Abstracts: Basic

MP1.01: Spinal cord injury-induced sprouting of bladder afferents is independent from the lumbosacral cord highly repulsive environment 2

MP1.02: Comparison of bacterial species and antimicrobial resistance between catheter and non-catheter associated urinary tract infections 3

MP1.03: Urodynamic findings of a pilot minipig study for direct bladder wall stimulation 4

MP1.04: Beyond the bladder: Evidence of histological rearrangement and urethral denervation after thoracic spinal cord injury 5

MP1.05: Aging associated changes in bioenergetic profiles of rodent urothelial cells 6

MP1.06: Pelvic gross neuroanatomy of the female Yucatan minipig: A surgical and functional approach. 7

MP1.07: Inhibition of distension-induced afferent firing by Botulinum neurotoxin serotypes A and B in the mouse bladder 8

MP1.01: Spinal cord injury-induced sprouting of bladder afferents is independent from the lumbosacral cord highly repulsive environment

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Introduction: Spinal cord injury results in impairment of bladder function, caused by abnormal lumbosacral extension of bladder afferents. Mechanisms regulating this aberrant sprouting are unresolved but may involve changes in expression of inhibitory cues at the lumbosacral cord that block axonal growth, such as Nogo-A and Phosphacan. It is unclear whether bladder afferents recognize these repulsive cues and respond by regulating the expression of their receptors and, if so, what triggers this process.

Methods: Female rats were left spinal intact (controls) or underwent T8/T9 spinal transection (SCT), recovering for 7 or 28 days. At each endpoint, lumbosacral cords (L5-S1) and associated DRG were processed by Western Blotting and qPCR, respectively, to evaluate repulsive cues and RNA levels of their respective receptor complexes. GAP43 levels were also evaluated. L5-S1 DRG from other control and SCT animals were collected and cultured for 22h with 0, 50 or 100 ng/mL of NGF.

Results: Lumbosacral cord expression of Nogo-A and Phosphacan had a 3-fold increase at 7 days post-injury (dpi) (Phosphacan: $p \leq 0.05$ vs. controls; Nogo-A: $p \leq 0.001$ vs. controls), returning to baseline at 28dpi. A significant increase in GAP43 expression at the lumbosacral cord was also found following SCI. In lumbosacral DRG, Nogo-A receptor complex NgR1/p75 demonstrated a time-dependent decrease in expression, compared to controls (NgR1: $p \leq 0.05$ controls vs. 7dpi, $p \leq 0.001$ controls vs. 28dpi). Phosphacan receptor complexes NgR1/NgR3 and Rptpσ/Lar also showed time-dependent decreases in expression, compared to controls (NgR1: $p \leq 0.05$ controls vs. 7dpi, $p \leq 0.001$ controls vs. 28dpi; NgR3: $p \leq 0.05$ 7 and 28dpi; Rptpσ and Lar: $p \leq 0.05$ controls vs. 28dpi). To assess whether alterations in RNA levels of these receptor complexes were NGF-dependent, lumbosacral DRG were cultured in 0, 50 or 100 ng/ml of NGF. Preliminary results indicate that neurite length increased in an NGF concentration-dependent manner in control and SCT groups. In contrast, RNA levels of receptor complexes decreased in an NGF concentration-dependent manner in all groups, in tandem with *in vivo* observations.

Conclusions: Results demonstrate that central sprouting of sensory afferents, within the repulsive spinal environment, results from downregulation of repulsive cue receptors, a process dependent on exposure to high NGF concentrations, known to occur after SCT.

MP1.02: Comparison of bacterial species and antimicrobial resistance between catheter and non-catheter associated urinary tract infections

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Introduction: Urinary tract infections (UTIs) represent more than 40% of all hospital-acquired infections, with the majority of cases being catheter-associated UTIs (CAUTI). Our goal was to evaluate whether uropathogen (UP) and antimicrobial resistance (AMR) characteristics vary depending on catheter association.

Methods: We analyzed a dataset containing 27'158 urine cultures data from the ANRESIS database from calendar year 2019. Group differences in the proportions of bacterial species and antibiotic-resistant isolates from CAUTI and non-CAUTI samples were investigated using test statistics. A two-sided p value of < 0.05 was considered statistically significant. Samples of unknown origin were excluded. We analyzed the 4 most common pathogens and clinically relevant antibiotics for each pathogen.

Results: Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Proteus mirabilis together represented 70% and 85% of pathogens identified in CAUTI and non-CAUTI samples, respectively. The overall resistance rate for often-prescribed empirical antibiotic treatments ciprofloxacin (CIP), norfloxacin (NOR), trimethoprim/sulfamethoxazole was between 13% and 31%. E. coli from CAUTI samples were more often resistant ($p \leq 0.048$) to non-CAUTI samples to all classes of antibiotics analyzed (including 3rd generation cephalosporines used as surrogate for extended spectrum beta lactamase), except to nitrofurantoin. For K. pneumoniae, the difference in susceptibility was significant for CIP (p=.001) and NOR (p=0.03), for P. mirabilis, for NOR (p=0.01), with a lower resistance rate for non-CAUTI samples. P. aeruginosa from non-CAUTI samples were less often resistant to cefepime (p=0.02) and piperacillin-tazobactam (p=0.04).

Conclusions: The pathogens found in CAUTI and non-CAUTI samples were similar, with the exception of P. aeruginosa, more often detected in CAUTI samples. CAUTI pathogens were more often resistant to antibiotics compared to non-CAUTI pathogens. The overall resistance rate for most commonly used empirical antibiotic treatments was relevant with up to 31%. This emphasizes the need for urine sampling and susceptibility testing before initiating a therapy in case of UTIs and the need for therapeutic alternatives.

MP1.03: Urodynamic findings of a pilot minipig study for direct bladder wall stimulation

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Introduction: To present and discuss the main urodynamic findings of pilot study performed on minipigs, during the testing of a newly designed stimulation electrode implanted in the bladder wall to induce contraction and promote voiding.

Methods: The helical-wire hook electrodes were implanted in the bladder wall of three minipigs, which had a surgically created lesion of the sacral spinal cord. Urodynamic testing was performed on postoperative days four, seven, nine and twenty-eight. We have measured the vesical pressure (in the awake animal) and the rectal and detrusor pressures (under anaesthesia) during filling cystometry and during bladder wall stimulation at different current intensities.

Results: During the cystometric measurements we have encountered several substantial issues, some of them due to the incomplete sacral spinal cord lesion, and some due to significant abdominal contractions during the bladder stimulation. All the obtained measurement results will be presented, and some specific urodynamic curves will be shown in more detail.

Conclusions: The experience gathered during our urodynamic tests could be valuable for further research in the field of animal bladder wall stimulation.

MP1.04: Beyond the bladder: Evidence of histological rearrangement and urethral denervation after thoracic spinal cord injury

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Introduction: Spinal cord injury (SCI) results in urinary impairment due to neurogenic detrusor overactivity (NDO) and detrusor-sphincter-dyssinergia (DSD), known to be accompanied by tissue and nerve fibre rearrangement in the bladder wall. Although efficient voiding also depends on the urethra, the consequences of SCI in urethral innervation were never assessed.

Methods: Female Wistar rats were divided in 3 groups: spinal intact, and animals left to recover 1 and 4 weeks after SCI (n=5/group), and submitted to largely incomplete spinal cord transection at T8/T9 level. All animals underwent 1h cystometry under urethane anaesthesia before euthanasia and urethral tissue collection. Longitudinal urethral sections were used for histologic analysis and immunohistochemistry.

Results: Bladder contractions were abolished 1 week after SCI. At 4 weeks, NDO was already established, as shown by increased frequency and amplitude of bladder contractions ($p<0.05$ versus intact animals). Haematoxylin-Eosin staining showed increases in the height of urethral epithelium and lamina propria in 4 weeks post SCI animals ($p<0.05$ versus intact animals). The internal urethral sphincter (IUS) demonstrated marked atrophy, as evidenced by a significant decrease of actin expression 4 weeks post-SCI ($p<0.001$ versus intact). Increased fibrosis of the external urethral sphincter (EUS) was evident at both SCI time-points ($p<0.01$; $p<0.05$ versus intact animals).

Analysis of B-III tubulin, showed in the IUS signs of denervation 4 weeks post-SCI, while in the EUS this was noted as early as 1-week post-SCI ($p<0.05$ versus intact animals). CGRP expression showed sensory denervation in the mucosa ($p<0.001$ versus intact animals), as well as in the EUS ($p<0.05$ versus intact animals) at both time points. Sympathetic denervation was seen only in the IUS, as shown by the reduction of TH expression at both time-points ($p<0.01$ versus intact animals). Parasympathetic innervation, measured by VAChT immunolabelling, was not affected.

Conclusions: These results provide the first evidence that, as in the bladder, SCI results in profound and time-dependent changes in urethral organization. It is likely that these changes will affect urethra function, contributing to SCI-induced urinary dysfunction.

MP1.05: Aging associated changes in bioenergetic profiles of rodent urothelial cells

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Introduction: Involuntary urine loss and lower urinary tract (LUT) dysfunction are major health problems and a burden to patients and their caregivers. The prevalence of these problems significantly increases with age, and are amongst the primary reasons for elderly people to move to institutionalized care. The lack of a complete understanding regarding aging-related changes in the LUT is a major limitation for the development of individualized and specific therapies. In the current study we aim to further elucidate aging-related changes in urothelial bioenergetics.

Methods: Mucosal tissue (separated from the detrusor muscle by blunt dissection) was obtained from young (3-4 months old) and aged (25-30 months old) Fischer 344 rats. Urothelial cells (UTCs) were collected by gentle scraping, and cultured according to a standardized protocol. Cells obtained from all animals (n = 4 rats per age group) were assayed using the Seahorse XF96 Extracellular Flux analyzer, which measures oxygen consumption rate (OCR) in real time in living cells.

After basal OCR was determined, oligomycin (2.5 µM) was administered to quantify OCR unrelated to ATP synthesis. The resulting OCR represents proton leak across the inner mitochondrial membrane. Next, UTCs were exposed to the uncoupler FCCP (0.7 µM) to stimulate maximal respiratory rate by separating oxygen consumption from ATP production. The resulting collapse of the mitochondrial membrane potential triggers rapid consumption of energy and oxygen and was utilized to calculate the spare respiratory capacity of the cell. Finally, the complex I inhibitor, rotenone (10 µM), was added to establish the non-mitochondrial OCR.

Results: The OCR measured at baseline ($p = 0.001$), maximal respiratory rate ($p = 0.013$), and the spare respiratory capacity ($p = 0.017$) all proved to be significantly decreased in aged animals when compared to young animals.

Conclusions: Our results suggest that the OCR (monitored in real time) is significantly decreased in aged rats, suggesting that these UTCs have decreased their activity. A healthy urothelium is crucial for the maintenance of optimal barrier and signaling properties. We postulate that the observed decreased bioenergetic profile may alter the capacities of the urothelium to optimally execute these barrier and signaling functions.

MP1.06: Pelvic gross neuroanatomy of the female Yucatan minipig: A surgical and functional approach.

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Introduction: Although rodent models are widely used for investigations of the pelvic floor and lower urinary tract, larger animal models offer additional advantages for studies having translational potential. Yucatan minipigs have been considered ideal for preclinical model systems with respect to the 3R principle (Replacement, Reduction, and Refinement). Although porcine models have been developed for the study of LUT, the information regarding the pelvic organs and innervation is nonspecific and contradictory between studies. The aim of this study was to explore anatomically the organs and nerves relevant for micturition and sexual functions and describe a surgical approach for the physiological study of the extra-pelvic organs.

Methods: Four carcasses of female Yucatan mini pigs weighing 25-28kg and fixed with 4% paraformaldehyde were used. In two animals a detailed open dissection was performed focused on the hypogastric, pelvic, and pudendal nerves. Origins, course, relative position of nerves to arteries, viscera, and ligaments were documented. In a subset of specimens (n=2) via infra-pubic incision, the extra pelvic organs and nerves were explored.

Results: The hypogastric, pelvic, and pudendal nerves were fully identified and tracked. For the hypogastric, the main reference was the promontory below the aortic bifurcation, running together with the internal iliac artery until becoming the inferior hypogastric plexus with the pelvic nerve branches. Sacral roots (S2-S5) provide branches to form the pudendal and pelvic nerve. The pudendal nerve leaves and re-enters the pelvis through the greater and then the minor sciatic foramen diverging into the perineal, dorsal nerve of clitoris and suprapubic branches. Some branches going to the urethra-vaginal compressor and external urethral sphincter muscles were identified. By the infra-pubic approach, the dorsal nerve of clitoris and suprapubic branches were identified, as well as, the urethra-vaginal compressor, ischiocavernosus and external anal sphincter muscles and clitoris. The intermediate third of the urethra was harvested for further histological analysis aimed to confirm the external urethral sphincter muscle based on the pudendal branch attached to this area.

Conclusions: This results show the anatomical references of the major structures involved in the urogenital systems paving the way for further surgical and physiological investigations.

MP1.07: Inhibition of distension-induced afferent firing by Botulinum neurotoxin serotypes A and B in the mouse bladder

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Introduction: Botulinum neurotoxin (BoNT) is a potent neurotoxin that silences cholinergic neurons through inhibition of vesicular release mechanisms. Intravesical injection of BoNT serotype A (BoNT/A) for patients with overactive bladder (OAB) unresponsive to anticholinergics was approved by the Federal Drug Administration (FDA) in 2013 (FDA, 2013). Despite its widespread use, the complete mechanism of action of BoNT/A is still unclear, as reduction of bladder sensation has been described in basic science and clinical literature. Most studies have investigated only BoNT/A. The aim of this study was to use an ex vivo bladder-nerve preparation to investigate the action of BoNT/A and BoNT/B on distension-induced afferent firing.

Methods: An ex vivo bladder-nerve assay that allows the concomitant recording of afferent nerve firing and intravesical pressure was used to define the effect of BoNT/A and BoNT/B on bladder afferent mechanosensitivity. Nerve responses to distension, 90 minutes post-BoNT treatment were normalized as a percentage of nerve activity of baseline distension prior to application of BoNT/A and BoNT/B at a concentration of 3 pM, or PBS in time control studies. All data are presented as mean +/- SEM, analysed using one-way and two-way ANOVA with Bonferroni post hoc test (Figure 1.)

Results: Time control experiments showed no difference in the afferent nerve response to distension after 90 minutes. (90-minute distensions retained 94.48% ± 4.35% of the nerve activity compared to baseline distension, $P = 0.3398$, 2-way ANOVA, $n = 11$). Intravesical application of BoNT/A (3 pM) significantly reduced bladder afferent nerve response to distension ($p < 0.0001$; 2-way ANOVA $n = 5$) 29.8% ± 10.2% compared to baseline. BoNT/B also significantly attenuated the bladder afferent response to distension ($p < 0.0001$, 2-way ANOVA, $n = 6$) 47.5 %±19.9% compared to baseline. BoNT/B inhibited distension-induced afferent firing more potently than BoNT/A ($p < 0.05$, one-way ANOVA).

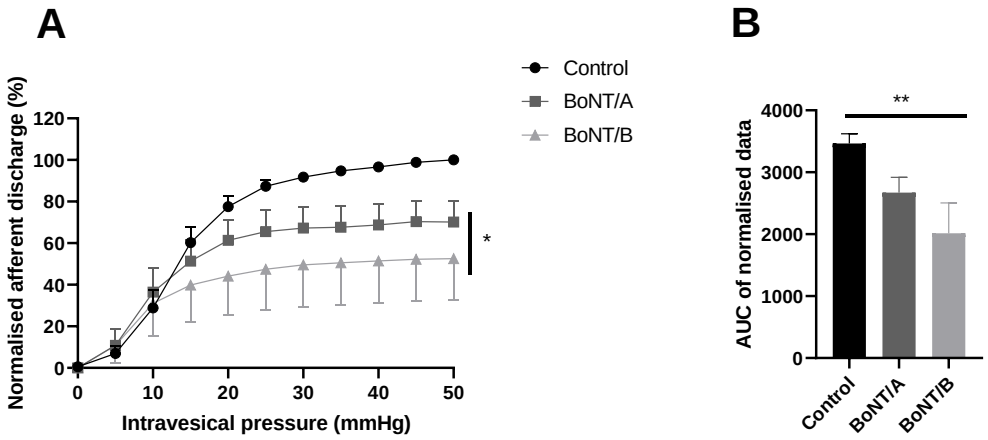

Figure 1: The effect of 3 pM BoNTs A and B on the bladder afferent response to distension

(A) Afferent nerve responses elicited by distension to 50 mmHg were significantly reduced 90 minutes after BoNT treatment, BoNT/B inhibiting afferent firing more potently than BoNT/A ($p<0.05$). (B) Afferent responses presented as area under curve (AUC). BoNTs significantly reduced the bladder afferent response to distension compared to control ($p=0.006$)

Conclusions: To our knowledge, this is the first study showing an effect of BoNT/B on bladder afferent nerve signaling. The results suggest that while both BoNT serotypes inhibit bladder mechanosensation, BoNT/B inhibits nerve firing more potently. Further studies are necessary to confirm and understand the underlying mechanism behind this effect.