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Neonatal amphetamine exposure and hippocampus-mediated behaviors

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ABSTRACT

Previous studies linking amphetamine use during pregnancy to changes in the behavioral development of affected infants have greatly increased society's level of concern regarding amphetamine use by women of reproductive age. The aim of this study was to investigate whether exposure to *d*-amphetamine sulfate during the brain growth spurt, the most dynamic period of brain development, alters hippocampus-mediated behaviors during both pre-adolescence and young adulthood. Sprague–Dawley rat pups were intragastrically administered a milk formula containing 0, 5, 15 or 25 mg/kg/day of amphetamine from postnatal day (PD) 4–9. Following weaning, the effects of neonatal amphetamine exposure on hippocampus-mediated behaviors were assessed using the open-field, the water maze, and the conditioned taste aversion behavioral tasks. Results from these behavioral tests revealed that while amphetamine exposure during the brain growth spurt alters behaviors in open-field testing, it does not interfere with performance in either the water maze or the conditioned taste aversion paradigm. These results offer speculation that the effects of neonatal amphetamine exposure on hippocampus-mediated behaviors may be related to interactions between the "temporal" (time of drug exposure) and "regional" (different regions of the hippocampus) vulnerability issues.

1. Introduction

Exposure to psychostimulants, such as amphetamine, methamphetamine and cocaine, has been shown to produce wide ranging effects on the developing fetus (Gingras, Weese-Mayer, Hume, & O'Donnell, 1992; Plessinger, 1998; Plessinger & Woods, 1998; Smith et al., 2006). For example, children born to mothers who abused psychostimulants during pregnancy suffer from low birth weight/somatic growth rates (Billing, Eriksson, Steneroth, & Zetterstrom, 1985; Cernerud, Eriksson, Jonsson, Steneroth, & Zetterstrom, 1996), reduced head circumference (Chomchai, Na Manorom, Watanarungsan, Yossuck, & Chomchai, 2004; Oro & Dixon, 1987), cardiac defects (Little, Snell, & Gilstrap, 1988; Zimmerman, 1991), cerebral hemorrhage (Eriksson, Larsson, & Zetterstrom, 1981; Spires, Gordon, Choudhuri, Maldonado, & Chan, 1989), and stillbirth (Dearlove, Betteridge, & Henry, 1992; Loebstein & Koren, 1997). One organ system of offspring that appears to be significantly and negatively influenced by the use of psychostimulants during gestation is the central nervous system (Chasnoff, 1992; Lester et al., 2001; Nulman et al., 1994), which may subsequently interfere with behavioral development (Billing, Eriksson, Jonsson, Steneroth, & Zetterstrom, 1994; Noland et al., 2005). Given the increasing use of psychostimulants by pregnant women (Substance Abuse and Mental Health Services Administration, 2004), the behavioral modifications among affected offspring represent a potential concern since their abilities to interact with others or to respond to environmental stimulation may be severely impeded.

Preclinical studies have further strengthened the notion that psychostimulant exposure during various stages of brain development alters behavioral manifestations (Acuff-Smith, Schilling, Fisher, & Vorhees, 1996; Cho, Lyu, Lee, Kim, & Chin, 1991; Holson, Adams, Buelke-Sam, Gough, & Kimmel, 1985). Interestingly, many behavioral deficits observed following developmental psychostimulant exposure appeared to be involved, at least partially, in hippocampal functions (Bashkatova, Meunier, Maurice, & Vanin, 2005; Morford, Inman-Wood, Gudelsky, Williams, & Vorhees, 2002; Onaivi, Bishop-Robinson, Motley, Chakrabarti, & Chirwa, 1996; White & Swartzwelder, 2004). The aim of the present study was to investigate the effects of *d*-amphetamine sulfate on performance in three behavioral tasks, the open-field, a modified version of the Morris water maze and conditioned taste aversion, thought to be partially modulated by the hippocampus.

The open-field, originally employed by Hall (1934), is designed to study emotional aspects of rodents' behavior. Bannerman et al. (2003) reported that cytotoxic lesion to the ventral hippocampus severely affected performance in behavioral tasks, including the open-field, that are associated with anxiety or fear. Similarly, Zhang, Pothuizen, Feldon, and Rawlins (2004) showed that infusion of N-methyl-D-aspartate (NMDA) to the ventral hippocampus

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resulted in behavioral alterations in open-field testing. In addition, the Morris water maze, originally developed by Morris (1984), is considered to be one of the standard behavioral assessments for hippocampal functions. Numerous studies, including an early one by Morris, Schenk, Tweedie, and Jarrard (1990) that used ibotenate (an NMDA receptor agonist) to induce excitotoxicity in the hippocampus, have demonstrated that lesions to the hippocampus interferes with spatial learning and memory as measured by this behavioral testing paradigm (Cimadevilla, Miranda, Lopez, & Arias, 2005). Conversely, conditioned taste aversion, demonstrated earlier by Garcia, Kimeldorf, and Koelling (1955), was thought to be mainly modulated by brain regions other than the hippocampus, such as the amygdala (Roldan & Bures, 1994; Yamamoto, Fujimoto, Shimura, & Sakai, 1995). However, evidence has emerged to show that the hippocampus may be partially involved in the acquisition of taste aversion (Gallo & Candido, 1995; Purves, Bonardi, & Hall, 1995; Reilly & Bornovalova, 2005). Based on the hypothesis that the hippocampus is a vulnerable target for developmental psychostimulant exposure, it was expected in the present study that amphetamine exposure during the brain growth spurt would lead to impairment in the performance of these three behavioral tasks.

One additional aim of the current study was to investigate whether brain development during the early postnatal period was sensitive to the potentially neurotoxic effects of amphetamine. In rats, the most dynamic growth stage of the developing brain is during the first few postnatal weeks, a period that is equivalent to the human third trimester, and one that has been termed the "brain growth spurt" by Dobbing and Sands (1979). It was hypothesized that the behavioral manifestations of the open-field, water maze and conditioned taste aversion would be impaired following amphetamine exposure during this vulnerable stage of brain development, and such behavioral impairments would be amphetamine-dose related, with animals that received the highest dose of amphetamine being the ones most affected.

2. Methods

2.1. Animals

One-hundred and forty-six Sprague–Dawley rat pups, derived from 24 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 AAALAC-International accredited vivarium with a 12/12-h light/dark cycle at the Texas A&M Health Science Center College of Medicine. For breeding purposes, one female was placed with one male at 5:00 p.m., left overnight, and a vaginal smear was performed at 9:00 a.m. the following morning. The day that a female was confirmed to be sperm-positive was considered gestational day (GD) 1. Pregnant females were housed singly, with food and water available *ad libitum*. Following parturition, all litters were culled to between 8 and 10 pups on postnatal day (PD) 1 (one day after birth), with cross-fostering techniques used as necessary. All experimental procedures described in this study were approved by the Institutional Animal Care and Use Committee (IACUC) at Texas A&M University.

On PD 4, pups were randomly assigned to one of five experimental treatment groups: AMPH 5 (5 mg/kg/day *d*-amphetamine sulfate; $n = 30$), AMPH 15 (15 mg/kg/day *d*-amphetamine sulfate; $n = 30$), AMPH 25 (25 mg/kg/day *d*-amphetamine sulfate; $n = 30$), IC (intubation control; milk formula with 0 mg/kg/day *d*-amphetamine sulfate; $n = 28$) and NC (normal control with no drug treatment or intubation; $n = 28$). *d*-Amphetamine sulfate (Sigma; St. Louis, MO) was administered to animals via intragastric intubation from PD 4 through PD 9. Given that amphetamine-receiving pups are likely to have excreted amphetamine in their urine, it is possible that dams of these pups may be exposed to amphetamine after cleaning the pups. Therefore, in order to prevent pups from the control groups from being indirectly exposed to amphetamine through dam breast milk, animals from the amphetamine-receiving groups (AMPH 5, AMPH 15 and AMPH 25) and control groups (IC and NC) were generated from separate litters. In total, 13 litters were used to generate all amphetamine-receiving animals, and 11 litters were used to generate all control animals. The doses of *d*-amphetamine sulfate employed in the current study have been used previously by our laboratory and others to demonstrate neonatal amphetamine-induced changes in development (Smith, Pappalardo, & Chen, 2008; Tavares & Silva, 1993). While the two highest doses of amphetamine do produce significant reductions in body weight (reaching a maximum on PD 15 of ~10 and 5% for the AMPH 25 and AMPH 15 groups, respectively), these changes are transient in nature, disappearing once the animals reach weanling age at PD 21 (unpublished observations). Further, no incidences of mortality were observed as a result of these doses. 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. Once the amphetamine treatment regimen was completed, animals were left with dams to be reared without further manipulation until weaning on PD 21, after which time they were housed in pairs with the same gender littermate until completion of behavioral testing. Open-field testing began on PD 24, a developmental stage that is equivalent to pre-adolescence, and both water maze and conditioned taste aversion testing began on PD 60, an age that is considered young adulthood in rats.

2.2. Intragastric intubation procedures

Intragastric intubation was used to deliver amphetamine in this study because (1) it is a relatively non-invasive and simple procedure that produces minimal stress to the animals, and (2) it effectively models an oral route of administration commonly found in abuse of amphetamine and amphetamine-like substances (Hegadoren, Baker, & Bourin, 1999). Each subject received two intubations daily with a 2-h inter-intubation interval. Intubation procedures were carried out at 10:00 a.m. and 12:00 noon each day. Litters, including dams, were removed from the vivarium and brought to a separate room for intubation procedures. Immediately prior to intubation procedures, the entire litter of pups was removed from the dam, placed into a container that was sitting on a heating pad until each subject from that litter had received their assigned treatment and then returned to the dam once all intubations had been completed. To intubate, a 15 cm piece of PE-10 tubing was attached to a 30 gauge needle on a 1 cc syringe, and the PE-10 tubing was advanced down the esophagus into the stomach. Each animal was fed two-twelfths of 33% of their body weight each day. This amount is based on previous studies showing adequate growth of the pups in artificial-rearing procedures (Allen, West, Chen, & Earnest, 2004; Chen & Harle, 2005; Smith, Zeve, Grisel, & Chen, 2005; Thomas, Goodlett, & West, 1998). The leading one-third of the intubation tubes was lubricated with mineral oil and the tip was smoothed to prevent damage to the esophagus. Each intubation episode was completed in approximately 20–30 s.

2.3. Open-field behavioral testing

On PD 24, animals ($n = 10$ and $n = 8$ for three amphetamine-treated and two control groups, respectively, with an equal number of males and females in all groups) began open-field behavioral testing using the SmartFrame Open-Field System from Kinder Scientific (Poway, CA). This system consists of a $40.6 \times 40.6 \times 38.1$ cm ($16 \times 16 \times 15$ in.) ($L \times W \times H$) Plexiglas chamber with 32 infrared