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SOMATIC NERVE TRANSFER TO PELVIC NERVE REINNERVATES THE DETRUSOR MUSCLE AFTER SPINAL ROOT INJURY.

Hypothesis / aims of study: To determine if transfer of a somatic nerve (genitofemoral, GF) to the anterior vesical branch of the pelvic nerve (PN) allows reinnervation of the detrusor muscle after spinal root injury. The return of function and reinnervation of the detrusor muscle was determined using functional electrical stimulation (FES), urodynamic observations and neuronal retrograde tracing methods. We hypothesized that new axons ingrowing from the transferred GF nerve through the anterior vesical branch of the PB would reinnervate the detrusor muscle of the bladder and restore function.

Study design, materials and methods: 23 mongrel hound female canines were divided into three groups: 1) sham/unoperated (Control; n=6); 2) denervated (n=5); and 3) denervated plus genitofemoral nerve transfer (GF-NT: n=12). A power analysis was performed to determine the number of animals needed per group. In order to detect differences with an alpha level of 0.05 and 80% power, our estimated sample size for neuron count comparisons was n=5 dogs/gp. Prior to any surgical procedure, dogs were sedated with 6 mg/kg of propofol i.v. to allow endotracheal tube insertion for inhalational anesthesia of isoflurane at 2-3% maximum alveolar concentration with 100% oxygen as the carrier gas. Bladder denervation was performed by bilateral transection of lumbosacral nerve roots inducing bladder contractions by intraoperative FES. Bladder reinnervation was performed by bilateral transfer of GF nerves to vesical branches of the PN. Nerve cuff electrodes interfaced to radiofrequency micro stimulators were placed on the transferred GF nerves. Bladder emptying during a 6 month recovery period was accomplished by abdominal vesicostomy. FES and urodynamic studies were performed every 4 weeks. Three weeks prior to euthanasia, dogs were anesthesized and a neuronal retrograde *in vivo* dye (FluoroGold, Fluorochrome, LLC) was injected into the bladder at 4 sites around each ureteral orifice (4% w/v in saline solution 0.9%, 50µL/injection, 400µL total per dog). We then reversed the vesicostomy concomitant with bladder catheterization for 5−7 days to allow bladder recovery. The dogs were allowed to recover from anesthesia and were returned to their home cages. Three weeks after dye injection and vesicostomy reversal, the dogs were anesthesized again, and FES and urodynamic studies performed. Intravesical, rectal, urethral and anal pressures were recorded. Detrusor pressure was determined by subtracting the rectal pressure from the intravesical pressure. Bladder and spinal cord tissue were harvested for histological analysis, fixed by immersion, and cyrosectioned into transaxial sections, before mounting on slides. FluoroGold labeled neuronal cell bodies was counted in a $1mm^2$ region of each ventral horn section assayed. Spinal cord segments assayed included L3, L4, L7, S1, S2 and S3.

Results: Of the 12 animals with GF-NT, 10 (83%) demonstrated functional bladder reinnervation as evidenced by increased bladder pressure during stimulation of the transferred genitofemoral nerve under isoflurane anesthesia, bilateral in 3, unilateral in 7. Activation of the radio frequency micro-stimulators interfaced with nerve cuff electrodes surrounding the transferred genitofemoral nerves induced increased bladder pressure in 7 animals (58%), bilateral in 2, and unilateral in 5. Although the nerve transfers were performed bilaterally, bilateral functional reinnervation was observed in only 3 of the 10 animals with return of bladder function (**Fig. 1**). Histological examination of lumbar and sacral segments showed that denervated dogs showed few to no fluorogold-labeled cell bodies in sacral cord segments, indicating successful denervation (**Fig 2**). In the control dogs, in which the pelvic nerves (PN) were intact, fluorogold-labeled cell bodies were observed in the zona intermedia of ventral horns in L7, S1, S2 and S3 cord segments (**Fig. 2**). In the GF-NT dogs, fluorogold-labeled neuronal cell bodies were observed in lamina IX of ventral horns in the L3 and L4 cord segments (**Fig. 2**), the site of origin of motor neurons that contribute to the GF nerve. Two-way ANOVA showed a significant difference in the origin of the control dog neurons in L7-S3 versus the origin of the GF-NT dog neurons in L3 and L4 ($p<0.01$), showing successful reinnervation of the detrusor muscle by a lumbar somatic nerve following nerve transfer.

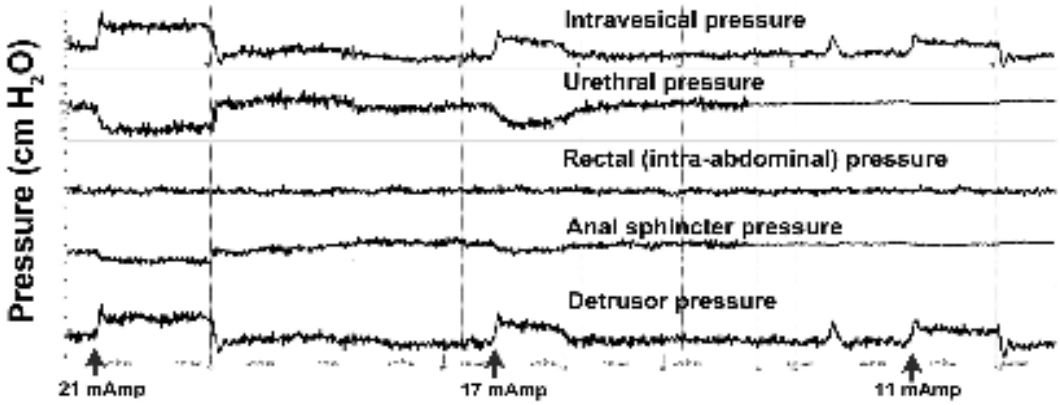

Figure 1. Functional electrical stimulation (FES) of a transferred Genitofemoral-Pelvic nerve at 184 days post operatively. Representative urodynamic pressure recordings during FES via the implanted RF micro-stimulator with a lead to a unipolar nerve cuff electrode surrounding the transferred genitofemoral nerve proximal to the site of anastomosis with the pelvic nerve branches.

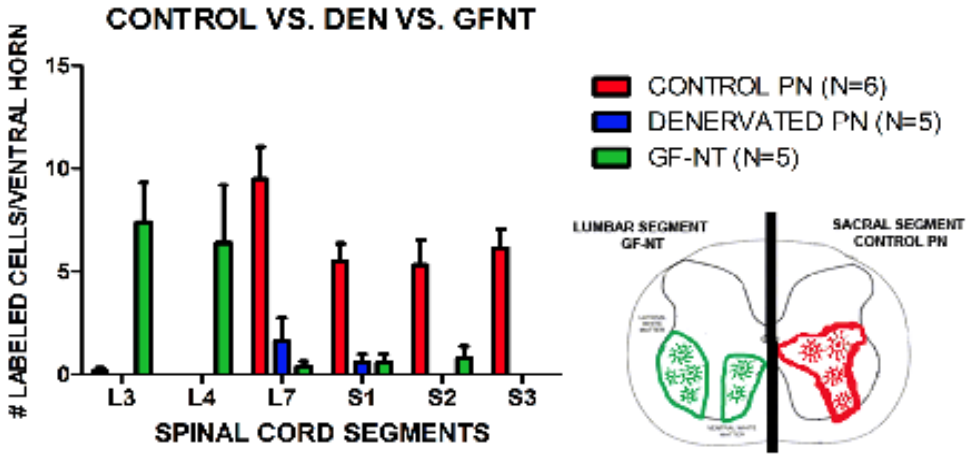

Figure 2. Quantative analysis of retrogradely labeled cells from the detrusor muscle to spinal cord ventral horns. Graph showing number of fluorogold-retrogradely labeled cells in each lumbar and sacral spinal cord segments in unoperated/sham control dogs (with intact pelvic nerve (PN)), denervated dogs, and dogs in which a genitofemoral-pelvic nerve transfer (GF-NT) was performed. Right panel: Diagram showing the typical location of the fluorogold-retrogradely labeled cells in the GF-NT and control groups.

Interpretation of results: These results indicated that the detrusor muscle can be reinnervated by transfer of peripheral genitofemoral nerves (L3, 4 origin) to the anterior vesical branch of the pelvic nerve. The evidence present in this model for bladder reinnervation includes: a) return of the detrusor contractions after 184 days post bilateral genitofemoral nerve transfer following electrical stimulation by activation of the cuff electrodes placed in the transferred nerve; and b) similar number of cell bodies labeled in the lumbar segments of the spinal cord that contribute to the origin of the genitofemoral nerve, in comparison with the sacral segments in the control dogs.

Concluding message: This surgical approach may be useful to patients with lower motor spinal cord injury by providing the possibility of having better control of micturition and urinary incontinence, improving their quality of life.

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Disclosures

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