Published in final edited form as:
Brain Res. 2008 December 30; 1246: 80–87. doi:10.1016/j.brainres.2008.09.053.

Somatic Innervation of the Feline Lower Urinary Tract

Paul B. Yoo, Ph.D, **John P. Woock, B.S**, and **Warren M. Grill, Ph.D**
Department of Biomedical Engineering, Duke University, Durham, North Carolina, USA

Abstract

Electrical stimulation of pudendal nerve sensory pathways can evoke excitatory bladder reflexes. However, the precise peripheral innervation pattern of these somatic fibers remains unclear. In adult male cats, we investigated pudendal nerve innervation of the lower urinary tract (LUT) by employing anatomical (Sihler's stain) and electrophysiological (selective electrical nerve stimulation) techniques. The stained specimens revealed differential innervation of the proximal and distal urethrae by fibers derived from the sensory branch of the pudendal nerve. Cranial sensory branch fibers penetrated the prostate to terminate along the intraluminal surface of the urethra, whereas the dorsal nerve of the penis primarily innervated the glans penis. Further examination of the proximal urethra showed a separate pathway (deep perineal nerve) that inserted directly into the external urethral sphincter. These observations were confirmed electrophysiologically by the measured urethral sphincter activity evoked in response to selective nerve stimulation. Electrical activation of the sensory pathway evoked only reflex (latency = 8.9 ± 1.1 ms) contractions of the urethral muscle, whereas stimulation of the perineal pathway elicited direct (latency = 1.3 ± 0.1 ms) responses. Our findings identify specific pudendal nerve sensory pathways that can be used to potentially restore bladder function in persons with spinal cord injury and also treat LUT symptoms such as urinary retention.

Keywords

Sihler's stain; urethral sensory; external urethral sphincter; prostate; reflex bladder contraction

Introduction

In most species, the somatic innervation to the lower urinary tract (LUT) is derived from sacral spinal nerves that converge to form the pudendal nerve. This multi-fasciculated nerve trunk provides efferent control of the striated pelvic floor muscles including the external urethral sphincter (EUS) for urinary function, and afferent sensation associated with sexual function. Evidence supporting the role of afferent (or sensory) pudendal nerve fibers in mediating pudendo-vesicle reflexes has also been described in cats (Barrington, 1931; Boggs et al., 2006; Shefchyk and Buss, 1998; Tai et al., 2007) and rats (Peng et al., 2008a; Peng et al., 2008b), and demonstrated by pudendal nerve stimulation in persons with spinal cord injury (SCI) (Gustafson et al., 2004; Kirkham et al., 2001; Yoo et al., 2007): low frequency (5–15 Hz) pulse trains evoke a robust bladder inhibitory reflex, whereas high frequency (20–50 Hz) pulse trains generate reflex bladder excitation. Recent work in cats has further differentiated

Please Address Correspondence to Paul B. Yoo, Duke University, Department of Biomedical Engineering, Hudson Hall, Room 136, Box 90281, Durham NC 27708-0281, Email: E-mail: paul.yoo@duke.edu.

the distal sensory branches of the pudendal nerve (innervating the pelvic urethra and penis) as independent pathways that can engage either a spinobulbospinal or direct spinally mediated micturition reflex, respectively (Woock et al., 2008; Yoo et al., 2008).

However, anatomical studies in both cats and humans (Martin et al., 1974; Schraffordt et al., 2004) have provided limited details of pudendal division and innervation distal to the ischiorectal fossa, thus resulting in conflicting reports regarding the source of excitatory bladder responses evoked by high frequency nerve stimulation: the deep perineal nerve (Boggs et al., 2005) vs. the sensory nerve (Woock et al., 2008; Yoo et al., 2008), as well as the origin of the dorsal nerve of the penis (Mariano et al., 2008; Martin et al., 1974). In humans, a similar debate regarding the innervation of the proximal urethra also remains despite various staining and reconstruction methods employed to visualize the distal branches of the pudendal nerve (Karam et al., 2005; Tanagho et al., 1982; Zvara et al., 1994).

The objectives of this study were to expand the current knowledge of feline pudendal nerve innervation (Martin et al., 1974), understand the anatomical origin of excitatory pudendo-vesicle reflexes (Yoo et al., 2008), and explore the potential clinical implications of pudendal nerve pathways. We documented the innervation of the lower urinary tract of male cats using a Sihler's technique for staining peripheral nerves combined with an electrophysiological approach that differentiated the frequency-dependent bladder activation patterns mediated by pudendal nerve afferents.

Results

Anatomy of the Feline Pudendal Nerve

Surgical and postmortem dissection demonstrated consistent division (9 of 10 cats) of the pudendal nerve trunk within the ischiorectal fossa into two primary nerve bundles labeled the sensory (SN) and rectal perineal (RP) branches (Figure 1A). In one cat, multiple smaller (secondary) branches were observed instead of a distinct bifurcation of the pudendal nerve trunk. Distal to this point, the SN branch of the pudendal nerve also bifurcated at the level of the bulbourethral gland, resulting in two secondary branches extending in opposite directions along the urethra. The cranial sensory (CSN) component traveled along the proximal urethra, whereas the dorsal nerve of the penis (DNP) coursed distally along the urethra towards the glans penis. Microscopic observation of the Sihler's stained specimens (Figure 1B and Figure 2A) revealed that both the CSN and deep perineal (dPN, derived from the RP branch) nerves projected into the proximal urethra at the level of the bulbourethral gland (BG). The stained specimens (Figure 1B) also showed division of nerve fascicles (e.g., CSN and DNP) occurring prior to the separation of these components into branches coursing in opposite directions along the urethra. The specimen shown in Figure 2 was representative of all cats and thus used to illustrate further somatic innervation (Figures 3–5).

Innervation of the Proximal Urethra and Prostate

In all 10 specimens, the CSN innervated the proximal urethra through lateral (L) and medial (M) components that coursed cranially along the external urethral surface (Figure 2A). The medial component generally consisted of a single branch that deviated proximally at the ventral midline of the urethra (Figure 2B and C), but could also include additional fibers (M^*). Both CSN components traveled along the membranous urethra, superficial to the dPN fibers (Figure 2A and B) until the lateral (L) branch (Figure 2A) and ventral projections of the medial (M) bundle (labeled 1 & 2 in Figure 2D) inserted into the ventro-lateral aspects of the prostate. The remaining medial CSN fibers continued proximally beyond the prostate and towards the pre-prostatic urethra (label 3 in Figure 2D), whereas in some cases the proximal extent of the CSN branch was near the cranial aspect (or base) of the prostate (label 4 in Figure 2D). The precise

proximal extent of the CSN fibers was frequently obscured by distally projecting autonomic fibers (label 5 in Figure 2D).

The pudendal nerve also innervated the proximal urethra via the deep perineal (dPN) nerve, which derived from the rectal perineal (RP) branch. From the ischiorectal fossa, dPN fibers projected ventrally towards the urethra, and approached the lateral aspect of the membranous urethra (Figure 2A and B). An early component of the dPN branch innervated the bulbourethral gland, while the remaining fibers inserted into the external urethral sphincter (EUS). As shown from a lateral view (Figure 2A), dense projection of the dPN fibers along the lateral and ventral aspects of the urethra extended up to the proximal aspect of the prostate.

Subsequent dissection of the superficial CSN fibers and a complete cut along the ventral midline of the urethra (from urethral meatus to the bladder neck) provided a complete view of the dPN projection pattern (Figure 3A). These fibers projected into the EUS primarily along the ventro-lateral surface of the urethra and extended distally to the base (between the crura) of the penis. Contralateral innervation by dPN fibers was also observed, but appeared confined to the medial aspect of the membranous urethra (white box in Figure 3A).

Intraurethral Projection of CSN fibers

Resection of the dPN fibers along the external surface of the urethra provided an unobstructed view of the intraluminal surface of the proximal urethra and complete visualization of CSN fiber projection (Figure 3B). The ascending superficial CSN fibers that inserted dorsally into the prostate (Figure 2B and Figure 3A) reversed direction and provided periurethral innervation at various locations along the prostatic and post-prostatic urethra. The physical characteristics of these CSN projections defined 3 regions exhibiting distinct patterns (Figure 4B): (a) prostatic, (b) post-prostatic and (c) membranous. Visualization of these projection patterns was possible in only 4 of 10 cats due to inconsistent staining within the intraurethral space. The prostatic region (Figure 4A) was densely innervated by CSN fibers that were characterized by very thin nerve endings projecting towards the urethral lumen. The disorganized path of these CSN processes was followed by minute focal adjustments of the microscope until the visible end-points of the stained fibers reached the intraluminal surface (indicated by arrows in Figure 4). The post-prostatic region (Figure 4B), which also exhibited a high density of nerve endings, expressed numerous CSN processes resembling pacinian corpuscles that aligned with the proximal-distal axis of the urethra. In contrast, innervation of the membranous region (Figure 4C) was sparse with nerve processes (nerve endings) aligned either parallel or perpendicular to the intraurethral surface. In this particular specimen, innervation of the membranous region (Figure 4C) was derived from the left lateral (L) CSN branch and also from a division of the right medial (M) CSN branch (Figure 3A and B).

Innervation of the Distal Urethra

The DNP branch separated from the SN near the base of the penis (Figure 1) and traveled distally past the ischiocavernosus muscle (IC), where it bifurcated into two pathways along the medial (M) and lateral (L) aspects of the penis (Figure 5A and B). The lateral branch provided periodic ventral projections that resulted in relatively sparse innervation of the penile urethra (arrows, Figure 5C). The remaining nerve fibers within these projections innervated the skin of the perineum. In contrast, the glans and preputial area of the penis exhibited dense innervation by both the lateral and medial branches of the DNP (Figure 5B).

Physiological Response to Electrical Nerve Stimulation

Selective electrical stimulation of the SN and RP branches verified the functional difference between the two primary nerve pathways (Figure 6A–B): RP branch stimulation elicited direct (latency = 1.3 ± 0.1 ms) EMGs in both the EAS and EUS followed by longer latency reflex

activity; SN branch stimulation evoked only reflex (latency = 8.9 ± 1.1 ms) EMGs. The proximal extent of CSN innervation was subsequently mapped by comparing the stimulus threshold required to evoke the pudendal-anal reflex (Figure 6A) in response to single pulses delivered at specific distances (0.5 cm intervals) from the middle of the prostate (inset in Figure 6). In all 5 cats, this activation threshold was consistent (0.3 ± 0.2 mA, mean ± SD, range = 0.1 to 0.7 mA) along the prostatic and membranous urethra (Figure 7A), but increased significantly beyond 0.5 cm proximal to the prostate ($p < 0.005$). Based on these results, excitatory bladder reflexes were measured in response to indirect CSN (needle tip at 1 cm distal to prostate) or direct DNP nerve stimulation. At bladder volumes above 70% of the threshold for distension evoked contractions (Figure 6C–D), selective electrical activation of the CSN and DNP pathways (20-second pulse trains) evoked sustained increases of 26.1 ± 8.9 cmH_2O and 36.5 ± 6.6 cmH_2O in bladder pressure, respectively. Furthermore, these electrically evoked responses were tuned to specific frequency ranges (Figure 7B): epiurethral CSN (2 – 5 Hz) and direct DNP (20 – 50 Hz).

Discussion

The results of this study provide a detailed account of the somatic innervation of the feline lower urinary tract (LUT). Using the Sihler's staining technique, we were able to differentiate between the deep perineal (dPN) nerve and cranial sensory (CSN) nerve fibers innervating the proximal urethra (Figure 3), while also confirming termination of dorsal nerve of the penis (DNP) fibers in the penile urethra, glans and preputial area of the penis (Figure 5). The particularly dense innervation of the feline glans penis by DNP fibers suggests a critical role of this structure in the neuromodulation of sexual (Johnson et al., 1986) and urinary (Tai et al., 2007;Woock et al., 2008) function. The excitatory bladder contractions evoked by selective electrical activation of the CSN and DNP fibers (Figure 6) verified the anatomical correlates of two distinct central micturition pathways (Yoo et al., 2008).

In contrast to the conventional use of Sihler's staining technique for studying neural innervation within homogenous muscle tissue (Mu and Sanders, 1999; Ryan et al., 2003), we applied this method for the first time to the entire feline LUT, which consists of various tissue types. The dense connective tissue of the penis, in particular, required modifications to the staining, de-staining and clearing times to maximize the contrast of the neural processes. In some cases, this resulted in the inconsistent staining of CSN fibers along the intraurethral surface, while in others it enabled visualization of very fine processes and apparent nerve fiber terminations. This technique is also limited by its non-selective staining characteristics, which resulted in images of the pudendal nerve obscured by autonomic nerve fibers projecting distally along the proximal urethra or anteromedially from the pelvic plexus. This made it difficult to verify the proximal extent of CSN fibers innervating the proximal urethra (Figure 2B), as was demonstrated electrophysiologically in cats (Bradley et al., 1973).

The gross anatomical features of the feline pudendal nerve trunk (Figure 1) were consistent with earlier work (Martin et al., 1974) and exhibited remarkable similarities with that in humans (Gustafson et al., 2005;Schraffordt et al., 2004), including the sensory (dorsal genital nerve), caudal rectal (external anal sphincter), and perineal (external urethral sphincter) components. The overall pudendal nerve branching patterns observed within the ischiorectal fossa resembled the classical one-trunk (type I) architecture reported in humans (Sikorski et al., 1987). Organization of the secondary pudendal nerve branches was also consistent across all cats and again exhibited characteristics similar to humans, including primary DNP innervation of fibers into the glans penis (Yang and Bradley, 1998), and dPN innervation of the external urethral sphincter (Narayan et al., 1995;Strasser et al., 1996).

The anatomical evidence obtained from the Sihler's stained specimens showed, for the first time, a dual innervation pattern of the proximal urethra by the dPN and CSN branches of the pudendal nerve. Intraluminal visualization of CSN fiber projections (Figure 4), the lack of any direct urethral EMG response to SN branch stimulation (Figure 6B), and the excitatory bladder responses evoked by epiurethral stimulation (Figure 6C) all indicate this specific nerve branch is the "urethral sensory" pathway. That this nerve branch diverged from the DNP, distal to the bifurcation point of the SN (Dorsal N. Penis or Clitoris) and RP (Deep Perineal and Caudal Rectal) branches, further supports the correct identity of this pathway (Martin et al., 1974). In contrast to a recent study in cats (Mariano et al., 2008), our results show that the "urethral sensory" component does not emerge as a nerve branch proximal to the point of SN/RP bifurcation. In fact, our dissections identified this branch as the obturator internus (OI) nerve (Figure 1A), which separated from the pudendal nerve trunk just caudal to the coccygeus muscle and innervated the obturator internus and gemellus muscles. Further, our data supports the original assertion (Martin et al., 1974) that the DNP arises from the sensory (SN) branch, and not from the deep perineal (dPN) nerve as recently suggested (Mariano et al., 2008).

This dual innervation of the proximal urethra by both the sensory (SN) and rectal perineal (RP) branches (Figure 2A) supports previous human anatomical studies indicating external urethral sphincter (EUS) innervation by perineal (Karam et al., 2005) or dorsal genital nerves (Zvara et al., 1994), and suggests a similar innervation pattern by analogous nerves (e.g., CSN and dPN) may be present in humans. The high density of CSN fiber projections along the intraluminal surface of the prostatic and post-prostatic urethra (Figure 4A–B) also corresponded anatomically to the distribution of urethral mechanoreceptors (e.g., pacinian corpuscles) in the feline urethra (Todd, 1964) and functionally to excitatory bladder responses evoked by low frequency (2 – 5 Hz) electrical stimulation of this nerve branch (Yoo et al., 2008). In humans, this nerve pathway most likely mediates the 'urethro-vesicle reflex' (Gustafson et al., 2004; Shafik et al., 2003).

Electrically evoked bladder contractions mediated by myelinated CSN afferents (Figure 6C) combined with the prostatic insertion and intraluminal projection of these fibers (Figure 3A) present a potential mechanism for generating certain lower urinary tract symptoms (e.g., frequency or urgency) under pathological conditions (e.g., prostatitis or benign prostatic hyperplasia). Although prostatic innervation involves sympathetic, parasympathetic and somatic components (Danuser et al., 1997), the absence of electrically evoked bladder responses by intraurethral stimulation of the prostatic urethra following bilateral pudendal nerve transection, suggests these autonomic components are not associated with micturition reflexes (Gustafson et al., 2004). Rather, these pelvic or hypogastric nerve fibers are considered efferent in nature and modulate both smooth and striated muscle components of the urethra (Elbadawi and Schenk, 1974; Morita et al., 1984).

Somatic innervation of the male feline urinary tract involves a complex network of pudendal nerve branches involved with mechanisms underlying urinary continence (dPN) and micturition (DNP and CSN) mechanisms. In this study, we verified the anatomical innervation targets of these branches (CSN: prostatic urethra, DNP: glans penis) and provided, for the first time, a detailed description of somatic innervation of the proximal urethra. Although remarkable similarities between the feline and human pudendal nerves are observed, urethral innervation by the CSN (or analogous) branch has yet to be demonstrated in humans.

Experimental Procedure

Neuroanatomy of the Lower Urinary Tract

Lower urinary tract innervation was investigated in 10 post-mortem male cats (weight = 3.3–5.5 kg) using a modified Sihler's technique (Mu and Sanders, 1999; Ryan et al., 2003). The

ureters, vas deferens, and ischiocavernosus muscles were cut to allow resection of the entire lower urinary tract. The location of pudendal nerve transection varied (i.e., proximal or distal to the SN/RP branching point) among each specimen. The staining process consisted of fixation in 10% unneutralized formalin (1 month), maceration in 3% KOH (3 weeks), decalcification in Sihler's solution I (2 weeks), staining in Sihler's solution II with Harris hematoxylin (3 weeks), destaining in Sihler's solution I (approximately 3–4 hours), brief immersion in 0.05% lithium carbonate (1 hour), clearing in 50% glycerin (2–3 days), and storage in 100% glycerin with thymol crystals. Each specimen was rinsed under running tap water (1 hour) between each step of the staining process.

The branching pattern of the pudendal nerve in each stained specimen was studied using a stereomicroscope (Nikon SMZ1000) by identifying each nerve branch and tracing these processes distally. The layered neural structure of the proximal urethra permitted successive micro-dissection of the CSN and dPN branches within each stained specimen, which resulted in enhanced visualization of the underlying nerves. Optimum contrast of neural tissue was achieved either by direct reflected light (RL) or transmitted light (TL), and is indicated in the figure captions. Digital photomicrographs were made throughout each dissection (ProgRes C5 digital camera, JENOPTIK Laser, Optik, Systeme GmbH).

Electrophysiology of the Lower Urinary Tract

In 5 of the 10 cats, the responses to electrical stimulation of pudendal nerve branches were measured. Anesthesia was induced with ketamine HCl (35 mg/kg I.M.) and maintained with I.V. α-chloralose (initial 65 mg/kg supplemented at 15 mg/kg). Artificial respiration (12–15 breaths per minute), fluid levels (I.V. saline at 55 ml/hr; cephalic vein) and core temperature (39°C) were maintained, while blood pressure (via a catheter in the carotid artery) and end tidal CO_2 (3–4 %) were continuously monitored. At the conclusion of the experiment, animals were euthanized with an overdose of sodium pentobarbital (I.V.). Animal care and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Duke University.

The bladder was exposed via a midline abdominal incision and catheterized through the apex with a 3.5 Fr polyethylene catheter. A syringe pump was used to fill the bladder with room temperature saline via the catheter, and the bladder pressure was measured with a solid state transducer (Deltran, Utah Medical). The abdomen was sutured closed in layers. The pudendal nerve trunk was exposed via a dorsal incision, whereas access to the cranial sensory (CSN) and dorsal penile (DNP) nerves was achieved by exposing the ventral surface of the lower urinary tract. This involved an incision from the lower abdomen to the penis, transection of the pubic symphysis and bisection of the levator ani muscle.

Individual nerve branches (RP, SN and DNP) were stimulated electrically by nerve cuff electrodes (1 mm × 1 mm platinum contact embedded within silicone elastomer cuff), delivering current pulses (cathodic, pulse width = 0.1 ms, frequency = 1 – 50 Hz) generated from a constant current source (Pulsar 6bp, FHC Inc., Bowdoin, ME). In contrast, CSN nerve fibers were stimulated by a 26 gauge concentric EMG needle electrode (Viasys Healthcare) placed along the ventral surface of the exposed urethra. The tip was positioned initially at the midpoint of the prostate and was subsequently moved rostro-caudally at 0.5 cm intervals. The counter (anodic) electrode was a 20 gauge needle inserted through a pinched skinfold, lateral to the lumbosacral spine. The evoked electromyographic (EMG) signals were recorded from the external anal sphincter (EAS) with a pair of insulated multi-stranded stainless steel wires (exposed tip = 2 mm, Cooner Wire Inc., Chatsworth, CA), and from the external urethral sphincter (EUS) with a custom-made 3.5 or 5 Fr polyethylene catheter containing a pair of platinum ring electrodes (each 1 mm in length and separated by 1 cm) positioned 3–5 cm from

the urethral meatus. All EMG signals were amplified, filtered (gain = 1000, filter = 10 Hz to 3 kHz) and recorded (sampling rate = 20 kHz; Astromed 8Xe, Astro-Med Inc).

Acknowledgments

This work was supported by a research grant from the National Institutes of Health (R01NS050514) and a National Science Foundation graduate research fellowship. The authors thank Ms. Gilda Mills for technical assistance.

Abbreviations

SN
Sensory Component of Pudendal Nerve

RP
Rectal Perineal Component of Pudendal Nerve

DNP
Dorsal Nerve of Penis

CSN
Cranial Sensory Nerve

dPN
Deep Perineal Nerve

CR
Caudal Rectal Nerve

IC
Ischiocavernosus Muscle

BG
Bulbourethral Gland

RL
Reflected Light

TL
Transmitted Light

References

Barrington FJ. The component reflexes of micturition in the cat. I and II. Brain 1931;54:177–188.

Boggs JW, Wenzel BJ, Gustafson KJ, Grill WM. Spinal micturition reflex mediated by afferents in the deep perineal nerve. J Neurophysiol 2005;93:2688–97. [PubMed: 15601736]

Boggs JW, Wenzel BJ, Gustafson KJ, Grill WM. Frequency-dependent selection of reflexes by pudendal afferents in the cat. J Physiol 2006;577:115–26. [PubMed: 16945977]

Bradley W, Griffin D, Teague C, Timm G. Sensory innervation of the mammalian urethra. Invest Urol 1973;10:287–9. [PubMed: 4683378]

Danuser H, Springer JP, Katofiasc MA, Thor KB. Extrinsic innervation of the cat prostate gland: a combined tracing and immunohistochemical study. J Urol 1997;157:1018–24. [PubMed: 9072536]

Elbadawi A, Schenk EA. A new theory of the innervation of bladder musculature. 2. Innervation of the vesicourethral junction and external urethral sphincter. J Urol 1974;111:613–5. [PubMed: 4856786]

Gustafson KJ, Creasey GH, Grill WM. A urethral afferent mediated excitatory bladder reflex exists in humans. Neurosci Lett 2004;360:9–12. [PubMed: 15082166]

Gustafson KJ, Zelkovic PF, Feng AH, Draper CE, Bodner DR, Grill WM. Fascicular anatomy and surgical access of the human pudendal nerve. World J Urol 2005;23:411–8. [PubMed: 16333625]

Johnson RD, Kitchell RL, Gilanpour H. Rapidly and slowly adapting mechanoreceptors in the glans penis of the cat. Physiol Behav 1986;37:69–78. [PubMed: 3016773]

Karam I, Droupy S, Abd-Alsamad I, Korbage A, Uhl JF, Benoit G, Delmas V. The precise location and nature of the nerves to the male human urethra: histological and immunohistochemical studies with three-dimensional reconstruction. Eur Urol 2005;48:858–64. [PubMed: 16230229]

Kirkham AP, Shah NC, Knight SL, Shah PJ, Craggs MD. The acute effects of continuous and conditional neuromodulation on the bladder in spinal cord injury. Spinal Cord 2001;39:420–8. [PubMed: 11512072]

Mariano TY, Boger AS, Gustafson KJ. The feline dorsal nerve of the penis arises from the deep perineal nerve and not the sensory afferent branch. Anat Histol Embryol 2008;37:166–8. [PubMed: 18479311]

Martin WD, Fletcher TF, Bradley WE. Innervation of feline perineal musculature. Anat Rec 1974;180:15–29. [PubMed: 4278149]

Morita T, Nishizawa O, Noto H, Tsuchida S. Pelvic nerve innervation of the external sphincter of urethra as suggested by urodynamic and horse-radish peroxidase studies. J Urol 1984;131:591–5. [PubMed: 6700010]

Mu L, Sanders I. Neuromuscular organization of the canine tongue. Anat Rec 1999;256:412–24. [PubMed: 10589027]

Narayan P, Konety B, Aslam K, Aboseif S, Blumenfeld W, Tanagho E. Neuroanatomy of the external urethral sphincter: implications for urinary continence preservation during radical prostate surgery. J Urol 1995;153:337–41. [PubMed: 7815577]

Peng CW, Chen JJ, Cheng CL, Grill WM. Role of pudendal afferents in voiding efficiency in the rat. Am J Physiol Regul Integr Comp Physiol 2008a;294:R660–72. [PubMed: 18077513]

Peng CW, Chen JJ, Cheng CL, Grill WM. Improved bladder emptying in urinary retention by electrical stimulation of pudendal afferents. J Neural Eng 2008b;5:144–54. [PubMed: 18430976]

Ryan S, McNicholas WT, O'Regan RG, Nolan P. Intralaryngeal neuroanatomy of the recurrent laryngeal nerve of the rabbit. J Anat 2003;202:421–30. [PubMed: 12739619]

Schraffordt SE, Tjandra JJ, Eizenberg N, Dwyer PL. Anatomy of the pudendal nerve and its terminal branches: a cadaver study. ANZ J Surg 2004;74:23–6. [PubMed: 14725700]

Shafik A, el-Sibai O, Ahmed I. Effect of urethral dilation on vesical motor activity: identification of the urethrovesical reflex and its role in voiding. J Urol 2003;169:1017–9. [PubMed: 12576835]

Shefchyk SJ, Buss RR. Urethral pudendal afferent-evoked bladder and sphincter reflexes in decerebrate and acute spinal cats. Neurosci Lett 1998;244:137–40. [PubMed: 9593508]

Sikorski A, Olszewski J, Miekos E. Anatomical considerations of selective pudendal neurectomy. Int Urol Nephrol 1987;19:159–63. [PubMed: 3667129]

Strasser H, Klima G, Poisel S, Horninger W, Bartsch G. Anatomy and innervation of the rhabdosphincter of the male urethra. Prostate 1996;28:24–31. [PubMed: 8545278]

Tai C, Wang J, Wang X, de Groat WC, Roppolo JR. Bladder inhibition or voiding induced by pudendal nerve stimulation in chronic spinal cord injured cats. Neurourol Urodyn 2007;26:570–7. [PubMed: 17304521]

Tanagho EA, Schmidt RA, de Araujo CG. Urinary striated sphincter: what is its nerve supply? Urology 1982;20:415–7. [PubMed: 6183812]

Todd JK. Afferent Impulses in the Pudendal Nerves of the Cat. Q J Exp Physiol Cogn Med Sci 1964;49:258–67. [PubMed: 14195114]

Woock JP, Yoo PB, Grill WM. Activation and inhibition of the micturition reflex by penile afferents in the cat. Am J Physiol Regul Integr Comp Physiol 2008;294:R1880–9. [PubMed: 18434446]

Yang CC, Bradley WE. Peripheral distribution of the human dorsal nerve of the penis. J Urol 1998;159:1912–6. [PubMed: 9598486]

Yoo PB, Klein SM, Grafstein NH, Horvath EE, Amundsen CL, Webster GD, Grill WM. Pudendal nerve stimulation evokes reflex bladder contractions in persons with chronic spinal cord injury. Neurourol Urodyn 2007;26:1020–3. [PubMed: 17480024]

Yoo PB, Woock JP, Grill WM. Bladder activation by selective stimulation of pudendal nerve afferents in the cat. Exp Neurol 2008;212:218–25. [PubMed: 18502417]

Zvara P, Carrier S, Kour NW, Tanagho EA. The detailed neuroanatomy of the human striated urethral sphincter. Br J Urol 1994;74:182–7. [PubMed: 7921935]

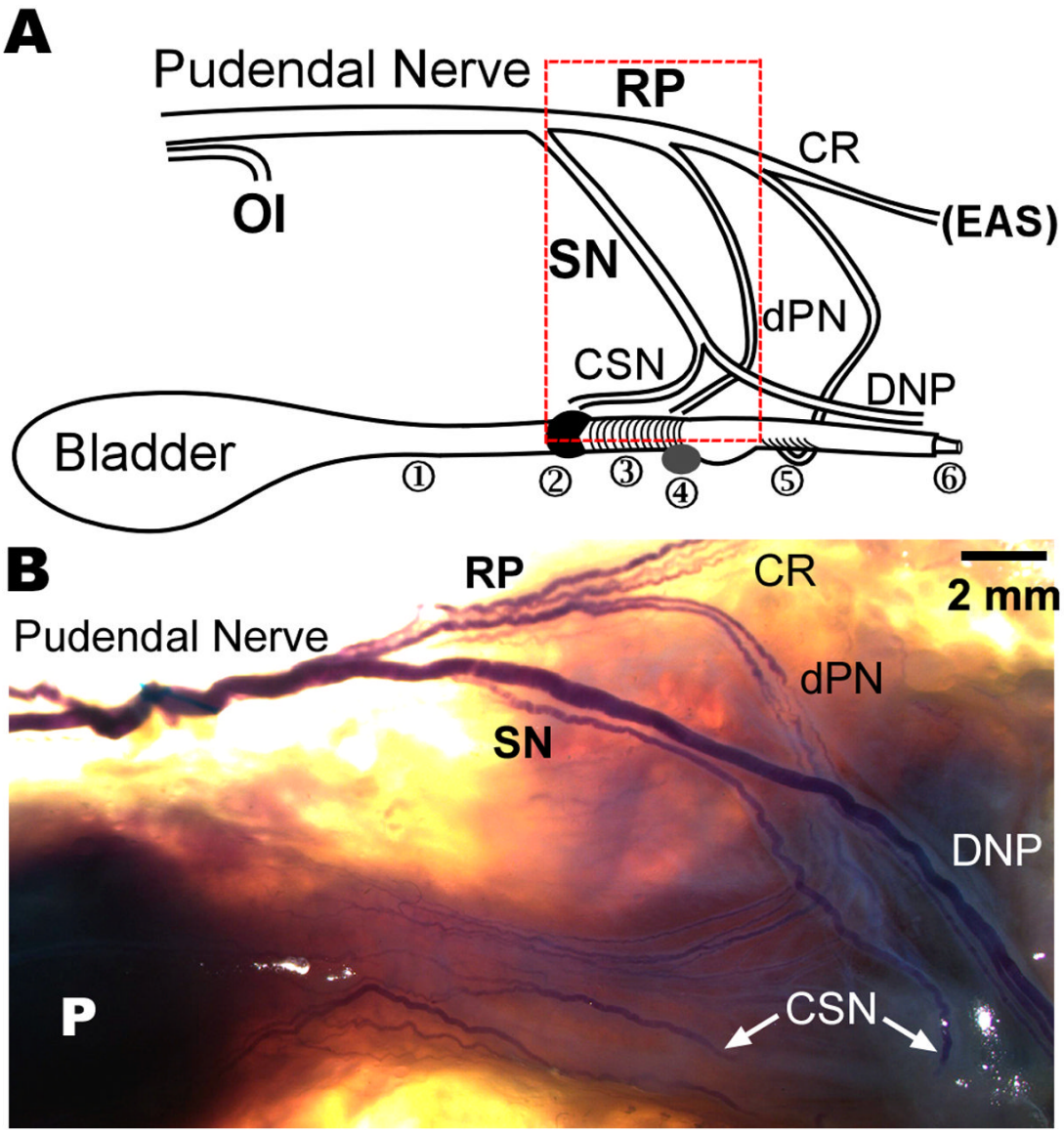

Figure 1.

(A) The schematic shows the typical branching pattern of the pudendal nerve within the ischiorectal fossa and the distal nerve projections toward the lower urinary tract (lateral view). The compound nerve trunk bifurcates into the sensory (SN) and rectal perineal (RP) branches, approximately 1cm caudal to the obturator internus (OI) nerve, which innervates the obturator and gemellus muscles. The SN further divides into the cranial sensory (CSN) and dorsal nerve of the penis (DNP); whereas the RP separates into the deep perineal (dPN) and caudal rectal (CR) branches. Nerve branch nomenclature was based on a previous study (Martin et al., 1974). **(B)** Image of the ventral surface of a stained specimen shows the corresponding nerve branches (CSN, DNP and dPN) of the pudendal nerve (dashed box in (A)). [① = bladder neck, ② = prostrate (P), ③ = external urethral sphincter (EUS), ④ = bulbourethral gland (BG), ⑤ = bulbospongiosus muscle, ⑥ = glans penis, EAS = external anal sphincter; Illumination: RL +TL]

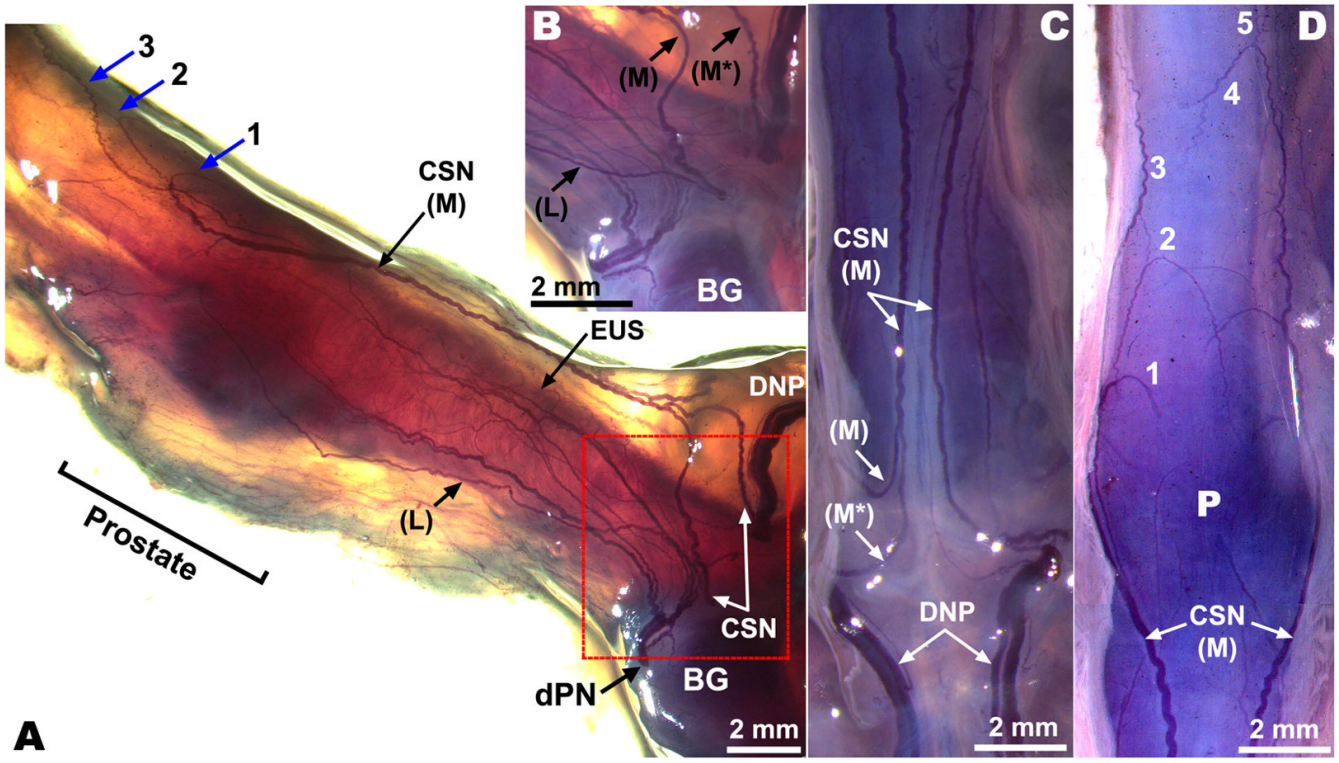

Figure 2.

Innervation of the prostatic and membranous urethra. **(A)** A lateral view of the urethra shows that the CSN branch has further divided into a lateral (L) and medial (M) component, both of which travel proximally along the urethra, superficial to the EUS and dPN fibers. **(B)** A closer image of the CSN branches (dashed box in (A)) shows the distinct lateral (L) component and **(C)** a late medial (M^*) branch that, in this example, merged with the medial (M) component of the CSN along the ventral surface of the membranous urethra. Urethral insertion of the lateral (L) branch occured at the level of the prostate. **(D)** Ventral view of the prostatic urethra in the same specimen shows the medial CSN (M) branch projecting into the prostate (labels 1 & 2) and the pre-prostatic urethra (label 3). In this example, the proximal extent of the left medial (M) CSN branch was cranial to the prostate (label 4) and partially obscured by descending autonomic fibers (label 5).

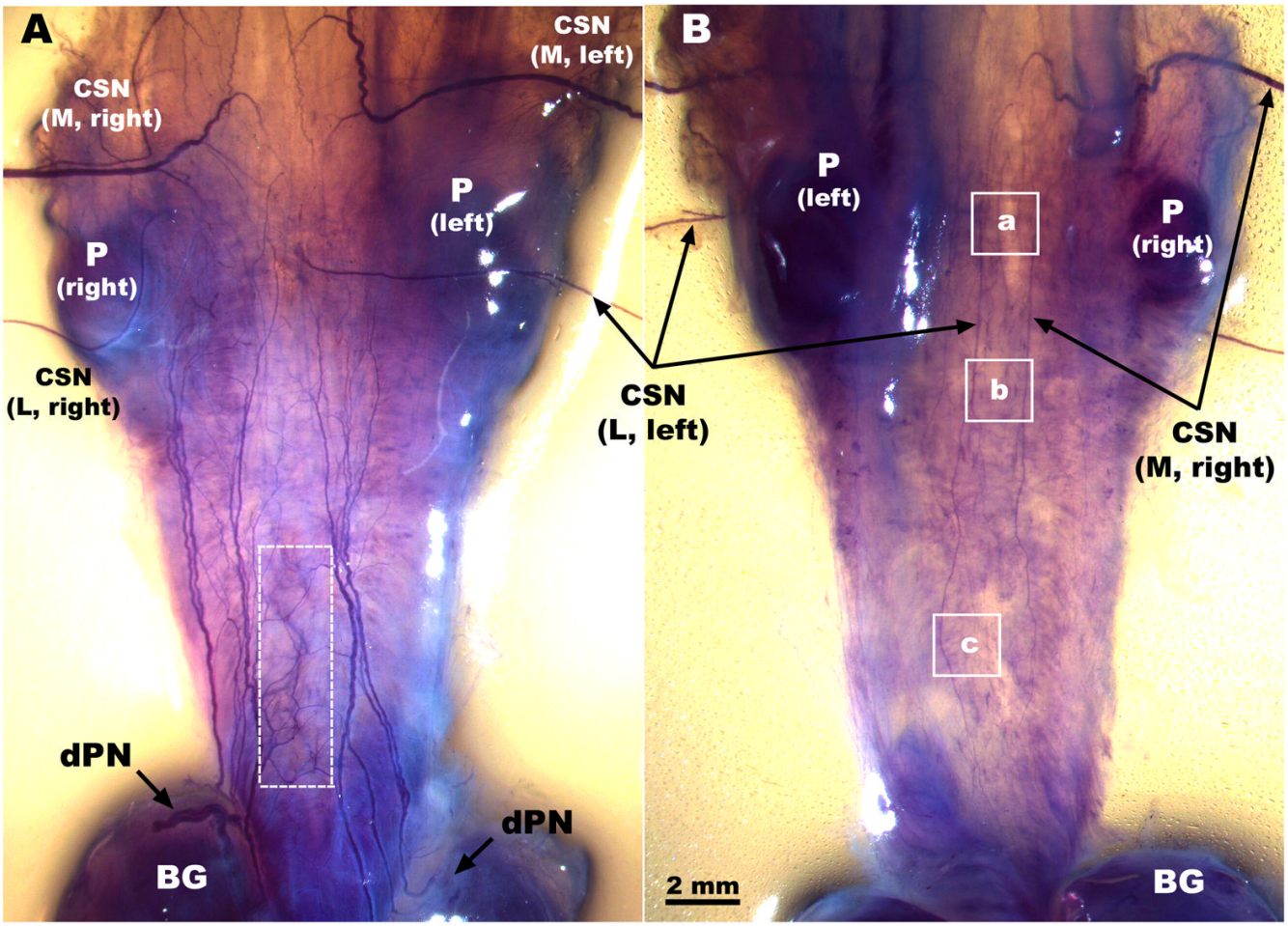

Figure 3.

Somatic innervation of the **(A)** external and **(B)** internal surfaces of the proximal urethra. **(A)** Dissection of the CSN fibers yielded an unobstructed view of the deep perineal (dPN) innervation of the dorsal (anterior) aspect of the prostatic and membranous urethra. Extensive contralateral innervation of the external urethral sphincter (EUS) by the dPN was observed in the distal half of the membranous urethra (white box). **(B)** Complete removal of the dPN fibers improved the visualization of intraurethral innervation of CSN fibers. The density and physical characteristics of the CSN fiber endings defined 3 regions along the intraurethral surface: (a) prostatic, (b) post-prostatic and (c) membranous. In this specimen, membranous (c) innervation was provided by the left lateral (L) and right medial (M) branches of the CSN. [Illumination: RL+TL, P = prostate, BG = bulbourethral gland]

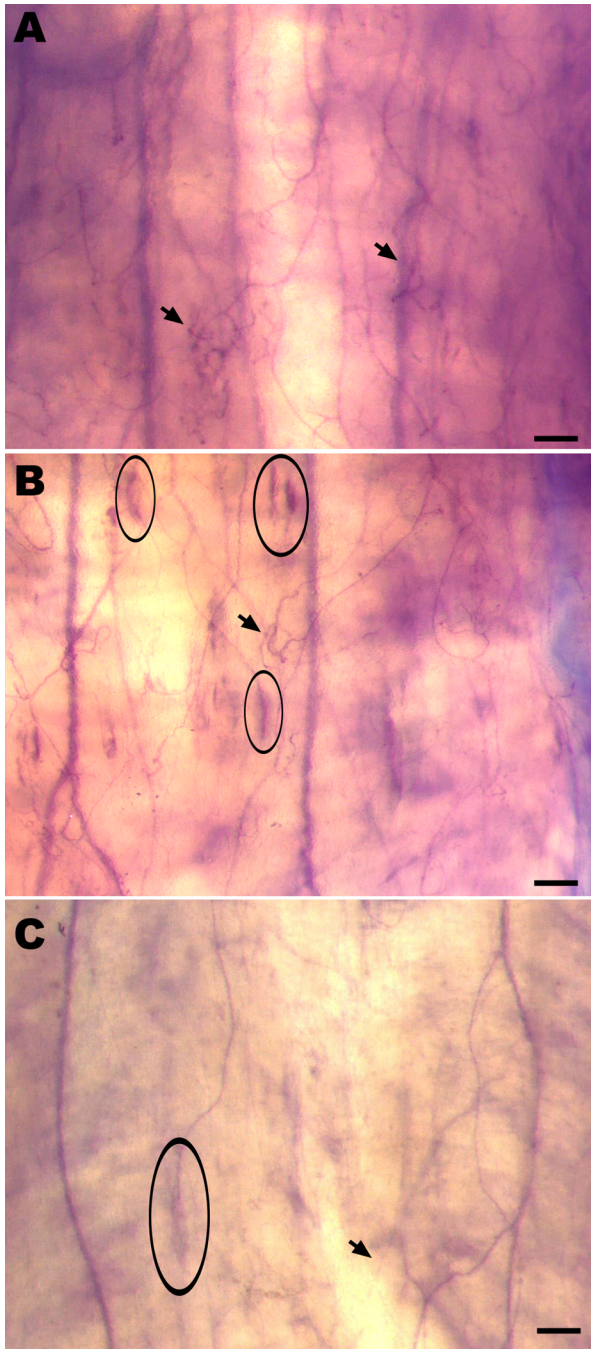

Figure 4.
Micrographs of the intraluminal surface along the proximal urethra. **(A)** The prostatic urethra exhibited a high density of CSN nerve fiber endings (➔) that projected onto the intraurethral surface. **(B)** The post-prostatic region was densely innervated by these nerve endings (➔) and also by fiber end-points that resembled pacinian corpuscles (O). In contrast, **(C)** the membranous urethra showed sparse innervation by both nerve endings (➔) and pacinian corpuscle-like structures (O). [Illumination: A (RL), B (TL), C (RL), scale = 200 μm]

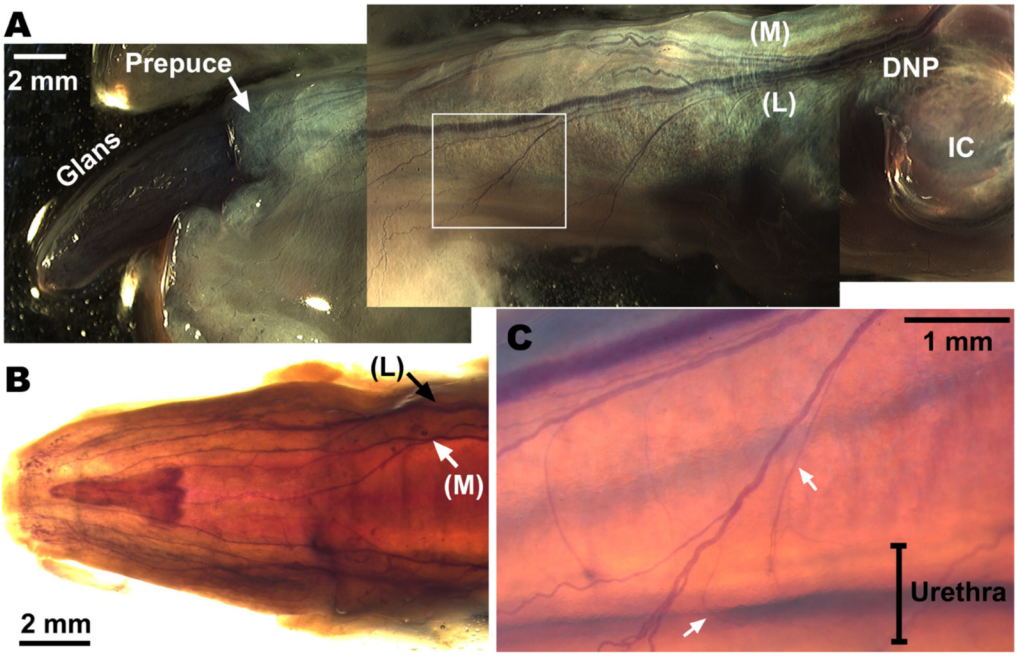

Figure 5.

Innervation of the distal (i.e., penile) urethra. **(A)** The lateral view of the cat penis shows the division of the dorsal nerve of the penis (DNP): the main lateral (L) division and the dorsal (M) branch. **(B)** From a dorsal view of the glans penis, dense innervation by both the lateral (L) and medial (M) branches is observed. The stained os penis is visible near the tip of the glans. **(C)** A small subset of the ventral projections from the lateral (L) branch (zoomed image of box in A) innervated the penile urethra (➔), whereas the majority of fibers terminate in the perineum. [IC: Ischiocavernosus muscle, Illumination: A (RL), B (TL) and C (TL)]

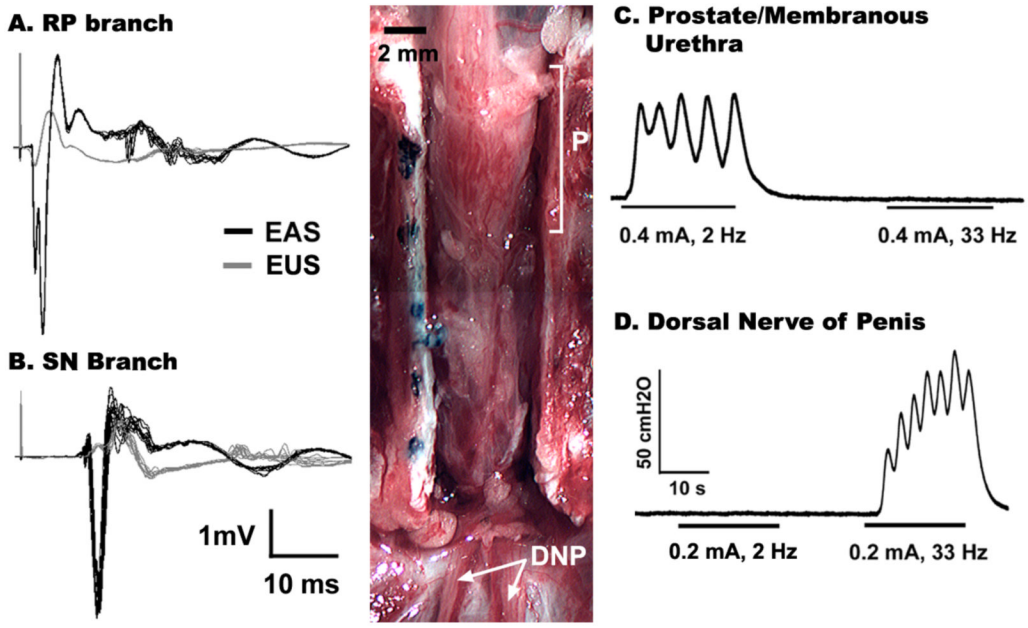

Figure 6.

Physiological responses evoked by electrical nerve stimulation of pudendal nerve branches. **(A, B)** The primary branches of the pudendal nerve were characterized by the electromyographic (EMG) responses of both the external anal (EAS) and external urethral (EUS) sphincters that were evoked by single current pulses (series of 10 pulses delivered at 1 Hz). Stimulation of the rectal perineal (RP) branch evoked primarily direct (latency = 1.3 ± 0.1 ms) EMGs followed by longer latency reflex activity, whereas stimulation of the sensory (SN) branch elicited only reflex (latency = 8.9 ± 1.1 ms) EMGs. **(C, D)** Application of stimulus trains (20 second duration) to the CSN (epiurethral) and DNP (direct nerve) evoked bladder activation dependent on the frequency of stimulation. Baseline bladder pressures ranged from 6 to 12 cmH_2O. [P = prostate, DNP = dorsal nerve of penis]

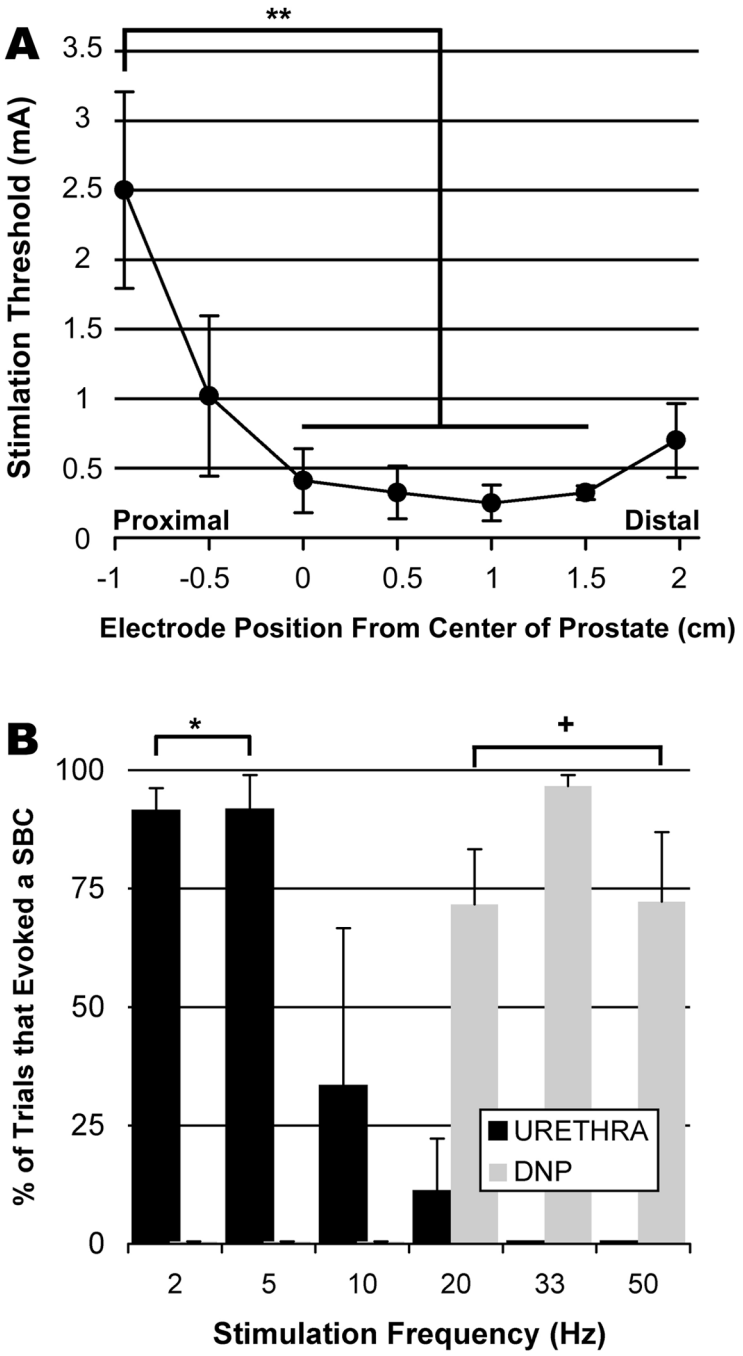

Figure 7.

Analysis of responses evoked by selective electrical stimulation of pudendal nerve branches. **(A)** Stimulation threshold to evoke reflex EAS EMGs (i.e., pudendo-anal reflex) in response to epiurethral stimulation of the dorsal (anterior) surface of the proximal urethra was consistent from the prostate (0 cm) to the distal part of the membranous urethra (1.5 cm). At 1 cm proximal to the prostate, the stimulation threshold increased significantly ($p^{**} < 0.005$). **(B)** The frequency tuning curves of the percentage of stimulation trials that evoked a sustained bladder contraction (SBC) for epiurethral (CSN) stimulation (1 cm distal to prostate, 2 – 5 Hz greater than 10 – 50 Hz, $p^{*} < 0.002$) and direct stimulation of the DNP (20 – 50 Hz greater than 2 – 10 Hz, $p^{+} < 0.001$).