Published in final edited form as:

Life Sci. 2010 March 27; 86(13-14): 482–487. doi:10.1016/j.lfs.2010.02.007.

Amphetamine treatment during early postnatal development transiently restricts somatic growth

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Abstract

Aims—Restricted somatic growth during fetal or early postnatal periods has been suggested to serve as a predictive indicator for neuroanatomical changes and behavioral impairments during adulthood. Here, the effects of d-amphetamine sulphate (AMPH) exposure during the brain growth spurt period on this potential indicator were evaluated.

Main Methods—Rats received 0, 5, 15 or 25 mg/kg/day of AMPH via two daily intragastric intubations from PD4-9. Body weight data were collected every other day from PD1 to 21, and then weekly until PD59. On PD9, a subset of animals was terminated 90 min after the last amphetamine treatment and the weights of the cortex, cerebellum, and brainstem were collected. Weights of these brain regions from young adult rats were also assessed on PD68.

Key Findings—AMPH exposure during early postnatal development limited somatic growth in a dose-related manner, with significantly lower body weights in animals assigned to the AMPH 25 and AMPH 15 groups. However, this was transient in nature, with no significant difference in weight observed after pups were weaned on PD21. Further, no differences in brain weight were observed at either age as a result of AMPH exposure.

Significance—These findings support the idea that developmental AMPH exposure transiently restricts somatic growth. Moreover, the lack of effect on brain weight shows that AMPH differentially affects somatic and brain growth. The current findings suggest that in addition to the immediate effects on body weight, amphetamine may alter the rate of growth, and increase the risk for weight-related adult diseases.

Keywords

amphetamine; brain growth spurt; intragastric intubation; serum amphetamine concentration; somatic growth

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

INTRODUCTION

Psychostimulants, such as amphetamine, methamphetamine and cocaine, are known to interfere with development of the central nervous system (Winslow et al., 2007; Berman et al., 2008; Thompson et al., 2009). Using preclinical models, developmental psychostimulant exposure has been shown to produce long-lasting changes in both behavior (Vorhees et al., 2000; Williams et al., 2004; Smith et al., 2009) and neuroanatomy (Tan and Costa, 1995; Campillo et al., 2004; Cui et al., 2006). In terms of more general developmental parameters, psychostimulant exposure has also been found to interfere with somatic growth (Acuff-Smith et al., 1996; Slamberova et al., 2006). For instance, prenatal methamphetamine exposure causes infants to be smaller for their gestational age and to have lower birth weights (Smith et al., 2006). These results are supported by evidence from the preclinical literature demonstrating a decrease in somatic growth parameters (Tavares and Silva, 1996; Tan et al., 2003; Slamberova et al., 2006).

While drug exposures throughout gestation are well known to alter somatic growth, there is a growing body of literature supporting the idea that exposure to certain drugs during only a portion of development is equally capable of altering somatic growth (Day et al., 1991; Cornelius et al., 1999; Bateman and Chiriboga, 2000; England et al., 2001). Bernstein and colleagues (2005) found that smoking during only the third trimester significantly lowered the overall birth weight of infants. Similar findings of stage-related effects on somatic growth have been shown in preclinical studies. In particular, drug exposure during the brain growth spurt, a period of rapid, transient growth in the brain that is vulnerable to teratogens (Dobbing and Sands, 1971), has been shown to cause changes in both body (Bonthius and West, 1988; Goodlett et al., 1998) and brain weight (Bonthius and West, 1988; Ieraci and Herrera, 2006). However, while brain growth spurt exposures to some psychostimulants, such as cocaine (Tan and Costa, 1995) and methamphetamine (Vorhees et al., 2000; Williams et al., 2000), have been shown to induce microcephaly, there are currently no studies that have examined the effects of amphetamine per se on somatic growth during this period of development.

In the current study, Sprague-Dawley rat pups received *d*-amphetamine sulphate via intragastric intubation during a portion of the brain growth spurt, in order to determine the effects amphetamine of this dynamic stage of development. Body weight measurements were made at regular intervals beginning the day after birth and continuing into adulthood. Further, the weight of the cortex, the cerebellum, the brain stem and the entire brain was measured both at the end of the amphetamine treatment period and in adulthood. Finally, serum amphetamine concentrations were analyzed in order to determine the effectiveness of intragastric intubation as a delivery method for amphetamine. It was hypothesized that neonatal exposure to *d*-amphetamine sulfate would limit the growth of both the body and the brain, as measured by weight, in the amphetamine-treated subjects, and such growth restrictions would be dose-related.

EXPERIMENTAL METHODS

Animals

Two-hundred and four Sprague-Dawley rat pups, derived from thirty-nine litters, served as subjects for this study. Male and female adult Sprague-Dawley rats obtained from Harlan, Inc. (Houston, TX) were used as breeders to generate these offspring, and all animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-International accredited vivarium with a 12/12-hour light/dark cycle at the Texas A&M Health Science Center College of Medicine. Breeding procedures were performed as described previously (Smith et al., 2008, Smith et al., 2009). All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Texas A&M University.

Experimental Design

Rat pups from each gender were randomly assigned to one of four experimental treatment groups on PD 4: AMPH 5 (5 mg/kg/day *d*-amphetamine sulfate; $n = 40$), AMPH 15 (15 mg/kg/day *d*-amphetamine sulfate; $n = 40$), AMPH 25 (25 mg/kg/day *d*-amphetamine sulfate; $n = 40$) and IC (intubation control; milk formula with 0 mg/kg/day *d*-amphetamine sulfate; $n = 38$). *d*-Amphetamine sulphate was obtained from Sigma Aldrich (St. Louis, MO). Pups received treatment from PD 4 through PD 9. The doses of *d*-amphetamine sulphate employed in the current study encompassed a wide range, and the use of these doses has been reported previously (Tavares and Silva, 1993; Smith et al., 2008). A normal control (NC; raised by dams with no drug treatment or intubation manipulation; $n = 46$) was introduced to specifically control for the effects of the intubation and other experimental manipulation (such as daily handling) on the dependent variables. Intragastric intubation procedures were used to deliver amphetamine as described previously (Smith et al., 2008, Smith and Chen, 2009). Briefly, milk formula, either with or without amphetamine, was delivered to rat pups by advancing a 15 cm piece of PE-10 tubing attached to a 30 gauge needle on a 1 cc syringe was advanced down the esophagus into the stomach. Subjects received two intubations per day, with a 2-hr inter-intubation interval, and each intubation contained one-half of the total daily dose of *d*-amphetamine sulphate. In order to determine the effects of amphetamine on somatic growth, body weights (g) for the animals were collected every other day from PD 1 until weaning at PD 21, after which time body weights were recorded once a week until PD 49, and once again 10 days later (PD 59) prior to commencement of behavioral testing (results not included in this study).

Given that amphetamine-treated pups are likely to have excreted amphetamine or its metabolites in their urine, it is possible that dams of these pups may be exposed to trace quantities of amphetamine through the grooming of the pups. Therefore, pups from the amphetamine-treated groups (AMPH 5, AMPH 15 and AMPH 25) and control groups (IC and NC) were generated from separate litters in order to prevent the control animals from being indirectly exposed to amphetamine through other means (e.g., dam's breast milk). No more than one male or one female pup from a litter was assigned to the same treatment group, and an effort was made to have an equal number of males and females within a treatment group. In total, 21 litters were used to generate all amphetamine-treated animals, and 18 litters were used to generate all control animals.

Once the amphetamine treatment regimen was completed, animals were left with dams to be reared without further experimental manipulation until weaning on PD 21. Animals used for determining the serum amphetamine concentration and the effects of amphetamine on the developing brain ($n = 10$ /group, a total of $n = 50$) were sacrificed 90 min after the beginning of the last amphetamine treatment on PD 9, and the brain from these animals were used for the determination of the brain weights on PD 9. All remaining animals ($n = 154$) were sacrificed after having completed behavioral testing on PD 68 (results published in Smith et al., 2009). In all instances, termination involved an overdose of pentobarbital followed by a transcardial perfusion of saline and 4% paraformaldehyde in phosphate buffer. Following perfusion, the brains of 58 animals ($n = 10$ for AMPH 5, AMPH 15 AMPH 25 and IC, and $n = 18$ for NC) were excised and weights (mg) for the cortex (CX), the cerebellum (CB), the brainstem (BS) and the total brain (sum of all three brain regions assessed) were determined. Brains from the remaining animals (total $n = 96$) were processed for another study and the weights of these brains were not available for brain weight analyses.

Intragastric Intubation Procedures

Measurement of Serum Amphetamine Concentration—On PD 9, fifty animals from all five experimental treatment groups were given an overdose of pentobarbital (Sigma; St.

Louis, MO) and a terminal cardiac blood draw was performed. Blood samples from each animal were then centrifuged at 2500 rpm for 30 min to collect blood serum, and frozen at -20°C until serum amphetamine analysis was performed by MEDTOX Scientific, Inc. (St. Paul, MN). In order to yield a sufficient serum level for analysis, serum samples from 4-5 rat pups in each of the three amphetamine-treated groups were combined to yield 4 samples (approximately 1.2 ml of serum/sample) in the AMPH 5, 15 and 25 treatment groups. Since animals within the two control groups did not receive amphetamine, all amphetamine-free serum samples from control animals (IC and NC groups) were pooled and a volume of this pooled serum equivalent to 25% of each amphetamine-containing serum sample volume was added to the sample. This procedure was carried out in order to increase the necessary serum volume for reliable analysis of the serum amphetamine concentration. The values of the serum amphetamine concentration reported in this study were corrected accordingly.

Statistical Analysis

Serum amphetamine concentration data were analyzed using a one-way analysis of variance (ANOVA) with “amphetamine dose” (AMPH 5, AMPH 15, AMPH 25) as the independent (between) factor. For somatic growth (body weight) data, a mixed ANOVA was used with “postnatal day” as the within-subject variable and “treatment” (AMPH 5, AMPH 15, AMPH 25, IC) and “gender” as two between-subject factors. The “pre-weaning” and “post-weaning” somatic growth data were analyzed separately given the nature of ontogenetic differences in body weights (significant increases after the weaning at PD 21). Additional ANOVAs were performed between the IC and NC groups to determine the effects of intubation or experimental manipulation on somatic growth. For each brain region, brain weight data were analyzed using a two-way ANOVA with “treatment” (AMPH 5, AMPH 15, AMPH 25, and IC) and gender as two between-subject variables. Fisher’s least significant difference (LSD) test was used for *post hoc* analyses if a significant main effect of any given variable was observed. Furthermore, if an interaction between any factors was observed, simple effect analyses were performed to identify the loci of such an interaction. The α level for all analyses was set at 0.05.

RESULTS

Serum Amphetamine Concentration

A one-way ANOVA conducted on the serum amphetamine concentration data revealed a main effect of treatment ($F_{2,9} = 25.20$; $p < 0.05$; Fig. 1). Further post hoc tests revealed significant differences among all treatment groups with mean serum amphetamine concentrations of 170.0 ± 26.8 , 362.2 ± 20.3 and 489.7 ± 44.2 ng/ml for the AMPH 5, 15 and 25 groups, respectively.

Amphetamine-Induced Alterations in Body Weights

Pre-Weaning Body Weight—The mixed ANOVA conducted on the body weight data showed main effects of treatment ($F_{3,110} = 7.92$; $p < 0.05$; Fig. 2), gender ($F_{1,110} = 12.07$; $p < 0.05$), and postnatal day ($F_{13,1340} = 7496.20$; $p < 0.05$; Fig 2). Additionally, there was an interaction between the postnatal day and treatment factors ($F_{39,1430} = 3.69$; $p < 0.05$) and the postnatal day and gender ($F_{13,1430} = 2.51$; $p < 0.05$). The observed main effect of gender and the lack of interaction between gender and treatment suggest that the body weight difference seen between male and female animals was neither significantly nor specifically affected by the amphetamine treatment. The results of simple effect analyses indicate that the body weights for animals in the AMPH 25 group were significantly lower than those animals in the IC and AMPH 5 groups beginning on PD 7 and lasting through the remainder of the pre-weaning period. In addition, animals in the AMPH 15 group had significantly lower body weights than the IC and AMPH 5 group subjects PD 9, 13 and 15. Moreover, body weights of the subjects in the AMPH 15 group were also found to be significantly different from subjects in the AMPH

25 group on PDs 13 and 15. The main effect of gender showed that the male pups had a higher overall body weight than the female pups across the pre-weaning period.

Post-Weaning Body Weight—The ANOVA performed on the body weight data during the post-weaning period yielded main effects of gender ($F_{1,110} = 619.66$; $p < 0.05$) and postnatal day ($F_{4,440} = 6801.01$; $p < 0.05$); however, no significant main effect of treatment was found. An interaction was observed between postnatal day and gender ($F_{4,440} = 535.04$; $p < 0.05$). The observed main effect of gender and the lack of interaction between gender and treatment suggest that the body weight difference seen between male and female animals was neither significantly nor specifically affected by the amphetamine treatment. Simple effect analyses revealed that this was due to male subjects increasing in body weight at a significantly faster rate than females during this period of time.

Comparison of Body Weight in Control Groups—In order to evaluate the potential intubation or manipulation effects on the dependent variables, an ANOVA was conducted between the IC and NC groups in both periods (pre- and post-weaning), and no significant main effect of treatment was obtained suggesting that intubation or daily handling/manipulation did not interfere with somatic growth. However, there were main effects of gender ($F_{1,60} = 7.10$; $p < 0.05$) and postnatal day ($F_{10,600} = 6709.80$; $p < 0.05$) during the pre-weaning period. Similarly, main effects of gender ($F_{1,60} = 482.54$; $p < 0.05$) and postnatal day ($F_{4,240} = 5485.13$; $p < 0.05$) were also observed during the post-weaning period. An interaction between gender and postnatal day was also observed during both the pre-weaning ($F_{10,600} = 2.52$; $p < 0.05$) and post-weaning periods ($F_{4,240} = 424.72$; $p < 0.05$). Simple effect analyses revealed that this interaction was a result of males increasing body weight at a significantly faster rate than females.

Brain Weights of Cortex, Cerebellum and Brainstem

The results of two-way ANOVAs did not reveal main effects of treatment on the weights (mg) in the cortex (CX), cerebellum (CB), brainstem (BS) or total brain weight either at either PD 9 (Table 1) or PD 68 (Table 2). However, main effects of gender were observed in all brain regions assessed at PD 68 ($F_{1,32} = 19.75, 26.19, 5.38$ and 23.91 for the CX, CB, BS and whole brain, respectively; p 's < 0.05 ; Table 2). Post hoc analysis showed that the male brain weights were significantly higher than the female ones. No interaction was observed between treatment and gender in any of the brain regions assessed at either age. The analyses conducted between the two control groups to determine the effects of experimental manipulations/intubations on brain weights did not reveal any statistical significance suggesting that such experimental procedures do not interfere with brain growth in terms of weight measurements.

DISCUSSION

In the current study, exposure to amphetamine during a postnatal stage equivalent to the third trimester produced a dose-related decrease in body weight, with animals in the AMPH 25 and AMPH 15 groups having significantly lower body weights than those in the IC or AMPH 5 groups prior to weaning at PD 21. However, these amphetamine-mediated decreases in body weight were transient in nature and disappeared following weaning. Conversely, neonatal amphetamine administration did not alter brain weight, either in the cortex, the cerebellum, the brainstem or the whole brain (sum of all three brain regions assessed). These findings suggest that brain development is less sensitive to the toxic effects of amphetamine when compared with general somatic growth when the current exposure regimen is employed. Given that in the current study, amphetamine was administered neonatally during a developmental period corresponding to the third trimester of brain development in humans, rather than the entire prenatal period, the clinical relevance of the current findings is somewhat limited. However,

the current amphetamine exposure regimen may mimic the indirect exposure to amphetamine that would occur through breastfeeding during early postnatal development; and importantly, the results showed that such an exposure paradigm is sufficient to induce changes in somatic growth patterns.

Previous studies have examined the effects of psychostimulants on fetal growth parameters, the vast majority of these studies have focused on drugs related to amphetamine, such as cocaine or methamphetamine (Church et al., 1995; Middaugh et al., 1996; Campillo et al., 2004; Williams et al., 2004; Slamberova et al., 2006), rather than amphetamine per se. As expected, the current findings that neonatal amphetamine exposure alters the somatic growth of animals are in agreement with previous studies of methamphetamine. Interestingly, neonatal amphetamine-induced decrements in body weight disappeared completely by PD 28, one week after weaning. Such a catch up in body weight within a very short period time was remarkable, but not surprising, given the significant increase in growth velocity observed in all groups between PD 21 and 28 (mean increase in body weight = 59%). Similar results have been observed in clinical studies of maternal smoking which showed that prenatal exposure to cigarette smoke resulted in a postnatal growth “catch-up” period (Larsen et al., 1997). Moreover, it is possible that the decreased body weights observed prior to weaning in this study may have reemerged if the animals been allowed to continue growing to a point beyond early adulthood (PD 68), an effect that has been observed with other psychostimulants (e.g., cocaine Church et al., 2004). Studies such as these suggest that psychostimulants may alter the rate of growth, and increase the risk for weight-related adult diseases, such as obesity and diabetes (Ong, 2006).

It should be noted that no pair-fed control group was introduced in this study to control for the potential anorexic effects from amphetamine treatment in these neonatal pups. This is due to the fact that milk intake from dams by amphetamine-treated pups between the completion of amphetamine administration procedures on one day and the commencement of these procedures the following day cannot be measured. Therefore, it could be hypothesized that the transient decrease in body weight seen in the amphetamine-treated animals in this study may be the result of decreased milk consumption during the period in between daily amphetamine administration. However, prior studies have found that amphetamine treatment does not alter feeding behavior in neonatal rat pups. Capuano and colleagues (1989) found that administration of amphetamine on postnatal days 3, 7 and 10 increased milk intake in rat pups that had been deprived of milk for 1 hr, and had no effect on rat pups that were milk-deprived for 24 hr. Conversely, milk intake was decreased following amphetamine administration on PD 15. Similarly, Raskin and Campbell (1981) found that administration of amphetamine on PD 5 had no effect on food-related behaviors, such as latency to attach to the nipple, time spent with the mother, duration of suckling, or weight gain; however, administration on PD15 slowed or reduced the frequency of attachment to the nipple, and decreased time spent nursing. Thus, amphetamine administration during the first ten postnatal days is unlikely to alter feeding patterns in neonatal rats, suggesting that decreased feeding or suckling cannot account for the decreases in body weight observed here. Unlike the effects on somatic growth, neonatal amphetamine exposure did not significantly reduce overall brain weight, nor did it reduce weight in the cortex, the cerebellum or the brainstem individually. However, a lack of change in brain weight does not necessarily indicate a lack of neuroanatomical and/or molecular changes in the brain. Studies showing no change in brain weight following developmental exposure to amphetamine-like substances found changes in other dependent measures such as diminished hippocampal volume (Tavares and Silva, 1993), and an increase in DNA damage (Jeng et al., 2005). Further, neonatal amphetamine exposure from postnatal days four through nine alters the rate of neuronal incorporation (i.e., the total rate of neuronal migration and/or insertion) in the hippocampus (Smith et al., 2008). Since the brain exhibits regional vulnerabilities in regards to different drug exposures (Brown and Molliver, 2000; Maier and

West, 2001; Slotkin, 2002), it may be possible to detect changes in the weight (or volume) of more specific brain regions, such as the hippocampus or thalamus. Thus, future studies should consider the possibility that the effects of amphetamine on cytoarchitecture in the brain may be too subtle to detect by assessing changes in gross neuroanatomy (i.e., brain weight).

The current study demonstrated the use of intragastric intubation as an effective method of drug delivery in developmental studies, as evidenced by the dose-related changes in serum amphetamine concentration of animals exposed to amphetamine. While there have been previous reports of serum methamphetamine concentrations (Capon and Vorhees, 2001), to our knowledge, this is the first report of serum amphetamine concentrations in rats following neonatal exposure to amphetamine via intragastric intubation. In comparison with the clinical literature, the current study produced serum amphetamine concentrations lower than those found in humans (Cody et al., 2003, 2004). However, the half life for amphetamine metabolism in the rat has been suggested to be 1.5 hr (Cho et al., 1973), thus, it is possible that in this study more than 50% of the total amphetamine in the blood had already been metabolized at the time the samples were collected. The fact that amphetamine has a much faster metabolism in rats (1.5 hr) than in humans (5.0 hr; Beckett and Rowland, 1965) may also explain the lower concentrations of serum amphetamine in rats seen here.

CONCLUSIONS

The current study found that neonatal amphetamine exposure transiently restricts somatic growth, but not brain development, as measured by weight. Conversely, previous studies of other drugs, such as alcohol (Goodlett et al., 1998; Ieraci and Herrera, 2006), have been shown to significantly alter both body and brain weights. Observation of changes in somatic growth in children of psychostimulant-abusing mothers may serve as an effective predictor of certain behavioral deficits that will develop as the offspring ages; however, such conclusions drawn from these somatic growth markers must be made carefully since the effects may be transient in nature. Further, the current findings suggest that in addition to the immediate effects on body weight, amphetamine may alter the rate of growth, and increase the risk for weight-related adult diseases, such as obesity and diabetes (Ong, 2006).

Acknowledgments

This research was supported by NIH grant DA018809 to A.M. Smith.

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Figure 1.

Serum amphetamine concentrations. Results demonstrated a dose-related increase in serum amphetamine concentration. * indicates significant difference from all other treatment groups. Bars indicate standard error of the means (SEMs); $p < 0.05$.

Figure 2.

Neonatal amphetamine administration decreases somatic growth in rat pups prior to weaning. Amphetamine produces a dose-related decrease in somatic growth (in grams) prior to weaning (collapsed across gender). # indicates significant difference from IC and AMPH 5 groups. * indicates significant difference from all other treatment groups. Bars indicate standard error of the mean (SEM); $p < 0.05$.

Table 1

Mean daily body weights (in grams) for male and female Sprague-Dawley rat pups in the intubation control (IC) and normal control (NC) groups

		<i>Pre-weaning Period</i>										<i>Post-weaning Period</i>									
	PD	PD	PD	PD	PD	PD	PD	PD	PD	PD	PD		PD	PD	PD	PD	PD	PD	PD	PD	PD
	1	3	4	5	6	7	8	9	11	13	15	17	19	21	28	35	42	49	59		
<i>Males</i>																					
IC	7.3 (0.1)	9.9 (0.1)	11.1 (0.2)	13.1 (0.2)	15.1 (0.2)	17.2 (0.3)	19.9 (0.2)	22.0 (0.5)	26.6 (0.4)	31.2 (0.5)	35.4 (0.5)	38.8 (0.6)	44.0 (0.7)	51.3 (0.9)	88.1 (1.1)	138. 9 (1.8)	191. 3 (1.8)	245. 3 (3.6)	301. 8 (5.9)		
N	7.4	9.9	11.5	13.1	15.3	17.0	19.5	21.3	25.8	30.6	34.8	37.8	43.8	50.9	88.1	137.	191.	246.	301.		
C	(0.1)	(0.1)	(0.3)	(0.2)	(0.3)	(0.3)	(0.3)	(0.4)	(0.4)	(0.5)	(0.6)	(0.7)	(0.8)	(0.7)	(1.1)	(1.4)	(1.8)	(2.4)	(4.3)		
<i>Females</i>																					
IC	6.9 (0.1)	9.3 (0.1)	10.8 (0.2)	12.4 (0.2)	14.7 (0.2)	16.3 (0.3)	19.5 (0.4)	21.1 (0.4)	25.6 (0.5)	30.3 (0.5)	34.3 (0.6)	37.4 (0.8)	42.7 (0.1)	49.2 (0.8)	80.2 (1.3)	119. 1 (2.2)	153. 5 (3.0)	177. 2 (3.4)	203. 5 (5.3)		
N	7.0	9.2	10.7	12.3	14.3	16.1	18.3	20.2	24.6	29.1	33.4	37.2	42.0	48.0	79.4	119.	152.	175.	202.		
C	(0.1)	(0.2)	(0.3)	(0.2)	(0.3)	(0.2)	(0.3)	(0.3)	(0.4)	(0.5)	(0.5)	(0.6)	(0.7)	(0.9)	(0.9)	(1.4)	(1.6)	(2.0)	(2.9)		

Numbers in parentheses indicate standard error of the means (SEMs).

* indicates a main effect of gender (the weights in male subjects were significantly higher than those in female subjects).

Table 2

Mean brain weights (in milligrams) at postnatal days 9 and 68 for male and female Sprague-Dawley rats

Postnatal Day 9										Postnatal Day 68													
Total Weight										Cortex		Cerebellum		Brainstem		Total Weight		Cortex		Cerebellum		Brainstem	
Males																							
NC		931.8 (18.9)		780.8 (18.1)		84.4 (5.6)		66.5 (3.9)		1615.2 (29.5)		1260.3 (20.0)		237.0 (6.8)		117.9 (3.5)							
IC		867.4 (16.6)		730.9 (13.3)		72.6 (4.8)		63.9 (4.7)		1623.2 (13.5)		1255.3 (8.0)		245.8 (5.3)		122.1 (5.6)							
AMPH 5		852.4 (18.3)		721.3 (21.9)		75.0 (4.9)		56.1 (1.5)		1610.0 (22.6)		1254.9 (20.7)		237.9 (2.9)		117.2 (3.9)							
AMPH 15		857.3 (19.3)		738.5 (24.1)		71.4 (2.2)		47.4 (1.9)		1617.8 (27.4)		1263.0 (21.1)		239.0 (5.4)		115.8 (3.4)							
AMPH 25		873.3 (19.3)		745.3 (11.9)		71.6 (4.0)		56.5 (5.4)		1610.8 (27.0)		1250.8 (17.3)		236.7 (6.7)		123.2 (6.9)							
Females *																							
*NC		879.9 (23.1)		736.3 (17.7)		80.5 (6.4)		63.1 (4.6)		1545.5 (30.0)		1208.9 (23.5)		224.3 (6.5)		112.2 (2.8)							
*IC		829.4 (27.0)		693.7 (29.0)		72.0 (5.5)		63.6 (3.8)		1557.8 (16.5)		1216.3 (9.9)		224.6 (5.6)		116.9 (5.7)							
*AMPH 5		854.9 (30.0)		722.8 (24.8)		74.7 (6.2)		57.4 (4.3)		1498.2 (16.8)		1170.5 (17.4)		218.9 (2.6)		108.8 (2.4)							
*AMPH 15		828.0 (31.0)		696.1 (32.8)		69.3 (7.6)		62.6 (5.7)		1499.6 (31.0)		1183.8 (22.2)		209.2 (7.9)		106.7 (3.1)							
*AMPH 25		842.8 (18.4)		711.9 (15.3)		72.4 (2.2)		58.5 (6.9)		1549.8 (40.4)		1217.8 (26.1)		218.3 (9.4)		113.7 (6.4)							

Numbers in parentheses indicate standard error of the means (SEMs).

* indicates main effects of gender between NC and IC, and among IC, AMPH 5, AMPH 15 and AMPH 25 at PD 68.