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Bladder reinnervation by somatic nerve transfer to pelvic nerve vesical branches does not reinnervate the urethra

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Abstract

Aims: We sought to determine whether somatic lumbar nerve transfer to the pelvic nerve's anterior vesical branch after sacral decentralization for detrusor muscle reinnervation also leads to aberrant innervation of the bladder outlet.

Methods: Twenty-six female mongrel hound dogs underwent transection of sacral dorsal and ventral spinal roots (ie, sacral decentralization). Immediately afterward, 12 received genitofemoral nerve transfer and 9 received femoral nerve branch transfer. Five were left sacrally decentralized. Controls included 3 sham-operated and 6 unoperated. Eight months postsurgery, the bladder and urethra were injected with retrograde tracing dyes cystoscopically. After 3 weeks, detrusor and urethral pressures were assayed electrophysiologically immediately before euthanasia and characterization of neural reinnervation.

Results: Electrical stimulation of spinal cords or roots did not lead to increased urethral sphincter pressure in nerve transfer animals, compared with decentralized animals, confirming a lack of functional reinnervation of the bladder outlet. In contrast, mean detrusor pressure increased after lumbar cord/root stimulation. In sham/unoperated animals, urethral and bladder dye injections resulted in labeled neurons in sacral level neural structures (dorsal root ganglia [DRG], sympathetic trunk ganglia [STG], and spinal cord ventral horns); labeling absent in decentralized animals. Urethral dye injections did not result in labeling in lumbar or sacral level neural structures in either nerve transfer group while bladder dye injections lead to increased labeled neurons in lumbar level DRG, STG, and ventral horns, compared to sacrally decentralized animals.

Conclusion: Pelvic nerve transfer for bladder reinnervation does not impact urethral sphincter innervation.

KEYWORDS

bladder reinnervation, nerve transfer, retrograde dye, somatic nerve, urethral sphincter

1 | INTRODUCTION

Previous studies established that surgical transfer of somatic lumbar nerves to the pelvic nerve's (PN) anterior vesical branch can restore contractile function and spinal cord motor reinnervation of the bladder detrusor muscle after sacral root decentralization in which the dorsal and ventral roots of sacral (S) 1 to 3 segments were transected.[1-4] For example, 8.5 months after immediate nerve transfer surgery, increased detrusor pressure was observed in 12 of 17 dogs that had received genitofemoral nerve transfer (GFNT) to the anterior vesical branch, and in 9 of 10 dogs in which a femoral nerve branch was similarly transferred (FNT).[1] Retrogradely-labeled neurons from the bladder were observed in ventral horns of lumbar spinal cord segments after GFNT and FNT, showing direct spinal cord reinnervation of the bladder from motor neurons located in spinal cord regions from which these nerves originated.[1] However, it has not been determined whether somatic lumbar nerve transfer to a pelvic nerve branch after sacral decentralization results in sprouting of the transferred nerves and aberrant innervation of the bladder outlet, which could result in bladder neck obstruction (detrusor sphincter dyssynergia), impairing efficient voiding.[5]

Therefore, we sought to determine if somatic lumbar nerve transfer to the anterior vesical branch of the pelvic nerve immediately after sacral decentralization leads to aberrant innervation of the bladder outlet. We expanded our past examination of reinnervation from motor neurons located in spinal cord ventral horns to also examine for reinnervation from neurons located in dorsal root ganglia (DRG) and sympathetic trunk ganglia (STG). We hypothesized that no aberrant urethral sphincter reinnervation would be observed as this end organ is innervated by the pudendal nerve[6-8] rather than the anterior vesical branch (the recipient of nerve transfer).

2 | MATERIALS AND METHODS

All studies were approved by the Institutional Animal Care and Use Committee and carried out in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and laboratory animal care guidelines of the United States Department of Agriculture and Association for Assessment and Accreditation of Laboratory Animal Care. Thirty-five female mongrel hound dogs, 6 to 8 months old, 16.7 to 20 kg (Marshall BioResources, North Rose, NY) were used. All dogs had free access to food and water and were maintained on a 12:12 hour light-dark cycle.

2.1 | Nerve transfer surgeries

Animals were anesthetized using isoflurane (0.5%-4% mean alveolar concentration) using oxygen as the carrier gas.[1,3] A lower motoneuron lesioned bladder model was created in 26 dogs by transecting all sacral nerve roots that increased detrusor pressure using intraoperative electrical stimulation (dorsal and ventral roots of S1-S3), and coccygeal roots, as previously described.[1,3] Immediately afterward, 12 underwent transfer of genitofemoral nerves to the pelvic nerve's vesical branch (GFNT) while 9 underwent a transfer of femoral nerve branches to this same branch (FNT), as previously described.[1,2,9] Five were left sacrally decentralized and served as sacral root transected controls.[1,2,9] Controls included three sham-operated and six unoperated animals.[1,2,9] Bladder emptying in animals was accomplished by the Credé maneuver as necessary during the 8-month recovery period (219 ± 6.78 days, mean ± standard error of the mean [SEM]). Because of the S1-S3 root transection and loss of both pelvic and pudendal nerve function, the sacrally decentralized animals were incontinent of urine and thus a Credé maneuver was not generally required for bladder emptying.

2.2 | Retrograde dye injection

Eight months after reinnervation surgery and 3 weeks before euthanasia, dogs were sedated.[1,3] The bladder muscle was cystoscopically injected in all 35 animals with Fluorogold (4%-5% w/v in 0.9% saline, Fluorochrome, LLC, Denver, CO) at four sites lateral to each ureteral orifice (50 μL/injection, 8 injections/dog, 400 μL/animal). In addition, dogs received True Blue (2% w/v in 74% dimethyl sulfoxide, Life Technologies Corporation, Grand Island, NY) injections into four different quadrants of the urethral tissue (50 μL/injection, 200 μL total per animal; analysis of the specific site is reported below). Dogs were allowed to recover from anesthesia before returning to home cages.

2.3 | Electrophysiology

On the day of euthanasia, animals were anesthetized as previously described.[1,3] The bladder was catheterized through the urethra with a dual balloon Foley catheter connected to external pressure transducers for recording bladder and ureteral sphincter pressures. Bladder capacity was determined by three successive filling cystometrograms using normal saline at 30 mL per minute.[6,10] The bladder was then emptied and filled to half of this capacity (approximately 30 mL). Intraoperative electrical stimulation of lumbar (L) 1 through sacral (S) 1 to 3 spinal cord segments and bilateral spinal roots was performed to

evaluate return of spinal cord or spinal nerve root evoked urethral sphincter or detrusor contractile function. Bladder and urethral sphincter pressures were recorded with external pressure transducers and dual balloon Foley catheters as previously described,[6,10] and the maximum pressure reported for 3 to 8 stimulations per dog.

2.4 | Euthanasia and tissue collection

After electrophysiological testing, all animals were euthanized by a terminal dose of euthasol (pentobarbital sodium 86 mg/kg and phenytoin sodium 11 mg/kg, IV). Tissues were collected, fixed by immersion, and sectioned, as previously described.[11,12] Spinal cord segments, DRG, and STG were collected, bilaterally, from thoracic (T) 10 through S3 or coccygeal (CG) 1 vertebral levels, when ganglia were present. Not all DRGs were present due to surgical transection-induced die back, and not all STG were present bilaterally either due to the irregular nature of canine STG, die back, or both (numbers of ganglia collected per segment and group is reported in Supplemetray Information Tables). All were fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 to 2 weeks. After equilibration in 30% buffered sucrose, tissues were cryosectioned (14 μm). Every fifth section of the spinal cord segment and every third section of DRG and STG was mounted onto slides and coverslipped with 80% glycerol/PBS. Urethra tissues were collected with surrounding tissues from the bladder neck and inferiorly, fixed by immersion, before cryosectioning (14 μm cross-sections; every 10th section was collected). Urethral sections were either stained with hematoxylin and eosin (H&E) and coverslipped or left unstained and coverslipped with 80% glycerol/PBS.

Quantification was performed with Nikon E800 microscopes outfitted with a digital camera interfaced with an image analysis program (Life Science, Bioquant, Nashville, TN). Sections from each DRG, STG, and spinal cord ventral horn were quantified, as previously described,[2-4,11] in every dog and vertebral level, from T10 to S3 or CG1 levels, bilaterally, for the number of retrogradely-labeled neuronal cell bodies per area. Data for right and left sides were averaged. The site of Fluorogold dye injection into urethral internal and external sphincters was assayed in unstained sections, and compared to nearby sections stained with H&E.

2.5 | Statistical analysis

Urethral and detrusor pressures were compared across groups using one-way analysis of variance. Two-way analysis of variance were used to compare number of dye-labeled neurons per structure, section, and group; with retrograde dye results compared to sacral roots transected group results. Analysis of variance were followed by Dunnett's post hoc multiple-comparison tests and adjusted P values reported; $P < .05$ was considered as statistically different. Means and SEM are reported.

3 | RESULTS

3.1 | Site of urethral sphincter labeling

Urethral sphincter cross-sections were assayed for site and depth of dye injection. Urethral sphincters from 6 of 13 dogs examined (46%) showed the site and depth of dye injections included only the internal urethral sphincter (Figure 1B,C). The remaining 7 showed deeper dye injection sites that included both the internal sphincter and the external urethral sphincter muscle layer (Figure 1D,E). Thus, retrograde results shown hereafter for innervation of the urethral sphincter relate to either the internal or the external urethral sphincter.

3.2 | Electrophysiology

Direct electrical stimulation of the sacral level spinal cord did not produce detectable urethral sphincter pressures in nonreinnervated sacral roots transected animals (sacral roots had been previously transected and were no longer present for stimulation; Figure 2A). These results were significantly lower than the robust urethral sphincter contractions produced in sham/unoperated animals (Figure 2A). In GFNT and FNT animals, no detectable urethral pressure was observed by either sacral cord stimulation (data not shown) or lumbar cord/root stimulation (Figure 2A).

In contrast, mean detrusor pressure in response to electrical stimulation of sacral spinal cord or roots was undetectable in 4 of 5 sacral roots transected animals; these responses were significantly lower than in the sham/unoperated animals (Figure 2B). Also, no detectable detrusor pressures were observed after sacral cord or sacral root stimulation in GFNT and FNT animals, further confirming sacral decentralization (data not shown). However, lumbar cord or lumbar root stimulation in GFNT and FNT animals induced similar detrusor pressures as observed in sham/unoperated animals after sacral spinal cord/root stimulation in the latter (Figure 2B).

3.3 | Retrograde dye labeling in DRG

After dye labeling into the urethral sphincter of sham/unoperated controls, a large number of labeled neurons were present in S1 DRG (Figure 3A). Sacral roots transected, GFNT and FNT animals showed a loss of

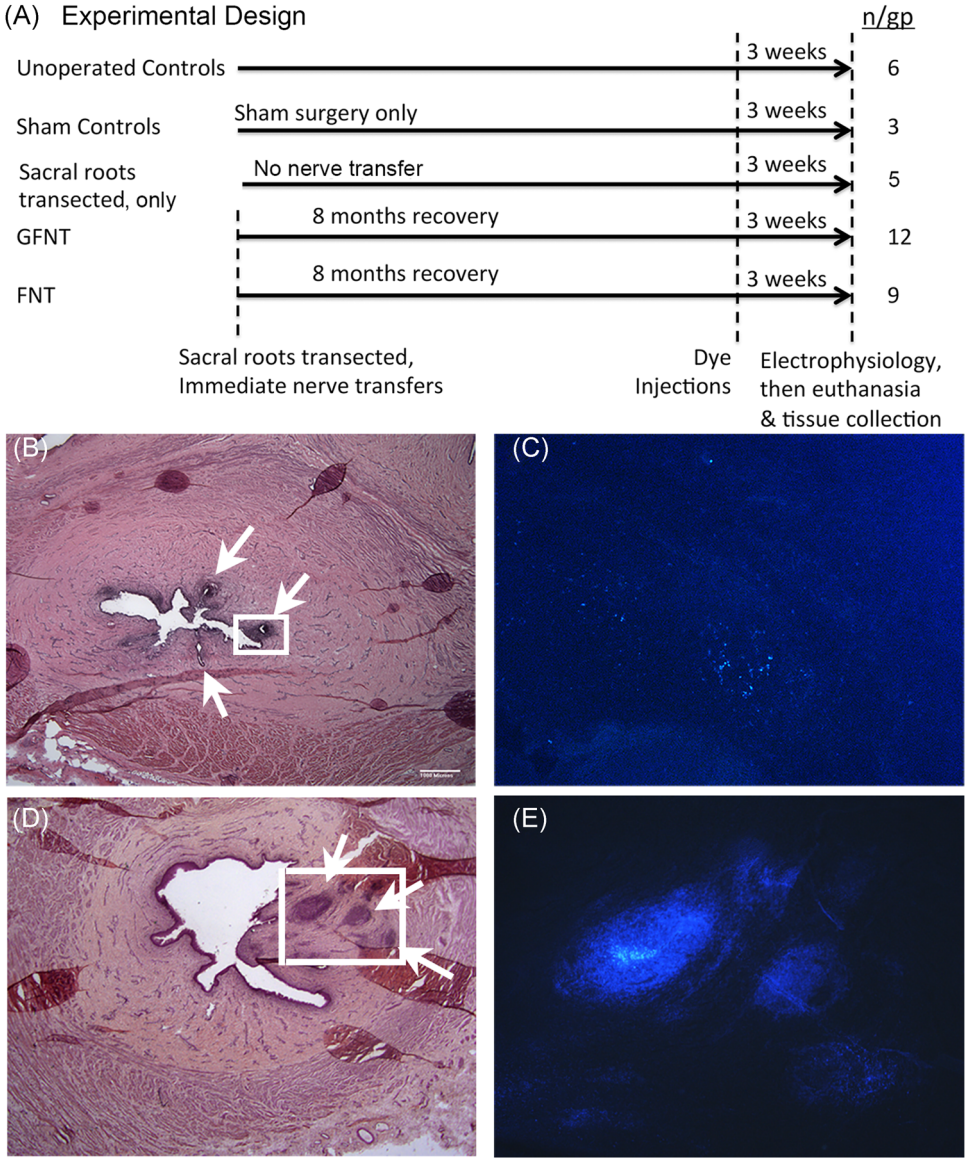

FIGURE 1 Experimental design and urethral sphincter injection sites (indicated by arrows). A, Experimental design showing time of recovery after nerve transfer surgery (8 months), then time of recovery after dye injection (3 weeks). B, Injection sites representative of 46% of animals showing that the depth of the injection did not reach the external urethral sphincter. C, Enlargement of box shown in A showing presence of True-Blue labeling. C, Injection sites representative of 54% of animals showing that the depth of the injection reached the inner boundaries of the external urethral sphincter in the area indicated by box. D, Enlargement of box shown in C showing presence of True-Blue labeling

retrograde labeling in sacral DRGs labeling compared with sham/unoperated controls ($P < .01$; Figure 3A). Bladder dye injections in sham/unoperated controls resulted in labeled neurons in L7-S3 DRGs (Figure 3B). Sacral roots transected animals that showed a loss of labeling in S1-S3 DRGs, compared with sham/unoperated controls ($P < .05$ each, Figure 3B). Bladder dye injections in nerve transfer groups resulted in increased numbers of labeled neurons in L3 and L4 DRGs in GFNT animals ($P < .01$), and in L3-L5 DRGs in FNT animals (and reduced numbers in L7 DRG) ($P < .05$ each), compared with sacral roots transected animals (Figure 3B).

3.4 | Retrograde dye labeling in sympathetic trunk ganglia

After urethral sphincter dye injections into sham/unoperated controls, labeled neurons were present in S1/S2 STG only, labeling lost in sacral roots transected animals ($P < .01$) and not recovered in nerve transfer groups (Figure 4A). Bladder dye injections in sham/unoperated controls resulted in labeled neurons in S1/S2 STG, compared to sacral roots transected animals that showed a loss of labeling in these STG ($P < .05$; Figure 4B). Yet, bladder dye injections in nerve transfer groups resulted in

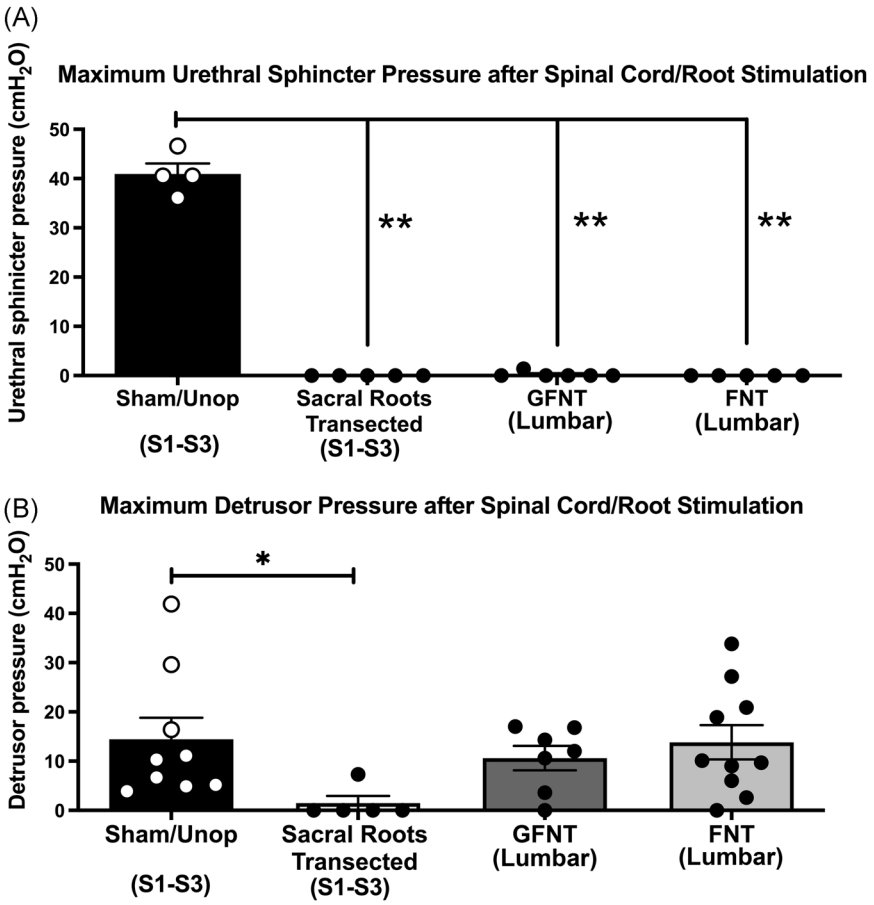

FIGURE 2 Maximum urethral sphincter pressure (A) and detrusor pressure (B) after spinal cord and root electrical stimulation. Results for electrical stimulation of sacral cord and/or root stimulation are shown for sham/unoperated (Sham/Unop) and sacral roots transected animals and for lumbar levels of GFNT and FNT animals. *$P < .05$, and **$P < .01$, compared with Sham/unoperated controls. FNT, femoral nerve branch transfer; GFNT, genitofemoral nerve transfer

increased labeled neurons in L3 and L4 STG in GFNT animals ($P < .01$ each) and in L5 STG in FNT animals compared with sacral roots transected animals ($P < .01$; Figure 4B).

3.5 | Retrograde dye labeling in spinal cord ventral horn

After dye injections into urethras of sham/unoperated controls, labeled neurons were present in S1-S3 cord

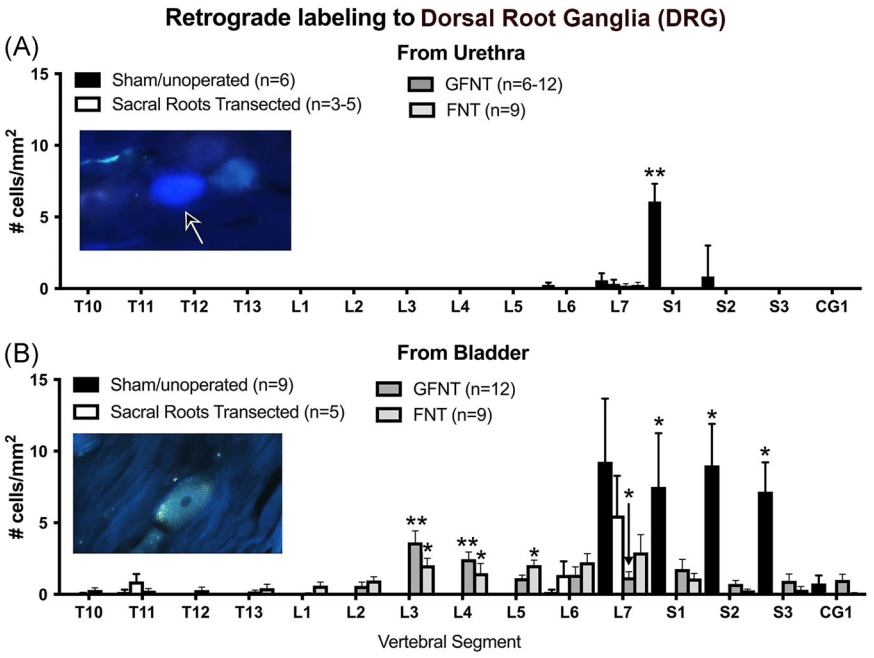

FIGURE 3 Numbers of retrogradely-labeled neurons in dorsal root ganglia (DRG) (n = animals assayed per group). A, Labeled neurons in DRG after dye injections into the urethral sphincter. B, Labeled neurons in DRG after dye injections into the bladder wall. Insets show a representative labeled neuron for each dye (True Blue in A (indicated with an arrow) and Fluorogold in B, 200× magnification. *$P < .05$, and **$P < .01$ compared with Sacral Roots Transected group

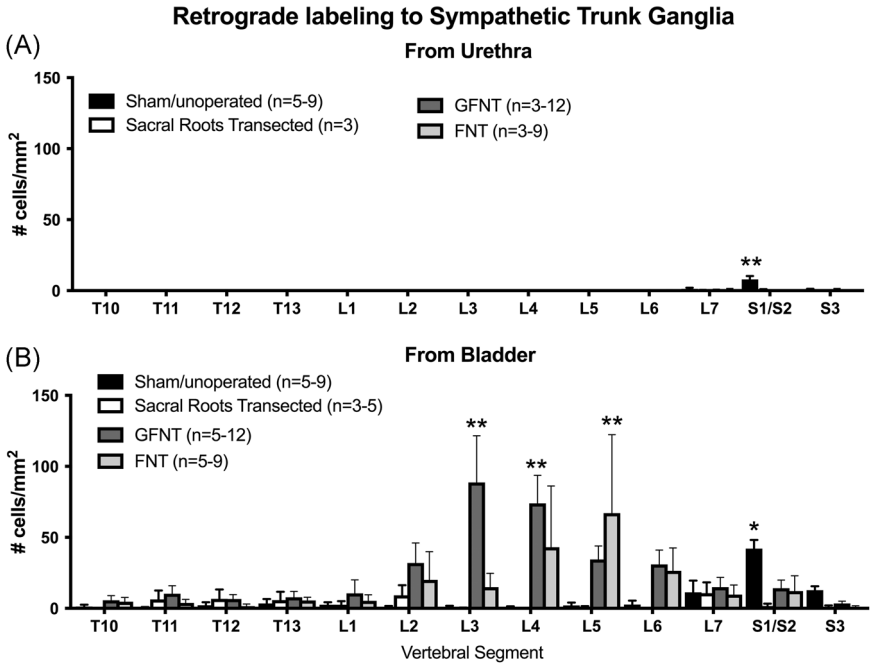

FIGURE 4 Numbers of retrogradely-labeled neurons in segmental sympathetic trunk ganglia (STG) (n = animals assayed per group). A, Labeled neurons in STG after dye injections into the urethral sphincter. B, Labeled neurons in STG after dye injections into the bladder wall. *$P < .05$, and **$P < .01$, compared with Sacral Roots Transected group

ventral horns, labeling lost in sacral roots transected animals ($P < .01$ each) and not recovered in nerve transfer groups (Figure 5A). In contrast, after dye injections into the bladders of sham/unoperated controls, labeled neurons were present in S1-S3 cord ventral horns, labeling lost in sacral roots transected animals ($P < .01$ each; Figure 5B). Yet bladder dye injections in nerve transfer groups resulted in increased labeled neurons in L3 and L4 ventral horns of GFNT animals ($P < .01$ and $P < .05$, respectively) and in L1-L4 ventral horns in FNT animals ($P < .05$ each) compared with sacral roots transected animals (Figure 5B).

4 | DISCUSSION

We have previously established that transfer and end-on-end anastomosis of lumbar originating nerves to anterior vesical branches of the pelvic nerve provided return of nerve-evoked detrusor muscle contraction in 9 of 10 FNT animals, and 12 of 17 GFNT animals.[1] Growth of motor axons into the bladder was observed from ventral horns of lumbar spinal cord segments from which genitofemoral and femoral nerves originate.[1] In a second study examining these same animals,[13] retrogradely-labeled cells were observed in nodose ganglia (sensory ganglia of the vagus) in 5 of 7 nerve transfer animals, and dorsal

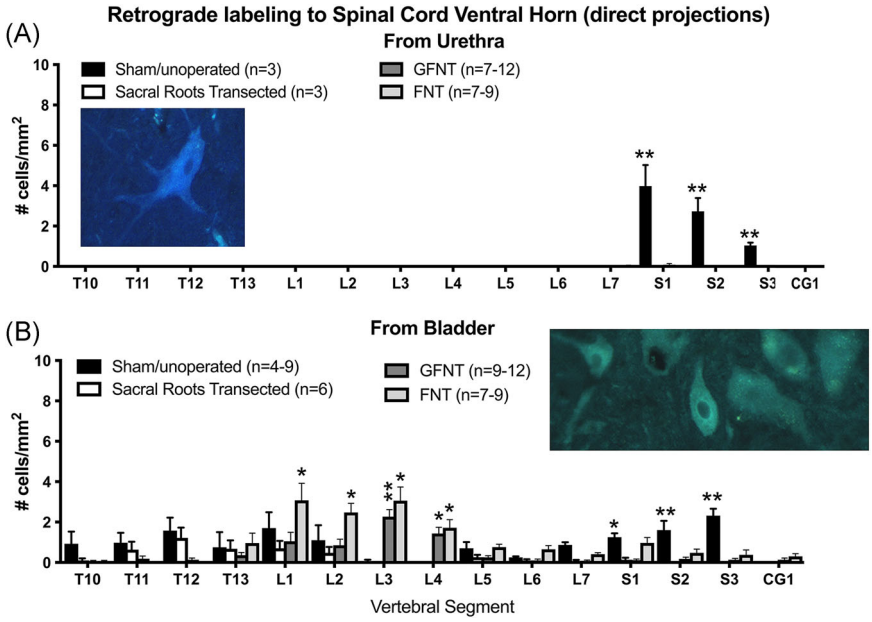

FIGURE 5 Numbers of retrogradely-labeled neurons in segmental spinal cord ventral horn regions (n = animals assayed per group). A, Labeled neurons in spinal cord ventral horn after dye injections into the urethral sphincter. B, Labeled neurons in spinal cord ventral horn after dye injections into the bladder wall. Insets show representative labeled neuron(s) for each dye (true blue in panel A and Fluorogold in panel B, 200× magnification. *$P < .05$, and **$P < .01$ compared with Sacral Roots Transected results

motor nucleus of the vagus in 3 of 5 nerve transfer animals, yet none in unoperated control animals, indicative of vagal nerve sprouting into the bladder after nerve transfer.

This sprouting prompted us to examine for aberrant sprouting into the urethral internal sphincter after GFNT or FNT to the pelvic nerve. Both canine and human urethra muscle has been shown to contract to cholinergic stimulation.[14,15] Given that the pelvic nerve contains both sympathetic and parasympathetic input[16] and the donor somatic axons are bringing in somatic motor input we were concerned that sprouting from the transferred nerves could create detrusor-internal sphincter dyssynergia (detrusor muscle contraction occurring with concomitant involuntary internal urethral sphincter activation upon activation of the axon source).[5] To confirm that the regrowing axons are not innervating the urethral sphincters, we extended our characterization of neural reinnervation to include sensory dorsal root ganglionic and sympathetic trunk ganglionic retrograde tracing studies. We report here for the first time that neither genitofemoral nor femoral nerve transfer to the pelvic nerve resulted in aberrant sensory or motor reinnervation of the urethral sphincter by 8 months after nerve transfer, tested both functionally and using retrograde tracing methods. Specifically, analysis of the urethral tissues indicated the injected dye was observed in the internal sphincter in 6 of the 13 dogs examined and in both the internal and external sphincters in the remaining 7 dogs.

In unoperated canines, cell bodies of afferents from the bladder are located primarily in DRGs in lumbar (L) 7 to sacral (S) 2 vertebral levels, with smaller numbers from thoracic (T) 11, L 6, and S3 DRG, while afferents from the urethral sphincter are located in S1 and S2 DRG.[11] Also in unoperated canines, sympathetic postganglionic efferents to the urinary bladder originate from lower thoracic, L1, L2, and L6-S3 STG (highest in S2 STG), while the urethral sphincter receives more restricted input from S1 and S2 STG.[11] Direct motor inputs from the spinal cord to both the bladder and urethral sphincter are observed in S1-S3 ventral horns.[11] In this study, through dye labeling, we observed anatomical evidence of bladder reinnervation in lumbar DRG, STG, and spinal cord ventral horns from which the transferred genitofemoral and femoral nerves originate (L3-L4 and L4-L6 in canines, respectively) compared with sacral root transected animals. These results are indicative of successful sensory and motor reinnervation of the bladder at 8 months after the transfer of these nerves to the pelvic nerve's anterior vesical branch. However, and importantly, because the urethral (internal and external) sphincter dye injections did not result in any neuronal labeling in any DRG, STG or ventral horn segment examined, at any level, in the GFNT or FNT animals, transfer of somatic nerves to the anterior vesical branch of the pelvic nerve does not result in aberrant reinnervation of the urethra.

Reduced numbers of labeled neurons in S1-S3 DRG of sacral roots transected, GFNT and FNT animals compared with sham/unoperated controls, indicates die back of neurons in DRG after the sacral roots transections performed proximally between the DRG and spinal cord. Yet, sacrally decentralized animals showed similar numbers of labeled neurons in L7 DRG as sham control animals. Future experiments in bladder decentralization animal models should transect the dorsal root of L7 in addition to all sacral roots to achieve more complete decentralization of the bladder from lower lumbar and sacral spinal cord segments, as performed recently for acute bladder decentralization studies,[10] and for 1 year decentralization studies in which bladder integrity and contractility was examined.[12]

We have reported that the rerouting procedures did not disrupt sympathetic projections to the bladder from caudal mesenteric ganglia.[13] We extend those results here to report that numbers of labeled neurons projecting to the urethral sphincter and bladder from sacral STG were significantly lower (yet not absent) in sacral decentralized groups compared with sham/unoperated controls. Thus, transecting sacral roots induced die back of many sacral sympathetic trunk neurons. The GFNT and FNT rerouting resulted in increased numbers of labeled neurons in lumbar STG compared with sham/unoperated results. Potential consequences of this increase need to be explored further, although that exploration is beyond the scope of this study focused on examining aberrant reinnervation of the urethral sphincter after somatic to pelvic nerve transfers.

Results in this study differ considerably from our previous femoral to pudendal nerve transfer study in which increased numbers of labeled neurons were observed in lumbar spinal cord ventral horns after urethral sphincter dye injections.[6] These same animals also showed return of urethral sphincter pressure at 183 and 200 days post femoral to pudendal nerve transfer surgery.[6] Thus, the urethral sphincter can be reinnervated after sacral decentralization, although only after nerve transfer to the pudendal nerve that we and others have shown to be feasible in humans as well.[6,17,18]

5 | CONCLUSIONS

Functional evidence for reinnervation of the bladder, but not the urethral sphincter, was observed in animals receiving GFNT or FNT to anterior vesical branches of the pelvic nerve. Anatomical evidence of bladder

reinnervation was present in lumbar DRG, STG, and spinal cord ventral horn segments in the reinnervated animals from which the transferred genitofemoral and femoral nerves originated (L3-L4, and L4-L6 in canines, respectively), yet no anatomical evidence of reinnervation of the urethral sphincter (internal or external) was detected. Thus, no aberrant reinnervation of the urethral sphincter was detected functionally or using retrograde dye labeling methods. Clinically, this indicates that nerve transfer to the pelvic nerve for bladder reinnervation is not likely to cause bladder neck obstruction due to internal sphincter activation. In cases of stress urinary incontinence, bladder reinnervation of the anterior vesical plexus must be accompanied by nerve transfer to the pudendal nerve for urethral sphincter reinnervation. Our findings indicate that somatic nerve transfer is feasible in establishing spontaneous voiding in persons with pure sacral denervation, such as may occur after resection of sacral cordoma involving the majority of the sacral nerve roots.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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