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MOTOR DEFICITS, IMPAIRED RESPONSE INHIBITION, AND BLUNTED RESPONSE TO METHYLPHENIDATE FOLLOWING NEONATAL EXPOSURE TO DECABROMODIPHENYL ETHER

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Abstract

Decabromodiphenyl ether (decaBDE) is an applied brominated flame retardant that is widely-used in electronic equipment. After decades of use, decaBDE and other members of its polybrominated diphenyl ether class have become globally-distributed environmental contaminants that can be measured in the atmosphere, water bodies, wildlife, food staples and human breastmilk. Although it has been banned in Europe and voluntarily withdrawn from the U.S. market, it is still used in Asian countries. Evidence from epidemiological and animal studies indicate that decaBDE exposure targets brain development and produces behavioral impairments. The current study examined an array of motor and learning behaviors in a C57BL/6/J mouse model to determine the breadth of the developmental neurotoxicity produced by decaBDE. Mouse pups were given a single daily oral dose of 0 or 20 mg/kg decaBDE from postnatal day 1 to 21 and were tested in adulthood. Exposed male mice had impaired forelimb grip strength, altered motor output in a circadian wheel-running procedure, increased response errors during an operant differential reinforcement of low rates (DRL) procedure and a blunted response to an acute methylphenidate challenge administered before DRL testing. With the exception of altered wheel-running output, exposed females were not affected. Neither sex had altered somatic growth, motor coordination impairments on the Rotarod, gross learning deficits during operant lever-press acquisition, or impaired food motivation. The overall pattern of effects suggests that males are more sensitive to developmental decaBDE exposure, especially when performing behaviors that require effortful motor output or when learning tasks that require sufficient response inhibition for their successful completion.

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Conflict of Interest Statement

The authors declare that they have no actual or potential conflicts of interest including any financial, personal or other relationships with other people or organizations that inappropriately influenced, could have influenced, or be perceived to influence, their work.

Keywords

behavior; development; DRL; flame retardant; decabromodiphenyl ether; learning; mouse; operant; PBDE; neonatal exposure

1. Introduction

Polybrominated diphenyl ethers (PBDE) are flame-retardant chemicals that were widely used in products such as textiles, plastics, and foam sold in the United States and Europe from the 1970's through the mid-2000s. Although the PBDE class consists of 209 possible congeners, the penta-, octa-, and decaBDE congeners had the most commercial value. Due to their toxicity, pentaBDE and octaBDE have not been produced in the United States or Europe since 2005. The sale of most products containing the fully-substituted decaBDE (2,2',3,3',4,4',5,5',6,6'-brominated diphenyl ether) was banned in Europe in 2008 and was voluntarily discontinued in the U.S. in 2013 [26,31].

Unfortunately, regulation of PBDEs began years after they had become global environmental contaminants. Many studies have detected PBDEs in the atmosphere, waterways, soils, biota, and sewage sludge. Levels are typically higher in urban areas and near manufacturing sites but PBDEs are also found in remote locations with little human activity such as the Arctic and Antarctica [8,25,9]. Although total PBDE levels in the environment have begun to decline in some European sampling sites where restrictions and bans have been in place for the longest time, PBDEs will persist in the environment for years to come as these chemicals migrate from aging or discarded products and enter the atmosphere, waterways, and sediments [7]. Furthermore, decaBDE continues to be used in products manufactured in China and Asian markets [41]. One of the most common uses for decaBDE is in the production of high-impact polystyrene plastic, which is often used as casing for televisions and other electronic equipment. Environmental levels measured in and around electronics-waste recycling sites in China are among the highest recorded [41]. After decaBDE is released into the environment, it appears to preferentially accumulate in freshwater sediments and there are concerns that this large reservoir will debrominate over time, producing metabolites that are more bioavailable and toxic [29].

DecaBDE is present throughout the food web [3,34,66] and can be found in lipid-rich foods that people commonly eat such as fish, meat, cheese, poultry and infant food [52,53,55,35]. DecaBDE is readily absorbed after oral exposure [40], crosses the placenta [33,65,68], and is excreted into breast milk [37,54]. Breast-fed infants appear to be exposed to some of the highest levels and animal studies have shown that decaBDE is more likely to be passed during lactation rather than across the placenta [4].

Perhaps of greater concern, decaBDE is also a dominant congener in house dust in the U.S. and very high levels have been found in breast milk and house dust samples collected near electronic-waste recycling sites in China [41,57]. DecaBDE is also the dominant congener in house dust in Sweden and the ingestion of contaminated house dust is the most important source of exposure for both mothers and their toddlers [51]. Children are exposed to higher levels of household decaBDE than adults because they spend a greater proportion of time

indoors, they are closer to floors, and they engage in greater hand-to-mouth behavior [36]. Consequently, infants and toddlers are viewed as the most susceptible to decaBDE's developmental neurotoxicity.

Clinical studies with at least five different cohorts of children have reported associations between elevated PBDEs during the prenatal period and neurobehavioral impairments in toddlers and school children. Attention problems and/or motor impairments appear to be the most common outcomes. For instance, maternal PBDE levels measured at 16 or 26 weeks of gestation were positively correlated with hyperactivity in a cohort of 5-yr olds in Ohio [5] and impaired attention and increased reports of ADHD in a cohort of 5-yr olds in California [17]. Attention problems in a NYC cohort of 4-yr olds were positively associated with PBDE levels in umbilical cord plasma collected at birth [6]. Ongoing investigation of the California cohort found persisting negative effects of prenatal PBDEs on attention at 9- and 12-yrs of age [50]. Investigation of a Danish cohort (where PBDE levels are typically lower) found that maternal PBDEs at 35 weeks were related to impaired attention and fine manipulative abilities in 5–6 yr. old children [48]. In all of the above studies, BDE-47, -99, -100, & -153 were the dominant congeners although it is not clear if the fully brominated BDE-209 was even examined. Given the propensity for decaBDE to attach to dust and be distributed to breast milk, the contribution of decaBDE to the clinical impairments could be underestimated since most of the above studies estimated gestational exposure. However, one study in Spain reported that among the 14 congeners found in colostrum, decaBDE was the most concentrated and was the only one associated with impaired mental development at 14-months of age [22].

Animal studies have confirmed the selective effects of decaBDE on motor and learning functions. A single oral dose of decaBDE administered to male or female mice on the 3rd postnatal day has been shown to impair motor habituation, a type of learning deficit. The effect is persistent, noted at 2-, 4-, and 6-months of age [2,30,63]. The same outcome was also observed in male rats [60]. The learning impairment could be the consequence of decaBDE's effects on the levels of key proteins that play critical roles in the development of the mouse hippocampus and cortex [2,64].

The current study was designed to extend our earlier investigations of neonatal exposure to decaBDE where we found reduced thyroxine levels in weanling mice, increased locomotor activity in young adults, and some indications of impaired response inhibition and perseverative behavior during operant tasks as the animals grew old [45,46]. In these studies, males were found to be more sensitive to decaBDE toxicity than females. Rather than examining a range of decaBDE doses in the current study, we opted to administer the single most active dose from our earlier studies but to a larger number of litters. These animals were then assigned to a wide range of behavioral tests in an effort to better understand the locomotor and response inhibition effects. For motor assessment we examined forelimb grip strength, coordination on an automated rotarod, circadian wheel running, and activity in operant chambers. Operant responding under differential reinforcement of low rates (DRL) schedules was used to examine response inhibition. After examining baseline DRL performance, we pretreated animals with a dose range of the dopamine reuptake transport inhibitor, methylphenidate, in an effort to shed some light on the underlying neurochemical

consequences of neonatal decaBDE exposure. Developmental exposure to other PBDEs as well as the polychlorinated biphenyls (PCBs) has been shown to perturb dopamine systems [12,24,38,43,49,58]. Finally, to determine whether altered food motivation accounted for differences in operant performance, we also examined responding during several progressive ratio schedules. We hypothesized that males would continue to show greater sensitivity to decaBDE and that their motor and learning deficits would persist throughout adulthood but be unrelated to underlying motivational impairments.

2. Materials and Methods

2.1 Breeding and DecaBDE Exposure

Adult male and female C57BL/6J inbred mice (The Jackson Laboratory, Bar Harbor, ME) were allowed to acclimate to the vivarium quarters at the State University of New York at Geneseo for two weeks before breeding. Mice were fed standard pellet chow (LabDiet 5001, Brentwood, MO) ad libitum and were maintained on a reversed 12-hr light:12-hr dark cycle in a room with an ambient temperature of 68 ± 2 °F and 40–60% humidity.

For breeding, two females were housed with one male. Females were examined every morning for the presence of a sperm plug. Sperm-positive females were then caged individually. The day of birth was considered postnatal day (PND) 0 and litters were culled to 3 male and 3 female pups on PND1, immediately prior to the onset of dosing. Litters with fewer than 6 pups or a skewed sex distribution were not used. Each litter was assigned in a randomized fashion to a 0 or 20 mg decaBDE/kg bodyweight exposure condition. Each pup was administered a single daily oral dose from PND1–21. A total of 39 control litters and 42 decaBDE litters were generated for the complete study although some of these litters were used to generate calvarial cells to determine whether decaBDE impairs osteogenesis and/or osteoblast function by antagonizing thyroid hormone mediated activity (to be reported in a future manuscript). After weaning on PND21, the pups assigned to the behavior tests were individually ear-marked for identification. Animals were housed with same-sex littermates in standard polycarbonate cages (45 cm long $\times$ 23 cm wide $\times$ 15 cm high). Males and females were housed in the same room throughout the experiment.

Since neonatal mouse pups are too small to safely dose by intragastric gavage, the decaBDE was administered as an emulsion using a micropipette with 200- μ l tips at a concentration of 25 μ l/g bodyweight. Small amounts of dosing emulsion were placed in each pup's mouth and the micropipette tip was used to gently stimulate the perioral region to promote suckling and swallowing. Although this was a time-consuming process (requiring 30–90-sec per animal), it was efficient, with minimal loss of dosing emulsion. The emulsion was meant to resemble breast milk [45]. It consisted of a stock solution of 1 mg L- α -phosphatidylcholine in 10 ml peanut oil. To this solution, 40 ml of sterile water and 40 mg of decaBDE (product 194425, 98% 2,2',3,3',4,4',5,5',6,6'-decabromodiphenyl ether, Sigma-Aldrich) were added, sonicated to create the emulsion, and hand-shaken throughout the dosing procedure. Control animals received the emulsion vehicle in the same manner and volume as the decaBDE-exposed pups. Fresh dosing solutions were prepared every other day.

All animal procedures complied with approved institutional animal care protocols and were in accordance with NIH guidelines [28]. Animal care and welfare were supervised by a veterinarian.

2.2 Developmental Milestones

Crown-rump length and anogenital distance were recorded every other day from PND1-21 (see Figure 1 for complete timeline). The ages at which both pinna detached, the upper and lower incisors erupted, and both eyes opened were also determined. The age of puberty was determined by vaginal opening or descent of the testes.

2.3 Grip Strength

Forelimb grip strength was evaluated using a meter equipped with a digital sensor (Columbus Instruments, Columbus, OH). Grip tests began after both eyes had opened. Each animal was held by the scruff and the base of the tail until they grasped the pull-bar with both forepaws. The scruff was then released and the tail was pulled steadily away from the bar until the animal released both forepaws. Every pup in each litter was examined on PND15, 17, 19 and 21. At least one male-female pair from each litter was examined on the day of puberty onset, young adulthood (PND55), and mid-adulthood (around PND230). On a given test day, each animal performed three trials that were later averaged.

2.4 Rotarod

A programmable rotating rod for mice (Rotamex-5, Columbus Instruments) was used to test motor coordination at three different ages: day of puberty, young adulthood (PND60), and mid-adulthood (PND242-250). In each litter, a different male-female pair was used for each test age. The rotarod procedure began with a training session where each animal received 2 trials with the rod moving at a fixed speed of 4 RPM. Animals had to make a forward walking motion to remain on the rod. Each trial lasted for 60-sec or until an animal fell. Trials were separated by at least 5-min. The day after the training session, each animal received 4 trials where the rod accelerated from 4–40 RPM over 360-sec. The 4 trials were later averaged together and 3 variables were examined: total run time, speed at the time of the fall, and passive rotation time. Passive rotation time represented brief absences from the photodetection beam caused by slips or partial falls.

2.5 Circadian Wheel Running

Around 3-months of age, mice were transferred from the homecage and housed individually in activity wheel chambers (model 80820, Lafayette Instrument Company, Lafayette, IN) for 4 consecutive 12-hr light:12-hr dark cycles. Each wheel chamber consisted of a 24 × 35 × 20-cm clear polycarbonate cage with a food hopper, water bottle, and a 13-cm diameter wheel with an electronic counter. Data collection was managed by Activity Wheel Monitor Software (model 86065, Lafayette Instrument Company). Data downloads occurred every 30-sec. Total wheel revolutions and average speed for the entire period were examined as well as revolutions and speed for each 12-hr light or dark period. Data from download periods when an animal failed to run were not included in the speed calculations.

2.6 Activity

Around 4-months of age, naïve animals were acclimated to commercial operant chambers (18 w × 18 d × 30-cm h; Coulbourn Instruments, Allentown, PA) that were controlled by Graphic State software (ver. 3.01, for Windows XP). One male and one female were selected from each available litter and placed individually in the chamber for a 2-hr session that began at least 2-hrs after the onset of the dark cycle in the vivarium. The number of movement episodes was recorded by an overhead infrared activity monitor (model H24-61, Coulbourn Instruments). The overhead houselight was illuminated during the 2-hr activity session but there were no other programmed consequences. The field of view of the activity monitor was calibrated to the interior dimensions of the operant cage and recorded movement at any elevation in the cage, including rearing or leaning against the cage wall. A movement episode was defined as contiguous motor output with inter-event intervals of less than 400 milliseconds. Prior to analysis, the number of movement episodes in the 2-hr activity session was grouped into eight consecutive 15-min time blocks.

2.7 Lever Press Acquisition and Fixed-Ratio Schedule

Training for operant behavior began around 5-months of age. Each chamber contained a single response lever on the left side of one chamber wall and a food bin centered on the same wall. A tri-colored LED display was positioned directly above the lever and served as the discriminative stimulus that a response would be reinforced. An overhead houselight was illuminated during sessions. Single food pellets (20-mg, Bio-Serv, Frenchtown, NJ) were automatically delivered into the food hopper to reinforce correct responses.

A continuous reinforcement schedule was used to train mice to press the lever. During training sessions, cue lights above the lever were illuminated and a lever press produced an audible click from the food dispenser, 3-sec of illumination in the food hopper, and delivery of a single food pellet. Initially, free food pellets were automatically delivered on a variable interval schedule until the subject performed 10 lever presses. After 10 responses, training continued on a fixed ratio 1 (FR1) schedule for 12-hrs or until a subject earned 60 food pellets. Subjects were considered to have learned the lever press response when they had completed a session with 40 responses.

After subjects acquired the lever press response, they were trained with 3 additional sessions under a FR1 schedule to stabilize lever pressing and build response rate. Each FR1 session lasted for 30 minutes or until a subject earned 60 food pellets. The following variables were examined: overall response rate, number of earned food pellets, total lever presses, start latency, postreinforcement pause, session duration and activity counts from the overhead infrared monitor. All operant sessions were run on consecutive days, 5 days per week, during the dark phase of the subjects' cycle.

2.8 Progressive Ratio Schedule

The day after the last FR1 session, food motivation was tested with 3 different progressive ratio (PR) schedules, where the response requirement for reinforcement (PR1, 2, or 5) increased by the respective value during each subsequent trial within a session. Sessions continued until a subject stopped responding for 5-min or earned 60 food pellets. The

following variables were examined: activity, break point (defined as the last completed ratio), overall response rate, number of earned food pellets, total lever presses, start latency, postreinforcement pause, and session duration.

2.9 Differential Reinforcement of Low Rate and Methylphenidate Drug Challenges

Following the PR, a series of differential reinforcement of low rate (DRL) schedules were used to examine timing and response inhibition. Testing began with 5 sessions of DRL10 where interresponse times (IRTs) had to be 10-sec to receive reinforcement. Shorter IRTs restarted the 10-sec delay. The DRL10 was followed by 3 sessions of DRL20 and then 3 sessions of DRL30. Operant testing concluded with 5 sessions of DRL30 that were preceded by a dose of 0, 2.5, 5.0, 10.0 or 20.0 mg/kg methylphenidate hydrochloride (MPH, Sigma-Aldrich product M2892) in sterile physiological saline, administered in a counterbalanced order. Animals were injected intraperitoneally and returned to their homecage for 15-min before being placed in the operant chamber.

2.10 Statistical Methods

All repeated measurements including offspring bodyweight, anogenital distance, crown-rump length, locomotor activity, and operant behavior were analyzed with repeated-measures analysis of variance (ANOVA) with PROC GLM SAS version 9.2 (SAS Institute Inc., Cary, NC). Prior to analysis, developmental data from individual pups were averaged with their same-sex littermates at each observation day.

For all endpoints, the litter served as the statistical unit of analysis, with the decaBDE dose as a between-litter factor and sex and daily sessions as within-litter factors. The Huynh-Feldt adjustment was used when appropriate. Newman-Keuls multiple range tests were used to make pairwise comparisons. A $P < 0.05$ was considered statistically significant.

3. Results

3.1 Offspring Body Measurements and Milestones

DecaBDE exposure did not affect offspring bodyweight gain, anogenital distance, or crown-rump length throughout the PND1-21 dosing period. For each of these variables there were the expected main effects of age ($P < 0.0001$ for each), and sex ($P < 0.01$ for each), as well as sex-by-age interactions for bodyweight ($P < 0.05$) and anogenital distance ($P < 0.0001$). DecaBDE did not affect the age that the pinna detached, incisors erupted, eyes opened, or age of puberty onset (see Table 1).

3.2 Forelimb Grip Strength

Male and female grip strength data were examined separately since the average age of the puberty test differed for the males and females. There were significant main effects of age as forelimb grip strength increased over the first 55 days [main effect of PND for males: $F(6,90)=787.28$, $P < 0.0001$; females: $F(6,90)=799.59$, $P < 0.0001$]. There were no other effects in the females. For the males, there was a significant main effect of exposure [$F(1,15)=8.09$, $P=0.01$; see Figure 2], where control males pulled with more force than the decaBDE males.

3.3 Rotarod

Data from the three test ages were examined separately since male-female pairs from different litters were assigned to the test ages. There were no significant effects of decaBDE or sex on any of the 3 variables (total run time, speed at the time of the fall, and passive rotation time), although there was a marginal sex-by-exposure interaction [$F(1,21)=3.99$, $P=0.06$] in the puberty test, where the decaBDE males had a shorter total run time than the other 3 groups. There were no effects in the young adult or mid-adult test ages.

3.4 Circadian Wheel Running

Animals were housed in the wheel chambers for 4 consecutive 12-hr light:12-hr dark cycles, however the first light:dark cycle was regarded as a habituation period and was not included in the data analysis. Total wheel revolutions and average revolution speed for the entire period were examined first. There was a significant main effect of sex on speed [$F(1,17)=6.87$, $P=0.02$] and a main effect of sex [$F(1,7)=21.46$, $P=0.0002$] and a sex-by-exposure interaction [$F(1,7)=6.32$, $P=0.002$] for the total revolutions. Post hoc tests indicated that both female groups and the decaBDE males ran more than the control males and that the females ran faster than the males.

A more fine-grained analysis was performed by comparing the number of wheel revolutions and speed for each of the 12-hr light and dark phases. For the number of wheel revolutions, there was a significant main effect of sex [$F(1,17)=21.45$, $P=0.0002$], where females ran more than males and a robust main effect of phase [$F(1,17)=222.63$, $P<0.0001$], with much more running occurring during the dark phases versus the light phases. There were also significant sex-by-exposure [$F(1,17)=6.31$, $P=0.02$], sex-by-day [$F(2,34)=3.18$, $P=0.05$], sex-by-phase [$F(1,17)=9.38$, $P=0.007$], and sex-by-phase-by-exposure [$F(1,17)=5.78$, $P=0.03$; see Figure 3] interactions. To probe this last interaction, data were averaged across the 3 light phases and 3 dark phases and the light phase and dark phase averages for the decaBDE males, control males, decaBDE females and control females were compared. Post hoc tests indicated that both female groups ran significantly more than both male groups during the light phase. Each group also ran significantly more during the dark phase than the light phase, however, there were unique exposure and sex effects during the more active, dark phase. The decaBDE females ran significantly less, while the decaBDE males ran significantly more than their same sex controls.

When the mean revolution speed was examined there was a significant main effect of sex [$F(1,16)=11.09$, $P=0.004$] and a robust main effect of phase [$F(1,16)=152.90$, $P<0.0001$]. However, there were no effects of decaBDE on speed.

3.5 Activity

The exploratory activity of naïve animals during their first 2-hrs in the operant chambers was significantly affected by sex [$F(1,12)=22.83$, $P=0.0004$] and time interval [$F(7,84)=28.54$, $P<0.0001$], but not decaBDE exposure. Post hoc tests indicated that females were more active than males. The activity of both sexes declined over the course of the 2-hr assessment period.

3.6 Fixed Ratio Operant Behavior

Three FR1 training sessions were run to stabilize the lever-pressing behavior. These sessions were averaged prior to analysis. There were no effects of decaBDE on any of the operant variables, although there was a significant main effect of sex [$F(1,13)=5.23$, $P=0.04$] on the number movement episodes recorded by the overhead activity monitors as females were more active than males.

3.7 Progressive Ratio Operant Behavior

For analysis, the three progressive ratio values (PR1, 2, & 5) were treated as a within-subject factor. There were no effects of decaBDE on any of the variables. There were main effects of sex on breakpoint [$F(1,13)=9.79$, $P=0.008$] and activity [$F(1,13)=6.71$, $P=0.02$]. Males had higher breakpoints and were more active in the chambers than females. There was also a main effect of PR value on activity [$F(2,26)=31.57$, $P<0.0001$] during the operant sessions where both sexes were more active during the PR1 sessions compared to PR2 and PR5.

3.8 Differential Reinforcement of Low Rates

Prior to analysis, the individual sessions were averaged for each DRL value (5 sessions of DRL10, 3 sessions of DRL20, and 3 sessions of DRL30) and the three DRL value means were treated as a within-subject factor. For the overall response rate variable, there was a main effect of DRL value [$F(2,26)=8.71$, $P=0.0056$] and a sex-by-DRL-by-exposure interaction [$F(2,26)=5.48$, $P=0.01$]. Post hoc tests found that the decaBDE males responded at higher rates than control males during the DRL30. There were no effects in the females.

The increased response rate in the decaBDE males was related to a significant main effect of DRL [$F(2,26)=66.41$, $P<0.0001$] and a sex-by-DRL-by-exposure interaction [$F(2,26)=6.03$, $P=0.0091$; see Figure 4] for the total errors variable. Post hoc tests indicated that decaBDE males made more errors than control males during the DRL30.

Other findings of interest included the total activity counts and the earned food pellets variable. For the activity counts variable there was a main effect of sex [$F(1,13)=8.82$, $P=0.01$] and a main effect of DRL value [$F(2,26)=46.99$, $P<0.0001$] but there was no effect of decaBDE exposure. Females were more active than males in the operant chambers overall and both sexes were less active during the DRL10 compared to the DRL20 and DRL30. For the earned food variable there was a main effect of DRL value [$F(2,26)=121.35$, $P<0.0001$], where mice earned less food during the DRL30 than the DRL10 and DRL20 schedules. There was also a marginal sex-by-exposure interaction [$F(1,13)=4.12$, $P=0.06$], where the decaBDE males tended to earn less food than the control males and the decaBDE females.

3.9 DRL30 Performance Following Acute Methylphenidate Drug Challenges

Administration of methylphenidate (MPH) 15-min before the DRL30 sessions produced sex-specific effects in the decaBDE vs. control mice. For both the overall response rate and the total errors variables, there were main effects of decaBDE [response rate: $F(1,9)=6.11$, $P=0.04$; total errors: $F(1,9)=6.11$, $P=0.04$], main effects of sex [response rate: $F(1,9)=6.68$, $P=0.03$; total errors: $F(1,9)=6.68$, $P=0.03$], main effects of MPH dose [response rate: $F(4,36)=3.65$, $P=0.03$; total errors: $F(4,36)=3.65$, $P=0.03$], and sex-by-dose-by-exposure

interactions [response rate: $F(4,36)=3.26$, $P=0.05$; total errors: $F(4,36)=3.26$, $P=0.05$; see Figure 5]. Post hoc tests of this last interaction indicated that control males responded at increasingly higher rates and made more errors as the MPH dose increased from 5 to 20 mg/kg. Control males were also significantly more active [$F(4,36)=3.88$, $P=0.02$] and they earned significantly less food [$F(4,36)=2.82$, $P=0.04$] following 5, 10, or 20 mg/kg MPH.

Because of the dramatic effect of MPH on total errors in the control males, the total burst responses and the burst response ratio were also examined. Burst responses were defined as lever presses with IRTs of ≤ 2 -sec and the burst response ratio was the percent of total responses. For both variables there were main effects of decaBDE [total: $F(1,9)=12.27$, $P=0.007$; ratio: $F(1,9)=9.51$, $P=0.01$], main effects of sex [total: $F(1,9)=9.29$, $P=0.01$; ratio: $F(1,9)=9.52$, $P=0.01$] and main effects of MPH dose [total: $F(4,36)=4.02$, $P=0.05$; ratio: $F(4,36)=5.70$, $P=0.02$]. Post hoc tests found that the control group performed more burst responses than the decaBDE group, males performed more bursts than females (see Figure 6), and the 10 mg/kg and 20 mg/kg doses of MPH produced more bursts than the other MPH doses.

4. Discussion

The current study found that a daily oral dose of 20 mg/kg decaBDE from PND1-21 selectively and permanently impaired four different motor or response inhibition behaviors in C57BL/6J mice. Males were more sensitive to decaBDE's developmental neurotoxicity than were females. Exposed males had impaired forelimb grip strength, altered wheel running output in a test of circadian rhythmicity, impaired response inhibition during an operant differential reinforcement of low rates (DRL30) procedure, and a blunted response to methylphenidate.

The first decaBDE-related effect to emerge was reduced forelimb grip strength (FGS) in the exposed males. The FGS deficit was already apparent during the first test age on PND15, while decaBDE administration was ongoing. However the deficit appeared to be permanent, persisting well into mid-adulthood. Female FGS was not affected by decaBDE. The FGS deficit in the males was not simply the result of a decaBDE-mediated growth impairment since there were no bodyweight, crown-rump length, or developmental milestone differences. This finding extends our earlier study, where we found a modest effect of 20 mg/kg decaBDE on male FGS [45]. However, the earlier study used a less sensitive measure of FGS, the latency to fall from a suspended rod, and only tested animals through PND20.

One potential mechanism for impaired FGS is a developmental thyroid hormone (TH) deficiency. A significant reduction of thyroxine was observed in male mice on PND21 in our earlier study [45] and was replicated in littermates in the current study (companion manuscript in preparation). The mutant TR α 1 mouse model of early life hypothyroidism presents a range of cognitive and motor impairments in adulthood, including fore- and hindlimb grip deficits. The grip deficits can be prevented if maternal and fetal TH levels are elevated enough to activate the aporeceptor throughout the perinatal period [67]. However, TH supplementation during gestation or adulthood alone is insufficient to rescue motor behavior. Interestingly, FGS is negatively correlated with advancing age, especially in the

male C57BL6/J mouse [27]. FGS at 22-months has been described as an accurate predictor of the remaining natural lifespan [18]. Although we did not examine senescent animals in the current study, we have previously observed long-term FGS deficits and shortened lifespan in male rats that were developmentally-exposed to the hexabromocyclododecane flame retardant [39].

FGS is frequently a component of the motor behavior test batteries that are used to examine animal models of Parkinson's disease. Exposure to manganese or administration of the dopaminergic neurotoxicant MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) reduces both FGS and striatal dopamine in the C57BL6/J mouse [1,11,32]. Although we did not directly examine central dopamine systems in the current study, several of the other behavioral deficits that we observed during the DRL procedure could also be the result of altered dopaminergic development.

In addition to FGS, three other, more complex motor behaviors were examined in the current study. Fine motor coordination measured on an accelerating rotarod was not significantly affected by decaBDE, nor was the exploratory activity of the mice during their first 2-hrs in the operant chambers. In our earlier study, we found that 2-month old decaBDE males were significantly more active in the operant chambers than male controls but also that the male exposure groups were no longer different at 1-yr [45]. In the current study, the male exposure groups did not differ at 4-months of age suggesting that this effect fades between 2–4 months of age. Indeed, visual comparison of the current data from the 4-month old males and females greatly resembles the data from the 1-yr old animals in our previous study. Still, this lack of effect at 4-months is at odds with many other reports that describe either motor hyperactivity or a failure to habituate following neonatal exposure to tetraBDE [15,23], pentaBDE [14,15,16,20,61], or hexaBDE [62]. Three other studies from the Eriksson laboratory tested decaBDE with the same spontaneous behavior procedure and found impaired motor habituation at 2-, 4- and 6-months of age. Collectively, these 3 studies found that PND3 was a more sensitive exposure period than PND10 or PND19 [63]. The lowest effective dose was a single dose of 6µmol/kg on PND3 [2,30]. Possible explanations for the divergent effects between the current study and these earlier works include the size of the test apparatus. Most of the earlier studies used an arena with the same dimensions as the home cage whereas the current study examined motor activity in a smaller, darkened operant chamber. Strain differences could also account for some of the discrepant findings since most of the earlier studies examined the NMRI mouse, although motor hyperactivity has been observed in the C57BL6/J following tetra- [23] or pentaBDE given on PND10 [61]. Finally, most of the earlier studies examined exploratory behavior during the light phase rather than the dark phase of the animals' cycle.

Although exploratory activity in the operant chamber was not affected by decaBDE, circadian wheel running was altered in a sex-specific manner. In all animals, wheel running was strongly entrained to the light cycle with much more running occurring during the 12-hr dark phases. Females accumulated more revolutions and their mean revolution speed was faster than the males. Exposure to decaBDE exerted sex-specific effects that diminished the baseline dimorphism. DecaBDE males ran more and decaBDE females ran less than their same-sex controls. Wheel rotation speed was not affected by decaBDE, which argues against

impaired motor coordination as the cause of the reduced wheel rotation output in the control males and the decaBDE females. The absence of any differences on the rotarod also rules out motor coordination impairments as an explanation for any of the behavioral differences in the current study.

We have only found one other example in the literature where a single manipulation produced opposite effects on wheel running in treated males vs. females. Thyroid- and parathyroidectomy led to significantly increased running in males but decreased running in female rats [56]. In an earlier study, neonatal hypothyroidism induced by propylthiouracil administration from PND1-24, also led to increased wheel running in adulthood, although this study only examined males [59]. Interestingly, the propylthiouracil manipulation also impaired motor habituation, reminiscent of the large body of work from the Eriksson lab.

In addition to the motor changes, cognitive deficits also emerged during the operant procedures. Male mice exposed to 20 mg/kg decaBDE responded at higher rates, made more errors, and earned fewer food pellets during the DRL30 procedure compared to the control males. Females were not affected by decaBDE during DRL30. Although they were more active on the lever, decaBDE males did not display general motor hyperactivity in the operant chambers since the activity counts recorded by the overhead monitors did not differ. However, when the control males were challenged with an acute dose of methylphenidate (MPH 5 mg/kg), their response rate and errors increased and their earned food pellets decreased in a dose-related fashion. The females and the decaBDE males did not respond to MPH. MPH also increased the general activity of the control males in the operant chambers. To further examine the effects of decaBDE and MPH on response inhibition during the DRL30, we examined a specific type of error, burst responses, with short interresponse times of 2-sec or less. The same general pattern was observed with burst responses as for total errors: males had more bursts than females, and the 10 and 20 mg/kg doses of MPH produced more bursts than 0, 2.5, or 5 mg/kg MPH. These effects were more robust in the control animals. Burst responding in the decaBDE animals was less affected by MPH. The overall pattern of decaBDE effects is similar to that reported by Sable et al. [49] in adult rats following developmental exposure to the Fox River PCB mixture. PCB-exposed males had an increased number of unreinforced responses on a DRL15 procedure but they were not as impaired as control males when challenged with acute amphetamine. PCB-exposed females did not differ significantly from controls.

The effect of MPH on the control male mice resembles earlier findings in male Sprague-Dawley rats. MPH in doses ranging from 2–33 mg/kg increased response rate, decreased reinforcement rate, and shortened the IRT during various DRL procedures [13,19,42]. Acute treatment with other dopamine reuptake inhibitors such as cocaine has also been shown to decrease the DRL IRT in mice [47]. However, the blunted response to MPH in the male decaBDE mice suggests that exposure produced long-term effects on the dopamine reuptake transporter and/or presynaptic dopamine stores. Although this mechanism has not been directly examined in vivo for decaBDE, Dreiem et al. [12] drew similar conclusions following an ex vivo exposure of striatum isolated from PND7, 14, or 21 rats. Striatal synaptosomes had reduced dopamine concentration while the medium had increased dopamine content, indicative of impaired dopamine reuptake. Fonnum et al. [21] had earlier

concluded that the higher-molecular sized PCBs such as those in the Fox River mixture, preferentially inhibit VMAT2 compared to the dopamine reuptake transporter thus facilitating cytosolic degradation of dopamine. Perhaps the fully-brominated decaBDE has similar effects on the development of central dopamine systems.

Prior to the MPH challenge procedure, the decaBDE males performed significantly worse on the DRL30. The integrity of the hippocampus is known to be critical for accurate performance in the DRL. For instance, NMDA receptor-mediated excitotoxic lesions of the hippocampus increased responding and reduced reinforcement during a DRL10 in C57BL/6J mice [44]. Flame retardant exposure has also been shown to target the hippocampus. A single low dose of tetraBDE given to male mice on PND10 reduced hippocampal long-term potentiation, perhaps by reducing specific glutamate receptor subunits [10]. The medial prefrontal cortex is another structure that regulates response inhibition. Infusion of the dopamine agonist bupropion into the medial prefrontal cortex improved DRL15 performance in controls but not in rats exposed to a PCB mixture throughout the perinatal period [38]. PCB exposure did not affect the behavioral response to selective postsynaptic D1 or D2 receptor agonists. Since both bupropion and MPH block the dopamine reuptake transporter, it is tempting to once again conclude that flame retardant exposure alters development of the dopamine transporter and/or presynaptic dopamine stores. Unlike the current study, the blunted response to bupropion was found in both PCB-exposed males and females. However, perinatal exposure to the same PCB mixture was previously shown to blunt the locomotor activating effects of repeated amphetamine administration and this effect was significantly greater in PCB-exposed males versus females [43]. Adult male rats have also been shown to have a greater reduction of striatal dopamine following developmental exposure to PCB153 [58].

In conclusion, mice exposed to decaBDE throughout the preweaning period showed an array of motor, learning, and response inhibition deficits in adulthood. Exposed males were more impaired, with greater forelimb grip strength deficits, more response errors on an operant DRL30 schedule, and a blunted response to acute methylphenidate treatment. Although decaBDE likely produces developmental neurotoxicity through multiple mechanisms mediated by factors such as dose, age, and sex, many of the behavioral effects observed in the current study could be the consequence of a developmental disruption of the dopamine reuptake transporter system. In this regard, decaBDE does not appear to be a safe alternative to other, banned PBDE congeners or the PCBs. Instead, it resembles them. Given the global distribution of decaBDE and its debrominated metabolites, efforts to extend regulation throughout Asia and Africa should continue.

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Highlights

- Exposure to decabromodiphenyl ether during early development produces long-term behavioral deficits.
- Males show more impairments than females following exposure to decabromodiphenyl ether.
- Developmental decabromodiphenyl ether exposure produces motor and response inhibition impairments but does not affect motivation.

Figure 1.
Timeline of the major events for the current project. The day of puberty is nominally represented as PND30 in this figure.

Figure 2.

Mean $\pm$ SEM forelimb grip strength (newtons) for male mice throughout the assessment period (upper panel). There was a significant main effect of exposure in males (* $P < 0.05$) but the female mice were not affected (lower panel). $n = 8, 9, 7, 10$ for control male, 20 mg/kg decaBDE male, control female, 20 mg/kg decaBDE female groups respectively.

Figure 3.

Mean $\pm$ SEM revolutions during the circadian wheel-running procedure. As anticipated, females ran more than males and all animals ran more during the dark phase of the 12h:12h cycle. DecaBDE exposure attenuated the sex-difference, significantly increasing running in males and decreasing running in females (* $P < 0.05$). $n = 13, 11, 12, 10$ for control male, 20 mg/kg decaBDE male, control female, 20 mg/kg decaBDE female groups respectively.

Figure 4.

Mean $\pm$ SEM total errors (unreinforced responses) during the DRL10-sec, 20-sec, & 30-sec operant schedules. Individual sessions were averaged for each DRL value. Males exposed to 20 mg/kg decaBDE performed significantly more errors than control males during the DRL30-sec schedule (* $P < 0.05$). $n = 8, 7, 9, 8$ for control male, 20 mg/kg decaBDE male, control female, 20 mg/kg decaBDE female groups respectively.

Figure 5.

Mean $\pm$ SEM total errors during the DRL30-sec schedule following pretreatment with acute methylphenidate. Methylphenidate (5, 10, or 20mg/kg) selectively increased errors in the control males but not the females or decaBDE-exposed males. * $P < 0.05$ for control males vs. all other groups per methylphenidate dose. $n = 6, 6, 9, 6$ for control male, 20 mg/kg decaBDE male, control female, 20 mg/kg decaBDE female groups respectively.

Figure 6.

An example of the interresponse time (IRT) distribution during the DRL30-sec schedule. The most frequent responses were “burst-type” errors with IRTs in the 0–2 second range. The IRT following acute pretreatment with 20mg/kg methylphenidate is shown here although the overall shapes of the distributions were the same following 5 or 10mg/kg methylphenidate (not shown). * $P < 0.05$ for control males vs. all other groups. $n = 6, 6, 9, 6$ for control male, 20 mg/kg decaBDE male, control female, 20 mg/kg decaBDE female groups respectively.

Table 1Mean $\pm$ SEM Postnatal Day of Emergence of Developmental Milestones During the Neonatal Period

Exposure-by-sex Group	Pinnae Detachment	Upper & Lower Incisor Eruption	Both Eyes Open	Puberty Onset
0 mg/kg male	3.9 $\pm$ 0.097 (n=24)	10.6 $\pm$ 0.14 (n=12)	13.9 $\pm$ 0.23 (n=8)	28.0 $\pm$ 0.32 (n=13)
0 mg/kg female	3.9 $\pm$ 0.091 (n=26)	10.5 $\pm$ 0.14 (n=11)	14.0 $\pm$ 0.21 (n=10)	33.7 $\pm$ 0.48 (n=12)
20 mg/kg male	4.0 $\pm$ 0.041 (n=29)	10.3 $\pm$ 0.20 (n=14)	13.7 $\pm$ 0.17 (n=13)	28.6 $\pm$ 0.26 (n=12)
20 mg/kg female	4.0 $\pm$ 0.038 (n=26)	10.1 $\pm$ 0.20 (n=14)	14.2 $\pm$ 0.20 (n=11)	34.6 $\pm$ 0.29 (n=8)