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Time course of neuroanatomical and functional recovery after bilateral pudendal nerve injury in female rats

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Abstract

The pudendal nerve innervates the external urethral sphincter (EUS) and is among the tissues injured during childbirth, which may lead to symptoms of stress urinary incontinence (SUI). To understand the mechanisms of injury and repair, urethral leak-point pressure (LPP) was measured 4 days, 2 wk, or 6 wk after bilateral pudendal nerve crush. Morphometric changes in the distal nerve and EUS were examined by light and electron microscopy. To determine whether recovery resulted from pudendal neuroregeneration, LPP was measured before and after pudendal nerve transection 2 wk after nerve crush. LPP was significantly decreased 4 days after pudendal nerve crush compared with sham-injured animals as well as 2 or 6 wk after nerve crush. LPP was not significantly different 2 or 6 wk after nerve crush compared with sham-injured animals, suggesting that urethral function had returned to normal. Four days after pudendal nerve crush, the EUS branch of the pudendal nerve distal to the injury site showed evidence of nerve degeneration and the EUS appeared disrupted. Two weeks after nerve crush, the distal nerve and EUS both showed evidence of both nerve degeneration and recovery. Two weeks after nerve crush, LPP was significantly decreased after nerve transection. Six weeks after nerve injury, evidence of neuroregeneration was observed in the pudendal nerve and the EUS. This study has demonstrated that functional recovery and neuroregeneration are significant 2 wk after nerve crush, although by anatomical assessment, recovery appears incomplete, suggesting that 2 wk represents an early time point of initial neuroregeneration.

Keywords

leak point pressure; urinary incontinence; voiding; pudendal nerve; urethra; Sprague-Dawley

STRESS URINARY INCONTINENCE (SUI) is the involuntary leakage of urine during moments of increased intra-abdominal pressure in the absence of detrusor contraction (1). It occurs when the intravesical pressure exceeds urethral resistance, such as when laughing, sneezing, or coughing. SUI is a significant medical problem affecting ~25 million Americans. It affects 40-50% of women over 55 years old (47) and has been shown to be a major concern among

geriatric women (17,48,61). Although SUI is not a life-threatening condition, its negative effects on quality of life and economic costs to society are substantial (22).

In women, vaginal delivery, parity, menopause, and pelvic surgery contribute to this common condition (62). Specifically, the development of SUI in women is strongly correlated with vaginal delivery of children, which can injure the nerves, muscles, and connective tissue responsible for maintaining continence (47). The pudendal nerve innervates the external urethral sphincter (EUS) and is among the tissues that may be injured during vaginal delivery (13,55,60). Because the EUS normally responds to increases in intra-abdominal pressure by contracting to maintain continence, denervation can lead to the leakage of urine and symptoms of SUI (4,60).

Pudendal nerve block has been shown to lead to decreased urethral resistance to leak (18), and pudendal nerve injury has been demonstrated in many women with SUI (60). Rat neuromuscular anatomy is similar to human in that female rats have both smooth and striated muscular components to the urethra (19,30,64) and the striated muscle component, the EUS, is innervated by the pudendal nerve (44). Pudendal nerve crush induces symptoms of SUI in female rats, likely due to urethral dysfunction, including decreased urethral resistance to leakage (3,15). The purpose of this study was to determine the time course and extent of anatomical and functional recovery after bilateral pudendal nerve crush in female rats. Urethral resistance to leakage was measured, and morphometric changes indicative of pudendal nerve regeneration were examined by light and electron microscopy 4 days, 2 wk, or 6 wk after bilateral pudendal nerve crush.

MATERIALS AND METHODS

Pudendal nerve crush

Fifty-nine female Sprague-Dawley rats (190-210 g body wt) were anesthetized intraperitoneally with a mixture of ketamine (100 mg/kg) and xylazine (15 mg/kg). This study was approved by the Institutional Animal Care and Use Committee of the Louis Stokes VA Medical Center. To minimize anatomical and functional variations due to prior vaginal deliveries (28), virgin rats were used, although estrus cycle was not standardized. Forty-eight of the rats then underwent bilateral pudendal nerve crush as previously described (15,28). In brief, the ischiorectal fossa was exposed by using a muscle-separating technique and the neurovascular sheath containing the pudendal nerve was located. All three branches of the pudendal nerve were crushed twice bilaterally for 30 s with a Castroviejo needle holder, just proximal to the branch point of the obturator nerve. The nerve injury was visualized immediately afterward by a marked transparency of the nerve sheath at the crush site. Muscle and skin were closed separately, and buprenorphine (0.1 ml/100 g body wt) was given subcutaneously as an analgesic. The rats were used in functional and anatomical studies 4 days (10 rats), 2 wk (25 rats), or 6 wk (13 rats) after injury. Eleven rats were used as sham controls and underwent identical anesthesia and skin and muscle incisions, but the ischiorectal fossa was not opened because even sham surgery can injure the pudendal nerve (28). Sham-injured rats were studied 2 wk after sham injury.

Bladder-catheter implantation

Two days before measurement of urethral-resistance to leakage via leak-point pressure (LPP) measurement, all rats underwent suprapubic bladder-catheter implantation, as previously described (15). In brief, the rats were anesthetized as described in *Pudendal nerve crush* and a longitudinal abdominal incision was made. A circular purse-string suture (4-0 silk) was placed on the bladder wall. A small incision was made in the bladder wall in the center of the purse string, and the catheter (polyethylene-50 tubing with a flared tip) was

implanted. The purse string was tightened around the catheter, and the catheter was tunneled subcutaneously to the neck, where it exited the skin. Buprenorphine was given as an analgesic as described in *Pudendal nerve crush*, and the catheter was plugged until it was used for LPP testing 2 days after catheter implantation.

Urethral-resistance LPP testing

Four days, two weeks, and six weeks after pudendal nerve crush, urethral-resistance LPP testing was performed as previously described (11,12,15). To determine if recovery was a result of pudendal nerve regeneration, LPP testing was performed both before and after bilateral pudendal nerve transection in six of the rats that received pudendal nerve crush 2 wk prior. These rats were not used for morphometric studies. Urethral-resistance LPP testing was performed 2 wk after sham surgery in the control group. Although no anesthetic agent maintains neuronal reflexes identical to those of a conscious state (67), urethane (1.2 g/kg ip) was selected for these studies because movement is highly disruptive to the measurements and urethane is among the best for maintaining micturition reflexes (11,34,36).

The bladder catheter was then connected via a stopcock to both a pressure transducer (model P300; Grass Instruments, West Warwick, RI) and a flow pump (model 100; KD Scientific, Holliston, MA). The transducer was connected to an amplifier, a polygraph (model MT 9500, Astro-Med, West Warwick, RI), and a computer that digitized pressure data at 10 samples/s. The rat was placed supine and underwent a 30-min accommodation period of filling (5 ml/h) and voiding. The bladder was palpated to empty and was filled with saline to 0.3 ml, approximately half of the bladder capacity of a 200 g rat (11). While bladder pressure was recorded and digitized, gentle manual pressure was applied externally over the abdomen to simulate a Crede maneuver. Pressure was slowly increased until the rat leaked saline through the urethra. At the first indication of leakage, the externally applied pressure was rapidly removed. By definition, SUI occurs in the absence of bladder contractions (65); therefore, if a bladder contraction occurred during urethral-resistance LPP measurement, those data were not included. The bladder was drained and refilled, and the test was repeated. Because of the slow nature of bladder compression, not all tests triggered an associated bladder contraction. Thus we were able to obtain an LPP measurement in the absence of bladder contraction in all animals. The bladder was drained and refilled, and the test was repeated three times in each rat.

The increase in bladder pressure due to the externally applied abdominal pressure for each trial was calculated as baseline bladder pressure subtracted from peak bladder pressure at leakage (LPP). The mean LPP value was calculated for each rat and was used to calculate mean and standard error for each experimental group.

Urethral and pudendal nerve histology

Immediately after urethral-resistance testing, the animals underwent intracardiac perfusion fixation as previously described (28). In brief, each rat was perfused with warm phosphate-buffered and heparinized saline (100 ml), followed by cold, dilute aldehyde fixative (400 ml of 2.5% cold glutaraldehyde, 0.5% paraformaldehyde in 0.1 M cacodylate buffer) and a cold concentrated fixative (400 ml of 2.5% cold glutaraldehyde, 0.5% paraformaldehyde in 0.1 M cacodylate buffer). The pudendal nerve was dissected free, osmicated (2% osmium tetroxide), and prepared for embedding with the sectioning face 5 mm distal to the nerve-crush site. Similarly, the urethra was then dissected free, and a portion containing the EUS, ~6 mm from the trigone, was selected for study and prepared identically to the nerve. At this location, the neurovascular tissues are at their maximal development (30). All tissues were postfixed, stained with aqueous 2% osmium tetroxide and 1% uranyl acetate dehydrated in

graded alcohol rinses, and embedded in epoxy resin. They were then transversely sectioned (1 μm) on an ultramicrotome and were stained with methylene blue-azure II to enable the identification of striated muscle, nerve fascicles, and myelinated axons under high-magnification light microscopy.

At the level of the crush injury, the rat pudendal nerve has three main branches: a sensory branch and a motor trunk that divides into two smaller motor branches, one that innervates the EUS (EUS branch) and one that innervates the external anal sphincter (26,37,38). The EUS branch of the pudendal nerve was identified in cross-section distal to the crush site, and the number of myelinated axons and endoneurial nuclei in it were counted. Counts were performed three times by a blinded investigator, and a mean value was calculated for each rat. Pudendal nerve morphometry studies were not performed in animals 4 days after nerve crush because nerve regeneration had not yet begun at that time. These specimens were analyzed qualitatively.

Morphometry of nerve-fascicle regeneration in the urethra was based on a method previously described (15,25). In brief, a single cross-section of each urethra ~ 6 mm from the trigone was selected for study and nerve fascicles near the EUS (< 20 μm) were examined for signs of neurodegeneration and regeneration as follows. Signs of nerve degeneration after injury used to categorize nerve fascicles as neurodegenerated included the visualization of irregular myelin sheaths, vacuoles, macrophages, myelin figures (indicative of myelin breakdown), and flocculent endoneurial material (15,25). Signs of nerve regeneration used to categorize nerve fascicles as regenerating included large unmyelinated axons (> 1 μm) and small myelinated axons within thin myelin sheaths, distorted shape of the axon and surrounding myelin sheath, and endoneurial nuclei (25). Normal axons were oval in shape with a smooth contour and an even, nondisrupted myelin sheath surrounding them, with no vacuoles present. As with the pudendal nerve, counts of normal, degenerating, and regenerating nerve fascicles were performed three times by a blinded investigator and a mean value was calculated for each rat.

To confirm the observations made with light microscopy ($\times 100$ magnification), selected regions of the EUS motor branch of the pudendal nerve were trimmed for ultrathin sectioning and qualitative analysis under electron microscopy. These sections were stained in lead citrate and uranyl acetate before examination with a 100CX transmission electron microscope (JEOL, Tokyo, Japan). Electron micrographs were analyzed qualitatively for more subtle signs of nerve degeneration and regeneration, including axonal profiles at both nodes of Ranvier and clefts of Schmidt-Lanterman.

Data analysis

Data are presented as a means $\pm$ SE for each experimental group. Statistical comparisons were made between the experimental groups with a one-way ANOVA on ranks followed by a Dunn's test. Statistical comparisons of LPP before and after pudendal nerve transection were made with a paired *t*-test. In all statistical tests, $P < 0.05$ was considered statistically significant. Electron-microscopy data was analyzed qualitatively.

RESULTS

Functional results

LPP values demonstrated the characteristic shape observed previously (15), showing a slow rise to peak and a rapid decrease in bladder pressure once the externally applied abdominal pressure was removed. LPP was significantly decreased 4 days after pudendal nerve crush (20.3 ± 2.1 cmH_2O) compared with sham-injured animals (38.3 ± 3.2 cmH_2O) or animals tested 2 wk (32.0 ± 2.1 cmH_2O) or 6 wk (31.4 ± 2.6 cmH_2O) after nerve crush (Fig. 1).

There were no significant differences in LPP between animals tested 2 or 6 wk after nerve crush and sham-injured animals, suggesting that urethral function had returned nearly to normal values by 2 wk after injury. To determine whether the functional recovery 2 wk after nerve crush was due to pudendal nerve regeneration, six rats underwent LPP testing before (29.6 ± 3.2 cmH₂O) and after (24.6 ± 3.5 cmH₂O) bilateral pudendal nerve transection, causing a statistically significant drop in LPP. This suggests that at least part of the functional recovery 2 wk after pudendal nerve crush results from pudendal nerve regeneration.

Structural results: pudendal nerve

In sham-injured animals, the EUS branch of the unoperated pudendal nerve appeared as a single fascicle bound by a perineurium with many myelinated and unmyelinated axons (Fig. 2A). Four days after pudendal nerve crush, the EUS branch of the pudendal nerve distal to the injury showed early evidence of nerve degeneration, including many distorted axons, myelin figures, and no normal myelinated axons, indicating that the crush injury was successful (Fig. 2B). Two weeks after nerve crush, the distal pudendal nerve showed many small myelinated axons (62 ± 5) but significantly fewer than in the sham-injured group (81 ± 2), suggesting that nerve regeneration was in progress. The number of endoneurial nuclei was also significantly increased (29 ± 4) compared with the sham-injured group (6 ± 1), further indicating nerve regeneration (Fig. 2, C and E). Six weeks after nerve crush, the regenerated axons appeared more mature (Fig. 2D). The number of myelinated axons (75 ± 6) was not significantly different from the number in the sham-injured group, indicating that by this time nerve regeneration had substantially progressed to near baseline levels. Compared with 2 wk after nerve crush, the number of endoneurial nuclei was also decreased 6 wk after nerve crush (14 ± 2), indicating that nerve regeneration was slowing. However, the number of endoneurial nuclei 6 wk after nerve crush was still significantly higher than values in sham-injured animals (Fig. 2E), suggesting that there remained some residual regenerative activity.

Structural results: electron microscopy

Electron-microscopic analysis confirmed the light-microscopic results and revealed ultrastructural details not as visible at the light-microscopic level, particularly 4 days after nerve injury. The normal unoperated EUS branch of the pudendal nerve in sham-injured rats showed primarily mature axons with thick myelin sheaths. The endoneurial nuclei were few in number and included fibroblasts and Schwann cells, with a multilayered perineurium forming the nerve fascicle (Fig. 3A). Four days after nerve crush, examination of the expanded endoneurium revealed many nucleated cells, including Schwann cells, fibroblasts, and macrophages associated with myelin figures. Axon profiles of various sizes were ensheathed by Schwann cell cytoplasm, and some were associated with growth cones (Fig. 3B). Two weeks after nerve crush, the EUS branch had regenerated many small myelinated and nonmyelinated axons, as well as showing an abundance of endoneurial nuclei, indicating nerve regeneration (Fig. 3C). By 6 wk after nerve crush, the EUS nerve fascicle had a mature appearance. There were some axonal profiles at the level of the node of Ranvier and others at the clefts of Schmidt-Lanterman, indicating nerve stability (Fig. 3D).

Structural results: urethra

The smooth muscle of the urethra is located in the submucosal layer surrounded by an extensive venous plexus, which was inflated by the perfusion process (Fig. 4a). The striated muscle of the EUS appeared as a band of dark striated fibers one to six fibers wide, compact and continuous around the urethral lumen. Nerve fascicles near the EUS in sham-injured rats containing healthy myelinated axons were identified in the region adjacent to the EUS (Table 1). Nerve fascicles in sham-injured rats had myelinated axons that were round with a

smooth contour and an undisrupted myelin sheath surrounding them. There were no irregular myelin sheaths, vacuoles, macrophages, myelin figures, endoneurial nuclei, or any other evidence of neurodegeneration in nerve fascicles of sham-injured animals (Fig. 4b, Table 1).

Four days after nerve crush, the EUS appeared disrupted (Fig. 4c). The total number of nerve fascicles and the number that appeared histologically normal were both reduced compared with sham-injured animals, although only the number of normal fascicles was significantly decreased (Table 1). Four days after injury, there were also significantly fewer normal-appearing smooth myelinated axons compared with sham-injured animals and a significantly increased percentage of nerve fascicles showing evidence of degeneration, including irregular myelin sheaths, vacuoles, macrophages, myelin figures, and flocculent endoneurial material (Table 1).

Two weeks after nerve crush, the EUS showed persistent evidence of nerve disruption (Fig. 4d). The total number of nerve fascicles, the number of normal-appearing fascicles, and the number of normal-appearing myelinated axons were between those observed in sham-injured animals and 4 days after injury and were not statistically different from either group (Table 1). The percentage of both normal-appearing and degenerated nerve fascicles, on the other hand, was significantly decreased compared with sham-injured animals. The trend suggests that although injury is present, recovery has begun. An intermediate morphology was also evident with respect to the reformation of neuromuscular junctions (Fig. 4d). Although only rarely seen, this field in the ventrolateral EUS has muscle fibers either with (Fig. 4e) or without (Fig. 4f) the presynaptic bouton.

Six weeks after nerve crush, the EUS showed histological evidence of neuroregeneration (Fig. 4g). There were no nerve fascicles near the EUS with evidence of degeneration, yet the percentage of normal-appearing nerve fascicles was significantly decreased compared with sham-injured animals. Like-wise, the number of normal-appearing nerve fascicles was reduced compared with 2 wk after nerve crush. This was because most of the nerve fascicles and most of the myelinated axons near the EUS showed evidence of regeneration, making this the time point studied with the best histological evidence of neuroregeneration (Fig. 4g, Table 1).

DISCUSSION

The pathophysiology of SUI in humans is still being explored. Although vaginal delivery, pelvic surgery, and hormonal changes have been implicated in both human and animal studies (2,16,45,60), it is apparent that a combination of factors is the cause. The pudendal nerve is among the tissues injured during vaginal childbirth, particularly with a prolonged second stage of labor (13,21,55). This may be the result of pressure-induced ischemic injury, avulsion, stretch, or crush of nerves, muscles, or other pelvic-floor support structures. Pudendal nerve injury can lead to EUS denervation, resulting in urethral dysfunction and symptoms of SUI (13,55,60). Women who become incontinent after vaginal childbirth have been shown to have more pudendal nerve damage than those who are continent (60), and multiparity leads to repeated injury and greater urethral dysfunction with each vaginal birth (56).

EUS biopsies from women with SUI show significantly less striated muscle and more connective tissue than those from continent women (19). Accordingly, EUS and pelvic floor electromyograms (EMG) in these women demonstrate evidence of pudendal nerve injury and regeneration (19,53,57,59). Finally, women with SUI show characteristic alterations in

urethral pressure, including a decrease in urethral closure pressure, indicative of reduced striated musculature activity leading to the symptoms of SUI (8).

Outcomes after peripheral nerve injuries are determined by the degree and location of the lesion, amount of connectivetissue disruption, vascular injuries, and the age and health of the patient (20). Following axotomy, there is an initial degeneration of myelin sheaths and distal axoplasm, accompanied by sealing of the proximal stump of the axon (32). Forty-eight to ninety-six hours after injury, the surrounding Schwann cells are invaded by phagocytic macrophages and Wallerian degeneration occurs (32). The distal fragmentation of crushed nerves in mice appears within a short time frame, 40-44 h following injury, and spreads retrogradely (6). The regenerating axons, or growth cones, then emerge from the proximal stump and elongate toward the distal stump within the Schwann cell tube (32). This process begins within hours of injury but may not traverse the gap between the nerve stumps until 4 wk after injury, depending on the extent of the injury (20). Daughter Schwann cells then remyelinate the regrowing axon, with the new myelin sheaths being thinner than uninjured ones (32).

Animal models have been used to study the pathophysiology of SUI and to investigate the roles of pregnancy, birth trauma, pudendal nerve damage, and estrogen on the development of this condition (3,15,25,28,44,50). The effect of pudendal nerve crush in rats on urinary function has been demonstrated through its effects on voiding behavior (28,50), sneeze testing (24,33,51), vertical tilt-table test (31), maximum urethral closure pressure (46), and LPP testing (5,11,12,15,46,51). The results of these studies have shown that symptoms of SUI develop after either bilateral (15,44) or unilateral (44) pudendal nerve injury or simulated birth injury using vaginal distension (15) and that pudendal nerve injury occurs during vaginal distension (15,33).

Although not required by ICS definition, LPP is clinically useful in the evaluation of SUI in that it corresponds with the severity of incontinence symptoms and serves as a quantitative indicator of the level of urethral dysfunction (52,65). LPP measures both smooth and striated muscle function as well as urethral mucosal closure and has been shown to be decreased in both humans and animal models with SUI (14,52). Although not universally accepted, LPP has been found to be 78% sensitive and 100% specific for SUI (58). It provides a baseline value of urethral function to improve diagnostic accuracy and the outcome of therapy and is generally accepted as an accurate surrogate for SUI in animal models.

Prior studies have given somewhat conflicting data regarding the length of voiding dysfunction after bilateral pudendal nerve injury. Voiding behavior patterns return to normal 2 wk after injury (50), whereas LPP data still show a significant deficit 2 wk after injury (3). In contrast, urethral resistance significantly decreased in our study 4 days after nerve crush but returned to near normal values after 2 wk and remained at normal values 6 wk after injury. Some studies evaluating functional outcomes after sciatic nerve crush demonstrated full recovery by 20 days (66), whereas others show that function does not return to normal until 32 days after injury; however, intermittent time points were not evaluated (39). Although the exact number of days with a functional deficit is variable, the overall range of functional impairment seems to be ~14-35 days (27,29). This discrepancy in recovery times is likely due to differences in the respective regeneration distances for the two models. Therefore, 2 wk after injury represents an early time point for initial functional recovery after pudendal nerve crush. This recovery stems at least in part from pudendal neuroregeneration, as demonstrated by our studies with animals tested before and after nerve transection.

Although there was no further improvement in voiding function from 2 wk to 6 wk after nerve crush, the number and percentage of degenerating fascicles decreased while regenerating ones increased, suggesting that the neuroregenerative process was still developing at 2 wk and was nearing completion by 6 wk. These results are consistent with prior studies investigating the course of events after pudendal nerve injury. Pan et. al. (42) recently showed that after 10 days, rats with a 1-h vaginal distention return to normal LPP levels, whereas rats that had undergone a 4-h vaginal distention have impaired LPP at the same time point. As in our study, at time points where there was complete functional recovery, there was not yet histological return to normal (42). Similarly, our prior studies (28) show that rat voiding behavior returns to normal 3 mo after pudendal nerve injury, although only half the motor neurons had fully regenerated at that time. It may be that, as the EUS is repairing from 2 to 6 wk after nerve crush, further regeneration adds redundancy but does not increase LPP. Regardless, the subsequent histological maturation that is observed seems to be primarily structural.

Multiple studies have shown that pudendal nerve injury leads to impaired EUS function (15,28,50). However, recent studies (44) using EUS-EMG have shown that there is still ~10% residual EUS function after bilateral pudendal nerve transection. This is consistent with our findings, which showed that 4 days after bilateral pudendal nerve crush, although a majority of nerve fascicles showed evidence of damage, rats are still able to maintain some level of urethral resistance to urinary flow. In addition, immediately after bilateral pudendal nerve transection, LPP was significantly decreased but was not equal to zero. This suggests that redundant mechanisms for maintaining continence exist, including contributions from urethral smooth muscle. Some of the EUS fibers may be innervated by extrapudendal nerve fibers, such as by motor nerves that innervate surrounding periurethral striated muscles and have branches that can contribute to the EUS (23,40,41). Alternatively, these fibers may represent autonomic innervation to the smooth muscle of the urethra (15,23,25,28,40,64), as supported by the finding that reflex increases in intraurethral pressure are not completely eliminated by pudendal nerve transection (23). There is evidence to suggest that in partial nerve injury, some denervated muscle fibers are reinnervated by terminal sprouts from surviving axons (9). The possibility of an altered innervation pattern is supported by altered EUS-EMG neural-bursting patterns after pudendal nerve transection (44).

Other nerves have been implicated in the development of SUI. Transection of the sensory component of the pelvic splanchnic nerve in the rat has been shown to interfere with parturition and to lead to bladder distention (10). Additionally, the expression of *c-fos*, a reactive proto-oncogene that appears in spinal neurons hours after nerve injury, has been shown to be upregulated in the dorsal horn neurons after vaginal distention (33), suggesting the involvement of sensory fibers and nociception in these processes. These results suggest that there are other nerves involved in continence in addition to the pudendal nerve.

One result of our study was the wide range in the number of nerve fascicles and myelinated axons. The total number of nerve fascicles ranged from 10 to 57, and the number of myelinated axons was 0 to 153. Although this is a large variation, similar results have been found in other studies evaluating human urethral biopsies (43). Nerve density has been found to correlate with the number of muscle cells as well as patient age (43) and is therefore a function of the individual being studied. Because the rats in this study were age matched at the time of injury, age cannot account for the observed variation. In most experimental studies involving nerve crush, the time course for the appearance of degeneration and regeneration with respect to functional and histological outcome measures are usually found to be in synchrony or only mildly staggered (7). However, the correlations are often weak and outcome measures may reflect disparate events (35).

Although rats are a useful model in which to conduct voiding studies, there are some mismatches compared with humans. SUI develops under a multifactorial influence, including endocrine and neural factors (3), which cannot be perfectly recreated in the rat model. Therefore, we did not synchronize the estrus cycle of the rats before pudendal nerve crush, which may have contributed to variability in results. Similarly, the infant head-to-birth canal ratio of the human, which is thought to be the cause of the traumatic injuries leading to the development of SUI, is not recreated in any other mammal (49). There are also neural differences between rat and human voiding in that the rat EUS contracts while voiding to promote urethral evacuation (63). Partly for this reason, we always measure LPP in the absence of voiding contraction. Finally, because rats are quadrupeds, the direction of the fetal compression injury is expected to be different from the force that a fetus exerts on a bipedal woman (51). However, valuable information regarding the role of pregnancy, birth trauma, pudendal nerve damage, and estrogen on the development of SUI has been obtained from rat models (3,15,25,28,44,50). Therefore, although rats are not a perfect model for human voiding, they are a good choice for preclinical studies, and when properly characterized, this animal model may be useful for preclinical testing of new treatments for urethral dysfunction and SUI (12,33,46,51).

Although SUI has been shown to be multifactorial in origin, there are currently no therapies aimed at treating its neurogenic causes. Because the symptoms of SUI do not appear until years after the initial insult, there is the potential for nerve rehabilitation to promote prevention. Similarly, the timing of pudendal nerve injury during vaginal delivery and at surgery is well known, and treatments aimed at preventing this injury or facilitating nerve repair or regeneration could be useful in preventing the development of SUI. In symptomatic cases, pudendal nerve damage can be diagnosed (54,56), but no treatments are currently available to address the problem once it has been recognized. Future studies should be aimed at clarifying the functional, anatomical, and molecular events occurring at the urethra in response to pudendal nerve injury, as well as identifying the mechanisms involved in functional recovery.

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Fig. 1. Urethral function after bilateral pudendal nerve crush as measured by leak-point pressure (LPP). Sham-injured animals are represented by the symbol at time 0. Symbols and error bars represent means $\pm$ SE of 10 to 19 animals per point. *Statistically significant difference compared with all other groups ($P < 0.05$).

Fig. 2.

Light microscopy of external urethral sphincter (EUS) branch of pudendal nerve from a sham-injured rat (A), a rat 4 days after nerve crush (B), a rat 2 wk after nerve crush (C), and a rat 6 wk after nerve crush (D) stained with methylene blue-azure II. MF, myelin figure; MA, myelinated axon; N, endoneurial nuclei. Bar = 10 μm. E: number of myelinated axons and endoneurial nuclei in sham-injured controls (placed at *time 0*) and 2 and 6 wk after bilateral pudendal nerve crush. Each symbol represents data from 6 animals and is presented as a mean ± SE. *Significant difference with respect to sham-injured group ($P < 0.05$).

Fig. 3.

Electron microscopy of EUS branch of the pudendal nerve distal to pudendal nerve-crush site from a sham-injured rat (A), a rat 4 days after nerve crush showing endoneurium expanded (B), a rat 2 wk after nerve crush (C), and a rat 6 wk after nerve crush (D). Sections were stained with lead citrate and uranyl acetate. F, fibroblasts; SC, Schwann cells; M, macrophages; BV, blood vessel; V, vacuole; P, multilayered perineurium; arrow, node of Ranvier; star, clefts of Schmidt-Lanterman. Bar = 2 μ m.

Fig. 4.

Light microscopy of urethra showing examples of normal, degenerated, and regenerated nerve fascicles. *a*: Low-power view of normal urethra shows dorsal position of the vagina (V) and a complete ring of striated muscle fibers in EUS (open arrow). Submucosal venous plexus is evident. *b*: In sham-injured control group, nerve fascicles (N) containing normal axons are abundant in vicinity of EUS. *c*: In 4-day group, nerve fascicles (N) typically contain myelin debris, more nuclei, and flocculent material, whereas EUS appears disrupted. *d*: In 2-wk group, an occasional nerve fascicle (solid arrow) approaches a muscle fiber, where a portion of neuromuscular junction (boxed area; see *e*) appears normal, whereas an adjacent fiber (open arrow; see *f*) has no presynaptic bouton and a ragged margin at

postjunctional folds. *g*: In 6-wk group, typical appearance of nerve fascicles is suggestive of neuroregeneration. Submucosa has smooth muscle (SM) surrounded by collagen and venous plexus. Semithin plastic sections were stained with methylene blue-azure II.

Table 1

Morphometric analysis of nerve fascicles near external urethral sphincter distal to site of crush injury

	Sham		4 Days NC		2 Wk NC		6 Wk NC	
	n		n	%Total	n	%Total	n	%Total
Total fascicles	34.2±6.0		20.8±4.0		26.8±5.4		22.5±3.9	
Normal-appearing fascicles	34.2±6.0		4.4±1.7*	21±8*	9.3±4.3	29±8*	3.0±0.7*	13±3*
Degenerating fascicles	0		16.4±3.2	79±8*	17.5±3.2	71±8*	0	
Regenerating fascicles	0		0		0		19.5±3.5	87±3
Normal myelinated axons	64.2±24.8		7.6±2.8*		20.5±11.7		10.7±3.9	
Regenerating myelinated axons	0		0		0		32.5±5.9	

Data are presented as means ± SE of 4-6 animals in each group. NC, nerve crush.

* Significant difference compared with corresponding sham-injured group ($P < 0.05$).