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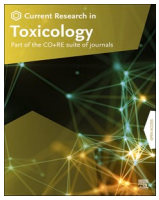

Developmental polychlorinated biphenyl (PCB) exposure impacts on voiding physiology persist into adulthood and influence sensitivity to bladder stimuli in mice

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ABSTRACT

Polychlorinated biphenyls (PCBs) are toxicants present in the environment, foodstuff, animal and human tissues. PCBs are linked to numerous adverse health effects; however, impacts of developmental PCB exposure on lower urinary tract function are a comparatively newer area of interest. We have previously found developmental exposure (*in utero* and lactational) to a human-relevant PCB mixture in mice leads to sex- and dose- dependent changes to urinary voiding physiology at 6 weeks of age. This study expands upon previous findings to investigate if developmental PCB-induced urinary voiding phenotypes persist or shift as mice age to 12 weeks of age. Urinary voiding physiology testing through void spot assays, uroflowmetry, and cystometry demonstrated several sex- and dose- dependent effects of PCB exposure at 12 weeks of age. Further, patterns of dysfunction were either maintained, newly acquired, or reversed compared to those from younger adult mice in a previous study. Here, developmental PCB exposure decreased number of small urine spots in adult male and female mice in a dose dependent manner, and female mice had more frequent voiding events assessed by anesthetized cystometry. Mice also had PCB dose-dependent changes to urinary voiding physiology when challenged with intravesical capsaicin infusion to target transient receptor potential cation channel subfamily V member 1 (TRPV1)-mediated pathways. PCBs either blocked or exacerbated capsaicin induced responses depending on the endpoint examined, suggesting this pathway may play a role in PCB-dependent changes in voiding. PCBs also had subtle impacts on prostate wet weight, with high PCB doses reducing tissue mass compared to low PCB doses, while none differed from vehicle. This study demonstrates developmental exposure to PCBs continues to impact lower urinary tract function in adulthood to at least 12 weeks of age both during homeostatic conditions and upon challenge of capsaicin. Better understanding of how early life stressors like PCBs contribute to aging-associated voiding dysfunction are imperative as these findings may help mitigate risk or improve treatment strategies for patients suffering from lower urinary tract symptoms.

1. Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous, synthetic, persistent organic pollutants that have been produced in the United States for over a century, and research continues to elucidate potential adverse health outcomes from PCB exposure. Despite the 1979 Toxic Substances Control Act banning intentional PCB production in the United States, studies estimate that in 2020 the United States has retained 1000 metric tons of pure PCBs and 13,755 metric tons of PCB materials in electrical transformers alone (Jahnke and Hornbuckle, 2019; Melymuk et al., 2022). In addition to legacy sources of PCBs produced before the bans, PCBs are unintentionally produced as by-products of manufacturing processes that use chlorine, salts, and hydrocarbons or chlorinated hydrocarbons at high temperatures such as pigment production (Grossman, 2013; Hu and Hornbuckle, 2010). Although the US Environmental Protection agency and US Food and Drug Administration set safety limits to mitigate PCB exposure, legacy and contemporary PCB sources continue to bioaccumulate and biomagnify in animal foodstuff resulting in chronic PCB exposure for humans (Melymuk et al., 2022; Guo et al., 2014; Koh et al., 2015) and the US-based production of

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contemporary PCBs in 2019 could have surpassed peak production of legacy PCBs in the 1970 s (Megson et al., 2024). In the environment, PCBs are ubiquitous and can be transported throughout air, water, and soil (Melymuk et al., 2022; Grimm et al., 2015; Anh et al., 2021). In biota, PCBs accumulate in adipose and other fatty tissues with an elimination half-life on the order of months to years (Quinn and Wania, 2012; Ritter et al., 2011). Thus, the adverse outcomes from legacy and contemporary PCB sources continue to be relevant for environmental and human health.

PCBs can transfer to the next generation through developmental exposure via transplacental transfer *in utero* and through lactational exposure via breast milk (Ando et al., 1985; Björvang et al., 2021; Eguchi et al., 2018; Jeong et al., 2018; Lancz et al., 2015; Needham et al., 2011). Developmental PCB exposures are hypothesized risk factors for adverse health effects later in life including neurodevelopmental disorders such as autism spectrum disorder (Panesar et al., 2020; Balalian et al., 2024). Several types of neurological dysfunctions and disorders, including autism spectrum disorder, are comorbid with the occurrence of lower urinary tract dysfunction (LUTD) (Gubbiotti et al., 2019; Gubbiotti et al., 2019; Moussa et al., 2020; Gubbiotti et al., 2024). However, a role for PCBs in LUTD etiology alone or in the context of neurological disorders is not well understood. Studying the etiology of LUTD has been historically challenging because the heterogeneity of symptom onset, progression, and severity in patients likely arises from a combination of risk factors which are not fully elucidated (Peterson and Vezina, 2022). This is further complicated by the temporal consideration where challenges to the lower urinary tract in adolescence may predispose or exacerbate the effects of aging and incidence of LUTD later in life (Peterson and Vezina, 2022; de Wall et al., 2021; Nishii, 2021; Suskind, 2017). While pharmacological interventions exist for palliative treatment, the incomplete understanding of LUTD etiology remains a barrier for targeted, more effective treatments (Abreu-Mendes et al., 2020; MacNevin et al., 2022).

In our investigation of risk factors for LUTD, we have identified the bladder as a novel target for PCB effects in rodent models (Keil Stietz et al., 2021; Kennedy et al., 2022; Kennedy et al., 2021). Using a human-relevant PCB mixture (termed the MARBLES PCB mixture) (Hertz-Picciotto et al., 2018), several developmental PCB exposure effects on mouse offspring have been observed. These include increased suburothelial nerve density (Keil Stietz et al., 2021), increased bladder CD45 + immune cells in ~ 4-week-old offspring (Kennedy et al., 2021); and altered voiding physiology with a phenotype reminiscent of overactive bladder in ~ 6-week-old offspring (Kennedy et al., 2022). These PCB effects were observed in the absence of signs of overt toxicity, with no changes to body mass, bladder mass, bladder muscle thickness, or urothelium composition (Keil Stietz et al., 2021; Kennedy et al., 2022; Kennedy et al., 2021). Whether the voiding alterations persist, return to control levels or new phenotypes arise as mice age, is completely unknown and the first goal of this study.

Together this work aims to better define the long-term outcomes of developmental PCB exposure on lower urinary tract function and begin to identify underlying mechanisms. This study fits into our greater understanding of PCB effects on lower urinary tract function by considering the temporal shifts associated with voiding as mice age and how PCBs could impact these processes. We found PCBs induced several sex- and dose- dependent effects on urinary voiding in 12 week old mice, and the overall phenotype is incongruent with previously described effects in younger mice at 6 weeks of age, signifying that PCB effects on urinary voiding are dynamic over time. Additionally, this is the first study to examine whether transient receptor potential cation channel subfamily V member 1 (TRPV1)-mediated pathways are involved in PCB effects on urinary voiding function. TRPV1 is involved in physiological and pathological processes throughout the body, especially in bladder mechanosensation (Storozhuk et al., 2019), making TRPV1-pathway activity of interest as a potential mechanism for PCB action in the bladder. By infusing the bladder with capsaicin, a TRPV1 agonist, during anesthetized cystometry, we reveal several capsaicin responses in voiding which are modified by PCB exposure. Since prostate morphology can impact voiding function (Lee and Kuo, 2017; Lee et al., 2015; Ruetten et al., 2019) and we previously observed PCB effects on prostate tissue mass at 6 weeks of age (Spiegelhoff et al., 2023), these tissues were collected to determine sex-specific effects of PCBs in male mice at this 12 week timepoint. PCBs had subtle impacts on prostate wet weight, with high PCB doses reducing tissue mass compared to low PCB doses, while none differed from vehicle. Together these results suggest that developmental PCB exposure can influence urinary voiding physiology at least through 12 weeks of age, and TRPV1 dependent pathways may be disrupted. Improving the basic understanding of how early life stressors such as PCBs can contribute to aging-associated urinary voiding dysfunction are imperative as these findings may help mitigate risk or improve treatment strategies for LUTD.

2. Materials and Methods

2.1. Animals

All procedures involving animals were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Wisconsin-Madison Animal Care and Use Committee. Wild type C57BL/6J mice (#000664, Jackson Labs, Bar Harbor, ME), were used in this study. All mice were housed in clear plastic cages containing corn cob bedding and maintained on a 12 h light and dark cycle at 22 ± 2°C. Feed (Diet 2019 (breeder) and 2020x, Teklad, Indianapolis, IN) and water were available ad libitum.

2.2. Developmental PCB exposures

The MARBLES PCB mixture was chosen since it is derived from the MARBLES (Markers of Autism Risk in Babies Learning Early Signs) study and represents a contemporary relevant mixture of PCBs found in pregnant women (Hertz-Picciotto et al., 2018) which was characterized for endocrine activity (Sethi et al., 2019) and has been used in prior mouse studies on neurological and urological function (Panesar et al., 2020; Keil Stietz et al., 2021; Kennedy et al., 2022; Kennedy et al., 2021; Spiegelhoff et al., 2023; Matelski et al., 2020; Sethi et al., 2021). This mix contains each of the following PCBs at the specified percentage: 28 (48.2 %), 11 (24.3 %), 118 (4.9 %), 101 (4.5 %), 52 (4.5 %), 153 (3.1 %), 180 (2.8 %), 149 (2.1 %) 138 (1.7 %), 84 (1.5 %), 135 (1.3 %), and 95 (1.2 %). PCBs were synthesized and authenticated by the Synthesis Core of the University of Iowa Superfund Research Program previously described (Sethi et al., 2019; Li et al., 2018), and mice were dosed as described previously (Kennedy et al., 2022). Briefly, female nulliparous adult mice were dosed orally daily with the MARBLES PCB mixture mixed in organic peanut oil and peanut butter (Spectrum Organic Products, LLC, Melville, NY, Trader Joe's, Monrovia, CA) or vehicle control (peanut oil and peanut butter only) starting two weeks prior to mating and continuing through gestation and lactation until pups were weaned at postnatal day (P) 21. The 0.1, 1, or 6 mg/kg body weight/day doses and dosing paradigm were chosen because it results in exposed offspring bearing detectable levels of PCBs in bladder and other organs at levels relevant to human health and lead to varied phenotypes in offspring (Keil Stietz et al., 2021; Sethi et al., 2021; Kania-Korwel et al., 2017; Yang et al., 2009). After weaning, male and female offspring were group housed with same sex and dose littermates. In an effort to reduce and refine animal numbers, mice were generated as part of a larger study (Kennedy et al., 2022), being allowed to age to 12 weeks while some littermates were collected at earlier timepoints. A total of 8–13 mice per group derived from 3 to 7 litters per group were used in this study.

2.3. Void spot assay (VSA)

VSA was conducted using best practices as described previously

(Kennedy et al., 2022; Wegner et al., 2018). Briefly, mice (84.4 ± 0.7 days old, n = 8–13 mice derived from 3 to 7 litters per group) were acclimated to the testing room for at least 1 h prior to starting. Mice were singly placed into an empty plastic cage with 3 MM chromatography paper (057163E, Fisher Scientific) lining the bottom and had access to food but not water during the 4-hour testing period. Filter papers were allowed to dry and then imaged using a UVP ChemStudio PLUS (Analytik Jena, Jena, Germany) under UV light with 7 s exposure with VisionWorks (Analytik Jena) software. VSA image analysis was performed using the Void Whizzard freely available analysis software designed for Image J using default parameters by an individual blinded to treatment conditions (Wegner et al., 2018).

2.4. Uroflowmetry

Uroflowmetry was conducted as described previously using Void Sorcerer open access design and software (Kennedy et al., 2022; Wang et al., 2019). Briefly, uroflowmetry was performed in the same room as VSA testing, and mice (86.1 ± 1.1 days old, n = 8–13 mice derived from 3 to 7 litters per group) were acclimated to the room for at least 1 h prior to testing. Mice were placed into uroflowmetry chambers with access to water but not food during the 4-hour testing period. Uroflowmetry data was analyzed by an individual blinded to treatment conditions. Only urine events that fell without inference from the grid floor bars or without co-occurrence of a fecal event were used in analysis. This resulted in a final n = 7–12 mice derived from 3 to 6 litters per group. To determine a stream rating, a scale from 1 to 3 was used where 1 was a drop pattern void and 3 was a strong stream void. Urine mass was converted to urine volume by dividing the change in urine mass by 1.0046 g/ml. Flow rate was then calculated as change in volume over change in time for urine events.

2.5. Cystometry

Anesthetized cystometry was performed essentially as described previously (Kennedy et al., 2022). Briefly, mice (87.5 ± 1.3 days old, n = 8–13 mice derived from 3 to 7 litters per group) were anesthetized with a subcutaneous injection of urethane dissolved in saline (AC32554-0500, Fisher, Waltham, MA) at a dosage of 1.43 g urethane/kg mouse from fresh stock 86 mg/ml. Approximately 30 min following urethane administration, the abdomen was opened and a purse string suture (6–0 Silk, 501180809, Fisher) was placed in the dome of the bladder. PE-50 tubing (NC9140178, Fisher) was used as a catheter and placed into the dome of the bladder using a 25 G 1.5-inch needle. The needle was removed and a purse string suture was tied to secure the catheter and prevent fluid from retrograde leaking from the bladder dome. The abdominal wall and skin were closed with a suture, and the mouse was placed on a heating pad for ~ 60 min. Mice were then connected to an in-line pressure transducer and infusion pump. Saline was infused at a rate of 0.8 ml/hr, and intravesical pressure was recorded using an MLT844 physiological pressure transducer (ADInstruments, Colorado Springs, CO) connected to an FE221 Bridge Amp (ADInstruments) with a Power lab 2/26 (PL2602) data acquisition system. Cystometrograms were recorded and analyzed using LabChart software (ADInstruments). Recordings were conducted for ~ 1 h to achieve a steady pattern. Immediately following saline infusion, mice were then infused with a fresh stock solution of capsaicin (CAS 404–86-4, Millipore Sigma, Burlington, MA) dissolved in saline (30 μM). While transitioning between the two solutions, the in-line tubing was disconnected from the catheter and was flushed with the 30 μM capsaicin solution to ensure capsaicin solution immediately entered the bladder upon reconnection of the tubing to the catheter. The cystometrogram was recorded for the first 5 consecutive voids during capsaicin infusion. 5 consecutive voids were selected from both saline and capsaicin infusions, analyzed, and averaged per animal by an individual blinded to treatment conditions. Parameters measured from the cystometrograms include void duration, intervoid interval, normalized threshold pressure, normalized maximum void pressure, non-voiding contractions (minimum 5 mmHg pressure change), and compliance (infused volume/change in pressure). If intravesical pressure did not build over the hour and was not recovered with a subsequent repositioning of the catheter, then the mouse was excluded from analysis. Additionally, if there was not a clear threshold pressure, that parameter was not analyzed, resulting in a final n = 5–11 mice derived from 3 to 7 litters per group. If disconnecting and switching to capsaicin resulted in removal of the catheter or failure to elicit voids, then that animal was excluded from the paired capsaicin comparisons, resulting in a final n = 4–10 mice derived from 3 to 6 litters per group.

The order of testing was void spot assay (VSA), uroflowmetry, and then anesthetized cystometry with first a saline infusion immediately followed by infusion of capsaicin in saline. Testing was carried out at the same time of day; however, cytometry can only accommodate 4 mice per day, so mice were randomly counterbalanced to start in the AM or PM. After cystometry, mice were euthanized with CO_2 and necropsy was immediately performed to collect prostate lobes, seminal vesicle, and testes for wet weight masses from male mice.

2.6. Tissue collection

Following CO_2 euthanasia, testes were removed, and seminal vesicle and prostate lobes were micro-dissected. Tissue wet weights were measured immediately after necropsy from n = 4–11 mice derived from 3 to 6 litters per group.

2.7. Statistics

Statistics were run using GraphPad Prism software version 10 or R. Data were assessed using mixed effects models to account for litter effects. In Prism this is termed a nested one-way ANOVA (littermates are entered into subcolumns within a treatment group), the same analysis can be conducted in R using the lme function from the nlme package. R was used for paired or repeated measures data since this structure could not be accounted for in nested Prism analyses. Nested one-way ANOVAs were followed by Tukey's multiple comparisons tests. The lme function in R followed by the emmeans package for pairwise comparisons with Tukey adjustment was used to determine PCB effects within urine spot size distribution in male and female mice (Fig. 1B-C). Pairwise analysis of saline and capsaicin infusion during anesthetized cystometry were limited to mice with endpoints collected in both tests. Each parameter analyzed the effect of 30 μM capsaicin infusion compared to saline infusion within a group using lme function in R (Fig. 4). Descriptive statistics of frequent spotting patterns (Table 1) were assessed with Fisher's exact tests. Statistical tests run on each data set and n values of animal and litter are stated within the figure legends, p values ≤ 0.05 were considered significant and are indicated by asterisks in the figures and reported in the results. Trends were noted only in the text for data in which $p \leq 0.09$. The test statistic details are provided in Supplemental Table 1. Data were assessed for normality using D'Agostino and Pearson test, Anderson-Darling test, Shapiro-Wilk test, Kolmogorov-Smirnov test or QQ plots. If a log or square root transformation improved normality the test was also performed on transformed data and is also included in Supplemental Table 1. For nonparametric paired data (Fig. 4) for which transformation did not restore normality, ranked values were used in the lmer function and are also included in Supplemental Table 1.

3. Results

3.1. PCBs decrease the number of voided small diameter urine spots

Voiding phenotypes are dynamic over the course of aging in mice (Keil et al., 2016). Therefore, it is important to assess voiding function throughout life in response to early life environmental hits. Here we focus on testing voiding function in male and female mice

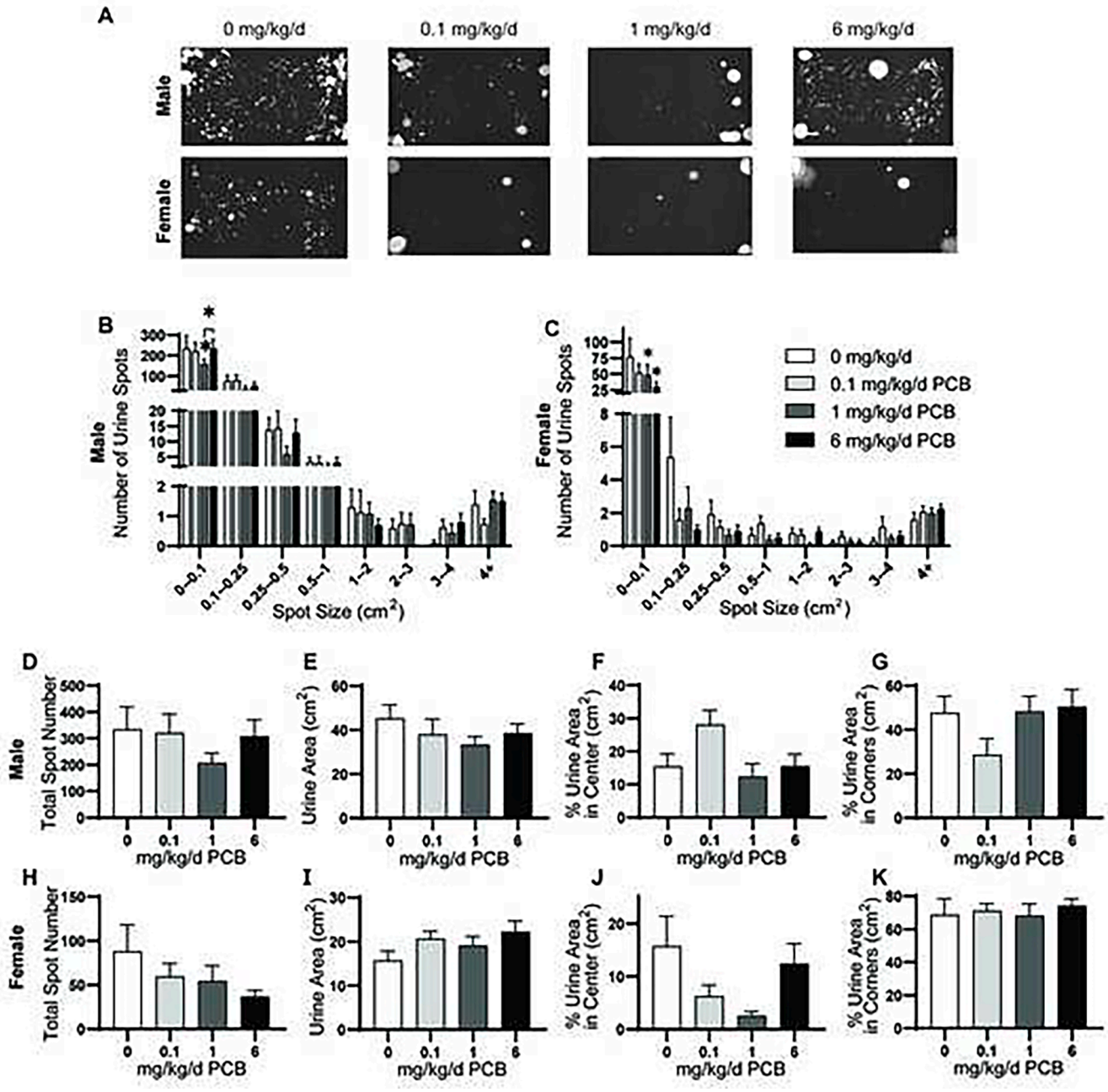


Fig. 1. Developmental PCB exposure decreased the number of small 0–0.1 cm^2 spots in male and female offspring while undergoing VSA. Mice were developmentally exposed to PCBs via the dam and VSA testing conducted on adult male and female mice. (A) Representative images of VSA for both male and female PCB dosage groups, paper length 16.5 x 27 cm. Parameters examined following the 4-hour VSA include for male and female include; (B,C) urine spot size distribution, (D,H) total urine spot number, (E,I) total urine area, (F,J) percent of the urine area in the center of the paper and (G,K) percent of the urine area in the corners of the paper. Results are mean ± SEM n = 8–11 males derived from 4 to 6 litters per treatment group, n = 8–13 females derived from 3 to 7 litters per treatment group. * indicates significant difference from vehicle control. Bar and * indicate significant differences between dosage groups. $p \leq 0.05$ were considered significant as determined by mixed effects models to account for litter: (B,C) Mixed effects model (R code: m = lme(Value ~ Treatment * Spot, random = ~ 1| Litter/Animal, data = data) followed by pairwise comparisons with Tukey adjustment (R code: c = emmeans(m, ~ Treatment | Spot); effects = pairs(c), (D-K) Nested one-way ANOVA to account for litter followed by Tukey's multiple comparisons test.

developmentally exposed (*in utero* and lactational) to PCBs and then aged to 12 weeks of age. To examine voiding physiology in a non-invasive manner, we first conducted void spot assay (VSA). Changes to distribution of spotting frequency were limited to small spot sizes 0–0.1 cm^2 (Fig. 1A-C, Supplemental Table 1). In male mice, there was a significant decrease in small urine spot sizes in the 1 mg/kg/d PCB group compared to vehicle control and 6 mg/kg/d PCB dose groups (Fig. 1B, p = 0.02, p = 0.03 respectively). In female mice, there was a significant decrease in small urine spot sizes in the 1 and 6 mg/kg/d PCB dose groups compared to the vehicle control group (Fig. 1C, p = 0.05, p = 0.003, respectively; the 0.1 mg/kg/d PCB group versus vehicle control group was trending p = 0.08). There were no significant differences in the total spot number, total urine area, or the percentage of urine area located in the center or corners of the cage in male or female mice (Fig. 1D-K). A normal effect of aging is for a subset of mice to acquire a 'frequent' spotter phenotype (Keil et al., 2016). We assessed this

Table 1
Urine spotting frequency unchanged by developmental PCB exposure in either sex.

	Male				Female			
mg/kg/d PCB	0	0.1	1	6	0	0.1	1	6
Frequent spotters (50 +)	8	8	11	10	5	6	2	3
Non-frequent spotters	2	0	0	0	8	7	8	5
Frequent spotters (100 +)	8	7	9	9	3	2	2	0
Non-frequent spotters	2	1	2	1	10	11	8	8
Frequent spotters (200 +)	6	5	4	5	2	1	0	0
Non-frequent spotters	4	3	7	5	11	12	10	8
Frequent spotters (300 +)	5	4	3	5	1	0	0	0
Non-frequent spotters	5	4	8	5	12	13	10	8

Developmental PCB exposure did not alter the distribution of a frequent spotter phenotype. Mice were developmentally exposed to PCBs via the dam and VSA testing conducted on adult male and female mice. Total number of urine spots were analyzed at each cutoff value and the number of mice in each category was assessed using Fischer's exact test, no significant differences from vehicle control were found. Results are from n = 8–11 male mice per group, n = 8–13 female mice per group derived from 3 to 7 litters per group.

parameter by determining the number of mice that produced a total number of urine spots greater than 50, 100, 200, or 300 (Table 1). No significant changes in proportion of mice with each spot frequency was observed.

Since VSA is unable to discriminate between discrete void events, we also conducted uroflowmetry on 12 week old male and female offspring following developmental PCB exposure. Uroflowmetry parameters of stream rating, void mass, void duration and flow rate were unchanged in male and female mice (Fig. 2, Supplemental Table 1). However, there was a trend for reduced male stream rating driven by 0.1 mg/kg/d PCB group compared to the vehicle control group (Fig. 2A, $p = 0.09$).

3.2. PCBs decrease the time between urinary void events during anesthetized cystometry in female mice

In order to examine voiding physiology in response to bladder filling in a controlled environment, we conducted anesthetized cystometry (CMG) on 12 week old male and female offspring following developmental PCB exposure (Fig. 3, Supplemental Table 1). No changes were observed for either sex regarding void duration, normalized peak pressure, non-voiding contractions, normalized threshold pressure, or compliance (Fig. 3A-D and Fig. 3G-L). In male mice, there were no PCB effects on intervoid interval (Fig. 3E). However, in female mice, intervoid interval was significantly decreased in the 1 mg/kg/d PCB and 6 mg/kg/d PCB groups compared to vehicle control (Fig. 3F, $p = 0.04$, $p = 0.01$ respectively). Therefore, developmental PCB exposure increases the frequency of urinary voiding events of female mice undergoing anesthetized cystometry at 12 weeks of age.

3.3. PCBs alter TRPV1-mediated voiding physiology

Since we observed PCB induced changes to urine spot sizes in male and female mice and intervoid intervals in female mice, we sought to test whether PCBs influence bladder sensitivity to filling. To test this, we examined the bladder's response upon challenge with intravesical instillation of capsaicin. Capsaicin is a specific agonist of TRPV1 (transient receptor potential vanilloid 1) receptors which are important for mechanosensation. Fig. 4 presents the results of comparing CMG parameters before (saline) versus after capsaicin (30 μM capsaicin in saline) infusion in the same animals. There were several effects of PCBs on capsaicin responses in a sex- and dose- dependent manner. There were three parameters, void duration, intervoid interval and non-voiding contractions, which displayed capsaicin effects in only one of the sexes. In male mice, void duration was significantly increased by capsaicin in the vehicle control group (Fig. 4A, $p = 0.004$, Supplemental Table 1) but not in any PCB groups (though a trend was noted for an effect in the 1 and 6 mg/kg/d PCB groups, $p = 0.08$), nor was this parameter altered in female mice (Fig. 4B). Likewise, nonvoiding contractions were significantly decreased by capsaicin in the vehicle control group (Fig. 4K, $p = 0.01$) but not in any PCB groups (though a trend was noted for an effect in the 1 mg/kg/d PCB group, $p = 0.07$), nor was this parameter altered in female mice (though a trend was noted for an effect in the 0.1 mg/kg/d PCB group, $p = 0.07$, and the 1 mg/kg/d PCB group, $p = 0.09$) (Fig. 4L). Conversely, male mice showed no changes in intervoid interval (Fig. 4C), while in female mice intervoid interval was decreased by capsaicin only in the 0.1 mg/kg/d PCB group (Fig. 4D, $p = 0.05$). Several parameters had effects of capsaicin in both males and females that varied by PCB dose. In male mice, normalized threshold voiding pressure was decreased by capsaicin in the vehicle control group (Fig. 4E, $p = 0.007$), but not in any of the PCB groups. In female mice,

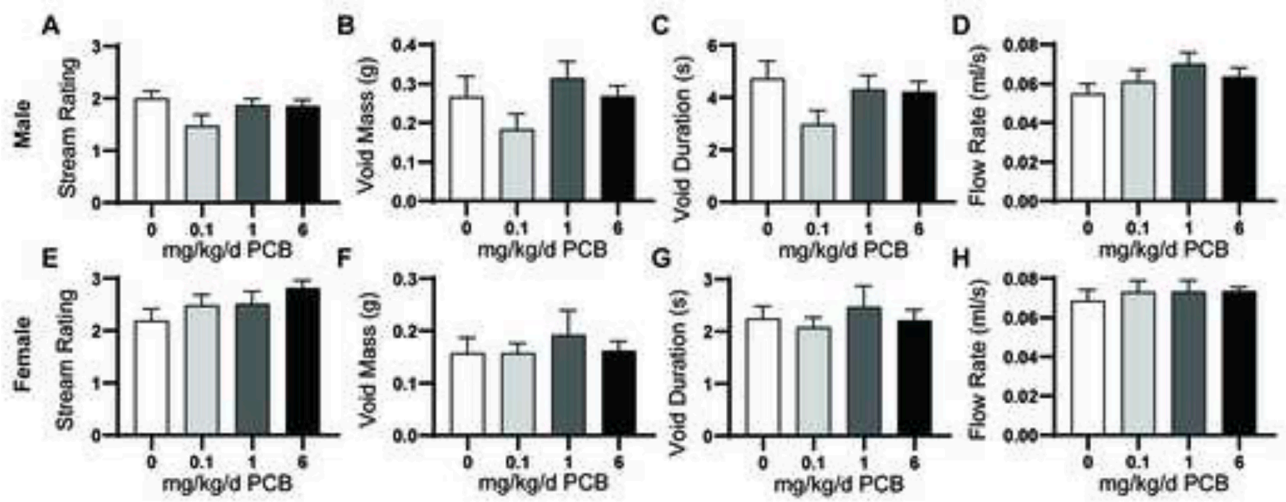


Fig. 2. Developmental PCB exposure did not alter urine flow rating, mass, duration, or flow rate while undergoing uroflowmetry. Mice were exposed to PCBs via the dam and uroflowmetry testing conducted on young adult male and female mice. Examined parameters include (A,E) urine stream rating, (B,F) void mass (g), (C,G) void duration (s), and (D,H) flow rate (mL/s). Results are mean ± SEM n = 8–10 males derived from 4 to 6 litters per treatment group, n = 7–12 females derived from 3 to 6 litters per treatment group. No significant differences, $p \leq 0.05$ as determined by nested one-way ANOVA to account for litter followed by Tukey's multiple comparisons test.

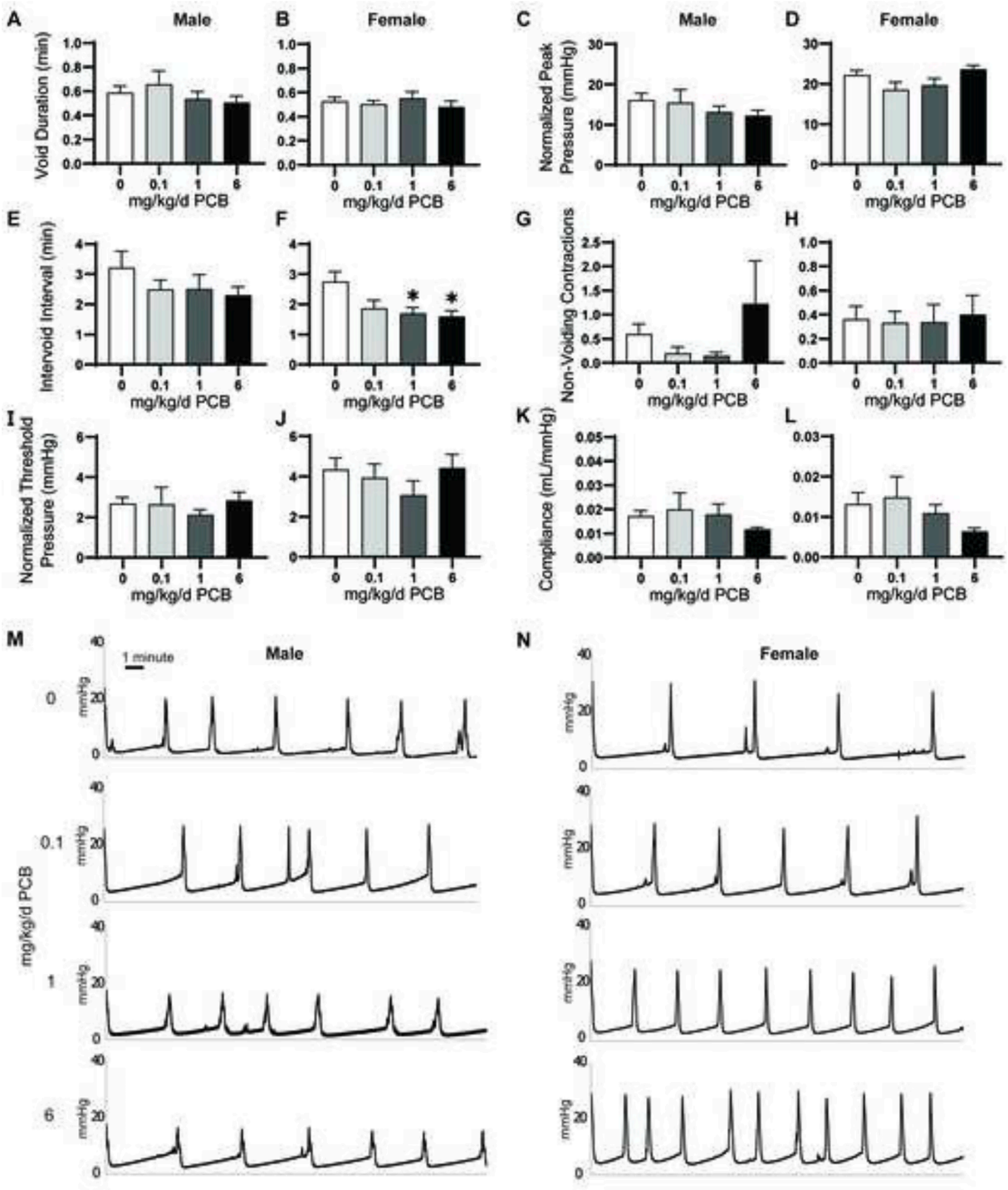


Fig. 3. Developmental PCB exposure decreases intervoid interval in female offspring. Mice were exposed to PCBs via the dam and anesthetized cystometry testing was conducted with saline infusion on young adult male and female mice. Parameters examine include (A, B) void duration (min), (C, D) normalized peak pressure (mmHg), (E, F) intervoid interval (min), (G, H) non-voiding contractions, (I, J) normalized threshold pressure (mmHg), and (K, L) compliance (ml/mmHg). (M, N) Representative cystometrograms. Results are mean ± SEM n = 5–11 males derived from 4 to 6 litters per treatment group, n = 8–11 females derived from 3 to 7 litters per treatment group. * indicates significant difference from vehicle control $p \leq 0.05$ as determined by nested one-way ANOVA to account for litter followed by Tukey's multiple comparisons test.

this parameter was also decreased by capsaicin in the vehicle control group, 0.1 mg/kg/d, 6 mg/kg/d PCB groups (Fig. 4F, p = 0.004, p = 0.03, p = 0.01 respectively) but not the 1 mg/kg/d PCB group. In male mice, maximum voiding pressure was increased by capsaicin only in the 1 mg/kg/d and 6 mg/kg/d PCB groups (Fig. 4I, p = 0.05, p = 0.007) (though a trend was noted for an effect in the vehicle control group, p = 0.07), while in female mice capsaicin decreased maximum voiding pressure only in the 6 mg/kg/d PCB group (Fig. 4J, p = 0.03). In male mice capsaicin also significantly increased compliance in the 1 mg/kg/d PCB group (Fig. 4G, p = 0.02) (though a trend was noted for an effect in the vehicle control group and 6 mg/kg/d PCB group both p = 0.08), while in female mice capsaicin increased compliance in the vehicle control group and 6 mg/kg/d PCB groups only (Fig. 4H, p = 0.05, p = 0.003 respectively). These results indicate that capsaicin infusion alone

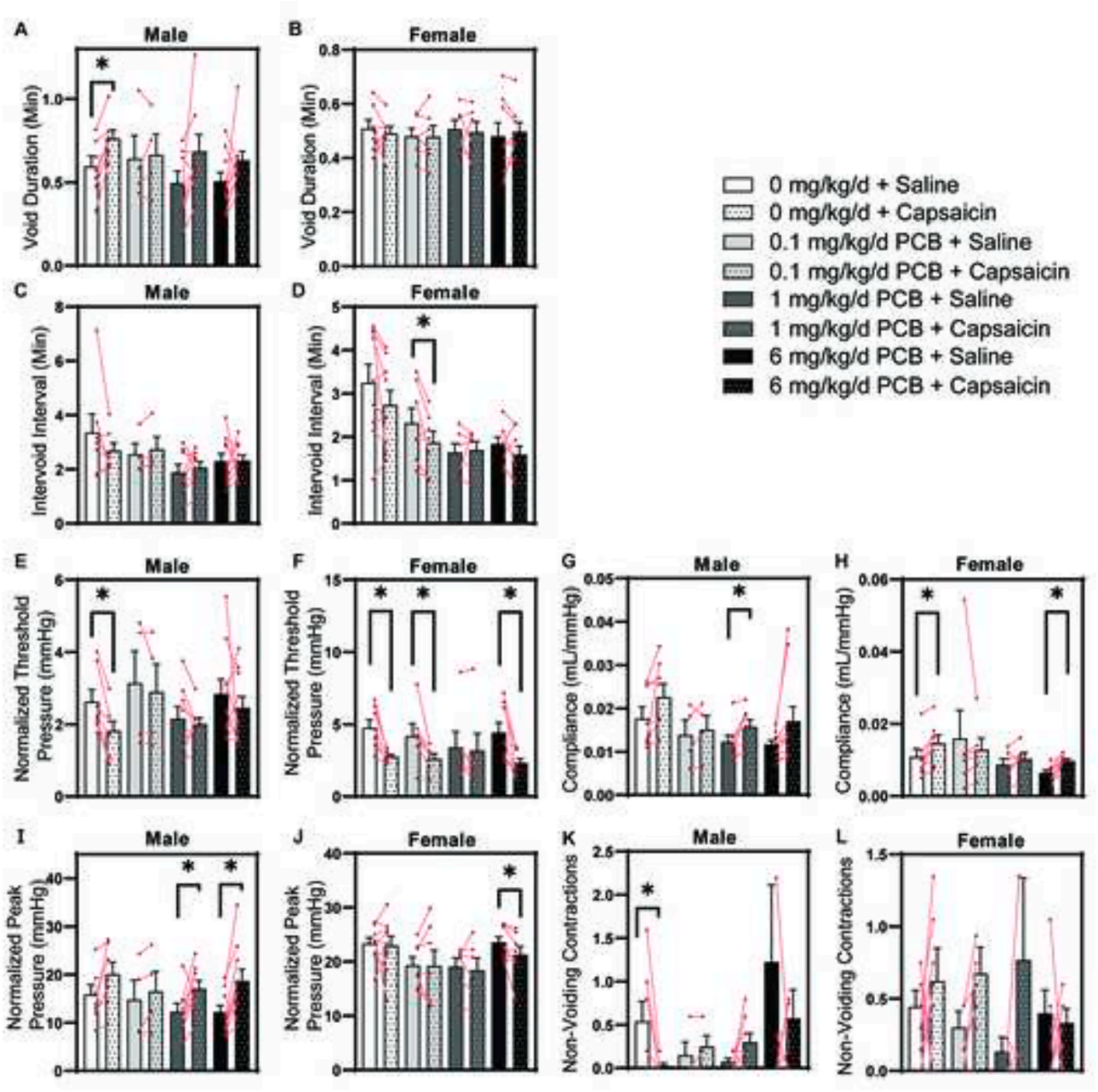


Fig. 4. Developmental PCB exposure had sex- and dose- dependent effects on CMG parameters when comparing saline to capsaicin infusion. Mice were exposed to PCBs via the dam and anesthetized cystometry testing was conducted with saline infusion followed by capsaicin infusion on young adult male and female mice. The average values for each mouse are shown as red points, and a red line connects data points collected from the same individual mouse. Parameters examine include (A, B) void duration, (C, D) intervoid interval, (E, F) normalized threshold pressure, (G, H) compliance, (I, J) normalized peak pressure, and (K, L) non-voiding contractions. Results are mean ± SEM n = 4–10 males derived from 3 to 6 litters per treatment group, n = 6–9 females derived from 3 to 6 litters per treatment group. * indicates significant difference from saline of that group $p \leq 0.05$ as determined by mixed effects model to account for litter using R lme function (R code: m = lme (Value ~ Treatment, random = ~ 1| Litter/Animal, data = data). For nonparametric data the mixed effects model using R lmer function (R code: m = lmer(ranked_values ~ Treatment + (1 | Animal) + (1 | Litter), data = data) on ranked values was used (C 0.1 mg; K 0 mg, 0.1 mg, 6 mg; L 1 mg). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

induces a measurable effect on bladder sensitization, and PCBs are capable of influencing this response in a sex- and dose- dependent manner, both blocking capsaicin responses seen in vehicle control animals and enhancing responses not otherwise seen in vehicle control animals. Table 2 summarizes all significant changes in voiding parameters.

3.4. PCBs decrease ventral prostate mass

Since we previously observed in younger (6 weeks of age) male mice that developmental PCB exposure led to a decrease in ventral prostate mass in the 1 mg/kg/d PCB dose group compared to the 0.1 mg/kg/d and 6 mg/kg/d PCB dose groups only (Spiegelhoff et al., 2023), we wanted to confirm whether changes in prostate persist to 12 weeks of age or could be responsible for any of the male voiding phenotypes observed. In this study, at 12 weeks of age, body mass of male and

Table 2
Summary of PCB effects on urinary voiding physiology.

	0 mg/kg/d		0.1 mg/kg/d		1 mg/kg/d		6 mg/kg/d	
Void Spot Assay	Male	Female	Male	Female	Male	Female	Male	Female
0–0.1 cm^2 spot size	–	–	ns	ns	↓	↓	ns	↓
0.1–0.25 cm^2 spot size	–	–	ns	ns	ns	ns	ns	ns
0.25–0.5 cm^2 spot size	–	–	ns	ns	ns	ns	ns	ns
0.5–1 cm^2 spot size	–	–	ns	ns	ns	ns	ns	ns
1–2 cm^2 spot size	–	–	ns	ns	ns	ns	ns	ns
2–3 cm^2 spot size	–	–	ns	ns	ns	ns	ns	ns
3–4 cm^2 spot size	–	–	ns	ns	ns	ns	ns	ns
4 + cm^2 spot size	–	–	ns	ns	ns	ns	ns	ns
Total spot number	–	–	ns	ns	ns	ns	ns	ns
Total urine area cm^2	–	–	ns	ns	ns	ns	ns	ns
Urine area in center cm^2	–	–	ns	ns	ns	ns	ns	ns
Urine area in corners cm^2	–	–	ns	ns	ns	ns	ns	ns
Uroflowmetry								
Stream rating	–	–	ns	ns	ns	ns	ns	ns
Void mass	–	–	ns	ns	ns	ns	ns	ns
Void duration	–	–	ns	ns	ns	ns	ns	ns
Flow rate	–	–	ns	ns	ns	ns	ns	ns
Cystometry – Saline								
Void duration	–	–	ns	ns	ns	ns	ns	ns
Intervoid interval	–	–	ns	ns	ns	↓	ns	↓
Normalized threshold pressure	–	–	ns	ns	ns	ns	ns	ns
Normalized peak pressure	–	–	ns	ns	ns	ns	ns	ns
Non-voiding contractions	–	–	ns	ns	ns	ns	ns	ns
Compliance	–	–	ns	ns	ns	ns	ns	ns
Cystometry – Saline then Capsaicin								
Void duration	↑	ns	ns	ns	ns	ns	ns	ns
Intervoid interval	ns	ns	ns	↓	ns	ns	ns	ns
Normalized threshold pressure	↓	↓	ns	↓	ns	ns	ns	↓
Normalized peak pressure	ns	ns	ns	ns	↑	ns	↑	↓
Compliance	ns	↑	ns	ns	↑	ns	ns	↑
Non-voiding contractions	↓	ns	ns	ns	ns	ns	ns	ns

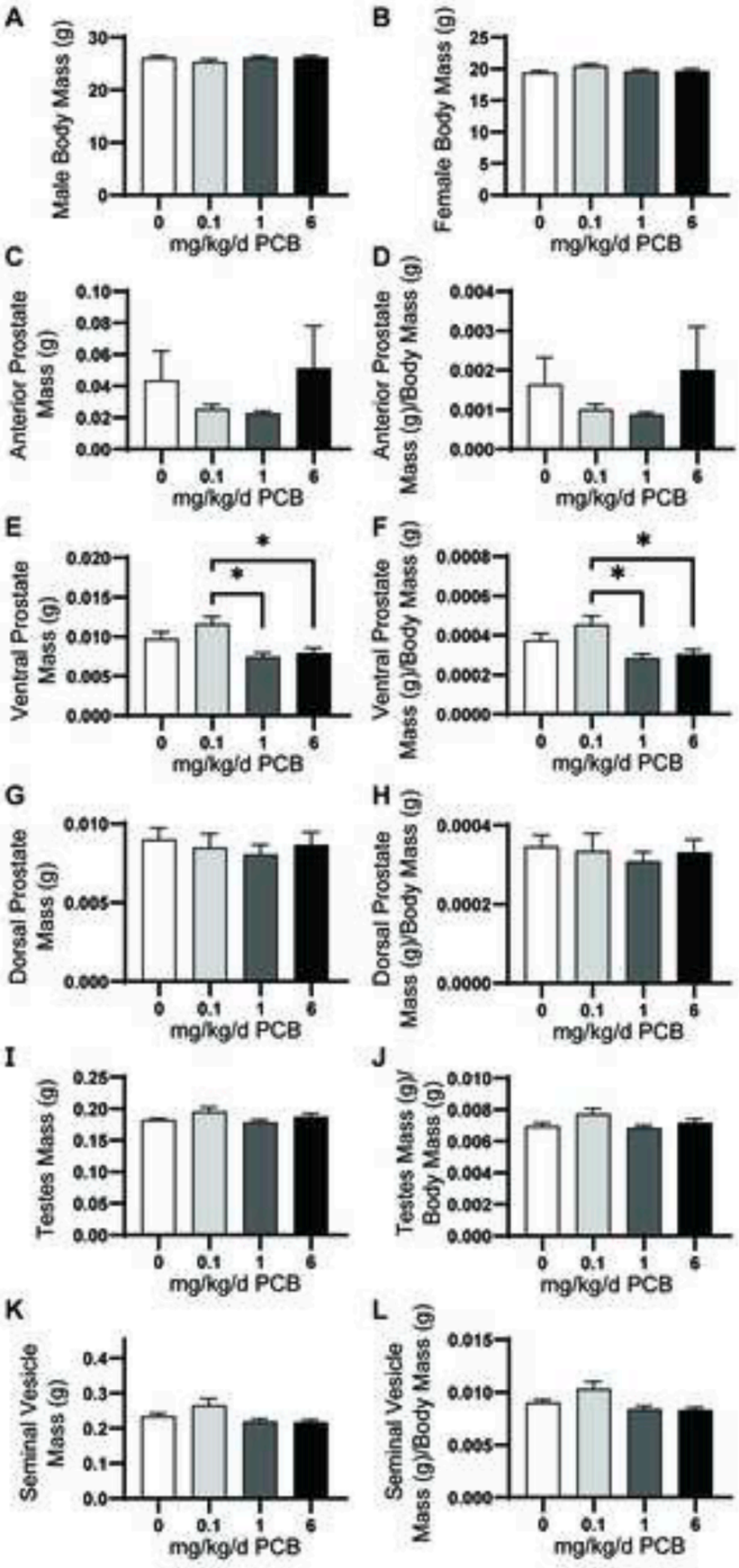
Developmental PCB exposure had sex- and dose- dependent effects on voiding physiology at 12 weeks of age. Summary of results where arrow indicates significant changes ($p \leq 0.05$), in direction indicated. Dash indicates comparisons were made to the vehicle control group and therefore not applicable. "ns" indicates "no significance.".

female mice was unchanged across all dose groups (Fig. 5A-B). Anterior prostate mass and dorsal prostate mass were unchanged both as raw wet weights and when normalized to body mass (Fig. 5C-D, G-H). Ventral prostate mass of both the 1 mg/kg/d and 6 mg/kg/d PCB groups were significantly decreased compared to the 0.1 mg/kg/d PCB group male mice both when comparing raw wet weight (Fig. 5E, $p = 0.01$, $p = 0.03$ respectively) and when normalized to body mass(Fig. 5F, $p = 0.01$, $p = 0.03$ respectively). Aside from changes in prostate mass, changes in wet weight to testes and seminal vesicle can also be influenced by hormone disruption. There were no significant differences in testes wet weight alone or normalized to body mass (Fig. 5I-J), a trend was noted for testes mass alone and normalized to body mass driven by the 0.1 mg/kg/d PCB group versus 1 mg/kg/d PCB group (Fig. 5I, $p = 0.08$, Fig. 5J, $p = 0.06$). Seminal vesicle mass was not significantly altered (Fig. 5K), but a trend was noted for a decrease in the 6 mg/kg/d PCB group compared to the 0.1 mg/kg/d PCB group when normalized to body mass (Fig. 5L, $p = 0.06$). Together, these data indicate that the higher doses of PCBs tend to have reduced wet weight of steroid hormone sensitive tissue compared to the low dose of PCBs. While PCB induced changes to ventral prostate tissue mass was reserved to between PCB groups and not the vehicle control, it suggests that different doses of PCBs may trigger different effects on steroid hormone dependent endpoints.

4. Discussion

This study investigated persistence of adult urinary voiding dysfunction resulting from developmental exposure to MARBLES PCBs. Previous work demonstrated developmental exposure to MARBLES PCBs results in sex- and dose- dependent voiding dysfunction, generally characterized as more frequent, drop-like voids (Spiegelhoff et al., 2023). Here we investigated whether detectable changes in voiding physiology persisted to 12 weeks of age, 9 weeks removed from the last possible lactational PCB exposure (summarized in Table 2). We demonstrate developmental PCB exposure is sufficient to decrease small urine spot sizes and intervoid interval in a sex- and dose-dependent manner. Strikingly, we provide evidence that upon subsequent challenge with capsaicin (TRPV1 agonist/desensitizer) PCB exposed mice do not respond similarly to vehicle control, suggesting subtle PCB induced changes in voiding physiology – which were not necessarily apparent under saline cystometry conditions – were revealed when hit with a second challenge. This is important as changes to voiding through normal aging or response to additional stressors later in life could be influenced by developmental PCB exposures.

Urinary voiding physiology is dynamic and can be influenced by genotype, age, sex, and xenobiotic exposures (Ruetten et al., 2019; Keil et al., 2016; Bjorling et al., 2015). This study illustrates the persistence of voiding dysfunction into adulthood following developmental MARBLES PCB exposure, highlighting the dynamic nature of urinary voiding abnormalities over time. While the PCB exposure paradigm is the same between the current study and a previous study of younger mice (Kennedy et al., 2022), the 6 week difference in age coincides with sexual maturity. Age is expected to shift baseline metrics of voiding phenotypes in both sexes (Keil et al., 2016; Bjorling et al., 2015). Therefore, this may also impact the maximum influence of PCBs as animals age. Two major differences in VSA outcomes were observed when comparing the effects of developmental PCB exposure between the previous (6 weeks of age (Kennedy et al., 2022) and current study (12 weeks of age) – urine spot count and size. At 6 weeks of age, PCBs increased frequent spotting in 0.1 mg/kg/d and 6 mg/kg/d PCB male mice and in 0.1 mg/kg/d PCB female mice, and PCBs increased number of small urine spots (0–0.1 cm^2) in 0.1 mg/kg/d and 6 mg/kg/d PCB male mice and all doses in female mice (Kennedy et al., 2022). In this



(caption on next page)

Fig. 5. Developmental PCB exposure decreased the mass of ventral prostate in 1 mg/kg/d and 6 mg/kg/d compared to 0.1 mg/kg/d PCB dose group in male offspring. Mice were exposed to PCBs via the dam and weighed prior to euthanasia. Prostate lobes, testes, and seminal vesicle were collected immediately following euthanasia and weighed. Results are mean ± SEM n = 8–11 males derived from 4 to 6 litters per treatment group (body mass), 4–11 males derived from 3 to 6 litters per treatment group (prostate/SV), n = 8–13 females derived from 3 to 7 litters per treatment group. Bar and * indicate significant differences $p < 0.05$ as determined by nested one way ANOVA to account for litter followed by Tukey's multiple comparisons tests.

study, no PCB effects on frequent spotting were observed. The number of small spots was not increased, rather fewer small urine spots were observed in the 1 mg/kg/d PCB male mice and the 1 and 6 mg/kg/d PCB group female mice when compared to vehicle controls. Since the quantity of urine spots in untreated wild type mice increases with age (Keil et al., 2016; Bjorling et al., 2015), these observed changes suggest two possibilities. 1) PCBs may have a protective effect on age associated increases in small urine spots, or 2) because PCBs increase small urine spots earlier in life, the difference with aging is masked in PCB exposed mice. The drop like stream of 0.1 mg/kg/d PCB male mice at 6 weeks of age undergoing uroflow (Kennedy et al., 2022) was not as severe at 12 weeks of age, though the same trend persisted. Anesthetized cystometry also demonstrated a shift in PCB effects over time. In male mice at 6 weeks of age, PCBs increased max void pressure in 0.1 mg/kg/d and 1 mg/kg/d PCB groups versus vehicle control (Kennedy et al., 2022). This was no longer observed at 12 weeks of age. In female mice at 6 weeks of age PCBs decreased intervoid interval at 0.1 mg/kg/d and 6 mg/kg/d PCB doses compared to the vehicle control group (Kennedy et al., 2022). Here, at 12 weeks of age, decreased intervoid interval in female mice was retained in the 6 mg/kg/d PCB group, no longer observed in the 0.1 mg/kg/d PCB group, and newly observed in the 1 mg/kg/d PCB group. These CMG results suggest developmental PCB exposure produces more severe effects in female mice versus male mice at 12 weeks of age. Overall, results of the three voiding assays clearly underline that PCBs continue to impact voiding at 12 weeks of age, generally resulting in fewer small urine spots in both sexes, and more frequent voiding in female mice (summarized in Table 2). Furthermore, by comparing PCB effects on voiding here at 12 weeks of age to previously reported mice at 6 weeks of age, this study demonstrates PCB effects on voiding can converge, diverge, or persist over the course of normal aging.

PCB effects on TRPV1-mediated voiding activity to our knowledge has never been studied. TRPV1 pathway signaling is involved in physiological and pathological processes throughout the body, especially bladder mechanosensation (Storozhuk et al., 2019). TRPV6, another calcium channel in the TRPV family, is implicated in PCB effects on skeletal development in zebrafish and rats (Ju et al., 2012; Ronis et al., 2020) and cytotoxicity in the osteoblast cell line MC3T3-E1 (An et al., 2012). Intravesical infusion of capsaicin is useful for studying TRPV1-mediated urinary voiding physiology; Capsaicin specifically binds to and activates TRPV1, sensitizing C-type afferent nerves which are generally silent in homeostatic conditions, causing a period of increased bladder sensitivity and subsequent refractory period of desensitized tachyphylaxis (Avelino et al., 2002; Fischer et al., 2020; Mohapatra and Nau, 2005; Sanz-Salvador et al., 2012; Smutzer and Devassy, 2016; Tian et al., 2019; Yoshimura and Chancellor, 2003). Interestingly, developmental PCB exposure is sufficient to alter responses to capsaicin which are sex- and dose-dependent (summarized in Table 2). In male mice PCB exposure at all doses prevented any capsaicin induced change that was seen in vehicle control mice on void duration, threshold pressure, and non-voiding contractions. Newly acquired sensitivity to capsaicin (not seen in vehicle control) was observed in 1 mg/kg/d and 6 mg/kg/d PCB male mice with capsaicin induced increases in max void pressure, and in 1 mg/kg/d PCB male mice with capsaicin induced increases in compliance. In female mice, the 1 mg/kg/d PCB exposure prevented any capsaicin induced change seen in vehicle control on threshold pressure and compliance. The 0.1 mg/kg/d PCB exposure also prevented capsaicin induced increases in compliance. Newly acquired sensitivity to capsaicin (not seen in vehicle control), was observed in the 0.1 mg/kg/d and 6 mg/kg/d PCB female mice with capsaicin induced decreases in intervoid interval and max void pressure respectively. Looking across all parameters during capsaicin challenge, a capsaicin effect was never observed in male 0.1 mg/kg/d PCB groups or female 1 mg/kg/d PCB groups (Table 2) suggesting these doses produce a capsaicin resistant/insensitive phenotype. Together these data demonstrate that PCBs are capable of inducing sex- and dose-dependent changes to sensitivity to capsaicin, enhancing or eliminating the sensitivity, suggesting that capsaicin sensitive pathways may be important targets mediating developmental PCB exposure on voiding function. With the ability of capsaicin to both sensitize and desensitize bladder TRPV1 receptors (Avelino et al., 2002; Fischer et al., 2020; Mohapatra and Nau, 2005; Sanz-Salvador et al., 2012; Smutzer and Devassy, 2016; Tian et al., 2019; Yoshimura and Chancellor, 2003), it is not surprising that PCB effects could prime the bladder to either be more sensitive or less sensitive to capsaicin. Since treatment with capsaicin or other agonists of this pathway are used in humans to desensitize C-fiber afferents thus reducing overactive bladder symptoms, whether PCBs can influence response to this treatment strategy is a future area of study (Andersson et al., 2022; Charrua et al., 2009; Liu et al., 2014). Overall, TRPV1-mediated signaling is directly and/or indirectly altered under this PCB dosing paradigm, which could underlie perturbed voiding physiology in 12 week old mice. Assessing this pathway further by quantifying expression, using TRPV1 knockout mice and determining whether TRPV1 in urothelium or sensory nerves are important in mediating PCB effects are areas of future study.

Hormone induced changes to prostate size are implicated in voiding dysfunction in mice, typically with increased prostate mass attributed to obstruction, large bladder volumes, and small voided urine volumes (De Falco and Laforgia, 2021; Nicholson et al., 2018; Turco et al., 2023). Given the absence of increased prostate mass (Fig. 5), prostate enlargement and bladder outlet obstruction are unlikely contributing to altered voiding in male mice seen here. Instead, we found males had decreased ventral prostate mass in the 1 mg/kg/d and 6 mg/kg/d PCB groups compared to the 0.1 mg/kg/d PCB group but not compared to vehicle control. The difference among PCB groups in prostate size was more extensive at this age compared to younger mice, which only showed a decrease in ventral prostate mass in the 1 mg/kg/d PCB group compared to the two other PCB groups (Spiegelhoff et al., 2023). While we did not examine prostate histology in this study, in the previous study of mice at 6 weeks of age there were no observed developmental PCB effects on prostate smooth muscle thickness, caspase 3 positive cells, Ki67 positive cells, bladder mass or bladder thickness (Kennedy et al., 2022; Spiegelhoff et al., 2023). While we cannot rule out these changes in the current study it is unlikely that these would arise in mice at 12 weeks of age when they were not present at 6 weeks of age (Kennedy et al., 2022; Spiegelhoff et al., 2023). Collecting prostate in PCB exposed mice at a younger timepoint could help to determine whether PCB induced reductions in prostate size occur early in development and are then maintained throughout adulthood or whether any histological changes are evident. Although the MARBLES PCB mixture itself has not yet been tested for pro- or anti-estrogenic or androgenic activity, individual congeners in the MARBLES PCB mix can mimic sex steroid hormones and disrupt hormone receptor dependent signaling (Bonefeld-Jørgensen et al., 2001; Plíšková et al., 2005; Sechman et al., 2016; Tam et al., 2022; Wang et al., 2021). Further, anogenital distance (an endocrine responsive endpoint) in postnatal day 7 male mice was found to be decreased following developmental exposure to MARBLES PCB at the 0.1, 1 and 6 mg/kg PCB groups vs vehicle control (Matelski et al., 2020). Since the prostate, seminal vesicle, and testes are sensitive

to changes in circulating hormones and endocrine disruption by exogenous chemicals, it is plausible that PCBs decrease steroid hormone levels leading to reduction in tissue size (Ruetten et al., 2019; De Falco and Laforgia, 2021; Nicholson et al., 2018; Turco et al., 2023; Bianco et al., 2002). Endocrine disruption can both decrease seminal vesicle and prostate lobe masses, and drive changes in voiding (Ruetten et al., 2019; Bianco et al., 2002). Androgens and estrogens, especially 5α-dihydrotestosterone and estradiol-17β, are both implicated in prostatic proliferation and maintenance (Nicholson et al., 2012; Nicholson and Ricke, 2011; Wynder et al., 2015), and exogenous endocrine disruptors including PCBs may interfere with these pathways (De Falco and Laforgia, 2021; Nicholson et al., 2018; Turco et al., 2023; Bianco et al., 2002). Finasteride, a type II 5α-reductase inhibitor, inhibits the transformation of testosterone to the more potent 5α-dihydrotestosterone, and finasteride treatment results in a 25 % reduction in total prostate volume in men with benign prostatic hyperplasia (Kaplan et al., 2008); PCBs have been shown to decrease 5α-reductase RNA and protein expression in rat testes (Thangavelu et al., 2018; Yamamoto et al., 2005), and reduce 5α-reductase activity in LNCaP cells (Endo et al., 2003). Likewise, activation of estrogen receptor beta (ER-β) inhibits prostate proliferation (Nicholson and Ricke, 2011; Wynder et al., 2015). PCBs and their hydroxylated metabolites can bind ER-β and induce estrogen receptor-mediated transcriptional activity (Bonefeld-Jørgensen et al., 2001; Tam et al., 2022; Kuiper et al., 1998). These are two examples of how changes to endocrine signaling could result in decreased prostatic tissue mass, and the potential for PCBs to interact with these mechanisms either early in development or in adulthood to alter lower urinary tract function is an ongoing area of study.

Taken together, this study demonstrates that urinary voiding dysfunction following developmental PCB exposure persists to at least 12 weeks of age and suggests a possible role for TRPV1 dependent mechanisms in mediating PCB effects on voiding. While changes in prostate mass were only seen between PCB groups and not vehicle control, this also highlights that low and high doses of PCBs may have divergent mechanisms in relation to endocrine disruption. This work highlights the importance of ongoing investigations into PCBs' and other xenobiotics' roles in the etiology of lower urinary tract symptoms throughout life, especially in elucidating mechanisms which may lend to identifying susceptible populations disproportionately affected by these toxicant exposures or improving therapeutic treatments. The MARBLES PCB mix was used here since it represents the top PCBs found in a contemporary cohort of pregnant women, thus these PCBs are present in these women and their offspring have been exposed (Hertz-Picciotto et al., 2018; Sethi et al., 2019). Whether PCBs impact the voiding phenotypes of individuals directly exposed through the environment, or if PCBs can predispose to LUTD from developmental exposure (*in utero* and lactational), remains to be determined. This study provides an area of opportunity; future work may assess voiding function in humans with known PCB exposure, alone or in combination with epidemiology studies focused on measuring PCB concentrations in human tissues. Further, examining PCB concentrations in patients with well documented lower urinary tract dysfunction could provide useful in determining risk factors for LUTD, and may provide insight for developing possible future treatment options for patients.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crtox.2025.100227.

Data availability

Data will be made available on request. Raw data is deposited in the Dryad database and can be found at the following DOI: https://doi.org/10.5061/dryad.ffbg79d36.

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