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Original Article

Developmental polychlorinated biphenyl (PCB) exposure alters voiding physiology in young adult male and female mice

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Abstract: The impact of developmental exposure to environmental chemicals on lower urinary tract function is not well understood, despite the fact that these chemicals could contribute to etiologically complex lower urinary tract symptoms (LUTS). Polychlorinated biphenyls (PCBs) are environmental toxicants known to be detrimental to the central nervous system, but their impact on voiding function in mouse models is not known. Therefore, we test whether developmental exposure to PCBs is capable of altering voiding physiology in young adult mice. C57Bl/6J female mice received a daily oral dose of the MARBLES PCB mixture for two weeks prior to mating and through gestation and lactation. The mixture mimics the profile of PCBs found in a contemporary population of pregnant women. Voiding function was then tested in young adult offspring using void spot assay, uroflowmetry and anesthetized cystometry. PCB effects were sex and dose dependent. Overall, PCBs led to increases in small size urine spots in both sexes with males producing more drop-like voids and greater peak pressure during a voiding cycle while females displayed decreases in void duration and intervoid interval. Together, these results indicate that developmental exposure to PCBs are capable of altering voiding physiology in young adult mice. Further work to identify the underlying mechanisms driving these changes may help develop more effective preventative or therapeutic strategies for LUTS.

Keywords: Lower urinary tract, bladder, persistent organic pollutants, developmental origins of health and disease

Introduction

Polychlorinated biphenyls (PCBs) are a class of persistent organic pollutants that are pervasive in the environment due to their resistance to degradation and continued production as unintentional byproducts from processes such as paint pigment production [1-4]. A major source of human exposure occurs though the diet with PCBs detected in dietary sources such as milk [5, 6], meat, and fish [7, 8]. Despite production bans, levels of PCBs in the environment [5, 6, 8-11] and human tissues [12-17] are still of concern and the presence of contemporary congeners, which were not produced as a part of commercial PCB mixtures prior to manufacturing bans [9], warrant continued research into their detrimental health effects. For example, levels of a contemporary congener, PCB 11, were found to be high in samples from cattle and pregnant women in California [5, 18, 19]. The mixture of PCBs used in this study is based on the contemporary levels found in the MARBLES cohort of pregnant women at risk of having a child with a neurodevelopmental disorder and contain both legacy as well as contemporary congeners of concern such as PCB 11 [18, 20, 21].

Developmental PCB exposures are associated with cognitive deficits in children [22, 23] and have been implicated as risk factors for neurodevelopmental disorders such as autism spectrum disorder [21, 24-27]. PCBs have also been linked to changes in neural structure and connectivity in animal models [28-33]. While the developing brain is a major target, other tissues are also impacted by PCB exposures. Other tar-

gets include the intestine and microbiome [34], immune system, and inflammatory pathways [35-41]. We have recently reported PCB dependent changes in nerve fiber density as well as inflammatory cells in the bladder of developmentally exposed juvenile mice [42, 43]. Whether PCB exposures contribute to altered voiding physiology in mice is unknown and the goal of the current study.

Symptoms such as overactive bladder, incontinence, nocturia, difficulty urinating and retained urine are significant LUTS that pose a serious detriment to quality of life [44]. While LUTS tend to increase with age, rates of incontinence are also higher among children with neurodevelopmental disorders [45-48]. This represents a physical and emotional burden to these children, as well as their caregivers [45, 47, 48]. Understanding whether PCBs could impact voiding function in neurotypical individuals, but especially in individuals with neurodevelopmental disorders, could lead to new more effective management or preventive strategies since PCB exposures are modifiable. This could greatly improve quality of life. Here we test whether developmental exposure to PCBs has impacts on voiding function when wild type mice reach young adulthood. Void spot assay, uroflowmetry and anesthetized cystometry are used to reveal sex and dose dependent effects of PCBs on voiding function.

Materials and methods

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*.

Developmental PCB exposures

Female nulliparous adult mice were dosed orally daily with the MARBLES PCB mixture or vehicle control for two weeks prior to mating and all through gestation and lactation as described previously [35, 42]. The MARBLES PCB mixture and doses used (0.1, 1 and 6 mg/kg/day) were chosen since this mixture mimics the proportion of PCBs found in a contemporary cohort of pregnant women at risk of having a child with a neurodevelopmental disorder [20] and the doses result in PCB levels in offspring relevant to human concentrations [42, 49, 50]. PCBs were synthesized and authenticated by the Synthesis Core of the University of Iowa Superfund Research Program with > 99% purity as reported previously [18, 50]. PCBs were dissolved in organic peanut oil (Spectrum Organic Products, LLC, Melville, NY), and mixed into organic peanut butter (Trader Joe's, Monrovia, CA) for oral consumption, vehicle contained peanut oil in peanut butter only. Male and female offspring were weaned at postnatal day (P) 21 and group housed with same sex and dose littermates until collection. Urine specific gravity was determined from free catch urine prior to testing by refractometry (Vet360-Check digital refractometer, feline setting, Reichert Technologies, Depew, NY). Order of testing was void spot assay (VSA), uroflowmetry, and anesthetized cystometry. Testing was carried out at the same time of day and for cystometry, which can only accommodate 4 mice per day, mice were randomly counterbalanced to start in the AM or PM. Mice were collected as part of a larger study so not all mice which underwent VSA (non-invasive) also underwent anesthetized cystometry (terminal and for which bladder cannot be collected for other endpoints such as histology). At the end of the study, mice were euthanized with CO_2 or isoflurane.

Void spot assay (VSA)

VSA was conducted using best practices as described previously [51, 52]. Briefly mice (43.1 ± 1.4 days old, n = 14-24 per group derived from 5-7 dams) were acclimated to the testing room for at least 1 hour prior to starting. A 3 MM chromatography paper (057163E, Fisher Scientific) was placed in the bottom of an empty plastic mouse cage. Mice were placed into the cage and had access to food but not water during the 4-hour testing period. Filter papers were allowed to dry and were then imaged under UV light. VSA analysis was per-

formed using the Void Whizzard freely available analysis software [52] designed for Image J using default parameters by an individual blinded to treatment conditions.

Uroflowmetry

Uroflowmetry was conducted as described using Void Sorcerer open access design and software [53]. Briefly, uroflowmetry was performed in the same room as VSA testing and mice (45.9 ± 2.3 days old, n = 13-24 per group derived from 5-7 dams) were acclimated for at least 1 hour prior to testing. Mice were placed into uroflowmetry chambers for a 4-hour testing period with access to water but not food. Uroflow data was analyzed by an individual blinded to treatment conditions. Only urine events which did not hit the bars of the grid floor were used in analysis. To determine a stream rating a scale from 1-3 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.

Cystometry

Anesthetized cystometry was performed essentially as described previously [54]. Mice (49.4 ± 2.7 days old, n = 6-14 per group derived from 3-7 dams) were anesthetized with a subcutaneous injection of urethane (AC32554-0500, Fisher) at a dosage of 1.43 g urethane/kg mouse. Mice were dosed using a fresh stock solution of urethane in saline at 86 mg/ml. Mice were placed back into cages for at least 30 min prior to beginning surgery. The abdomen was opened and a purse string suture (6-0 Silk, 501180809, Fisher) 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 in needle. The needle was removed and the purse string suture tied around the catheter. The body wall and skin were closed with a suture and the mouse was allowed to recover on a heating pad for ~60 min. Following recovery, mice were connected to an in-line pressure transducer and infusion pump. Saline was infused at a rate of 0.8 ml/hr and pressure recorded using an MLT844 physiological pressure transducer (ADInstruments) 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 hour or until a steady pattern was achieved. For data analysis 5 consecutive voids were analyzed and averaged per animal and were selected by an individual blinded to treatment conditions. Parameters measured are described in detail previously [54] and include void duration (time between threshold pressure and baseline pressure after a void), void interval (time between baseline pressure to baseline pressure during a void cycle), normalized threshold pressure (threshold pressure - baseline pressure), normalized peak void pressure (peak void pressure - baseline pressure), non-voiding contractions (spikes in pressure before a void not leading to release of urine) and compliance (infused volume/change in pressure (threshold-baseline)).

Statistics

Statistics were run using GraphPad Prism software version 9.2. Data were assessed for normality and variance. Normal data were assessed using one-way ANOVA followed by Dunnett's multiple comparison test. If data failed to pass normality, log transformation was applied followed by one-way ANOVA and Dunnett's multiple comparison test, if transformation failed to restore normality data were assessed using Kruskal Wallis test followed by Dunn's multiple comparisons test. If data were normal but did not display equal variance, a Welch's ANOVA followed by Dunnett's multiple comparison test was used. Two-way ANOVA followed by Dunnett's multiple comparisons test was used to determine differences in urine spot size distribution in male and female mice. Fisher's exact test was used to determine differences in percentage of the frequent spotter populations. Sample sizes for each endpoint are indicated in the figure legends.

Results

Developmental PCB exposure does not have overt effects on pregnancy or litter size

Previous studies demonstrate that MARBLES PCB exposure does not change the amount of time dams take to become pregnant, however

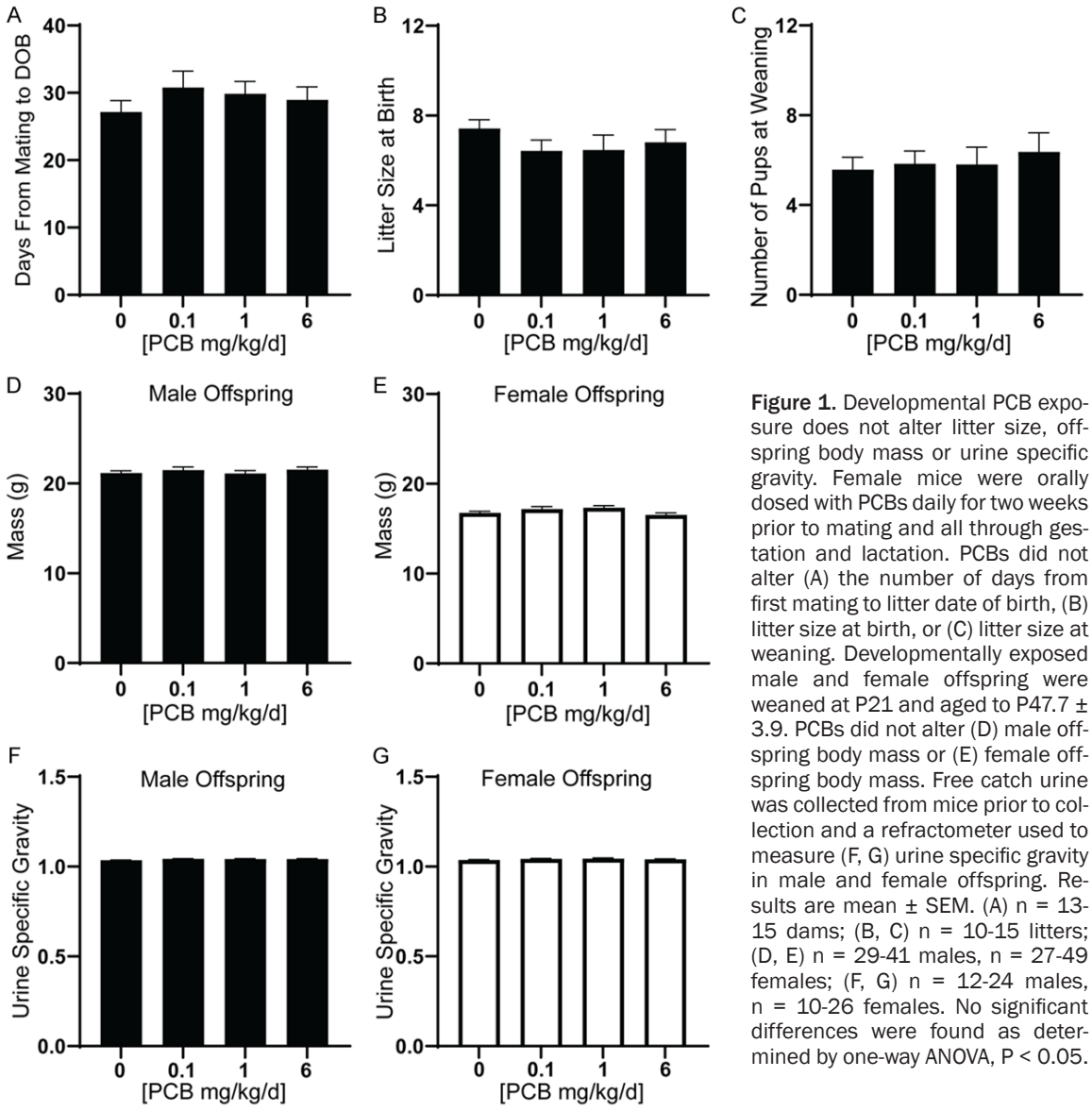

Figure 1. Developmental PCB exposure does not alter litter size, offspring body mass or urine specific gravity. Female mice were orally dosed with PCBs daily for two weeks prior to mating and all through gestation and lactation. PCBs did not alter (A) the number of days from first mating to litter date of birth, (B) litter size at birth, or (C) litter size at weaning. Developmentally exposed male and female offspring were weaned at P21 and aged to P47.7 ± 3.9. PCBs did not alter (D) male offspring body mass or (E) female offspring body mass. Free catch urine was collected from mice prior to collection and a refractometer used to measure (F, G) urine specific gravity in male and female offspring. Results are mean ± SEM. (A) n = 13-15 dams; (B, C) n = 10-15 litters; (D, E) n = 29-41 males, n = 27-49 females; (F, G) n = 12-24 males, n = 10-26 females. No significant differences were found as determined by one-way ANOVA, $P < 0.05$.

this was done in mixed background wild-type dams at a different University [35]. Therefore, we first confirmed that this same dosing paradigm in our hands did not alter reproductive outcomes in a straight C57Bl/6J wild type background. PCBs did not alter the average number of days from first mating to litter date of birth (**Figure 1A**). There were no differences in the litter size at birth (**Figure 1B**) and for live litters, there was no effect of PCBs on the number of pups at weaning (**Figure 1C**). There were also no effects of PCBs on body mass in either male or female offspring at time of collection (**Figure 1D**, **1E**) or on urine specific gravity (**Figure 1F**, **1G**). Together these data indicate that the PCB dosing paradigm used here does not lead to overt health effects on the dam or offspring and further that urine concentration is not altered by PCB exposure.

PCB's influence voiding behavior in a dose and sex-dependent manner

Developmental exposure to PCBs resulted in significant dose- and sex-dependent difference in void spot assay (VSA) parameters in young adult mice (**Figure 2**). The relative frequency of urine spot number is presented in **Figure 2B**, **2C**. There is a considerable rightward shift in the urine spot frequency distribution with PCB exposed mice more frequently displaying higher urine spot counts in both male and female

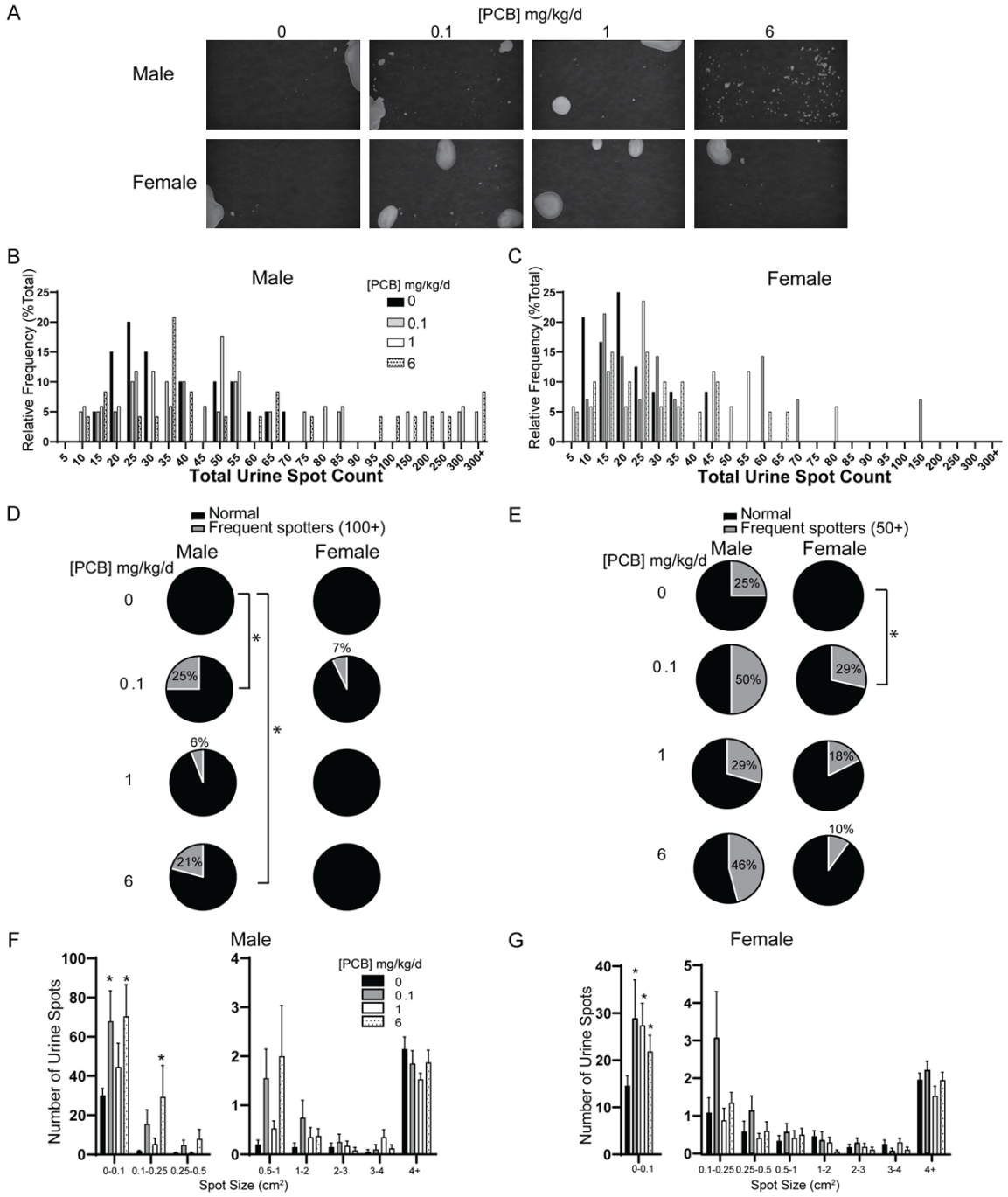

Figure 2. Developmental PCB exposure increases the proportion of frequent spotters and the number of small urine spots in male and female offspring. Mice were developmentally exposed to PCBs via the dam and VSA testing conducted on young adult male and female mice aged to P43.1 ± 1.4. Images of VSA for both male and female PCB dosage groups with urine spots in white (A). Parameters examined following the 4-hour VSA include (B, C) relative frequency (in % total), (D, E) frequent spotter percentage defined as 100 or 50+ urine spots, and (F, G) the urine spot size distribution. Results are mean ± SEM n = 17-24 males, n = 14-24 females. * indicates significant difference from vehicle control. $P \leq 0.05$ were considered significant. A bar and * indicate significant differences as determined by: (D, E) Fisher's exact test, and (F, G) Two-way ANOVA followed by Dunnett's multiple comparisons test. The second y-axis denotes a change in scale so all data can be visualized on one graph.

mice (**Figure 2B**, **2C**). As mice age, urine spot frequency distribution also shifts to the right and subpopulations of mice begin to show a frequent spotter phenotype, which can be cat-

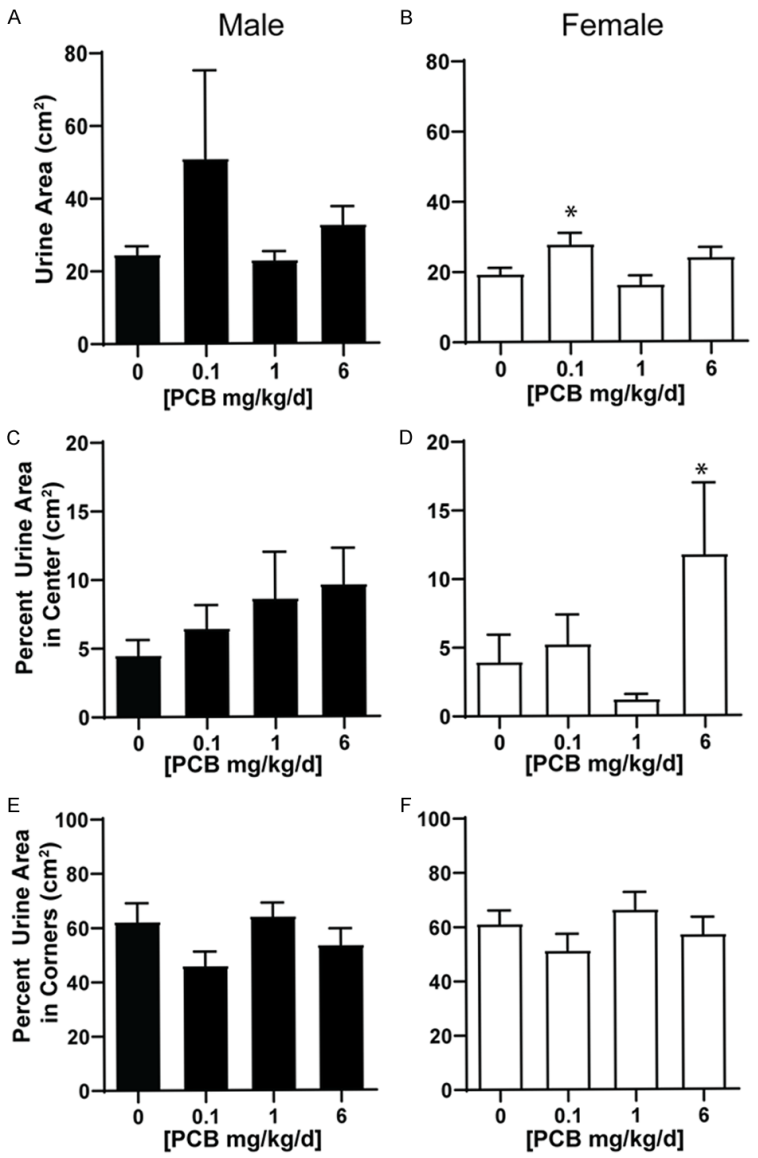

Figure 3. Developmental PCB exposure alters urine area and percent urine area in center of female mice undergoing void spot assay (VSA). Mice were developmentally exposed to PCBs via the dam and VSA testing conducted on young adult male and female mice aged to P43.1 ± 1.4. Parameters examined following the 4-hour VSA include (A, B) total urine area, (C, D) percent of total urine area within center of VSA paper, (E, F) percent of total urine area within corners of VSA paper. Results are mean ± SEM n = 17-24 males, n = 14-24 females. * indicates significant difference from vehicle control. $P < 0.05$ as determined by: (A, D-F) Kruskal Wallis test followed by Dunn's multiple comparisons test, (B) one-way ANOVA followed by Dunnett's multiple comparison test, (C) one-way ANOVA on log transformed data.

egorized as mice which have greater than 50 or 100 spots at 6 and 9 weeks respectively [51]. We examined the number of mice which produced greater than 50 or 100 urine spots during the 4-hour VSA testing period. Developmental PCB exposure significantly increased the percentage of male mice which produced more than 100 spots in the 0.1 (25%) and 6 (21%) mg/kg/d dose groups compared to vehicle control (0%) (**Figure 2D**). There were no PCB effects on the number of mice producing more than 50 spots in male mice as all groups contained a percentage of mice with this phenotype. However, in female mice developmental PCB exposure at the 0.1 mg/kg/d dose group resulted in significantly more mice with over 50 urine spots compared to vehicle controls (**Figure 2E**). We next tested whether developmental PCB exposure altered the size of the urine spots produced. In male mice there was a significant increase in the smallest urine spots (0-0.1 cm^2) in the 0.1 and 6 mg/kg/d PCB dose groups and an increase in the next smallest urine spots (0.1-0.25 cm^2) in the 6 mg/kg/d PCB dosing groups compared to vehicle control (**Figure 2F**). There was also a significant increase in the smallest urine spots in female mice at all PCB dose groups compared to vehicle control (**Figure 2G**). Total urine area was unchanged in male mice but was significantly greater in the female 0.1 mg/kg dose group versus vehicle control (**Figure 3A**, **3B**). Mice tend to void in corners of the paper thus changes in where voiding occurs can indicate voiding dysfunction [51, 55]. The percent urine area in the center of the paper following PCB exposure was significantly increased in the female 6 mg/kg/d dose group compared to vehicle control (**Figure 3C**, **3D**). The percent area in the corners was unchanged by PCB exposure in male and female offspring (**Figure 3E**, **3F**). Together these results indicate that PCBs tend to increase the number of small urine spots

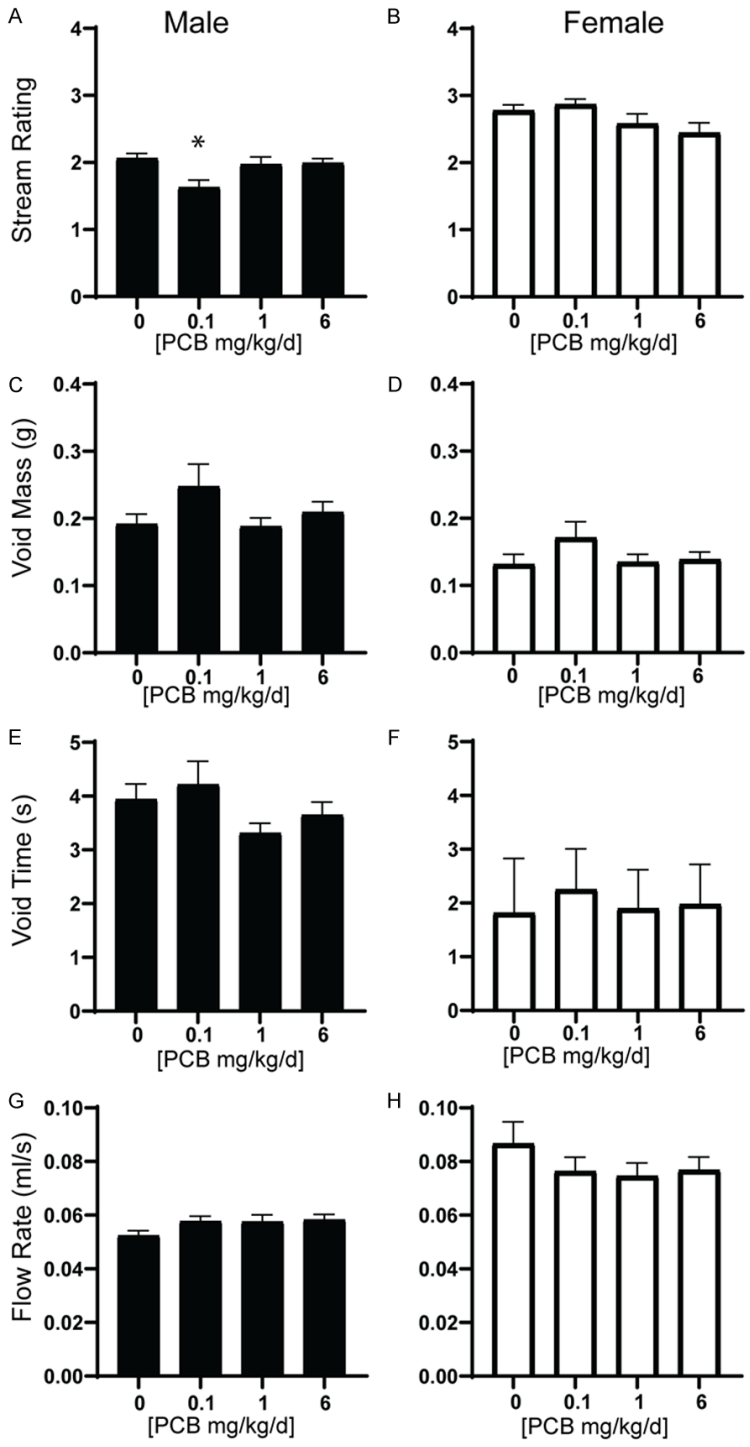

Figure 4. Developmental PCB exposure decreases the stream rating in the male offspring in the 0.1 mg/kg males compared to 0 mg/kg males. Mice were developmentally exposed to PCBs via the dam and uroflowmetry testing conducted on young adult male and female mice aged to P45.9 ± 2.3. Examined parameters include (A, B) urine stream rating, (C, D) void mass, (E, F) void time, and (G, H) flow rate in mL/s. Results are mean ± SEM n = 15-24 males, n = 13-21 females. * indicates significant difference from vehicle control. $P < 0.05$ were considered significant as determined by: (A, B) Kruskal-Wallis test followed by Dunn's multiple comparisons test, (C) Welch's ANOVA on log transformed data, (D) Welch's ANOVA, (E, H) One-way ANOVA on log transformed data, (F, G) One-way ANOVA.

produced in a sex- and dose-dependent manner.

Developmental PCB exposure alters voiding stream in male mice

We also used uroflowmetry to assess voiding function in developmental PCB exposed mice as it allows for the visualization of individual void events [53]. However, unlike VSA, uroflowmetry is conducted with mice suspended over a scale on a grid, both suspension and the grid footing are known to decrease urine spot count [52]. Nevertheless, there are several unique endpoints that can be assessed with this method. Stream rating was used to assess the drop or stream-like pattern of voiding during a 4-hour testing period. PCB exposure decreased the stream rating - thus mice produced more drop like voids - in male mice exposed to the 0.1 mg/kg PCB dose compared to vehicle control (**Figure 4A**). There were no significant changes in this parameter in female mice or in void mass, void time or flow rate for males or females (**Figure 4B-H**).

Developmental PCB exposure acts in a sex- and dose-dependent manner to alter parameters of voiding in mice undergoing anesthetized cystometry

In order to remove behavioral components of voiding, as well as changes in voiding that occur when mice are elevated on a grid (uroflowmetry), we also assessed voiding function in anesthetized mice using cystometry [55]. PCBs altered voiding function in a sex- and dose-dependent manner. Void duration and void interval were only altered in female mice,

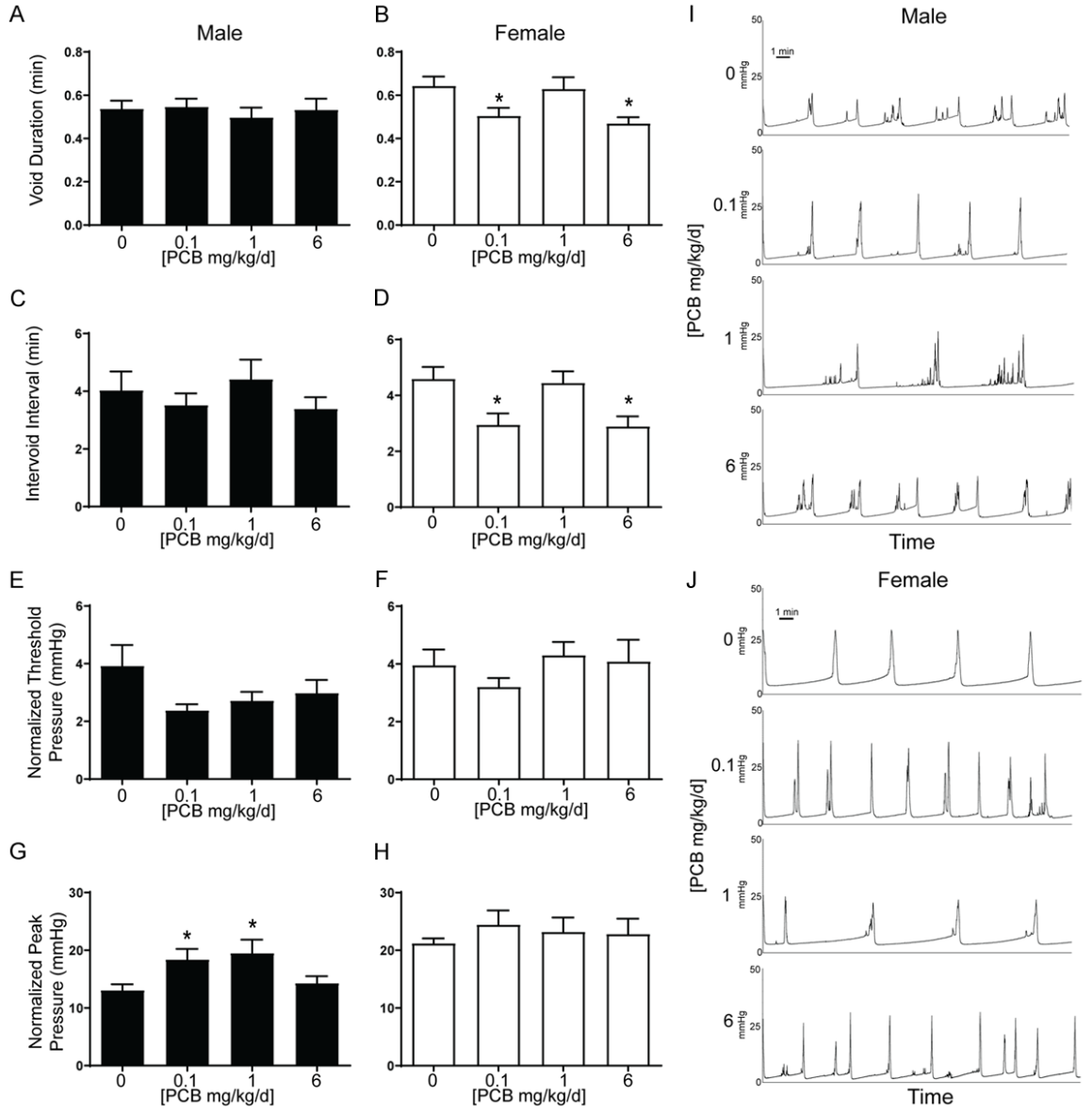

Figure 5. Developmental PCB exposure decreases intervoid interval in female offspring and increases peak pressure in male offspring. Mice were developmentally exposed to PCBs via the dam and anesthetized cystometry conducted on young adult male and female mice aged to P49.4 ± 2.7. Examined parameters include (A, B) void duration, (C, D) intervoid interval, (E, F) normalized threshold pressure (threshold subtracted from baseline pressure), and (G, H) normalized peak pressure (peak subtracted from baseline pressure). (I, J) Representative cystometrograms. Results are mean ± SEM n = 6-10 males, n = 8-14 females. * indicates significant difference from vehicle control. $P < 0.05$ were considered significant as determined by: (A, C, G) One-way ANOVA followed by Dunnett's multiple comparisons test, (B, F) One-way ANOVA followed by Dunnett's multiple comparisons test on log transformed data, (D, H) Kruskal-Wallis test followed by Dunn's multiple comparisons test, (E) Welch's ANOVA.

with the 0.1 and 6 mg/kg PCB dose decreasing these parameters compared to vehicle control (**Figure 5A-D**). In contrast, pressure changes were observed only in male mice. While normalized threshold pressure was unchanged by PCBs (**Figure 5E**, **5F**), normalized peak pressure during a void cycle was increased in the 0.1 and 1 mg/kg PCB dose compared to vehicle control (**Figure 5G**, **5H**). There were no significant differences in the number of non-voiding contractions or compliance (**Figure 6A-D**), however there was a trend for decreased compliance in female mice of the 6 mg/kg PCB dose (**Figure 6D**).

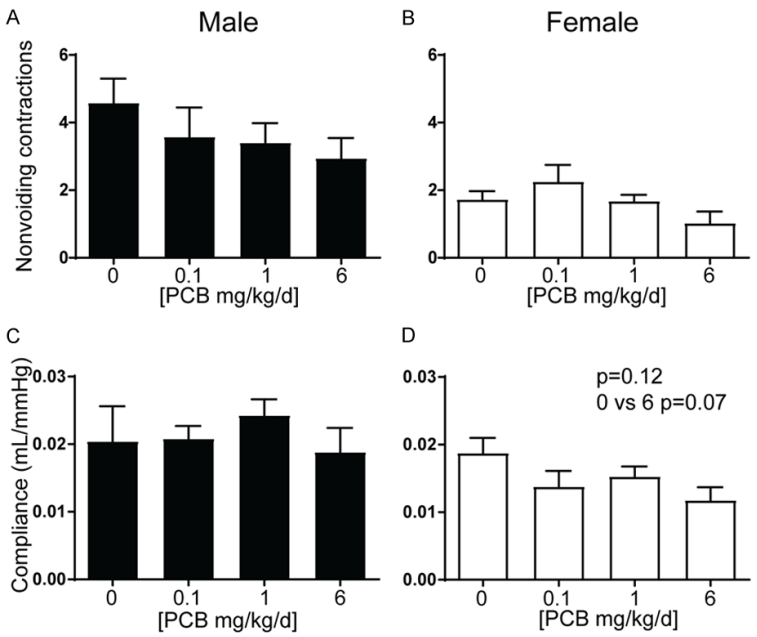

Figure 6. Developmental PCB exposure does not alter non-voiding contractions and trends to decrease compliance in female offspring. Mice were developmentally exposed to PCBs via the dam and anesthetized cystometry conducted on young adult male and female mice aged to P49.4 ± 2.7. Examined parameters include (A, B) number of non-voiding contractions, (C, D) bladder compliance (change in volume/change in pressure). Results are mean ± SEM n = 6-10 males, n = 8-14 females. $P < 0.05$ were considered significant as determined by: (A, B, D) One-way ANOVA followed by Dunnett's multiple comparisons test, (C) Welch's ANOVA.

Table 1 summarizes the major PCB effects observed for each endpoint examined and illustrates the sex and dose dependent differences in voiding function as a result of developmental PCB exposure. Overall PCBs tend to decrease spot size, increase frequency and can increase peak pressures achieved during a voiding cycle.

Discussion

In this study, we demonstrate that developmental exposures to PCBs are capable of altering voiding behavior in young adult offspring. While changes are sex- and dose-dependent, the overall phenotype indicates the production of smaller, more frequent voids and in male mice these are also associated with increases in peak pressures achieved during a voiding cycle. Together, these data provide evidence that voiding can be altered by an environmental chemical and that these phenotypes are present in young adults - weeks after the last possible developmental exposure. The long-term consequences of these exposures and the mechanisms underlying these phenotypes are unknown but of considerable interest for future study as these pathways may lead to improved treatment strategies or therapeutics.

Our findings that developmental PCB exposure can influence voiding function later in life are consistent with other studies. The persistent organic pollutant dioxin, or TCDD, has been linked to alterations in prostate development, growth and altered voiding function in mice with aging hormone-induced urinary dysfunction [56-58]. Recently, it has been demonstrated that developmental exposure to TCDD is sufficient to alter voiding function in wild type untreated mice including decreasing intervoid interval [59]. These findings are of particular interest as a subset of PCBs are classified as dioxin-like based on the structural similarity and ability to bind the aryl hydrocarbon receptor (AhR) [9]. In the current study we use the MARBLES PCB mixture which contains only one dioxin-like congener, PCB 118, which makes up only 4.9% of the mixture. The contribution of this dioxin-like congener compared to the remaining non-dioxin like congeners within the mixture on the voiding phenotypes observed here is not known. However, the possibility exists that PCB 118 could have effects on the prostate, similar to TCDD, which contribute to the voiding phenotypes in male mice. On the other hand, there is evidence to suggest this is not the main driver of the observed phenotypes. First, PCB effects of increasing small diameter urine spots occur in both males and females. As female mice do not have appreciable prostate, it is unlikely the prostate is the main driver of this phenotype. Second, TCDD exposure caused a decrease in intervoid interval in wild type male mice and no change in peak void pressures [59], in our study, PCBs did not change intervoid interval in male mice but did significantly increase peak void pressure - this is the opposite phenotype

Table 1. Summary of developmental PCB exposure effects on voiding

Endpoint	[MARBLES PCB mg/kg/d]		
	0.1	1	6
Urine Specific Gravity	-	-	-
Number of small urine spots (0-0.1 cm)	↑, ↑	↑	↑, ↑
Number of small urine spots (0.1-0.25)	-	-	↑
Number of larger urine spots (0.25-4+)	-	-	-
Urine area (cm^2)	↑	-	-
Percent Urine Area in Center (cm^2)	-	-	↑
Percent Urine Area in Corners (cm^2)	-	-	-
Stream Rating	↓	-	-
Void Mass (g)	-	-	-
Void Time (s)	-	-	-
Flow Rate (ml/s)	-	-	-
Void Duration (min)	↓	-	↓
Intervoid Interval (min)	↓	-	↓
Normalized Threshold Pressure (mmHg)	-	-	-
Normalized Peak Pressure (mmHg)	↑	↑	-
Nonvoiding Contractions	-	-	-
Compliance (mL/mmHg)	-	-	-

Statistically significant changes ($P < 0.05$) are indicated by blue arrows for males, and pink arrows for females relative to sex-matched vehicle control. Dash represents no statistically significant differences.

observed with the TCDD exposure [59]. The precise contribution of each dioxin-like and non-dioxin like PCB on the prostate, bladder and ultimately voiding function is an area of future study. Nonetheless, these findings are important as they establish that developmental exposures to chemicals such as TCDD and PCBs during development can have persistent effects on voiding function into adulthood.

One of the major differences in voiding observed following developmental PCB exposure was an increase in small diameter urine spots in both male and female mice. Males exhibited this phenotype at the 0.1 and 6 mg/kg dose and females at all three doses. This study alone cannot determine the underlying mechanisms driving these changes. However, there are several factors which could give rise to an increase in the number of small urine spots including but not limited to; obstruction, detrusor overactivity, urethral dysfunction, incontinence, inflammation or behavioral changes. The behavioral aspect is interesting in that we have previously observed that as mice age, it is normal for the number of urine spots to increase, with a subset of male mice displaying a distinct phenotype of 'frequent voiding' which may be territorial marking [51]. Using parameters of 50+ spots for males 6 weeks of age or 100+ spots for males 9 weeks of age during a 4 hour VSA these frequent voiding mice were found to represent 5 and 10% of the population respectively [51]. What is striking is that with PCB exposure, the frequent voiding phenotype of 100+ spots is significantly greater in the 0.1 and 6 mg/kg PCB groups with the percentage of the population over 20% - thus even if PCB effects are behavioral they lead to these changes in the frequent voiding phenotype in 6 week old animals that is expected of untreated animals that are at least three weeks older.

Changes in bladder contractility and/or sensation of filling could also contribute to PCB effects on voiding function. In mice undergoing anesthetized cystometry, pressure changes were observed in male mice with PCBs increasing the peak pressure during the voiding cycle in the 0.1 and 1 mg/kg PCB groups. This effect was not observed in female mice. The greater contractile pressure in males at the 0.1 mg/kg PCB dose may void with greater force but they still produce more small voids. One possibility is that the bladder does not empty with each void. It is also possible that obstruction in males contributes to the higher pressure voiding but with smaller volume voids elicited. Obstruction via changes in density to the prostate or urethra could be factors, along with the contractile properties themselves of the urethral sphincter or bladder [60-63]. Future research will be necessary to determine the role of obstruction via prostatic growth, urethral narrowing, altered bladder/urethral coordination and contractility on the phenotypes observed here. The possibility of altered sensation or nerve evoked contractility is intriguing as we have previously demonstrated that developmental PCB exposure can increase total nerve fiber density within the submucosa of the bladder in 4 week old male mice of the 6 mg/kg PCB group [42]. While this is younger than the timepoint examine here, it raises the question as to whether developmental PCB exposure

alters bladder innervation which may contribute to contractility. This hypothesis is also supported by the recent evidence that developmental TCDD exposure is capable of increasing noradrenergic nerve density within the prostate and enhances prostate contractility into adulthood [56].

The phenotypes induced by developmental PCB exposure (small spot size, decreased intervoid interval) also bear some resemblance to those of mouse models of induced inflammation. For example, cyclophosphamide-induced cystitis is associated with more frequent urination events and smaller urine spots [64]. In addition, cyclophosphamide treated mice show decreased intervoid intervals in a similar manner to the effect seen in female mice developmentally exposed to PCBs [65]. While developmental PCB exposures are not likely as severe as cystitis models, the underlying mechanism of inducing inflammation is possible. We have previously demonstrated an increase in immune cells in younger animals (P28) exposed developmentally to the same mix of PCBs used here [43]. At the 0.1 mg/kg PCB dose CD45+ immune cells were higher in the bladder compared to controls [43]. This is consistent with small more frequent voids in the 0.1 mg/kg treatment groups. Macrophages were found to be abundant in PCB groups but only in female mice [43] - here only female mice showed small urine spots along with decreased intervoid interval. Examining inflammation as a mediator of some of the voiding phenotypes observed here is an area of future study.

Voiding patterns are known to differ between male and female mice [51, 55], our results indicate that PCBs also act on these inherent differences to produce sex-dependent effects on voiding function (**Table 1**). While both male and female mice display increases in small area urine spots, female mice display a significant decrease in intervoid interval while males had increased void cycle peak pressures and had a reduced stream rating assessed via uroflowmetry. Sex differences in response to PCBs are consistent with others, including recent studies using the same PCB mixture, which demonstrate sex impacts the effects of PCBs on dendrite morphology and behavioral tests in young adult mice [49, 66-68]. These data further highlight the importance of considering sex as a biological variable and understanding how PCBs act to confer these phenotypes.

Dose-dependent effects are also observed in voiding parameters examined here and are mainly concentrated in the 0.1 mg/kg and the 6 mg/kg PCB groups (**Table 1**). When looking across voiding assays and doses there are several consistent results, for example in male mice at the 0.1 mg/kg PCB dose there was an increase in small urine spots, decrease in stream strength and increase in voiding pressure. Similarly, in female mice at the 0.1 mg/kg and 6 mg/kg dose, there was an increase in small urine spots, no change in stream rating and a corresponding decrease in void duration and intervoid interval. On the other hand, there were also dose effects which only occurred in one voiding assay. In males at the 1 mg/kg PCB dose there were no changes in void spot size or stream strength, but there was an increase in peak pressure. In female mice at the 1 mg/kg dose and male mice at the 6 mg/kg dose group, there was an increase in small size spots but no changes in any other parameter. These types of non-linear dose responses are common for PCBs, including this same mixture [28, 35, 42, 49, 67]. Thus, effects at lower doses do not always predict effects at higher doses. The underlying mechanisms are not understood, but they likely arise from the targeting of different pathways related to signaling or metabolism. For example, PCBs have been shown to upregulate ryanodine receptors (RyR), which are responsible for mediating the effects of non-dioxin like PCBs in the brain [28, 29]. In the brain of developmentally exposed offspring, an Aroclor mixture of PCBs at the 1 mg/kg increased RyR1 expression to a greater extent than the 6 mg/kg treatment group [28]. In the same study, PCBs were also found to increase cytochrome p450 content in liver at the 6 mg/kg group but not the 1 mg/kg group [28]. While it is difficult to compare different mixtures of PCBs despite the same doses used, it is possible that in our study, PCBs differentially upregulate target genes such as RyRs or change metabolism (such as Cyp induction), which could account for some dose effects observed. Future study will be necessary to understand these dose-response relationships. Examination of RyR effects is of considerable interest as non-dioxin like PCBs are known to bind these receptors, and the

MARBLES PCB mix has demonstrated affinity for RyRs [18]. RyRs are also important in regulating contractility of the bladder [61, 69] and have been shown to be upregulated after TCDD exposure in aging mouse models [57]. Nonetheless, these results clearly indicate the importance of examining a range of doses when studying effects of environmental contaminants on voiding function.

Overall, we demonstrate that developmental exposure to PCBs can influence voiding physiology in young adult mice using several rodent urinary function tests which each can reveal unique insight into voiding phenotypes. Considering that PCBs can result in effects across all tests indicates that PCBs disrupt voiding physiology in a sex- and dose-dependent manner. Overall, mice exposed to PCBs at the lowest 0.1 mg/kg dose produce smaller urine spot voids during the VSA test, a more drop-like vs stream pattern of voiding assessed by uroflow (males), higher pressure voids as assessed by cystometry (males) and a shorter time between void events as assessed by cystometry (females). A subset of these phenotypes are also observed in the 1 or 6 mg/kg dose. While the mechanisms underlying each dose response are an area of future study, the phenotype of more frequent or smaller voids is reminiscent of phenotypes associated with incontinence and overactive bladder. Therefore, better understanding of the pathways leading to these unique changes may help identify prevention and/or treatment strategies for patients suffering from LUTS. Future studies may help inform whether decreasing PCB exposures in susceptible individuals or during critical windows of development could help prevent LUTS.

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Disclosure of conflict of interest

None.

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References

[1] Hu D and Hornbuckle KC. Inadvertent polychlorinated biphenyls in commercial paint pigments. Environ Sci Technol 2010; 44: 2822-2827.

[2] Koh WX, Hornbuckle KC and Thorne PS. Human serum from urban and rural adolescents and their mothers shows exposure to polychlorinated biphenyls not found in commercial mixtures. Environ Sci Technol 2015; 49: 8105-8112.

[3] Jahnke JC and Hornbuckle KC. PCB emissions from paint colorants. Environ Sci Technol 2019; 53: 5187-5194.

[4] Herrick RF. PCBs in school-persistent chemicals, persistent problems. New Solut 2010; 20: 115-126.

[5] Sethi S, Chen X, Kass PH and Puschner B. Polychlorinated biphenyl and polybrominated diphenyl ether profiles in serum from cattle, sheep, and goats across California. Chemosphere 2017; 181: 63-73.

[6] Chen X, Lin Y, Dang K and Puschner B. Quantification of polychlorinated biphenyls and polybrominated diphenyl ethers in commercial cows milk from california by gas chromatography-triple quadruple mass spectrometry. PLoS One 2017; 12: e0170129.

[7] Barone G, Storelli A, Busco A, Mallamaci R and Storelli MM. Polychlorinated dioxins, furans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) in food from Italy: estimates of dietaryintake and assessment. J Food Sci 2021; 86: 4741-4753.

[8] Schecter A, Colacino J, Haffner D, Patel K, Opel M, Päpke O and Birnbaum L. Perfluorinated compounds, polychlorinated biphenyls, and organochlorine pesticide contamination in composite food samples from Dallas, Texas, USA. Environ Health Perspect 2010; 118: 796-802.
[9] Klocke C, Sethi S and Lein PJ. The developmental neurotoxicity of legacy vs contemporary polychlorinated biphenyls (PCBs): similarities and differences. Environ Sci Pollut Res Int 2020; 27: 8885-8896.
[10] Howell NL, Suarez MP, Rifai HS and Koenig L. Concentrations of polychlorinated biphenyls (PCBs) in water, sediment, and aquatic biota in the Houston Ship Channel, Texas. Chemosphere 2008; 70: 593-606.
[11] Herrick RF, Stewart JH and Allen JG. Review of PCBs in US schools: a brief history, an estimate of the number of impacted schools, and an approach for evaluating indoor air samples. Environ Sci Pollut Res Int 2016; 23: 1975-1985.
[12] Björvang RD, Vinnars MT, Papadogiannakis N, Gidlöf S, Mamsen LS, Mucs D, Kiviranta H, Rantakokko P, Ruokojärvi P, Lindh CH, Andersen CY and Damdimopoulou P. Mixtures of persistent organic pollutants are found in vital organs of late gestation human fetuses. Chemosphere 2021; 283: 131125.
[13] Li AJ, Feldman SM, McNally RK and Kannan K. Distribution of organohalogen and synthetic musk compounds in breast adipose tissue of breast cancer patients in Ulster County, New York, USA. Arch Environ Contam Toxicol 2019; 77: 68-78.
[14] Herrick RF, Meeker JD, Hauser R, Altshul L and Weymouth GA. Serum PCB levels and congener profiles among US construction workers. Environ Health 2007; 6: 25.
[15] Heiger-Bernays WJ, Tomsho KS, Basra K, Petropoulos ZE, Crawford K, Martinez A, Hornbuckle KC and Scammell MK. Human health risks due to airborne polychlorinated biphenyls are highest in New Bedford Harbor communities living closest to the harbor. Sci Total Environ 2020; 710: 135576.
[16] Chu S, Covaci A and Schepens P. Levels and chiral signatures of persistent organochlorine pollutants in human tissues from Belgium. Environ Res 2003; 93: 167-176.
[17] Ampleman MD, Martinez A, DeWall J, Rawn DF, Hornbuckle KC and Thorne PS. Inhalation and dietary exposure to PCBs in urban and rural cohorts via congener-specific measurements. Environ Sci Technol 2015; 49: 1156-1164.
[18] Sethi S, Morgan RK, Feng W, Lin Y, Li X, Luna C, Koch M, Bansal R, Duffel MW, Puschner B, Zoeller RT, Lehmler HJ, Pessah IN and Lein PJ. Comparative analyses of the 12 most abundant PCB congeners detected in human maternal serum for activity at the thyroid hormone receptor and ryanodine receptor. Environ Sci Technol 2019; 53: 3948-3958.
[19] Sethi S, Keil KP, Chen H, Hayakawa K, Li X, Lin Y, Lehmler HJ, Puschner B and Lein PJ. Detection of 3,3'-dichlorobiphenyl in human maternal plasma and its effects on axonal and dendritic growth in primary rat neurons. Toxicol Sci 2017; 158: 401-411.
[20] Hertz-Picciotto I, Schmidt RJ, Walker CK, Bennett DH, Oliver M, Shedd-Wise KM, LaSalle JM, Giulivi C, Puschner B, Thomas J, Roa DL, Pessah IN, Van de Water J, Tancredi DJ and Ozonoff S. A prospective study of environmental exposures and early biomarkers in autism spectrum disorder: design, protocols, and preliminary data from the MARBLES study. Environ Health Perspect 2018; 126: 117004.
[21] Granillo L, Sethi S, Keil KP, Lin Y, Ozonoff S, Iosif AM, Puschner B and Schmidt RJ. Polychlorinated biphenyls influence on autism spectrum disorder risk in the MARBLES cohort. Environ Res 2019; 171: 177-184.
[22] Schantz SL, Widholm JJ and Rice DC. Effects of PCB exposure on neuropsychological function in children. Environ Health Perspect 2003; 111: 357-576.
[23] Koh WX, Hornbuckle KC, Wang K and Thorne PS. Serum polychlorinated biphenyls and their hydroxylated metabolites are associated with demographic and behavioral factors in children and mothers. Environ Int 2016; 94: 538-545.
[24] Lyall K, Croen LA, Sjödin A, Yoshida CK, Zerbo O, Kharrazi M and Windham GC. Polychlorinated biphenyl and organochlorine pesticide concentrations in maternal mid-pregnancy serum samples: association with autism spectrum disorder and intellectual disability. Environ Health Perspect 2017; 125: 474-480.
[25] Xi T and Wu J. A review on the mechanism between different factors and the occurrence of autism and ADHD. Psychol Res Behav Manag 2021; 14: 393-403.
[26] Panesar HK, Kennedy CL, Keil Stietz KP and Lein PJ. Polychlorinated biphenyls (PCBs): risk factors for autism spectrum disorder? Toxics 2020; 8: 70.
[27] Mehri F, Bashirian S, Khazaei S and Jenabi E. Association between pesticide and polychlorinated biphenyl exposure during pregnancy and autism spectrum disorder among children: a meta-analysis. Clin Exp Pediatr 2021; 64: 286-292.
[28] Yang D, Kim KH, Phimister A, Bachstetter AD, Ward TR, Stackman RW, Mervis RF, Wisniewski AB, Klein SL, Kodavanti PR, Anderson KA, Wayman G, Pessah IN and Lein PJ. Developmental exposure to polychlorinated biphenyls interferes with experience-dependent dendritic plasticity and ryanodine receptor expression in

weanling rats. Environ Health Perspect 2009; 117: 426-435.
[29] Wayman GA, Yang D, Bose DD, Lesiak A, Ledoux V, Bruun D, Pessah IN and Lein PJ. PCB-95 promotes dendritic growth via ryanodine receptor-dependent mechanisms. Environ Health Perspect 2012; 120: 997-1002.
[30] Wayman GA, Bose DD, Yang D, Lesiak A, Bruun D, Impey S, Ledoux V, Pessah IN and Lein PJ. PCB-95 modulates the calcium-dependent signaling pathway responsible for activity-dependent dendritic growth. Environ Health Perspect 2012; 120: 1003-1009.
[31] Lesiak A, Zhu M, Chen H, Appleyard SM, Impey S, Lein PJ and Wayman GA. The environmental neurotoxicant PCB 95 promotes synaptogenesis via ryanodine receptor-dependent miR132 upregulation. J Neurosci 2014; 34: 717-725.
[32] Lee CM, Sadowski RN, Schantz SL and Llano DA. Developmental PCB exposure disrupts synaptic transmission and connectivity in the rat auditory cortex, independent of its effects on peripheral hearing threshold. eNeuro 2021; 8: ENEURO.0321-20.2021.
[33] Bandara SB, Eubig PA, Sadowski RN and Schantz SL. Developmental PCB exposure increases audiogenic seizures and decreases glutamic acid decarboxylase in the inferior colliculus. Toxicol Sci 2016; 149: 335-345.
[34] Rude KM, Pusceddu MM, Keogh CE, Sladek JA, Rabasa G, Miller EN, Sethi S, Keil KP, Pessah IN, Lein PJ and Gareau MG. Developmental exposure to polychlorinated biphenyls (PCBs) in the maternal diet causes host-microbe defects in weanling offspring mice. Environ Pollut 2019; 253: 708-721.
[35] Matelski L, Keil Stietz KP, Sethi S, Taylor SL, Van de Water J and Lein PJ. The influence of sex, genotype, and dose on serum and hippocampal cytokine levels in juvenile mice developmentally exposed to a human-relevant mixture of polychlorinated biphenyls. Curr Res Toxicol 2020; 1: 85-103.
[36] Svensson BG, Hallberg T, Nilsson A, Schütz A and Hagmar L. Parameters of immunological competence in subjects with high consumption of fish contaminated with persistent organochlorine compounds. Int Arch Occup Environ Health 1994; 65: 351-358.
[37] Stølevik SB, Nygaard UC, Namork E, Haugen M, Meltzer HM, Alexander J, Knutsen HK, Aaberge I, Vainio K, van Loveren H, Løvik M and Granum B. Prenatal exposure to polychlorinated biphenyls and dioxins from the maternal diet may be associated with immunosuppressive effects that persist into early childhood. Food Chem Toxicol 2013; 51: 165-172.
[38] Spector JT, De Roos AJ, Ulrich CM, Sheppard L, Sjödin A, Wener MH, Wood B and McTiernan A. Plasma polychlorinated biphenyl concentrations and immune function in postmenopausal women. Environ Res 2014; 131: 174-180.
[39] Leijs MM, Koppe JG, Olie K, van Aalderen WM, de Voogt P and ten Tusscher GW. Effects of dioxins, PCBs, and PBDEs on immunology and hematology in adolescents. Environ Sci Technol 2009; 43: 7946-7951.
[40] Horváthová M, Jahnová E, Palkovičová L, Trnovec T and Hertz-Picciotto I. Dynamics of lymphocyte subsets in children living in an area polluted by polychlorinated biphenyls. J Immunotoxicol 2011; 8: 333-345.
[41] Heilmann C, Grandjean P, Weihe P, Nielsen F and Budtz-Jørgensen E. Reduced antibody responses to vaccinations in children exposed to polychlorinated biphenyls. PLoS Med 2006; 3: e311.
[42] Keil Stietz KK, Kenny CL, Sethi S, Valenzuela A, Nunez A, Wang K, Wang Z, Wang P, Spiegelhoff A, Puschner B, Bjorling DE and Lein PJ. In utero and lactational PCB exposure drives anatomic changes in the juvenile mouse bladder. Curr Res Toxicol 2021; 2: 1-18.
[43] Kennedy CL, Spiegelhoff A, Wang K, Lavery T, Nunez A, Manuel R, Hillers-Ziemer L, Arendt LM and Stietz KPK. The bladder is a novel target of developmental polychlorinated biphenyl exposure linked to increased inflammatory cells in the bladder of young mice. Toxics 2021; 9: 214.
[44] Rubin EB, Buehler AE and Halpern SD. States worse than death among hospitalized patients with serious illnesses. JAMA Intern Med 2016; 176: 1557-1559.
[45] von Gontard A, Hussong J, Yang SS, Chase J, Franco I and Wright A. Neurodevelopmental disorders and incontinence in children and adolescents: attention-deficit/hyperactivity disorder, autism spectrum disorder, and intellectual disability-A consensus document of the International Children's Continence Society. Neurourol Urodyn 2022; 41: 104-112.
[46] von Gontard A, Niemczyk J, Borggrefe-Moussavian S, Wagner C, Curfs L and Equit M. Incontinence in children, adolescents and adults with Williams syndrome. Neurourol Urodyn 2016; 35: 1000-1005.
[47] von Gontard A. Urinary incontinence in children with special needs. Nat Rev Urol 2013; 10: 667-674.
[48] Radstaake M, Didden R, Giesbers S, Korzilius H, Peters-Scheffer N, Lang R, von Gontard A and Curfs LM. Incontinence in individuals with Angelman syndrome: a comparative study. Res Dev Disabil 2013; 34: 4184-4193.
[49] Sethi S, Valenzuela AE, Klocke CR, Silverman JL, Puschner B, Pessah IN and Lein PJ. Developmental exposure to a human-relevant polychlorinated biphenyl mixture causes behavioral phenotypes that vary by sex and genotype in

juvenile mice expressing human mutations that modulate neuronal calcium. Front Neurosci 2021; 15: 766826.
[50] Li X, Holland EB, Feng W, Zheng J, Dong Y, Pessah IN, Duffel MW, Robertson LW and Lehmler HJ. Authentication of synthetic environmental contaminants and their (bio)transformation products in toxicology: polychlorinated biphenyls as an example. Environ Sci Pollut Res Int 2018; 25: 16508-16521.
[51] Keil KP, Abler LL, Altmann HM, Bushman W, Marker PC, Li L, Ricke WA, Bjorling DE and Vezina CM. Influence of animal husbandry practices on void spot assay outcomes in C57BL/6J male mice. Neurourol Urodyn 2016; 35: 192-198.
[52] Wegner KA, Abler LL, Oakes SR, Mehta GS, Ritter KE, Hill WG, Zwaans BM, Lamb LE, Wang Z, Bjorling DE, Ricke WA, Macoska J, Marker PC, Southard-Smith EM, Eliceiri KW and Vezina CM. Void spot assay procedural optimization and software for rapid and objective quantification of rodent voiding function, including overlapping urine spots. Am J Physiol Renal Physiol 2018; 315: F1067-F1080.
[53] Wang Z, Guzman EC, Nimunkar A, Keil KP, Vezina CM, Ricke WA, Macoska J and Bjorling DE. Void sorcerer: an open source, open access framework for mouse uroflowmetry. Am J Clin Exp Urol 2019; 7: 170-177.
[54] Ruetten H, Wegner KA, Zhang HL, Wang P, Sandhu J, Sandhu S, Mueller B, Wang Z, Macoska J, Peterson RE, Bjorling DE, Ricke WA, Marker PC and Vezina CM. Impact of sex, androgens, and prostate size on C57BL/6J mouse urinary physiology: functional assessment. Am J Physiol Renal Physiol 2019; 317: F996-F1009.
[55] Bjorling DE, Wang Z, Vezina CM, Ricke WA, Keil KP, Yu W, Guo L, Zeidel ML and Hill WG. Evaluation of voiding assays in mice: impact of genetic strains and sex. Am J Physiol Renal Physiol 2015; 308: F1369-1378.
[56] Turco AE, Oakes SR, Stietz KPK, Dunham CL, Joseph DB, Chathurvedula TS, Girardi NM, Schneider AJ, Gawdzik J, Sheftel CM, Wang P, Wang Z, Bjorling DE, Ricke WA, Tang W, Hernandez LL, Keast JR, Bonev AD, Grimes MD, Strand DW, Tykocki NR, Tanguay RL, Peterson RE and Vezina CM. A neuroanatomical mechanism linking perinatal TCDD exposure to lower urinary tract dysfunction in adulthood. Dis Model Mech 2021; 14: dmm049068.
[57] Turco AE, Thomas S, Crawford LK, Tang W, Peterson RE, Li L, Ricke WA and Vezina CM. In utero and lactational 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure exacerbates urinary dysfunction in hormone-treated C57BL/6J mice through a non-malignant mechanism involving proteomic changes in the prostate that differ from those elicited by testosterone and estradiol. Am J Clin Exp Urol 2020; 8: 59-72.
[58] Ricke WA, Lee CW, Clapper TR, Schneider AJ, Moore RW, Keil KP, Abler LL, Wynder JL, Lopez Alvarado A, Beaubrun I, Vo J, Bauman TM, Ricke EA, Peterson RE and Vezina CM. In utero and lactational TCDD exposure increases susceptibility to lower urinary tract dysfunction in adulthood. Toxicol Sci 2016; 150: 429-440.
[59] Turco AE, Oakes SR, Keil Stietz KP, Dunham CL, Joseph DB, Chathurvedula TS, Girardi NM, Schneider AJ, Gawdzik J, Sheftel CM, Wang P, Wang Z, Bjorling DE, Ricke WA, Tang W, Hernandez LL, Keast JR, Bonev AD, Grimes MD, Strand DW, Tykocki NR, Tanguay RL, Peterson RE and Vezina CM. A mechanism linking perinatal 2,3,7,8 tetrachlorodibenzo-p-dioxin exposure to lower urinary tract dysfunction in adulthood. Dis Model Mech 2021; 14: dmm049068.
[60] Nicholson TM, Ricke EA, Marker PC, Miano JM, Mayer RD, Timms BG, vom Saal FS, Wood RW and Ricke WA. Testosterone and 17β-estradiol induce glandular prostatic growth, bladder outlet obstruction, and voiding dysfunction in male mice. Endocrinology 2012; 153: 5556-5565.
[61] Herrera GM, Heppner TJ and Nelson MT. Regulation of urinary bladder smooth muscle contractions by ryanodine receptors and BK and SK channels. Am J Physiol Regul Integr Comp Physiol 2000; 279: R60-68.
[62] de Groat WC and Yoshimura N. Afferent nerve regulation of bladder function in health and disease. Handb Exp Pharmacol 2009; 91-138.
[63] de Groat WC, Fraser MO, Yoshiyama M, Smerin S, Tai C, Chancellor MB, Yoshimura N and Roppolo JR. Neural control of the urethra. Scand J Urol Nephrol Suppl 2001; 35-43.
[64] Lee TG, Sanderson D, Doyle P, Li D and Wood RW. High-definition ultrasound characterization of acute cyclophosphamide-induced cystitis in the mouse. Investig Clin Urol 2020; 61: 75-80.
[65] Chen YH, Chen CJ, Wang SJ, Lin YN, Chen WC, Tsai MY and Chen HY. Downregulation of tight junction protein zonula occludens-2 and urothelium damage in a cyclophosphamide-induced mouse model of cystitis. Taiwan J Obstet Gynecol 2018; 57: 399-406.
[66] Keil KP, Sethi S and Lein PJ. Sex-dependent effects of 2,2',3,5',6-pentachlorobiphenyl (PCB 95) on dendritic arborization of primary mouse neurons. Toxicol Sci 2018; 168: 95-109.
[67] Keil Stietz KP, Sethi S, Klocke CR, de Ruyter TE, Wilson MD, Pessah IN and Lein PJ. Sex and genotype modulate the dendritic effects of de-

velopmental exposure to a human-relevant polychlorinated biphenyls mixture in the juvenile mouse. Front Neurosci 2021; 15: 766802.

[68] Sethi S, Keil KP and Lein PJ. Species and sex differences in the morphogenic response of primary rodent neurons to 3,3'-dichlorobiphenyl (PCB 11). Toxics 2017; 6: 4.

[69] Jiang HH, Song B, Lu GS, Wen QJ and Jin XY. Loss of ryanodine receptor calcium-release channel expression associated with overactive urinary bladder smooth muscle contractions in a detrusor instability model. BJU Int 2005; 96: 428-433.