A New Mechanism Linking I*n Utero* Environmental Chemical Exposure To Prostatic Innervation And Urinary Voiding Dysfunction

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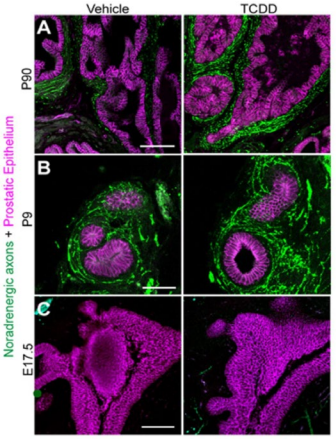
Introduction

We recently found that the in utero environment exerts a lifelong influence over male voiding function and can sensitize to LUTD later in life.

- We have shown that developmental exposure to TCDD, a widespread environmental contaminant, increases noradrenergic axon density in the smooth muscle of the prostate and changes voiding function.



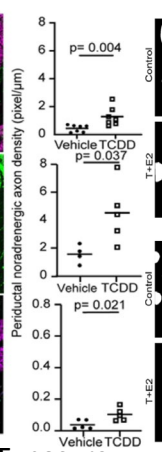


Figure 1: In Utero TCDD Exposure Increases Prostatic Noradrenergic Axon Density.

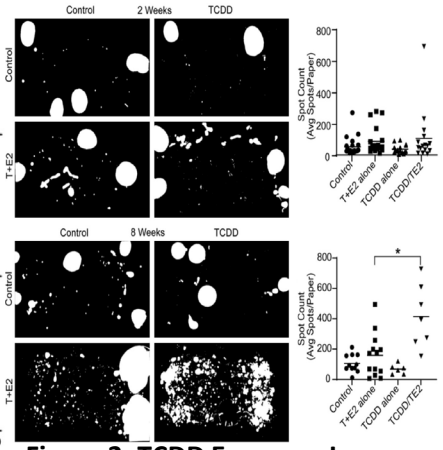


Figure 2: TCDD Exposure In Utero and Lactation Exacerbates T+E2 Treatment.

- TCDD exerts most of its effects through the Aryl Hydrocarbon Receptor (AHR)
- Activation of the AHR, a transcription factor which binds to many xenobiotics, during LUT development in utero increases prostatic noradrenergic axon density and sensitizes prostatic smooth muscle to adrenoreceptor stimulation, leading to excessive prostatic urethral tone throughout life.
- In zebrafish, the long non-coding RNA *Slincr* has been shown to mediate many of the teratogenic effects of TCDD through modulation of *Sox9* transcription, a pivotal gene which influences the fate of neural crest cells and influences gliogenesis. However, the role of *Sox9* in neurons innervating the prostate is relatively unknown and *Slincr*'s influence over Sox9 has not been tested in mice.
- *We hypothesize that* fetal AHR activation increases prostatic noradrenergic axon innervation by inducing the expression of a long-noncoding RNA (SlincR) and repressing Sox9. *We hypothesize that Slincr* null mouse fetuses will resist AHR mediated increases in prostatic noradrenergic axon density.

Materials and Methods

Creation of *Slincr* Knockout Mouse

- Use CRISPR/Cas9 to create *Slincr* null hemizygous mice.

Dosing Paradigm

- Expose wild type and null fetuses to (25ug/kg) TCDD or Vehicle (5mL/kg corn oil) on Embryonic day 10.5 before prostate innervation begins.
- Collect prostates on E 18.5

Tissue Analysis

- Compare SlincR abundance, Sox9 abundance, and noradrenergic axon quantity in prostate smooth muscle and pelvic ganglia through RNA Scope and IHC staining , respectively.

Results and Discussion

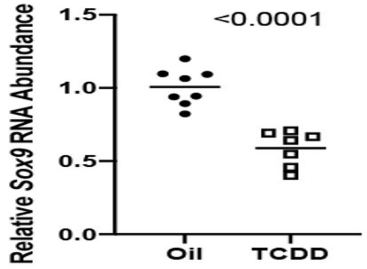


Figure 3: In Utero TCDD Exposure Decreases *Sox9* Expression in the Pelvic Ganglia and Urogenital Sinus

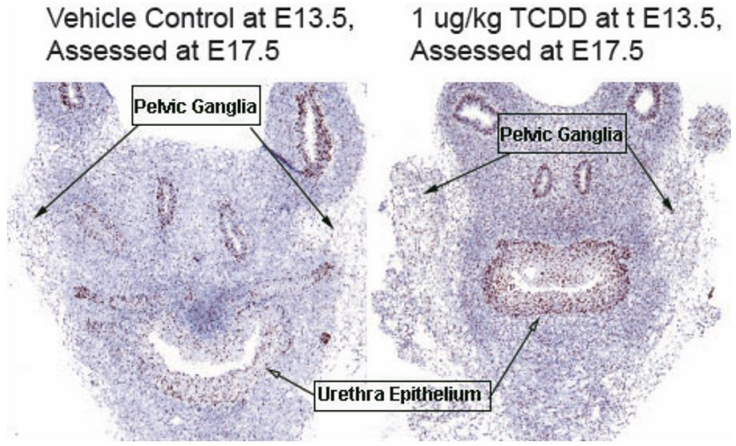


Figure 4: In Utero TCDD Exposure Increases *Slincr* Abundance.

- We already found that TCDD increases the number of *Slincr+* cells in the pelvic ganglia, urethral epithelium, seminal vesicle and ductus deferens.
- We also found that TCDD reduces *Sox9* RNA abundance in the urogenital sinus + pelvic ganglia
- We expect to find that *SlincR* null mice are deficient in *SlincR* RNA, resistant to TCDD-mediated decreases in *Sox9*+ pelvic ganglia cells, and resistant to the TCDD-mediated increase in prostatic density of TH+ axons.

Conclusions

These findings will be significant because they will identify a potential mechanism linking perturbations in prostatic neuroanatomical development to lifelong hyperactivity of prostatic smooth muscle, increased prostatic urethra tone, and impaired bladder emptying.

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