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FUNCTIONAL URINARY BLADDER REINNERVATION IN A CANINE MODEL

Aims of Study

The overall goal of this project is to use a canine model to determine the feasibility of nerve transfer for reinnervation of the urinary bladder and achieving bladder emptying with functional electrical stimulation (FES) of the transferred nerves. The goal of this initial study is to transect and immediately repair the bladder motor nerves to determine the feasibility and time course of normal bladder reinnervation.

Methods

After an L7 laminectomy the sacral nerves mediating bladder contraction were identified with unipolar electrical stimulation (2V, 20Hz, 1ms square wave trains) while monitoring bladder pressure. After neurorrhaphy of the transected bladder nerves a tripolar nerve cuff electrode was placed around the nerves proximal to the reanastomosis site. At monthly postoperative intervals these electrodes were stimulated under isoflurane anesthesia while monitoring bladder pressure to determine return of bladder function. One year after surgery the bladder was injected with fluorogold and the animals were euthanized 3 weeks later. For the nerve tracing studies, the unfixed spinal cord with the nerves attached was labeled with Fast DiO (green; Molecular Probes, Eugene, OR). The spinal cord and nerve specimen was then postfixed 5 days in 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). On the 5th day, CM-DiI cell labeling paste (red; Molecular Probes, Eugene, OR) was placed on the distal cut ends of the four surgically re-anastomosed nerves (2 on each side), 2-2.5 cm distal to the nerve surgery sites. The cord was incubated in fixative for 3 months at 37C. At the end of the 3 months, the cord and nerves were removed and cut with a vibratome into 180 m longitudinal sections. All tissues were examined using epi-fluorescence microscopy for DiI and DiO retrograde and anterograde labeled nerve processes, respectively, the cord sections were examined for fluorogold and DiI retrogradely labeled neuronal cell bodies.

Results

Three out of 4 animals showed return of bladder function 6 months post-surgery as evidenced by increased bladder pressure and bladder emptying during electrical stimulation of the bladder nerves (1.2mA, 20 Hz, 0.5 ms quasi trapezoidal wave trains). DiI labeled processes (red) were visualized in nerve roots proximal to the nerve surgery site (Figure 1A), indicating that axons had grown through the surgery site proximally toward the cord. Figure1B shows DiO labeled processes (green) in the same nerve root, indicating that the correct spinal cord segments had been labeled. There was some overlap of the dyes within the nerve root (Figure 1C), further indicating that motor neurons from the cord had regrown through the surgery site toward the bladder. A number of fluorogold labeled neuronal cell bodies were observed in the ventral horn of the sacral cord (Figure 1D), proving reinnervation of the bladder from the spinal cord. A small number of these fluorogold labeled neuronal cell bodies were also retrogradely labeled with CM-DiI (Figure 1E). The retrograde labeling with CM-DiI of these same neuronal cell bodies further confirms that nerve processes have regrown through the surgery site toward the bladder.

Conclusions

Transected bladder motor nerves in the sacral spine can be reanastomosed and are capable of functionally reinnervating the urinary bladder. These reinnervated bladder nerves can induce bladder emptying via functional electrical stimulation. This feasibility study paves the way for future studies utilizing other more proximal motor nerves to bypass the transection site for bladder reinnervation.

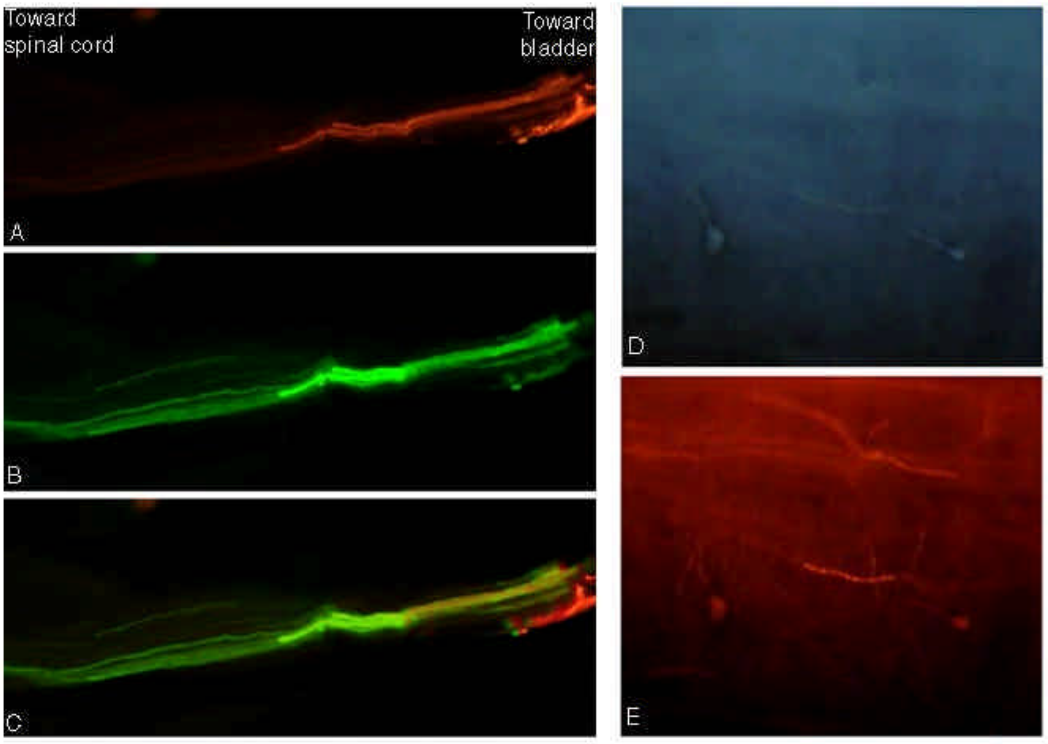

Figure 1. (A-C) Bladder motor nerve immediately proximal to the site of reanastomosis shows both labeling from the cord (B; green; DiO) and from nerve regions distal to the anastomosis (A; red; DiI), and neuronal axons labeled with both dyes (C). (D-E) Spinal cord from sacral segments showing fluorogold labeled motor neurons that were retrogradely labeled from the bladder (D). These same neurons are also labeled with DiI from the re-anastomosed nerve (E).