A Novel Cystometric Model of Pelvic Floor Dysfunction After Rabbit Pelvic Floor Noxious Electrical Stimulation

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Objectives: Although a relationship between pelvic floor dysfunction and lower urinary tract symptoms is described in the literature, the mechanism and pathways need further characterization. We developed an animal model of pelvic floor dysfunction after noxious stimulation of the pubococcygeus (PC) muscle.

Methods: Fifteen female adult rabbits were evaluated with cystometry (CMG) and electromyography (EMG) recordings from the PC muscle. Cystometry/EMG was performed before and after treatment animal (n = 11) received noxious pelvic floor electrical stimulation through the PC EMG electrode, and controls (n = 4) underwent sham needle placement. Two animals underwent S3 dorsal rhizotomy to demonstrate that the observed results required afferent innervation.

Results: Voiding changes were demonstrated in 9 of 11 rabbits after stimulation. Most of the rabbits (7/9) exhibited a prolonged-dysfunctional voiding pattern with larger capacity (mean, 17 mL [SEM, ±8 mL]), longer intercontractile interval (227% [SEM, ±76%]) and duration (163% [SEM, ±20%]), and increased postvoid residual (24 mL [SEM, ±6 mL]). The remaining dysfunctional rabbits (2/9) exhibited an overactive-dysfunctional voiding pattern with lower capacity (−26 mL [SEM, ±6 mL]), shortened intercontractile interval (16% [SEM, ±9%]) and duration (56% [SEM, ±30%]), and decreased postvoid residual (−27 mL [SEM, ±6 mL]). Nonresponder rabbits (2/11) were relatively unchanged in their micturition cycles after stimulation. Rhizotomy animals were acontractile and filled until overflow incontinence occurred.

Conclusions: Using noxious electrical stimulation of the pelvic musculature, we were able to produce an animal model of pelvic floor dysfunction in most rabbits as hallmarked by a larger bladder capacity, an increased intercontractile interval, and prolonged contraction duration.

Key Words: basic research, female urology, animal model, bladder dysfunction

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The pelvic floor musculature serves a central role in modulating voiding and defecatory behavior, and clinicians have recently recognized an association between lower urinary symptoms and pelvic floor tone.[1] In the clinical realm, complaints related to pelvic organ dysfunction fall into 2 categories and typically depend on the tonicity of the pelvic floor.[2] For instance, in a patient with a high-tone pelvic floor, obstructive voiding symptoms may predominate along with complaints of urinary hesitancy, weak stream, and incomplete emptying. However, in this same patient, pelvic floor hypertonicity may also manifest as storage symptoms similar to overactive bladder and include the complaints of urinary urgency, frequency, and urgency urinary incontinence. On the opposite end of the spectrum, symptoms related to a low-tone pelvic floor may include stress urinary incontinence, pelvic organ prolapse, and fecal incontinence. Furthermore, the elements of acute or chronic pelvic pain, pelvic floor spasticity, dyspareunia, and bladder pain may complicate the clinical ascertainment of complaints related to pelvic floor function.[3] Consistent with the heterogeneity of seemingly unrelated complaints of the urinary and bowel system, in our experience, many patients may undergo up to a decade of diagnostics and interventions before a simple physical examination directed at the levator ani pelvic floor complex. Identification and treatment of dysfunctional pelvic floor musculature has been shown to offer significant relief beyond commonly used therapies directed at the bladder or bowel.[4–6]

Weakness or laxity of the pelvic floor muscles has been well described in stress incontinence and pelvic organ prolapse; however, the literature delineating the specific mechanisms and pathways linking dysfunction of the pelvic floor muscles and urinary complaints needs further development.[5–8] One of the obstacles to reaching this goal is lack of an effective animal model. Rodents, the most commonly used laboratory animal models, lack well-defined perineal musculature and demonstrate activation of the external urethral sphincter during voiding, opposite in physiologic function to the human sphincter in which relaxation occurs.[9,10] In contrast to the rodent pelvic floor, the female rabbit has a well-defined pelvic floor pubococcygeus (PC) muscle, which is homologous in innervation to the human pelvic floor. The role of the PC muscle during normal micturition in the rabbit has been well characterized by Corona-Quintanilla et al,[11] where they demonstrated activation of the PC muscle complex during urine storage and silencing of activity during voiding.[12] To further highlight the role of the rabbit pelvic floor in modulating micturition, similar in function to the PC muscle, Rajasekaran et al[13] demonstrated that puborectalis stimulation generates urethral pressure that would be important for the maintenance of continence during bladder filling. Despite the rabbit's quadruped state, the function of pelvic floor musculature in the rabbit is synonymous in function to the levator ani pelvic floor muscle complex in humans and therefore an ideal target to study the effect of pelvic floor dysfunction.

In our current experiment, we present a pilot study to evaluate the cystometric (CMG) and electromyographic (EMG) effects of noxious pelvic musculature stimulation on spontaneous voiding behavior during filling CMG in rabbits. We hypothesize that noxious stimulation of the PC musculature in the rabbit, in the form of tetanizing electrical current, will result in an animal phenotype consistent with pelvic floor dysfunction.

MATERIALS AND METHODS

Experimental Design

All methods were approved by our Institutional Animal Care and Use Committee (Stratton VA Medical Center, Albany, NY; see overall study design as outlined in Fig. 1). Fifteen adult female

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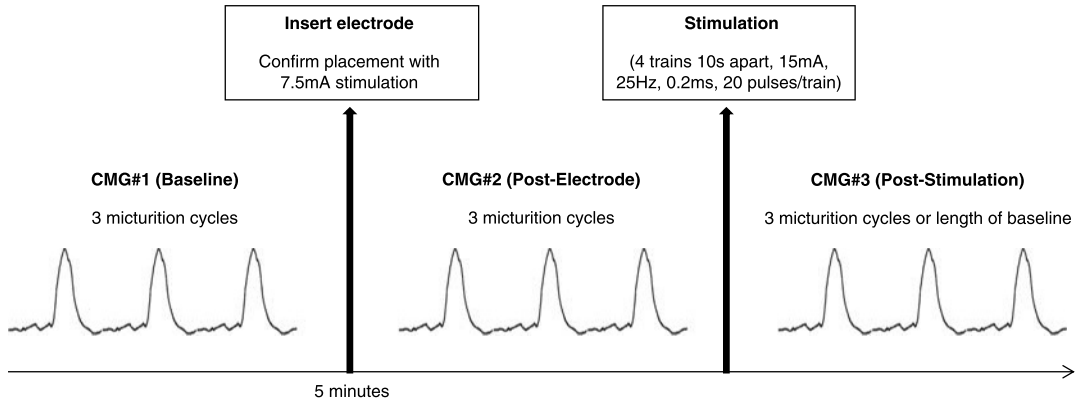

FIGURE 1. Study design.

virgin white New Zealand rabbits, approximately 3.5 kg each, were chosen for the well-developed pelvic floor musculature. Animals were anesthetized using ketamine/xylazine sedation, and a urethral catheter was placed. Baseline CMG (CMG 1) was performed using the XLTEC NeuroMax 1002 machine (Natus Medical Inc, Oakville, ON, Canada) at a continuous filling rate of 2 mL/min until the rabbit's third productive voiding detrusor contraction, with subsequent measurement of postvoid residual. Cystometric pressure was recorded continuously using a Grass polygraph, and EMG tracings were monitored and qualitatively scored using an integrated visual and sound unit. Cystometry was performed and interpreted by a urologist board certified in both urology and female pelvic medicine and reconstructive surgery, with subspecialty training in voiding dysfunction and urodynamics (E.J.B.D.). Performance and interpretation of the EMG was by a board-certified physiatrist with subspecialty boards in EMG and clinical specialization in pelvic EMG (A.H.D.). Both were present for all animal studies.

After the completion of CMG 1, a thin insulated EMG needle was placed into the left and right PC muscle groups. Position within the PC was then confirmed by a single test stimulation (7.5 mA, 0.1 millisecond), which demonstrated the characteristic ipsilateral abduction of the tail, vagina, and rectum as detailed by previous investigators.[12,14] Left and right needles were then used to obtain EMG data. A second CMG (CMG 2) was then performed until the third spontaneous micturition was observed, thus identifying any changes in CMG parameters that might occur after EMG needle placement.

After CMG 2, treatment animals (n = 11) then received noxious tetanizing stimulation (4 trains, 10 seconds apart; 15 mA, 25 Hz, 0.2 millisecond, 20 pulses per train) through the previously placed EMG needles to both the left and right PC muscles. Control animals (n = 4) did not receive noxious electrical stimulation. All animals (n = 15) were then subjected to a poststimulation CMG (CMG 3) until the third spontaneous productive void was observed. After the completion of CMG 3, rabbits were then euthanized using phenobarbital in accordance with local Institutional Animal Care and Use Committee guidelines, and pelvic floor dissection was performed to confirm EMG electrode position within the PC muscle.

As a further control and to evaluate the effect of field stimulation from the PC electrodes, dorsal rhizotomy was performed on 2 additional rabbits (n = 2). Baseline CMG 1 was performed as previously described. After this, the dorsal sacral nerve roots under the S3 vertebrae were surgically exposed and transected. Electromyographic needles were then placed into the left and right PC muscles as previously described, and then bilateral tetanizing needle stimulation was administered (4 trains, 10 seconds apart; 15 mA, 25 Hz, 0.2 millisecond, 20 pulses per train). After noxious stimulation, a second CMG was performed until spontaneous micturition was observed.

Statistical Analysis

Cystometry data were recorded for each animal and included measurement of the baseline CMG pressure (basal pressure [centimeters of water]) at the start of bladder filling, the pressure at which spontaneous micturition occurred (threshold pressure [centimeters of water]), the maximum CMG pressure during productive micturition (peak pressure [centimeters of water]), the interval between contractions (intercontractile interval [minutes]), volume infused into the bladder at time of the first productive contraction (capacity [milliliters]), and the catheterized volume of the bladder after completion of the third productive contraction (postvoid residual [milliliters]). Electromyography tracings were not stored; however, the EMG activity was observed and subjectively scored at the time of CMG using a graded system (1, minimal motor unit potentials [MUPs]; 2, moderate MUPs; 3, many MUPs]. Mean, standard error of the mean (SEM), and Student *t* test were used to make comparisons between animals and treatment groups. A *P* value of less than 0.05 was defined as statistically significant.

Cystometrograms were also subjectively interpreted according to the observed representative contraction patterns noted on CMG 3, and contractions were graded as overactive-dysfunctional or prolonged-dysfunctional in reference to each animal's baseline CMG 1 (Fig. 2). If there was no change in contraction pattern after noxious stimulation, then the animal's CMG 3 was categorized as a nonresponder. Mean and SEM were used to describe CMG parameters. Results were then reported based on voiding patterns and the variables recorded, which were used to separate animals into groups; therefore, additional statistical analysis was not performed to make comparisons between treatment groups.

RESULTS

The baseline CMG 1 in the control (n = 4), stimulation (n = 11), and rhizotomy (n = 2) groups were not significantly different from each other (Table 1). No rabbit had unproductive contractions during baseline CMG 1 or CMG 2. The control group (n = 4) had micturition cycles that were not significantly different over the 3 CMG cycles (CMG 1, CMG 2, and CMG 3), with amplitude, duration, and intervals of all 3 micturition contractions within 20% of each rabbit's baseline. There were no unproductive contractions in the control group. A summary of all recorded

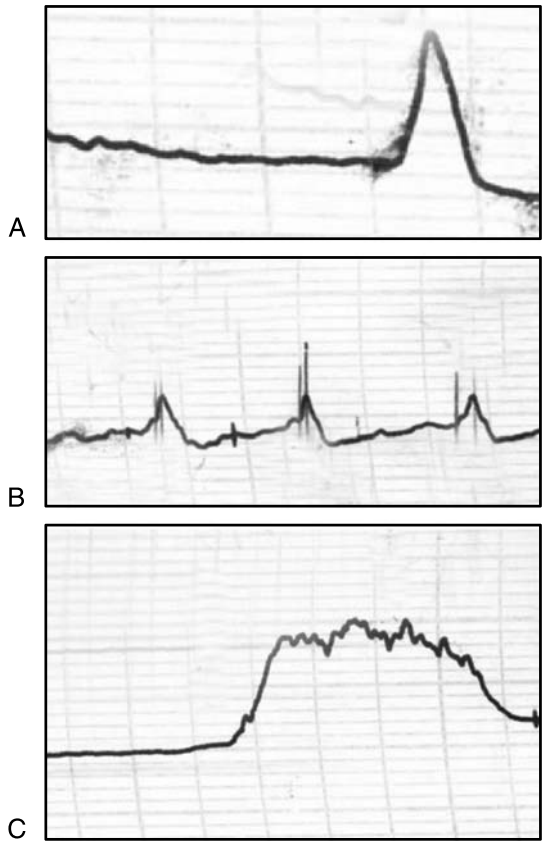

FIGURE 2. Examples of representative CMGs: (A) prestimulation baseline, (B) overactive-dysfunctional pattern, and (C) prolonged-dysfunctional pattern.

CMG parameters is tabulated for CMG 1, CMG 2, and CMG 3, with results grouped according to treatment allocation (sham needle placement vs noxious PC stimulation; Table 1). When grouped by treatment allocation, there was no statistically significant difference noted between treatment groups after noxious pelvic floor stimulation (Table 1). When comparisons were made within each animal group, compared with prestimulation parameters, the only statistically significant changes in CMG 3 parameters were noted as follows: a decrease in threshold pressure for the control animals (4.7 cm H_2O, $P < 0.05$, vs CMG 1 and CMG 2) and an increase in basal pressure for the treatment animals (3.0 cm H_2O, $P < 0.05$, vs CMG 2). Limited statistical significance was noted between treatment groups based on treatment allocation; therefore, data were also presented for all animals using box plot for CMG 1, CMG 2, and CMG 3 for control and treatment animals (Fig. 3).

Cystometric data were then grouped according to the representative contraction patterns noted on CMG 3 (Fig. 2), which were compared with each animal's baseline CMG 1. Results according to this CMG grouping are summarized (Table 2). Of the rabbits that underwent stimulation, 9 of 11 (82%) demonstrated voiding dysfunction after stimulation. The remaining 2 of 11 rabbits were relatively unchanged overall in their micturition cycles after stimulation and were classified as nonresponders. The only difference between this group and the control animals was the presence of poststimulation unproductive contractions. Of the 9 rabbits that demonstrated voiding dysfunction after noxious stimulation, there were 2 clear groups based on their micturition pattern on CMG 3 after noxious stimulation (Table 2). Most (n = 7) of the rabbits exhibited a prolonged-dysfunctional voiding pattern with capacity increase of 17 mL (SEM, ±8 mL), a 227% (SEM, ±76%) longer interval between contractions, and a 163% (SEM, ±20%) longer contraction duration in the poststimulation phase (Table 2). Two rabbits exhibited an overactive-dysfunctional voiding pattern with a lower bladder capacity and mean decrease in postvoid residual of 27 mL (SEM, ±6 mL), a 16% (SEM, ±9%) shortened interval between contractions, and 56% (SEM, ±30%) shorter duration of contraction after stimulation. There were no differences in peak pressure or bladder compliance between the 9 rabbits that demonstrated voiding dysfunction.

In the 2 additional rabbits that underwent rhizotomy, no detrusor contraction was demonstrated on CMG 3 after the S3 sacral roots were transected. In these animals, each bladder was filled until overflow incontinence occurred. Capacity (measured at incontinence) increased from a baseline of 17 up to 72 mL in the first rabbit and from 46 up to 90 mL in the second rabbit.

The EMG patterns were similar among nonrhizotomy rabbits on CMG 2 after simple electrode placement with flat EMG activity demonstrated during filling and increase in MUP activation during voids. After noxious stimulation, on CMG 3, the MUP activity increased during both filling and voiding in all but 2 rabbits. The opposite pattern was seen in the latter 2 rabbits, one in the overactive group and one in the nonresponder group. The rhizotomy group did not show any increase in MUP activity after stimulation.

DISCUSSION

After noxious tetanizing electrical stimulation of the PC muscle group and no direct intervention to the bladder, we were able to produce an animal model of voiding dysfunction in 9 of 11 rabbits. Consistent with the heterogeneous clinical presentation of pelvic floor dysfunction seen in humans, we observed 2 distinct changes in voiding patterns (overactive-dysfunctional and prolonged-dysfunctional) after stimulation. For instance, in humans with hypertonic pelvic floor musculature, one patient may complain of urinary frequency and urgency, whereas another patient could paradoxically describe obstructive voiding symptoms. The standard hypothesis underlying pelvic floor dysfunction is an initial insult followed by propagation in a susceptible individual.[2] Patients may have similar events, such as difficult childbirth or recurrent urinary infections, but may manifest varying degrees of pelvic floor dysfunction after initial insult.[15] This is analogous to the 2 rabbits that had little change in overall pattern after stimulation except for a few nonproductive contractions, in contrast to those rabbits that demonstrated prolonged inefficient contractions (prolonged-dysfunctional) versus overactive voiding behavior (overactive-dysfunctional). Although premature to extrapolate many conclusions regarding the applicability to humans, we found the patterns in our animal model fairly analogous to clinical presentation.

The mechanism of pelvic floor dysfunction in relation to urination in humans is hypothesized to be related to both increased tone and elevated afferent signals from S2 to S4 neural innervation.[2,4,16] In a patient with urinary hesitancy and difficulty with emptying, analogous to the prolonged-dysfunctional group, the pelvic floor acts at the level of the external urethral sphincter by either obstructing the bladder and/or providing activation of the spinal guarding reflex. This is consistent with the findings of previous investigators, whereupon Rajasekaran et al[13] demonstrated that stimulation of the rabbit pelvic floor puborectalis muscle resulted in a dose-dependent increase in urethral pressure. We hypothesize that, in our experiment, noxious pelvic floor stimulation creates low-level cross-talk from the levator ani pelvic floor musculature and external sphincter parasympathetic afferents as well as the pudendal nerve to the pontine storage center. This would be analogous to humans with storage symptoms and an overactive

TABLE 1. CMG Parameters Grouped According to Treatment Allocation (Noxious PC Stimulation [Treatment, n = 11]) Versus Sham Needle Placement [Control, n = 4])

	Control Group (n = 4)		Treatment Group (n = 11)		
CMG 1 (baseline)	**Mean**	**±SEM**	**Mean**	**±SEM**	***P***
Basal pressure, cm H_2O	3.4	±0.4	2.9	±0.1	0.330
Threshold pressure, cm H_2O	6.1	±0.5	4.9	±0.2	0.114
Peak pressure, cm H_2O	9.3	±1.0	7.3	±0.5	0.147
Contraction duration, s	33	±11	43	±8	0.469
Intercontractile interval, min	5.3	±2.7	7.2	±1.3	0.545
Bladder capacity, mL	19	±13	26	±4	0.639
Postvoid residual, mL	32	±18	26	±7	0.774
CMG 2 (postelectrode)	**Mean**	**±SEM**	**Mean**	**±SEM**	
Basal pressure, cm H_2O	3.3	±0.4	2.4	±0.3	0.133
Threshold pressure, cm H_2O	6.0	±0.6	5.0	±0.3	0.205
Peak pressure, cm H_2O	8.9	±1.1	7.5	±0.5	0.316
Contraction duration, s	46	±10	52	±9	0.668
Intercontractile interval, min	3.7	±1.1	7.8	±2.0	0.106
Bladder capacity, mL	31	±10	24	±5	0.552
Postvoid residual, mL	20	±10	34	±11	0.382
	Mean	**±SEM**	**Mean**	**±SEM**	
CMG 3 (poststimulation)	**(*P* vs CMG 1)** **(*P* vs CMG 2)**		**(*P* vs CMG 1)** **(*P* vs CMG 2)**		
Basal pressure, cm H_2O	2.7	±0.1	3.0	±0.2	0.260
	0.180		0.755		
	0.136		**0.031***		
Threshold pressure, cm H_2O	4.7	±0.3	4.8	±0.2	0.778
	0.036*		0.646		
	0.047*		0.497		
Peak pressure, cm H_2O	8.0	±0.8	7.2	±0.5	0.382
	0.160		0.727		
	0.267		0.415		
Contraction duration, s	40	±10	56	±11	0.312
	0.270		0.267		
	0.544		0.721		
Intercontractile interval, min	7.2	±3.8	9.3	±2.4	0.666
	0.320		0.176		
	0.947		0.503		
Bladder capacity, mL	23	±6	31	±6	0.344
	0.694		0.381		
	0.251		0.070		
Postvoid residual, mL	—	—	44	±19	—
	—		0.186		
	—		0.332		

*$p < 0.05$.

bladder pattern, whereupon like our overactive-dysfunctional group, they may have enough ascending neural cross-talk activity from the levators along the parasympathetics to activate the voiding reflex.[17]

As expected, rabbits in the control group did not have significant differences in CMG values throughout the 3 CMG cycles. Furthermore, the rhizotomy group lost detrusor contraction after dorsal rhizotomy, and there was no increase in MUP activity after PC muscle stimulation, further evidence that the electrical field stimulation of the muscles alone was not causing detrusor contractions.

Our EMG results show some interesting comparisons with previously published studies. Two studies from the same group show the virgin rabbit PC muscle to be active during storage and relaxed during micturition,[11] with a loss of this coordination seen in multiparous rabbits.[18] At baseline, our virgin rabbits showed increased EMG activity during bladder contraction. Our results demonstrated a general increase in MUP of the PC muscle after stimulation, which may correspond to the multiparous rabbits with presumed obstetric insult to their pelvic floor muscles or nerves. The aforementioned study also found that, after anesthetizing

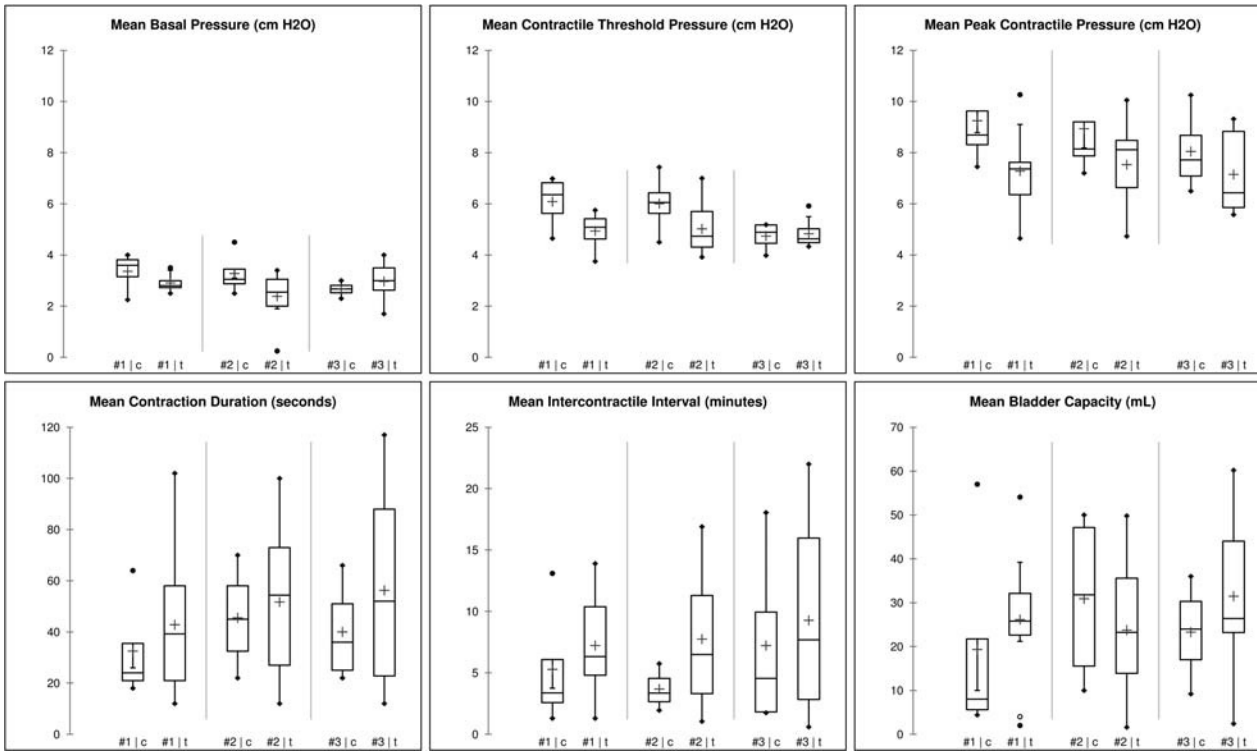

FIGURE 3. Box plot of CMG parameters for all CMG studies (CMG 1 [#1], CMG 2 [#2], and CMG 3 [#3]) grouped according to noxious stimulation (treatment [t], n = 11) versus sham needle placement (control [c], n = 4).

the PC muscle with lidocaine injection, the rabbits experienced less efficient voids with decreased contraction amplitude and increased duration.[11] This constellation of findings suggests that, at least in a rabbit model, both hypotonicity and hypertonicity of the PC muscle can impair efficient voiding and that voiding dysfunction may be due to more than only an obstructive effect of hypertonic musculature.

Our animal model of pelvic floor dysfunction has many limitations that need to be considered including issues related to study design, methodology, and data analysis. With regard to study design, in our pilot study, to evaluate the effect of noxious pelvic floor stimulation, we sought to use the minimum number of animals possible with the expectation of finding a statistically significant difference between groups based on treatment allocation. Unfortunately, the animals had a great degree of heterogeneity within each treatment group; however, much internal consistency was demonstrated within each animal. To minimize this limitation, animals were grouped subjectively according to our interpretation of the voiding pattern demonstrated on CMG 3. A further limitation with respect to the methodology of our model, which may explain the heterogeneity noted between animals, is with regard to the noxious PC electrical stimulation. A minimal

TABLE 2. CMG Parameters Grouped According to CMG Interpretation of CMG 3

	Noxious Pelvic Floor Stimulation (n = 11)					
	Dysfunctional (n = 9)					
	Prolonged (n = 7)		Overactive (n = 2)		Nonresponder (n = 2)	
	Mean	±SEM	Mean	±SEM	Mean	±SEM
CMG 1 (baseline)						
Contraction duration, s	27	±2	37	±11	15	±3
Intercontractile interval, min	11.0	±0.5	9.8	±6.2	1.1	±0.5
Bladder capacity, mL	28	±3	26	±8	3	±1
Postvoid residual, mL	38	±14	26	±17	4	±2
CMG 3 (poststimulation)						
Change in contraction duration, %	163%	±20%	56%	±30%	83%	±17%
Change in intercontractile interval, %	227%	±76%	16%	±9%	114%	±24%
Change in bladder capacity, mL	17	±8	−26	±6	7	±8
Change in postvoid residual, mL	24	±6	−27	±6	−6	±2
Unproductive contractions	Present		Present		Present	

level of current was used to confirm EMG needle position based on characteristic musculature contractions after CMG 1; however, it is still difficult to ascertain whether a precise amount of electrical current was delivered to each animal in the same way. Despite this limitation, there was no significant difference from baseline noted between treatment groups on CMG 2, and most animals (9/11 [82%]) demonstrated a change in their voiding pattern on CMG 3 after noxious stimulation of the PC muscle. In our experiment, there were no animals excluded from the final data analysis. However, to demonstrate a clear difference after noxious stimulation, animals needed to be grouped according to our observer-assigned treatment groups (Table 2), thus limiting the value of further statistical analysis between treatment groups.

Further technical limitations related to the methodology of our animal model, which may impair generalizability, include the elements of pain, the ketamine/xylazine sedation, our descriptive modality of recording EMG data, and absence of urethral pressure profile measurement during the filling and voiding phases of CMG. With respect to pain, this is a missing data point that we are unable to assess in our animal model, however certainly a significant component of the assessment of pelvic floor function in humans presenting with pelvic floor dysfunction. Although not necessary for the diagnosis, in patients with pelvic pain and voiding dysfunction, it can be difficult to separate pain from urinary discomfort.[19] It is significant however that, even in an anesthetized animal, without volitional control, the bladder still demonstrated dysfunction after noxious stimulation of the pelvic floor. This implies that the dysfunction is due to factors beyond consciously perceived pain. A further limitation is the known effect that ketamine has on altering CMG parameters. However, despite this effect, the degree of sedation should be relatively the same because all animals were sedated according to body weight. With respect to our data reporting, our EMG equipment did not allow continuous recording of output, and our EMG data are purely descriptive in nature. As summarized in our results, after noxious stimulation, during CMG 3, MUP activity increased during both filling and voiding in all but 2 rabbits. These findings suggest paradoxical activation of the PC musculature that would not be expected during the normal voiding phase of rabbit CMG. However, as a further limitation, without a urethral pressure profile or perineal EMG measurement, our findings are limited to the CMG bladder pressure parameters recorded on CMG as reported in our results. A further limitation of our animal model of voiding dysfunction is the anatomical quadruped state of rabbits, in which, unlike humans, the pelvic floor serves a different requirement than for upright animals. However, despite these anatomic differences, previous investigators have demonstrated the normal function of the PC and puborectalis pelvic floor musculature in rabbits in the maintenance of continence, with increased activity noted during urine storage and silencing during micturition, much like the levator ani pelvic floor musculature during normal urine storage and emptying in humans.[11,13]

Despite the limitations we have outlined, which are mostly related to the pilot study nature of our experiment, our study clearly demonstrated changes in voiding parameters in most animals after noxious tetanizing pelvic floor electrical stimulation. Although our study offers proof of concept and a model for acute testing, further investigation is needed to elucidate the exact mechanism underlying the changes in voiding parameters as well as the effects of varying levels and chronicity of electrical stimulation.

CONCLUSIONS

Our findings are consistent with our hypothesis that noxious stimulation of the PC musculature in the rabbit, in the form of tetanizing electrical current, results in an animal phenotype consistent with pelvic floor dysfunction. A resultant prolonged-dysfunctional pattern was demonstrated in most animals as hallmarked by a larger bladder capacity, increased intercontractile interval, and prolonged contraction duration. Future studies are needed to further characterize the long-term effects of pelvic floor dysfunction after noxious electrical stimulation.

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