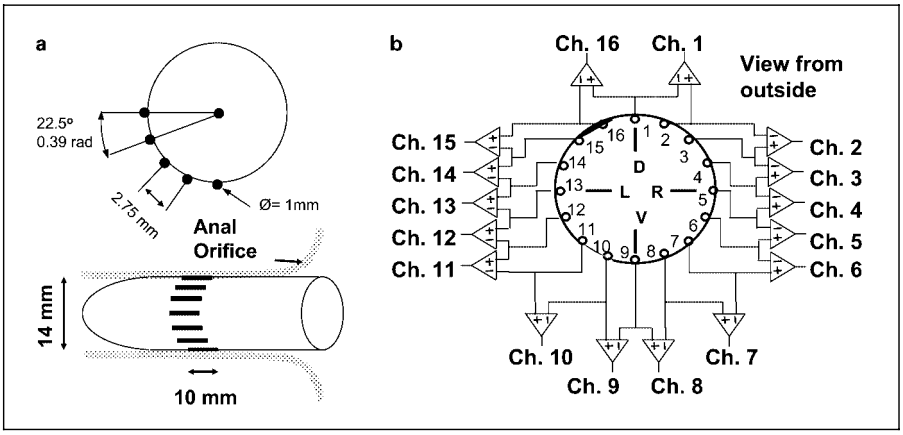

Fig. 1. a Rectal probe with electrode array. **b** Amplifier connections. D = Dorsal; V = ventral; R = right; L = left.

sification of single motor unit action potentials (MUAPs) or identification of the location of the innervation zone(s) (IZ) of the sphincter muscle is not possible, but also not necessary due to the limited purpose of the task.

Information concerning the motor unit (MU) structure would, however, be very useful in a number of clinical situations: for description of asymmetry, for investigation and quantification of muscle activity during stress, relaxation, and fatigue, for the differentiation between myogenic and neurogenic pathologies of the muscle, and for the identification of areas that may be more vulnerable to trauma and/or surgery because of the resulting possible denervation [8–11] or because of increased risk of post-intervention symptoms due to asymmetry of sphincter innervation [12].

A previous paper demonstrated the possibility to extract this information from the external anal sphincter (EAS) [13] by means of S-EMG via an array of electrodes placed along the direction of the muscle fibers. Such methodology is based on an anal probe comprising an array of 16 equally spaced electrodes placed around the circumference of the probe and able to detect the generation, propagation and extinction of MUAPs produced by the MUs of this muscle [13]. Proper conditioning hardware and signal processing software amplify, filter and display the signals on the screen of a PC. The location of the innervation zones (IZs), the individual MUAPs and their conduction velocity can then be estimated visually or automatically with suitable software (under development).

The main aims of this study were to identify the location of the IZ at three different depths (1, 2 and 3 cm) along the anal canal in a number of healthy males and nulliparous females.

Materials and Methods

Probes and Subjects

Cylindrical probes, 14 mm in diameter and carrying an array of 16 silver bars (1 mm diameter, 10 mm length, 2.75 mm and 0.39 rad apart, as shown in fig. 1a) were specifically designed and built (by Ottino Bioengineering, Rivarolo, Torino, Italy) to record MUAPs circumferentially along the muscle fibers of the EAS during voluntary contractions in 52 healthy subjects with no history of neurological or pelvic floor problems (37 nulliparous females and 15 males, 20–55 years old) recruited in three centers (Department of General Surgery, University Hospitals Tübingen, Germany; Department of Gynecology, Vivantes Klinikum Neukölln Berlin, Germany; Division of Digestive Disease, University Hospital Val d'Hebron, Barcelona, Spain). All subjects signed an informed consent form approved by the local Ethical Committee.

Signal Detection and Conditioning

Signals were recorded differentially between adjacent electrodes of the same array (fig. 1b), during rest and during maximal voluntary contractions of the EAS, at 10, 20 and 30 mm depth from the anal orifice, as indicated in figure 2. Since the array is circular, 16 differential signals are obtained from the 16 electrodes, the last pair being made by electrodes 16 and 1 (fig. 1b). The EMG signals were amplified (Prima Srl amplifier model EMG-16 with gain variable from 1,000 to 50,000 in 6 steps, 10–500 Hz 3 dB bandwidth, roll-off of 40 dB/decade, noise level of <1 μV_{rms}), sampled at 2,048 Hz per channel and stored on a PC after 12 bit A/D conversion. The bandwidth of the EMG amplifiers was designed to accommodate the spectrum of the striated muscle EMG. Slow signals produced by active smooth muscles (if any) were rejected because of the high pass filter at 10 Hz. Further noise reduction was obtained by digital moving average low pass filtering with 350 Hz cut-off frequency.

Experimental Procedure

Subjects were lying on their side and the probe was held in position by the operator. A drop of glycerine was applied to the tip of the probe to facilitate insertion. Conductive gel or gel for ultrasonic applications produced electrode shorting and could not be used as a lubricant because of high conductivity. Each subject was instructed to relax completely and to produce three maximal voluntary contrac-

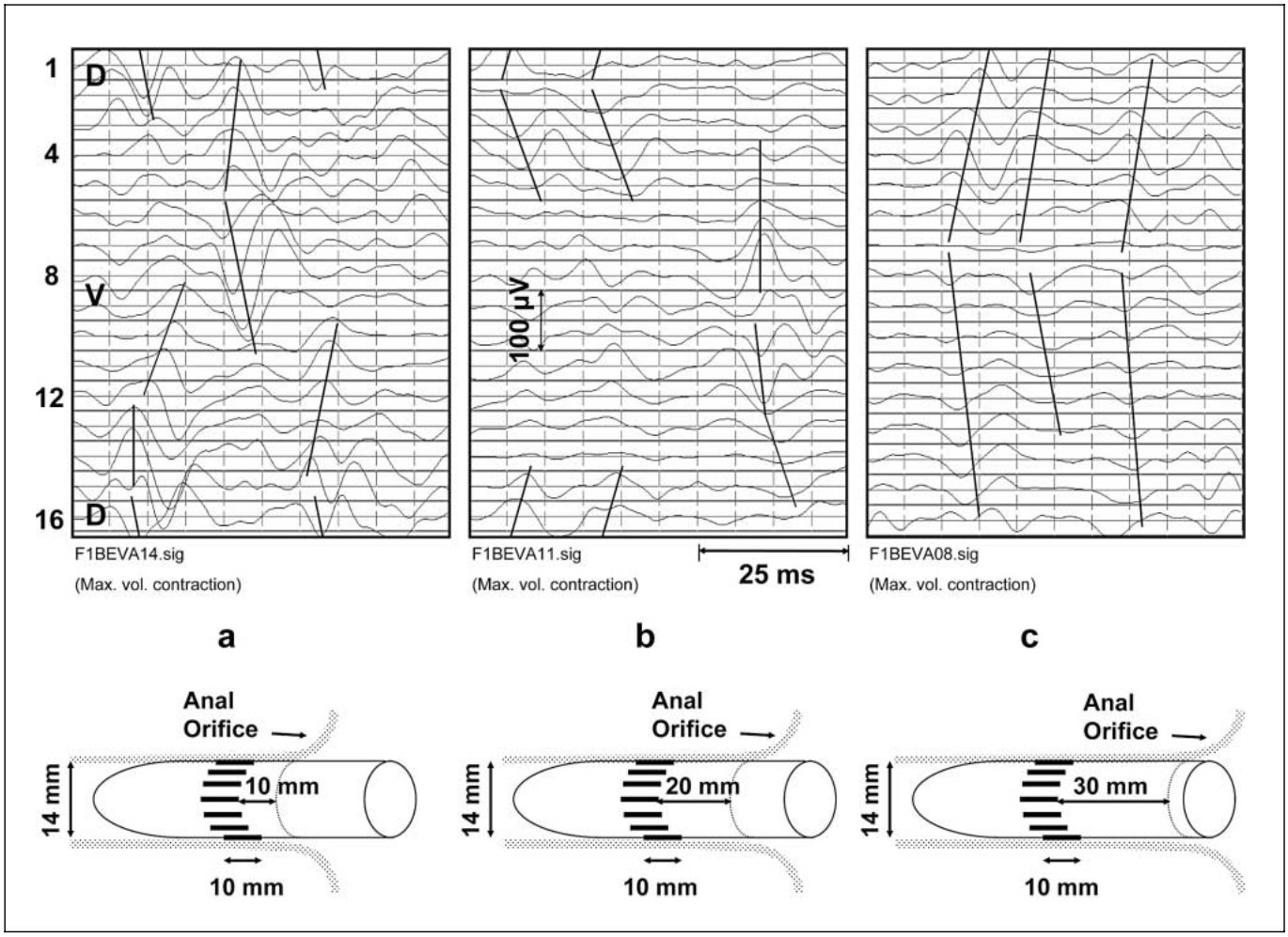

Fig. 2. Examples of raw S-EMG signals detected at three different depths into the anal canal during maximal voluntary contractions. The characteristic 'signatures' of motor unit action potentials are evident. Two innervation zones can be identified in (**a**) and in (**b**) and one in (**c**).

tions (MVC), each sustained for 10 s. S-EMG signals were recorded in the two conditions. Rest time between MVC contractions was about 2 min.

Identification of the Innervation Zones

The IZ is defined as the region of a MU including the end-plates (or neuromuscular junctions) and its location is defined as the position of the electrode(s) under which the MUAPs are generated and begin their propagation in two opposite directions toward the fiber endings. The signals propagating in opposite directions appear with opposite phase at the output of the amplifiers since the potential wavefront propagating in one direction is detected first by the inverting inputs (–) and then by the non-inverting inputs (+) of the amplifiers while the opposite happens for the other wavefront propagating in the other direction (fig. 1b, 2). In some cases, a MUAP appears as propagating in a single direction suggesting that a MU might be innervated at one end. In some other cases, the MUAPs are aligned on the different channels (no propagation delay) suggesting that the signal is generated by a remote source, outside the EAS, possibly within the pelvic floor or the glutei. These two cases have not been considered for the purpose of this work and their nature requires further investigation.

The operator identifies two-directional propagating patterns of individual firings and finds the IZ of each. Figure 2 depicts examples of raw signal segments obtained from the same subject at three different depths and at the MVC contraction level. The skewed lines are added for clarity. The left panel (1 cm depth) clearly shows three different MUs, one innervated between channels 5 and 6 and two between channels 15 and 16 (consider that channel 1 follows channel 16 around the circle). The central panel (2 cm depth) also shows three different MUs, two innervated under channel 2 and one between channels 9 and 10. The right panel (3 cm depth) shows three different MUs all innervated under channel 7. An IZ shared by many MUs (as in fig. 2c) is counted as one for the purpose of this paper. A sharp

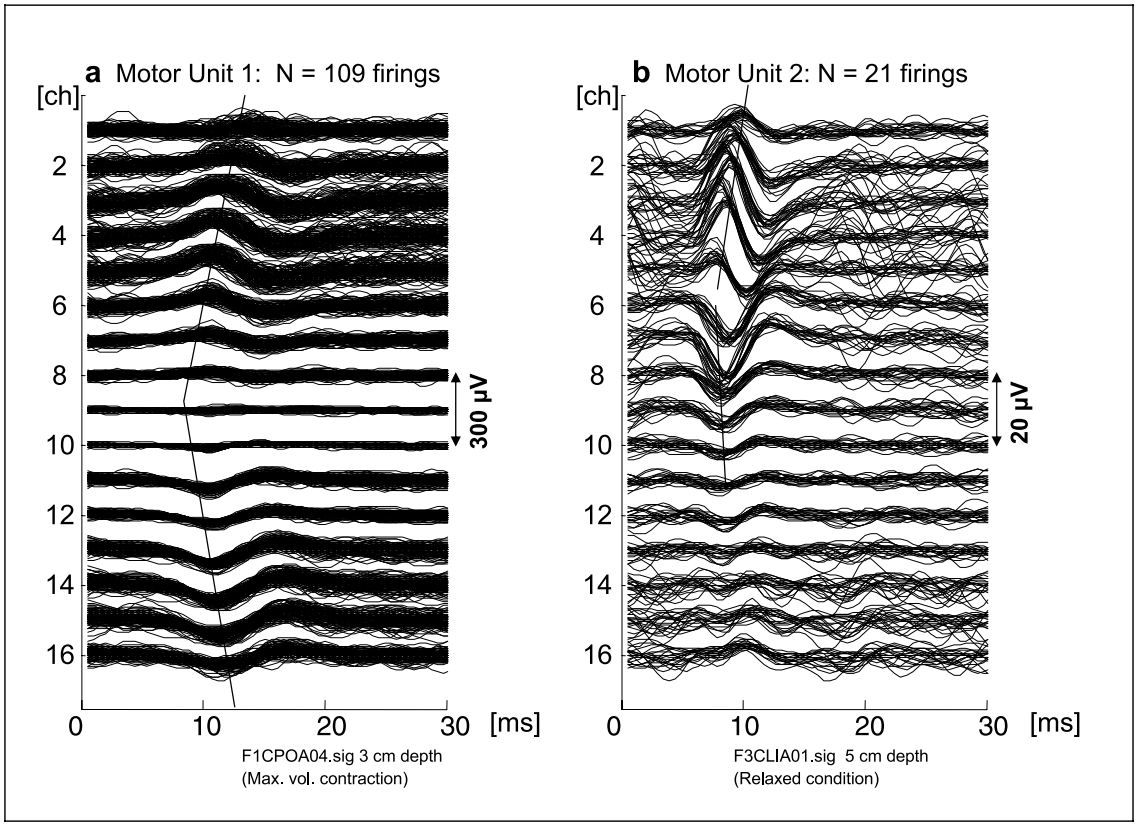

Fig. 3. a Example of 109 superimposed MUAPs generated by the same motor unit, innervated under Ch. 9, with fibers extending to the entire circle, recorded during a maximal voluntary contraction. **b** Example of 21 superimposed MUAPs generated by the same motor unit, innervated between Ch. 5 and 6 with fibers extending to Ch. 1 and 11, recorded in relaxed conditions.

phase inversion between one channel and the next indicates a very narrow IZ. The presence of a few channels with low amplitude and unclear propagation at the center of the pattern suggests a wide IZ. To be sure that a signal is a MUAP and not an artifact its repetition must be checked. Figure 3 depicts superimposed MUAPs generated by two MUs in different subjects. The MU in figure 3a has a wide IZ between Ch. 8 and 10 (its center is assumed to be under Ch. 9) whereas the MU in figure 3b has a narrow IZ between Ch. 5 and 6, that is under electrode 6. For the purpose of this work, on the basis of visual analysis of the signals, it was decided to define an IZ as the zone corresponding to the generation of at least 10 MUAPs, showing two-directional propagation, in 10 s. These MUAPs may of course belong to different MUs, as evident in figure 2. This arbitrary threshold was defined to insure positive IZ identification; it might be more or less liberal in the future as experience with the technique will increase and automatic IZ recognition will be developed and tested against visual recognition. Only few recordings in relaxed conditions met this criterion and will not be described in this work; only maximal contractions will be discussed.

Results

From the recordings shown in figures 2 and 3, it is clear that the technique allows the identification of individual MUAPs and of the IZs of the EAS.

At the MVC level, all the 15 male subjects met the IZ identification criterion at each of the three depths. Out of the 37 female subjects 34 met the criterion at 1 and 2 cm depth (in 3 cases no IZ could be clearly identified at either 1 or 2 cm depth), and 35 met it at the depth of 3 cm. In most cases only one IZ could be detected clearly, according to the criterion given in 'Material and Methods' (fig. 2c), whereas in some cases two or three IZs were identified (as in fig. 2a and b).

The left column of figure 4 shows the histograms of the IZ distributions at the three depths in the 15 male subjects. At all three levels the IZs are rather scattered showing a large interindividual variability. If the three histograms are added, the plot of figure 5a is obtained. An

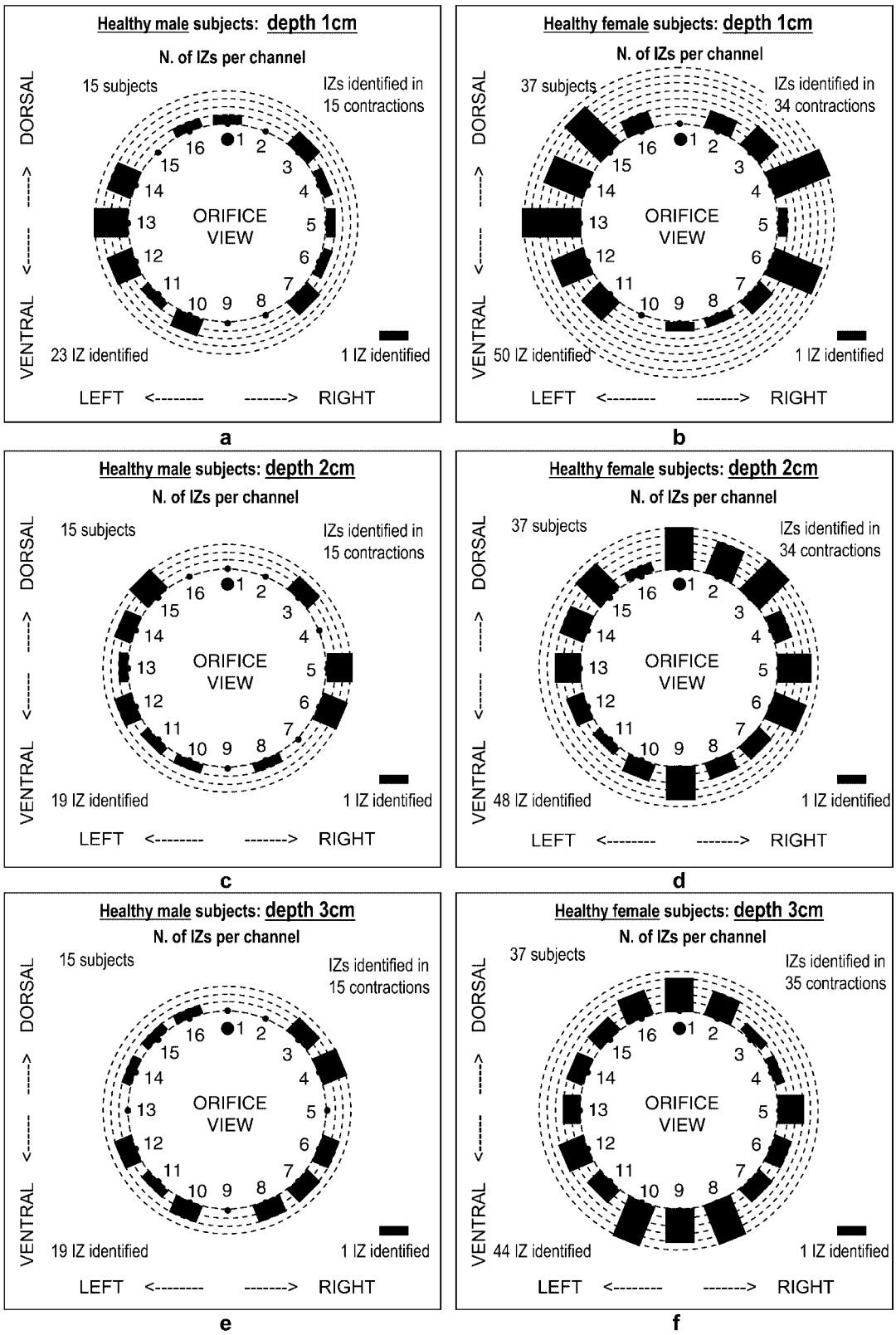

Fig. 4. Histograms of the number of innervation zones (IZ) found under each channel in 15 males (left column) and 37 females (right column) at the depth levels of 1, 2 and 3 cm.

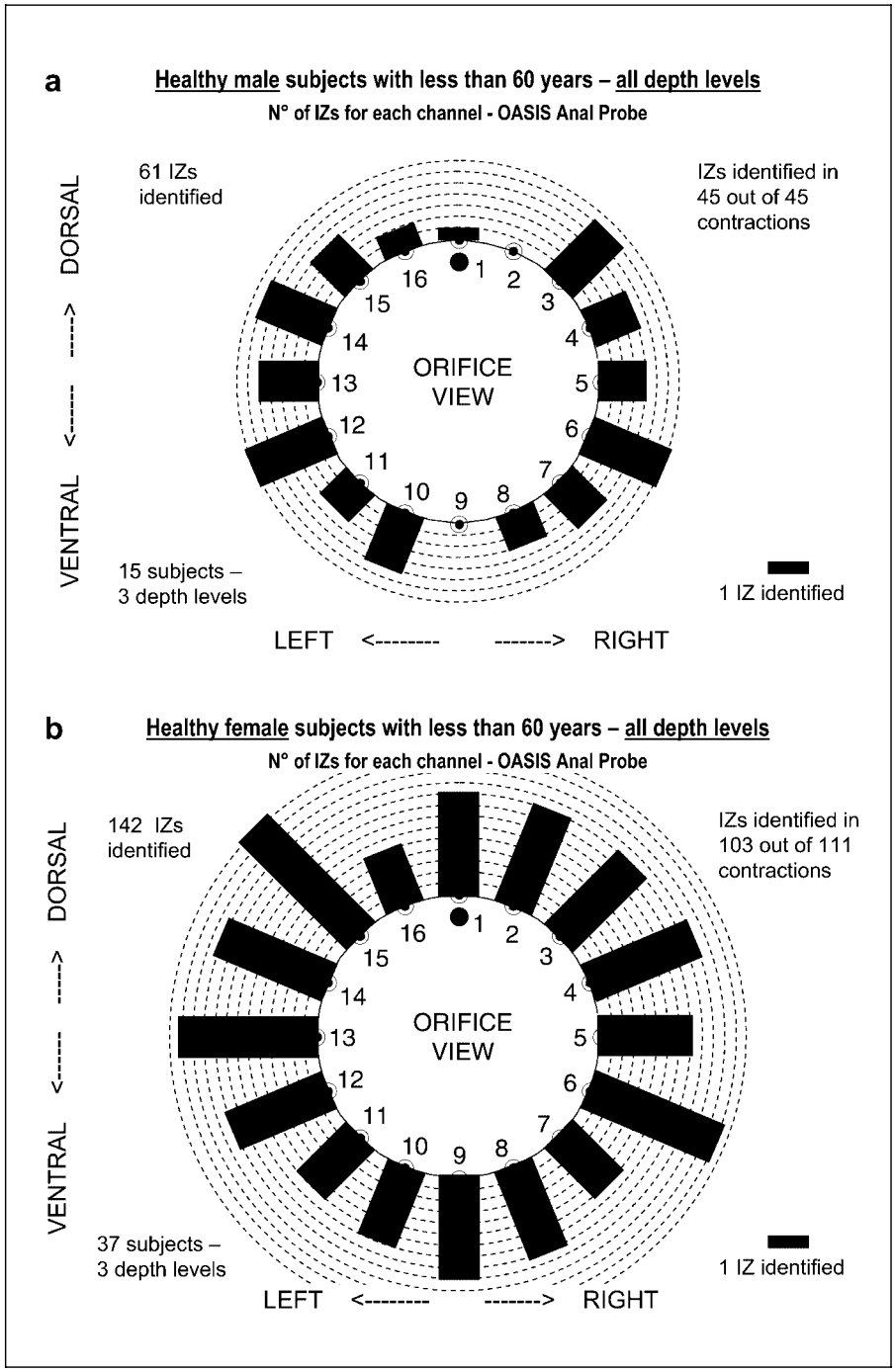

Fig. 5. Cumulative histogram of the number of innervation zones (IZ) found under each channel in 15 males and 37 females. The IZs at different depth levels are pooled together.

apparent predominance of right-left innervation with respect to ventral-dorsal innervation can be detected but is not confirmed by ANOVA.

The right column of figure 4 shows the histograms of the IZ distributions at the three depths in the 37 nulliparous female subjects. An innervation pattern similar to that observed in males appears only at the 1 cm level. At the 2-cm level the distribution of IZs is more uniform around the circle and at the 3 cm level IZs are mostly in the dorsal and ventral regions with predominance on the ventral side. The ANOVA detects a correlation between the IZs in the ventral quadrant and depth ($p = 0.017$). The

Newman-Keuls test detects a statistically significant difference ($p = 0.013$) between the number of IZs in the ventral quadrant at depths 1 and 3 cm.

The Kolmogorow-Smirnoff test indicates that there is insufficient data to detect an effect of either depth or gender on the statistical distribution of IZs.

Discussion

A considerable amount of information can be extracted from the S-EMG of the EAS. This study deals only with the location of the IZs detectable at 1, 2 and 3 cm depth levels in the anal canal during maximal voluntary contractions. Except for our recently reported work showing that it is possible to identify MUAPs of the EAS and that there is large interindividual variability [13], no previous experience on this topic has been reported. Although IZ can be identified from a single MUAP, at least 10 MUAPs starting at the same location and showing bidirectional propagation were required in this study to identify such location as an IZ. The MUAPs generated in such location could belong to different MUs. This criterion is rather arbitrary and has the purpose of guaranteeing a positive identification of an IZ. Relaxing it would increase the number of IZs by including those where fewer MUAPs are generated, but would also increase the risk of wrong detection due to artifacts; this may be appropriate once more experience with this technique has been gathered. This criterion therefore underestimates the total number of innervation zones observed in the EAS with the electrode array. Two additional limitations should be considered: (a) the IZs of MUs that are far enough from the electrodes, so that their MUAPs are near the noise level, are not detected, and (b) the MUAPs showing unidirectional propagation are not considered. For the reasons indicated above these results should be considered preliminary. More solid evidence will come with the development of automatic procedures for detecting IZs and with a larger number of subjects.

These data may allow to close a missing link between different clinical findings with respect to the pelvic floor. First, the incidence and prevalence of urinary and fecal incontinence is known to be significantly higher in women as compared to men, especially at higher age [14]; this is usually attributed to specific risks for pelvic floor trauma women undergo with pregnancy and childbirth. The pathophysiology of incontinence, as discussed in the papers by Enck et al. [15] and by Heesakkers and Gerretsen [16] in this issue of *Digestion*, has recognized these gender-specific risk factors, as has the investigation of the role of asymmetry in incontinence [7]. It is conceivable that the different probability of innervation of the ventral region at different depths – as found in our study – may be a developmental consequence of higher risks for birth trauma in this region of the pelvic floor, and is 'aimed' at preventing or reducing the risk of incontinence in such case.

Second, pelvic anatomy has also shown significant differences between men and women, especially since new imaging technologies (endoanal ultrasound, MRI) have been applied to the study of pelvic floor anatomy [17, 18]. Among the many findings, one important fact is that at deeper muscle layers of the EAS, the muscle body becomes thinner, and less distinguishable from the surrounding connective tissue especially in the ventral region [19]. Interestingly, this is the same area in which the data from this study showed a higher likelihood of innervation (i.e. more frequent IZs). However, in light of the increased risk of the ventral and superficial part of the anal sphincter for damage during childbirth, increased ventral innervation in the deep part and increased lateral innervation in the superficial part may be nature's mechanisms to reduce the consequences of trauma.

Finally, measurement of pelvic floor functions such as anorectal manometry has long been noted to show significant differences between the genders: women exhibit lower resting pressures (a function of the internal anal sphincter) and squeeze pressures (attributed to the EAS) than men, irrespective of age [20], but ultimate reasons for this have never been disclosed. Usually, this is discussed with respect to a different muscle composition (e.g. the ratio of type 1 to type 2 fibers) in women [21], but solid data are missing. With the data from the study presented here, it becomes conceivable that an altered innervation pattern in women – a less homogenous innervation in the circumference – may change the function of the sphincter apparatus during contractions as measured by the pressure inside the anal canal. Final proof of the hypothesis will, however, require further studies.

Conclusions

The conclusions of this preliminary study are the following: (a) one, occasionally two, IZs can be identified, during a MVC contraction of the EAS, using a circumferential electrode array; (b) there is no standard innervation pattern of the EAS and interindividual variability is large, and (c) the available data are insufficient to establish if the distribution of IZs is significantly related to depth or gender. However, (d) the number of IZs in the ventral quadrant of the EAS of healthy nulliparous females increases with depth.

Further research is necessary to (a) associate the findings of this work to possibly different EAS anatomical structures in male and female subjects [22]; (b) understand the nature and origin of MUAPs showing either aligned potentials or unidirectional propagation [23]; (c) identify asymmetry indexes [24–26]; (d) identify fatigue indexes based on myoelectric manifestations of muscle fatigue, and (e) identify correlations with age and pathologies.

Acknowledgements

The multichannel EMG amplifier system was manufactured by Prima Srl, Treviso, Italy. The probe prototypes were designed by LISiN and manufactured and distributed by Ottino Bioengineering, Rivarolo Canavese, Torino, Italy, and FORNIX Medical Systems, Beunigen, The Netherlands. This work was supported by the European shared cost project On ASymmetry In Sphincters (OASIS, Contract No. QLK6-CT-2001-00218), the Regional Health Aministration of Piemonte, Fondazione Cassa di Risparmio di Torino, Compagnia di San Paolo, Torino, Italy, and the Deutsche Forschungsgemeinschaft (DFG En 50/21).

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