Functional Reinnervation of the Canine Urinary Bladder

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Abstract

This study was performed to determine whether nerve transfer after nerve root transection leads to bladder reinnervation. In one animal, the T12 intercostal nerve was mobilized on the left side and attached to the to the severed ends of the nerve roots inducing bladder contraction through a graft from the T11 intercostal nerve. On the right side and bilaterally in 2 other dogs, coccygeal nerve roots innervating the tail musculature were attached to the severed bladder nerve roots (CG NT). In addition, bladder nerve roots were transected and the genitofemoral nerve was transferred to the pelvic nerve (GF NT) either immediately (N=4), 1 month (N=4) or 3 months (N=6) after denervation. Nerve cuff electrodes were implanted on the transferred nerves proximal to the pelvic nerve transfer site. Because of differences in the distance of nerve regrowth, reinnervation proceeded for 14 months for CG NT and 4.5 months for GF NT. FES induced bladder pressure and urethral fluid flow in the majority of the nerve transfer sites. Reinnervation was further shown by retrograde labeling of spinal cord neurons following fluorogold injections into the bladder wall. These results clearly demonstrate functional bladder reinnervation by immediate nerve transfer using either somatic nerves or a primarily sensory nerve (the genitofemoral nerve) up to 3 months after denervation.

1 Introduction

One of the major medical and social problems of the vast majority of spinal cord injured (SCI) patients is neurogenic bladder dysfunction. A recent survey of SCI patients indicates that restoration of bladder function is an important priority and in paraplegics is considered more important than recovery of walking movement [1].

Bladder stimulation strategies with neuroprosthetic implants that can achieve bladder emptying have been developed for patients with spastic bladders resulting from “upper motor neuron” lesions in which the sacral cord cell bodies innervating the bladder are damaged or their axons are severed. The goal of this investigation is to develop a strategy for bladder reinnervation and restoration of emptying function in this latter group of patients.

In the immediate management of patients with spinal cord injuries, patients are typically observed for a period of time to determine whether voluntary control of bladder function returns. For this reason, bladder reinnervation surgeries are not likely to be performed immediately after the injury. Therefore reinnervation surgery was performed 1 and 3 months after bladder denervation to determine whether this type of bladder reinnervation surgery has potential clinical feasibility.

2 Methods

For bladder denervation, the S1 and S2 ventral roots innervating the bladder were exposed with a 30 degree V-laminectomy of the T7 vertebral body and a partial laminectomy of the T6 and S1 vertebral bodies. The two bilateral ventral roots that induced increased bladder pressure upon intraoperative electrical stimulation were transected. Completeness of bladder denervation was confirmed by root 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.

For 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 transected bladder nerve roots in the lumbosacral spine (2 per side, 4 total). In one of the 3 animals, nerve transfer on the left side was done using intercostal nerves. Nerve cuff electrodes were placed around the 2 root bundles on each side, proximal to the anastomosis site.

For GF NT, after bladder denervation, the animal was repositioned to the supine position and the GF nerves were located and attached to the pelvic nerves as they emerged from the pelvic plexus towards the urinary bladder. The implanted electrodes were stimulated approximately 4 months postoperatively (1.2mA, 20 Hz, 0.5 ms quasi trapezoidal wave trains of 20 second duration) while monitoring bladder pressure to determine return of bladder function.
Immediately before the animals were euthanized, the pelvic nerves leading to the bladder were also evaluated for their ability to induce bladder pressure and urine flow by intraoperative electrical stimulation using a unipolar probe electrode (15V, 20Hz, 1ms square wave trains).

Fluorogold retrograde neuronal tracing from the urinary bladder to the spinal cord was performed on all animals as previously described [3]. For the CG NT animals, a post nerve transfer period of approximately 14 months was allowed. For the GF NT animals, a post nerve transfer period of only 4-5 months was allowed before the animals were euthanized. Spinal cord sections were analyzed quantitatively for the presence of fluorogold retrogradely labeled motor neuronal cell bodies. In addition, postmortem neurotracing from the pelvic nerve to the bladder was performed using the lipophilic dialkylcarbocyanine dye, Neurotrace DiI.

3 Results

Representative traces of the bladder pressure responses to FES of the implanted electrodes are shown in figure 1. This data along with the fluorogold retrograde tracing results are summarized in figure 2.

FES of the implanted electrodes induced bladder contraction bilaterally in one of the 3 CG NT animals and one of the 2 immediate GF NT animals. Of the 8 electrodes implanted in the 4 animals with GF NT performed one month after denervation, FES induced bladder pressure increases in 3 (bilaterally in one and right side of one). However, FES of the pelvic nerve induced bladder pressure and urethral fluid flow on the intercostal nerve transfer side, in 5 of the 5 CG NT, 6 of 8 immediate GF NT, 6 of 8 one month delay GF NT and in 10 of 12 three month delay GF NT sites. This indicates electrode failure in 18 of the 30 implanted electrodes. Failure to induce bladder contraction with FES of either the implanted electrodes or the pelvic nerve prior to euthanasia was only observed in 3 animals – one animal in each of the GF NT groups (figure 2).

**Figure 2.** Overall 14 of the 17 (82%) animals with nerve transfer shown in table 1 showed functional reinnervation by increased bladder pressure with functional electrical stimulation (FES) of the pelvic nerve prior to euthanasia. ICNT: intercostal nerve transfer, CGNT: coccygeal nerve transfer, GFNT: genitofemoral nerve transfer.

**Figure 3.** Bars are mean ± SEM for n=3 sham, n=6 sacral root repair, n=4 GF NT and n=3 CG NT animals. Results of Bonferroni t-tests are indicated as significantly different from sham (*=p<0.05, **=p<0.01) and from root repair (#= p<0.05, ##=p<0.01). The bladder becomes reinnervated by motor neuronal cell bodies in the spinal cord section specific for the repaired roots or transferred nerves – the sacral cord for the repaired sacral roots, the lumbar cord for the GFNT and the coccygeal cord for the CGNT.

**Figure 4.** Bars represent the mean ± standard error of the mean for 4 fields for each animal. 1 sacral root repair animal, 1 denervated control, 1 sham (root-intact) control, 2 coccygeal nerve transfer animals, and 2 genitofemoral nerve transfer animals. Results of Bonferroni t-tests are indicated as statistically significantly different from sham (***=p<0.05, ****=p<0.01), denervated (##=p<0.05, ###=p<0.01) and transected and repaired (++=p<0.05, +++=p<0.01).
The number of nerve cell bodies per section retrogradely labeled with fluorogold are shown in figure 3 for the CG NT and immediate GF NT groups compared with sham operated controls as well as our previous study of animals with nerve transection followed by immediate repair [3].

Figure 4 displays the quantitation of bladder myocytes versus ganglia that became labeled with the lipophilic dialkylcarbocyanine dye, Neurotrace DiI applied to the pelvic nerves. This demonstrated innervation of only intramural ganglia in sham operated controls but also myocytes in repaired roots. GF NT and CG NT reinnervated both myocytes and ganglia to a lesser extent.

4 Discussion and Conclusions

While a cure for spinal cord injury induced paralysis is certainly one of the ultimate goals of research in this area, an improvement in the quality of life by promoting functional recovery in the short term is a more realistic goal that may be achievable in a more immediate time frame. The results of our study clearly demonstrate that after transection of the spinal roots innervating the urinary bladder, reinnervation can be accomplished 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. Successful reinnervation by GF NT 1 and 3 months after denervation provide initial proof of concept that genitofemoral to pelvic nerve transfer is a potentially viable clinical approach to reinnervation of the lower motor neuron lesioned urinary bladder. The 1 month time point was chosen as the shortest practical time point to allow the animal to completely recover from the first surgical procedure. The 3 month time point was chosen since in human patients, following a traumatic spinal injury, this is the amount of time that some return of bladder function might be expected to occur if it is to occur at all [4, 5].

In our previous report [3] and in the present study, in all animals only 2 nerve roots on each side of the spinal cord (S1 and S2) could be identified that induced increases in bladder pressure upon intraoperative electrical stimulation during the denervation procedure. In our previous study as well as in this present study, stimulation of the entire conus medullaris with an epidural spinal electrode was capable of inducing bladder contraction before transection of these nerve roots but did not induce contraction after the roots were severed. Although this finding demonstrates functional denervation of the bladder, it is possible that in some animals a few motor fibers exit the sacral cord and reach the urinary bladder through nerve roots other than S1 and S2. However, these would be insufficient in number to induce a measurable increase in bladder pressure upon conus medullaris stimulation. The possibility exists that these few fibers may gain a greater functional effectiveness during the recovery period such that they may be capable of inducing bladder contraction.

In the GF NT group there were a very few number of fluorogold labeled coccygeal cord cell bodies. It is possible that these coccygeal nerves were present before the nerve transection. The possibility exists for the few number of labeled coccygeal cell bodies as well as for the few labeled sacral cord cell bodies, that these cells sprouted axons in the lumbosacral cord and reinnervated the bladder through the severed S1 and S2 roots following the denervation. However, because there were 2-18 fold more labeled cell bodies in the lumbar than the sacral cord and 9-50 fold more labeled cell bodies in the lumbar than the coccygeal cord in these animals, bladder reinnervation by the genitofemoral nerve is the most likely explanation for the successful pelvic nerve stimulation induced bladder pressure increases in these animals.

The findings of this study paves the path for clinical application of this nerve transfer surgery in human patients with LMN lesions resulting in flaccid bladder paralysis. This would provide some degree of motor control of urinary bladder function but would likely not provide motor control of the function of the bladder outlet that is involved in controlling urinary continence. To achieve voluntary control of urinary continence as well as bladder emptying will require development of additional strategies for reinnervation of the pudendal nerve supplying innervation to the external urethral sphincter striated muscle.

References


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