Repeatability of innervation zone identification in the external anal sphincter muscle

REPEATABILITY OF INNERVATION ZONE IDENTIFICATION IN THE EXTERNAL ANAL SPHINCTER MUSCLE

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Abbreviations: AI, asymmetry index; CC, coefficient of correlation; CMC, coefficient of multiple correlation; EAS, external anal sphincter; EMG, electromyography; IED, inter electrode distance; IZ, innervation zone; MU, motor unit; MUAP, motor unit action potential; MVC, maximal voluntary contraction; SD, single differential; STD, standard deviation

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ABSTRACT

Aims: Knowledge of the distribution of the innervation zones (IZs) of the external anal sphincter (EAS) may be useful for preventing anal sphincter incompetence during vaginal delivery. A method proposed for the automatic estimation of the distribution of IZs of EAS from high-density surface electromyography (EMG) was evaluated for repeatability of the estimation of IZ distribution in continent volunteers.

Methods: In thirteen healthy female subjects (age: 35 ± 11 years) surface EMG signals were acquired using an anal probe with three circumferential electrode arrays (of 16 contacts each) at different depths within the anal canal (15 mm distance between the centers of adjacent arrays), during four independent experimental sessions. Three maximal voluntary contractions (MVC) of 10 s were performed for each session for a total of 12 contractions per subject. Repeatability of the estimation of the distribution of IZ was tested by evaluating the coefficient of multiple correlations (CMC) between the IZ distributions estimated from the signals recorded from each subject.

Results: A high repeatability (CMC>0.8) was found comparing IZ distributions estimated from signals recorded by each array within the same session. A slightly lower value was obtained considering signals recorded during different sessions (CMC>0.7), but a higher value (CMC>0.8) was obtained after aligning the estimated IZ distributions. The realignment compensates the operator's error in repositioning the probe in the same place during different sessions.

Conclusion: This result justifies clinical studies using high density surface EMG in routine examinations, providing information about IZs of EAS and assessing the possibilities of preventing neuronal trauma during vaginal delivery.

Keywords: electromyography, innervation zone, external anal sphincter, episiotomy

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INTRODUCTION

Reliable methods for identifying women at risk to become incontinent after delivery are lacking, and often have low sensitivity and specificity. Anorectal manometry shows great inter-individual variance of external and internal anal sphincter functions among continent subjects [1], and even more among patients with incontinence.[2] Endoanal ultrasound allows identification of delivery-related muscle trauma [3,4] but has poor correlation to functional findings such as with needle electromyography (EMG) [5] and manometry. [6]

Needle EMG is used in clinical practice to investigate the neuronal control of the external anal sphincter (EAS) in patients with defecation disorders.[7] Surface EMG signals detected by electrodes incorporated in a probe and inserted into the anal canal have also potential applications in the analysis of pelvic floor muscles.[8] Although surface EMG is less selective than needle EMG, it is less invasive and provides information about a greater tissue volume. Pairs of electrodes have been applied in previous works.[9-12]

The analysis of the EAS muscles by surface EMG has recently suggested the possibility of locating the innervations zones (IZ) of the EAS and, therefore, its principle ability to prevent neuronal damage during elective episiotomy, especially in cases of asymmetric sphincter innervation.[7] Some preliminary results based on two channel detection of surface EMG indicated a correlation between childbirth-related sphincter injuries, asymmetric sphincter innervation, and faecal incontinence symptoms.[12]

Recently developed multi-electrode probes provide more selective information compared to bipolar probes used in most clinical studies so far. The electrodes of the probe are arranged on circular arrays following the presumed arrangement of muscle fibers. Several motor unit (MU) action potentials (MUAP) travelling across different channels can be identified [13] and the position of the IZ of the identified MUAPs along the circumference can be estimated.[13-15]

Great attention has recently been devoted to the assessment of the distribution of IZ of EAS (see Digestion, volume 69, 2004, section on the European Community Project OASIS, pp. 84-130). The IZ distribution can be estimated from multi-channel surface EMG signals by visual analysis,[13] or by an automatic method [15], and the reliable performance of this method was validated on simulated signals.[16]

Our method is completely different from other techniques, which do not measure electrophysiological variables, such as the location of the IZ and its asymmetry. It is expected that this totally new information extracted from multi channel surface EMG can support the development of a technique of clinical relevance. In order to evaluate the clinical applicability of our method, the repeatability of the automatic estimation of the distribution of the IZ in EAS muscles is a prerequisite. Repeatability of the estimation of IZ distribution is also a pre-requisite to continuing our research, applying the method on a larger set of subjects and providing clinical evidence of its importance. The repeatability of IZ estimation together with the accuracy of the estimate supported by the simulation study 16 would provide the indication that a single measure is sufficient to get reliable information. This would support the development of a fast, minimally invasive and reliable method with potential clinical relevance.

This work is focused on the assessment of this repeatability. In addition, two parameters indicating asymmetry and non-homogeneity of IZ distribution are investigated.

METHODS

Subjects

Fifteen healthy female subjects participated to the measurements. Two subjects were discarded due to unstable electrode-mucosa contact, resulting in poor signal quality. Thus,

thirteen subjects were considered for subsequent processing (mean ± SD: age: 35 ± 11 years, height: 163 ± 6 cm, body mass: 61 ± 11 kg). The study was carried out at the University of Tübingen (Tübingen, Germany) and was approved by the Local Ethics Committee. A written informed consent was obtained from each participant prior to the measurement session. All subjects were free from neuromuscular diseases, and all were nulliparous.

Surface EMG measurement

The surface EMG from the EAS was acquired using the system shown in Figure 1a. The cylindrical anal probe is shown in Figure 1b. The surface EMG differential signals were detected with 48 electrodes (silver wires, 10x1 mm) grouped into three circular arrays of 16 electrodes equally spaced along the circumference (probe diameter of 14 mm). The three arrays were spaced of 15 mm (measured from the center of electrodes). The surface EMG signals were conditioned to reduce common mode interference and amplified (EMG-USB amplifier, LISiN-OTBioelettronica, Torino, Italy; CMRR > 96 dB, gain adjustable from 100 to 10000, 10–750 Hz 3 dB bandwidth, roll-off of 40 dB/decade, equivalent input noise level less than 1 μVrms), sampled at 2048 Hz per channel and stored on a PC after 12 bit A/D conversion. Single differential (SD) signals were detected between adjacent electrodes so that 16 signals were recorded from each array for a total of 48 signals.

Experimental procedure

Subjects were lying on their left side and the probe was held in position by the operator. A drop of glycerin was applied to the tip of the probe to facilitate insertion. The probe was placed by the operator using the visual reference provided by the fin of the anal probe (Figure 1b). This reference was always placed in dorsal position. The first electrode of each

array was identified by a triangular marker, pointing in the direction of increasing electrode number (clockwise in the rear view).

The protocol is shown in Figure 1c. Measurements were performed in two days. For each day, the following procedure was followed. Each subject was instructed to relax completely and to produce maximal voluntary contractions (MVC) on request. Signals were acquired during a rest condition, followed by three MVCs and a final rest condition, with 2 minutes rest between each MVC. The probe was then removed, cleaned and reinserted. The sequence of five acquisitions was then repeated.

Each insertion of the probe can be considered as a separate experimental session, resulting in four independent different sessions per subject.

Signal Processing

Each signal was digitally filtered off-line with a band-pass 4^{th} order Butterworth filter (15-400 Hz band) using an anti-causal implementation without phase distortion. The algorithm discussed in [16] was used to extract the IZ distributions of the filtered signals. The algorithm detects propagating MUAPs and estimates the IZ for each detected MUAP under the channel from which propagation starts, therefore obtaining a distribution of IZ. For each of the three arrays, the IZ distribution was automatically determined, as shown in Figure 2a which refers to the most external array. The IZ distribution IZ_{dist} was normalized to have unit area and considered as a 16 bin probability density histogram showing the percentage of MUAPs initiating under each electrode pair.

Two parameters were extracted from the estimated IZ distribution.

1) The asymmetry index (AI) was defined as the distance of the barycenter (centroid of the distribution) from the center of the probe normalized with respect to the radius of

the probe. Symmetric distributions of IZs correspond to AI=0; in the case in which the IZs of all MUs were located under one single channel, AI=1.[16]

2) AI can be small when the subject either has two or more IZs in opposite locations with respect to the probe axis or a homogeneous distribution of IZs. Thus, a second index that describes whether the IZ has peaks or is homogeneously distributed was introduced. This index is the standard deviation (STD) of the 16 values of the IZ distribution Its mathematical definition is provided in the Appendix.

Placing the probe exactly in the same position in different experimental sessions may be difficult since the operator can rely only on the probe fin to correctly reposition the probe. To estimate this error an off-line realignment (based on crosscorrelation of the patterns) was performed to compare IZ distributions obtained from processing signals recorded in different sessions. The relative probe rotation between different sessions can be deducted from the estimated IZ distributions, which showed similar shapes affected by a small shift (see Figure 3). The realignment of two estimated distributions of IZ was obtained shifting IZ distributions relative to one taken as a reference by the optimal phase delay (which was limited to 1/16 of the circle and obtained in frequency domain to avoid resolution error [17]) providing the minimum mean square error between the two distributions.

Assessment of repeatability of the estimated IZ distribution

Twelve IZ distributions were computed using the signals acquired in each of the four sessions (three repetitions in four independent sessions) for each subject and array. Shapiro-Wilk test of normality indicated that the IZ distribution on each channel was normally distributed across measurements. Correlation between estimated distributions was assessed by the Coefficient of Multiple Correlation (CMC), which requires the hypothesis of normal

distribution of the data. The mathematical definition of the CMC is provided in the Appendix.

Assessment of repeatability of the estimation of asymmetry and homogeneity of IZ distribution

AI and STD computed for all estimated IZ distributions were analyzed to find significant relationships for the following four factors: subjects, sessions, arrays and repetitions. This data set was tested against the hypothesis of normality (Shapiro-Wilk test). The test revealed that AI was not normally distributed if grouped by session, repetition and array; only AI grouped by subjects showed a significantly normal distribution. According to the results of the Shapiro-Wilk test, the Kruskal-Wallis nonparametric four-way analysis of variance (ANOVA) was performed to investigate the effect of the four factors on the AI values.

Repeatability of AI and STD was assessed using the Interclass Correlation Coefficient (ICC).[18] ICC is defined as the ratio of the variance due to the variability of the subjects and the sum of the variances due to subjects, sessions and repetitions. Its mathematical definition and interpretation are provided in the Appendix.

RESULTS

Repeatability of the IZ distributions estimated from signals acquired within the same session

For each subject and for each session the repeatability of IZ distribution was assessed by evaluating the CMC for the three IZ distributions of a given session. Figure 2 provides an example of IZ distribution in one session, Figure 3 describes the STD of IZ distributions across different sessions in an individual. The results for each session of the thirteen subjects are shown in Figure 4a.

IZ distributions with a CMC greater than 0.8 can be considered very similar.[19] Considering 13 subjects and 4 sessions of measure per subjects, 52 CMC values were available for each array. Considering the internal, intermediate and external array, the CMC value computed on IZ distributions of the same session was greater than 0.8 in 44 (array 1), 43 (array 2), and 45 (array 3), out of 52 cases respectively.

The 52 CMC values computed on the three IZ distribution of each session had mean and median value of 0.89 and 0.92 respectively on the internal array, 0.84 and 0.91 on the intermediate array, 0.90 and 0.95 on the external array.

Repeatability of the IZ distribution computed for all the acquisitions over different sessions within the same subject

The repeatability of the IZ distribution between different sessions was assessed by computing the CMC for the 12 IZ distributions (four sessions of three measures each) obtained from each subject. The CMC computed without realignment of the distributions was affected by the operator error discussed previously, resulting in lower values of CMC.

This error could be due to the operator or to the different population of MUs activated in different sessions. The fact that the error was compensated by correcting the spatial shift between the IZ distributions evaluated on signals from different sessions proves that it was due to the operator who reinserted the probe with slightly different orientation. For each subject and array the realigned IZ distributions were then used to compute the CMC. CMC medians and quartiles are shown in Figure 4b.

After realignment the median of the CMC between different sessions for the internal array increased from 0.79 to 0.85, from 0.67 to 0.72 for the intermediate array, from 0.76 to 0.83 for the external array.

The absolute value of the misalignment between IZ distribution computed from different session was 44.8 ± 51.1% of the IED (mean ± SD) with a median of 25.5% of the IED, equivalent to about 6^{o}.

The coefficient of correlation (CC) computed between the mean IZ distribution of the internal and the intermediate array had a median of 0.31 (lower quartile = -0.16; upper quartile = 0.59; n=13); the CC computed between the mean IZ distribution of the intermediate and the external array had a median of 0.23 (lower quartile = -0.01; upper quartile = 0.76; n=13).

Repeatability of AI

Results of Kruskal-Wallis ANOVA are shown in Table 1a. AI was found to be dependent on the subjects ($p<0.001$, d.f.=12) and on the arrays ($p<0.001$, d.f.=2), but not on sessions ($p= 0.275$, d.f=3) and repetitions ($p=0.897$, d.f.=2). Figure 5a shows AI for all the subjects and arrays (median, lower and upper quartile values, considering the AI estimated from each signal). The AI varies at different depths (internal array AI = 19.16 ± 8.90%; intermediate array AI = 19.16 ± 8.91%; external array AI = 25.03 ± 10.11%; n = 13). ICC computed for each array is shown in Table 2a, in the case of considering the AI as a scalar (distance from the center of the probe) or as a vector (two coordinates with respect to the center of the probe).

Repeatability of STD

STD was found to be dependent on the subjects ($p<0.001$, d.f.=12) and on the arrays ($p<0.001$, d.f.=2), but not on sessions ($p= 0.481$, d.f=3) and repetitions ($p=0.929$, d.f.=2) as shown in Table 1b. The STD (normalized with respect to 0.242, which is the maximal STD possible for a single IZ) varies at different depths (internal array STD = 16.8 ± 6.4%;

intermediate array STD = 14.7 ± 5.6%; external array STD = 19.0 ± 3.5%; n = 13). ICC of the STD index computed for each array is shown in Table 2c.

DISCUSSION

Ever since the first reports of significant muscle defects following delivery, [20] identified by endoanal ultrasound [21] and linked to increased incidence of urinary and fecal incontinence after childbirth, [22,23] functional investigations of the pelvic floor continence apparatus by means of anal manometry, electromyography and endoanal ultrasound have received increasing attention for the potential ability to identify disturbed sphincter anatomy and predict postpartal incontinence – however, between-subject variability of findings have remained high and associations between muscle function and muscle anatomy have remained poor. [1,2,24]

Identification of substantial asymmetry of anal sphincter muscle function – and the respective innervation – in health and in incontinence [7,13] has linked this feature to the possibility of unilateral trauma of the dominant innervation side during delivery.[12] In this case, reliable identification of cases of asymmetric sphincter innervation prior to delivery would be a prerequisite for preventive obstetric measures such as the selection of the site of elective episiotomy. However, needle EMG evaluation of the anal sphincter muscle – despite its correlation with sonographically identified muscle lags [5] – offers little chances to become routine screening tool during pregnancy due to its invasive nature.

A recent development of detection systems for surface EMG signals indicates new potential applications in the evaluation of EAS. Partners of the European Project OASIS used multi-electrode arrays in anal and urethral sphincters to identify the location of IZs along the sphincter muscle circumference and established their asymmetric distribution in many

healthy subjects and its clinical importance in cases of pelvic floor trauma or incontinence.[13] However, before this technique may become a gold standard in pelvic floor screening, it needs to be established that measures of IZ location and distribution are repeatable within subjects; it is also of value to know whether more general or rather individual patterns of innervation exist across subjects. This was the purpose of the present study.

Repeatability of the IZ distribution

Four independent experimental sessions were considered. Reinsertion of the probe in different sessions occasionally resulted in a small relative rotation between IZ distributions. This problem was corrected by alignment of the estimated IZ distributions (see Figure 3) demonstrating that the shift should be attributed to the operator and not to different distributions of MUs in different sessions.

The repeatability of the estimation of the IZ distribution was high (CMC near 0.8), between different trials and sessions within each subject. This indicates that a single trial is sufficient to locate the IZ of the EAS and that the method is suitable for clinical routine, as the procedure is fast and minimally invasive. This conclusion indicates that the proposed technique provides the surgeon with knowledge of the location of the IZs of the EAS and with the information about the potential risk (or the optimal modality) of performing elective episiotomy. Since IZ distribution varies considerably among subjects, a general rule concerning the location of the IZ cannot be provided and individual testing is required.

Repeatability of parameters extracted from the IZ distribution

Differences between IZ distributions of healthy subjects have been assessed. Differences between IZ distributions of healthy and pathological subjects or of healthy females before and after delivery are expected. The two parameters suggested in this work to quantify these

differences (AI as the asymmetry of IZ distribution; STD as a measure of homogeneity of IZ distribution) were repeatable in the same subject, so that differences in their values among subjects could be associated to differences in the anatomy of EAS and not to chance or noise.

The asymmetry of the innervation of EAS can provide an estimation of the risk for faecal incontinence in case of trauma.[7,12] AI was also found to be significantly different between the arrays (Table 1a). Figure 5 shows the great inter subject variability of the IZ barycenter from which the AI is computed.

Values of ICC of the order of 70% were obtained considering the two components of the barycenter of the distribution of IZ. The AI based on both coordinates of the barycenter is preferable to that based on the radial distance only.

While AI gives information about the asymmetry of the IZ of the EAS, STD provides information about the homogeneity of such a distribution. The ICC of STD computed on the IZ distribution of the external array is 34%: this value is lower than that for the other arrays because of the smaller inter subject variance in the external portion of the EAS. This suggests that different subjects have the same type of non-homogeneity of the IZ on the external part of EAS. In particular, two main IZs (corresponding to two peaks of the estimated IZ distribution) were found on 11 out of 13 subjects (see Figure 2a).

Different IZ at different depths

IZ distribution is different at different depths along the anal canal. This difference was assessed by the CC between pairs of estimated IZ distributions, which was very low. In general, the IZ of the external part of the EAS is the most asymmetric and the most non-homogeneous.

Limitations of the study

Some technical problems in the detection of surface EMG signals from EAS muscle are expected. The technique works well if the mucosa is clean. If this is not the case the contacts may be intermittent. The same thing applies to surface EMG in general, e.g. if the skin is hairy. The improvement of the detection system is an important goal to be reached with our future research activity. For example, the diameter of the probe may be optimized on the basis of experience. Slightly lager diameter and higher pressure may improve and stabilize the contact.

Unidirectional propagation of MUAPs is observed often in the EAS muscle (Figure 2a). The IZ of a MU showing unidirectional propagation is assumed to be under the electrode pair showing the earliest potential. This assumption might be incorrect since the MU fibers might extend away from the electrode array and the true location of IZ may not be detected.

The IZs detected by the internal array might belong to MUs of the puborectalis muscle. However, these considerations are not relevant for the issue of the repeatability of the IZ.

The automatic method adopted in this work cannot resolve superposition of MUAPs and is more sensitive to large or superficial MUs associated to MUAPs with high amplitude. Thus, the method has limitations similar to those of visual analysis, as the identification of MUAPs by human inspection is also affected by their amplitude, superposition and signal to noise ratio. Nevertheless, the application on simulated signals indicated that the automatic method provides a precise estimate of the position of detectable IZs, with resolution related to the IED.[16] Despite these limitations, indications can be provided from the estimated IZ to guide surgery and reduce the risk of incontinence in episiotomy and other surgical trauma.[25]

CONCLUSIONS

Inter-subjects variability of the innervation of the EAS muscle confirms that each subject must be individually studied. Detection systems for high density surface EMG of the EAS and an automatic method for the estimation of the distribution of IZs are available. In a given subject the estimation of the distribution of IZ is repeatable both considering different trials within the same session and independent experimental sessions in different days. Therefore, one measurement is representative.

Our results indicate the potentiality of a fast and minimally invasive test to identify the location of the IZs of the EAS muscle. The importance of this method in providing the surgeon with the information required to reduce the risks of neuronal damage related to episiotomy will be further tested in the near future before and after child delivery in order to prove clinical relevance.

APPENDIX

Definition of the index AI

$$AI_x = \sum_{i=1}^{n_{ch}} \left(IZ_{dist}(i) \cos\left(\frac{2\pi i}{n_{ch}} \right) \right)$$

$$AI_y = \sum_{i=1}^{n_{ch}} \left(IZ_{dist}(i) \sin\left(\frac{2\pi i}{n_{ch}} \right) \right)$$

$$AI = \sqrt{AI_x^2 + AI_y^2}$$

where n_{ch} is the number of channels ($n_{ch} = 16$) and AI_x and AI_y are the two components of AI considered as a vector.

Definition of the index STD

$$STD = \sqrt{\frac{1}{n_{ch}} \sum_{i=1}^{n_{ch}} \left(IZ_{dist}(i) - \frac{1}{n_{ch}} \sum_{j=1}^{n_{ch}} IZ_{dist}(j) \right)^2}$$

as standard deviation of the 16 values of the IZ distribution. STD can range from 0 for a uniform IZ distribution to $\sqrt{15}/16$ for a single IZ. In Figure 2b, c, d, e the IZ "signal" is scaled in the range of 0-100%.

The Coefficient of Multiple Correlation (CMC) is an indicator of similarity between patterns or profiles defined as:

$$CMC = \sqrt{1 - \frac{\sum_{i=1}^{m} \sum_{j=1}^{n} \left(Y_{ij} - \overline{Y_j} \right)^2}{\sum_{i=1}^{m} \sum_{j=1}^{n} \left(Y_{ij} - \overline{\overline{Y}} \right)^2} \cdot \frac{(mn-1)}{n(m-1)}}$$

where $i=1,\ldots,m$ is the index of the computed IZ distribution (m=3 within session, m=12 between sessions), $j=1,\ldots.n$ is the index the SD channel of the considered array (n=16), $\overline{Y_j}$ is the mean value at the j^{th} channel over the m IZ distributions, and $\overline{\overline{Y}}$ is the grand mean over the n =16 channels and the m IZ distributions. CMC can range from 0 to 1. Values of CMC close to 0 suggest no similarity of the distributions, whereas values close to 1 indicate that the distributions can be considered similar[19]. Negative values of CMC between two patterns indicate that the patterns are varying in opposite phase.

The Interclass Correlation Coefficient (ICC) [18] is defined as the ratio of the variance due to the variability of the subjects and the sum of the variances due to subjects, sessions and repetitions.

$$ICC = \frac{\sigma^2{}_{subjects}}{\sigma^2{}_{subjects} + \sigma^2{}_{sessions} + \sigma^2{}_{repetitions}} \cdot 100$$

ICC near 100% means that the variability due to sessions and repetitions is negligible with respect to the variability due to the subjects (that is the subjects are different and the measurements are repeatable). ICC near 0% means that the variability due to sessions and repetitions is predominant with respect to the variability due to the subjects (that is the measurements are not repeatable for the same subject).

Repeatability of AI was assessed considering AI both as a scalar value and as a vector of two Cartesian coordinates using a bi-variate statistic. In the case in which AI was considered as a vector, its variance can be represented as the sum of the variance of the two independent components of the bi-variate statistic (corresponding to the X and Y coordinates of AI):

$$\sigma_i^2 = \sigma_{x,i}^2 + \sigma_{y,i}^2 ,$$

where i indicates subject, session, or repetition.

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Disclosure of Interests

No conflict of interest accounted

Contribution to Authorship

PE and HF initiated the study and locally organised the recording, and contributed to the interpretation of the data and wrote the clinical parts of the manuscript; ED and FM performed the data recording and analysis, LM and RM developed the algorithms for data analysis, RM wrote the technical part of the paper

Ethical approval

The study was carried out at the University of Tübingen (Tübingen, Germany) and was approved by the Local Ethics Committee.

REFERENCES

1. Freys SM, Fuchs KH, Fein M, Heimbucher J, Sailer M, Thiede A. Inter- and intraindividual reproducibility of anorectal manometry. Langenbecks Arch Surg 1998;383:325-9.

2. Schäfer R, Heyer T, Gantke B, Schäfer A, Frieling T, Häussinger D, Enck P. Anal endosonography and manometry: comparison in patients with defecation. problems. Dis Colon Rectum 1997;40:293-7.

3. Karoui S, Savoye-Collet C, Koning E, Leroi AM, Denis P. Prevalence of anal sphincter defects revealed by sonography in 335 incontinent patients and 115 continent patients. AJR Am J Roentgenol 1999;173:389-92.

4. Sultan AH, Kamm MA, Talbot IC, Nicholls RJ, Bartram CI. Anal endosonography for identifying external sphincter defects confirmed histologically.

5. Enck P, von Giesen HJ, Schäfer A, Heyer T, Gantke B, Flesch S, Arendt G, Schmitz N, Frieling T. Comparison of anal sonography with conventional needle electromyography in the evaluation of anal sphincter defects. Am J Gastroenterol 1996;91:2539-43.

6. Titi MA, Jenkins JT, Urie A, Molloy RG. Correlation between anal manometry and endosonography in females with faecal incontinence. Colorectal Dis 2008;10:131-7.

7. Enck P, Hinninghofen H, Wietek B, Becker HD. Functional asymmetry of pelvic floor innervation and its role in the pathogenesis of faecal incontinence. Digestion 2004;69:102-11.

8. Enck P, Vodusek DB. Electromyography of pelvic floor muscles. J Electromyogr Kinesiol 2006;16:568-77.

9. Binnie NR, Kawimbe BM, Papachrysostomou M, Clare N, Smith AN. The importance of the orientation of the electrode plates in recording the external anal sphincter EMG by non-invasive anal plug electrodes. Int J Colorectal Dis 1991;6:5-8.

10. Gee AS, Jones RS, Durdey P. On-line quantitative analysis of surface electromyography of the pelvic floor in patients with faecal incontinence. Br J Surg 2000;87:814-8.

11. O'Donnell P, Beck C, Doyle R, Eubanks C. Surface electrodes in perineal electromyography. Urology 1988;32:375-9.

12. Wietek BM, Hinninghofen H, Jehle EC, Enck P, Franz HB. Asymmetric sphincter innervation is associated with faecal incontinence after anal sphincter trauma during childbirth. Neurourol Urodyn 2007;26:134-9.

13. Enck P, Franz H, Azpiroz F, Fernandez-Fraga X, Hinninghofen H, Kaske-Bretag K, Bottin A, Martina S, Merletti R. Innervation zones of the external anal sphincter in healthy male and female subjects. Preliminary results. Digestion 2004;69:123-30.

14. Enck P, Hinninghofen H, Merletti R, Azpiroz F. The external anal sphincter and the role of surface electromyography. Neurogastroenterol 2005;17:60-7.

15. Merletti R, Bottin A, Cescon C, Farina D, Gazzoni M, Martina S, Mesin L, Pozzo M, Rainoldi A, Enck P. Multichannel surface EMG for the non-invasive assessment of the anal sphincter muscle. Digestion 2004;69:112-22.

16. Mesin L, Gazzoni M, Merletti R, Automatic localisation of innervation zones: a simulation study of the external anal sphincter. J Electromyogr Kinesiol (submitted)

17. McGill KC, Dorfman LJ. High-resolution alignment of sampled waveforms. IEEE Trans Biomed Eng 1984;31:462-8.

18. Rainoldi A, Galardi G, Maderna L, Comi G, Lo Conte L, Merletti R. Repeatability of surface EMG variables during voluntary isometric contractions of the biceps brachii muscle. J Electromyogr Kinesiol 1999;9:105-19.

19. Neter J, Kutner MH, Nachtscheim CJ, Wasserman W. Applied Linear Statistical Models. Forth Edition. Chicago: Irwin, 1989.

20. Sultan AH, Kamm MA, Hudson CN, Thomas JM, Bartram CI. Anal-sphincter disruption during vaginal delivery. N Engl J Med 1993;329:1905-11.

21. Law PJ, Bartram CI. Anal endosonography: technique and normal anatomy. Gastrointest Radiol 1989;14:349-53.

22. Guise JM, Morris C, Osterweil P, Li H, Rosenberg D, Greenlick M.^ Incidence of fecal incontinence after childbirth. Obstet Gynecol 2007;109:281-8.

23. Dudding TC, Vaizey CJ, Kamm MA. Obstetric anal sphincter injury: incidence, risk factors, and management.Ann Surg 2008;247:224-37.

24. Enck P, von Giesen HJ, Schäfer A, Heyer T, Gantke B, Flesch S, Arendt G, Schmitz N, Frieling T. Comparison of anal sonography with conventional needle electromyography in the evaluation of anal sphincter defects. Am J Gastroenterol 1996;91:2539-43.

25. Nelson R. Epidemiology of fecal incontinence. Dimension of the problem; prevalence and impact. Gastroenterology 2004;126:3-7.

Legends of Tables and Figures

Table 1. a) Kruskal-Wallis non-parametric analysis of variance (ANOVA) on asymmetry index (AI) for the following effect: subject, array, session, repetition. **b)** Kruskal-Wallis non-parametric analysis of variance (ANOVA) on standard deviation index (STD) for the following effect: subject, array, session, repetition.

Table 2. a) Interclass correlation coefficient (ICC) computed for the asymmetry index (AI) for each array. **b)** ICC computed on AI considered as a vector of Cartesian coordinates. **c)** ICC computed for the standard deviation index (STD). σ^2 is the variance of the AI and STD computed for the subject, session and repetition and is presented as the percentage of the probe radius.

Figure 1. a) Acquisition setup. The surface EMG signal acquired by the anal probe is amplified by the 48 ch EMG-USB amplifier, converted using a 12 bit A/D and then stored on a laptop. **b)** Three array anal probe. Each array is made of 16 equally spaced Ag electrodes (1x10 mm). **c)** Experimental protocol. The four experimental sessions are divided into two days. In each session three maximum voluntary contractions are performed.

Figure 2. a) Epoch (250 ms) of surface EMG on the external array during a maximum voluntary contraction (MVC) and the propagating potentials from which the IZs are extracted. The decomposition algorithm computes the innervation zone (IZ) distribution of MUs from the given signal. The probability of finding an IZ on a given channel is defined as the percentage of the identified traveling potentials that originate from that channel. The IZ distribution is then used to compute the barycenter of the IZ. Equal masses are attributed to the MUAPs. **b) c) d) e)** Examples of IZ distributions, corresponding asymmetry indexes (AI) and standard deviation indexes (STD). AI is defined as the distance of the barycenter of the IZ distribution from the center of the probe normalized with respect to the radius of the

probe. STD is the standard deviation of the 16 values of the IZ distribution and provides information about the homogeneity if the IZ distribution.

Figure 3. IZ distributions on the external array for a typical subject are shown for each session and then are used to compute the coefficient of multiple correlation (CMC) among IZ profiles (n=3) for each session. CMC is computed among all IZ distributions estimated for the subject, with and without realignment between the IZ distributions. The operator error in positioning the probe is estimated by realigning all the IZ distributions.

Figure 4. Coefficient of multiple correlation for the three arrays. **a)** The boxes have lines at the lower quartile, median, and upper quartile values. The whiskers (lines extending from each end of the box) show the range of the data. For each session and array, the coefficient of multiple correlations (CMC) is computed between the IZ distributions of each subject, session and array. **b)** CMC is computed for each subject and array using all the IZ distributions with and without realignment.

Figure 5. a) The non-homogeneity of the IZ is measured by the standard deviation (STD) of the estimated IZ distribution. The boxes have lines at the lower quartile, median, and upper quartile values. The whiskers (lines extending from each end of the box) show the range of the data. **b)** The asymmetry index (AI) in percentage of the radius of the probe is shown for each subject and array, providing box and whickers as in a). **c)** IZ barycenters computed for each subject and for each array. The ellipses are centered in the mean IZ barycenter of each subject and array and the semi-axes of such ellipses represent the standard deviations of the barycenters of the IZ distributions on the X and Y axes for each subject.

Fig 1

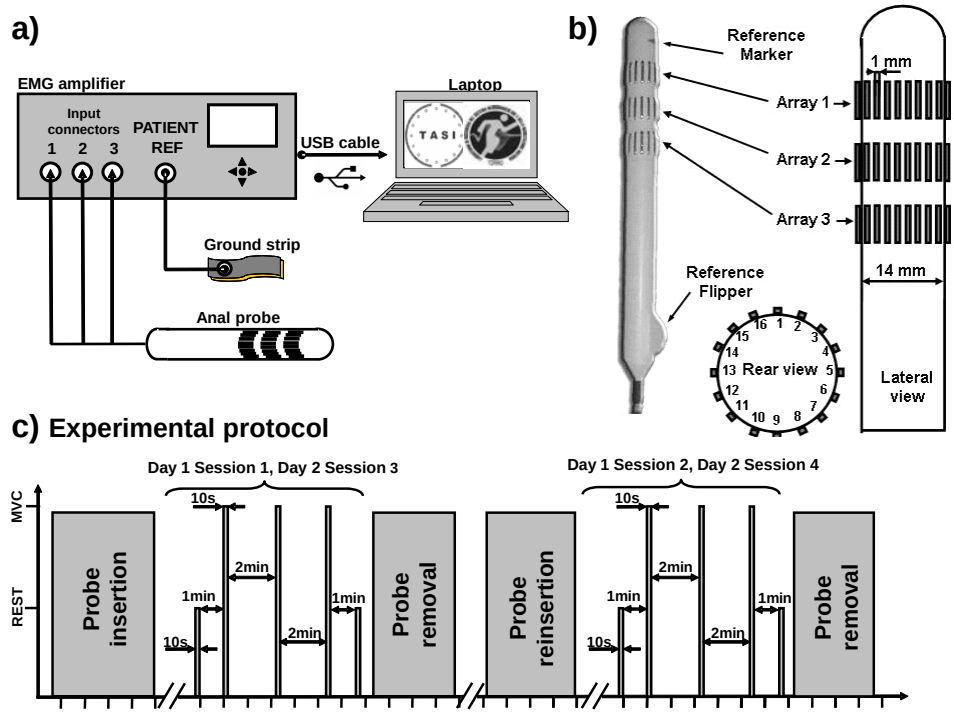

a)
EMG amplifier
Input connectors
1 2 3
PATIENT REF
USB cable
Laptop
TASI
Ground strip
Anal probe
b)
Reference Marker
Array 1
Array 2
Array 3
1 mm
14 mm
Reference Flipper
Rear view
Lateral view
c) Experimental protocol
Day 1 Session 1, Day 2 Session 3
Day 1 Session 2, Day 2 Session 4
MVC
REST
Probe insertion
Probe removal
Probe reinsertion
Probe removal
10s
1min
2min

Fig 2

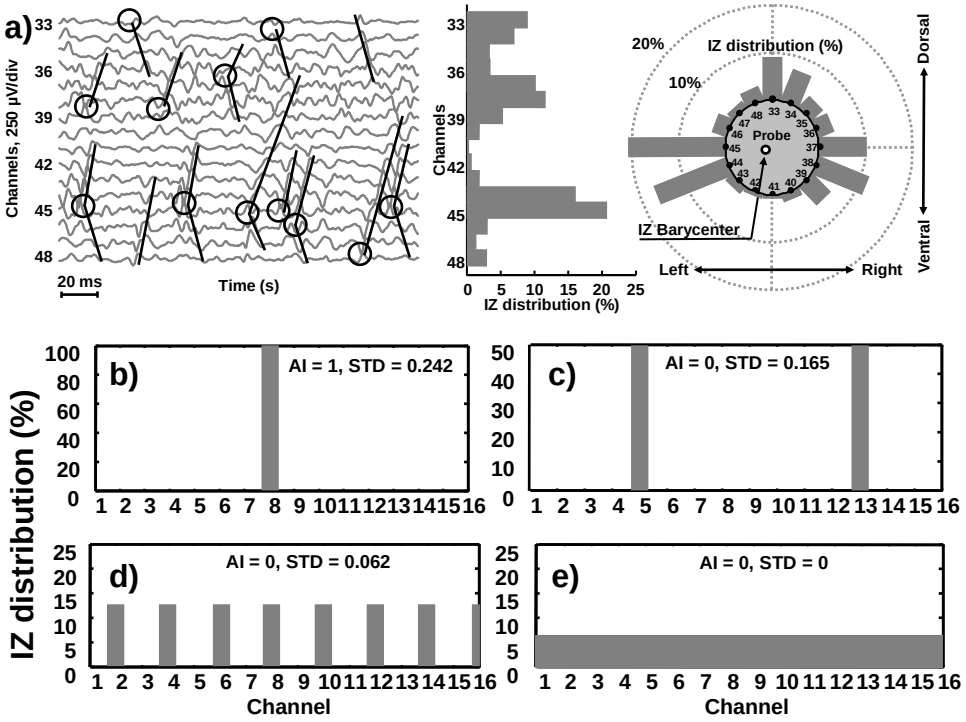

a)
Channels, 250 µV/div
20 ms
Time (s)
Channels
IZ distribution (%)
20%
10%
Probe
IZ Barycenter
Left
Right
Dorsal
Ventral
b)
AI = 1, STD = 0.242
c)
AI = 0, STD = 0.165
d)
AI = 0, STD = 0.062
e)
AI = 0, STD = 0
IZ distribution (%)
Channel

Fig 3

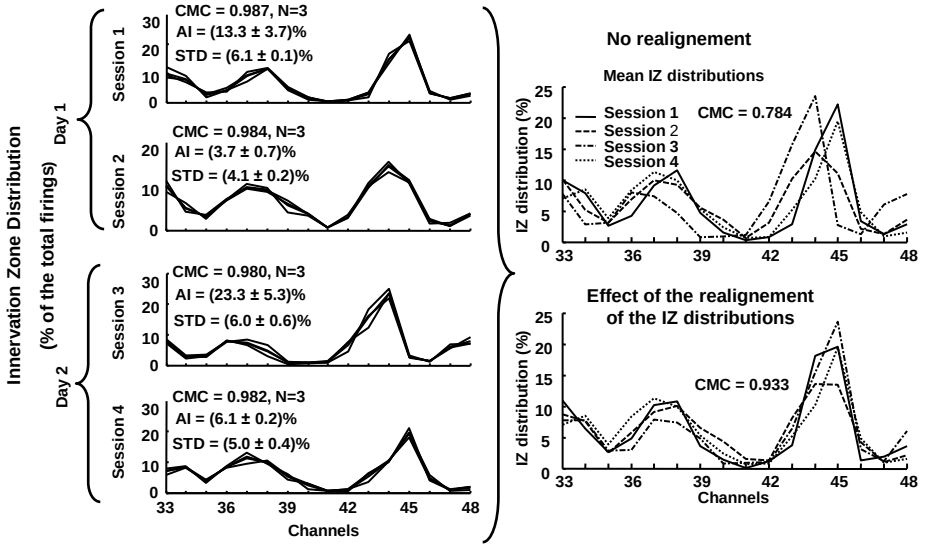

Innervation Zone Distribution
(% of the total firings)
Day 1
Day 2
Session 1
Session 2
Session 3
Session 4
CMC = 0.987, N=3
AI = (13.3 ± 3.7)%
STD = (6.1 ± 0.1)%
CMC = 0.984, N=3
AI = (3.7 ± 0.7)%
STD = (4.1 ± 0.2)%
CMC = 0.980, N=3
AI = (23.3 ± 5.3)%
STD = (6.0 ± 0.6)%
CMC = 0.982, N=3
AI = (6.1 ± 0.2)%
STD = (5.0 ± 0.4)%
Channels
No realignement
Mean IZ distributions
Session 1
Session 2
Session 3
Session 4
CMC = 0.784
IZ distribution (%)
Effect of the realignement
of the IZ distributions
CMC = 0.933
Channels

Fig 4

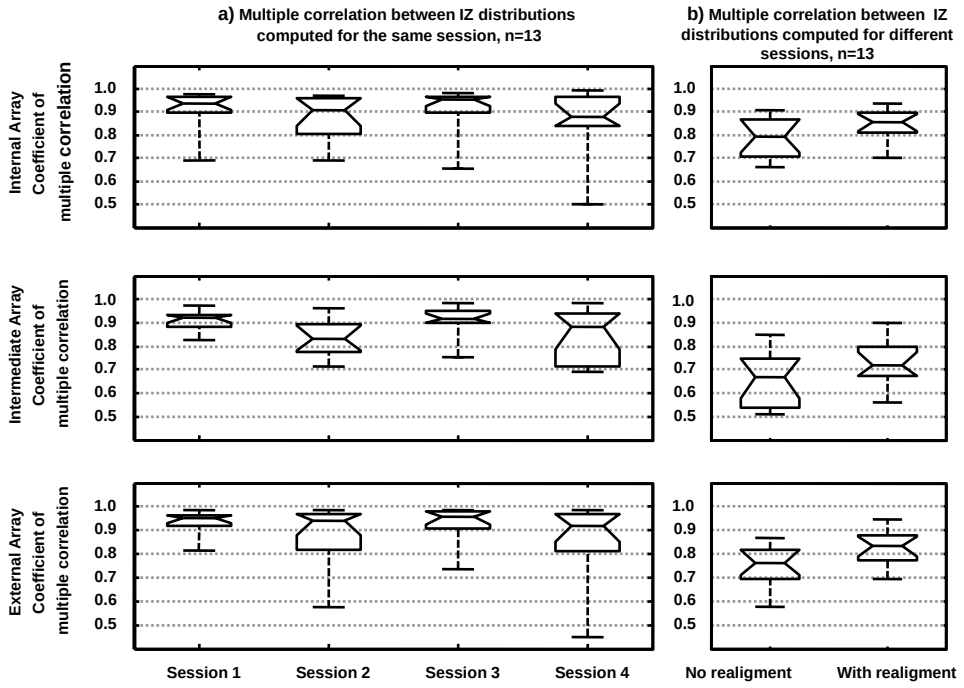
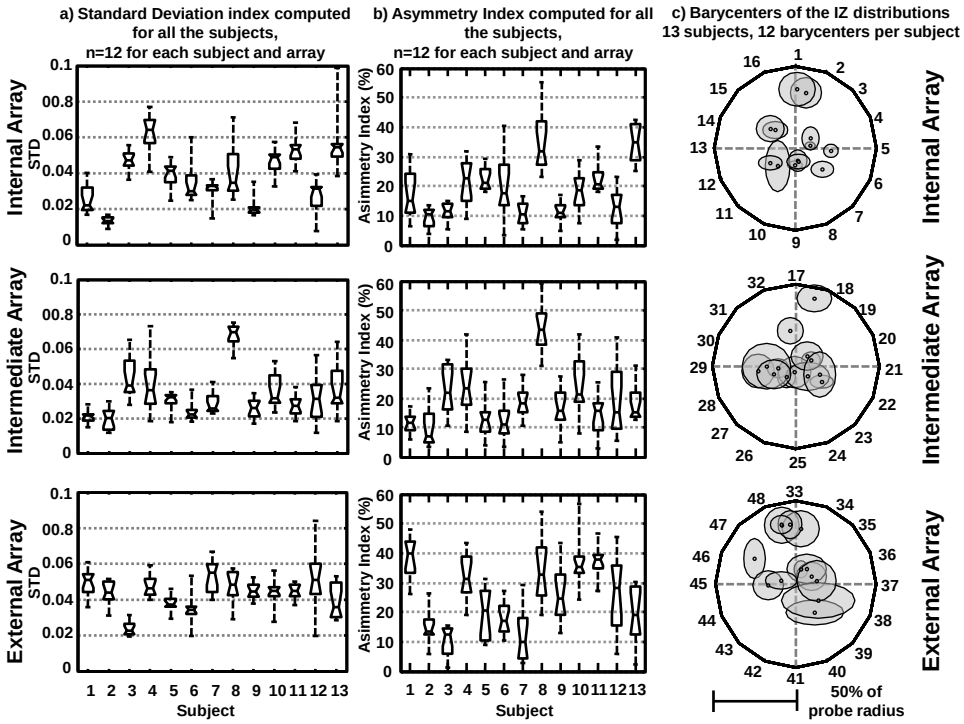

a) Multiple correlation between IZ distributions computed for the same session, n=13
b) Multiple correlation between IZ distributions computed for different sessions, n=13
Internal Array Coefficient of multiple correlation
Intermediate Array Coefficient of multiple correlation
External Array Coefficient of multiple correlation
Session 1
Session 2
Session 3
Session 4
No realigment
With realigment

Fig 5


a) Standard Deviation index computed for all the subjects, n=12 for each subject and array
b) Asymmetry Index computed for all the subjects, n=12 for each subject and array
c) Barycenters of the IZ distributions 13 subjects, 12 barycenters per subject
Internal Array
Intermediate Array
External Array
STD
Asimmetry Index (%)
Subject
50% of probe radius