Published in final edited form as:

Urology. 2014 August ; 84(2): 490.e1–490.e6. doi:10.1016/j.urology.2014.04.024.

Assessment of bladder sensation in mice with a novel device

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Abstract

Objective—To develop and test the efficacy of an implantable bladder electrode device that can be used with the Neurometer® electrodiagnostic stimulator to assess fiber-specific afferent bladder sensation in the mouse.

Methods—We constructed a ball-tipped platinum electrode and surgically implanted it into the mouse bladder. The Neurometer® was connected to the electrode to apply selective nerve fiber stimuli (250 Hz for Aδ fibers and 5 Hz for C fibers) of increasing intensities to the bladder mucosa in the mouse to determine bladder sensory threshold (BST) values. Using 58 female C57BL/6J mice, we measured the temporal and interobserver consistency of BST measurements, the effects of intravesical administration of lidocaine and resiniferatoxin on the BST, and the effects of our device on voiding behavior and bladder mucosal integrity.

Results—BST values at 250 and 5 Hz did not vary significantly when measured 2, 4, and 6 days after device implantation, or when obtained by two blinded, independent observers. Intravesical lidocaine yielded a transient increase in BST values at both 250 Hz and 5 Hz, whereas resiniferatoxin yielded a significant increase only at the 5 Hz stimulus frequency after 24 hours. Moderately increased micturition frequency and decreased volume per void were observed 4 and 6 days after device implantation. Histology revealed mild inflammatory changes in the area of the bladder adjacent to the implanted BST device.

Conclusion—Assessment of neuroselective bladder sensation in mice is feasible with our device, which provides reproducible BST values for autonomic bladder afferent nerve fibers.

Keywords

Bladder Sensory Threshold; Afferent Nerve; Electrode; Resiniferatoxin; Lidocaine

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FINANCIAL CONFLICT OF INTEREST

None

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INTRODUCTION

Afferent impulses from the lower urinary tract contribute to a network of reflexes that mediate urinary continence and micturition.¹ The pathogenesises of several diseases of the bladder such as interstitial cystitis, bladder outlet obstruction, and diabetic cystopathy have been attributed to afferent neuropathy in the lower urinary tract.²⁻⁵ Despite a strong body of literature supporting the involvement of altered autonomic sensitivity in these bladder conditions, it remains difficult to assess afferent bladder sensation objectively.

Afferent bladder innervation consists of small myelinated A δ and unmyelinated C fibers.⁶ In the micturition reflex, mainly A δ fibers convey bladder wall tension and volume.⁶ In neuropathological conditions, selective injury to a specific subtype of afferent fibers leads to bladder dysfunction.^{2,7} Neuroselective stimulations of A δ and C fibers have been achieved by sinusoidal wave electrical stimulation provided by a commercially available neurodiagnostic device (Neurometer®, Neurotron Inc., Baltimore, MD). This transcutaneous/transmucosal stimulator delivers sine wave stimuli of output intensities from 0.01 to 9.99 milliamperes at frequencies of 2000, 250, and 5 Hz, which have been reported to selectively stimulate large myelinated A β fibers, A δ fibers, and C fibers, respectively,^{8,9} although some evidence suggests a more complex pattern of neuroselectivity.^{10,11} This easy to conduct electrodiagnostic procedure has been used to determine current perception thresholds at various cutaneous and mucosal sites in humans,^{12,13} including in the bladder,^{14,15} and in rats^{9,10} and mice.^{16,17} Studies of the pathogenesis of bladder dysfunction in small rodent disease models can benefit greatly from the development of bladder electrodes suitable for use with the Neurometer® in those animals. We first reported a novel implantable bladder device that can be used with the Neurometer® to assess autonomic afferent bladder sensation in the rat,¹⁸ and have designated the current perception threshold in the bladder, detected by a light startle response, as the bladder sensory threshold (BST). Another group subsequently performed similar measurements with the Neurometer® electrodes attached to a balloon catheter inserted into the rat bladder.³ In light of the widespread use of transgenic mouse models in translational research to elucidate the pathogenesis of disease, development of an electrode for the Neurometer® suitable for implantation into the much smaller mouse bladder would also be valuable. Here we report the development of such a device and the results of testing the efficacy of the device combined with the Neurometer® for the assessment of bladder sensation in mice.

MATERIALS AND METHODS

Animals and Experimental Design

Fifty-eight female C57BL/6J mice 14 to 16 weeks of age and weighing 25 to 30 gm were used in this study. The animals were maintained with free access to laboratory chow and tap water in an animal facility with a 12:12-hour light/dark cycle. Mice with electrodes implanted in the bladder were used to assess: 1) the consistency of BST measurements with time (2, 4, and 6 days after implantation, n = 8 per day) and between different readers 4 days after implantation, 2) the impact of lidocaine (n = 8) or resiniferatoxin instillation (n = 8) on bladder BST values 4 days after implantation, 3) the effects of the device on urinary behavior 4 and 6 days after implantation (n = 6 per day) in comparison to 4 days after sham

implantation (n = 6), and 4) histology of the bladder in the region surrounding the implanted electrode. All protocols were preapproved by the Institutional Animal Care and Use Committee of Case Western Reserve University.

BST Electrode Device

We designed and constructed a prototype implantable bladder electrode device for use in mice (Figure 1). The electrode consists of a platinum wire (0.005 inch diameter, custom built by Hoover & Strong, N. Chesterfield, Virginia) with a platinum ball attached to the tip. The electrode is inserted into a PolyE polyethylene tubing catheter with a flared tip (Poly E-140, OD: 0.031 inch, Harvard Apparatus, Holliston, MA) via the side-port, with the platinum ball protruding from the catheter opening. Detailed The procedure for construction of the device is as follows: A 30 G ½ inch syringe needle is used to cut a small hole with a flap in the catheter, and then the platinum wire is inserted into the hole using forceps and fed through the catheter until it extends about 1 inch beyond the catheter tip. Under microscopic observation, a laser (PulsePoint Studio Plus 100 Laser Welder, Item Number: 710006, Italy) is used to melt the tip of the wire until it forms a ball (0.030–0.035 inch). The wire is retracted until the ball is 0.02 inch from the flared tip of the catheter, and then medical grade Loctite 4013 (Henkel Corp, Düsseldorf, Germany) is used to seal the hole where the wire was inserted, in order to prevent leakage. Saline solution (0.9%) can be injected through the catheter. For sensory testing, the platinum wire is connected to a battery operated Neurometer® (Neurotron, Baltimore, MD) capable of generating quartz crystal-calibrated stimuli of constant alternating current with intensities from 0.001 and 9.99 mA, and at sinusoid waveform frequencies of 5, 250, and 2000 Hz for selective stimulation of small unmyelinated C, small myelinated A δ , and large myelinated A β fibers, respectively. Since A β fibers have not been detected innervating the bladder in previous neuroanatomical and neurofunctional studies, we did not use the 2000 Hz stimulation frequency in our study.

BST Device Implantation

Implantation of the device in the bladder was performed with the mice under general anesthesia with 8–10 mg/kg ketamine/0.1 mg/kg xylazine. A small lower abdominal incision was created and the bladder was exposed. A purse-string stitch was placed around the bladder dome wall using 6-zero chromic gut. A small incision was made through the bladder wall in the middle of the purse, through which the device tip was placed in the bladder lumen. The purse string suture was tightened around the catheter and the flared tip was secured. The catheter was tunneled subcutaneously and externalized at the back of the neck. The distal end of the tubing was sealed, and the incisions were closed. Cephazolin (10 mg/kg, i.m.) was administered after surgery to prevent infection.

BST Measurement

Two, four, or six days after BST electrode device implantation each mouse was placed in a restraint device, where it remained conscious during the entire procedure. The tail of the mouse was cleaned with an alcohol pad, and a 2 × 2-inch SDE44 skin patch dispersion electrode (Neurotron, Baltimore, MD) was wrapped around the whole tail. Both the implanted BST electrode and the skin patch electrode were connected to the Neurometer. The bladder was emptied by withdrawing the urine through the catheter of the BST device

using a 1 ml syringe, and then 0.1 ml of 0.9% saline was injected into the bladder through the catheter to facilitate conduction of the electrical current from the electrode tip to the bladder mucosa by diffusion. Sine wave stimuli of 250 and 5 Hz were then applied to the bladder mucosa at increasing intensity increments of 0.01 mA. Each stimulus was applied for 1 second, followed by 30 seconds off before the next stimulus, until a light startle response (sudden movement of partial or whole body, with or without vocalization) was observed. The minimum intensity at which that response was observed was defined as the BST.

Interobserver Reproducibility of BST Measurement

To test the reproducibility of bladder sensory threshold measurements, BST values were determined in 8 mice, 4 days after implantation of a bladder electrode, by two different investigators. The two investigators determined BST values in the same 8 mice using the technique described above, and each investigator was blinded to the results of the other.

Intravesical Instillation of Lidocaine and Resiniferatoxin

In this set of experiments, BST values were obtained 4 days after device implantation, followed by intravesical instillation of 0.1 ml of lidocaine (4%, $n = 8$), a general anesthetic, or resiniferatoxin (1 μM $n = 8$), a highly potent analog of capsaicin that transiently activates and then desensitizes capsaicin-sensitive C fibers,^{19,20} for 30 minutes. For drug instillation, mice were anesthetized with 2% inhaled isoflurane and the drug solutions were injected transurethrally using a syringe and a 22 gauge angiocatheter. BST measurements were obtained 1 and 24 hours following instillation in fully conscious mice. The investigator obtaining BST values was blinded to the type of drug instilled.

24 hour Micturition Measurements

Mice were randomized to undergo implantation of the BST device or a sham operation (abdominal incision without bladder manipulation) ($n = 8$ per group). Four and six days after surgery, each mouse was placed in a metabolic cage (Med Associates Inc. St. Albans, VT) and given one hour to adapt before measuring 24-hour voiding frequency and volume per void in a 12-h light, 12-h dark cycle, as we described previously.²¹ Beginning 24 hours before the experiment until the end of measurements, dry food for the mice was replaced with a residue-free diet of Lactaid® lactose-free whole milk. This strategy substantially reduces the frequency and weight of feces generated during testing and thereby prevents aberrations of data analysis.²² Data including the number of voids and volume per void were collected at a sampling speed of 10 times/sec (Origin 7.5, OriginLab Corporation, Northampton, MA).

Histology

To assess the effect of BST device implantation on the condition of the bladder, on the 2nd, 4th, and 6th days after implantation, 2 hours after determination of BST values, mice were euthanized in a carbon dioxide chamber and the region of the bladder surrounding the implanted electrode was excised. The specimens were fixed in formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). The stained bladder

sections were examined under a light microscope, and representative images were photographed with a digital camera mounted on the microscope.

Statistics

Comparisons of BST measurements at different time points and before or after injection of lidocaine or resiniferatoxin, and of voiding parameters in device-implanted vs. sham implantation groups were performed by one-way ANOVA followed by multiple pair-wise post hoc comparisons, using Prism 4 (GraphPad Software, Inc., La Jolla, CA). The BST measurements by two investigators in the same 8 mice were compared using paired Student's *t* tests. *P* values <0.05 were considered to indicate statistical significance.

RESULTS

Time-Dependent and Interobserver Variability in BST Measurement

BST values did not differ significantly when obtained 2, 4, or 6 days after electrode device implantation, either at the 5 Hz or the 250 Hz frequency (Figure 2). BST values obtained in the same 8 mice, 4 days after electrode implantation, by two investigators blinded to each other's results were consistent (Supplemental Table).

Effects of Intravesical Lidocaine or Resiniferatoxin

BST values at both 5 and 250 Hz were significantly higher 1 hour after instillation of lidocaine, returning to pre-instillation levels after 24 hours (Figure 3A). Instillation of resiniferatoxin led to a significant increase in BST values at the 5-Hz, but not 250-Hz, stimulation frequency, and only at 24 hours after instillation (Figure 3B).

24-hour Micturition Measurements

The BST device had no significant effect on total 24-hour urine output 4 or 6 days after implantation (29.04 ± 1.3 and 26.39 ± 2.1 ml, respectively) compared with 4 days after sham implantation (27.7 ± 1.9 ml) (Figure 4). However, mean voiding frequencies were significantly higher and mean volumes per void were significantly lower in device implanted mice at 4 and 6 days compared with sham implantation mice at 4 days.

Histology

Review of the bladder specimens revealed evidence of mild inflammatory changes in the area surrounding the implanted device 2 and 4 days after implantation. These changes appeared to decrease 6 days after implantation. The mucosal layer appeared well preserved and it was without significant pathological change (Supplemental Figure).

DISCUSSION

The Neurometer® has been used clinically to measure abnormalities in peripheral somatic sensory perception in several neurological disorders, such as diabetic neuropathy and carpal tunnel syndrome.^{8,23,24} Following an initial report demonstrating differential stimulation of C, A δ , and A β fibers by the Neurometer® applied to the rat plantar surface,⁹ others have used cutaneous application of the device to assess neuropathy in rat models of neuropathic

pain²⁵ and diabetes,²⁶ and in a mouse model of central post-stroke pain.¹⁷ The Neurometer® has also been used recently to assess autonomic sensory function of urethral and bladder mucosa in humans.^{3,14,27,28} In a small early study, Ukimura et al found decreased current perception thresholds in patients with detrusor hyperreflexia and increased values in patients with underactive neurogenic bladders compared with healthy controls.¹⁴

The ability to use the Neurometer® to study bladder sensory function in small rodent models of lower urinary tract disorders should greatly enhance our ability to understand the mechanisms of bladder dysfunction in those conditions, as well as enable monitoring of the effects of potential treatments for bladder sensory dysfunction. We first developed a novel electrode device implantable in the rat bladder, which we used with the Neurometer® to assess fiber specific afferent bladder sensation in rats.^{18,29} Later, Hayashi et al used the Neurometer® with electrodes attached to a balloon catheter and inserted into the rat bladder to quantify increased bladder afferent sensation in hydrochloric acid-induced cystitis.³

In the current study, we used platinum wire to design an electrode device small enough to be implanted in the mouse bladder. Injection of 0.9% saline into the bladder through the catheter immerses the platinum electrode and ball tip in the solution, facilitating completion of the conduction of electrical stimulation from the Neurometer® to the bladder mucosa by diffusion. The consistency and reproducibility of BST values at both frequencies, when obtained 2, 4, and 6 days after device implantation and by two observers 4 days after implantation, support the dependability of our device and technique.

The ability to diagnose dysfunction in specific subsets of bladder afferent fibers provides a particular advantage for uncovering mechanisms of neurogenic bladder and guiding therapeutic intervention. Bladder afferent innervation consists mainly of small myelinated A δ fibers, which convey mechanosensory signals in response to bladder distension,³⁰ and small unmyelinated C fibers, which respond mainly to noxious stimuli and are minimally responsive to mechanosensation.²⁰ In mice with our BST device implanted in the bladder, intravesical instillation of resiniferatoxin, which transiently activates and then after several hours causes desensitization of C fibers,³¹ resulted in a significant increase in the BST generated by 5 Hz Neurometer® stimulation 24 hours after instillation, but not by 250 Hz stimulation at either 1 hour or 24 hours (Figure 3B). That result, consistent with a previous report in humans² and our report on the BST device in rats,¹⁸ provides support for the selectivity of 5 Hz Neurometer® stimulation with the mouse BST device for C afferent fibers in the bladder.

Other studies with the Neurometer® in rodents have led to challenges to the reported neuroselectivity of the 2000, 250, and 5 Hz frequencies for large myelinated A β fibers, A δ fibers, and C fibers, respectively. Koga et al¹⁰ used intracellular recordings from dorsal root ganglion neurons to show that 2000 Hz Neurometer® stimulation generated action potentials only in A β fibers, whereas action potentials were generated in A β and A δ fibers by 250 Hz. Given the paucity of A β fibers innervating the bladder, those results suggest that 250 Hz Neurometer® stimulation of the bladder specifically activates A δ fibers. Koga et al¹⁰ also found that all three classes of afferent neuron were activated by 5 Hz Neurometer® stimulation, however, they concluded that the action potentials generated in

A β and A δ fibers by 5 Hz stimulation were likely of insufficient frequency to induce functional sensation. The same conclusions were reached by another group using computer simulations based on models of nerve fiber excitation.³² In addition, we recently showed by c-Fos immunostaining that Neurometer® stimulation of the bladder at 5 and 250 Hz induced c-Fos expression in regions of the L6 segment of the spinal cord in which there is a predominance of C and A δ fibers, respectively.²⁹ Thus, based on the results of others and our own published and current results, we are confident that Neurometer® stimulations of the bladder at 250 and 5 Hz are selective for A δ and C fibers, respectively.

We also observed the effects of the implanted BST electrode device on bladder function, as measured by 24-hour micturition behavior. The increased voiding frequencies and decreased mean voiding volumes we observed in BST device-implanted mice compared with sham implantation mice, despite similar total 24-hour urine outputs, may have reflected a reduction in bladder capacity due to the implantation surgery. Implantation of the BST device requires making an incision in the dome of the bladder and tightening the incision with a suture, which may reduce the bladder capacity. In addition, implantation-related mild inflammatory changes in the bladder may have contributed to the changes in 24-hour micturition behavior.

CONCLUSIONS

In the current urologic practice, clinicians still rely on patients' self-reported bladder sensation during the filling phase of urodynamic studies. Therefore, a diagnostic tool to objectively measure bladder sensory dysfunction would allow clinicians to elucidate the degree of neuropathy and to evade numerous measurement biases. Our mouse BST measurement device can be used with the Neurometer® electrodiagnostic stimulator for the assessment of afferent function of the bladder in mice. These results warrant future studies to explore the potential use of this device in the investigation of changes of bladder sensory nerve fibers in mouse models of pathological conditions such as acute and interstitial cystitis, bladder outlet obstruction, overactive bladder, and spinal cord injury, as well as in testing potential treatments for bladder sensory dysfunction.

Histology of bladder sections from a sham implantation mouse (A) and from representative BST device-implanted mice at 2 days (B), 4 days (C), or 6 days (D) after implantation. The arrows indicate the sites of device implantation. H & E staining, magnification 40 $\times$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Grant: This study was supported by a Case Coulter Translational Research Partnership (CCTRP) Award (to GL) and NIH/NIDDK grant R01 DK083733 (to FD)

We thank C. Thomas Powell, PhD for his medical editorial assistance. Steven Torontali, Engineer, Case Western Reserve University School of Medicine, established the methodology for fabricating the electrodes.

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Figure 1.

Design of the mouse implantable bladder sensory threshold (BST) measurement device. A, photograph and B, diagram of the BST device (units, inches). C, detailed diagram of the BST device: (a) platinum ball, (b) connection of platinum ball to platinum wire, (c) flared tip of the (d) PolyE polyethylene tubing, (e) exit of the platinum wire sealed with medical grade Loctite 4013 adhesive, (f) platinum wire, (g) the end of the PolyE polyethylene tubing.

Figure 2.

BST values at 250 and 5 Hz stimulation frequencies measured 2, 4, or 6 days after implantation of the BST device (n = 8 mice per time point). Results are expressed as mean $\pm$ SE.

Figure 3.

BST values at 250 and 5 Hz before, and 1 and 24 hours following intravesical administration of 4% lidocaine (A, * $p < 0.001$ vs. pre-lidocaine) or 1 μ M resiniferatoxin (B, * $p = 0.01347$ vs pre-resiniferatoxin). Results are expressed as mean + SE.

Figure 4. 24-hour urination frequency (A) and mean void volume (B) 4 and 6 days after BST device implantation (n = 6 mice per time point) compared with 4 days after sham implantation (n = 6). Results are expressed as mean + SE. A, * $p < 0.0001$ and § $p = 0.0016$ vs. sham. B, * $p = 0.0021$ and § $p = 0.018$ vs. sham.