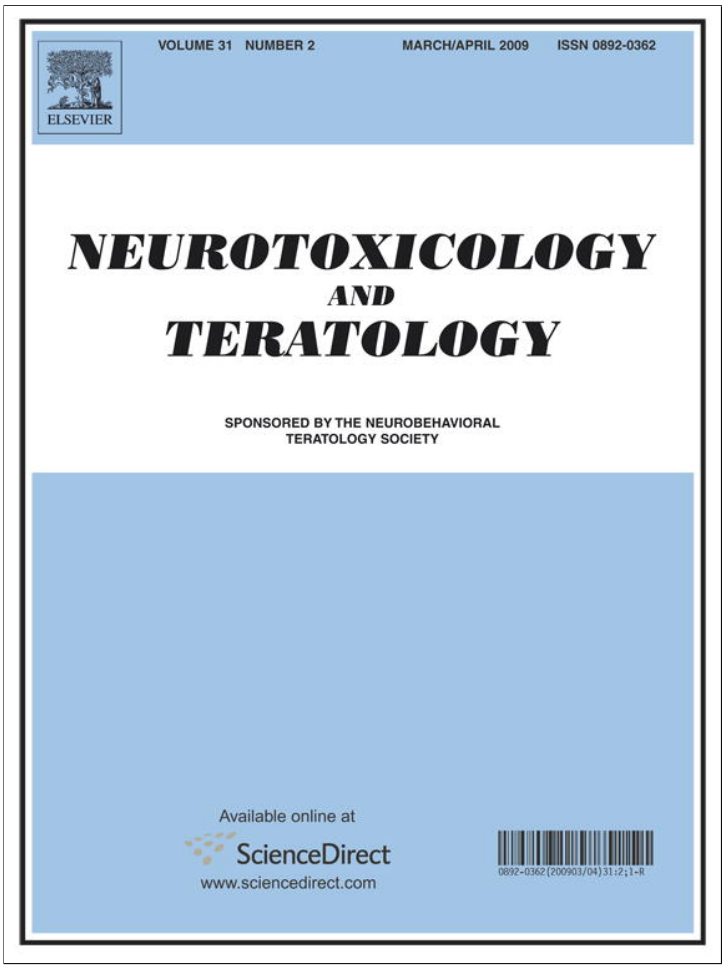

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Chronic postnatal DE-71 exposure: Effects on learning, attention and thyroxine levels☆

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ABSTRACT

Polybrominated diphenyl ethers (PBDEs) are ubiquitous, bioaccumulative flame retardants. Much remains to be learned about their developmental toxicological properties, particularly with regards to chronic exposure. In two experiments, male Long–Evans rats ingested the commercial pentaBDE mixture DE-71 from birth onward, first through the milk of lactating dams (who ingested 5 or 7.5 mg DE-71/day in a custom-mixed chow), then directly via chow consumption (at a dose of 3 or 4.5 mg/day). Control rats consumed the same brand of chow without DE-71. As adults, the rats were assessed for learning and attention using a series of five-choice serial reaction time tasks. A challenge with the muscarinic cholinergic antagonist scopolamine (0, 0.01, 0.03, or 0.05 mg/kg injected s.c.) was conducted on the final attention task. Serum total thyroxine (T4) levels were obtained at the end of testing. Total T4 was significantly lower in both DE-71 groups than in controls. Visual discrimination learning was unaffected by DE-71, but rats ingesting 4.5 mg/day DE-71 demonstrated significant impairments in sustained attention and inhibitory control, as evidenced by increased premature responding and decreased accuracy of responding in Attention Task 1. However, the DE-71-exposed rats did not respond differentially to the effects of scopolamine on attention compared to controls. These effects of chronic developmental DE-71 exposure differ from effects seen with brief postnatal exposure, suggesting that more research needs to be done on the more environmentally relevant chronic exposure model.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are synthetic flame retardant chemicals added to polymers for the manufacture of electrical appliances, carpets, and polyurethane foam. The 209 possible PBDE congeners are named according to the number and position of bromine atoms attached to the aromatic rings; for example, the pentabromodiphenyl ether molecules PBDE 99 and PBDE 100 each have five bromine atoms bound to the ten possible binding sites on the aromatic rings. Commercial flame retardant mixtures contain several congeners: the pentaBDE mixture contains primarily tetra- and penta-BDEs; the octaBDE mixture contains mostly octaBDEs; and the decaBDE mixture contains primarily PBDE 209, the only decabromodiphenyl ether.

PBDEs are easily released into the environment and are commonly detected in sediment, surface waters, sewage sludge, house dust, air, and on computers and electronics (e.g., [20,30,40]). Because they are lipophilic, PBDEs accumulate in fatty tissue, and they are found in high concentrations in blood, milk, and adipose tissue in wildlife [8,21] and humans [3,32,41,45]. PBDE tissue levels in humans increased exponentially throughout Europe from the 1970s through the late 1990s, with a doubling time of approximately five years [28]. Although tissue PBDE levels have begun to plateau in Europe, they continue to increase in North America [41,43], and PBDE levels in North America are currently one to two orders of magnitude higher than they are in Europe and Japan [22,32,41]. This is particularly true of lower-brominated congeners such as BDE 47 (a tetraBDE), and BDE 99 and 100 (pentaBDEs). Until 2003, the U.S. utilized approximately 95% of the world's tetraBDEs and pentaBDEs [21] in the form of the commercial BDE mixture DE-71. Although the U.S. manufacturer of the pentaBDE mixture DE-71 has voluntarily ceased production of this chemical, lower brominated congeners will be persistent in biota for decades to come, and the health effects of these compounds remain to be determined.

PBDEs are structurally similar to polychlorinated biphenyls (PCBs) and share some of their toxicological properties. Commercial PBDE mixtures induce phase I (EROD and PROD) and phase II (UDGPT) hepatic enzyme activities [15,47], increase protein kinase C translocation, inhibit microsomal and mitochondrial Ca^{2+} uptake [24], and bind to both androgen and estrogen receptors [26,46]. Also, as do polychlorinated biphenyls (PCBs), PBDEs disrupt the activity of thyroid hormones. Rodents briefly exposed to PBDEs during postnatal development demonstrate significant decreases in plasma T4 levels [19,60]. The decrease may be due in large part to PBDEs' upregulation

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of hepatic enzymes, leading to an increased catabolism of T4 [59], or to disruption of thyroid hormone transport [27].

Of particular interest for the sake of public health is how these effects of PBDEs influence developing organisms, which absorb PBDEs *in utero* [39,40] and consume them in high doses in breast milk [41,42], in addition to inhaling and ingesting them in house dust [23,58]. For example, the PBDE-induced disruption of thyroid hormone homeostasis can potentially have serious consequences for nervous system development because thyroid hormones serve an important developmental role in regulating many other hormones and growth factors in the brain, in effect modulating the developmental timing of neuronal and glial proliferation, migration, and differentiation [2]. As such, rodents with experimentally-induced hypothyroidism in the perinatal period exhibit general neural abnormalities such as delayed myelination and decreased synaptic connectivity [14], and aberrations in the development of the dopaminergic [50] and cholinergic [38] systems.

Given the *in vitro* and *in vivo* neural and hormonal effects of PBDEs, it is not surprising that rodents briefly exposed to commercial PBDE mixtures or single congeners during the perinatal period demonstrate neurobehavioral effects, such as altered locomotor activity [4,16,55,56] and learning impairments in the Morris water maze [55] and a food-motivated visual discrimination task [11]. Brief exposures also appear to perturb behavioral responses to cholinergic drugs, such as locomotor responses to nicotine in mice [54] and attentional impairments in response to the muscarinic cholinergic antagonist scopolamine in rats [11]. It remains to be determined whether functional alterations in other neurotransmitter systems also occur in response to PBDE exposure.

This emerging information on the neurobehavioral effects of acute developmental PBDE exposure is valuable for understanding the initial mode of action of PBDEs on the nervous system. However, brief exposure paradigms may not accurately model the lower-level chronic environmental exposure that is experienced by humans and wildlife. Very few studies of chronic effects have appeared in the literature. In the ranch mink, the pentaBDE mixture DE-71, administered in feed at doses of 0.1, 0.5, or 2.5 μg/g of chow to dams prior to gestation and in pups through 27 weeks of age, did not alter nicotinic or muscarinic receptor binding or acetylcholine or cholinesterase levels [6]. However, further examination of potential effects of chronic exposure should be conducted, particularly with higher doses, to fill the void in our understanding of the effects of acute and chronic PBDE exposures.

The current experiments were designed to explore the effects of chronic postnatal PBDE exposure on cognitive functions modulated by the cholinergic system, including the detection, sustained attention to, and processing of visual stimuli [5,25], and the ability to learn visuospatial discrimination problems [35]. In the current experiments, rodents were administered the pentaBDE mixture DE-71 in their laboratory chow; dams of the experimental subjects began ingestion 24 hours following birth, and the subjects themselves were weaned onto the same chow, which they consumed throughout the experiment. In adulthood, visual learning and attention tasks were administered, followed by a cholinergic challenge on the final attention task with the muscarinic antagonist scopolamine. Serum total thyroxine (T4) was measured in a subset of the animals at the end of behavioral testing. Because chronic DE-71 exposure may impose longer-term and more extensive neurobehavioral effects than brief exposure, it was hypothesized that both learning and attentional deficits would be observed in the current experiments, and that the DE-71-exposed animals would demonstrate altered cholinergic modulation of attentional processes. It was also expected that the exposed animals, as adults, would demonstrate significantly reduced total T4 levels compared to controls. Because adult-onset hypothyroidism, even at a subclinical level, can disrupt attention, working memory, and other aspects of executive functioning in humans (57,61), it is possible that any behavioral effects of chronic DE-71 exposure observed in this study could be due in part to this mixture's effects on thyroid hormone homeostasis.

Described below are two separate experiments conducted in separate cohorts of animals. In Experiment 1, animals exposed to a lower daily dose of DE-71 in their chow were compared to controls; in Experiment 2, the exposed animals received 1.5 times the daily DE-71 dose as did the animals in Experiment 1. These experiments do not comprise a true dose–response study, as the data from the two cohorts could not be combined and compared directly. However, each exposed group could be compared to its own littermate-matched control group.

2. Methods

2.1. Animals and DE-71 exposure

Nulliparous female Long Evans rats (Harlan Sprague–Dawley, Blue Spruce stock; Indianapolis, IN) were bred with male Long Evans rats that were born at Colorado College. Dams were housed singly and given unlimited access to tap water and standard laboratory chow (LabDiet 5001; PMI Nutrition International, Richmond, IN). Twenty-four hours following parturition, on postnatal day 1 (PND1), the litters were culled to nine pups, while attempting to maintain a fairly equal sex ratio. Also at this time, one male pup from each biological litter was cross fostered to a litter of the same age, and the litter receiving this pup contributed one male pup to the donor litter. Therefore, each biological litter contributed two male pups for the behavioral testing: one that remained in its original litter, and one that was fostered to the sister litter. In this way, matched biological littermates were represented in both treatment groups, and litter was treated as the unit of analysis. All animal care and experimental procedures were conducted in accordance with guidelines published in the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

Immediately following fostering, the primary investigator (LLD) assigned each litter (which now contained one foster pup in addition to the original pups) to a treatment group and coded the litters so that the testers remained blind to subjects' treatment status. The control dams ($n=8$) received an *ad libitum* diet of standard laboratory rat chow and tap water. The experimental dams ($n=8$) were placed on an *ad libitum* diet of the same rodent chow (LabDiet 5001; Dyets, Inc; Bethlehem, PA) adulterated with the commercial pentaBDE mixture DE-71 at a concentration of 300 mg/kg (Experiment 1) or 450 mg/kg (Experiment 2) of chow. These concentrations resulted in dams' daily consumptions of approximately 4.5–5.5 mg (Experiment 1) or 7.0–8.0 mg (Experiment 2) DE-71. The DE-71 (lot 7550OK20A), generously donated by Dr. Kevin Crofton of the U.S. Environmental Protection Agency, contains approximately 25% tetra-BDE, 50–60% penta-BDE, and 4–8% hexa-BDE [44].

Due to concern that the DE-71-adulterated chow would not be palatable to the exposed dams, all dams' bodyweights were monitored daily. At no point were bodyweights, or the changes in bodyweight from the beginning to the end of lactation, different between the two groups (data not shown). Although specific food consumption amounts were not directly measured, colony keepers reported that food hoppers were replenished at equal rates for the two treatment groups.

2.2. Maintaining motivation

On the day of weaning (PND 21), each pup to be used for behavioral testing was moved to a cage with its non-biological, same-treatment littermate. Extra pups were retained for breeding, blood sampling, or pedagogical purposes. Pups were gradually food restricted to 15 g of chow per day on PND 25 and 10 g of chow per day on PND 28; therefore, by adulthood, the subjects were consuming 3 mg

(Experiment 1) or 4.5 mg (Experiment 2) DE-71 per day. From PND 40 onward, all pups were housed singly.

From the onset of behavioral testing (PND 40) through the end of the experiment (ranging from PND 115–PND 125), daily food allotments, consisting of a combination of the control or DE-71-adulterated chow and non-adulterated reward pellets received during testing, were adjusted individually based on behavioral trial completion rates to maintain motivation while still allowing for normal growth (at least 2 g per day). This food restriction procedure resulted in bodyweights that were ≥85% of ad libitum weights. By the end of the study, 84% (27/32) of the rats were receiving 13 g of food daily, and the remaining rats (3 controls and 2 exposed) were receiving 12 g daily. However, for most of the DE-71-exposed rats, the amount of adulterated chow consumed daily was still approximately 10 g, as the mean weight of non-adulterated reward pellets consumed per day was 2.5 g.

2.3. Apparatus

Testing was conducted in four Plexiglas chambers (ENV-008, Med Associates, Inc.; St. Albans, VT), each measuring 30.5×24×34.5 cm and enclosed in an opaque sound-attenuating exterior box. The chambers were modeled after the 5-choice serial reaction time task chambers [7]. Embedded in one wall of the main chamber, 2.5 cm above the floor, was a square alcove (5 cm tall×5 cm wide), into which a sweetened 45 mg reward pellet (Noyes formula AIN-76A; Research Diets, Inc.; Lancaster, PA) was dispensed (automated pellet dispenser ENV-203-451R, Med Associates) on each trial in which a correct port nosepoke was made. A motorized guillotine-type door controlled nosepokes into the alcove. Extending 1 cm deep from the curved wall opposite the alcove were five square ports (2.5×2.5 cm), 2.5 cm above the floor. Nosepokes into the alcove and the ports were detected by infrared photodiodes positioned just inside the openings of each. Each port was outfitted with a yellow light-emitting diode (LED) embedded in the back wall. Illumination of these LEDs constituted the visual cue to which animals were trained to respond. A house light, situated above and to the left of the alcove, was illuminated for most of the session but was extinguished for 5 s upon the commission of an error (an event termed a "timeout"). If the rat made a nosepoke in a port during a timeout, the 5 s timer was reset, the house light remained extinguished, and no rewards were delivered. An infrared video camera was located above each chamber to monitor behavior.

2.4. Behavioral testing

Testing began on PND40 and took place six days per week, with each testing session lasting 100 trials or 60 min, whichever came first. Each trial began with the opening of the guillotine door and the rat's nosepoke into the alcove (initiation) and ended with either a timeout or the rat's retreat from the alcove after reward delivery.

Each rat was first given a series of four training tasks to shape the sequence of responses that constituted a trial: initiation of the trial by poking into the alcove, followed by turning around and making a nosepoke into one of the ports, followed by receipt of reward in the alcove. The criterion to proceed from one step to the next was the attainment of 100 reward pellets within a single 60-minute session. In the first task, the animal was trained to poke its head into the alcove to obtain reward, with the door remaining open throughout the session. In the second task, the animal learned that the opening of the door signaled availability of reward. The door closed 3 s after the pellet was dispensed and reopened 2 s later for the beginning of the next trial. The third shaping task trained the animal to initiate a trial at the alcove, turn around, and make a nosepoke into any of the five ports before being rewarded. The purpose of the fourth and last shaping task was to give the rat equal experience with each of the five ports, so that port biases (i.e., responding preferentially to some ports over others) could be minimized. Four of the five ports were covered during each session, forcing the rat to poke into the same port for all 100 trials. A different port was uncovered on each subsequent training session until the rat reached the performance criterion of 100 total responses for each of the five ports.

Once reliable nosepoke responding had been established, the rats progressed through a series of five-choice visual discrimination tasks. For these and subsequent tasks, initiation at the alcove was followed by a 2 s delay before any visual cues were presented in order to allow the animal time to turn around and orient itself toward the ports. For the first discrimination task, the light cue appeared inside one of the five ports for 15 s or until a response was made, whichever came first. To receive a reward, the rat was required to poke in the illuminated port at any time between the cue onset and within 5 s after the cue offset (the "limited hold" period). The location of the visual cue on a given trial was pseudo-randomized such that each port was chosen randomly without replacement until all five ports were chosen; the cycle was then reset for the next five trials. The animals were required to obtain a performance criterion of at least 80% correct for two out of three sessions of 100 trials each before advancing. The two subsequent tasks were similar to the visual discrimination task but had briefer cue durations of 5 s and 1 s. The rat received one and three sessions on each of these tasks, respectively.

In the sustained attention task that followed (Sustained Attention Task 1), the 1 s visual cue occurred unpredictably, with a pre-cue delay of 0, 3, or 6 s (in addition to the 2 s "turnaround" period), thus requiring the animal to sustain attention across the five ports for an indeterminate period of time. The delay on a given trial varied pseudo-randomly, as did the location of the cue. The animals were given ten sessions on this task. The final sustained attention task, Sustained Attention Task 2, included the same variable pre-cue delays but also incorporated variable cue durations of 200 ms, 500 ms, and 800 ms. After ten sessions on this task, the rats were administered the drug challenge.

2.5. Scopolamine challenge

For the drug challenge phase, the rats were injected subcutaneously with scopolamine hydrochloride (0, 0.01, 0.03, or 0.05 ml/kg body weight; Sigma-Aldrich), dissolved in sterile water and filtered through a 2 μm filter, then run on Sustained Attention Task 2. The injections, administered at a volume of 1 ml/kg body weight, took place 30 min prior to testing to allow time for peak plasma levels to be achieved [1]. Each animal received each of the four doses twice; the dosing order was randomized according to a Latin-square design. Because receptor upregulation can occur with closely-spaced scopolamine administrations [49], drug administrations took place only twice per week (on Tuesday and Friday). Animals completed one testing session without drug administration the day before each drug testing session. Differences in the response to scopolamine between control and DE-71-exposed rats were interpreted as evidence for DE-71-induced alterations in the cholinergic modulation of performance in the task.

2.6. Performance measures

Percentages of specific response types were calculated in each behavioral task. A premature response was one made before the onset of the cue light, indicating a failure of inhibitory control. A response made after the onset of the cue light but at a non-illuminated port was termed an inaccurate response. Finally, a failure to make a nosepoke response into any port within 5 s after cue onset was scored as an omission error. Both omission errors and inaccurate responses reflected lapses of attention. Percent accuracy was defined as the number of correct responses divided by the number of all "timely" responses (i.e., responses made after cue onset and before 5 s following

cue offset). In addition to the above measures, total trials and errors to criterion were calculated for the Visual Discrimination task.

Three latency measures were also recorded. Initiation (alcove) latency, the duration of time between the end of the previous trial and the rat's initiation of a new trial, reflected the animal's motivation, as did the reward latency, the time between the animal's correct response and its retrieval of the reward pellet. The time between cue onset and the animal's nosepoke in the correct port, the correct response latency, revealed the animal's information processing speed and motivation.

2.7. Blood serum total thyroxine (T4) levels

Following behavioral testing, blood samples to test for serum total thyroxine (T4) levels were taken via cardiac puncture from a randomly selected subset of the rats ($n=7$ per treatment) following anesthesia overdose with pentobarbital sodium (100 mg i.p.; Sigma-Aldrich, Inc; St. Louis, MO). Determination of total T4 was conducted by the Colorado State University Veterinary Diagnostic Laboratory using the Immulite chemiluminescent immunoanalyzer system (Siemens Healthcare Diagnostics, Inc.; Deerfield, IL). The Veterinary Diagnostic Laboratory is accredited by the American Association of Veterinary Laboratory Diagnosticians, and acts as a consultant to the United States Animal Health Association on uniform diagnostic criteria involved in regulatory animal disease programs.

2.8. Statistical analyses

The Statistical Package for the Social Sciences (version 15 for Windows; SPSS Inc., Chicago, IL) was used to analyze the data. Litter was used as the unit of analysis, with one DE-71/control pair per litter. Paired *t*-tests were used to analyze treatment differences in bodyweights, total T4 levels, and criterion measures (mean number of errors and trials to criterion) for the Visual Discrimination task. Repeated measures analyses of variance (ANOVAs) were used on performance measures for the first four sessions of the Visual Discrimination task to examine treatment differences in learning rate. Repeated measures ANOVAs were also used to analyze the dependent measures for Sustained Attention Tasks 1 and 2 and the Drug Challenge, with the following included as within-subjects factors: treatment, session block (mean performance across every two days to illustrate improvement in performance), and pre-cue delay for Attention Task 1; treatment, pre-cue-delay, and cue duration for Attention Task 2; and treatment, pre-cue delay, cue duration, and scopolamine dose for the Drug Challenge. All significant main effects in ANOVA were followed by Bonferroni-corrected post hoc tests. When significant effects involving treatment were found, effect sizes (partial eta squared or partial η^2) were calculated in addition to traditional *F* and *p* values.

3. Results

3.1. Bodyweights

For both Experiment 1 and Experiment 2, bodyweights of DE-71-exposed rats did not differ significantly from those of controls at weaning (PND21), nor did they differ at any point during behavioral testing (weights compared once per day from PND21-PND40, then once per week through PND105, all $p>.05$; data not shown).

3.2. Acquisition of visual discrimination

3.2.1. Experiment 1

Performance improved steadily as the rats acquired the visual discrimination task, as evidenced by a significant increase across session (data for the first four sessions only) for response accuracy, $F(3,15)=23.51$, $p<.001$ (see Fig. 1A), and significant decreases across session for percent premature responses, $F(3,15)=6.02$, $p<.01$, and percent omission errors, $F(3,15)=8.26$, $p<.01$. The total number of trials required to reach criterion on the visual discrimination task (2 out of 3 sessions with at least 80% correct) did not differ between the DE-71-exposed rats and the control rats, $t(15)=-1.13$, $p>.05$, nor did the number of errors to criterion, $t(15)=-1.50$, $p>.05$ (data not shown). There were also no main treatment effects or treatment by session interactions for accuracy (see Fig. 1A), percent premature responses, percent omission errors, initiation latency, correct response latency, or reward latency (all $p>.05$).

3.2.2. Experiment 2

Visual discrimination task performance improved across sessions, although the animals in this cohort acquired the task more slowly than did the animals in the previous cohort. Main effects of session were seen for accuracy, $F(3,15)=29.05$, $p<.001$ (see Fig. 1B), percent omission errors, $F(3,15)=6.06$, $p<.01$, and percent premature responses, $F(3,15)=8.82$, $p<.001$. As before, the treatment groups did not differ in number of errors to criterion, $t(15)=.91$, $p>.05$, or trials to criterion, $t(15)=.45$, $p>.05$ (data not shown). There were also no main treatment effects or treatment by session interactions for accuracy (see Fig. 1B), percent premature responses, percent omission errors, initiation latency, correct response latency, or reward latency (all $p>.05$).

3.3. Attention Task 1

3.3.1. Experiment 1

In Attention Task 1, the imposition of unpredictable 0, 3, or 6 s pre-cue delays affected the rats' ability to wait for, detect, and respond

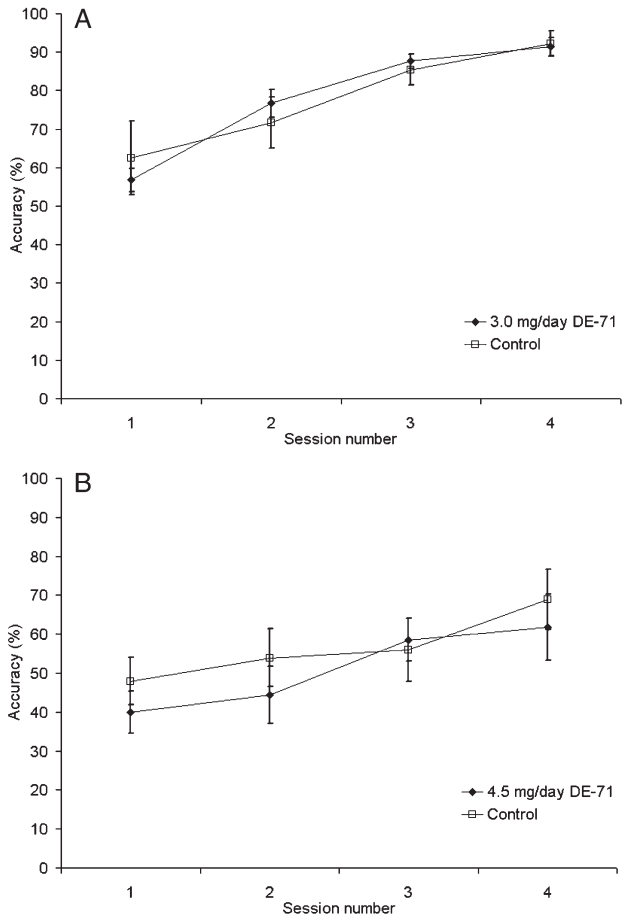

Fig. 1. Percentage of accurate responses in the Visual Discrimination Task as a function of task session for control rats and rats exposed to DE-71 at daily doses of 3.0 mg/day (A) or 4.5 mg/day (B). No acquisition deficits were observed in the DE-71-exposed rats compared to controls. Error bars=+\−2 SEM.

accurately to the 1 s visual cues. Declines in accuracy, $F(2,30)=37.87$, $p<.001$ (see Fig. 2A), and increases in percent premature responses, $F(2,30)=16.13$, $p<.001$ (see Fig. 3A), and percent omission errors, $F(2,30)=235.12$, $p<.001$ (data not shown) were seen with increasing pre-cue delay. However, the effect of this manipulation on performance decreased as the rats progressed through the task, as was evidenced by main effects of block on accuracy, $F(4,60)=24.14$, $p<.01$, percent premature responses, $F(4,60)=3.30$, $p<.05$, and percent omission errors, $F(4,60)=60.53$, $p<.001$ (data not shown). Block by delay interactions were seen for accuracy, $F(8,120)=2.53$, $p<.05$, and for percent omission errors, $F(8,120)=34.93$, $p<.001$, indicating that the effects of delay on these performance measures were largest in the first block of sessions and became attenuated as the blocks progressed.

There were no main effects of treatment on any performance or latency measure in this task, nor were there any interactions between treatment and block, treatment and delay, or treatment, block, and delay (all $p>.05$).

3.3.2. Experiment 2

Improvement in performance across the five blocks of the task was evidenced by significant effects of block on accuracy, $F(4,60)=33.08$, $p<.001$, percent premature responses, $F(4,60)=67.45$, $p<.001$, and percent omission errors, $F(4,60)=9.32$, $p<.001$ (data not shown). In addition, main effects of pre-cue delay were observed for accuracy, $F(2,30)=79.76$, $p<.001$ (see Fig. 2B), percent premature responses, $F(2,30)=283.04$, $p<.05$ (see Fig. 3B), and percent omission errors, $F(2,30)=9.40$, $p<.01$ (data not shown), indicating that the rats had difficulty waiting for and attending to the unpredictable visual cues. There were also block by delay interactions for accuracy, $F(8,120)=2.55$, $p<.05$, percent prematures, $F(8,120)=44.75$, $p<.001$, and omission errors, $F(8,120)=2.20$, $p<.05$. In all three cases, performance improved and error types decreased from the first to the final block of sessions.

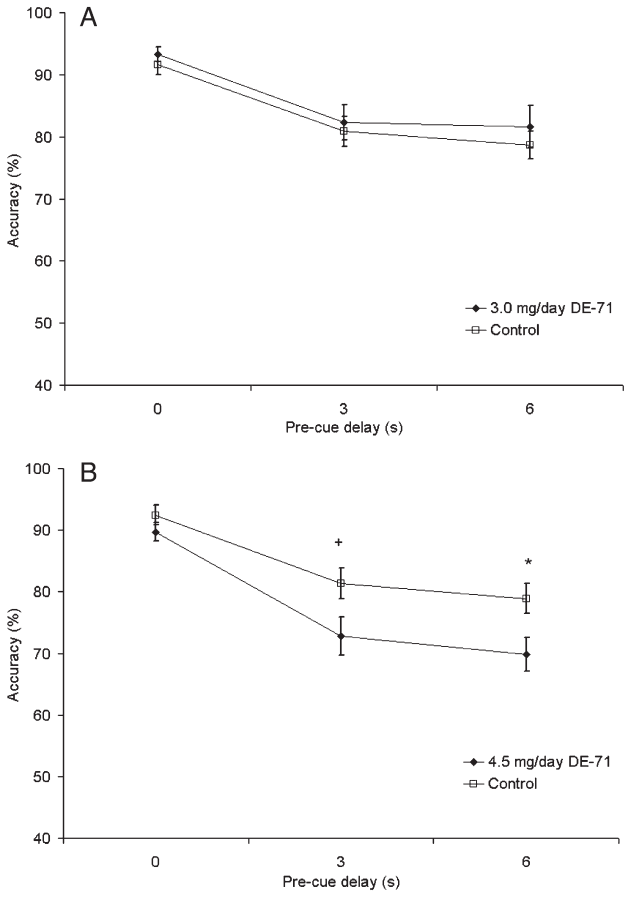

Fig. 2. Percentage of accurate responses in Attention Task 1 as a function of the delay between trial onset and 1 s cue presentation. Overall, response accuracy declined on trials in which a delay preceded the cue. (A) Rats exposed to 3.0 mg/day DE-71 (Experiment 1) responded as accurately as controls for all pre-cue delay conditions. (B) Rats exposed to 4.5 mg/day DE-71 (Experiment 2) performed as well as controls when there was no delay between trial onset and cue presentation, but their response accuracy was worse than that of controls on trials with a 6 s pre-cue delay (*$p<.05$), and marginally worse than that of controls on trials with a 3 s pre-cue delay (+$p=.077$). Error bars=+\−2 SEM.

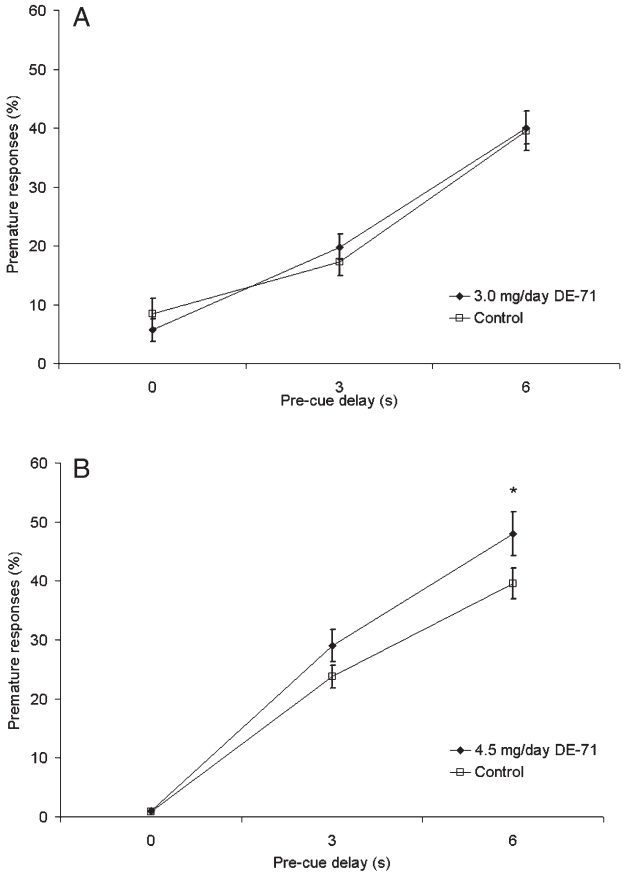

Fig. 3. Percentage of premature responses in Attention Task 1 as a function of the delay between trial onset and 1 s cue presentation. Overall, premature responses increased on trials in which a delay preceded the cue. (A) Premature response rate did not differ between controls and rats exposed to 3.0 mg/day DE-71 (Experiment 1). (B) Rats exposed to 4.5 mg/day DE-71 (Experiment 2) made a higher percentage of premature responses than control rats specifically on trials in which a 6 s delay preceded the visual cue (*$p<.05$). Error bars=+\−2 SEM.

The DE-71-exposed rats demonstrated significantly lower accuracy in Attention Task 1 than did controls, $F(1,15)=4.61$, $p<.05$, partial $\eta^2=.24$ (see Fig. 2B). Treatment also interacted with pre-cue delay, $F(2,30)=4.11$, $p<.05$, partial $\eta^2=.22$, and pairwise comparisons revealed that the deficit in accuracy in the DE-71-exposed rats was significant at the 6 s delay ($p=.033$), marginally significant at the 3 s delay ($p=.077$), and nonsignificant at the 0 s delay ($p=.34$) (Fig. 2B). The treatment by block interaction was not significant.

Overall, the DE-71-exposed rats tended to make a higher percentage of premature responses than controls, $F(1,15)=3.70$, $p=.074$ (see Fig. 3B). There was also a significant treatment by delay interaction for premature responses, $F(2,30)=4.79$, $p<.05$, partial $\eta^2=.21$; pairwise comparisons revealed significantly increased premature responding in the exposed rats compared to controls at the longest pre-cue delay of 6 s ($p=.047$), but not at the 0 s or 3 s delays ($p=.77$ and .12, respectively) (Fig. 3B). Treatment did not interact with block on this measure.

No main effects of treatment, and no interactions including treatment, were observed for percent omission errors, initiation latency, correct response latency, or reward latency in Attention Task 1.

3.4. Attention Task 2

3.4.1. Experiment 1

As was the case with Attention Task 1, the parameter of pre-cue delay in this task significantly affected all performance measures ($p<.05$ for accuracy, percent premature responses, and percent omission errors). In addition, performance varied significantly with the duration of the visual cue: accuracy decreased on trials with shorter cue durations, $F(2,30)=145.94$, $p<.001$, and percent omission errors increased, $F(2,30)=115.61$, $p<.001$ (data not shown).

As with Attention Task 1, there were no significant differences between the DE-71-exposed and control groups on any of the performance or latency measures, nor were there any interactions of delay or duration with treatment (all $p>.05$).

3.4.2. Experiment 2

As before, accuracy, percent premature responses, and percent omission errors were significantly affected by the delay prior to cue onset (main effects of delay, all $p<.05$). Performance also varied as a function of cue duration, with lower accuracy, higher percent premature responses, and higher omission errors on trials with the shortest cue durations (all $p<.05$). Delay by duration interactions were seen for accuracy, $F(4,60)=11.74$, $p<.001$, and percent omission errors, $F(4,60)=12.31$, $p<.001$; in both cases, the effect of duration was strongest at the shortest pre-cue delay (data not shown).

The DE-71-exposed rats were not significantly impaired relative to controls in this task. There were no main effects of treatment on any performance or latency measure (all $p>.05$). There was a significant treatment by duration interaction for accuracy, $F(2,30)=4.12$, $p<.05$, partial $\eta^2=.22$. However, the treatment groups did not differ significantly at any of the cue durations ($p=.25$, .55, and .85 for the 200, 500, and 800 ms cues, respectively). No other interactions with treatment were observed on any measure in this task.

3.5. Attention Task 2 with scopolamine challenge

3.5.1. Experiment 1

Scopolamine dose-dependently impaired accuracy, $F(3,39)=16.29$, $p<.001$, and increased the percentage of premature responses, $F(3,39)=15.19$, $p<.001$, in Attention Task 2 (data not shown). It did not significantly alter the percentage of omission errors ($p>.05$). There was a significant effect of dose on initiation latency, $F(3,39)=8.24$, $p<.001$, but no scopolamine dose differed significantly from the vehicle dose. Correct response latency was affected by scopolamine as well, $F(3,39)=11.33$, $p<.001$; the .03 and .05 mg/kg doses produced faster latencies than those seen with the vehicle dose ($p<.05$ and $p<.001$ for the medium and high doses vs. vehicle, respectively) (data not shown). The high dose of scopolamine also decreased reward latencies with respect to the vehicle, $p<.05$.

There were no treatment by dose interactions on any performance or latency measures, indicating that the DE-71-exposed rats were not differentially sensitive to scopolamine compared to controls. No higher order interactions involving treatment and dose were seen.

3.5.2. Experiment 2

Scopolamine impaired accuracy, $F(3,39)=6.19$, $p<.01$ and increased premature responding, $F(3,39)=3.62$, $p<.05$, in a dose-dependent manner (data not shown). No significant effects of the drug were observed for omission errors or initiation latencies, but scopolamine affected correct response latencies, $F(3,39)=5.23$, $p<.01$, with the highest dose producing significantly faster latencies than the vehicle ($p<.05$). A main effect of drug dose was also observed for reward latencies, $F(3,39)=7.56$, $p<.001$, although none of the scopolamine doses differed significantly from vehicle (data not shown).

As was the case in Experiment 1, the DE-71-exposed rats did not show differential sensitivity to scopolamine compared to controls: there were no significant treatment by dose interactions for any dependent measure, nor were there any higher-order interactions involving treatment.

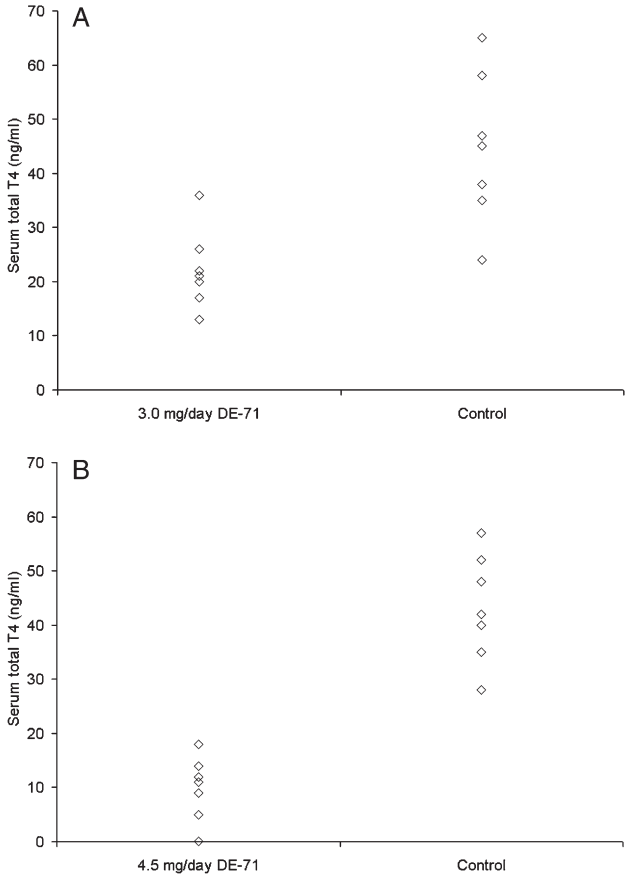

Fig. 4. Serum total thyroxine (T4) measured at the end of behavioral testing (approximately PND120). (A) Experiment 1 T4 levels in controls were significantly higher than in rats exposed to 3.0 mg/day DE-71 ($p=.011$). (B) Experiment 2 T4 levels in controls were significantly higher than in rats exposed to 4.5 mg/day DE-71 ($p<.001$). Error bars = +\−2 SEM.

3.6. Serum total T4 levels

3.6.1. Experiment 1

Serum total T4 levels, measured in a subset of the animals at the end of the experiment, were significantly lower in the DE-71 exposed rats than in the control rats, $t(6)=-3.59$, $p=.011$, $r^2=.68$ (see Fig. 4A). Mean T4 levels in the exposed animals (22.14 ng/ml) were approximately 50% of the mean control levels (44.57 ng/ml).

3.6.2. Experiment 2

Total T4 levels were significantly lower in the DE-71-exposed rats than in controls at the end of behavioral testing, $t(6)=-7.92$, $p<.001$, $r^2=.91$ (see Fig. 4B). Mean levels in the exposed animals (9.86 ng/ml) were approximately 25% of the mean levels in controls (42.75 ng/ml).

4. Discussion

In the current experiments, the behavioral effects of chronic postnatal DE-71 exposure, first via the dams' milk, and then directly through chow consumption, were examined in adult rats. The lower concentration of DE-71 (300 mg/kg chow), which resulted in consumptions of approximately 5.0 mg DE-71 per day by dams and 3.0 mg per day by subjects, produced no deficits (compared to controls) in learning, attention, or inhibitory control. Rats exposed to the higher concentration of DE-71 (450 mg/kg chow, with an

approximate dose of 7.5 mg per day for dams and 4.5 mg per day for subjects) also demonstrated no learning impairments. However, on Sustained Attention Task 1, which featured unpredictable pre-cue delays of 0, 3, and 6 s, these rats made a lower percentage of accurate responses and a higher percentage of premature responses to the visual cues than did controls, particularly on trials in which the pre-cue delay was long. These deficits were specific to Sustained Attention Task 1; they did not persist in the more attentionally demanding Sustained Attention Task 2, in which both pre-cue delay and cue duration were randomly variable. In addition, neither the lower nor the higher DE-71-exposed rats demonstrated an altered sensitivity to the attention-impairing effects of scopolamine on Sustained Attention Task 2.

The pattern of behavioral effects observed in this study differs from the pattern of effects observed in animals exposed briefly (daily from PND 6–12 via oral gavage) to 30 mg/kg/day DE-71 in early postnatal life [11], which included intact attention but impaired learning and a blunted response to the effects of the muscarinic cholinergic antagonist scopolamine on sustained attention. The reason for these differences is unclear, but two key differences in the exposure paradigms may provide important information about relative sensitivities of different processes to different types of exposure. First, it is possible that a chronic exposure and/or a higher lifetime body burden of DE-71, such as that provided by the higher dose in Experiment 2, is required to produce attentional deficits in adulthood. Second, even though the overall lifetime exposure to DE-71 was higher in the current study than in Dufault et al. [11], it is very likely that the exposure during the early postnatal period, particularly during the sensitive period known as the "brain growth spurt" [9], was greater in Dufault et al. [11] than in the current study. In Experiment 2, the dams, which weighed approximately 275 g, consumed approximately 7.5 mg of DE-71 per day, which is a dose of approximately 27 mg/kg body weight per day. However, each pup likely received a lower dose than this indirectly through the dam's milk. Therefore, the higher "pulse" of DE-71 administered directly via gavage in Dufault et al. [11] (30 mg/kg/day) during early postnatal life, and particularly during the brain growth spurt, may be necessary to produce learning impairments and lasting effects on the cholinergic modulation of attention.

In the current experiments, the impairments in attention and inhibitory control in the high dose DE-71 group were observed in Sustained Attention Task 1 but not Sustained Attention Task 2, which is interesting given that the only difference between the tasks was the increasing challenge of shorter and variable cue durations. It is possible that the unpredictable delays in Attention Task 1 taxed the attentional resources of the exposed rats more than the control rats, but the addition of shorter and unpredictable cue durations in Attention Task 2 was necessary to sufficiently tax attentional resources of both groups. However, it is also possible that the impairments in sustained attention and inhibitory control seen during Sustained Attention Task 1 in the higher dose DE-71 rats were actually due to slowed acclimation to the new attentional requirements of the task (i.e., the cue appears after a delay of unpredictable length on 2/3 of the trials instead of immediately on all trials) rather than to difficulties with attention or inhibitory control per se. The fact that the deficits were specific to trials with the longer pre-cue delays supports this hypothesis. However, if this were the case, interactions between treatment and session block in Sustained Attention Task 1 should have occurred, and they did not.

The DE-71-exposed animals in the current experiments showed no deficiencies in motor functioning, information processing speed, or motivation, as their latencies to initiate trials, respond to cues, and collect rewards, respectively, did not differ from control latencies. The increase in premature responding in Attention Task 1 could have been due to hyperactivity, which would be consistent with reports of increased locomotor activity observed in animals exposed to PBDE mixtures and individual congeners for a brief period in postnatal life [12,13,16,51–54,56], or it could also be indicative of impaired inhibitory control. The latter is a more likely explanation, given that the treatment groups did not differ in latencies to initiate trials, respond to visual cues, or collect rewards.

Significant decreases in total serum T4 levels were observed at the end of the study in both the 3 and 4.5 mg/day DE-71 groups compared to controls. Total T4 levels were 50% of control levels in the former group and 25% of control levels in the latter group. These reductions are consistent with previous reports of significantly reduced T4 levels in rats exposed to PBDE congeners and commercial mixtures in the early postnatal period [33,59,60] and in adulthood [15,18,19,34]. However, the reductions are noticeably more dramatic in this study than have been reported previously. For example, after four days of oral exposure to 100 mg/kg/day of BDE 47, 9-week-old mice demonstrated reductions in serum T4 of only 43% when compared to controls [34]. The greater reductions observed in the current study may be due to the fact that the animals consumed DE-71 chronically from birth, resulting in both developmental and concurrent effects on systems responsible for the maintenance of thyroid hormone homeostasis. It is also possible that long-term bioaccumulation of DE-71 resulted in greater effects on T4 than have been seen with much briefer exposures. To our knowledge, no other studies have examined total T4 levels in adult laboratory animals exposed to DE-71 developmentally as well as chronically, so replication of the current data would be useful.

Adult-onset hypothyroidism in humans has long been known to produce a wide spectrum of cognitive deficits, from reductions in general intelligence and psychomotor functioning (see [10] for review) to alterations in attention, working memory, and other executive functions [57,61]. In rodents, experimentally-induced hypothyroidism, resulting in very dramatic reductions of T4 and sometimes also T3, produces depressive-like behaviors [29] and perturbations in hippocampal neuronal morphology [36] and physiology [48]. Therefore, the attentional deficits observed in the animals exposed to the higher dose of DE-71 in the current study could potentially be due to the effects of this mixture on thyroid hormone homeostasis. In fact, it is surprising that greater deficits were not seen, and that the animals exposed to the lower DE-71 dose in Experiment 1 showed no behavioral deficits. Unfortunately, assessments of higher cognitive functioning in animals with less severe forms of induced hypothyroidism (i.e., T4 levels that are 50% of control levels or more) have not been conducted, so a direct comparison of such effects with those seen in our DE-71-exposed animals cannot be made.

It is also interesting that this chronic postnatal exposure paradigm affected T4 but had no detectable effect on the muscarinic cholinergic modulation of sustained attention, given that early hypothyroidism can produce lasting aberrations in cholinergic system development [17,31,38]. However, the propylthiouracil-induced hypothyroidism in most of these studies resulted in the near elimination of T4 levels, whereas the reductions in T4 in the current experiments were far less dramatic, even after several months of DE-71 consumption. Measurements of T4 levels during lactation and weaning would have provided important information about how dramatic the DE-71-induced reductions in T4 were during development, but these measurements were not taken in the current study, so the severity of the effects is unknown.

Because the deficits in attention and inhibitory control in the 4.5 mg/kg/day DE-71 group were not accompanied by an altered sensitivity to the effects of scopolamine on performance in Sustained Attention Task 2, it seems likely that the neural bases of DE-71's effects with this exposure paradigm were not cholinergic in nature. Similarly, chronic DE-71 from gestation through adulthood, albeit at a lower dose, also failed to affect cholinergic parameters, including muscarinic and nicotinic receptor binding, acetylcholinesterase activity, and acetylcholine activity, in the cerebral cortex of ranch minks [6]. It is

unexpected, then, that much briefer exposures to PBDE mixtures or individual congeners are capable of altering behavioral sensitivity to cholinergic drugs [11,51,54] and cholinergic receptor numbers [53]. There is no clear explanation for the discrepancy, as some of the studies employing the brief exposure paradigm used a single PBDE dose that was likely to be in the range of the dose consumed by the pups in the current experiments. However, because lactational DE-71 intake was not measured in the current experiments, it cannot be stated with certainty that the pups consumed daily doses that would be sufficient to perturb development of cholinergic parameters during the period encompassing the brain growth spurt.

Although DE-71 is composed almost entirely of PBDE congeners, it is important to note that these commercial mixtures also contain small amounts of compounds such as dioxins and furans that have their own biological effects (see [37]). Therefore, it is possible that any or all of the effects reported here are due in part to these impurities as well as to one or more of the PBDE congeners themselves.

Conclusions regarding dose–response relationships cannot be made with the current experiments because the two DE-71 doses were administered at different time points in separate cohorts of animals. Great care was taken to conduct the experiments as similarly as possible, but as can be seen in Fig. 1, learning rates differed between the two cohorts, which further limits the ability to compare data across the two experiments. In addition, because the DE-71 was administered in the chow, doses were approximate rather than exact, and they varied slightly between individual animals. Despite these limitations, the current experiments provide important information about the impact of chronic postnatal DE-71 exposure on neurobehavioral outcomes in adulthood, and suggest that inferences about the impact of PBDEs on health and behavior should not be made solely on data from acute exposure studies, as humans and non-human animals are exposed to these compounds throughout life. Additional investigations of this environmentally-relevant exposure paradigm are warranted.

Conflict of Interest

Nothing declared.

References

[1] T. Ali-Melkkila, J. Kanto, E. Iisalo, Pharmacokinetics and related pharmacodynamics of anticholinergic drugs, Acta Anaesthesiol. Scand. 37 (1993) 633–642.

[2] E. Auso, R. Lavado-Autric, E. Cuevas, F.E. del Rey, G.M. de Escobar, P. Berbel, A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration, Endocrinology 145 (2004) 4037–4047.

[3] A. Bradman, L. Fenster, A. Sjodin, R.S. Jones, D.G. Patterson, B. Eskenazi, Polybrominated diphenyl ether levels in the blood of pregnant women living in an agricultural community in California, Environ. Health Perspect. 115 (2007) 71–74.

[4] G. Branchi, F. Capone, A. Vitalone, F. Madia, D. Santucci, E. Alleva, L.G. Costa, Early developmental exposure to BDE 99 or Aroclor 1254 affects neurobehavioural profile: interference from the administration route, Neurotoxicology 26 (2005) 183–192.

[5] D.J. Bucci, P.C. Holland, M. Gallagher, Removal of cholinergic input to rat posterior parietal cortex disrupts incremental processing of conditioned stimuli, J. Neurosci. 18 (1998) 8038–8046.

[6] K. Bull, N. Basu, S. Zhang, J.W. Martin, S. Bursian, P. Martin, L.H.M. Chan, Dietary and in utero exposure to a pentabrominated diphenyl ether mixture did not affect cholinergic parameters in the cerebral cortex of ranch mink (*Mustela vison*), Toxicol. Sci. 96 (2007) 115–122.

[7] M. Carli, T.W. Robbins, J.L. Evenden, B.J. Everitt, Effects of lesions to ascending noradrenergic neurones on performance of a 5-choice serial reaction task in rats; implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal, Behav. Brain Res. 9 (1983) 361–380.

[8] D. Chen, B.X. Mai, J. Song, Q.H. Sun, Y. Luo, X.J. Luo, E.Y. Zeng, R.C. Hale, Polybrominated diphenyl ethers in birds of prey from northern China, Environ. Sci. Technol. 41 (2007) 1828–1833.

[9] J. Dobbing, J. Sands, Comparative aspects of the brain growth spurt, Early Hum. Dev. 3 (1979) 79–83.

[10] A.T. Dugbartey, Neurocognitive aspects of hypothyroidism, Arch. Intern. Med. 158 (1998) 1413–1418.

[11] C. Dufault, G. Poles, L.L. Driscoll, Brief postnatal PBDE exposure alters learning and the cholinergic modulation of attention in rats, Toxicol. Sci. 88 (2005) 172–180.

[12] P. Eriksson, E. Jakobsson, A. Fredriksson, Brominated flame retardants: a novel class of developmental neurotoxicants in our environment? Environ. Health Perspect. 109 (2001) 903–908.

[13] P. Eriksson, H. Viberg, E. Jakobsson, U. Orn, A. Fredriksson, A brominated flame retardant, 2,2′,4,4′,5-pentabromodiphenyl ether: uptake, retention, and induction of neurobehavioral alterations in mice during a critical phase of neonatal brain development, Toxicol. Sci. 67 (2002) 98–103.

[14] B.C. Figueiredo, U. Otten, S. Strauss, B. Volk, D. Maysinger, Effects of perinatal hypo- and hyperthyroidism on the levels of nerve growth factor and its low-affinity receptor in cerebellum, Brain Res. Dev. Brain Res. 72 (1993) 237–244.

[15] J.R. Fowles, A. Fairbrother, L. Baechersteppan, N.I. Kerkvliet, Immunological and endocrine effects of the flame-retardant pentabromodiphenyl ether (DE-71) in C57Bl mice, Toxicology 86 (1994) 49–61.

[16] J.R. Gee, V.C. Moser, Acute postnatal exposure to brominated diphenylether 47 delays neuromotor ontogeny and alters motor activity in mice, Neurotoxicol. Teratol. 30 (2008) 79–87.

[17] E. Gould, L.L. Butcher, Developing cholinergic basal forebrain neurons are sensitive to thyroid hormone, J. Neurosci. 9 (1989) 3347–3358.

[18] S. Hallgren, P.O. Darnerud, Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and chlorinated paraffins (CPs) in rats – testing interactions and mechanisms for thyroid hormone effects, Toxicology 177 (2002) 227–243.

[19] S. Hallgren, T. Sinjari, H. Hakansson, P.O. Darnerud, Effects of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) on thyroid hormone and vitamin A levels in rats and mice, Arch. Toxicol. 75 (2001) 200–208.

[20] S. Harrad, S. Hazrati, C. Ibarra, Concentrations of polychlorinated biphenyls in indoor air and polybrominated diphenyl ethers in indoor air and dust in Birmingham, United Kingdom: Implications for human exposure, Environ. Sci. Technol. 40 (2006) 4633–4638.

[21] R.A. Hites, Polybrominated diphenyl ethers in the environment and in people: a meta-analysis of concentrations, Environ. Sci. Technol. 38 (2004) 945–956.

[22] K. Inoue, K. Harada, K. Takenaka, S. Uehara, M. Kono, T. Shimizu, T. Takasuga, K. Senthilkumar, F. Yamashita, A. Koizumi, Levels and concentration ratios of polychlorinated biphenyls and polybrominated diphenyl ethers in serum and breast milk in Japanese mothers, Environ. Health Perspect. 114 (2006) 1179–1185.

[23] H.A. Jones-Otazo, J.P. Clarke, M.L. Diamond, J.A. Archbold, G. Ferguson, T. Harner, G.M. Richardson, J.J. Ryan, B. Wilford, Is house dust the missing exposure pathway for PBDEs? An analysis of the urban fate and human exposure to PBDEs, Environ. Sci. Technol. 39 (2005) 5121–5130.

[24] P.R.S. Kodavanti, T.R. Ward, Differential effects of commercial polybrominated diphenyl ether and polychlorinated biphenyl mixtures on intracellular signaling in rat brain in vitro, Toxicol. Sci. 85 (2005) 952–962.

[25] J. McGaughy, M. Sarter, Effects of chlordiazepoxide and scopolamine, but not aging, on the detection and identification of conditional visual stimuli, J. Gerontol., A. Biol. Sci. Med. Sci. 50 (1995) B90–96.

[26] I.A.T.M. Meerts, R.J. Letcher, S. Hoving, G. Marsh, A. Bergman, J.G. Lemmen, B. van der Burg, A. Brouwer, In vitro estrogenicity of polybrominated diphenyl ethers, hydroxylated PBDEs, and polybrominated bisphenol A compounds, Environ. Health Perspect. 109 (2001) 399–407.

[27] I.A.T.M. Meerts, J.J. van Zanden, E.A.C. Luijks, I. van Leeuwen-Bol, G. Marsh, E. Jakobsson, A. Bergman, A. Brouwer, Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro, Toxicol. Sci. 56 (2000) 95–104.

[28] D. Meironyte, K. Noren, A. Bergman, Analysis of polybrominated diphenyl ethers in Swedish human milk. A time-related trend study, 1972–1997, J. Toxicol. Environ. Health, Part A 58 (1999) 329–341.

[29] A. Montero-Pedrazuela, C. Venero, R. Lavado-Autric, I. Fernández-Lamo, J.M. García-Verdugo, J. Bernal, A. Guadaño-Ferraz, Modulation of adult hippocampal neurogenesis by thyroid hormones: implications in depressive-like behavior, Mol. Psychiatry 11 (2006) 361–371.

[30] D.R. Oros, D. Hoover, F. Rodigari, D. Crane, J. Sericano, Levels and distribution of polybrominated diphenyl ethers in water, surface sediments, and bivalves from the San Francisco estuary, Environ. Sci. Technol. 39 (2005) 33–41.

[31] A.J. Patel, M. Hayashi, A. Hunt, Selective persistent reduction in choline-acetyltransferase activity in basal forebrain of the rat after thyroid-deficiency during early life, Brain Res. 422 (1987) 182–185.

[32] M. Petreas, J.W. She, F.R. Brown, J. Winkler, G. Windham, E. Rogers, G.M. Zhao, R. Bhatia, M.J. Charles, High body burdens of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) in California women, Environ. Health Perspect. 111 (2003) 1175–1179.

[33] D.C. Rice, E.A. Reeve, A. Herlihy, R.T. Zoeller, W.D. Thompson, V.P. Markowski, Developmental delays and locomotor activity in the C57BL6/J mouse following neonatal exposure to the fully brominated PBDE, decabromodiphenyl ether, Neurotoxicol. Teratol. 29 (2007) 511–520.

[34] V.M. Richardson, D.F. Staskal, D.G. Ross, J.J. Diliberto, M.J. DeVito, L.S. Birnbaum, Possible mechanisms of thyroid hormone disruption in mice by BDE 47, a major polybrominated diphenyl ether congener, Toxicol. Appl. Pharmacol. 226 (2008) 244–250.

[35] R.M. Ridley, H.C. Barefoot, C.J. Maclean, P. Pugh, H.F. Baker, Different effects on learning ability after injection of the cholinergic immunotoxin ME20.4IgG-saporin into the diagonal band of Broca, basal nucleus of Meynert, or both in monkeys, Behav. Neurosci. 113 (1999) 303–315.

[36] J. Sala-Roca, E. Estabanez-Perpina, F. Balada, A. Garau, M. Assumpció Martí-Carbonell, Effects of adult dysthyroidism on the morphology of hippocampal neurons, Behav. Brain Res. 188 (2008) 348–354.

[37] J.M. Sanders, L.T. Burka, C.S. Smith, W. Black, R. James, M.L. Cunningham, Differential expression of CYP1A, 2B, and 3A genes in the F344 rat following

exposure to a polybrominated diphenyl ether mixture or individual components, Toxicol. Sci. 88 (2005) 127–133.
[38] S. Sawin, P. Brodish, C.S. Carter, M.E. Stanton, C. Lau, Development of cholinergic neurons in rat brain regions: dose-dependent effects of propylthiouracil-induced hypothyroidism, Neurotoxicol. Teratol. 20 (1998) 627–635.
[39] A. Schecter, S. Johnson-Welch, K.C. Tung, T.R. Harris, O. Papke, R. Rosen, Polybrominated diphenyl ether (PBDE) levels in livers of US human fetuses and newborns, J. Toxicol. Environ. Health, Part A. 70 (2007) 1–6.
[40] A. Schecter, O. Papke, K.C. Tung, J. Joseph, T.R. Harris, J. Dahlgren, Polybrominated diphenyl ether flame retardants in the U.S. population: current levels, temporal trends, and comparison with dioxins, dibenzofurans, and polychlorinated biphenyls, J. Occup. Environ. Med. 47 (2005) 199–211.
[41] A. Schecter, M. Pavuk, O. Papke, J.J. Ryan, L. Birnbaum, R. Rosen, Polybrominated diphenyl ethers (PBDEs) in U.S. mothers' milk, Environ. Health Perspect. 111 (2003) 1723–1729.
[42] A. Schecter, O. Papke, T.R. Harris, K.C. Tung, A. Musumba, J. Olson, L. Birnbaum, Polybrominated diphenyl ether (PBDE) levels in an expanded market basket survey of US food and estimated PBDE dietary intake by age and sex, Environ. Health Perspect. 114 (2006) 1515–1520.
[43] J.W. She, M. Petreas, J. Winkler, P. Visita, M. McKinney, D. Kopec, PBDEs in the San Francisco Bay area: measurements in harbor seal blubber and human breast adipose tissue, Chemosphere 46 (2002) 697–707.
[44] A. Sjodin, Occupational and dietary exposure to organohalogen substances, with special emphasis on polybrominated diphenyl ethers, Doctoral dissertation, Stockholm University (2000).
[45] A. Sjodin, R.S. Jones, J.F. Focant, C. Lapeza, R.Y. Wang, E.E. McGahee, Y.L. Zhang, W.E. Turner, B. Slazyk, L.L. Needham, D.G. Patterson, Retrospective time-trend study of polybrominated diphenyl ether and polybrominated and polychlorinated biphenyl levels in human serum from the United States, Environ. Health Perspect. 112 (2004) 654–658.
[46] T.E. Stoker, R.L. Cooper, C.S. Lambright, V.S. Wilson, J. Furr, L.E. Gray, In vivo and in vitro anti-androgenic effects of DE-71, a commercial polybrominated diphenyl ether (PBDE) mixture, Toxicol. Appl. Pharmacol. 207 (2005) 78–88.
[47] T.W. Stoker, S.C. Laws, K.M. Crofton, J.M. Hedge, J.M. Ferrell, R.L. Cooper, Assessment of DE-71, a commercial polybrominated diphenyl ether (PBDE) mixture, in the EDSP male and female pubertal protocols, Toxicol. Sci. 78 (2004) 144–155.
[48] L. Sui, F. Wang, B.M. Li, Adult-onset hypothyroidism impairs paired-pulse facilitation and long-term potentiation of the rat dorsal hippocampo-medial prefrontal cortex pathway in vivo, Brain Res. 1096 (2006) 53–60.
[49] E.L. Sutin, P.J. Shiromani, J.R. Kelsoe, F.I. Storch, J.C. Gillin, Rapid-eye movement sleep and muscarinic receptor-binding in rats are augmented during withdrawal from chronic scopolamine treatment, Life Sci. 39 (1986) 2419–2427.
[50] A. Vaccari, Z.L. Rossetti, G. Demontis, E. Stefanini, E. Martino, G.L. Gessa, Neonatal-hypothyroidism induces striatal dopaminergic dysfunction, Neuroscience 35 (1990) 699–706.
[51] H. Viberg, A. Fredriksson, P. Eriksson, Changes in spontaneous behaviour and altered response to nicotine in the adult rat, after neonatal exposure to the brominated flame retardant, decabrominated diphenyl ether (PBDE 209), Neurotoxicology 28 (2007) 136–142.
[52] H. Viberg, A. Fredriksson, P. Eriksson, Neonatal exposure to the brominated flame-retardant, 2,2′,4,4′,5-pentabromodiphenyl ether, decreases cholinergic nicotinic receptors in hippocampus and affects spontaneous behaviour in the adult mouse, Environ. Toxicol. Pharmacol. 17 (2004) 61–65.
[53] H. Viberg, A. Fredriksson, P. Eriksson, Neonatal exposure to polybrominated diphenyl ether (PBDE 153) disrupts spontaneous behaviour, impairs learning and memory, and decreases hippocampal cholinergic receptors in adult mice, Toxicol. Appl. Pharmacol. 192 (2003) 95–106.
[54] H. Viberg, A. Fredriksson, P. Eriksson, Neonatal exposure to the brominated flame retardant 2,2′,4,4′,5-pentabromodiphenyl ether causes altered susceptibility in the cholinergic transmitter system in the adult mouse, Toxicol. Sci. 67 (2002) 104–107.
[55] H. Viberg, A. Fredriksson, E. Jakobsson, U. Orn, P. Eriksson, Neurobehavioral derangements in adult mice receiving decabrominated diphenyl ether (PBDE 209) during a defined period of neonatal brain development, Toxicol. Sci. 76 (2003) 112–120.
[56] H. Viberg, N. Johansson, A. Fredriksson, J. Eriksson, G. Marsh, P. Eriksson, Neonatal exposure to higher brominated diphenyl ethers, hepta-, octa-, or nonabromodiphenyl ether, impairs spontaneous behavior and learning and memory functions of adult mice, Toxicol. Sci. 92 (2006) 211–218.
[57] E.M. Wekking, B.C. Appelhof, E. Fliers, A.H. Schene, J. Huyser, J.G.P. Tijssen, W.M. Wiersinga, Cognitive functioning and well-being in euthyroid patients on thyroxine replacement therapy for primary hypothyroidism, Eur. J. Endocrinol. 153 (2005) 747–753.
[58] B.H. Wilford, M. Shoeib, T. Harner, J.P. Zhu, K.C. Jones, Polybrominated diphenyl ethers in indoor dust in Ottawa, Canada: implications for sources and exposure, Environ. Sci. Technol. 39 (2005) 7027–7035.
[59] T. Zhou, M.M. Taylor, M.J. DeVito, K.M. Crofton, Developmental exposure to brominated diphenyl ethers results in thyroid hormone disruption, Toxicol. Sci. 66 (2002) 105–116.
[60] T. Zhou, D.G. Ross, M.J. DeVito, K.M. Crofton, Effects of short-term in vivo exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic enzyme activities in weanling rats, Toxicol. Sci. 61 (2001) 76–82.
[61] D.-F. Zhu, Z.-X. Wang, D.-R. Zhang, Z.-L. Pan, S. He, X.-P. Hu, X.-C. Chen, J.-N. Zhou, fMRI revealed neural substrate for reversible working memory dysfunction in subclinical hypothyroidism, Brain 129 (2006) 2923–2930.