Published in final edited form as:

Int Urogynecol J. 2017 July ; 28(7): 1049–1056. doi:10.1007/s00192-016-3223-1.

Impaired contraction and decreased detrusor innervation in a female rat model of pelvic neuropraxia

Johanna L. Hannan^{1,2}, Shelby A. Powers¹, Vinson M. Wang², Fabio Castiglione^{3,4}, Petter Hedlund^{3,5}, and Trinity J. Bivalacqua²

¹Department of Physiology, Brody School of Medicine, East Carolina University, 600 Moye Boulevard, Greenville, NC, 27834, USA

²The James Buchanan Brady Urological Institute, and Department of Urology, The Johns Hopkins School of Medicine, 600 North Wolfe Street, Marburg 420, Baltimore, MD, 21287, USA

³Unit of Urology, Division of Oncology, Urological Research Institute, IRCCS Ospedale San Raffaele, Milan, Italy

⁴Laboratory for Experimental Urology, Organ Systems, Department of Development and Regeneration, University of Leuven, Leuven, Belgium

⁵Department of Clinical and Experimental Pharmacology, Lund University, Lund, Sweden

Abstract

Introduction and hypothesis—Bilateral pelvic nerve injury (BPNI) is a model of post-radical hysterectomy neuropraxia, a common sequelae. This study assessed the time course of changes to detrusor autonomic innervation, smooth muscle (SM) content and cholinergic-mediated contraction post-BPNI.

Methods—Female Sprague-Dawley rats underwent BPNI or sham surgery and were evaluated 3, 7, 14 and 30 days post-BPNI (n=8/group). Electrical field stimulated (EFS) and carbachol-induced contractions were measured. Gene expression was assessed by qPCR for muscarinic receptor types

Corresponding Author: Johanna L. Hannan, PhD, hannanj14@ecu.edu, 600 Moye Blvd, Mail Stop 634, Greenville, North Carolina 27834-4354, USA, Phone: +1-252-744-2099, Fax: +1-252-744-3460.

Author Contributions:

JL Hannan: Project development, Data collection and analyses, Drafting article, Revision and final approval.

SA Powers: Data collection and analyses, Drafting article, Revision and final approval.

VM Wang: Data collection and analyses, Revision and final approval.

F Castiglione: Project development, Revision and final approval.

P Hedlund: Project development, Revision and final approval.

TJ Bivalacqua: Project development, Data analyses, Revision and final approval.

A portion of this work was previously presented as a poster presentation at the American Urology Association Annual Meeting in San Diego, CA in April 2013.

Ethical/IRB Approval: Human subjects were not used in this study. All animal experiments were performed ethically in accordance with Johns Hopkins University School of Medicine guidelines for animal care and use.

Financial disclaimers/conflict of interest

None.

Financial Disclaimers/Conflict of Interest Statement

The production of the manuscript was supported by the National Institutes of Health NIDDK K08 Career Development Award (TJB), Urology Care Foundation (JLH, TJB) and ECU startup funds (JLH). The funding sources have only supported the project financially and have not been involved in the design, production or handling of the intellectual content of the manuscript.

2 (M2) and 3 (M3), collagen type 1 α 1 and 3 α 1 and SM actin. Western blots measured M2 and M3 protein expression. Bladder sections were stained with Masson's trichrome for SM content and immunofluorescence staining for nerve terminals expressing vesicular acetylcholine transporter (VACHT), tyrosine hydroxylase (TH) and neuronal nitric oxide synthase (nNOS).

Results—BPNI caused larger bladders with less SM content and increased collagen type 1 α 1 and 3 α 1 gene expression. At early time points, cholinergic-mediated contraction increased, while EFS-mediated contraction decreased and returned to baseline by 30 days. Protein and gene expression of M3 was decreased 3 and 7 days post-BPNI while M2 was unchanged. TH nerve terminals surrounding the detrusor decreased in all BPNI groups while VACHT and nNOS terminals decreased 14 and 30 days post-BPNI.

Conclusions—BPNI increased bladder size, impaired contractility and decreased SM and autonomic innervation. Therapeutic strategies preventing nerve injury-mediated decline in neuronal input and SM content may prevent the development of a neurogenic bladder and improve quality of life after invasive pelvic surgery.

Keywords

radical hysterectomy; neuropraxia; neurogenic bladder; autonomic innervation; detrusor

Introduction

Hysterectomies are the most frequent non-obstetric surgical procedure performed on American women [1]. Indications include benign conditions such as endometriosis and uterine leiomyoma or malignancies like cervical cancer, the third most common female cancer worldwide [2]. Radical hysterectomy (RH), the most invasive form of hysterectomy, is recommended treatment for early-stage cervical cancer [3]. RH includes removal of the uterus, parametrium and upper vagina. Evaluations comparing women who have or have not undergone hysterectomy demonstrate an increased incidence of lower urinary tract dysfunction (LUTD) following the procedure [4–6]. Post-RH, women experience transient bladder dysfunction and 30–75% have long-term LUTD [7–9]. These sequelae have significant detrimental physical and psychological impacts on quality of life [10,11].

Peri-operative and long-term LUTD following hysterectomy and other invasive pelvic procedures can be caused by damage or disruption of autonomic nerves innervating pelvic viscera. Bladder compliance and storage is principally controlled through sympathetic nerves releasing norepinephrine (NE) onto β -3 adrenergic receptors of the bladder detrusor muscle resulting in bladder relaxation and onto α -1 adrenergic receptors of the bladder neck, resulting in smooth muscle contraction and blocked urine outflow [12]. Tyrosine hydroxylase (TH), a key enzyme catalyzing NE production, is a marker for adrenergic neurons. The parasympathetic postganglionic release of acetylcholine (ACh) onto bladder muscarinic receptors produces contraction through cholinergic muscarinic type 2 (M2) and type 3 (M3) receptors [13]. Vesicular acetylcholine transporter (VACHT) loads ACh into presynaptic vesicles for release and is a marker for cholinergic neurons. Although the main role of bladder parasympathetic innervation is contraction and micturition, postganglionic parasympathetic nerves can also contribute to bladder relaxation and filling through the

release of nitric oxide (NO) via nitrergic neurons and NO production by neuronal nitric oxide synthase (nNOS) [14].

Autonomic axons directing bladder responses branch from the pelvic plexus lateral to the rectum, vaginal wall, and urethra before they subdivide to supply the detrusor smooth muscle wall of the bladder [14]. Extensive dissection and wide tissue margins during RH increases the likelihood that these pelvic nerves may be stretched, compressed or transected. Nerve-sparing RH attempts to avoid the pelvic plexus and associated nerves. Differences in outcomes between nerve-sparing and conventional RH as measured through urodynamic studies, length of postoperative catheterization, and validated questionnaires support the important role of these nerves in bladder function following hysterectomy [15–17]. Although nerve-sparing RH is associated with better pelvic function, not all RH is nerve-sparing due to variations in tumor invasion, patient anatomy, and surgeon skill or preference. Nerve injury is a possible outcome of RH and changes in bladder innervation, function and smooth muscle morphology following injury require further characterization.

To simulate RH neuropraxia, a nerve clock-crush technique bilaterally injures the pelvic nerves supplying the vagina and bladder in female rats. This model has previously demonstrated impaired voiding, overflow incontinence and non-voiding contractions [18]. The present study builds on this work by using a modified nerve clock-crush to model RH injury and assesses bladders at several post-operative time points. Based on the data from the previous study, we hypothesize that bladders from these animals will have decreased autonomic innervation and less contraction to electrical field stimulation and muscarinic agonists [18]. In order to assess bladder function, detrusor contractile response to muscarinic agonist and EFS was completed. Using histological and molecular techniques, we examined detrusor smooth muscle area and bladder autonomic innervation (sympathetic, parasympathetic and nitrergic), as well as gene and protein expression of cholinergic receptors, alpha-smooth muscle actin (α SMA) and collagen.

Material and Methods

Experimental design

Female nonparous Sprague-Dawley rats (12 weeks; n=65) weighing 275–300g were housed 2 per cage with 12 hr light/dark cycle and free access to water and regular chow. All animal experiments were performed ethically in accordance with Johns Hopkins University School of Medicine guidelines for animal care and use. Animals were divided into 5 groups (n=13/group): Sham and 3 days, 7 days, 14 days and 30 days post-bilateral pelvic nerve injury (BPNI). Within each group, 8 animals were used for functional studies of bladder strips (distal half) and the remaining (remaining upper half) tissue was saved for Western blots analysis. The other 5 animals were used for histology (upper portion) and qPCR gene expression.

Bilateral pelvic nerve injury

BPNI was performed as previously described [18]. Briefly, under isoflurane anesthesia, a midline laparotomy was performed and the pelvic plexus was identified on the dorsolateral

wall of the vagina below where the ureters enter the bladder. BPNI was induced by applying a forceps for 3×15 seconds to the pelvic nerves and the vesicogenital branches of the pelvic plexus supplying the urinary bladder, outflow region, and vagina, 2–3 mm distal to the pelvic plexus. Animals undergoing sham surgery had the pelvic plexus identified without injury.

Functional studies of isolated tissue

Rats were euthanized at 3, 7, 14 and 30 days post-BPNI by isoflurane anesthesia and thoracotomy. Vaginal smears were assessed to determine each rat's estrus phase. Shams were used with the 14 day BPNI group which was the halfway point for all groups. Bladders were carefully removed, drained of urine and wet weight measured. The bladder was evenly divided sagittally and the upper portion was formalin fixed and paraffin embedded. The bottom portion was opened longitudinally and the trigone region removed. Strips (approximately 2 × 10mm) from the distal ventral and dorsal sides of the bladder body were mounted in the tissue bath (DMT 820M, Denmark) containing a physiological salt solution (in mM: 130 NaCl, 4.7 KCl, 14.9 NaHCO₃, 5.5 dextrose, 1.18 KH₂PO₄, 1.17 MgSO₄, 1.6 CaCl₂) aerated with 95% O₂ and 5% CO₂ (37°C, pH 7.4). The tissue was stretched to a resting tension of 2mN and equilibrated for 45 minutes. High concentration potassium chloride solution (KCl; 120mM) assessed detrusor contraction and viability. Cumulative concentration-response curves to carbachol (10⁻⁸M to 10⁻⁵M) and EFS evaluated cholinergic nerve-mediated contraction at 20V, 3-ms pulse width at increasing frequencies (0.5–32Hz) for 10 seconds at 2 minute intervals (Grass Instruments, Quincy, MA). Carbachol and EFS responses were recorded using Lab Chart 7 software and Powerlab acquisition hardware (ADInstruments, Colorado Springs, CO). Data were measured as percentage of the maximal response to KCl and the effective concentration for 50% of maximal response (EC₅₀) was calculated with GraphPad Prism 5.0. All compounds and drugs were obtained from Sigma Chemical Co. (St. Louis, MO).

Quantitative PCR (qPCR)

Real-time qPCR was used to determine relative expression of collagen, smooth muscle actin and muscarinic receptors in bladders from sham, 3, 7, 14, and 30 day BPNI rats. Frozen bladders were homogenized and total RNA purified using the RNeasy system (Qiagen, Hilden, Germany), quantified and then reverse transcribed. Real-time qPCR was performed using the StepOnePlus system (Applied Biosystems, Foster City, CA, USA). TaqMan gene expression assays for cholinergic muscarinic receptor type 2 (*chrm2*) and 3 (*chrm3*), collagen type 1 alpha 1 (*Col1a1*) and 3 alpha 1 (*Col3a1*), smooth muscle actin (*acta2*) and hypoxanthine phosphoribosyltransferase 1 (*hprt1*) were used (Applied Biosystems). HPRT1 was unchanged between groups and used as an endogenous control. All experiments were performed in triplicate (n=8/group).

Western Blot Analysis

Bladders from sham, 3, 7, 14 and 30 day BPNI rats were homogenized in cell lysis buffer (Cell Signaling, Beverly, MA, USA) and from the cytosolic fraction muscarinic receptors type 2 and 3 were isolated for Western blot analysis. Protein amounts were determined by the BCA kit (Pierce, Rockford, IL, USA), and equal amounts of protein (30 µg) were loaded

to 4–20% Tris-HCl gel (Bio-Rad, Hercules, CA, USA). Proteins were transferred to polyvinylidene fluoride membranes and incubated with primary antibodies (muscarinic acetylcholine receptor 2 (1:500, rabbit), muscarinic acetylcholine receptor 3 (1:500, rabbit) and glyceraldehyde-3-phosphate dehydrogenase (1:2000, mouse, GAPDH); Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C. The membranes were incubated with a horseradish peroxidase-linked secondary antibody and visualized using an enhanced chemiluminescence kit (Amersham Biosciences Corp., Piscataway, NJ, USA). The densitometry results were normalized by GAPDH expression and quantified by using Image J Software (NIH, Bethesda, MD, USA). All results were expressed relative to sham values.

Histology

Bladders fixed in 10% formalin and paraffin-embedded were cut into 6 µm sections and deparaffinized. Antigen retrieval was performed using a modified citrate buffer (Target Retrieval Solution; Dako, Carpinteria, CA, USA) and endogenous peroxidases were quenched (Dual Endogenous Enzyme Block; Dako, Carpinteria, CA, USA). Non-specific background staining was inhibited through a protein blocker (Protein Block, Serum Free; Dako, Carpinteria, CA, USA). Primary antibodies for alpha-smooth muscle actin (1:500; Rabbit; Abcam, Cambridge, MA, USA or Mouse; Sigma) and one of three markers of interest for nerve terminals TH (1:250; Rabbit; EMD Millipore, Billerica, MA, USA), nNOS or VACht (1:50; Mouse; 1:200; Rabbit; Santa Cruz, Dallas, TX) were incubated overnight at 4°C. Species appropriate fluorescing secondary antibodies (Alexa Fluor 488, Alexa Fluor 594; Invitrogen, Carlsbad, CA, USA) were applied and incubated for 60 minutes at room temperature. Slides were cover slipped with mounting medium containing 4,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA, USA). Fluorescent images were obtained of 4 separate areas within each bladder cross section using an Olympus IMT-2 fluorescent microscope and Spot digital camera system (Diagnostic Instruments, Sterling Heights, MI). Two persons, blinded to experimental groups, counted individual positive nerve terminals for the antibody of interest within the smooth muscle layer stained with alpha-smooth muscle actin in each 20× detrusor images. The number of positive nerve terminals from the 4 bladder images were averaged to reach the number of nerve terminals for each bladder.

Additional sections were stained using Masson's trichrome and photographed using Olympus microscope. Image J software was used to measure smooth muscle area. The smooth muscle area was quantified and presented as a ratio of smooth muscle area to total bladder surface area for each section.

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM) or standard deviation as appropriate. Groups were compared using one-way analysis of variance (ANOVA) and Dunnett's multiple comparison post hoc test. For western blots, we used a modified t-test to compare the experimental groups with the normalized control ratio. P values of less than 0.05 were used as criteria for statistical significance (Graph Pad Prism 5.0).

Results

Animals

There was no significant difference in body weight between groups (Table 1). Whole bladder weight was markedly increased in all rats following nerve injury (Table 1). A significant increase in the percent bladder to body weight ratio was evident at 3, 7, and 14 days post-BPNI ($p<0.05$, Table 1). Representative immunofluorescent images are provide in the electronic supplemental material section.

Functional studies of isolated bladder tissue

Detrusor contractions to increasing carbachol concentrations were significantly increased 3 and 7 days after injury ($p<0.05$, Fig. 1A) and the EC50 was decreased at 3, 7 and 30 days (Fig. 1A inset). When compared to sham, contraction to EFS was significantly decreased 7 days post-BPNI ($p<0.05$) with other time points showing a slight decrease in contraction (Fig. 1B).

Analysis of bladder smooth muscle and collagen content

Representative images illustrate the marked changes in bladder morphology following BPNI (Fig. 2A). Overall, the bladder detrusor layer appears to be disorganized with less smooth muscle and increased collagen deposition. Quantification of detrusor cross sectional smooth muscle area confirmed significantly reduced smooth muscle area post-BPNI across all groups ($p<0.05$, Fig. 2B). Gene expression of smooth muscle actin was significantly declined 3 days following injury ($p<0.05$, Fig. 2C). BPNI lead to an increase in the gene expression collagen type 1 α 1 ($p<0.05$, Fig. 2D) 7 days post-BPNI and collagen type 3 α 1 ($p<0.05$, Fig. 2E) expression showed a temporal increase at early time points with a significant peak at 7 days post-BPNI.

Protein and gene expression of muscarinic receptors

Western blot analyses showed a decrease in the quantity of cholinergic muscarinic type 2 receptors (CHMR2) across all time points following pelvic nerve injury ($p<0.05$, Fig. 3A). Cholinergic muscarinic receptor type 3 was 50% lower than control values at 3 days post-BPNI and gradually increased to higher values than controls by 30 days post-BPNI ($p<0.05$, Fig. 3B). Although not significant, the relative mRNA expression of CHRM2 was slightly elevated following BPNI (Fig. 3C). Gene expression of CHRM3 was lower at all time points following injury when compared to sham and was significant 3 and 30 days post-BPNI ($p<0.05$, Fig. 3D).

Nitrenergic, adrenergic and cholinergic innervations

All post-BPNI bladders showed significantly fewer TH nerve terminals around detrusor smooth muscle compared to sham bladders ($p<0.05$, Fig. 4A). VACHT positive nerves did not significantly change in the week following BPNI, but significantly decreased at later injured time points ($p<0.05$, Fig. 4B). Nerve terminals expressing nNOS declined in all BPNI bladders and were significantly lower 14 and 30 days post-injury ($p<0.05$, Fig. 4C).

Discussion

This study characterized bladder function, morphology and innervation following pelvic nerve injury in female rats. BPNI increased relative bladder weight and type I and III collagen gene expression. Detrusor smooth muscle area and smooth muscle actin gene expression were decreased following injury. These structural changes increased markers of fibrosis and decreased detrusor smooth muscle content. Functional data revealed greater contraction to cholinergic agonist and decreased contraction to EFS in the week following injury. Nerve terminals positive for nNOS and TH were decreased across all time points, while VACHT nerve terminals were slightly elevated at 3 days and decreased at later time points. These findings may explain the mix of impaired filling, storage and voiding function seen in patients following RH as nerves controlling bladder relaxation and contraction are affected [19]. One month post-BPNI, the bladder's contractile response to a muscarinic agonist or muscarinic neurotransmitter release had returned to sham levels. This response may be the result of a compensatory increase in M3 receptors and improved function of nerve terminal release in the remaining lower number of automatic nerve terminals present. Furthermore, the morphology did not recover leaving the bladder at an increased susceptibility to additional dysfunction with age.

Cholinergic contractile responses were increased in our early time points similar to those demonstrated in a BPNI 10 day group in a previous study [18]. Interestingly, the significant decrease in M3 receptor protein and gene expression following BPNI conflicts with the increased contractile response to carbachol at 3 and 7 days post-injury. Detrusor M3 receptors are considered the primary mediators of contraction in healthy bladders [20]. These findings may be explained by the lack of significant change in the gene and protein expression of M2 receptors. Normally M2 receptors enhance contraction by inhibiting relaxation, but in other rat models of neurogenic bladders and in human spinal cord injury the dominant contractile force switched from M3 to M2 receptor mediated contraction [21–23]. A comparable switch following BPNI may explain increased carbachol contractile response during periods of decreased M3 receptor expression. In contrast, a male rat model of cavernous nerve crush injury found increased M2 and no change in M3 protein expression at 1, 2 and 4 weeks post-crush [24]. Further studies are required to elucidate the role of M2 versus M3 receptor following BPNI. The role of other signaling pathways in the bladder should also be assessed in this RH model as changes to purinergic signaling or downstream pathways such as rho-kinase may also lead to increased contraction early post-injury [24,25]. Overall, it appears that the bladder is compensating for the decreased innervation caused by nerve injury by increasing its ability to contract to cholinergic agonist in order to maintain the ability to void.

The detrusor's response to EFS-mediated contraction paralleled previous findings, revealing decreased contraction to midrange and high frequencies at early time points following BPNI [18]. Contraction at later time points successively increased to closely approximate sham contraction by 30 days post-nerve crush. In male rats, decreased detrusor contraction to both low and high frequency EFS was evident 1, 2 and 4 weeks post-injury [24]. These findings suggest a decline in available bladder cholinergic nerve activation following BPNI which is also supported by the decrease in markers specific to cholinergic nerve terminals 7–30 days

following BPNI. The nerve terminal data parallel previous studies showing decreased VACHT expression in bladder wall and pelvic plexus 10 days post-injury [18]. Additionally, in male rats undergoing cavernous nerve injury VACHT gene expression was transient in the major pelvic ganglia as it initially decreased early (2, 7 days) and at late time points (30, 60 days) following injury [26]. In this study, at 14 and 30 days following injury there were also significant declines in nNOS and adrenergic (TH positive) nerve terminals. This decreased innervation related to relaxation and bladder filling may contribute to impaired bladder function through an imbalance of contractile and inhibitory inputs.

Following BPNI, the bladder to body weight ratio was increased at 3, 7 and 14 day time points. Additionally, an increase in gene expression of collagen type I and III were observed while smooth muscle content decreased. Changes towards a fibrotic bladder morphology are commonly observed in models of bladder dysfunction such as spinal cord injury, bladder outlet obstruction, or diabetes [27]. Importantly, by 30 days bladder contraction appears to return to sham levels even though decreased neuronal input, loss of smooth muscle content and increased bladder fibrosis persist. Similar changes in neuronal input are evident in the male model of cavernous nerve injury in which nNOS and VACHT gene expression are much lower than sham animals but erectile function is regained and the penis remains fibrotic [26]. We believe that the neuronal connections that remain intact after injury are fewer but much more functional and allow the end organ to operate normally. Although the bladder these compensations allow the bladder to function in the short-term, these neuronal and structural changes will predispose bladders to dysfunction with additional insults, injuries or aging. Of note, all animals assessed in the present study were young, hormonally intact females who may benefit from the neuroprotective effects of estrogen [28]. Examining the impact of this RH-induced neuropraxia model in ovariectomized and/or aged animals would likely lead to further impaired bladder function and increased fibrosis and smooth muscle loss [28].

There were several limitations to this study. Cystometry to compare in vivo bladder dysfunction to changes in contractile response in isolated tissue were not done, but would provide practical understanding of the effects of neuropraxia. The previous study modeling RH nerve injury demonstrated increased non-voiding contractions and overflow incontinence at 10 days post-BPNI [18]. The origin of bladder dysfunction may be more central than the peripheral nerves investigated in this study; however, changes in the control of bladder function above the pelvic plexus were not studied. The role of urothelial dysfunction was not addressed in this study, but future investigations into urothelial changes following nerve injury may provide additional understanding of LUTD. Another limitation to our study was that the contractile responses via M3 versus M2 receptors were not determined which merits further investigation. Furthermore, functional bladder relaxation to adrenergic and NANC components was not assessed. Lastly, the purinergic contribution to bladder contraction was not assessed in this study.

Conclusions

A rat model of neuropraxia resulted in detrusor smooth muscle with decreased innervation and resulting EFS contraction but increased cholinergic-mediated contraction, indicating the bladder was compensating to continue voiding in a denervated state. Nerve injury also

increased markers of fibrosis and decreased smooth muscle content leading to a stiff and less compliant bladder. These data demonstrate that 1–2 weeks following injury is a critical time in which functional deficits, alterations to tissue structure and innervation occur and these pathological changes can persist up to 30 days after the initial injury. These findings support the use of this rat model to investigate the mechanisms and therapies related to bladder dysfunction following invasive pelvic surgeries such as RH.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by supported by the National Institutes of Health NIDDK K08 Career Development Award (TJB), Urology Care Foundation (JLH, TJB) and ECU startup funds (JLH). The authors would like to thank Xiaopu Liu and Elena Pak for their assistance in data collection.

References

- DeFrances CJ, Lucas CA, Buie VC, et al. 2006 National hospital discharge survey. *Natl Health Stat Rep.* 2008; 5:1–20.
- Jemal A, Bray F, Center MM, et al. Global cancer statistics. *CA Cancer J Clin.* 2011; 61:69–90. [PubMed: 21296855]
- Bansal N, Herzog TJ, Shaw RE, et al. Primary therapy for early-stage cervical cancer: radical hysterectomy vs radiation. *Am J Obstet Gynecol.* 2009; 201(5):485.e1–9. [PubMed: 19879394]
- Altman D, Granath F, Cnattingius S, et al. Hysterectomy and risk of stress-urinary-incontinence surgery: nationwide cohort study. *Lancet.* 2007; 370:1494–9. [PubMed: 17964350]
- Kudish BI, Shveiky D, Gutman RE, et al. Hysterectomy and urinary incontinence in postmenopausal women. *Int Urogynecol J.* 2014; 25:1523–1531. [PubMed: 24964761]
- Brown JS, Sawaya G, Thom DH, et al. Hysterectomy and urinary incontinence: a systemic review. *Lancet.* 2000; 356:535–9. [PubMed: 10950229]
- Scotti RJ, Bergman A, Bhatia NN, et al. Urodynamic changes in urethrovesical function after radical hysterectomy. *Obstet Gynecol.* 1986; 68:111–120. [PubMed: 3725241]
- Zullo MA, Mancini N, Angioli R, et al. Vesical dysfunctions after radical hysterectomy for cervical cancer: a critical review. *Crit Rev Oncol Hematol.* 2003; 48:287–293. [PubMed: 14693341]
- Benedetti-Panici P, Zullo MA, Plotti F, et al. Long-term bladder function in patients with locally advanced cervical carcinoma treated with neoadjuvant chemotherapy and type 3–4 radical hysterectomy. *Cancer.* 2004; 100:2110–7. [PubMed: 15139052]
- Sinclair AJ, Ramsay IN. Review: the psychosocial impact of urinary incontinence in women. *Obstet Gynecol.* 2011; 13:143–8.
- Leanza V, Passanisi A, Leanza G. Urinary incontinence: quality of life and psychological aspects. *Urogynaecologia International Journal.* 2013; 27:6–8.
- Fowler CJ, Griffiths D, de Groat WC. The neural control of micturition. *Nat Rev Neurosci.* 2008; 9(6):453–466. [PubMed: 18490916]
- Hirose H, Aoki I, Kimura T, et al. The subtypes of muscarinic receptors for neurogenic bladder contraction in rats. *Eur J Pharmacol.* 2002; 452:245–253. [PubMed: 12354576]
- Andersson KE, Arner A. Urinary bladder contraction and relaxation: physiology and pathophysiology. *Physiol Rev.* 2004; 84:935–986. [PubMed: 15269341]
- Aoun F, van Velthoven R. Lower urinary tract dysfunction after nerve-sparing radical hysterectomy. *Int Urogynecol J.* 2015; 26(7):947–957. [PubMed: 25432634]
- Kanao H, Fujiwara K, Ebisawa K, et al. Various types of total laparoscopic nerve-sparing radical hysterectomies and their effects on bladder function. *Gynecol Oncol.* 2014; 25(3):198–205.

17. Kim HS, Kim TH, Suh DH, et al. Success factors of laproscopic nerve-sparing radical hysterectomy for preserving bladder function in patients with cervical cancer: a protocol-based prospective cohort study. *Ann Surg Oncol*. 2015; 22:1987–1995. [PubMed: 25465377]
18. Castiglione F, Bergamini A, Bettiga A, et al. Perioperative betamethasone treatment reduces signs of bladder dysfunction in a rat model for neurapraxia in female urogenital surgery. *Euro Urol*. 2012; 62(6):1076–1085.
19. O'Callaghan D, Dwyer PL. Clinical and urodynamic follow-up of women with established urinary dysfunction after radical hysterectomy. *Aust NZ J Obstet Gynaecol*. 1994; 34:557–561.
20. Andersson KE, Arner A. Urinary bladder contraction and relaxation: physiology and pathophysiology. *Physiol Rev*. 2004; 84:935–984. [PubMed: 15269341]
21. Nakamura T, Kimura J, Yamaguchi O. Muscarinic M2 receptors inhibit Ca^{2+} -activated K^{+} channels in rat bladder smooth muscle. *Int J Urol*. 2008; 9:689–696.
22. Hedge SS, Choppin A, Bonhaus D, et al. Functional role of M2 and M3 muscarinic receptors in the urinary bladder of rats in vitro and in vivo. *Br J Pharmacol*. 1997; 120:1409–1418. [PubMed: 9113359]
23. Pontari MA, Braverman AS, Ruggieri MR. The M_2 muscarinic receptor mediates in vitro bladder contractions from patients with neurogenic bladder dysfunction. *Am J Physiol*. 2004; 286(5):R874–R880.
24. Kim SJ, Lee DS, Bae WJ, et al. Functional and molecular changes of the bladder in rats with crushing injury of nerve bundles from major pelvic ganglion to the bladder: role of rhoA/rho kinase pathway. *Int J Mol Sci*. 2013; 14:17511–17524. [PubMed: 23985824]
25. Burnstock G. Purinergic signaling in the urinary tract in health and disease. *Purinergic Signal*. 2014; 10:103–155. [PubMed: 24265069]
26. Hannan JL, Matsui H, Sopko NA, Liu X, Weyne E, Albersen M, et al. Caspase-3 dependent nitroergic neuronal apoptosis following cavernous nerve injury is mediated via RhoA and ROCK activation in major pelvic ganglion. *Sci Rep*. 2016; 6:29416. [PubMed: 27388816]
27. Aitken KJ, Bagli DJ. The bladder extracellular matrix. Part I: architecture, development and disease. *Nat Rev Urol*. 2009; 6:596–611. [PubMed: 19890339]
28. Lleul P, Palea S, Barras M, Grndadam F, Heudes D, Bruneval P, et al. Functional and morphological modifications of the urinary bladder in aging female rats. *Am J Physiol Regul Integr Comp Physiol*. 2000; 278:r964–R972. [PubMed: 10749785]

Figure 1.

Functional assessment of cholinergic mediated and electrical field mediated contraction of bladder tissue from sham and bilateral pelvic nerve injured (BPNI) female rats. (a) Concentration response curves to carbachol (10^{-8} – 3×10^{-5} M) and the inset graph shows an increase in contraction and decrease in half maximal effective concentration (EC₅₀) of the bladder to carbachol at 3, 7 and 30 days post BPNI. (b) Electrical field stimulated contractions (1–32 Hz) were decreased in 7 day BPNI bladders. Values normalized to maximum contraction to high potassium chloride (KCl) and presented as mean $\pm$ standard error of the mean (n=5–8 animals/group). *p<0.05 vs sham.

Figure 2.

Analysis of bladder smooth muscle and collagen content following bilateral pelvic nerve injury (BPNI). (a) Representative bladder cross sections stained with Masson's trichrome. (b) Smooth muscle area of the bladder was quantified with Image J as a percentage of whole bladder cross sectional area. The gene expression of whole bladder (c) smooth muscle actin was lower in 3 day BPNI rats and relative expression of collagen type 1 α 1 and 3 α 1 was increased 7 days post BPNI. Values are presented as mean $\pm$ standard error of the mean (n=5 animals/group). *p<0.05 vs sham.

Figure 3.

Western blot and gene expression analyses of cholinergic muscarinic receptors types 2 (M2) and 3 (M3) in sham and bilateral pelvic nerve injury (BPNI) bladders (a–d). For quantification, densitometric values of sham bladders were set to 100% and values of BPNI rats were expressed as a percent of sham rats. Representative blots are shown and values are normalized to GAPDH and presented as mean $\pm$ standard error of the mean (n=5 animals/group). *p<0.05 vs sham.

Figure 4.

Time course of the change in detrusor nerve endings following bilateral pelvic nerve injury (BPNI). The number of sympathetic (TH, a), parasympathetic (VACHT, b) and nitregeric (nNOS, c) neurons were counted by 2 blinded observers for 4 different sections of the bladder. Each bar represents mean $\pm$ SEM (n=3–5/group). *p<0.05 vs sham.

Table 1

Body and bladder weights of rats following bilateral pelvic nerve injury.

	Body weight (g)	Bladder weight (mg)	Bladder to Body weight (%)
Sham	300 ± 33.3	93.9 ± 12.64	0.031 ± 0.0027
BPNI 3 days	294 ± 19.4	125.7 ± 26.07 *	0.043 ± 0.0013 *
BPNI 7 days	301 ± 14.9	105.4 ± 11.11	0.041 ± 0.0013 *
BPNI 14 days	280 ± 11.1	118.9 ± 26.43	0.042 ± 0.0023 *
BPNI 30 days	335 ± 11.1	124.7 ± 26.17	0.037 ± 0.0034

*
p<0.05 vs Sham.

BPNI: bilateral pelvic nerve injury.