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Innervation of parasympathetic postganglionic neurons and bladder detrusor muscle directly after sacral root transection and repair using nerve transfer

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Abstract

Aims—This is a continuation of studies examining the effectiveness of root repairs and nerve transfers for bladder reinnervation. Our previous retrograde fluorogold tracing studies from the bladder to the spinal cord found regrowth of axons from the spinal cord through the nerve repair site to the bladder which was confirmed electrophysiologically (Ruggieri et al., 2006). The current study determines whether the pattern of axonal regrowth from the repaired nerves or roots to the bladder is different between the surgical reanastomosis methods.

Methods—The canine bladder was denervated by transection of all nerve roots from the sacral spinal cord mediating bladder contraction. Reinnervation surgeries included end-on-end repair of transected sacral ventral roots, transfer of coccygeal to sacral ventral roots (CG NT), or transfer of genitofemoral to pelvic nerves (GF NT).

Results—Postmortem dialkylcarbocyanine dye tracing with Neurotrace DiI from the distal pelvic nerve to the bladder wall, combined with PGP9.5 neuronal immunohistochemistry, demonstrated innervation by DiI-labeled axons of only parasympathetic postganglionic intramural ganglia in normal controls and sham operated controls, but reinnervation of both intramural ganglia and detrusor muscle directly after repair of sacral ventral roots. GF NT and CG NT also resulted in reinnervation of both intramural ganglia and detrusor muscle, although to a lesser extent than repaired roots.

Conclusions—Bladder reinnervation with either the same nerve (orthotopic reinnervation) or with either a primarily somatic nerve (coccygeal) or a primarily sensory nerve (genitofemoral) results in reinnervation of both intramural ganglia as well as direct innervation of detrusor muscle.

Keywords

Urinary bladder; carbocyanines; nerve regrowth; denervation

INTRODUCTION

As previously shown by retrograde horseradish peroxidase tracing, the normal innervation of the canine bladder is similar to other mammals (Sundin and Carlsson, 1972), including humans (Bradley et al., 1974), and consists of preganglionic autonomic nerve fibers that emanate from the sacral spinal cord to the bladder via the pelvic nerve (Kokotas et al., 1978; Petras and Cummings, 1978; Shefchyk, 2002) to postganglionic neuronal cell bodies located in the bladder wall (i.e. intramural ganglia). These postganglionic neurons then send axons to the bladder wall into connective tissue spaces located between detrusor muscle bundles, and then to the detrusor muscle (de Groat et al., 1981; Watanabe and Yamamoto, 1979). Most of these terminal nerve fibers are unmyelinated and are organized into small bundles with no more than 3 axons in the finest branches (Watanabe and Yamamoto, 1979).

Using a canine model, we previously showed that transection of the sacral ventral roots innervating the bladder followed immediately by end-on-end repair results in functional reinnervation as evidenced by increased bladder pressure upon functional electrical stimulation up to one year after ventral root or nerve transection and repair. Retrograde fluorogold tracing from the bladder along with postmortem lipophilic dye tracing studies from the repair site confirmed regrowth of axons from the spinal cord through the ventral root repair site to the bladder wall (Ruggieri et al., 2006). We also showed that functional bladder reinnervation after sacral root transection can be achieved by coccygeal nerve transfer (CG NT) to the severed sacral roots (Ruggieri et al., 2008b) as well as the transfer of genitofemoral nerves (GF NT) that originate from the lumbar spinal cord within the abdomen to the pelvic nerve (Ruggieri et al., 2008a). Retrograde fluorogold tracing from the bladder to the spinal cord confirmed the regrowth of axons from coccygeal spinal cord segments or lumbar spinal cord segments, respectively (Ruggieri et al., 2008a; Ruggieri et al., 2008b).

This current study was performed to determine for the first time in our reinnervation studies whether the pattern of axonal regrowth from the repaired nerves or roots to the bladder differed across the surgical methods. Although bladder reinnervation was previously confirmed by both functional electrical stimulation of the repaired and transferred nerves and retrograde tracing from the bladder to the spinal cord, exactly which cells in the bladder become reinnervated by the regrowing axons was not previously determined. We examined this using postmortem lipophilic dialkylcarbocyanine dye tracing with Neurotrace DiI from the distal pelvic nerve to the bladder wall, combined with PGP9.5 neuronal immunohistochemistry. We also examined the general histology of the intramural ganglia and larger myelinated nerves in the bladder wall using hematoxylin and eosin (H&E) staining and neurofilament 200 immunohistochemistry. Sacral ventral root repair was accomplished by end-on-end reanastomoses. Nerve transfer was accomplished using either coccygeal (CG) ventral roots to the transected sacral ventral roots, or genitofemoral (GF) nerves in the lower abdomen to the pelvic nerve proximal to its innervation of the bladder. Results were compared to sham operated controls.

MATERIALS AND METHODS

The Temple University Institutional Animal Care and Use Committee approved all studies in accordance with the laboratory animal care guidelines of both the United States Department of Agriculture and the Association for Assessment and Accreditation of Laboratory Animal Care. The study subjects were fully conditioned female mongrel hounds 6–12 months of age and 18–22 kg body weight. A total of 27 canine pelvic nerve-bladder specimens that had been collected during our past reinnervation studies (Ruggieri et al., 2008a; Ruggieri et al., 2008b; Ruggieri et al., 2006) were examined: 2 normal controls, 2

denervated controls, 5 sham operated (root-intact) controls, 5 sacral root repairs, 6 coccygeal nerve transfers, and 7 genitofemoral nerve transfers.

Surgical preparation

Animals were fasted the day prior to surgery and covered with antibiotics (30 mg/kg trimethoprim and 6 mg/kg sulfadiazine P.O.). A fentanyl patch (75–100 mg/hr for a 20 kg dog) was adhered to the shaved skin of the inner thigh and left in place for 3 days. Perioperative pain management included morphine (10 mg/l) in the intravenous Ringers lactate delivered at 60–100 ml/hr. Postoperative pain management included 2 mg/kg ketoprofen IM for 2 days beginning on the second day post surgery. Propofol (6 mg/kg iv) was administered to allow insertion of an endotracheal tube for isoflurane anesthesia (0.5–4% mean alveolar concentration) using oxygen as the carrier gas. For postoperative management of the neurogenic bladder, an abdominal vesicostomy was created as previously described (Ruggieri et al., 2006).

Bladder denervation was performed as previously described (Ruggieri et al., 2006). Briefly, with the animal in the prone position, a 30 degree V-laminectomy of the T7 vertebral body and a partial laminectomy of the T6 and S1 vertebral bodies was done so that the S1 and S2 ventral roots innervating the bladder could be stimulated with a unipolar probe electrode. The two bilateral ventral roots that induced increased bladder pressure upon intraoperative electrical stimulation were transected. In the initial animals, completeness of bladder denervation was confirmed by nerve transection-induced disappearance of bladder contractions upon stimulation of the entire conus medullaris with an epidural electrode placed in the midline under the T5 vertebral body.

Repair of bladder roots, denervated controls, sham operated controls, and normal controls

For repair of the severed sacral ventral roots to the bladder, the proximal and distal segments of the transected roots were approximated end-on-end. They were then reanastomosed microscopically by pulling the epineurium of the two cut root stumps gently together using 8-0 nylon microsutures at 3–4 sites around the nerve circumference as described and depicted previously (Ruggieri et al., 2006). The roots were transected but not repaired for the denervated controls. Sham operated controls received the lumbosacral laminectomy, intraoperative identification of bladder nerve roots with electrical stimulation and the abdominal vesicostomy but did not receive nerve transection or nerve transfer. Normal controls did not receive the laminectomy, root transection and repair, or nerve transfer.

Coccygeal nerve transfer and genitofemoral nerve transfer

For CG nerve transfer (CG NT), nerve roots inducing only tail movement upon electrical stimulation were transected and the proximal ends were sutured to the distal ends of the bladder sacral ventral roots by end-on-end anastomosis using 8-0 nylon sutures at 3–4 sites around the nerve circumference as described and depicted previously (Ruggieri et al., 2008b). For GF nerve transfer (GF NT), approximately 15 mm lengths of the bladder sacral ventral roots in the lumbosacral spine were removed to prevent spontaneous orthotopic reinnervation. The lumbosacral incision was then closed and the animal was repositioned to the supine position. After surgically prepping and sterile draping the lower abdomen, the GF nerves were located along the psoas muscle through a lower abdominal midline incision. The distalmost 3 cm of the GF nerves up to the point at which they exit the abdominal wall were mobilized. The pelvic nerves were transected as they emerged from the pelvic plexus towards the urinary bladder and the GF nerves were attached bilaterally by end-to-end anastomosis using 10-0 nylon sutures as described and depicted previously (Ruggieri et al., 2008b).

Postmortem DiI tracing of pelvic nerves and spinal cord neurons

Four months postoperatively, dogs were euthanized with intravenous injections of 360 mg/kg sodium pentobarbital. Bladders with pelvic nerves still attached were collected. To determine whether the repaired roots and nerves reinnervated the bladder, a postmortem dialkylcarbocyanine dye, Neurotrace DiI, was utilized. Neurotrace DiI (N-22990; Molecular Probes of Invitrogen, CA; an axonal labeling paste), was thinly coated onto small pieces of pulled glass micropipettes, which were inserted carefully using a dissecting microscope into the distal pelvic nerves approximately 1 cm proximal to the bladder wall at the time of tissue collection in all dogs. Specifically, the DiI was placed into the vesicular branch of the pelvic nerve after it emerges from the pelvic plexus. In the animals receiving GF NT, DiI was placed at least 5 mm proximal to the end-on-end anastomosis site at the time of tissue collection, which was located in this same vesicular branch using the previously placed sutures as a guide for the site of surgery. Thus, for these animals, the DiI had to pass through the anastomosis site (i.e., the repair site) in order to enter into the bladder wall. The bladder specimens were then postfixed 6 weeks in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 37°C. At the end of the 6 weeks, the glass microelectrodes were removed and bladder-nerve complex washed to remove any free particles of DiI or glass. Typically, vibratome sectioning is recommended for DiI labeled tissues. Unfortunately, the fixed bladder muscle proved too dense to section using a vibratome. Instead, the labeled nerves and an adjacent region of bladder wall 20 mm² to 103 mm² in size was dissected from the rest of the bladder, frozen on dry ice without a sucrose incubation step, cryo-sectioned into 15 µm sections through the depth of the bladder wall then mounted immediately onto coated slides pre-chilled to -35°C (Fisher Plus), and kept frozen at -80°C until use, as described by Tsai et al (Tsai et al., 2001). At the time of microscope examination, the slides were washed for 3 minutes and cover-slipped with 80% glycerol in 0.1M phosphate buffer. The sections on slides were examined, analyzed and photographed within 2 hours of thawing to avoid the problem of DiI diffusion. The bladder sections were examined using epifluorescence microscopy for DiI labeled nerves and the structures that they innervated.

PGP9.5 and neurofilament immunohistochemistry

After DiI quantification, the coverslips were removed from selected slide sections, the sections (on the slides) were washed in phosphate buffered saline (PBS), digested with 0.5% pepsin in 0.01 N HCl for 20 min at room temperature, and then incubated with goat serum (4%) in PBS for 30 min at room temperature. Sections were then incubated with either anti-PGP9.5 (Abcam, Cambridge, MA, 1:50 dilution in 10% goat serum in PBS) or anti-dog neurofilament-200 (Chemicon, Temecula, CA, USA, 1:250 dilution in 10% goat serum in PBS), overnight at room temperature. Sections were incubated with appropriate secondary antibodies conjugated to Cy2 (green fluorescent tag, Jackson Immuno) for PGP9.5, or horseradish peroxidase (HRP, Jackson Immuno) for neurofilament visualization. The peroxidase was visualized with diaminobenzidine (Fast DAB, Sigma). The neurofilament labelled antigen appeared brown under the light microscope, with eosin used as a counterstain. Slides were then re-coverslipped with 80% glycerol in PBS for Cy2 visualization or with DPX for HRP-DAB. Adjacent sections were stained with H&E.

Quantitative analysis of DiI labeling and statistics

For the DiI innervation pattern, 4 non-adjacent bladder sections (150 micrometers apart from each other) were analyzed per animal. The number intramural bladder wall ganglia and/or the number of bladder detrusor muscle cells associated with DiI labeled axons was counted within a 0.065 mm² region of interest (at ×400 magnification). All microscope fields per section were analyzed systematically using an XY motorized microscope stage, so that total section areas ranging from 22 to 135 mm² (mean ± SD: 54 ± 34 mm²) were measured using a Nikon E800 microscope interfaced with a computerized quantification system (Bioquant,

Nashville, TN) that runs the motorized stage. Intramural ganglia were identified based on morphology (a group of at least 5 DAPI+ neuronal cell bodies that were also PGP9.5+). Identification was verified in adjacent sections stained with H&E, and neurofilament 200 staining. Bladder detrusor muscle cells were identified based on morphology (DAPI, a blue nuclear stain was used), with verification in adjacent sections stained with H&E.

Means and standard error of the mean (SEM) of DiI innervated intramural ganglia and/or detrusor muscle cells per cm² are presented for each surgical group. The percent difference in the number of parasympathetic ganglia versus detrusor muscle innervated by the DiI labeled axons was compared using two-way ANOVA in which reinnervation pattern and surgical group were the two factors. The Bonferroni method of post hoc analysis for multiple comparisons with adjusted p values was used to compare the differences between individual groups. Adjusted probability values of 0.05 or less were considered statistically significant.

RESULTS

H&E staining and neurofilament immunohistochemical staining revealed healthy looking intramural ganglia and large myelinated nerve bundles traversing the detrusor muscle wall in each surgical group (Figure 1). No qualitative differences were observed between the groups using these two methods.

However, after DiI labeling of the pelvic nerve, DiI labeled axons were associated with either nerve bundles within the detrusor muscle wall (Figure 2), small DiI labeled axons associated with the detrusor muscle itself in the bladder wall (Figure 2; Figure 3A–E), or DiI labeled axons in intramural ganglia (Figure 3), depending on the animal's surgical treatment. In the sham operated controls, DiI labeled axons were visible only as large nerve bundles (Figure 2A–B) or entering intramural ganglia (Figure 3F–H). Smaller PGP9.5 axon processes in sham animals (arrowheads in Figure 2C) were not DiI labeled (compared to Figure 2A), suggesting no direct innervation of the detrusor muscle in sham operated control animals. Similar results were seen in normal controls (data not shown). In contrast, Figure 2D–E shows DiI labeling in not only larger nerve bundles but also in smaller axons in association with the detrusor muscle in a root repair animal. These smaller axons were PGP-9.5 immunopositive (Figure 2F). Representative examples of double-labeled axons are shown with arrowheads in Figure 2D–F. Likewise, in GF-NT animals, DiI labeling and PGP9.5 double labeling of both larger nerve bundles and smaller axons in association with the detrusor muscle was observed (Figure 2G–I). A higher power of DiI and PGP9.5 double labeling of smaller axons in association with the detrusor muscle is shown in Figure 3A–D. Similar results were seen in CG-NT animals as in GF-NF animals (Figure 2J–L).

The quantitative results of the DiI labeling are shown in Figure 4. The sham operated animals contained many intramural ganglia receiving DiI labeled axons. No difference was observed between sham operated controls and normal controls (normal control data not shown). There were no DiI labeled axons observed in the denervated animals, suggesting complete denervation of the bladder in this group. However, the sham operated controls had many PGP9.5 immunostained small axons that were directly innervating detrusor muscle, none of which were DiI labeled (Figure 2C). The number of intramural ganglia receiving DiI labeled axons was not different between the sham operated controls and the transected and end-on-end sacral root repaired animals. In contrast, the number of individual detrusor muscle bundles receiving DiI +/PGP9.5+ axons was significantly higher in the root repaired animals compared to the sham operated controls ($p < 0.001$). There were approximately equal numbers of intramural ganglia receiving DiI labeled axons and detrusor muscle bundles associated with DiI +/PGP9.5+ axons in the GF NT animals, although the numbers were

statistically significantly lower than in the root repaired animals ($p < 0.001$ for both). There were also fewer ganglia with DiI labeled axons in the GF NT animals compared to sham operated controls ($p < 0.05$). The number of intramural ganglia with DiI labeled axons in the CG NT animals were also statistically significantly lower than the number of ganglia with DiI labeled axons in either the sham operated controls ($p < 0.001$) or root repaired animals ($p < 0.001$). Like the GF NT, there were fewer DiI +/PGP9.5+ axons associated with detrusor muscle bundles in the CG NT animals compared to sham controls ($p < 0.05$) or root repaired ($p < 0.05$) animals.

DISCUSSION

We previously showed that transection of the spinal roots innervating the bladder followed immediately by end-on-end repair results in functional reinnervation as evidenced by increased bladder pressure upon functional electrical stimulation one year after nerve transection and repair (Ruggieri et al., 2006). We also reported that bladder reinnervation can be achieved by immediate nerve transfer using either intercostal nerves or coccygeal nerve roots in the lumbosacral spine as well as peripheral genitofemoral nerves in the lower abdomen (Ruggieri et al., 2008a; Ruggieri et al., 2008b). The present report was designed to determine which cells in the bladder wall become reinnervated following these procedures. Our results show that, in contrast to the normal bladder innervation state in which all neurons running in the pelvic nerve innervate intramural postganglionic (parasympathetic) ganglia cells, after bladder reinnervation by either the same nerve root that normally innervates the bladder or by nerve transfer using different nerves, both bladder detrusor muscle as well as intramural ganglia become reinnervated.

There were over twice as many bladder detrusor muscle as intramural ganglia innervated by DiI labeled axons in animals with CG NT than with GF NT in which approximately equal numbers of detrusor muscle and ganglia were innervated (Figure 4). Thus it appears that reinnervation from a somatic nerve that formally innervated primarily skeletal muscle, such as the coccygeal nerve, results in a preferential direct reinnervation of bladder detrusor muscle over indirect bladder reinnervation via intramural postganglionic cells (which would not be DiI labeled since the DiI labeling is present only in the preganglionic axons). On the other hand, bladder reinnervation by a nerve that primarily serves a sensory function, such as the genitofemoral nerve (although there are autonomic motor nerves in the GF nerve that innervate blood vessels), results in an approximately equal degree of direct bladder detrusor muscle and indirect bladder intramural ganglia reinnervation. It is well known that nerve sprouting occurs in skeletal muscle after damage to the somatic nerve; however, we do not know whether the direct smooth muscle innervation that we observed is comparable to this skeletal muscle nerve sprouting.

It is possible that the DiI labeled axons that are directly innervating the detrusor muscle in the bladder wall are afferents, since DiI is a lipophilic dye that diffuses in the lipid bilayer and since nerve afferents have been shown to innervate bladder muscle directly in humans and rats (Dixon and Gilpin, 1987; Gabella and Davis, 1998). However, these same sensory axons should also be present in the sham operated and non-operated controls; however, no DiI labeling of muscle bundles were observed in the controls. In the bladder, it has been shown that ablation or destruction of the sensory nerve fibers abolishes the voiding reflex, even though these afferents constitute only a small part of the total innervation of the detrusor muscular layers (Gabella and Davis, 1998). There are even suggestions that bladder afferents have efferent roles necessary for micturition (de Groat et al., 1981). Interestingly, in small intestines, DiI labeling of the deep muscular plexus demonstrates that a non-ganglionated, Substance P immunoreactive population of nerve fibers innervates the circular muscle layer directly (Brookes et al., 1991). This population is postulated to function as

excitatory motor neurons and appears to be a subset of cholinergic neurons (Brookes et al., 1991). In future studies, in order to fully identify the functional type of nerves that are directly innervating the smooth muscle cells, we will characterize these subsets of neurons using immunohistochemistry in combination with an *in vivo* method of DiI labeling. We attempted calcitonin-gene related peptide (CGRP) immunohistochemistry on these sections (data not shown); unfortunately, the long incubation in fixative for 6 weeks at 37°C used for the current post mortem method of DiI labeling hindered antigenicity and antibody labeling. Nevertheless, even if some of the observed re-grown axons are afferents, our use of root repair and/or nerve transfer allows the regrowth of pelvic nerve axons into the bladder wall, some of which are reinnervating intramural ganglionic cells.

CONCLUSION

These current results and our recent findings (Ruggieri et al., 2008a; Ruggieri et al., 2008b) support and expand previous findings showing that transferred somatic motor axons can successfully reinnervate bladder ganglia after denervation in animal models (Conzen and Sollmann, 1982; Hou et al., 2000; Xiao et al., 1999) and in human spinal cord injured patients using either the L7 (Xiao et al., 2005; Xiao and Godec, 1994) or the S1 ventral root (Lin et al., 2009) or the 11th and 12th intercostal nerves (Livshits et al., 2004). In this study, we show that a variety of nerves can be used fairly successfully in nerve transfer surgeries to reinnervate the bladder. This gives surgeons more options to choose nerves or roots that bypass the area of spinal cord or nerve damage. We do not know yet the effect of the altered pattern of muscle innervation except that the bladder empties when the nerves are electrically stimulated (Ruggieri et al., 2008a; Ruggieri et al., 2008b; Ruggieri et al., 2006). It is not known whether the new pathway induces bladder contraction normally with neuronally released acetylcholine activating bladder smooth muscle muscarinic receptors or whether the direct innervation of detrusor smooth muscle involves other neurotransmitters and receptors. Future studies will address this and determine whether spontaneous voiding might appear in animals following nerve repair and reinnervation.

Skeletal muscle has been shown to alter morphologically after heterotopic nerve transfer, a change that still allows adaptive motor readjustment with positive clinical outcomes (Zhang et al., 2004; Zhang et al., 2006), so perhaps bladder detrusor muscle can also undergo adaptive motor readjustment. Also, as shown by Malessy et al. (Malessy et al., 1998), in a human study in which intercostal nerves were transferred to musculocutaneous or medial pectoral nerves following brachial plexus injuries, the central nervous system appears able to “rewire” over time to meet the functional needs of the neuromuscular system.

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Abbreviations

CG NT	coccygeal nerve transfer
GF NT	genitofem

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Figure 1.

Representative transverse histological sections of the detrusor muscle of the bladder. A> Sham control intramural ganglia stained with H&E. B> A serial section of the same ganglia stained for neurofilament (NF) 200. C> Low power micrograph of NF stained nerve bundle (arrow) in detrusor muscle wall. D> Higher power of area indicated with arrow in C. Similar photomicrographs are shown for a root repair animal (F–I), genitofemoral nerve transfer (GF-NT) animal (J–M), and a coccygeal nerve transfer (CG-NT) animal (N–Q). E> A negative control section showing a nerve bundle (n) in which the primary anti-neurofilament antibody was not included. Abbreviations: n -nerve bundle, ct – connective tissue, m – muscle bundle. Scale bars in C,H, L and P are 100 μ m; the remainder are 10 μ m.

Figure 2.

Representative transverse histological sections of the detrusor muscle showing DiI or PGP9.5 labeled axons, and DAPI staining, a general nuclear stain. A> Sham operated control dog in which only a DiI-labeled larger nerve bundle is seen (arrow). B> DiI and DAPI labeling in same field as A showing no detrusor muscle bundles (M) receiving DiI labeled axons directly. C> PGP9.5 labeling in same field showing smaller axons on detrusor muscles (arrowheads) that are not DiI labeled and the larger DiI labeled nerve bundle shown in A and B (arrow). D> Root repair animal showing both DiI labeled nerve bundles (arrow) and DiI labeled smaller axons in association with detrusor muscle bundles (arrowheads). E> DiI and DAPI labeling of same field as D. F> Same field showing PGP9.5+ axons in same locations as the smaller DiI labeled axons on detrusor muscles (arrowheads) and the larger DiI labeled nerve bundle (arrow). G> GF-NT animal showing both DiI labeled nerve bundle (arrows) and DiI labeled smaller axons in association with detrusor muscle bundles (arrowheads). H> DiI and DAPI labeling of same field as shown in G. F> PGP9.5 labeling in the same field showing double labeling with the smaller DiI labeled axons on the detrusor muscles (arrowhead) and the larger DiI labeled nerve bundle shown in G and H (arrows). J> CG-NT animal showing DiI labeled smaller axons in association with detrusor muscle bundles (arrowheads). K> DiI and DAPI labeling of same field as shown in J. L> PGP9.5 labeling in the same field showing double labeling with the smaller DiI labeled axons on the detrusor muscles (arrowheads). Scale bars = 50 μ m. All photomicrographs taken at same magnification. Abbreviations: GF-NT = genitofemoral nerve transfer, CG-NT = coccygeal nerve transfer; M=muscle.

Figure 3.

Higher power photomicrographs of detrusor muscle and intramural ganglia showing DiI or PGP9.5 labeled axons, and DAPI staining, a general nuclear stain in a GF-NT animal (A–E) and sham operated control animal (F–H). A> DiI labeling in a representative transverse histological section of detrusor muscle fibers (M as indicated in panel D) from a GF-NT animal. The detrusor muscle fibers are adjacent to a portion of an intramural ganglion (arrow; G as indicated in panel D). B> PGP9.5 labeling in the same field. C> Merged DiI and PGP9.5. D> DAPI staining in the same field. E> Merged DAPI and DiI. F> DiI labeled axons in an intramural ganglia from a sham operated animal. G> DAPI staining in the same field. H> Merged DAPI and DiI. Scale bars = 50 μ m.

Figure 4.

Number of DiI innervated intramural ganglia or detrusor muscle fibers following insertion of Neurotrace DiI into pelvic nerves proximal to their entry into the bladder. Bars represent the mean $\pm$ standard error of the mean for each surgical procedure. 0 = no innervated ganglia or detrusor muscle cells counted. Bonferroni post hoc analysis shows: a: $p < 0.05$ and b: $p < 0.001$ compared to sham, c: $p < 0.05$ and d: $p < 0.001$ compared to root repair. Abbreviations: DEN = denervated control animals, GF-NT = genitofemoral nerve transfer, CG-NT = coccygeal nerve transfer.