Hypothesis / aims of study
Pelvic floor muscle trauma and pudendal nerve injury following vaginal childbirth have been implicated in the development of female stress urinary incontinence (SUI). A dual injury model of vaginal distention (VD) and pudendal nerve crush (PNC) in female rats has been shown to significantly delay functional recovery of urinary continence compared to VD or PNC alone [1]. The aim of this study was to assess the effect of different simulated childbirth injury models in female rats on neuromuscular junctions and innervating motor neurons of the external urethral sphincter (EUS), in comparison to control animals as well as animals that underwent sham and pudendal nerve transection (PNT) injuries. We hypothesized that immunohistochemical appearance would mirror functional recovery and that a more severe injury would be demonstrated in the dual injured animals (PNC+VD) in comparison to either individual injury.

Study design, materials and methods
Thirty age-matched, virgin, Sprague-Dawley rats were divided into 6 groups. Equal numbers of rats underwent dual injury (PNC+VD), VD alone, PNC alone, PNT alone, sham dual injury, or served as unmanipulated controls. En bloc urethra and anterior vaginal wall segments were harvested following intracardiac saline perfusion two weeks after injury. Conventional methods for fluorescent microscopy and immunohistochemistry were applied to snap frozen longitudinal 15 μm thick sections. Fluorescently labeled α-bungarotoxin (α-Bgt) provided red staining of Acetylcholine receptors (AChRs) at the neuronal motor endplate. Labeled phalloidin stained muscle fibers blue. The binding of labeled monoclonal anti-neurofilament 200 and anti-neurofilament 68 to neuron specific proteins allowed for visualization of innervating neurons as green.

Results
Immunofluorescence of the EUS in unmanipulated control animals consistently displayed thick innervating axons (green in panel A) with sharp, organized motor endplates (red in panel A) on a background of striated skeletal muscle (blue). This same pattern was observed in the sham dual injury group as well. Immunofluorescence of the EUS in animals that underwent VD was characterized by fewer normally innervated neuromuscular junctions, and diffusion of AChRs in non-innervated motor endplates (panel C). Animals that underwent PNC also displayed fewer normally innervated neuromuscular junctions. Additionally, thinner and more tortuous innervating axons (panel D) were observed, often localizing with diffusing endplates. This same pattern was also identified in the PNT group however, axons were less commonly seen successfully innervating neuromuscular junctions. The pattern of AChR diffusion was more severe in animals that underwent PNT (panel B). Finally, animals that underwent PNC+VD exhibited characteristics of both PNC and VD, showing fewer normally innervated neuromuscular junctions as well as tortuous, thinner distal axons and areas of extensive AChR diffusion (panel E). Muscle striation was more often observed in the unmanipulated control and dual injury groups than in any of the injured groups.
Interpretation of results

Previous histological studies have established that VD results in muscle injury [2]. We have demonstrated that at the level of the neuromuscular junction, this damage is demonstrated by denervated motor endplates, which also exhibit a loss of organization resulting in a more diffuse pattern of AChR staining in comparison to normal endplates. Motor endplates are composed of numerous AChRs and rely on axonal input to preserve this formation [3]. Diffusion of these receptors away from the organized structure further demonstrates distal motor neuron injury and loss of innervation. The presence of normally innervated junctions in addition to denervated junctions is evidence that VD represents an incomplete injury. PNC is a model of a recoverable nerve injury and at the 2 week time point chosen for this study, functional return of continence and likewise nerve recovery has been shown to be present. Reinnervation preferentially follows the path of the degenerated nerve provided that the appropriate trophic and nerve growth factors are present. The tortuous and thin innervating axons found in these animals represent the regeneration and reinnervation process occurring in the rat EUS after PNC. Mild motor endplate diffusion was also found and was expected in light of the recoverable denervation injury in PNC. PNT is a complete neural injury and was found to result in a more profound injury with the thin regenerating axons less frequently finding an endplate to innervate. Consequently, with less reinnervation, there was a more severe effect at the motor endplates in these animals with more diffusion and loss of organization. This correlates with lack of recovery of urethral function after PNT. Animals that underwent PNC+VD exhibited immunofluorescence of the EUS most resembling the complete injury after PNT, despite being subjected to two separate recoverable injuries. These results suggest that PNC and VD act in concert to create a more severe injury, supporting the earlier finding of delayed return of functional continence [1].

Concluding message

The consistency of our observations lends support to our hypothesis and interpretation of the changes in the rat EUS following various simulated birth injuries. Continued investigation of this model may prove useful in the study of the pathophysiology of SUI and the potential for functional recovery. Treatments facilitating reinnervation and recovery of NMJs after childbirth may enhance recovery or even potentially prevent the sequelae of events that lead to SUI.

References


Specify source of funding or grant
NIH R01 HD38679

Is this a clinical trial?
No

What were the subjects in the study?
ANIMAL

Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?
Yes

Name of ethics committee
Institutional Animal Care and Use Committee at Cleveland Clinic