Published in final edited form as:

Neurol Urodyn. 2018 January ; 37(1): 153–162. doi:10.1002/nau.23394.

Lumbar to sacral root rerouting to restore bladder function in a feline spinal cord injury model: urodynamic and retrograde nerve tracing results from a pilot study

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Abstract

Introduction & objectives—Lumbar to sacral rerouting surgery has the potential to allow voiding via a skin-central nervous system-bladder reflex pathway. Here, we assessed if this surgery was effective in treating neurogenic bladder in felines.

Methods—Eight cats underwent spinal cord transection (SCT) at thoracic level 10/11. Unilateral L7 to S1 ventral root anastomosis was performed 1 month later in 6 cats. Two cats served as transection-only controls. Electrical and manual stimulation of L6-S1 dermatomes, and urodynamics were performed at 3, 5, 7 and 9/10 months post transection. At 9/10 months, cats were also evaluated by direct electrophysiological testing of anastomosed roots with urodynamics, then tissue collection and examination of the root anastomosis site and lumbosacral cord ventral horns for cells retrogradely labeled from tracer dye injected two weeks earlier into the bladder wall.

Results—At 9/10 months, 4 of 6 rerouted cats exhibited increased detrusor pressure provoked by cutaneous stimulation, one cat bilaterally. Two cats presented with a voiding stream after ipsilateral cutaneous stimulation at 7 and 9 months. All 6 rerouted animals showed regrowth of axons from the L7 ventral horn to the bladder, although some aberrant axonal regrowth was also observed.

Conclusion—L7 to S1 ventral root rerouting below the level of SCT showed successful axonal regrowth to the bladder from the L7 spinal cord segment in all rerouted animals, and induced increased detrusor pressure response to cutaneous stimulation in a subset. This feasibility study paves the way for future animal studies for bladder reinnervation.

Keywords

Spinal cord injury; spinal cord transection; rerouting surgery; neurogenic bladder; voiding dysfunction; cat

Introduction

Spinal cord injuries (SCIs) induce voiding dysfunctions including retention, infections, incontinence, urolithiasis and renal impairment (1). These impact patients' quality of life and increase costs (2). Therapies can include: clean intermittent catheterization, anti-muscarinic drugs, botulinum toxin injections (3), Brindley neuro-stimulator implantation requiring dorsal rhizotomy (3–5) and chronic urethral or supra-pubic catheterization.

Neural re-innervation is effective in the peripheral nervous system (6). Lumbar to sacral rerouting theoretically allows voiding via a skin-spinal cord-bladder reflex pathway and may allow volitional voiding (7). Human experiments with this technique report either high, 67%–100% rates of success (1) or total inefficacy (8–16). Studies in animal models show promise (17–20) but methodological limitations include transection of the spinal cord a few months after rerouting rather than before, as would occur in SCI patients and not measuring voiding as an endpoint, preventing extrapolation SCI patients. Recent international guidelines do not mention nerve-rerouting surgery for neurogenic bladder management (3).

We sought to assess whether lumbar to sacral ventral root rerouting is effective in treating neurogenic bladder after SCI in a feline model, using a protocol that mimics the clinical situation.

Materials and methods

Study design

Figure 1A summarizes the design using young, healthy, female, adult cats, 2–3 years old, 2.8 to 6.1 kg. Initial cystometry coupled with electromyography (EMG) of the urinary sphincter excluded one cat based on spontaneous bladder contraction during filling and <40 ml bladder capacity.

Spinal cord transection

Cats were anesthetized with Butorphanol (0.4 mg/kg), and Isoflurane 3.0–5.0% (1.5–2.0% MAC) then the spinal cord was transected at T10 or 11 vertebrae with interposition of Gelfoam (Fig. 1A). Postoperative drugs included buprenorphine (5–10 µg/kg Q 8–12 hrs) for 2 days and amoxicillin trihydrate and clavulanate potassium (15–20 mg/kg) for 7 days.

Rerouting surgery

One month after spinal cord transection, animals were either rerouted (n=6) or not (n=2). The nerve rerouting procedure was performed intradurally, as described (18), (Figure 1A). The sacral recipient root was chosen on the basis of cysto manometric bladder contraction in response to electrical stimulation of the S1 through S3 nerve roots. S1 gave the highest response typically and was chosen as the sacral recipient nerve root. The L7 lumbar donor nerve root was chosen on the basis of lower extremity contraction in response to ventral root electrical stimulation. To prevent neuronal cell death in the spinal cord ventral horns, root transection was performed at least 1 cm away from the spinal cord. The proximal ventral root of the donor nerve (L7) was then sutured end-on-end to the distal ventral root (S1). All remaining roots below L7 were transected bilaterally, as were L6 roots (Figure 1A), to isolate the functional components of the sensory-motor reflex arc, as described by Xiao et al (18).

Follow-up testing

Throughout the study, voiding was induced using the Crd maneuver, 3 times daily which was also used to assess post void residual (PVR) volume, confirmed by catheterization at each cutaneous stimulation evaluation. Animals were evaluated at 3, 5, 7, 9 and 10 months post-transection surgery by monitoring increased detrusor pressure or triggered voiding in response to manual scratching and electrical cutaneous stimulation of L6-S1 dermatomes. Filling urodynamics employed a 10 ml/min fill rate in un-anesthetized animals using a 6 French catheter with rectal catheterization and urethral sphincter electromyography. With the bladder filled to between 50% and 75% of capacity, the presence, frequency and amplitude of spontaneous detrusor contractions and resting bladder pressure was first assessed. Several bilateral scratching or electrical stimulations of L6, L7 and S1 dermatomes were delivered. This included 15 to 20 seconds of scratching stimulation with a wooden stick and 5 to 10 seconds of electrical stimulation of the same cutaneous region (5–15 volts, 40 Hz, Grass stimulator, bipolar electrodes). We also monitored incidence of a triggered voiding stream (TVS) during the cutaneous scratching or electrical stimulation. Delayed voiding streams were considered artifacts secondary to uninhibited contractions. The presence of detrusor-sphincter synergy was also monitored and was defined as urethral sphincter electromyographic silence during voiding streams provoked by scratching. Three weeks before euthanasia, animals were anesthetized and 4 injections of 4% (w/v) Fluorogold (Setareh Biotech, Eugene, OR, USA) were made lateral to each ureteral orifice (50 µL/injection).

At euthanasia (9 or 10 months post transection), ventral roots of L6-S1 spinal cord segments were stimulated electrically (1 to 4 mA, 40 Hz, 2-mS trains, 6–12 seconds) to evaluate nerve-evoked detrusor contractile function. The roots were then transected and nerve-evoked detrusor contraction was reassessed by stimulation of the distal and proximal ends of the transected roots. For the L7-to-S1 anastomosed roots, care was taken to transect proximal to the anastomosis site.

Tissue collection and analysis

Animals were euthanized with Euthasol (pentobarbital and phenytoin) then perfused intracardially with saline then with 4% Phosphate buffered paraformaldehyde. The spinal columns and bladders were immersion fixed for at least 3 days. The bladders were then weighed and the width, length and height was measured after removal of all fat and loose connective tissues. Sidedness of rerouting procedure, location of the anastomosis site, and presence of any scar tissue were noted and recorded as shown in Figure 1A. The spinal cord from lumbar (L) 1- L7, sacral (S) 1-S3 to coccygeal (CG) regions were removed from the vertebral column with roots and dorsal root ganglia attached. The anastomosis site was photographed again using the Olympus BH-2 stereomicroscope before spinal cord-root complexes were cryopreserved in 30% sucrose and cryosectioned into 20 μm longitudinal sections. Sequential sections were mounted onto charged slides. Every third section was analyzed for presence of Fluorogold labeled neurons in ventral horns of spinal cord segments of L6-S2, using previously described stereological methods (21). The mean and standard error of estimated number of Fluorogold labeled neurons per volume (mm^3) of spinal cord segment is reported. Adjacent sections were stained with Hematoxylin and eosin (H&E) and examined for spinal cord integrity or integrity of the anastomosis site using an E600 Nikon Bright-field microscope with a digital camera (DS-Ri2, Nikon) and imaging analysis system (NIS-Elements: Basic Research).

Statistical analysis

Given the conflicting results of investigations in humans (0 to 100% efficacy), it was not possible to assume a success rate in our series and, therefore, the number of cats that needed to be included. We decided to study 9 cats in this pilot study, with 6 as re-routing cases and the remaining as spinal cord transected-only controls. These numbers were decided *a priori*; thus, when one cat had to be excluded due to the presence of a baseline neurogenic or neurological bladder disorder, that animal was excluded from the control group. Two way ANOVAs were used for the statistical analyses (Prism 7, Graphpad, La Jolla, CA, USA). The maximum detrusor pressure (MDP) following scratching or electrical dermatomal stimulation results were compared using the factors: group and time post transection surgery. Sidedness was compared as part of the post hoc assays (Fishers LSD test). The MDP following electrical stimulation of roots at the terminal surgery was compared using the factors: side and nerve root segment. The number of Fluorogold labeled neurons was compared using the factors: group and cord ventral horn segment.

Results

Three cats underwent intradural anastomosis of the ventral root of L7 to the ventral root of S1 (L7-to-S1; Figure 1) on the right side, and two cats received this same rerouting on the left side, as confirmed post-euthanasia during histological analyses. In Cat 3 that underwent right-sided rerouting, the left L7 proximal ventral root attached itself to the right distal S1 root during the healing process so that both the right and left L7 ventral roots were attached to the right S1 ventral root by 9 months post rerouting surgery. Therefore, the data from the left side of Cat 3 is included with the right side data from this same cat, rather than with other contralateral data. All cats survived the 9 or 10 months postoperatively. Five rerouted

animals (88.33%) experienced at least 1 urinary tract infection (10 total) all resolved by antibiotic treatment.

Response of bladder pressure to cutaneous stimulation of L6-S1 dermatomes

ANOVAs revealed significant differences in MDP in rerouted versus transected animals during scratching ($p=0.03$) and electrical stimulation ($p<0.0001$). Post hoc analyses showed that MDP was elevated in 4 of 6 cats (67%) with ipsilateral L7-S1 dermatome stimulation at the 9/10-month end point, while no increases were observed in transected-only controls. The mean MDP (cm H₂O) at 9/10 months with stimulation of L6-S1 dermatomes was: rerouted cats ($n=5$) 37.2 ± 10.5 ipsilateral scratching and 42.2 ± 5.8 ipsilateral electrical stimulation and 9.8 ± 7.6 contralateral scratching and 14.7 ± 6.1 contralateral electrical stimulation. There were no responses in the 2 transection control cats (Figure 2).

Occurrence of triggered voiding during cutaneous stimulation of L6-S1 dermatomes

At 7 and 9/10 months post transection, a triggered voiding stream (TVS) was observed in Cats 5 and 6 with both scratching and electrical stimulation of the ipsilateral L6-S1 dermatomes. Cat 6 also showed a TVS with manual stimulation of the contralateral L6-S1 dermatomes at both 7 and 9 months post transection. A voiding stream was observed simultaneously with detrusor-sphincter synergy. Credé revealed no PVR in these cats. No triggered voiding was noted in the transected only controls.

Electrical stimulation of spinal roots

Electrical stimulation of the ipsilateral L7 root induced the greatest increases in detrusor pressure in Cats 1, 2, 4 and 6. There was evidence of aberrant regrowth across some of the transected roots such that stimulation of ipsilateral L6 and S1 roots in Cat 1 increased detrusor pressure, as did stimulation of the ipsilateral S2 root in Cat 3, and ipsilateral S1 root in Cat 4 (Fig. 3A left panel). There was evidence of aberrant regrowth across some of the contralateral transected roots (Fig. 3B right panel), such as increased detrusor pressure following stimulation of the contralateral L6 and L7 roots in Cat 1, and contralateral L7 and S1 roots in Cat 4. Thus, no significant differences were observed per side or segment. The mean MDP (cm H₂O) at 9/10 months with electrical stimulation of the L7 root in rerouted cats was: 17.7 ± 9.0 ipsilaterally ($n=6$) and 18.0 ± 8.3 ($n=5$) contralaterally (Fig. 4B). The roots of transected only controls were not tested at euthanasia.

These increases in detrusor pressure were reduced to near zero following intradural transection of nerve roots that had previously induced an increase (Fig. 4C). Stimulation of the distal end of these cut roots showed maintenance of nerve-evoked bladder contraction. After all L6 to S1 roots were cut bilaterally, direct stimulation of the spinal cord was tested in two animals and showed a loss of nerve-evoked bladder contraction from 60 to 7 (Cat 1) and from 15 to 0 (Cat 2).

Histological and retrograde tracing results

H&E staining of the L7-to-S1 anastomosis site revealed root repair of varying degrees in each animal, some excellent (Fig. 4A–C) and some with scar tissue (Fig. 4D). As expected, the transection sites of unrepaired roots (L6 and S2 roots, bilaterally, and contralateral L7

and S1 roots) did not show evidence of the 0.5 cm Gelfoam placed there during the original surgery at 8–9 months before tissue collection (Fig. 4E). Instead, the cut ends of unrepaired roots were in close proximity and contained a large number of disordered axons and scar tissue (Fig. 4E). H&E staining of the spinal cord was used to determine if there was any damage in the lumbosacral spinal cord segments as a result of the earlier spinal cord transection or laminectomy procedures that might affect the dye-tracing or electrophysiological results (Fig. 4F). None were observed. Longitudinally cut spinal cords of all animals showed intact undamaged ventral horn segments from L6 to S1 and the presence of many hematoxylin-stained neuronal cell bodies within the ventral horns (Fig. 4F).

Fluorogold labeled neuronal cell bodies in the L7 ventral horn retrogradely labeled from the bladder were observed in all 6 rerouted animals (Fig. 4G & 5). Some aberrant axonal regrowth was observed in adjacent S1 and S2 segments and even contralaterally in several animals (Fig. 5B and C), although only Cat 6 showed a large number of retrogradely labeled cells in contralateral S1 and S2 segments. One of the spinal cord transected-only control animals showed dye labeling in all segments and their vasculature, perhaps due to intravenous dye injection, and was thus excluded from this comparison. A two-way ANOVA was significant by group and segment ($p=0.04$). Post hoc analyses revealed statistically significantly higher labeling on the ipsilateral side of the L7 segment in rerouted animals versus the spinal cord transected control animal ($p=0.01$, Figure 5C). There were also higher numbers of retrogradely labeled cells in the ipsilateral side of rerouted animals, compared to their contralateral side ($p=0.02$).

There were no significant differences in bladder volume or weight in the four rerouted animals in which this was assessed, and in the two transection-only control animals. Bladder volume and weight were significantly correlated ($r^2=0.70$, $p=0.04$).

Discussion

Although nerve rerouting for voluntary voiding is an attractive concept with potential to benefit neurogenic bladder patients, there is currently no clinically applicable technique (7). The aim of our study was to further evaluate a somatic-to-autonomic nerve transfer technique. Xiao's animal experiments, published in the 1990's, led to his clinical application of his technique in patients who lacked volitional voiding, including adults with spinal cord injury and children with spina bifida (1, 17, 18, 22–24). Xiao et al reported 67% to 86% of patients had voluntary control of micturition after undergoing nerve rerouting (1, 23). Other clinical investigators have not reproduced these results. Two meticulously performed trials of Xiao's technique in adults with spinal cord injury and one randomized, double blind trial in children with spina bifida failed to result in any meaningful, reproducible voiding in any patient. (11, 13–16). Because of these conflicting clinical results we studied Xiao's nerve rerouting technique in spinal transected cats. Unlike Xiao (18), we induced experimental spinal cord injury before the nerve transfer, to simulate the clinically relevant scenario.

Ipsilateral dermatome stimulation induced detrusor pressure elevation beginning 6 months after rerouting surgery in all six rerouted but none of the 2 SCT controls. At 9/10 months

even greater detrusor pressures were induced in 4 of 5 rerouted animals with scratching dermatomal stimulation, and in all 4 rerouted animals with electrical dermatomal stimulation. This timeframe is compatible with the speed of axonal growth and re-innervation. These results are cautiously consistent with those of Xiao et al. in 4 cats (18) and Lin et al. in 6 dogs (19, 20). Urodynamic testing In those studies was done much longer after rerouting (1 and 2 years, respectively), the spinal transection was performed after the rerouting surgery (over 2 years) and the statistical significance of the success rate was not reported.

In most mammals, including in cats, leg dermatomes overlap (25). Thus we combined the L6-S1 dermatomes in the cutaneous stimulation studies. We cannot exclude that some positive results may be the consequence of regrowth in the transected sacral ventral roots, since the transected/repared roots were in close proximity and since we observed electrophysiological and retrograde tracing results suggestive of aberrant regrowth. Root rhizotomy is also known to alter detrusor activity, although it seems unlikely to provoke micturition. Future studies should include methods to prevent regrowth across unrepaired roots such as ligation and larger distances between cut ends.

We were unable to uncover evidence of detrusor-sphincter synergy in one (Cat 5) of the cats with triggered voiding streams. This has to be balanced with our limited sample size and possible interference of our electro-stimulation protocol with electromyographic monitoring. Evidence of synergy concomitant with an elevation in detrusor pressure was observed during dermatomal scratching stimulation in Cat 6, indicating that this is possible in rerouted cats.

The small number of rerouted and control cats in our series is a limitation, but, it supports continuation of research in this field. Our control group included spinal cord injured cats, but it would have also been interesting to have a third group of cats that underwent complete sectioning of all lumbosacral roots, on both sides, from L6 all the way through the sacral roots. Investigators performing the scratching and electrical stimulation maneuvers were not blinded to which treatment arm the cat was assigned. We also acknowledge that our quantification of the PVR in the 2 voiding cats was performed by Crd maneuver only. We confirmed the accuracy of this technique, yet in future larger scale animal studies, a more accurate technique could be considered, such as a bladder scan.

A strength of our study is the first investigation in animals where the spinal cord transection was performed before rerouting, as would be the case in clinical situations. Our assessments included longitudinal neuro-urological methods, both ipsi- and contralateral dermatome testing, terminal electrophysiologic assays of the roots, histological assays and retrograde dye tracing methods.

In conclusion, our study demonstrates that L7-to-S1 rerouting surgery below T10/11 spinal cord transection induces elevated bladder pressure in all rerouted cats and voiding in some cats after ipsilateral dermatome stimulation. These results are encouraging and justify a larger investigation with more animals, a control group with rhizotomy and longer follow-up (2 years). Only similar or better results, in a larger animal cohort, will support progression to a clinical study.

Acknowledgments

Research reported in this publication was supported by Lady Davis Research Institute and McGill University to JC, by the National Institute of Neurological Disorders and Stroke of the National Institutes of Health under award number NS070267 to MRR and MFB, and by the French Association of Urology and Groupe Pasteur Mutualité to OLVB. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Figure 1.

Design and a rerouted root example. (A) Spinal cord surgery methods. Spinal cords were transected at T10 or T11. One month post spinal transection, the proximal ventral root of L7 was cut and transferred to the distal ventral root of S1, and anastomosed end-on-end (termed rerouted), intradurally. The remaining dorsal and ventral roots of L6, S1 and below were transected proximal to the dorsal root ganglia, thereafter. The L7 dorsal root ganglion (DRG) was left intact. (B) An example (Cat 6) showing a left L7 ventral root transfer with anastomosis to a left S1 ventral root. The transferred L7 root was cut proximal to the

anastomosis site during terminal electrophysiological testing. The intact L7 dorsal root and its DRG is shown at left, the caudal end of the cord is shown at right and is marked blue with a histological tissue marker.

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Figure 2. Maximum detrusor pressure (cm H₂O) occurring in response to stimulation of anterior thigh dermatomes L6-S1 at 7 and 9/10 months post transection surgery. Cat 1 did not undergo scratching or electrical stimulation at 9 months. (A) Scratching stimulation of these dermatomes. (B) Electrical stimulation of these dermatomes. Results for each cat is shown individually, ipsilaterally and contralaterally. Incidence of a triggered voiding stream (TSV) in response to stimulation is noted on occurrence. Results of two-way ANOVAs and post hoc tests are shown. (C) Cysto manometry coupled with electromyography of the urethral

sphincter showing increased detrusor pressure associated with detrusor sphincter synergy and a triggered voiding stream (TSV; also indicated as a “Leak” on the figure) that was triggered by scratching stimulation of the left L7 dermatome in Cat 6.

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Figure 3.

Maximum detrusor pressure (cm H₂O) occurring in response to direct electrical stimulation of L6 to S1 ventral roots at 9/10 months post transection surgery. Testing was performed both extradurally and intradurally following a laminectomy. (A) Results for each cat shown individually for ventral roots, bilaterally, except for Cat 3 for which only ipsilateral data is presented here, as described in the text. (B) Mean results for direct electrical stimulation of intact roots are shown, prior to terminal root transection. There were no significant (n.s.) differences between groups in using the factors, transection group, side or segment. (C) The roots were transected and retested via direct electrical stimulation of their proximal end (not all animals or roots were assayed post root transection). Previously increased bladder pressure in these same roots is now mostly absent, although a low level of firing in the proximal end of the left L6 root was preserved in Cat 1.

Figure 4. Representative microscope images showing the root anastomosis site in three different L7-to-S1 rerouted animals. (A) A representative image from Cat 1 showing regrowth of axons across the ventral root anastomosis site in a H&E stained longitudinal section of the roots. The arrows indicate sutures. (B) Higher power photograph of the sutures indicated in panel A. (C) An image from Cat 2 showing a similar scenario as Cat 1 of clear regrowth across the anastomosis site. The sutures auto fluoresce in the ultraviolet wavelength allowing further confirmation of the surgery site. (D) An image from another cat (Cat 5) showing a kink at the nerve anastomosis site, yet regrowth of nerve axons to the right of suture site (a blue suture was used, arrow). (E) An image of a contralateral transected roots showing scar tissue between the cut ends, although close proximity and a large number of disordered axons. (F) Representative low power figure of longitudinal sections of the lumbosacral spinal cord

showing an intact undamaged ventral horn region from the L7 segment through the upper part of S1 and the presence of many hematoxylin-stained neuronal cell bodies. (G) Representative figure of Fluorogold labeled neuronal cell bodies in the L7 spinal cord segment. Inset shows a higher power image of Fluorogold labeled neuronal cell bodies.

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Figure 5.

Fluorogold labeled neurons in ventral horn regions of L6-S2 spinal cord segments at 9/10 months post transection. (A) Estimated number of Fluorogold labeled neuronal cell bodies is shown for each cat and segment analyzed on the side ipsilateral to the anastomosed roots. Bilateral results from the transected control animal in which the Fluorogold labeling was successful (Cat 7) is shown as mean $\pm$ SEM. Data from the cat in which both right and left ventral roots were annealed to the right S1 by the time of tissue collection (Cat 3) is shown with the ipsilateral data, as mean $\pm$ SEM. (B) Contralateral data. (C) Same data as in panels

A and B reorganized by segment and side. Results of two-way ANOVA and posthoc results are shown in panels A and C. * $p < 0.05$, compared to the transected control animal.

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