Neurotoxicology and Teratology 45 (2014) 70–74

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Effects of developmental methylphenidate (MPH) treatment on monoamine neurochemistry of male and female rats

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ARTICLE INFO

Article history:
Received 7 July 2014
Received in revised form 28 July 2014
Accepted 4 August 2014
Available online 15 August 2014

Keywords:
Methylphenidate
Rat
Monoamines
Development
Dopamine

ABSTRACT

Attention Deficit Hyperactivity Disorder (ADHD) is estimated to affect 4–5% of the adult human population (Kessler et al., 2006; Willcutt, 2012). Often prescribed to attenuate ADHD symptoms (Nair and Moss, 2009), methylphenidate hydrochloride (MPH) can have substantial positive effects. However, there is a paucity of literature regarding its use during pregnancy. Thus, adult women with ADHD face a difficult decision when contemplating pregnancy. In this study, pregnant Sprague–Dawley rats were orally treated a total of 0 (water), 6 (low), 18 (medium), or 42 (high) mg MPH/kg body weight/day (divided into three doses) on gestational days 6–21 (i.e., the low dose received 2 mg MPH/kg body weight 3×/day). Offspring were orally treated with the same daily dose as their dam (divided into two doses) on postnatal days (PNDs) 1–21. One offspring/sex/litter was sacrificed at PND 22 or PND 104 (n = 6–7/age/sex/treatment group) and the striatum was quickly dissected and frozen. High Performance Liquid Chromatography (HPLC) coupled to a Photo Diode Array detector (PDA) was used to analyze monoamine content in the striatum of one side while a sandwich ELISA was used to analyze tyrosine hydroxylase (TH) from the other side. Age significantly affected monoamine and metabolite content as well as turnover ratios (i.e., DA, DOPAC, HVA, DOPAC/DA, HVA/DA, 5-HT and 5-HIAA); however, there were no significant effects of sex. Adult rats of the low MPH group had higher DA levels than control adults ($p < 0.05$). At both ages, subjects of the low MPH group had higher TH levels than controls ($p < 0.05$), although neither effect (i.e., higher DA or TH levels) exhibited an apparent dose–response. PND 22 subjects of the high MPH treatment group had higher ratios of HVA/DA and DOPAC/DA than same-age control subjects ($p < 0.05$). The increased TH levels of the low MPH group may be related to the increased DA levels of adult rats. While developmental MPH treatment appears to have some effects on monoamine system development, further studies are required to determine if these alterations manifest as functional changes in behavior.

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1. Introduction

Methylphenidate (MPH) is classified by the U.S. FDA as a Category C risk for use during pregnancy (http://inside.fda.gov:9003/downloads/CDER/OfficeofNewDrugs/ImmediateOffice/ucm033583.pdf). Briefly, Category C is representative of drugs for which there are no laboratory animal reproduction studies *or* such studies have shown adverse effects and there are no adequate and well-controlled studies in humans. Use of MPH during pregnancy may be acceptable if the benefits outweigh potential risks. If Attention Deficit Hyperactivity Disorder (ADHD) was still considered to be solely a childhood disorder, women of reproductive age would have no concerns. However, MPH is commonly prescribed to treat the symptoms of adult ADHD, which is estimated to affect 4–5% of the population (Kessler et al., 2006; Willcutt, 2012). The substantial benefits of therapeutic MPH use in adults with ADHD indicate its effectiveness.

The National Toxicology Program-Center for the Evaluation of Risks to Human Reproduction (Golub et al., 2005) reviewed the developmental toxicity of MPH, noting there were insufficient data for the evaluation of developmental toxicity in humans and/or developmental neurotoxicity in laboratory animals. Because development of the central nervous system can be a particularly sensitive period, Andersen (Andersen, 2005) noted that the effects of stimulants, such as MPH, on development cannot easily be inferred from studies which treat adult animals. The panel cited several critical data needs, one of which was developmental neurotoxicity studies in laboratory animals using routes of exposure consistent with those of humans and use of multiple dose levels (Golub et al., 2005).

Earlier publications from our large MPH study indicated few and/or mild effects of pre- and postnatal treatment on body weight and behaviors using a paradigm designed to mimic adult human use (Panos et al., 2014a, 2014b) (Ferguson et al., submitted). However, a lack of behavior and/or body weight effects does not preclude MPH-induced effects on

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http://dx.doi.org/10.1016/j.ntt.2014.08.001
0892-0362/Published by Elsevier Inc.

neurochemical endpoints. A small number of studies have examined neurochemical differences after early postnatal MPH treatment (Wagner et al., 1981; Schaefer et al., 2006; Gray et al., 2007). However, those MPH exposure paradigms were not reflective of the typical human treatment. Further, the majority of preclinical studies of the neurochemical effects of MPH have focused on the acute or chronic effects in juvenile and adult rodents (e.g., (Gray et al., 2007; Sadasivan et al., 2012)).

Here, monoamine levels were examined in rodent offspring of dams that had received oral MPH treatment during gestation with direct oral treatment to pups beginning at birth and continuing through weaning. The treatment was designed to parallel potential human therapeutic MPH use during pregnancy. Continuing MPH treatment during the neonatal period was included to reflect potential human exposure during the last trimester. Use of a wafer administration method for MPH treatment during pregnancy (see (Ferguson and Boctor, 2009)) reduces the potential confounding variable of stress-induced changes associated with repeated gavage or injections. This is a particularly important issue since gestational stress can alter development of the dopaminergic system (Baier et al., 2012). Further, our previous study described serum MPH levels in pregnant dams (Panos et al., 2014a, 2014b) and one of those previous doses is used here and mimics the levels of humans taking recommended therapeutic doses.

2. Materials and methods

2.1. Animals and MPH treatment

Detailed descriptions of animals and housing environments, MPH treatment, body weight and behavioral outcomes have been described (Panos et al., 2014a, 2014b) (Ferguson et al., submitted) and will only be briefly summarized here. All animal procedures followed the "Guide for the care and use of laboratory animals" (National Research Council, 1996) and were approved in advance by the NCTR Institutional Animal Care and Use Committee. Sperm plug positive Sprague–Dawley dams obtained from the NCTR Breeding Colony were housed individually with ad lib access to food (NIH-41 formulated diet, irradiated pellets, Harlan, Madison, WI) and water. Methylphenidate hydrochloride (MPH) was obtained as the racemic mixture (50/50 mix of the *d*-threo- and *l*-threo-enantiomers) (Mallinckrodt, Inc., St. Louis, MO) and mixed with sterile water. Total daily doses were 0 (water control), 6 (low MPH), 18 (medium MPH), or 42 (high MPH) mg/kg/day. Beginning on gestational day (GD) 6 and continuing through GD 21, each rat was given a small wafer piece (Mini Nilla Wafers, Nabisco, Kraft Foods, Northfield, IL) three times daily (at 8:30 am, 11:30 am, and 2:30 pm). Each time, the wafer was treated with 0, 2, 6, or 14 mg MPH/kg (i.e., 1/3 of the total daily dose). Beginning on postnatal day (PND) 1 and continuing through PND 21, all offspring/litter (after culling to 4/sex/litter on PND 1) were orally treated with the same total daily doses as their dam had received, but divided into two doses administered at 8:30 am and 2:30 pm (i.e., the low, medium, and high MPH pups received 3, 9, or 21 mg/kg at each of the two treatments).

2.2. Tissue collection

One offspring/sex/litter was sacrificed via CO_2 euthanasia at PND 22 or PND 104 (n = 6–7/age/sex/treatment group) and the brain was rapidly removed. The striatum was quickly dissected, flash frozen on dry-ice, and stored at −80 °C until analysis.

High Performance Liquid Chromatography (HPLC) coupled to a Photo Diode Array detector (PDA) was used to analyze monoamine content in one side of the striatum while a sandwich ELISA (Sriram et al., 2004; O'Callaghan et al., 2014) was used to analyze tyrosine hydroxylase (TH) from the other side.

2.3. Data analyses

Statistical analyses were conducted using SigmaPlot (version 11.0, Systat Software, Inc., San Jose, CA). Separate three-way ANOVAs (with factors of treatment, age, sex, and all interactions) were conducted on concentration levels of two monoamine neurotransmitters (dopamine (DA) and serotonin (5-HT)), three metabolites (3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA)), two ratios (DOPAC/DA and HVA/DA) and tyrosine hydroxylase (TH). Significant main effects or interactions (i.e., $p < .05$) were further analyzed using multiple comparison Holm–Sidak post-hoc analyses.

3. Results

3.1. DA

There was a significant treatment × age interaction ($F(3,92) = 2.84$, $p < 0.0422$) detected in the analysis of DA levels (Fig. 1). Pairwise comparisons indicated higher DA levels in adult rats of the low MPH treatment group relative to control adults ($p < 0.05$). There were no significant differences between the control PND 22 group and any PND 22 MPH treatment group.

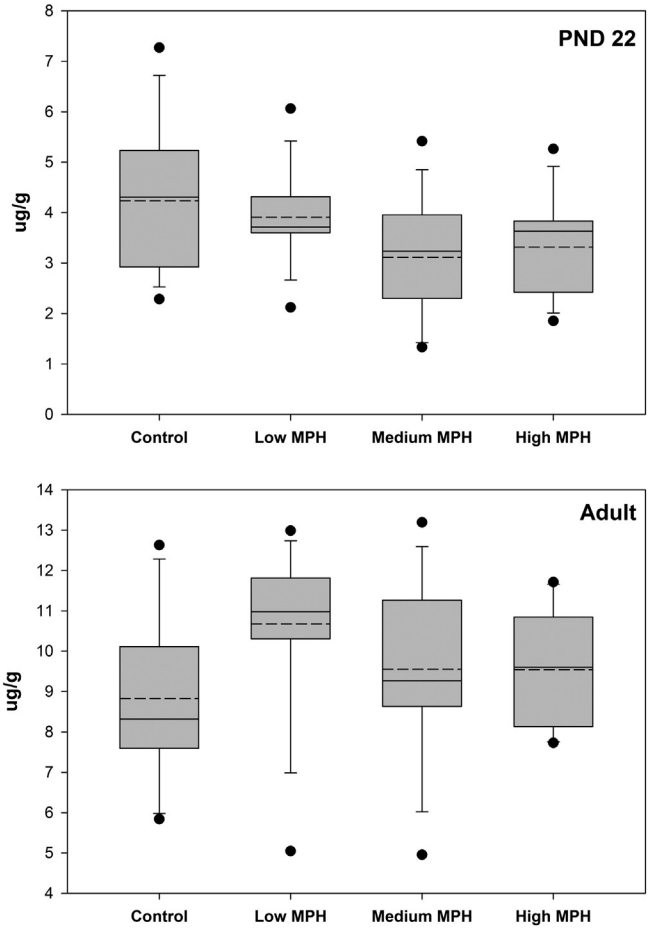

Fig. 1. DA concentrations by treatment and age. Top: PND 22 rats. Bottom: adult rats. Pairwise comparisons of the significant treatment × age interaction indicated no significant comparisons between the PND 22 control group and any PND 22 MPH treatment group. However, the low MPH treatment group had significantly higher levels of DA than controls as adult rats ($p < 0.05$). Each box shows the 25th and the 75th percentiles and the "whiskers" (bars) show the 10th and 90th percentiles. The solid and dashed lines inside the box show the median and mean, respectively. Solid points are datapoints falling outside the 10th and 90th percentiles.

3.2. DOPAC

Analysis of DOPAC levels indicated significant main effects of treatment ($F(3,92) = 2.91$, $p < 0.0386$) and age ($F(1,92) = 42.59$, $p < 0.0001$). Pairwise comparisons, however, did not indicate that any MPH treatment group was significantly different from the control group. Overall, adult rats had higher DOPAC levels than PND 22 rats (see Table 1).

3.3. HVA

There was a significant main effect of age detected in the analysis of HVA levels ($F(1,92) = 20.26$, $p < 0.0001$) which indicated higher levels on PND 22 than adulthood (see Table 1).

3.4. DOPAC/DA ratio

One PND 22 female rat of the medium MPH treatment group had a ratio of 240.00 which was clearly an outlier and that datapoint was deleted prior to analysis. This outlier ratio appeared to result from the DOPAC level for this subject which was 3.63 μg/g, the highest of the PND 22 medium MPH group, but not in the range of an outlier value. Analysis of DA turnover as indicated by the ratio of DOPAC to DA indicated a significant interaction of treatment × age interaction ($F(3,91) = 3.00$, $p < 0.0349$). Pairwise comparisons indicated that the PND 22 high MPH treatment group had a higher DOPAC/DA ratio than the PND 22 control group ($p < 0.05$). There were no significant comparisons between the adult control group and any MPH treatment group (see Table 1).

3.5. HVA/DA ratio

Analysis of DA turnover as indicated by the ratio of HVA to DA indicated significant main effects of treatment ($F(3,92) = 3.04$, $p < 0.03230$) and age ($F(1,92) = 176.82$, $p < 0.0001$). Pairwise comparisons indicated higher ratios in the high MPH treatment group relative to the control group; however, this appeared to be almost entirely due to the PND 22 differences. The age effect indicated that HVA/DA ratios were higher at PND 22 than at adulthood (see Table 1).

3.6. 5-HT

The analysis of 5-HT levels indicated a significant effect of age ($F(1,92) = 27.31$, $p < 0.0001$) which indicated that adult levels were higher than PND 22 levels (Table 1).

3.7. 5-HIAA

The significant effect of age ($F(1,92) = 5.76$, $p < 0.0184$) detected in the analysis of 5-HIAA indicated higher levels at adulthood than on PND 22 (Table 1). There was a marginally significant effect of treatment ($F(3,92) = 2.61$, $p < 0.0564$) and inspection of the data indicated somewhat higher 5-HIAA levels in the medium MPH treatment group than the control group (see Table 1).

3.8. TH

Analysis of TH indicated significant main effects of treatment ($F(3,92) = 11.73$, $p < 0.0001$) and age ($F(1,92) = 33.63$, $p < 0.0001$). Pairwise comparisons indicated higher TH levels in the low MPH treatment group than in the control group ($p < 0.05$) (Fig. 2). The age effect indicated higher levels in adulthood than on PND 22.

4. Discussion

The effects of oral MPH treatment during development on striatal neurochemistry were investigated in weanling and adult male and female Sprague–Dawley rats. Low MPH treatment (2 mg/kg/day) had mild but enduring effects on concentrations of tyrosine hydroxylase and dopamine, causing higher TH levels at both ages and higher DA levels in adult rats. Striatal neurochemistry concentrations were similar in both sexes and MPH treatment did not differentially affect males and females. The lack of substantial effects of developmental MPH treatment on striatal neurochemistry is similar to that described for MPH-induced behavioral alterations (Panos et al., 2014a, 2014b) (Ferguson et al., submitted).

All monoamine, metabolite, and turnover ratio endpoints measured here indicated significant effects of age. This is consistent with previous findings describing rapidly increasing DAT and SERT proteins from PND 0 through PND 21–35 (Coulter et al., 1997; Tarazi et al., 1998). Further, the majority of striatal development may be complete by PND 45 as there were no significant differences in transporter proteins for DA, 5-HT or NE in male rats between that age and PND 70 (Moll et al., 2001). Similarly, although direct age comparisons were not done, the density of striatal DAT immunoreactivity was somewhat similar in PND 35 and PND 135 male rats (Gray et al., 2007). No endpoint measured here demonstrated significant sex differences. Similar striatal measurements in adult male and female rats in our lab have also indicated no sex differences (Ferguson et al., 2005). However, there are brief periods during development (e.g., PND 40) when there are

Table 1
Mean (+/- SE) for PND 22 and Adult Rats.

	Age	Control	Low MPH	Medium MPH	High MPH
DA (μg/g)	PND 22	4.2309 ± 0.3924	3.9079 ± 0.2331	3.1141 ± 0.3138	3.3149 ± 0.2813
	Adult	8.8210 ± 0.5296	10.6723 ± 0.5240[a]	9.5492 ± 0.5802	9.5357 ± 0.4008
DOPAC (μg/g)	PND 22	2.6338 ± 0.1520	2.5818 ± 0.1254	2.3700 ± 0.1728	2.9592 ± 0.1671
	Adult	3.2556 ± 0.1893	3.0911 ± 0.1292	3.7016 ± 0.1865	3.7122 ± 0.2072
HVA (μg/g)	PND 22	1.2749 ± 0.0796	1.3606 ± 0.0669	1.2335 ± 0.0951	1.4730 ± 0.0776
	Adult	1.0386 ± 0.0439	1.0873 ± 0.0590	1.1148 ± 0.0540	1.1681 ± 0.0763
DOPAC/DA[b]	PND 22	68.10 ± 6.52	67.97 ± 4.08	74.45 ± 4.60	95.89 ± 8.85
	Adult	38.14 ± 2.75	29.71 ± 1.50	41.56 ± 4.49	39.81 ± 2.99
HVA/DA[c]	PND 22	32.45 ± 2.60	36.27 ± 2.88	44.38 ± 5.92	47.52 ± 4.30
	Adult	12.11 ± 0.61	10.36 ± 0.51	12.33 ± 1.16	12.49 ± 0.96
5-HT (μg/g)	PND 22	0.3207 ± 0.0316	0.4381 ± 0.0491	0.3646 ± 0.0305	0.3068 ± 0.0211
	Adult	0.5433 ± 0.0524	0.5184 ± 0.0188	0.5375 ± 0.0667	0.4476 ± 0.0298
5-HIAA (μg/g)	PND 22	0.6236 ± 0.0458	0.7103 ± 0.0294	0.6423 ± 0.0480	0.6666 ± 0.0294
	Adult	0.6816 ± 0.0343	0.7431 ± 0.0382	0.8455 ± 0.0646	0.6518 ± 0.0264

All endpoints indicated significant effects of age (see text for details).

[a] Pairwise comparisons of the significant treatment × age interaction indicated high DA levels in adults of the low MPH treatment group than control adults ($p < 0.05$).

[b] Pairwise comparisons of the significant treatment × age interaction indicated higher ratios in the high MPH treatment group at PND 22 than the control group at PND 22 ($p < 0.05$). There were no significant comparisons among the adult groups.

[c] Pairwise comparisons of the significant treatment effect indicated higher ratios in the high MPH group than controls ($p < 0.05$). However, this appears to be due to a stronger effect in the PND 22 groups.

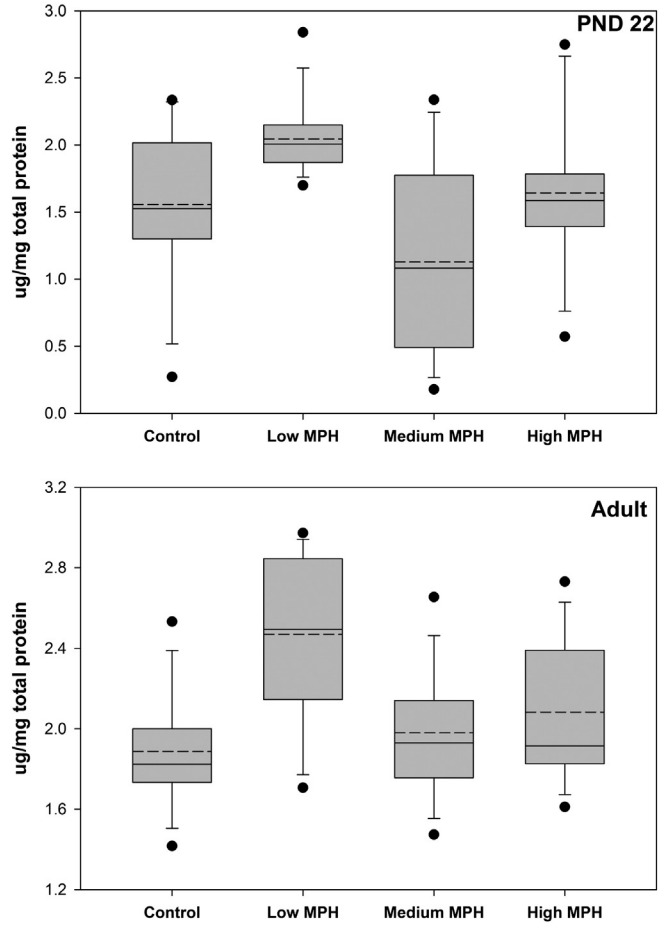

Fig. 2. TH concentrations by treatment and age. Top: PND 22 rats. Bottom: adult rats. Pairwise comparisons of the significant treatment effect indicated higher TH concentrations in the low MPH treatment group than the control group ($p < 0.05$). Each box shows the 25th and the 75th percentiles and the "whiskers" (bars) show the 10th and 90th percentiles. The solid and dashed lines inside the box show the median and mean, respectively. Solid points are datapoints falling outside the 10th and 90th percentiles.

significant sex differences in D1 and D2 receptor concentrations (Andersen and Teicher, 2000).

Low dose MPH treatment caused increased TH levels in adulthood and on PND 22 and increased DA levels in adult rats. Interestingly, similar effects were not apparent at higher MPH doses. However, the highest MPH treatment group (i.e., 42 mg/kg/day) exhibited increased HVA/DA ratios. This effect seems entirely due to differences at the weanling age, as ratios in adult rats of the high MPH treatment groups were only 3% higher than controls. However, it should be noted that effects at PND 22 could be pharmacological in nature as the last MPH treatment occurred within 24 h of the HVA/DA measurement. Literature comparisons for similar striatal measures are not directly available. However, Torres-Reveron et al. (Torres-Reveron et al., 2009) described no treatment-related differences in striatal TH or DAT after MPH treatment of 5 mg/kg twice daily on PNDs 7–35 in rats. Oral administration of MPH on PNDs 25–39 caused decreased striatal DAT levels on PND 45 and PND 70 (Moll et al., 2001). However, MPH treatment on PNDs 7–35 did not alter striatal DAT levels measured on PND 35 or PND 135, but TH immunoreactivity was decreased on PND 35 (Gray et al., 2007). Van der Marel et al. (van der Marel et al., 2014) noted only small MPH treatment effects with no uniformity in measures of striatal volume after MPH treatment on PNDs 25–46. Still, it is clear that acute MPH treatment can induce gene expression changes in the adult rat striatum (Yano and Steiner, 2005a, 2005b; Yano et al., 2006), but whether those changes result in alterations in monoamine or metabolite concentrations is less clear.

Low MPH treatment caused an increase of 21% for DA concentrations in adult rats and an increase of 31% for TH concentrations in weanlings. While this effect must be replicated, it is this treatment group which resulted in MPH serum levels in pregnant dams within the range of adult humans (Panos et al., 2014a, 2014b). The low MPH treatment group, however, exhibited few significant behavioral alterations (Panos et al., 2014a, 2014b) (Ferguson et al., submitted) and it is unclear what sort of alterations might be apparent with those neurochemical alterations. Perinatal studies of high fat diets have described increased striatal TH and DA which resulted in behavioral sensitization to amphetamines in adulthood (Naef et al., 2008). Interestingly, female subjects of the low MPH group did exhibit decreased sensitivity to later MPH treatment (Ferguson et al., submitted); however, there was no similar effect in male rats and DA levels in males and females of that treatment group were within 5% of each other. MPH treatment has also been shown to increase striatal TH immunoreactivity in the rodent ADHD model, the Spontaneously Hypertensive Rat (Kim et al., 2011).

This study examined only one brain region, the striatum. There were several reasons for selecting this region for measurement. MPH inhibits the DA transporter (DAT) and DAT is regionally distributed with high striatal concentrations (Ciliax et al., 1995; Emond et al., 2008). Additionally, some studies have suggested that the DAT gene (DAT1) may be a susceptibility gene for ADHD (reviewed in (Roman et al., 2004)). Finally, DAT can be detected as early as embryonic day 14 in rats (Fujita et al., 1993), indicating the potential sensitivity to MPH treatment. However, the current results should not be interpreted to indicate that other brain regions may be altered by developmental MPH treatment as well.

To summarize, developmental MPH treatment with a clinically relevant dosing regimen produced effects on striatal DA and TH concentrations and the turnover ratio, HVA/DA. The effects were not sex-specific and were apparent in both weanlings and adult rats. These results complement and extend our previous studies (Panos et al., 2014a, 2014b) (Ferguson et al., submitted).

Transparency document

The transparency document doi: http://dx.doi.org/10.1016/j.ntt.2014.08.001 associated with this article can be found in the online version.

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Acknowledgments

This work was supported by the National Center for Toxicological Research/U.S. Food and Drug Administration [Protocol #E7318 to S.A.F.]. The authors are grateful for the technical expertise provided by the animal care staff of the Priority One Corporation and appreciate the careful study supervision by Mr. C. Delbert Law of the Division of Neurotoxicology.

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