

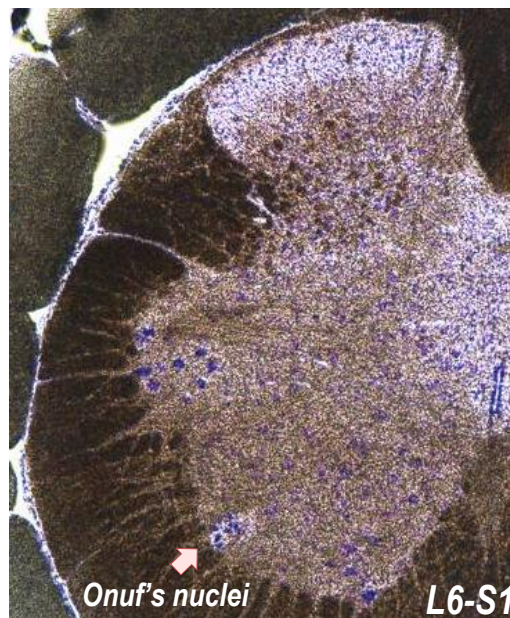
## PUDENDAL NERVE STIMULATION FOR SIMULATED CHILDBIRTH INJURY INCREASES NEUROTROPHINS EXPRESSION IN ONUF'S NUCLEUS

### Hypothesis / aims of study

Injury to both the urethra and pudendal nerve can occur during childbirth, resulting in stress urinary incontinence (SUI). It has been previously demonstrated that dual simulated childbirth injury, consisting of pudendal nerve crush (PNC) and vaginal distension (VD), results in slowed recovery of both continence & pudendal nerve function [1]. Expression of brain-derived neurotrophic factor (BDNF) was not upregulated after this combinatorial injury, contributing to the slowed recovery [2]. Electrical stimulation has been shown to upregulate BDNF in motor neurons and facilitate nerve regeneration after injury [3]. In the study, we electrically stimulated the pudendal nerve proximal to the injury site after a dual simulated childbirth injury, consisting of VD & PNC, to determine if this upregulates BDNF in Onuf's nucleus as a potential treatment to facilitate nerve recovery after childbirth.

### Study design, materials and methods

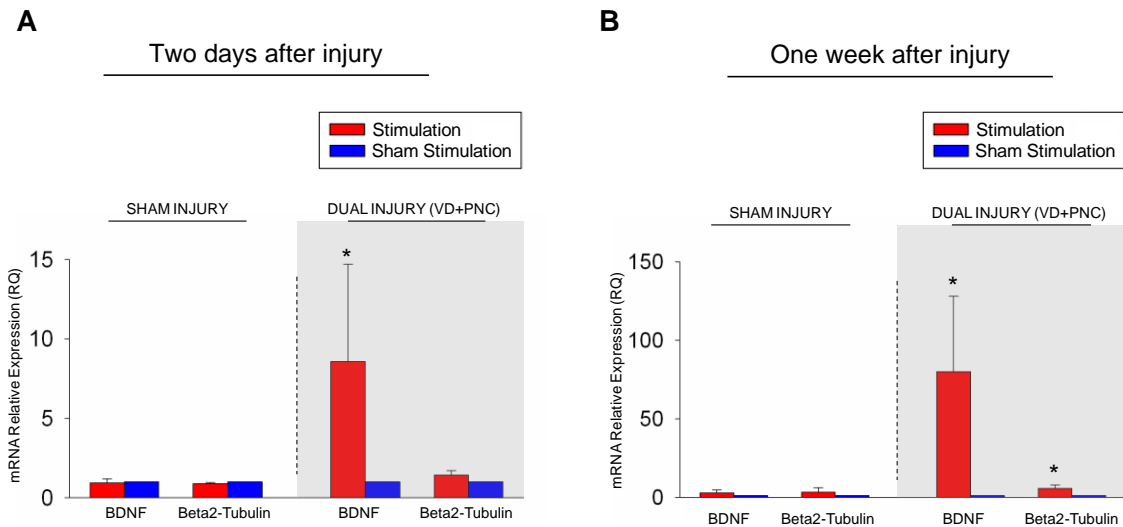
Twenty-five female Sprague-Dawley rats weighing 250-300g were randomized into groups of Control (Ctr), VD+PNC (Dual injury), and sham VD+PNC (Sham Injury). Rats in the dual injury group received 4 hours of VD immediately followed by bilateral PNC. Rats in the sham injury group underwent sham procedures. The rats in the sham and injury groups underwent 1 hour of electrical stimulation (20 Hz, 0.3mA, 0.1 ms duration) of the left pudendal nerve and sham stimulation of the right pudendal nerve immediately after injury. The lumbosacral spinal cord was harvested either 2 days (n=5) or 1 week (n=5) after stimulation and was flash frozen, sectioned at L6/S1, and laser dissected to isolate Onuf's nucleus (Fig. 1). The Onuf's nuclei are an independent group located in the anterolateral column of the grey matter. Cells in Onuf's nucleus from six sections were isolated and collected with the right sided specimens (sham stimulation) in one container and left sided specimens (stimulation) in another for each animal. BDNF and  $\beta_2$ -tubulin mRNA expression in Onuf's nucleus was determined by real-time RT-PCR and was normalized to expression of S-18, used as an internal control.  $\beta_2$ -tubulin is a marker of successful regenerative response and axon regrowth after injury.



**Figure 1.** Onuf's nuclei group (arrow) in anterolateral column of L6-S1 spinal cord in female Sprague-Dawley rat (thionin stain).

### Results

Two days after dual injury, BDNF expression in Onuf's nucleus was significantly increased on the stimulated (left) side compared to the sham stimulated (right) side, while  $\beta_2$ -tubulin expression was not significantly increased on the stimulated side ( $p < 0.05$ ). Neither BDNF nor  $\beta_2$ -tubulin was significantly increased after sham injury (Fig. 2A). One week after dual injury, both BDNF and  $\beta_2$ -tubulin expression in Onuf's nucleus were significantly increased on the stimulated side compared to the sham stimulated (right) side ( $p < 0.05$ ). Neither BDNF nor  $\beta_2$ -tubulin was significantly increased after sham injury (Fig. 2B).



**Figure 2.** BDNF and  $\beta_2$ -tubulin mRNA relative expression after simulated childbirth injury and pudendal nerve electrical stimulation. \* indicates significant difference compare to the sham stimulated (right) side. Data is presented as mean  $\pm$  standard error of the mean of each group (n=5). RQ, relative quantification; BDNF, brain-derived neurotrophic factor; VD+PNC, vaginal distension and pudendal nerve crush.

Interpretation of results

Electrical stimulation of the pudendal nerve proximal to the crush site upregulates BDNF expression in Onuf's nucleus two days and one week after dual injury, suggesting that it has the potential to facilitate neuroregeneration and improve recovery after injury. Electrical stimulation of the pudendal nerve proximal to the crush site also upregulates  $\beta_2$ -tubulin expression in Onuf's nucleus one week after dual injury, providing evidence of accelerated recovery after injury with stimulation.

Concluding message

Developing electrical stimulation to facilitate recovery of the pudendal nerve after childbirth provides a potential preventative strategy for SUI.

References

1. Neurourol. Urodyn. 28, 229 (2009)
2. Am. J. Physiol Renal Physiol 296, F277 (2009)
3. Dev. Neurobiol. 67, 158 (2007)

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<b>Is this a clinical trial?</b>	No
<b>What were the subjects in the study?</b>	ANIMAL
<b>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</b>	Yes
<b>Name of ethics committee</b>	Cleveland Clinic Institutional Animal Care and Use Committee