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Nerve transfer for restoration of lower motor neuron–lesioned bladder and urethra function: establishment of a canine model and interim pilot study results

Ekta Tiwari, PhD^{1,*}, Danielle M. Salvadeo, BS², Alan S. Braverman, PhD², Nagat A. Frara, PhD², Lucas Hobson, CVT², Geneva Cruz, BS², Justin M. Brown, MD³, Michael Mazzei, MD⁴, Michel A. Pontari, MD⁵, Amanda R. White, PhD², Mary F. Barbe, PhD², Michael R. Ruggieri Sr., PhD^{1,2,6}

¹Department of Computer and Electrical Engineering, College of Engineering, Temple University, Philadelphia, Pennsylvania

²Department of Anatomy and Cell Biology, Lewis Katz School of Medicine, Temple University, Philadelphia, Pennsylvania

³Department of Neurosurgery, Neurosurgery Paralysis Center, Massachusetts General Hospital, Boston, Massachusetts

⁴Department of Medicine, and Temple University, Philadelphia, Pennsylvania

⁵Department of Urology, Temple University, Philadelphia, Pennsylvania;

⁶The Shriners Hospital of Philadelphia, Pennsylvania

Abstract

Correspondence: Michael R. Ruggieri Sr.: Lewis Katz School of Medicine at Temple University, Philadelphia, PA. michael.ruggieri@temple.edu.

Author Contributions

Conception and design: Ruggieri, Tiwari, Salvadeo, Braverman, Brown, Barbe. Acquisition of data: Ruggieri, Tiwari, Frara, Cruz, Barbe. Analysis and interpretation of data: Ruggieri, Tiwari, Salvadeo, Braverman, Barbe. Drafting the article: Ruggieri, Tiwari, Salvadeo, Braverman, Barbe. Critically revising the article: Ruggieri, Tiwari, Salvadeo, Barbe. Reviewed submitted version of manuscript: Ruggieri, Tiwari, Salvadeo, Braverman, Frara, Brown, Pontari, Barbe. Approved the final version of the manuscript on behalf of all authors: Ruggieri. Statistical analysis: Ruggieri, Tiwari, Salvadeo, Barbe. Administrative/technical/ material support: Ruggieri, Tiwari, Salvadeo, Frara, Barbe. Study supervision: Ruggieri, Tiwari, Salvadeo, Braverman, Barbe. Help in experiments, and animal postoperation care: Hobson. Surgery: Mazzei, White.

*E.T. and D.M.S. contributed equally to this work.

Previous Presentations

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Disclosures

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Supplemental Information

Online-Only Content

Supplemental material is available with the online version of the article.

Supplemental Table 1. <https://thejns.org/doi/suppl/10.3171/2019.8.SPINE19265>.

OBJECTIVE—Previous patient surveys have shown that patients with spinal cord or cauda equina injuries prioritize recovery of bladder function. The authors sought to determine if nerve transfer after long-term decentralization restores bladder and sphincter function in canines.

METHODS—Twenty-four female canines were included in this study. Transection of sacral roots and hypogastric nerves (S Dec) was performed in 6 animals, and 7 animals underwent this procedure with additional transection of the L7 dorsal roots (L7d+S Dec). Twelve months later, 3 L7d+S Dec animals underwent obturator-to-pelvic nerve and sciatic-to-pudendal nerve transfers (L7d+S Dec+Reinn). Eleven animals served as controls. Squat-and-void behaviors were tracked before and after decentralization, after reinnervation, and following awake bladder-filling procedures. Bladders were cystoscopically injected with Fluoro-Gold 3 weeks before euthanasia. Immediately before euthanasia, transferred nerves were stimulated to evaluate motor function. Dorsal root ganglia were assessed for retrogradely labeled neurons.

RESULTS—Transection of only sacral roots failed to reduce squat-and-void postures; L7 dorsal root transection was necessary for significant reduction. Three L7d+S Dec animals showing loss of squat-and-void postures post-decentralization were chosen for reinnervation and recovered these postures 4–6 months after reinnervation. Each showed obturator nerve stimulation–induced bladder contractions and sciatic nerve stimulation–induced anal sphincter contractions immediately prior to euthanasia. One showed sciatic nerve stimulation–induced external urethral sphincter contractions and voluntarily voided twice following nonanesthetized bladder filling. Reinnervation was confirmed by increased labeled cells in L2 and the L4–6 dorsal root ganglia (source of obturator nerve in canines) of L7d+S Dec+Reinn animals, compared with controls.

CONCLUSIONS—New neuronal pathways created by nerve transfer can restore bladder sensation and motor function in lower motor neuron–lesioned canines even 12 months after decentralization.

Keywords

bladder reinnervation; voiding behavior; awake urodynamic; Fluoro-Gold; retrograde dye; spinal cord injury

IN canines, most sensory, motor, and autonomic axons innervating the lower urinary tract originate from the thoracolumbar (T10–L2) and sacral (S2–4) regions of the spinal cord and travel via the hypogastric, pelvic, and pudendal nerves to the bladder and external urethral sphincter (EUS).³ Urine storage and voiding are controlled by coordination of these neural pathways.⁶

Spinal cord injuries, cauda equina injuries, and other neurological disorders can disrupt the neurocircuitry that controls lower urinary tract function, resulting in neuro-genic bladder.⁷ Such disruptions can lead to dysfunction that can present as urinary retention, incontinence, and detrusor spasticity, which can diminish one's quality of life and self-esteem and limit social contacts.¹⁰ Restoration of bladder function is consistently identified as a top recovery priority for people with spinal cord injury^{1,19} and therefore is an emphasis of investigation.

We performed an extensive review that discussed the diverse studies focused on different nerve transfer strategies to restore lower urinary tract functions.⁹ We previously showed the

ability to restore motor function of the bladder in canines with sacral root transection after surgical transfer of genitofemoral or femoral nerves to the anterior vesicle branch of the pelvic nerve.⁸ Both procedures resulted in increased detrusor pressure when electrical stimulation was applied proximal to the site of coaptation in 21 of 28 animals.⁸ A pilot study showed evidence of new sensory pathways based on neuronal tracing results and observation of squat-and-void postures in the reinnervated animals.¹⁴ However, in these animals, decentralization was achieved by bilateral transection of the S1–3 roots alone. No attempt was made to eliminate bladder innervation from hypogastric nerves or L7 dorsal root–mediated sensory innervation of the bladder, as the latter was only recently identified.³ Therefore, it was not clear whether sensation was transmitted through sensory fibers in the newly reinnervated pathway, via fibers hitchhiking on hypogastric nerves, or through remaining L7 dorsal root afferents. Furthermore, behavioral analysis of squat-and-void postures is needed to confirm if sensation of bladder fullness can be restored via nerve transfer.

This study aimed to determine if decentralization using more extensive spinal root transection strategies can eliminate bladder function, and then, if surgical reinnervation after a year of decentralization results in a return of bladder sensation and voluntary voiding in canines. This was addressed by regular behavioral observation of squat-and-void postures and measurement and characterization of voided volume (percentage squat-and-void volume or percentage leaked volume) following awake bladder filling. Immediately prior to euthanasia, functional electrical stimulation of transferred nerves was performed to evaluate motor function. Sensory innervation was assessed using retrograde neurotracing techniques.

Methods

All animal studies were approved by the Institutional Animal Care and Use Committee and were compliant with the National Institutes of Health, United States Department of Agriculture, and American Association for Assessment of Laboratory Animal Care guidelines. Twenty-four female mixed-breed hounds, 6–8 months of age, weighing 20–25 kg, from Covance Research Products Inc. or Marshall BioResources were used.

Decentralization and Nerve Transfer Surgeries

Animals were anesthetized as previously described⁸ and randomized into 5 groups (Fig. 1). Sacral root–decentralized animals (S Dec, $n = 6$) underwent extradural sacral decentralization in which all spinal roots below L7 were exposed and transected bilaterally, in addition to bilateral transection of hypogastric nerves (Fig. 2A).⁸ Seven animals underwent similar sacral root and bilateral hypogastric nerve transections, plus bilateral transection of L7 dorsal roots (L7d+S Dec; Fig. 2B). To ensure no regrowth of spinal roots, each root was transected twice, at the dorsal root ganglia (DRGs) and at the emergence from the dura mater, and the remaining transected spinal root was removed. Thus, the DRGs remained intact but were disconnected from their connections with the spinal cord. The same decentralization procedure was used for the ventral roots. Three L7d+S Dec animals showing few to no squat-and-void postures across the 12 months after decentralization were chosen for reinnervation surgeries (L7d+S Dec+Reinn, $n = 3$). For nerve transfer procedures

intended to restore both sensory and motor functions, animals were anesthetized, and the obturator nerves were identified and divided into 2 fairly even longitudinal fascicles using a microscalpel. Half was left intact to retain innervation of hindlimb adductor muscles. The other half was transected, transferred, and sutured end-to-end to the transected anterior vesical branch of the pelvic nerve, bilaterally, as previously described (Fig. 2C).⁸ These 3 animals were reanesthetized 3 weeks later, the pudendal nerves were identified, and bilateral transfer of the semimembranosus branch of the sciatic nerves to the pudendal nerves was performed for reinnervation of urethral and anal sphincters. Controls included 8 sham-operated (lumbosacral laminectomy with no nerve root transections) and 3 unoperated animals.

Behavioral Observation of Squat-and-Void Postures

The frequency of squat-and-void postures, defined by the position exhibited by female canines during urination, was recorded for 24 hours at monthly intervals in 6 of 8 sham-operated control, S Dec, L7d+S Dec, and L7d+S Dec+Reinn groups using video surveillance cameras placed over housing cages. For each animal, at least 1 recording was performed during the acclimation period, prior to the first surgery. These videos were assessed by observers who were not aware of the animals' treatment allocation. The Credé maneuver (i.e., pressure applied to the abdomen to eliminate urine) was performed twice daily to empty the bladder as necessary during the 12-month post-decentralization recovery period or 9-month postreinnervation recovery period.

Observation of Squat-and-Void Postures With Full Bladder

Perception of bladder distention and ability to empty a full bladder was assessed in L7d+S Dec, L7d+S Dec+Reinn, and unoperated control animals. Each of these animals was placed in a sling 2–3 times/wk, 10 min/ session, for 1 week prior to the first awake bladder-filling procedure for acclimation to the setup. Prior to filling, they were anesthetized with propofol (intravenous, 6 mg/kg) for insertion of bladder, urethra, rectal, and anal sphincter catheters. After recovery from propofol, pressures were monitored with the fully awake animal in the sling, and bladder-filling curves were recorded using external pressure transducers interfaced with the PowerLab multichannel data acquisition system and LabChart software (ADInstruments). The bladder was filled with 0.9% normal saline solution to cystometric capacity, defined as the infused volume inducing a marked increase in the slope of the volume-pressure curve. In the absence of an inflection point in the pressure-time curve (as seen in decentralized animals), the bladder was filled to 60 cm of water pressure. Following recording of filling curves, animals were transferred to a transport cage, the Foley catheter was removed while the bladder remained full, and behaviors were video recorded for 10 minutes to assess bladder emptying via voluntary squat-and-void posture or leakage only. Any bladder contents expelled during squat-and-void postures were collected, measured, compared to the cystometric capacity volume previously infused into the bladder, and recorded as percent recovered volume (percentage squatand-void volume). Leaked bladder volume from animals that did not show squat-and-void postures was also collected, measured, and compared to the volume previously infused into the bladder (percentage leaked volume). Residual volume was calculated (percentage calculated residual volume) by subtracting squat-and-void volume or leaked volume from total infused volume.

Retrograde Dye Injection

Three weeks prior to euthanasia, the bladder wall was cystoscopically injected around the ureterovesical junction with Fluoro-Gold (4%–5% wt/vol in 0.9% saline, Fluorochrome, LLC), as previously described.³ True Blue (2% wt/vol in 0.9% saline, Life Technologies Corp.) was also injected into the EUS at 4 different sites. No dye injections were performed in unoperated controls. One animal in the S Dec group died during the postsurgery recovery period and did not undergo injection; 2 other S Dec animals underwent different procedures after the 12-month decentralization period and were excluded from this analysis.

In Vivo Functional Electrical Stimulation

Immediately prior to euthanasia, the animals were reanesthetized as previously described.⁸ Bladder, EUS, rectal, and anal sphincter pressures were monitored throughout the surgeries, as were vital signs. Three successive filling cystometrograms were obtained to determine bladder capacity using the previously described setup. Transferred nerves (obturator-to-pelvic and sciatic-to-pudendal) were stimulated (3–10 mA, 20 Hz, 0.2 msec) using monopolar or bipolar electrodes. Changes in detrusor pressure were recorded. Strength of nerve-evoked bladder, EUS, and anal sphincter contractions were derived from differences between the resting baseline pressure and the peak pressure obtained during stimulation.

Euthanasia and Tissue Collection

Three weeks after dye injections, animals were euthanized by a terminal dose of Euthasol (pentobarbital sodium 86 mg/kg and phenytoin sodium 11 mg/kg intravenously). Tissues were collected, fixed, sectioned, and counted as previously described.³ Briefly, T12–S3 DRGs were collected and fixed by immersion in 4% buffered paraformaldehyde for 4 hours. Ganglia were cryosectioned at 20 μ m. Every third section of DRGs was mounted, coverslipped with 80% glycerol/phosphate-buffered saline, and evaluated for retrogradely labeled neuronal cell bodies.

Statistical Analysis

Means and SEMs are reported. Retrograde labeling was analyzed using a two-way ANOVA with Tukey post hoc multiple comparisons; $p < 0.05$ was considered statistically significant.

Results

L7 Dorsal Root, Sacral Roots, Plus Hypogastric Nerve Transections Are Needed to Reduce Squat-and-Void Postures

Frequencies of squat-and-void postures are reported based on preoperative and monthly postoperative behavior analysis over 24-hour periods in housing cages. Squat-and-void postures were consistently observed in S Dec animals (Fig. 3A) at similar frequencies as sham-operated animals (Fig. 3B). Of the L7d+S Dec animals (Fig. 4), animal 7 showed only 1 posture at 5 and 10 months (150 and 300 days), animal 8 showed no postures across the 12-month post-decentralization period (350 days), and animal 9 showed increased frequency of greater than 10 postures per day only at 2 and 4 months post-decentralization (60 and 120 days). Animals 9 and 10 showed unusual postures (intermediate between squat-and-void and

defecation postures with longer duration) that coincided with cultureconfirmed bacteriuria, which disappeared with antimicrobial treatment (Fig. 4). One of these 4 animals (animal 10) was euthanized at the 11th postoperative month (330 days) due to kidney and bladder stones. The remaining animals (animals 11, 12, and 13) showed consistent squat-and-void postures at monthly observation periods and were euthanized at 8–9 months postoperatively (Fig. 4).

Nerve transfer surgeries were only performed in the 2 animals (animals 7 and 8) with few to no incidences of squat-and-void postures and in the animal (animal 9) that showed unusual postures only during culture-confirmed bacteriuria (Figs. 4 and 5). Importantly, at 4–6 months (120–180 days) after the reinnervation surgery, squatand-void postures were observed in all 3 reinnervated animals (Fig. 5). During video recording in the housing cages, most of the time urine was seen after these events, and screen capture shots were taken for validation of the event occurrence for all 3 animals. Additionally, during daily care, bladders were usually empty ($67\% \pm 12\%$ of the time over the 2-month span prior to euthanasia, $n = 3$) when performing the Credé maneuver during this postreinnervation period.

Observation of Bladder Fullness Sensation and Squat-and-Void Posture Events With Full Bladder

All 3 unoperated control animals showed squat-andvoid postures over the 10-minute video recording with full bladder. The measured squat-and-void volume was recorded as $71.1\% \pm 2.9\%$ of their cystometric bladder capacity (Supplemental Table 1). In contrast, squat-andvoid postures were absent in the L7d+S Dec animals after removal of the Foley catheter in the transport cage (Supplemental Table 1). The leaked volume percentage in L7d+S Dec animals was measured as $29.5\% \pm 6.0\%$. Interestingly, following the awake bladder-filling procedure, upon return to the transport cage, one of the L7d+S Dec+Reinn animals (animal 8) assumed a squat-and-void posture and partially emptied its bladder voluntarily on 2 occasions (Fig. 5). The recovered squat-and-void volume percentage for this animal was measured as $7.1\% \pm 4.3\%$ of its capacity in the transport cage (Supplemental Table 1). The other 2 L7d+S Dec+Reinn animals (animals 7 and 9) did not show any squat-and-void postures after bladder filling during the 10-minute video recording period after their return to the transport cage with the bladder filled. In these 2 reinnervated animals, the leaked volume percentage was measured as $43.1\% \pm 5.5\%$. The calculated residual volume percentage was $28.9\% \pm 2.9\%$ in unoperated controls, $70.5\% \pm 6.0\%$ in L7d+S Dec animals, $56.9\% \pm 5.5\%$ in L7d+S Dec+Reinn animals (animals 7 and 9) with no squat-and-void postures, and $92.9\% \pm 4.3\%$ in an L7d+S Dec+Reinn animal (animal 8) with squat-and-void posture (Supplemental Table 1).

Muscle Contractions Were Induced After In Vivo Electrical Stimulation of the Transferred Nerves

Immediately prior to euthanasia, L7d+S Dec+Reinn animals were reanesthetized, and bladder, EUS, rectal, and anal sphincter pressures were continuously monitored during 3 successive filling cystometrograms. Obturator nerve stimulation induced detrusor muscle contractions up to 10 cm H₂O in animals 7 and 8 (Fig. 6A). However, only a slight increase in detrusor pressure (2 cm H₂O) was observed during obturator nerve stimulation of animal 9 (Fig. 6A). When the sciatic nerve was stimulated in these 3 reinnervated animals, only 1

animal (animal 8) showed EUS contractions (15 cm H₂O; Fig. 6B), although high anal sphincter pressures were induced in all 3 animals (Fig. 6C).

Retrograde Dye Labeling of Transferred Nerves Shows Integrity of Anastomosis Sites

Fluoro-Gold labeling from the bladder was observed in the transferred obturator nerves immediately proximal to their site of anastomosis to the pelvic nerve branch, at 6–9 months after nerve transfer (Fig. 7A). Similarly, True Blue labeling from the urethra was observed in the transferred sciatic nerves immediately proximal to their site of anastomosis to the pudendal nerves (Fig. 7B).

Retrograde Dye Labeling in DRGs Confirms Growth of Sensory Axons to the Bladder in Transferred Nerves

Examples of retrogradely labeled neurons in DRGs are shown in Fig. 8A–C for sham-operated controls (Fig. 8A), L7d+S Dec animals (Fig. 8B), and L7d+S Dec+Reinn animals (Fig. 8C). Quantification of these labeled neurons showed a decrease in S1–2 DRGs of S Dec and L7d+S Dec animals, compared with sham-operated controls (Fig. 8D–F). In contrast, there were increased numbers of labeled cell bodies in L5 and L6 DRGs of L7d+S Dec+Reinn animals (Fig. 8G), compared with L7d+S Dec animals, as well as increases in L2 and the L4–6 DRGs of L7d+S Dec+Reinn animals compared with sham-operated controls.

Discussion

Bilateral somatic nerve transfer to the anterior vesicle branch of the pelvic nerve and pudendal nerve reversed year-long decentralization-induced loss of squat-and-void postures in the home cage 4–6 months after reinnervation in all 3 animals. One animal also clearly showed evidence of voluntary voiding after awake bladder filling, due to either a functional bladder contraction or a voluntary Valsalva maneuver. Stimulation of the surgically transferred obturator and sciatic nerves, which induced bladder and EUS contractions, respectively, in this animal, suggests recovery of motor function via the newly established pathways. Although there are many outcome measures in this study, we consider the most relevant outcomes to be the proportion of animals that show global sensory and motor assessments of functional recovery, which are binary outcomes in that they show the behavior or they do not. Given the binary outcome of our experiments (i.e., squat posture vs no squat posture), we assess success as a binomial proportion. As a binomial proportion, the small sample size ($n = 3$) clearly influences the precision of our estimate of the true success rate of surgery. Nevertheless, with 3 of 3 successes (100% success where all reinnervated dogs were squatting), we can be 95% confident that the true success rate of surgery in a large population of dogs would be greater than 30% success. Increasing the sample size to 9, with 9 successes, would increase our minimum 95% confidence to greater than 67% success.

While 2 L7d+S Dec+Reinn animals did not perform squat-and-void postures following awake bladder filling, they showed the behavior in their home cages. This difference may be due to lack of EUS reinnervation, as indicated by the absence of sciatic nerve stimulation induced EUS contractions. On removal of the catheter, the dogs immediately leaked the

infused volume, suggesting a deficiency of EUS tone. When the bladder is allowed to fill at a physiological rate, the smooth muscle of the bladder neck may provide enough tone for urine storage.

Two L7d+S Dec animals exhibited unusual postures, and 4 exhibited increased frequency of squat-and-void postures concurrent with culture-confirmed bacteriuria, which disappeared on treatment with antibiotics, suggesting sensation of discomfort or pain in the bladder induced by the bacteriuria.¹³ Squat-and-void behaviors despite decentralization indicates that they either retained or regained some bladder sensation following decentralization, perhaps due to activation of silent C fibers by the bacteriuria, sensory nerve sprouting, or variations in bladder sensory innervation as we previously reported.³

We previously reported that genitofemoral or femoral nerve transfer to the anterior vesical branch of the pelvic nerve results in increased Fluoro-Gold–labeled neurons in ventral horn regions of lumbar spinal cord segments from which the donor nerves originated after retrograde dye injection of the detrusor around the ureterovesical junctions. The labeling was not observed in sham or unoperated control animals, confirming that distal ends of the transferred nerves sprouted into the bladder end organ.^{8,15} Further-more, pelvic-plexus–induced stimulation was not disturbed following femoral nerve transfer, suggesting that somatic nerve transfer may maintain motor function to the bladder.⁸

It is important to note that the decentralization strategy previously implemented did not include transection of hypogastric nerves, which would reduce sympathetic and some sensory input,⁶ or L7 dorsal roots, which would reduce sensory input to the bladder.^{3,18} In this current study, squat-and-void postures were retained in animals that underwent hypogastric nerve and sacral root transections, while leaving the L7 dorsal roots intact (S Dec), with frequencies similar to those of sham-operated animals. In contrast, these postures were eliminated in 4 of the 7 animals in which L7 dorsal roots were transected in addition to the hypogastric nerve and sacral root transections. Despite this extensive decentralization, 3 of the 7 animals continued to show squat-and-void postures after decentralization, presumably due to either diversity in bladder sensory innervation or postoperative sensory nerve sprouting resulting in spontaneous bladder sensory reinnervation. In an additional group of 8 animals in which both the L7 and L6 dorsal roots were transected in addition to the hypogastric nerve and sacral root transections, none showed squat-and-void postures in their housing cages (data not shown). However, they suffered from multiple postoperative complications, including bacteriuria in 6, hindlimb self-mutilation in 6, and ambulation inability in 1 that required them to be euthanized 1–6 months after decentralization, prior to our ability to perform nerve transfer surgery or retrograde neurotracing studies. These findings provide further support that the lower lumbar dorsal roots also provide sensory innervation to the bladder and, therefore, at least L7 must be transected for a more complete motor and sensory decentralization.³

The closest human condition that parallels our animal model of lower motor neuron–lesioned pelvic viscera is in patients with sacral-coccygeal chordoma tumors in which the tumor engulfs the S2–4 nerve roots that must be removed with the tumor, leading to an acontractile bladder, weak external urethral and anal sphincters, sexual dysfunction, and

sensory deficits. Because this is a rare tumor accounting for only 1%–4% of all primary bone tumors, there are no large-scale clinical studies. However, continence is maintained in the majority of patients with preservation of S2 and at least one S3 root, which may result in incomplete resection of the tumor.¹² Thus, we propose that employing a split portion of the obturator nerve to the vesical plexus will provide effective reinnervation for restoration of voluntary voiding. The patient's bladder contraction would be activated via the split obturator nerve by sustained adduction of the thighs (which would be feasible when sitting on a toilet). Normal walking would not be compromised with the remaining portion of the obturator nerve still functional. In appropriate patients, future surgical reinnervation of the pudendal nerve is envisioned by repurposing nonessential branches from the saphenous nerve (sensory) and the nerve to the vastus medialis (motor) to restore tone to the sphincters in order to restore continence. Based on our canine studies, this reinnervation by nerve transfer appears to be feasible when the reinnervation surgery is performed immediately after the decentralization,¹⁷ at 1 and 3 months,¹⁶ and now at 1 year after decentralization.

The increased Fluoro-Gold–labeled cells in the L5–6 DRGs of L7d+S Dec+Reinn animals (Fig. 8G), compared with L7d+S Dec animals (Fig. 8F), and in L4–6 DRGs, compared with sham-operated controls (Fig. 8D), indicate that axons from these DRGs, which normally contribute to the obturator nerve (L4–6 in canines), very likely reinnervated the bladder. Importantly, a future study will include age-matched decentralized animals that do not display squat-and-void postures for comparison to reinnervated animals to confirm that the increase in labeling is due solely to the reinnervation rather than de novo sprouting. Although there were significantly decreased numbers of labeled cells in S1 and S2 DRGs in S Dec and L7d+S Dec groups compared with controls, we still observed low levels of retrogradely labeled neurons in DRGs of roots that had been previously transected. This is likely due to the fact that the DRGs were spared during the initial decentralization. If they had not become resorbed by the time of collection, they were harvested for assessment and showed retrogradely labeled neurons. Future decentralization studies will also include removal of DRGs in addition to transection of spinal roots.

Conclusions

All 3 animals that were selected for reinnervation exhibited a disappearance or marked reduction of squat-and-void postures during the 12-month decentralization period. Following reinnervation, they demonstrated a restoration of posture frequency 4–6 months after reinnervation, indicating recovery of sensory innervation of the bladder. They each showed bladder and anal sphincter contractions, while one showed EUS contractions during in vivo electrical stimulation of the transferred nerves, which also supports return of motor function. Most notably, one animal also showed voluntary voiding after awake bladder filling with saline. These findings indicate that nerve transfer can induce recovery of sensation of bladder fullness and possibly the ability to empty the bladder, and thus may be an appropriate surgical treatment for patients who sustain lower motor neuron lesion–induced bladder dysfunction. Cadaver studies by us^{2,4,5} and others^{11,20} confirmed that nerve transfer for bladder and urethra reinnervation is feasible and generalizable for reinnervation of the lower motor neuron–lesioned human pelvic viscera. This nerve transfer surgery is

considered to be well within the technical range of practicing peripheral nerve reconstruction neurosurgeons or plastic surgeons.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

DRG	dorsal root ganglion
EUS	external urethral sphincter
L7d+S Dec	S Dec plus bilateral transection of L7 dorsal roots
L7d+S Dec+Reinn	L7d+S Dec plus reinnervation
S Dec	sacral root decentralization with exposure and bilateral transection of spinal roots below L7 and hypogastric nerves

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FIG. 1.

Study design. Sacral decentralization animals in which S1–3 roots were transected (S Dec; n = 6) for decentralization and then a 1-year observation period before euthanasia. L7 dorsal root + S1–3 root–transected animals (L7d+S Dec; n = 3) for decentralization and then an 8-month to 1-year observation period before euthanasia. L7 dorsal root transected + S1–3 root transected + reinnervation animals (L7d+S Dec+Reinn; n = 3) underwent decentralization and then a 1-year observation period before reinnervation, followed by another 9-month observation period before euthanasia. All decentralization procedures included bilateral hypogastric nerve transection. Sham-operated animals (n = 8) underwent laminectomy followed by a 2- to 8-month observation period before euthanasia. Unoperated animals were used for awake bladder filling only (n = 3). N = number of animals/group; N. = nerve.

FIG. 2.

Decentralization and nerve transfer surgical approach. Transection included the removal of an approximately 1-cm-long segment of the root while leaving approximately 2 mm of root attached to the DRGs (D) intact. **A:** All roots caudal to L7 were transected bilaterally. **B:** All roots caudal to L7 in addition to L7 dorsal roots were transected bilaterally. **C:** Nerve transfers (obturator-to-pelvic and sciatic-to-pudendal) were performed 12 months after bilateral transection of all roots caudal to L7 as well as bilateral L7 dorsal root transection. In panels A and B, decentralization procedures included bilateral hypogastric nerve transection (not shown in diagram).

A Sacral Decentralization (all roots caudal to L7)

B Sham-operated

FIG. 3.

Squat-and-void postures observed in sacral decentralized and sham-operated animals during monthly 24-hour recording periods in home cages. **A:** Sacral decentralization included bilateral transection of all roots caudal to L7 (i.e., S1–3) and bilateral transection of hypogastric nerves. **B:** Sham-operated animals underwent lumbosacral laminectomies without root transection. *Squares* denote squat-and-void postures per day.

FIG. 4.

Squat-and-void postures observed in L7d+S Dec animals during monthly 24-hour recording periods in home cages. L7 dorsal + sacral decentralization included bilateral transection of all roots caudal to L7 (i.e., S1–3) and bilateral transection of hypogastric nerves, with additional bilateral transection of the L7 dorsal roots. *Squares* denote squat-and-void postures per day, and *triangles* indicate unusual postures between squat-and-void and defecation. PO = postoperatively.

FIG. 5.

Squat-and-void postures observed in L7d+S Dec+Reinn animals during monthly 24-hour recording periods in home cages. Three animals in the L7d+S Dec group with few or no voiding behaviors at 1 year post-decentralization underwent obturator-topelvic and sciatic-to-pudendal nerve transfers, followed by further observation as shown. *Squares* denote squat-and-void postures per day; *triangles* indicate unusual postures between squat-and-void and defecation; and *hexagons* denote bladder emptying in less than 10 minutes after awake bladder filling.

FIG. 6.

Representative traces of recordings from transferred nerve stimulations from L7d+S Dec +Reinn animals. **A:** Obturator nerve stimulation induced bladder contractions. **B:** Sciatic nerve stimulation induced EUS contractions. **C:** Sciatic nerve stimulation induced anal sphincter contractions. Period of stimulation indicated by “ON” and “OFF.”

FIG. 7.

Retrograde dye injected into the bladder was observed in transferred obturator and sciatic nerves immediately proximal to the site of anastomoses. **A:** Whole mount phase-contrast image of mesenteries (*left*) containing the Fluoro-Gold–labeled obturator nerve (*right*) immediately proximal to the site of anastomosis to the anterior vesicle branch of the pelvic nerve. **B:** Histological sections containing True Blue–labeled sciatic nerve semi-membranosus branch, immediately proximal to the site of anastomosis to the pudendal nerve; the *arrow* indicates suture at the pudendal end near the EUS. These images are from animal 8; the results of animals 7 and 9 were similar.

FIG. 8.

Labeling of cells in DRGs after cystoscopic injection of Fluoro-Gold retrograde dye into bladder wall. **A:** Representative retrogradely labeled cells in L7 DRGs of a sham control. **B:** Retrogradely labeled cells in the L7 DRGs of an L7d+S Dec animal. **C:** Retrogradely labeled cells in the L5 DRGs of an L7d+S Dec+Reinn animal. **D–G:** Number of cells/mm² counted in DRGs at spinal level of sham-operated control (D), S Dec (E), L7d+S Dec (F),

and L7d+S Dec+Reinn (G) animals. * $p < 0.05$ and ** $p < 0.01$ compared with sham-operated controls; && $p < 0.01$ compared with L7d+S Dec animals.

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