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## ABSTRACT

The role of nitric oxide (NO•) signalling in the urinary bladder is incompletely understood. It has been demonstrated that NO• mediates relaxation of the smooth muscle in the bladder neck and urethra, with nominal effects on the detrusor [1]. However, there is also robust expression of soluble guanylate cyclase (sGC) and NO•-induced cyclic guanosine monophosphate (cGMP) in afferent nerves, urothelial cells, myofibroblasts and vascular smooth muscle in the bladder [2]. The activity of sGC depends upon the presence of a reduced heme (Fe<sup>2+</sup>); it is hypothesized that stress can oxidize this group (Fe<sup>3+</sup>) and impair NO•-mediated cGMP production [3] (Figure 1).

The aim of our study was to investigate the acute effects of BAY 58-2667, a small molecule sGC activator that preferentially acts on oxidized/heme free sGC on bladder afferent nerves and detrusor smooth muscle in mice with irradiation-induced cystitis; a chronic inflammatory condition.

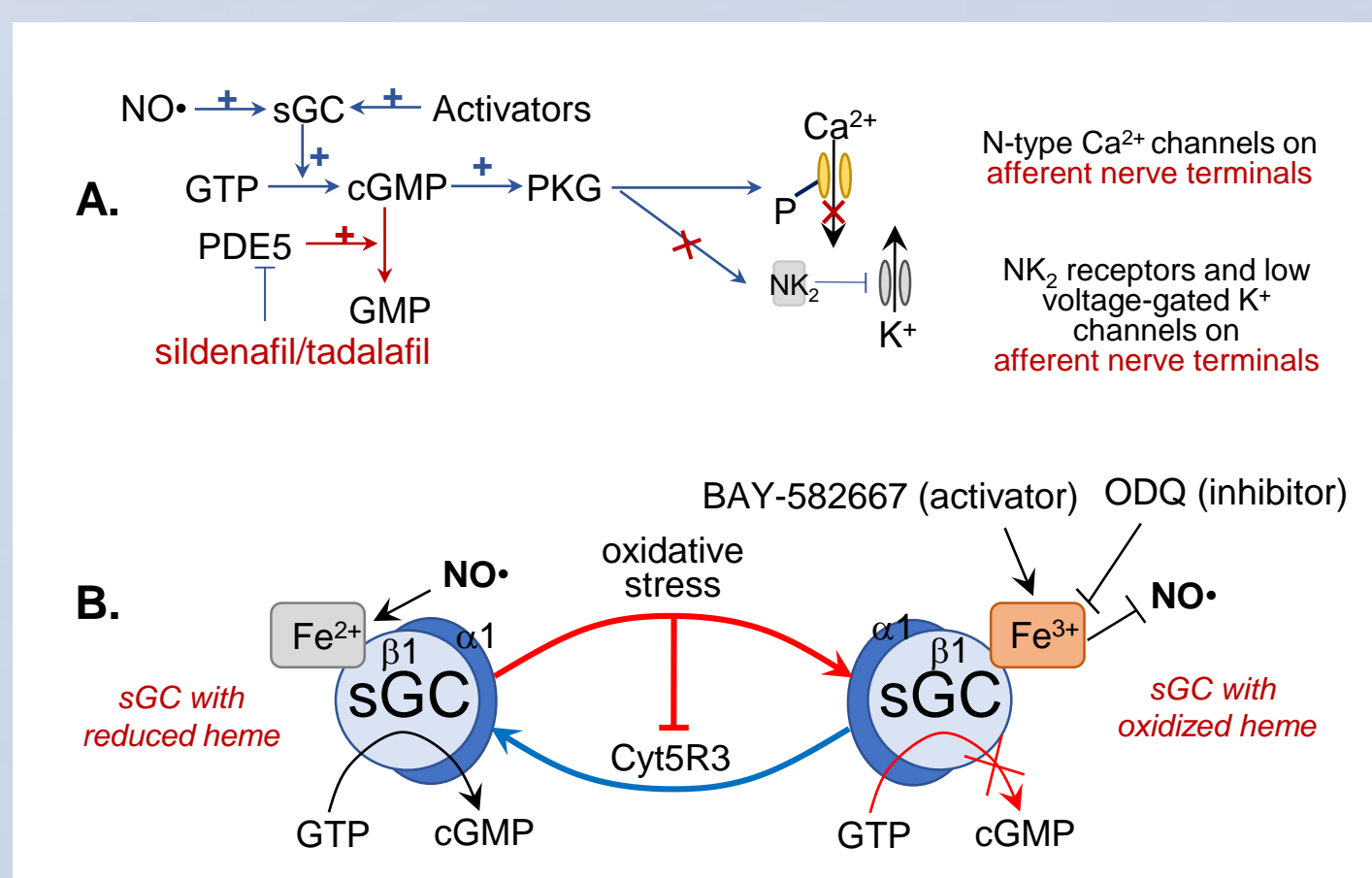


Figure 1. Schematic of sites of action for NO•-sGC signaling on bladder afferent pathways and regulation of sGC activity by the heme redox state

## METHODS

**Selective bladder irradiation:** Adult female C57BL/6 mice were anesthetized with avertin (2,2,2-tribromoethanol, 300 mg/kg via intraperitoneal injection) and a lower midline incision was made into the abdomen. Mice had their urinary bladders externalized and selectively exposed to a collimated beam of ionizing irradiation (10 Gy, 320 KV X-ray irradiator) to prevent exposure to other pelvic structures and prevent cross-sensitization (Figure 2A). The surgical wound was sutured, and mice were allowed to recover on prophylactic antibiotics and analgesics.

**In vitro single-unit bladder afferent nerve recordings:** Three to seven days following irradiation, mice were euthanized and bladders with associated spinal (L6-S2) nerves were dissected. The preparation was placed in a tension recording chamber with oxygenated Krebs solution for recording bladder wall tension and the spinal nerves passed into adjacent oil chambers for afferent nerve recordings (Figure 2B). Mechanosensitive afferent firing was elicited in response to varying stretches applied through a computer-controlled stepper motor in line with the tension transducer.

Experiments were carried out on  $n \geq 4$  mice. Data are expressed as mean  $\pm$  standard deviation. Unpaired student t-test determined differences between irradiated versus control groups or parameters before and after treatment.

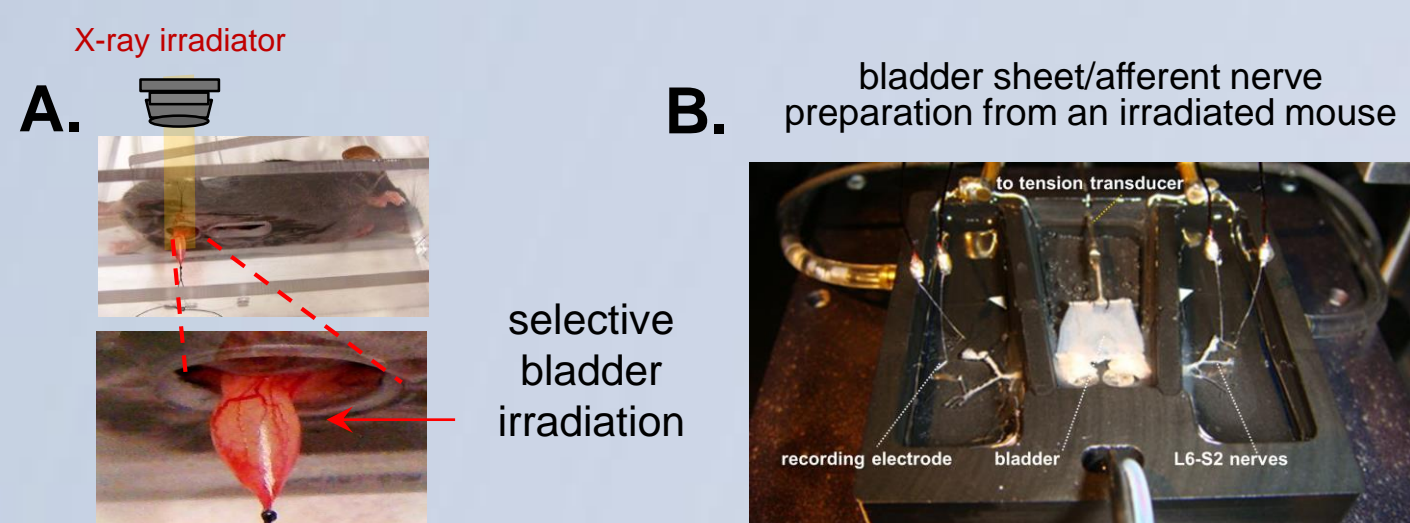


Figure 2. Photo images of mouse bladder irradiation procedure and in vitro single-unit mouse bladder afferent nerve recording preparation.

## RESULTS

In vitro afferent recordings were obtained using mouse bladder preparations where nerve firing was elicited in response to stepper-motor controlled stretch. Afferent recordings from irradiated mouse bladders exhibited spontaneous discharges not associated with changes in bladder tension. Spontaneous firing was not observed in non-irradiated mouse bladders. The addition of BAY 58-2667 (0.01-1  $\mu$ M) to the perfusate dose-dependently dampened afferent activity, but at higher concentrations, significantly decreased stretch-induced tension changes (Figure 3 and Table 1).

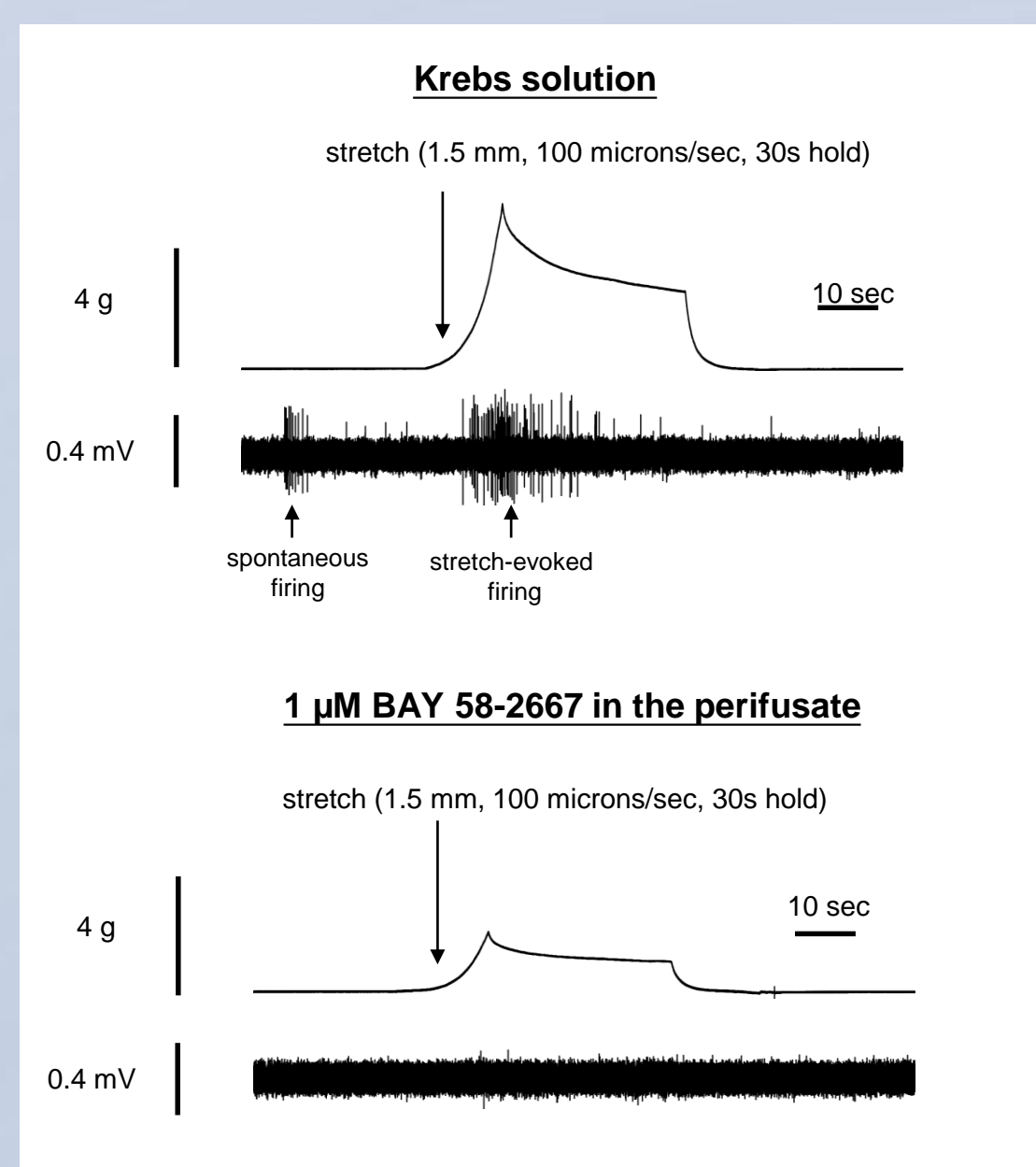


Figure 3. Example trace of simultaneous tension and single unit afferent activity from a mouse bladder three days post irradiation and the effect of acute BAY 58-2667 (1  $\mu$ M) administration.

Response to 1.5 mm stretches	Non-irradiated control	Acute irradiation	Irradiated + BAY 58-2667
Firing rate (units/second)	1.3 $\pm$ 0.2	4.1 $\pm$ 0.4*	2.5 $\pm$ 0.2*,#
Total counts per stretch	31 $\pm$ 4.0	147 $\pm$ 47*	108 $\pm$ 2.4

Table 1. Comparable afferent firing responses from non-irradiated, acute irradiated mouse bladders and the acute effect of 1  $\mu$ M BAY 58-2667. \*  $p < 0.05$  compared to non-irradiate, # compared to Krebs only.

## CONCLUSIONS

Acutely following bladder irradiation, there was increased sensitivity of afferent nerves and evidence of spontaneous firing. There was no indication of spontaneous detrusor contractions as a result of irradiation at the time point examined. The decrease of spontaneous and stretch-induced afferent firing as well as baseline tension *in vitro* demonstrates the direct action of BAY 58-2667 on afferent nerves and detrusor smooth muscle. Oxidation of sGC heme following irradiation may be responsible for decreased NO•-mediated inhibition, contributing to afferent sensitization.

sGC is highly expressed in the bladder neck and urethra where it is responsible for relaxation, whereas detrusor smooth muscle relaxes readily to  $\beta$ -adrenergic receptor stimulation. Our data demonstrate that activation of sGC in the urinary bladder wall decreases afferent excitability and intriguingly relaxes detrusor smooth muscle through a mechanism yet to be determined. These data support the therapeutic potential of sGC activator for bladder afferent sensitization and detrusor overactivity.

## REFERENCES

- Andersson KE., Int Neurourol J, 21(1):6-11, 2017
  - Groneburg D et al., Circulation, 121:401-409, 2010
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- Funding awards from NIH/NIDDK; R01 DK071085 (Kanai), R01 DK098361 (Kanai/Drake), P01 DK093424 (Kanai) and Department of Defense SC170171 (Kanai/Ikeda). Ethics approval: University of Pittsburgh Institutional Animal Care and Use Committee