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Discrimination and avoidance learning in adult mice following developmental exposure to diisopropylfluorophosphate

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Abstract

Exposure to acetylcholinesterase inhibitors during development was shown in the past to induce sex-dependent changes in locomotion and specific cognitive and emotional tests in rodents. Adult mice that had been treated with 0.5 mg/kg diisopropylfluorphosphate (DFP), on post-natal days 14–20 were tested on active avoidance and a set-shifting task. DFP pre-treatment did not affect the active avoidance task, but impaired performance on the extra-dimensional shift task. DFP-treated females showed more general deficits in the acquisition of simple discrimination, intra-dimensional shift, extra-dimensional shift and reversal learning. These data suggest that pre-weanling exposure to cholinesterase inhibitors may have long-term consequences on attentional capabilities.

Keywords: Acetylcholine; Active avoidance attention; Cholinesterase inhibitor; Discrimination; Sex differences

1. Introduction

Exposure to sub-toxic levels of acetylcholinesterase inhibitors (AChEI's) in pesticides poses a health hazard to which infants and toddlers are particularly vulnerable because of their tendency to explore objects orally (Eskenazi et al., 1999). In adult humans, chronic exposure to pesticides leads to long-term cognitive impairments in vigilance, concentration, attention, information processing, psychomotor speed, memory and language (Rosenstock et al., 1991; Keifer and Mahurin, 1997). Pre-natal exposure to pesticides was associated with an increased number of abnormal reflexes in babies tested after age 3 days (Young et al., 2005) and impaired performance on the Stanford Binet drawing task in school aged children (Grandjean et al., 2006). Long-term follow-up studies of pesticide exposure in children found impairments in short-term memory, attention (Ruckart et al., 2004), inhibitory motor control and rate of list learning (Kofman et al., 2006). Chronic low-dose exposure in children was associated with slower reaction times (Grandjean et al., 2006).

In rodents, it was shown that exposure to AChEI's during gestation and the pre-weanling period may be more deleterious than exposure in adulthood because of the critical role of acetylcholine (ACh) and acetylcholinesterase (AChE) in cortical differentiation synaptogenesis and neurite growth (Hohmann and Berger-Sweeney, 1998; Lauder and Schambra, 1999; Small et al., 1995). Some of these effects may be related to non-catalytic effects of AChE (Small et al., 1995). Basal forebrain cholinergic projections influence dendritic arborization (Villabos et al., 2000) and the development of normal cytoarchitecture (Ricceri et al., 2002; Zhu et al., 2002). The first 2–3 weeks after birth are characterized by a rapid increase in muscarinic receptor binding (Hohmann and Ebner, 1985; Tang et al., 2003; Qiao et al., 2003). Previous studies showed that gestational or pre-weanling exposure to AChEIs resulted in long-term down-regulation of muscarinic receptors (Ahlbom et al., 1995; Chakraborti et al., 1993; Chanda and Pope, 1996; Qiao et al., 2003, Stone et al., 2000; Tang et al., 2003). In addition, ACh is critical during development for the induction of AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic

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acid) glutamate receptors, which are critical for behavioural plasticity, and this effect can be reversed by DFP (Olivera et al., 1999). Diisopropylfluorophosphate (DFP) is an irreversible AChE inhibitor that was found to be equally effective at post-natal days (PND) 3, 10 or 19 after a single oral dose in mice (Ahlbom et al., 1995). Long-term effects of exposure during the pre-weanling period included deficits in operant behaviour (Gupta et al., 1985), and altered locomotion (Ahlbom et al., 1995; Dam et al., 2000).

Almost all the studies on long-term effects of exposure to pesticides in adult humans have been conducted in males (reviewed in Colosio et al., 2003); whereas the few studies in infants or children did not make gender comparisons, possibly due to limited samples (Ruckart et al., 2004; Young et al., 2005; Grandjean et al., 2006; Kofman et al., 2006). On the other hand, several pre-clinical studies that examined the long-term behavioural effects of administering AChE inhibitors during the pre-weanling period in rodents found significant interactions between treatment and sex. Chlorpyrifos on PND 1–4 impaired spatial memory in the radial arm maze was found in males, but improved performance in females (Levin et al., 2001; Aldridge et al., 2005), reversing the normal sex differences in rats. No sex differences were found following chlorpyrifos exposure on days PND 1–4 or 11–14 on a number of behavioural tests ranging from neonatal to adult age; however, in general the chlorpyrifos exposure did not affect the behaviours measured. Male mice who had been given chlorpyrifos on days 1–4 or 11–14 showed more agonistic behaviour to another male, but this was not tested in females (Ricceri et al., 2003).

A previous study in our lab showed that injections of a sub-toxic dose of diisopropylfluorphosphate (DFP) on PND 14–20 led to impaired performance on a passive avoidance (PA) retention test 24, but not 72 h after acquisition in female, but not male adult mice. In fact, the male mice treated with DFP showed better retention than the control group at 72 h. In addition, both male and female mice spent significantly less time in the open arms of the elevated plus maze on the second exposure, despite having spent more time in the open arms during the first exposure (Kofman and Ben-Bashat, 2006). This pattern of behaviour suggested that male mice that had were injected with DFP during the pre-weanling period had enhanced learned fear, as evident by the better retention in the 72 h PA test and greatly reduced in open arm exploration of the elevated plus maze. However, there was also evidence for an impaired ability to inhibit behaviour in a conflict situation, particularly in the female mice. This manifested itself in increased open arm exploration in the elevated plus maze in both sexes and impaired PA at 24, but not 72 h in females.

Improved learned fear and poor behavioural inhibition might be advantageous in a two-way active avoidance test (AA), in which the mouse has to actively run from one side of a shuttle box to another. Therefore, mice pre-treated with DFP on PND 14–20, were tested on AA. It was hypothesized that DFP-treated mice would show an advantage in AA learning compared to saline-treated mice and that this advantage would be greater for female mice.

In order to test if DFP during the pre-weanling period also leads to a deficit in behavioural inhibition in appetitive and not just aversive learning tasks, the affective and attentional set-shifting task, developed to test frontal lobe functions in rats (Birrell and Brown, 2000) and adapted to mice (Colacicco et al., 2002) was used. The prototypical test for attentional set shifting is the Extra-Dimensional Shift (EDS), which requires a shift of attention across different stimulus dimensions, for example, shape instead of color (Pantelis et al., 1999). On the other hand, affective set shifting entails the reversal of the stimulus-reward mapping within the same dimension (Nagahama et al., 2001; Chen et al., 2004). Thus, this test, which is based on a series of discriminations of increasing complexity, can distinguish between discrimination learning, affective and attentional set shifting. Inability to inhibit responses to previously relevant reward contingencies leads to impaired performance. In adult rats, scopolamine impaired reversal (Raffaele et al., 1990; Chen et al., 2004) and attentional set shifting (Chen et al., 2004) but the effect of early perturbation of cholinergic innervation has never been tested.

The cholinergic system plays a critical role in attention (McGaughy et al., 1996, 2002; Himmelheber et al., 2000; Davidson et al., 1999) and in sensory discrimination learning (Fine et al., 1997; Linster et al., 2001; Winters et al., 2004). In humans, the AChE inhibitor physostigmine improved reaction time on a working memory task for faces, and reduced activation of right prefrontal cortex, suggesting that less effort was required to perform the task (Freo et al., 2005). Administration of AChE inhibitors during early brain development is likely to be associated with long-term sequelae on discrimination learning. A deficit related to reward contingencies would be expected to affect reversal learning, whereas a deficit in cognitive flexibility would be predicted to impair the extra-dimensional shift. Finally, a generalized discrimination deficit would affect even the simple discrimination task.

Mice were pre-treated with DFP at ages 14–20 days, a period that parallels the human brain growth spurt from mid-gestation to 18 months and is characterized by enhanced synaptogenesis and myelination (Vidair, 2004). The relevance of this exposure period to humans is supported by the high incidence of significant levels of pesticides found in the blood of pregnant women and their newborns in urban (Whyatt et al., 2005) and agricultural areas (Castorina et al., 2003).

2. Materials and methods

2.1. Subjects and pre-treatment

80 male and female C57BL/6 mice, derived from 30 litters, were bred in our animal colony from mice purchased from Harlan, Israel. The temperature of the animal colony was 21C°, and there was 12:12 h light–dark cycle with lights on at 6 a.m. Mice had *ad libitum* access to food and water. Drug treatment on days 14–20 was conducted according to a split-litter design. Half the males and half the females in each litter were injected subcutaneously with 0.5 mg/kg DFP (Sigma, Israel) diluted in 0.9% saline in a volume of 1 ml/100 g, and the other half was

injected with saline, such that each litter contributed at most 1–2 mice/group. Each mouse was tested in only one experiment, either AA or set shifting. The dose of DFP did not lead to lethality, seizures or any peripheral cholinergic symptoms. At 22 days, mice were separated from the dams and housed in cages containing same-sex groups, until testing at ages 4–6 months. The protocol was approved by the Institutional Committee for Ethics in Animal Experimentation of Ben-Gurion University of the Negev in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Active avoidance

Testing was conducted in the latter half of the light phase (approximately between 1 p.m. to 6 p.m.) under low illumination. There were 20 DFP-treated mice (10 male, 10 female) and 20 saline-treated mice (10 male, 10 female) in the study.

Two-way AA testing was done in a Gemini avoidance system shuttle box, 52.6×17.3×21.3 cm (San Diego Instruments, San Diego, CA). An electric shock (0.5 mA) was delivered through the grid floor. The mouse's location was detected by 8 infrared photo beams per chamber located 13 mm above the grid floor. The day before the experiment, habituation to the apparatus and buzzer took place for 15 min during which every 3 min the warning signal was given. Avoidance training took place on 5 consecutive days with 30 trials per day in a random order, by an experimenter who was blind to the pre-treatment condition. After each mouse, the apparatus was thoroughly cleaned with a dilute solution of ethanol and water.

On the first trial the mouse was placed into one compartment of the apparatus, with the door between the chambers closed for 20 s. The door opening coincided with an auditory stimulus for 5 s, followed 5 s later by the shock for 20 s. Thirty trials were run with an inter-trial interval of 14–26 s. There were three possible responses: 1) *No response* — if the mouse didn't escape to the other side of the box; 2) *Escape* — if the mouse ran to the opposite chamber after onset of the shock; 3) *Avoidance* — if the mouse ran to the opposite chamber after the onset of the tone, but before the onset of the shock. The number of avoidance and escape responses was counted for each mouse each day for five days.

2.3. Discrimination and set shifting

The testing apparatus was made of black Perspex (30×24×32 cm) and was divided into a waiting compartment (24×15 cm), and two equally-sized choice compartments (12×15 cm). A removable guillotine door divided the waiting compartment from the other two compartments and two small guillotine doors in the back of the choice compartments allowed the experimenter to exchange the bowls without disturbing the mice.

Mice were trained to dig in small bowls to retrieve a food reward hidden underneath a digging medium such as sawdust (e.g. Colacicco et al., 2002). During each trial, two bowls (diameter 8.3 cm; depth 2.8 cm) filled with a scented digging medium, were placed in each choice compartment. One of the bowls was baited with a small piece of sweetened cereal (Shugi ®, Telma, Israel), placed at the bottom of the bowl, and the mice had to dig in order to get the reward.

Food intake was restricted 3 days before the training, to 85% initial body weight. Thus, each mouse got 3–5 gm/day of regular chow, titrated according to changes in body weight from the previous day. In addition to the regular chow, several morsels of the cereal reward were placed in bowls in the home cages in order to familiarize the mice to the taste and to the bowls. The discrimination testing involved the following stages: 1) Two days habituation to the experimental apparatus for 10 min with free access to both choice compartments. 2) Shaping to dig for the reward in the medium until 6 successful reward retrievals were made. In the shaping session, the medium was the same for both bowls and no odor was added. This medium was not used again in the experiment. The reward was placed on top of the digging medium on the first trial and thereafter was buried and not visible. 3) Days 1–6 discrimination trials as detailed below and in Table 1.

A trial began by raising the guillotine door to give the mouse access to the two digging bowls, only one of which was baited. The first 4 trials of each discrimination were 'open' trials. If the mouse dug in the unbaited bowl, it was allowed to explore the adjacent chamber and dig in the other bowl, but an error was recorded. Starting from Trial 5, if the mouse began to dig in the unbaited bowl or did not dig at all, an error was recorded and the trial was terminated. The first 10 trials were limited to 2 min and subsequent trials to 1 min. If the mouse did not retrieve the reward within this time, an error was recorded. Testing continued until the mouse reached a criterion of six consecutive correct trials (maximum 50 trials per day for each discrimination).

Table 1
Order of the discrimination learning

Day	Discrimination	Stimulus dimension		Combination of exemplars	
		Relevant	Irrelevant	S+ (correct)	S− (incorrect)
1	Simple discrimination (SD)	Odor		**O1**	O2
2	Compound discrimination (CD)	Odor	Medium	**O1**–M1	O2–M1
				O1–M2	O2–M2
2	Compound reversal (CD Rev)	Odor	Medium	**O2**–M1	O1–M1
				O2–M2	O1–M2
3	CD Rev repetition (CD Rev Rep)	Odor	Medium	**O2**–M1	O1–M1
				O2–M2	O1–M2
3	Intra-dimensional shift (IDS)	Odor	Medium	**O3**–M3	O4–M3
				O3–M4	O4–M4
4	IDS repetition (IDS Rep)	Odor	Medium	**O3**–M3	O4–M3
				O3–M4	O4–M4
4	IDS reversal (IDS Rev)	Odor	Medium	**O4**–M3	O3–M3
				O4–M4	O3–M4
5	IDS Rev repetition (IDS Rev Rep)	Odor	Medium	**O4**–M3	O3–M3
				O4–M4	O3–M4
6	Extra-dimensional shift (EDS)	Medium	Odor	**M5**–O5	M6–O5
				M5–O6	M6–O6

The order of the discriminations was the same for all mice, but half of the mice switched from odor (O) to medium (M), and half from medium to odor. The correct exemplar (shown in bold for an odor discrimination) can be paired with one of the two exemplars of the irrelevant dimension.

Table 2
Pairs of exemplars utilized in the experiment

Dimension	Pair 1		Pair 2		Pair 3	
	Exemplar 1	Exemplar 2	Exemplar 3	Exemplar 4	Exemplar 5	Exemplar 6
Odor	Curry	Cloves	Cinnamon	Garlic salt	Tumeric	Hyssop
Medium	Sawdust	Polystyrene	Gravel	Sand	Shredded paper	Dry leaves

The discriminations (except for SD) were based on combinations of three odor-medium pairs that were identical for all mice. The sequence of combinations was varied such that no two animals in the same group received the same sequence. Each sequence was performed by a mouse from the DFP and saline groups.

In the simple discrimination (SD), the bowls differed in only one of the two dimensions (odor or medium). For the compound discrimination (CD), the second dimension was introduced, but the correct and incorrect exemplars remained the same. For both the intra-dimensional shift (IDS) and extra-dimensional shift (EDS), new exemplars of both dimensions were used. In IDS, the relevant dimension was the same as before, whereas in EDS the previously relevant dimension became the irrelevant dimension. For the CD reversal and IDS reversal (CDRev, IDSRev), all exemplars and the relevant dimension remained the same, but the previously incorrect stimulus was correct. Each day, the task learned on the preceding day was repeated (CD, CD RevRep, IDS Rep, IDS Rev Rep) to criterion before starting the new task. CD is considered to be a repetition of SD, since the same stimulus is correct, but the second irrelevant dimension is added.

The order of the testing and exemplars was the same for all mice and is summarized in Table 1. The discrimination learning was based on tactile cues (digging medium) for half the mice in each group, or olfactory cues for the other half, and the extra-dimensional shift involved changing to the opposite dimension. The exemplars (Table 2) were varied within groups, but each set of exemplars was tested in parallel for a same-sex mouse pre-treated with DFP or saline.

2.4. Data analysis

Statistical analysis was done using the Statistica program (version 7.0), with significance at $p<.05$. AA was analyzed using 3-way repeated-measures ANOVA for the effect of sex (male, female), treatment (Saline, DFP) and day (5 days, repeated measure). Discrimination learning was analyzed with a 3 way repeated measures ANOVA for the effects of sex and treatment with discrimination as a repeated measure (SD, CD, CD Rev, CD Rev Rep, IDS, IDS Rep, IDS Rev, IDS Rev Rep, EDS). To study the effect of the initial relevant dimension (odor or medium) on the discriminations learning, a repeated-measures ANOVA with four factors was used, with three between-subjects (sex, treatment and initial relevant dimension) and one within-subject factor (discrimination). Planned comparisons between pre-weanling treatment groups (DFP vs. Saline) were made for males and females to test the source of the significant 3-way interaction.

3. Results

3.1. Active avoidance

A significant two-way interaction was found between sex and days of training, [F (4, 196)=3.51, $p<.01$]. The female mice showed more avoidance trials than the males on Day 5, [$F(1, 49)=6.58$, $p<.01$], as well as the expected main effect of Days, [$F(4, 196)=6.58, p<.000001$], indicating that avoidance learning took place. There was no effect of DFP pre-treatment (Fig. 1).

3.2. Discrimination and set shifting

A preliminary analysis was done to determine if the initially relevant stimulus dimension (medium or odor) affected discrimination learning. There was no main effect of the initial relevant

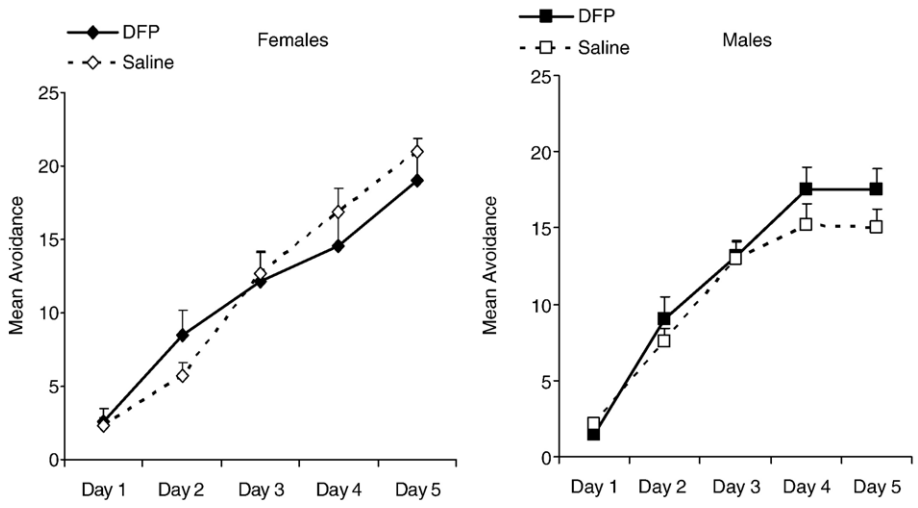

Fig. 1. Mean+SEM number of avoidance responses in male and female mice treated with 0.5 mg/kg DFP or saline on post-natal days 14–20.

dimension, [F (1, 32)=0.97, p=0.33], although a significant interaction between discrimination and initial relevant dimension, [F (8, 256)=2.04, p<0.05], was found. Subsequent analysis revealed that for the CD, [F (1, 32)=8.73, p<0.005], and IDS, [F (1, 32)=5.69, p<0.05] tasks, the criterion was reached in fewer trials when odor was the initially relevant dimension than when medium was initially relevant. However, since there were no interactions with treatment, and since the initial dimension was counterbalanced between the treatment groups, the subsequent analyses did not distinguish between 'odor first' and 'medium first' discriminations.

The interaction between sex, treatment and discrimination was significant, [F (8, 288)=2.63, p<0.01]. All the main effects and other interactions were significant as well. There was a significant main effect of sex, [F (1, 36)=13.13, p<0.001], treatment, [F (1, 36)=18.58, p<0.0005] and of discrimination, [F (8, 288)=24.08, p<0.0001]. The interaction between sex and treatment, [F (1, 36)=9.02, p<0.01], the interaction between sex and discrimination, [F (8, 288)=3.12, p<0.005] and the interaction between treatment and discrimination, [F (8, 288)=2.71, p<0.01] were also significant.

Planned comparisons revealed that both DFP-treated males and DFP-treated females required more trials to learn the EDS task, in comparison to saline-treated males [F (1, 36)=4.25, p<0.05]. DFP impaired acquisition of each of the initial discriminations in female mice, whereas there was no impairment on the retention trials which were performed 24 h later. Thus, DFP-treated females required more trials than saline-treated females for the acquisition of SD [F (1, 36)=18.7, p<0.0005], CD Rev [F (1, 36)=4.91, p<0.05], IDS [F (1, 36)= 6.28, p<0.05], IDS Rev [F (1, 36)=8.44, p<0.01] and EDS [F (1, 36)=9.77, p<0.05], but not for acquisition of CD [F(1, 36)= 1.4, p=0.24] (Fig. 2).

In order to validate that the discrimination tasks elicited similar results to those reported in the literature, the different tasks were compared within the group treated with saline. Saline-treated mice learned the discriminations and retained each task the following day since fewer trials to criterion were required for the repeat discriminations (CD Rev Rep, IDS rRep, IDS Rev Rep) than for the initial discriminations, [F (1,19)=14.88, p<0.005]. Reversal learning (CD Rev and IDS Rev) was the most difficult discrimination to acquire, as saline-treated mice needed more

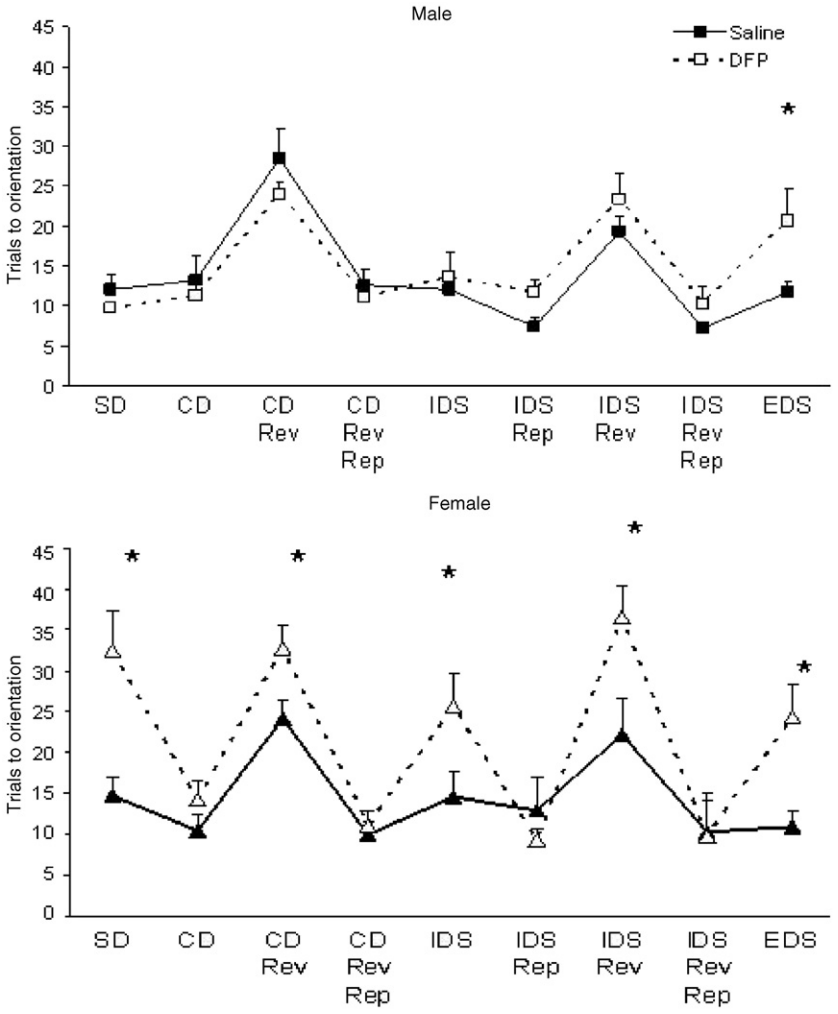

Fig. 2. Mean±SEM of trials to criterion (six consecutive correct trails) for each of the discriminations. SD — simple discrimination, CD — complex discrimination, IDS — intra-dimensional shift, EDS — extra-dimensional shift. Rev — reversal of previous rule. Rep — repeat of previous task. Exposure to DFP impaired the acquisition of SD, CD Rev, IDS, IDS Rev and EDS in females and the acquisition of EDS in males. *p<.05.

trials in order to learn the reversal learning as opposed to the rest of the discriminations, [$F(1, 36)=138.229$, $p<0.0001$]. No difference was observed in the saline-treated mice between the acquisition of EDS and the IDS, [$F(1, 36)=0.39$, $p=0.53$].

4. Discussion

Repeated exposure of mice to DFP on PND 14–20 led to significant sex-dependent changes in complex learning and high-order cognitive functions in adulthood, but did not affect AA learning, in contrast to its effect on PA performance in females (Kofman and Ben-Bashat, 2006). The dissociation between effects of DFP on AA and PA could be due to the learning and response requirements. First, AA uses a discrete auditory warning stimulus which might facilitate learning if there is a deficit in attention. Lesions of the right parietal lobe in rats impaired PA, but actually improved AA performance in the absence of other cognitive, motor and anxiety deficits (Hogg et al., 1998). Second, PA requires behavioural inhibition, whereas AA involves escape behaviour. The dissociation between AA and PA suggests that mechanisms controlling the separate brainstem circuits mediating freezing or escape (reviewed in Gray and McNaughton, 2000) are differentially affected by early treatment with DFP.

It was predicted that a specific impairment in behavioural inhibition in conflict situations would manifest itself in the reversal and EDS task. Indeed, both male and females treated with DFP showed impaired performance on EDS. However, the DFP-treated females showed deficits in each new acquisition, including SD, IDS, EDS and all the reversals, suggesting a more generalized impairment. Nonetheless, they were not impaired on repeat performance the day after the acquisition, suggesting that consolidation and retention were not affected by the early DFP treatment.

It is commonly accepted that EDS assesses attentional set shifting on the basis of a significant difference between performance on EDS and IDS (Birrell and Brown, 2000; Garner et al., 2006). In contrast to findings in rats (Birrell and Brown, 2000) and humans (Pantelis et al., 1999; Rogers et al., 2000), but in accord with other studies in mice (Colacicco et al., 2002; Laurent and Podhorna, 2004; Brigman et al., 2005), in the current study EDS did not prove to be more difficult than IDS. Garner et al. (2006) were able to demonstrate that the EDS was more difficult to learn than IDS only after overtraining on the IDS reversal stage. Although the performance of mice on the EDS suggests that they may not form a strong attentional set, compared to higher mammals, the fact that the only the EDS shift was impaired by DFP suggests that this task might have involved a more difficult discrimination even for mice. It is also possible, that DFP-treated male mice solved the discrimination problem in a different manner than saline-treated males. The slower learning on the EDS task suggests that they might have formed an attentional set in the previous discriminations and were therefore slower to learn the EDS.

Alternatively, the use of open trials in this task for the first 4 trials of each discrimination may facilitate learning the new rule. The more generalized impairment on each new discrimination, observed in the female mice treated with DFP, is similar to those reported in adult rodents following perturbation of the cholinergic system (Fine et al., 1997; Winters et al., 2004).

The differential response between the two sexes following DFP treatment extends previous findings on the sex difference in the long-term behavioural effects of AChE inhibitors (Aldridge et al., 2005; Kofman and Ben-Bashat, 2006). It is not known whether there is a difference in kinetics of DFP uptake between males and females at this age. In adult mice, DFP was found to have similar effects on AChE inhibition in males and females although female mice had higher levels of other esterases, such as butyrylcholinesterase and carboxylesterase (Tuovinen et al., 1997). This suggests that DFP has more binding sites in females than in males; however, it is not known whether this is relevant for pre-weanling mice. Moser and Padilla (1998) found no difference in the time course of AChE inhibition, or in the extent of muscarinic receptor down-regulation between males and females following oral chlorpyrifos at PND 17, except for down-regulation of muscarinic receptors in the pons, which was evident in female pups only. Similarly, there were no differences in the time course of peripheral and behavioural cholinergic symptoms between male and female pups at age PND 17. In contrast, sex differences were apparent in adult rats treated with a single dose of oral chlorpyrifos. The adult female rats showed greater AChE inhibition than males, a different pattern of receptor down-regulation and a protracted time course of behavioural results. Thus, there do not appear to be substantial sex differences in the effects of cholinesterase inhibitors at the pre-weanling age, although more comparisons between the sexes are required. Further research will be required to elucidate the mechanism of the increased sensitivity of females. The female basal forebrain cholinergic system matures at a different rate than that of the males and is affected by cholinergic deprivation in a different manner (Ricceri et al., 2002), so that during a particular post-natal treatment period, there might be significant anatomical variability between males and females. In addition, several studies have shown that estrogens affect synaptic plasticity and interact with the development of basal forebrain cholinergic nuclei (Farr et al., 2000; Gibbs, 2000), suggesting that during development males and females may be differentially affected by AChEIs.

A limitation of the study is the use of a single dose of DFP. In previous studies in our lab, a higher dose (1 mg/kg) was used without any overt signs of toxicity; however, in the present experiment, this dose was lethal in a few pups after the fourth or fifth injection (possibly due to a different stock of the drug). A pilot study was then run to test 0.5 mg/kg and 0.75 mg/kg, but only the lower dose showed no signs of toxicity in any mice. Future studies should examine a range of doses of DFP to determine if there is a dose response in the discrimination deficit.

In conclusion, this report extends previous findings suggesting that pre-weanling administration of DFP has long-term consequences on cognition. The results do not support a specific deficit in behavioural inhibition in the females, as even simple discrimination tasks were affected. Rather, they suggest that females are more vulnerable on both cognitive and emotional dimensions following developmental exposure, and that males also show deficits in more complex discrimination tasks.

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