INTRODUCTION AND OBJECTIVE: This study determined the effect of pelvic organ long-term decentralization and reinnervation one year later on the contribution of muscarinic and purinergic receptors to ex vivo, nerve-evoked, bladder smooth muscle contractions.

METHODS: Nineteen canines underwent decentralization by bilateral transection of all coccygeal and sacral (S) spinal roots, dorsal roots of lumbar (L)7 and hypogastric nerves. After exclusions, 8 were reinnervated 12 months post-decentralization with obturator-to-pelvic nerve and sciatic-to-pudendal nerve transfers then euthanized 8-12 months later; four served as long-term decentralized only animals. Controls included six sham-operated and three unoperated animals. Bladder tissues were assessed for contractile responses to potassium chloride (KCl) and electric field stimulation (EFS) before and after purinergic receptor desensitization with atropine, beta-methylened adenosine triphosphate (β,β-mATP), muscarinic receptor antagonism with atropine, or sodium channel blockade with tetrodotoxin.

RESULTS: Atropine inhibition of EFS-induced contractions increased by 26% in decentralized and 34% in the reinnervated animals compared to controls. Maximal contractile responses to β,β-mATP did not differ between groups. In strips from decentralized and reinnervated animals, the contractile response to EFS was enhanced by 52% at lower frequencies and the response to KCl was increased compared to normal controls.

CONCLUSIONS: The observation of increased blockade of nerve-evoked contractions by muscarinic antagonist with no change in responsiveness to purinergic agonist suggests either decreased ATP release or increased ectoATPase activity in detrusor muscle as a consequence of the long-term decentralization. The reduction in the frequency required to produce maximum contraction following decentralization may be due to enhanced nerve sensitivity to EFS or a change in the effectiveness of the neurotransmission.

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MP08-09
NERVE TRANSFER FOR RESTORATION OF LOWER MOTOR NEURON-LESIONED BLADDER FUNCTION. CORRELATION BETWEEN HISTOLOGICAL CHANGES AND NERVE EVOKED CONTRACTIONS

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INTRODUCTION AND OBJECTIVE: Our main objective was to determine the effects of long-term decentralization of 9-13 months and then reinnervation (followed by an 8-12 month post-reinnervation recovery period), versus the effects of 11-21 months of decentralization, on the histology of the urinary bladder, compared to sham/unoperated control animals. Our second objective was to correlate the histological findings with functional findings from ex vivo smooth muscle strip contractions in response to KCl and electric field stimulation, and with in vivo increases in bladder pressure evoked by electrical stimulation of intact pelvic nerves, transferred peripheral nerves and spinal roots.

METHODS: Twelve dogs underwent decentralization by bilateral transection of coccyygeal and sacral (S) spinal roots, dorsal roots of lumbar (L)7, and hypogastric nerves. Eight were reinnervated 9-13 months post-decentralization with obturator-to-pelvic nerve and sciatic-to-pudendal nerve transfers, then euthanized 8-12 months later; the remainder served as long-term decentralized only animals. Controls included 11 sham-operated and 3 unoperated animals. Before euthanasia, pelvic or transferred nerves and L1-S3 spinal roots were stimulated and maximum detrusor pressure (MDP) recorded. Bladder specimens were collected for histological and ex vivo smooth muscle contractility studies.

RESULTS: Decentralized and reinnervated animals showed less urothelium or a denuded urothelium, fewer intramural ganglia, and more inflammation and collagen in the bladder wall, than controls, although percent muscle was maintained. In reinnervated animals, pgp9.5+ axon density was higher, compared to decentralized animals. Ex vivo smooth muscle contractions in response to KCl correlated positively with submucosal inflammation, detrusor muscle thickness and pgp9.5+ axon density. In vivo, decentralized and reinnervated animals showed lower MDP than controls after stimulation of transferred or pelvic nerves and L7-S3 roots. Reinnervated animals showed higher MDP after stimulation of L1-L6 roots, compared to L7-S3 roots. MDP correlated negatively with detrusor collagen and inflammation, and positively with pgp9.5++ axon density and intramural ganglia numbers.

CONCLUSIONS: Although percent muscle and some bladder smooth muscle function was maintained in reinnervated animals, perhaps due to the innervation change, enhanced collagen deposition and inflammation were associated with decreased contractile function.

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MP08-10
LATERALIZATION OF BLADDER FUNCTION IN NORMAL FEMALE CANINES

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INTRODUCTION AND OBJECTIVE: Since the bladder receives innervation from the right and left sides through the spinal cord and the pelvic plexus, this study aimed to examine if stimulations of either the spinal roots or pelvic nerve cause a greater bladder contraction on the left versus the right side.

METHODS: Forty-four female canines were included in this study. Functional electrical stimulation (3-5 second trains at 20Hz, 0.02 msec, 0.5-10 mA) of bilateral lumbar (L)6 through sacral (S)3 spinal cord roots and the left and right pelvic plexuses were performed for all animals. Changes in detrusor pressure were continuously recorded during stimulations. Strength of nerve-evoked bladder contractions after spinal root and pelvic plexus stimulations were derived from differences between the resting baseline pressure and the peak pressure obtained during the stimulation. The dominant side for each spinal root and pelvic nerve in each animal was determined by calculating the percent difference (25%) between the left and right stimulation. Bladders are considered left or right sided if differences are greater or less than 25%, respectively. If differences are within 25%, bladders are considered bilaterally innervated.

RESULTS: Functional bilateral spinal root stimulation in 2/3 of the 44 dogs examined indicated that the maximum detrusor pressure was shown to be associated with either the left or right side of the spinal cord that was stimulated. Bladders were left side dominant in seventeen dogs (38.6%), right side dominant in 12 dogs (27.2%) and bilateral in 15 dogs (34%). Functional electrical stimulation of pelvic nerve revealed that about 3/4 of the 19 dogs tested, changes in detrusor pressure was shown to be associated with the side that was stimulated. Bladders were left side dominant in 8 dogs (42.1%), right side dominant in 6 dogs (31.6%) and bilateral in the remaining 5 dogs (26.3%).

Source of Funding:
CONCLUSIONS: Overall, these data provide evidence for asymmetry of the bladder function in the normal female dogs. Although, the current observations were obtained from normal animals, the determined left and right dominance of the bladder function might provide the basis for understanding the consequences of lateralization of bladder innervation in patients with bladder dysfunctions.

| Root/Plexus | Animals Tested | Left Dominance | Right Dominance | Bilateral |
|-------------|----------------|----------------|----------------|-----------|
| Any Spinal Root | 44             | 38.6%          | 27.2%          | 34.0%     |
| Pelvic Plexus | 19             | 42.1%          | 31.6%          | 26.3%     |

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MP08-12
GUANYLATE CYCLASE-C AGONIST IW-3300 ATTENUATES CHRONIC COLONIC AND BLADDER HYPERSENSITIVITY IN A RAT MODEL OF PELVIC RADIATION INJURY
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INTRODUCTION AND OBJECTIVE: Radiotherapy for pelvic cancers can lead to radiation-induced visceral organ injuries that develop into persistent chronic radiation proctitis, colitis, and cystitis. One key symptom associated with these conditions is pain associated with the affected organ. Currently, no recommended guidelines for the treatment of visceral pain associated with radiotherapy exist, making the management of these conditions extremely challenging. Here, we test the hypothesis that guanylate cyclase-c (GC-C) engagement via GC-C agonist IW-3300 relieves pain in a rat model of pelvic radiation injury.

METHODS: Adult male Lewis rats received a fractionated dose of radiation (FR: 48 Gy total over 8 days), a single high dose of radiation (HR: 25 Gy), or sham irradiation. On day 42 colonic sensitivity was assessed via a visceromotor response (VMR) to isobaric colorectal distension (CRD, 20-60 mmHg) and bladder sensitivity was assessed via quantification of suprapubic withdrawal reflex to von Frey filaments (SWR). Rats exposed to fractionated radiation then received intracolonic IW-3300 (3µg/kg) or vehicle on days 56-72 and rats exposed to a single high dose of radiation received IW-3300 or vehicle on days 42-72. Colonic and bladder sensitivity was reassessed in both groups on days 56 and 72.

RESULTS: Overall, 2 rats in both radiation groups exhibited an increased VMR to CRD (p < 0.0001 at 60 mmHg) and suprapubic withdrawal reflex (p < 0.000115g) that persisted until day 72. IW-3300 decreased the VMR to CRD and SWR (VMR 60mmHg = 14.6±1.1 vs. 23.3±0.58 contractions; SWR 15g = 4.2±0.5 vs. 7.2±0.8 withdrawal frequency) of FR rats on day 72 compared to vehicle. Similarly, HR rats that received IW-3300 demonstrated an attenuated VMR to CRD and SWR on days 56 (VMR 60mmHg = 14.0±0.6 vs. 23.8±1.2 contractions; SWR 15g = 4.2±0.3 vs. 7.8±0.3 withdrawal frequency) and 72 (VMR 60mmHg = 13.8±0.5 vs. 23.6±0.5 contractions; SWR 15g = 4.3±0.3 vs. 7.2±0.3 withdrawal frequency).

CONCLUSIONS: Irradiation of the pelvic region induces persistent colonic and bladder hypersensitivity in adult male rats that was significantly attenuated by IW-3300 treatment. These results suggest IW-3300 has the potential to help manage radiation-induced visceral hypersensitivity and support the further evaluation of IW-3300 in humans for this indication.

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MP08-13
MICRORNA-29A REGULATES TGF-β1-INDUCED EXPRESSION OF COLLAGEN 1A1 IN HUMAN UROTHELIAL CELLS
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INTRODUCTION AND OBJECTIVE: microRNA-29 (miR-29) exerts potent anti-fibrotic effect in various organs by suppressing mRNAs encoding many extracellular matrix molecules, including collagens. Urothelial cells line the lumen of the bladder and respond to various stimuli by producing and releasing a variety of biologically active molecules. Transforming growth factor-β1 (TGF-β1), a potent profibrotic cytokine, and TGF-β receptors 1 and 2 are present in urothelial cells. Bladder inflammation increases expression of these proteins, suggesting a functional role of TGF-β1 in both physiological and pathophysiological conditions in bladder. In the present study, we examined the regulatory role of miR-29a on TGF-β1–induced expression of collagen 1a1 in primary human urothelial cells.

METHODS: Primary human urothelial cells (HUCs) were transfected with 40 nM miR-29a mimics or an inhibitor of miR-29a

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