MOTOR UNIT NUMBER ESTIMATION OF THE EXTERNAL ANAL SPHINCTER OF FEMALE RATS

Hypothesis / aims of study
The myogenic control of the external anal sphincter (EAS) is essential for maintaining fecal continence. There is an unmet need of reliable and less-invasive approaches to evaluate the neuromuscular function of the EAS [1]. The aim of this study was to develop a novel motor unit number estimation (MUNE) approach to provide an estimation of the functioning motor units of the EAS in a rodent model. We used intravaginal stimulation together with intrarectal electromyography (EMG) recordings to mimic the clinical setup.

Study design, materials and methods
Female Sprague-Dawley rats (225-250 grams) were anesthetized with urethane. A graphic description of the experimental setup is shown in Fig 1 (a). The intravaginal probe, mounted with two ring-shaped electrodes, was inserted to apply bipolar electrical stimulation to the pudendal nerve. The intrarectal probe, equipped with four bar-shape copper recording electrodes, was inserted to record the evoked compound muscle action potential (CMAP) of the EAS in response to the intravaginal stimulation. The optimal stimulation site for the intravaginal probe was determined by temporary stimulations, and fixed in position. A series of electrical stimulation with incremental intensities was applied to determine the maximal CMAP. Then, the standard statistical MUNE was performed. Briefly, a series of 30 submaximal CMAPs were recorded within three different recording windows: 10%-20%, 25-35% and 40%-50% (set 0% is the highest stimulus intensity that does not activate any motor unit and 100% as the lowest intensity that activates all motor units) [2]. The variance in the CMAP sizes provides an estimate of the single motor unit potential size, and thus an estimate of the total number of motor units.

Results
The CMAP signals were successfully recorded with the intrarectal EMG probe at three stimulation intensities, as shown in Fig. 1 (b). The averaged estimated number of motor units of the EAS is 52 /rat.

Interpretation of results
This study represents the first effort to estimate the number of functioning motor units in the EAS muscle of rats. The recorded CMAP responses of the EAS from the intrarectal EMG probe indicate the successful effects of a non-invasive stimulation in the pudendal nerve. The estimated numbers of motor units in the tested rats are very close (51 and 53). Nonetheless, more experiments are necessary to compensate for the current small sample size. The immunohistochemical staining using acetylcholine-transferase and fluorogold will be performed to further consolidate the accuracy of this novel approach for EAS MUNE.

Concluding message
This proof-of-concept study demonstrates the feasibility of estimating the number of motor units of the EAS in a rodent model by combining intravaginal pudendal nerve stimulation and intrarectal surface EMG recording. This EAS MUNE approach represents the first effort to quantify the number of motor units of the EAS, and provides a theoretical basis for future tests during pathological conditions of fecal incontinence.

References

Disclosures
Funding: Brown Foundation and the Houston Methodist Foundation, NIH R00DK082644 and University of Houston Clinical Trial: No Subjects: ANIMAL Species: Rat Ethics Committee: Animal experiments were approved by the IACUC at Houston Methodist Research Institute, and performed in accordance with the NIH’s Guide for the Care and Use of Laboratory Animals.
Fig. 1 (a) System configuration (b) Overlaying plot for submaximal CAMPs under three stimulation levels.