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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.

Keywords

Amphetamine; Brain Growth Spurt; Hippocampus-Mediated Behaviors; Open-Field; Water Maze; Conditioned Taste Aversion

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, and O'Donnell, 1992; Plessinger, 1998; Plessinger and Woods, 1998; Smith, LaGasse, Derauf, Grant, Shah, Arria, Huestis, Haning, Strauss, Della Grotta, Liu, and Lester, 2006). For example, children born to mothers who abused psychostimulants during pregnancy suffer from low birth weight/somatic growth rates (Billing, Eriksson, Steneroth, and Zetterstrom, 1985; Cernerud, Eriksson, Jonsson, Steneroth, and Zetterstrom, 1996), reduced head circumference (Chomchai, Na Manorum, Watanarungsan, Yossuck, and Chomchai, 2004; Oro and Dixon, 1987), cardiac defects (Little, Snell, and Gilstrap, 1988; Zimmerman, 1991), cerebral hemorrhage (Eriksson,

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Larsson, and Zetterstrom, 1981; Spires, Gordon, Choudhuri, Maldonado, and Chan, 1989), and stillbirth (Dearlove, Betteridge, and Henry, 1992; Loebstein and 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, ElSohly, Wright, Smeriglio, Verter, Bauer, Shankaran, Bada, Walls, Huestis, Finnegan, and Maza, 2001; Nulman, Rovet, Altmann, Bradley, Einarson, and Koren, 1994), which may subsequently interfere with behavioral development (Billing, Eriksson, Jonsson, Steneroth, and Zetterstrom, 1994; Noland, Singer, Short, Minnes, Arendt, Kirchner, and Bearer, 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, and Vorhees, 1996; Cho, Lyu, Lee, Kim, and Chin, 1991; Holson, Adams, Buelke-Sam, Gough, and Kimmel, 1985). Interestingly, many behavioral deficits observed following developmental psychostimulant exposure appeared to be involved, at least partially, in hippocampal functions (Bashkatova, Meunier, Maurice, and Vanin, 2005; Morford, Inman-Wood, Gudelsky, Williams, and Vorhees, 2002; Onaivi, Bishop-Robinson, Motley, Chakrabarti, and Chirwa, 1996; White and 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 and colleagues (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 and co-workers (2004) showed that infusion of N-methyl-D-aspartate (NMDA) to the ventral hippocampus 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 and colleagues (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, and Arias, 2005). Conversely, conditioned taste aversion, demonstrated earlier by Garcia and colleagues (1955), was thought to be mainly modulated by brain regions other than the hippocampus, such as the amygdala (Roldan and Bures, 1994; Yamamoto, Fujimoto, Shimura, and Sakai, 1995). However, evidence has emerged to show that the hippocampus may be partially involved in the acquisition of taste aversion (Gallo and Candido, 1995; Purves, Bonardi, and Hall, 1995; Reilly and 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 twenty-four 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-hr 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 sulphate employed in the current study have been used previously by our laboratory and others to demonstrate neonatal amphetamine-induced changes in development (Smith, Pappalardo, and Chen, 2008; Tavares and 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, and Bourin, 1999). Each subject received two intubations daily with a 2-hr 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, and Earnest, 2004; Chen and Harle, 2005; Smith, Zeve, Grisel, and Chen, 2005; Thomas, Goodlett, and 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 sec.

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 beams (16 beams in both the x- and y-axis). All activity was recorded using the MotorMonitor software program, from Kinder Scientific (Poway, CA). Animals underwent two 3-min trials per day (except the first day of testing, see below) for 3 consecutive days with a 2-min inter-trial interval. Prior to each trial, the internal surface of the open-field was cleaned with Nolvasan (1:10 ratio of Nolvasan to water) to eliminate any olfactory cues from previous trials. Animals underwent an additional trial that preceded all others on the first day of testing to examine their responses to a novel environment (naïve trial). The parameters measured each trial were: 1) basic movements (subject changes its entire body position on the grid), 2) fine movements (subject makes a movement without changing body position on the grid; e.g. moving head, cleaning), 3) rearing and 4) total distance travelled (cm). While the duration of time used in each open-field trial is relatively short (3 min), previous studies by both our laboratory and others have demonstrated that trials lasting between 2–5 min in length are sufficient to observe locomotor activity (Meng and Drugan, 1993; Romero and Chen, 2004). For open-field testing, the SmartFrame Open Field System from Hamilton-Kinder (San Diego, CA) was used. Data were analyzed using the MotorMonitor software program, also available from Hamilton-Kinder.

2.4. Modified Morris Water Maze

Beginning on PD 60, the same set of animals ($n = 46$) used in the open-field behavioral testing paradigm were subjected to the modified Morris water maze task to assess developmental amphetamine's ability to alter spatial learning capabilities during young adulthood. For testing purposes, a black bottom water tank (157.5 cm in diameter and 61 cm in height) was filled with water ($21 \pm 1^\circ\text{C}$) to a depth of 32.5 cm, and surrounded by curtains in the center of a room. Distinctive visual cues (4 total) that remained constant throughout the entire course of testing were placed on each curtain. A transparent escape platform (Plexiglas; 100 cm^2 in surface area and 31.5 cm tall) was then submerged 1 cm below the surface of the water, where it was not visible to animals. Animals underwent 5 training trials per day, with a 5-min inter-trial interval, for 6 consecutive days. For each trial, an experimenter placed an individual animal into the water facing the wall of the tank, and allowed the animal to swim until it located the escape platform. At the beginning of each trial, the animal was placed into the tank at the same location

which was in the quadrant opposite of the location of the escape platform. While this method is different from the classic Morris water maze behavioral task, inasmuch as start positions were not varied, the presence of distinctive distal cues served to differentiate the surroundings of the tank, allowing the animals to determine spatial coordinates in the tank relative to the escape platform. If the animal failed to locate the platform within 60 sec, it was manually guided to the platform by the experimenter. After the animal had located the escape platform (either through individual effort or assistance), it was allowed to rest for 10 sec before being removed from the tank, towel dried and placed in an inter-trial holding cage where a heating source was provided in order to maintain the animal's body temperature during the inter-trial interval. Following conclusion of the acquisition phase (training days 1–6), animals underwent a probe trial, where the escape platform was removed and each animal was allowed to swim for 90 sec. This probe trial took place 5 min after the completion of the last training trial on the last training day (day 6). A second probe trial was performed 24 hr after the previous one. During these probe trials, as well as during the acquisition phase, measurements were made concerning latency to the platform (sec) and pathlength taken to locate the escape platform (cm). In addition, during the probe trial, the number of passes through the target quadrant (the quadrant of the tank that previously contained the escape platform) and the mean distance of the animal from the escape platform (i.e., the averaged distance between the location of the animal and the platform, and this distance was measured every 0.2 sec for the entire probe trial; the lower the mean distance, the closer the animal was to the target) were measured. For water maze testing, the SMART Video Tracking System (San Diego Instruments; San Diego, CA) was employed to track the animals' movements and to analyze the resulting data.

2.5. Conditioned Taste Aversion

For the conditioned taste aversion paradigm, 10 males and 10 females ($n=20$) were used in all five treatment groups. Beginning on PD 60, animals were housed singly and underwent a partial water deprivation (access to water limited to 30-min per 24-hr period) for 3 days. This water deprivation phase was designed to train the animals to drink when water was available only during a short period of time. The following 3 days consisted of the conditioning phase, during which animals were given 30 min daily access to water containing 0.1% saccharin (conditioned stimulus, CS). Immediately following the 30 min drinking period, one-half of the animals in each treatment group received an intraperitoneal (IP) injection of 0.2 ml/100 g of body weight of 0.5 M lithium chloride (unconditioned stimulus, US) and the other half received saline (IP). Lithium chloride (LiCl) induces a gastric malaise (unconditioned response, UR) in the animals. Generally, the animal will form an association between this unpleasant response induced by LiCl and the consumption of the saccharin-containing water, causing the animals to develop an aversion to the taste of saccharin (conditioned response, CR). Following the third day of the conditioning schedule, animals were given full access to water for 24 hr to recover from any potential confounding effects of dehydration. Subsequent to this period of rehydration, water access was removed from the animals for 24 hr, and at the conclusion of this water deprivation period, animals were given a choice between two drinking tubes, one containing regular tap water and the other containing saccharin (0.1%). The amount of each fluid consumed over 30-min and 24-hr was measured to evaluate whether early postnatal amphetamine exposure interferes with the formation of a taste aversion to saccharin.

3. RESULTS

3.1. Locomotor Activity in the Open-Field

The two-way analysis of variance (ANOVA), with amphetamine treatment and gender as the two between factors, performed on the naive trial data resulted in no significant main effects of, nor an interaction for the mean number of basic movements, fine movements, rearings, or the total distance traveled. Further, no difference was found for any of the measured parameters

between the first and second daily trials, indicating that no habituation took place between the two testing sessions each day. As such, only the data from the first trial on each day was analyzed in this study. When the data from the first trial on all three days of testing were analyzed using a three-way mixed ANOVA with amphetamine treatment (NC, IC, AMPH 5, AMPH 15, AMPH 25) and gender (M, F) as the between (non-repeated) factors, and testing day (Day 1, Day 2, Day 3) as the within (repeated) factor. For basic movement, the analysis yielded main effects of amphetamine treatment ($F_{4,36} = 2.88$; $p < 0.05$) and testing day ($F_{2,72} = 6.79$; $p < 0.01$). For fine movement and mean total distance traveled, the data analyses performed showed similar statistical outcomes to those seen in the basic movement, with significant main effects of amphetamine treatment ($F_{4,36} = 3.36$; $p < 0.05$ for fine movement and $F_{4,36} = 2.82$; $p < 0.05$ for total distance traveled) and testing day ($F_{2,72} = 8.52$; $p < 0.001$ for fine movement and $F_{2,72} = 7.00$; $p < 0.01$ for mean total distance traveled). As to the data from the number of rearings, the only significant statistical outcome observed was the main effect of amphetamine treatment ($F_{4,36} = 3.63$; $p < 0.05$). Interestingly, no significant main effect of gender and interactions between/among any factors were obtained in any dependent measure. In order to further examine the main effect of amphetamine treatment, Newman-Keuls *post hoc* tests were performed on all dependent measures across both gender and all three testing days. The results showed that animals in the AMPH 25 group had fewer movements (basic, fine or rearings) and lower total distance traveled compared with animals in all other treatment groups (Fig. 1). Further analyses on the main effect of testing day showed that the mean number of movements (basic or fine) and total distance traveled in the open-field apparatus was higher on Day 2 or Day 3 of testing compared with that on Day 1. No difference was observed between Day 2 and Day 3 in any dependent measure.

3.2. Spatial Learning and Memory in the Modified Morris Water Maze

The data from both the acquisition phase and the probe trial of the water maze were analyzed using three-way mixed ANOVAs with amphetamine treatment and gender as two between factors, and the training day within the acquisition phase (six training days) or probe trial (probe trial 1, probe trial 2) as a within factor. For the acquisition phase, the ANOVAs resulted in a significant main effect of training day on latency to the escape platform ($F_{5,180} = 49.95$; $p < 0.0001$) and mean pathlength to the escape platform ($F_{5,180} = 49.36$; $p < 0.0001$) (Fig. 2 and 3). Neither the main effects of amphetamine treatment and gender, nor the interactions between/among the factors, were statistically significant. Regarding probe trials, the analyses performed on latency and pathlength did not reveal any significant outcomes suggesting that amphetamine treatment and gender did not significantly affect the performance in either probe trials. Additional analyses conducted on the time spent in the target quadrant and the mean distance to the target yielded a significant main effect of probe trial on the time spent in the target quadrant ($F_{1,36} = 89.67$; $p < 0.0001$) showing that the animals spent much less time in the target quadrant in the second probe trial compared to the first (Table 1).

3.3. Acquisition of a Conditioned Taste Aversion

For the conditioning phase of the conditioned taste aversion procedures, a four-way mixed ANOVA was performed on the amount of saccharin ingested (ml) with amphetamine treatment, gender and conditioning (LiCl, SAL) as between factors and conditioning day (three conditioning days) as a within factor. This analysis revealed significant main effects of gender, conditioning and conditioning day ($F_{1,80} = 56.78$, $F_{1,80} = 41.92$, and $F_{2,160} = 13.48$; p s < 0.0001 for gender, conditioning and conditioning day, respectively) (Fig. 4). In addition, the analysis yielded interactions between gender and conditioning ($F_{1,80} = 4.45$, $p < 0.05$), and between conditioning and conditioning day ($F_{2,160} = 33.69$, $p < 0.0001$). Further simple effect analyses showed that the animals assigned to the LiCl group ingested more saccharin solution on conditioning day 1 (prior to first LiCl injection) compared with other conditioning days, and gender did not significantly affect this ingestion pattern even though the overall analysis

indicated an interaction between gender and conditioning. Two-bottle testing phase data were analyzed using three-way ANOVAs with amphetamine treatment, gender and conditioning as between factors. For the amount of saccharin ingested at 30 min, the analysis showed a significant main effect of conditioning ($F_{1,80} = 188.57, p < 0.0001$). The animals that received LiCl treatment ingested significantly less saccharin, regardless of gender or amphetamine treatment, compared with animals receiving the saline injection. Similarly, there was a significant main effect of conditioning on the amount of saccharin ingested at 24 hr ($F_{1,80} = 262.71, p < 0.0001$) (Fig. 5). In order to evaluate whether the decrease in the amount of saccharin ingested in the LiCl groups was a function of an overall decrease in ingestion of solution (both saccharin and water), an analysis was performed on the total solution consumed at the end of the 24 hr testing phase. This statistical analysis showed a main effect of gender ($F_{1,80} = 11.91, p < 0.001$) and an interaction between gender and conditioning ($F_{1,80} = 5.54, p < 0.05$) (Fig. 6). Simple effect analysis showed that male animals receiving LiCl injections, regardless of amphetamine treatment, drank more solution within the 24-hr testing period than animals assigned to the saline (SAL) groups, and such a relationship was reversed in female animals. Nonetheless, no significant main effect of conditioning in total solution intake was observed, suggesting that the decrease in saccharin ingestion observed in the LiCl-treated animals was not simply due to a decrease in solution consumption.

4. DISCUSSION

The current study used three behavioral tasks, the open-field, the water maze and the conditioned taste aversion, to study how neonatal amphetamine exposure affects performance of behaviors that are shown to be related, at least in part, to hippocampal function. The results showed that amphetamine exposure during this developmental stage produced a dose-related effect on some, but not all, of the hippocampus-mediated behaviors measured by these tasks. Animals that received the highest dose of amphetamine (AMPH 25) showed significantly lower levels of locomotor activity in some open-field testing parameters. However, amphetamine exposure during the brain growth spurt did not affect the performance in the water maze or conditioned taste aversion paradigms. These findings indicate a differential effect of amphetamine exposure during the brain growth spurt in regards to the behavioral processes being evaluated.

It is generally believed that increased locomotor activity is indicative of lower anxiety levels in rodents (Holmes, Wrenn, Harris, Thayer, and Crawley, 2002). Based on the present findings, it appears that animals increase their locomotor activity as a function of testing day within the open-field suggesting that the animals have learned that this previously novel environment is not associated with a negative consequence (Romero and Chen, 2004). The present findings showed that animals from both the control groups, as well as those in the AMPH 5 and AMPH 15 groups increased their level of activity over testing days. However, animals that received the highest dose of amphetamine (25 mg/kg/day; AMPH 25) did not seem to show the same pattern of activity over the course of the three testing days with similar levels of activity across all three testing days. These results are in agreement with those demonstrating that animals exposed to methamphetamine during the first ten days of postnatal life showed decreased levels of locomotor activity (Vorhees, Ahrens, Acuff-Smith, Schilling, and Fisher, 1994b). Similarly, studies examining the effects of gestational cocaine exposure have found lower locomotor activity levels in response to developmental psychostimulant exposures (Campbell, Bliven, Silveri, Snyder, and Spear, 2000; Kunko, Wallace, and Robinson, 1996). Given that there was no difference in locomotor activity between any treatment group in either the naive trial or on the first day of testing, it is possible that the lack of changes in activity levels by the AMPH 25 animals resulted from a failure in their ability to discern whether this open-field environment was a novel or a familiar one. This, in turn, may be related to anxiety-induced stress interfering with learning.

Stress has been suggested to negatively impact proper development of learning and memory (Diamond, Campbell, Park, and Vouimba, 2004). Induction of long-term potentiation (LTP), one of the physiologic foundations of learning and memory (Eichenbaum, 1996; Martinez and Derrick, 1996), is inhibited by stress (Foy, Stanton, Levine, and Thompson, 1987). Since the novel environment of the open-field produces anxiety-related stress in animals, it is possible that the inability of animals to learn that this open-field setting is not associated with any negative consequences is initially blocked by the stressed-related factors. Furthermore, exposure to certain psychostimulants (e.g. cocaine) during either postnatal (Magalhaes, Tavares, and de Sousa, 2002) or adolescent periods (Carlezon, Mague, and Andersen, 2003) has been shown to impede an animal's ability to cope with stressful stimuli. It may therefore be surmised that amphetamine exposure during a period of time corresponding to the brain growth spurt in rats causes an inability to cope with the stress produced by a novel environment in these pre-adolescent rats leading to their failure of learning that the open-field environment was indeed safe (i.e., free from negative consequences).

Unlike the results of the open-field study, amphetamine exposure during the brain growth spurt did not interfere with spatial learning during young adulthood as measured using a modified water maze with invariant start and goal positions. Neonatal animals treated with amphetamine were able to locate the escape platform as effectively as those that received control treatment. In other words, the amount of time necessary for animals to locate the target (latency) and the pathlength traveled to reach it were similar across all treatment groups. These findings are contradictory to those reported by Vorhees and his colleagues (Vorhees, Inman-Wood, Morford, Broening, Fukumura, and Moran, 2000; Vorhees, Skelton, and Williams, 2007) showing that methamphetamine exposure during postnatal days 11–20 produces spatial learning deficits that persist into adulthood. Interestingly, an earlier report by the same research group (Vorhees) has also shown that exposure to methamphetamine during postnatal days 1–10 has no effect on this spatial learning paradigm (Vorhees, Ahrens, Acuff-Smith, Schilling, and Fisher, 1994a). Additional effort by the Vorhees group further identified postnatal days 11–15, not postnatal days 16–20, being the most vulnerable and sensitive developmental stage to methamphetamine (Williams, Moran, and Vorhees, 2003). It should also be noted that there are a few differences between the classic Morris water maze paradigm, and the modified one used in the present study. In the majority of Morris water maze paradigms found in the literature, the starting location for entry to the water maze is varied so that the animal begins at a different point on each trial of each day. In the current study, animals were placed in the same starting position each day. As a result, it is possible that the ability of the animals to locate the escape platform was the result of the animals “recognizing” or “memorizing” a specific path, rather than learning the actual location of the escape platform. However, as was discussed above, the immediate surroundings of the water maze were identical except for a distinct distal cue on all four sides of the maze, it is still possible that the animals were able to locate the specific path by learning the relationship of the escape platform relative to the distal cues. This outcome would suggest that learning did take place, and is supported by the findings that all animals, regardless of prior amphetamine treatment, were able to locate the escape platform. When taken together with findings from other studies (Acevedo, de Esch, and Raber, 2007), the current results suggest that there is a window of temporal vulnerability in psychostimulant-induced deficits that may be specific to spatial learning and memory.

In the present study, open-field behavioral testing was performed during a period of time corresponding to late pubescence/early adolescence (PD 24 – 26), while the Morris water maze task was performed in animals that were considered to be young adults (PD 60 – 66). Given that the intention of this study was the effects of neonatal amphetamine exposure on the development of hippocampus-mediated behaviors, it is important to examine behavioral development at several different ages. As was described above, the water maze was used to measure spatial learning. In addition, the water maze also measures locomotor activity, as

evidenced by the total distance travelled parameter that we measured. As a result, it was determined that performing locomotor activity assessments using the open-field behavioral paradigm in young adult animals was unnecessary; therefore, in order to keep the number of animals required to complete this study at a minimum, which is in accordance with Public Health Services (PHS) recommendations/guidelines, only the water maze was used at the young adulthood time point. Conversely, since the majority of studies using the water maze task do not use animals that are less than 35 days of age, the open-field behavioral paradigm was used to measure locomotor activity in younger animals. The fact that differences are seen between the locomotor activity seen in the open-field results performed during late pubescence and the water maze performed in young adulthood suggests that the effects of amphetamine exposure are either 1) transient in nature, or 2) dependent upon the task which is used to examine them. Similar to the results of the water maze testing, neonatal amphetamine exposure did not impair the acquisition of a conditioned taste aversion in young adult rats. In general, animals in all experimental treatment groups were able to form the association between saccharin and gastric malaise induced by LiCl. It is unclear at present whether there is a window of temporal vulnerability for amphetamine to interfere with the acquisition of conditioned taste aversion that is similar to spatial learning and memory. However, one potential explanation for the lack of amphetamine effect may be related to the strength of the aversion produced by the dose/concentration of LiCl used in this study. Such a salient aversion may have potentiated the learning of this taste aversion among amphetamine-treated animals. Future studies, using a lower dose/concentration of LiCl or increasing the interval between the drinking of saccharin solution and the LiCl injection (such as trace conditioning), may be warranted to advance the understanding of how neonatal amphetamine exposure affects the performance of classical conditioning paradigms.

The present findings illustrate that exposure to amphetamine during a time period that corresponds to the early portion of the brain growth spurt has limited effects on the performance of the behavioral tasks used in this study, with the exception that the highest dose of amphetamine resulted in behavioral alteration in open-field testing. One possible explanation for these results is the disassociation within the hippocampus of anxiety- and memory-related behaviors (Bertoglio, Joca, and Guimaraes, 2006; Callaerts-Vegh, Beckers, Ball, Baeyens, Callaerts, Cryan, Molnar, and D'Hooge, 2006). Evidence has suggested that anxiety-related behaviors, such as those measured in the elevated plus maze, are heavily dependent on activity within the ventral hippocampus (McHugh, Deacon, Rawlins, and Bannerman, 2004; Pentkowski, Blanchard, Lever, Litvin, and Blanchard, 2006), while functions related to spatial learning rely primarily on the functions of the dorsal hippocampus (Cimadevilla et al., 2005; Moser, Moser, and Andersen, 1993; Zhang et al., 2004). Although there are currently no empirical studies that have specifically examined the effects of neonatal amphetamine exposure on the specific poles of the hippocampus, adult studies have shown that methamphetamine significantly decreases the volume of the ventral, but not dorsal, hippocampus (Shishido, Shishido, Kato, Watanabe, Ono, Kobayashi, Horikoshi, and Niwa, 2000) showing a regional vulnerability of methamphetamine-induced hippocampal injury. However, this regional vulnerability issue is further complicated by its interaction with the concept of a window of temporal vulnerability, since it appears that the developing hippocampus is more vulnerable to methamphetamine (or amphetamine-like substances) during the latter portion of the brain growth spurt (postnatal days 11–15). In sum, while only one of the behavioral tasks assessed in this study was impacted by neonatal amphetamine exposure, these findings provide a strong argument that future studies examining the effects of developmental amphetamine (or amphetamine-like drugs) on brain/behavioral development ought to be designed, in particular, with a consideration of the complex interaction between the “temporal” (e.g., time of exposure) and “regional” (e.g., ventral vs. dorsal hippocampus) factors in order to elucidate the developmental neurotoxicity produced by amphetamines.

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Figure 1.

Locomotor activity in the open-field. Mean number of basic movements (A), fine movements (B), rearings (C) and total distance traveled (D). The analyses showed main effects of amphetamine treatment with AMPH 25 significantly different from all other treatment groups across gender and testing day (p 's < 0.05; as indicated by “*”) Furthermore, the data from Day 2 and Day 3, across gender and amphetamine treatment, were significantly different from those from Day 1 (p 's < 0.05; as indicated by “#” in Figures 1A, 1B and 1D). There were no differences between male and female rats in any dependent variable, thus, the data presented in this figure were collapsed between male and female rats. Vertical bars represent standard errors of the means (SEMs).

Figure 2. Learning in the modified water maze. Representation of the path to the escape platform for one animal from the NC group. Tracks represent the first trial each day of training in the modified water maze. Each value represents the total pathlength traveled on the first trial for each day (cm).

Figure 3.

Target acquisition training in the modified water maze. Mean latency to escape platform (A) and pathlength traveled (B) over the six training days in the modified water maze. The overall analyses revealed significant main effects of training day in both latency and pathlength during the acquisition period (p 's < 0.05). There were no significant main effects of amphetamine and gender. The data presented in this figure were collapsed between male and female rats. Vertical bars indicate standard errors of the means (SEMs).

Figure 4.

Acquisition of a conditioned taste aversion. Change in saccharin solution consumption (ml) by LiCl-treated males (A) and females (C), and saline-treated males (B) and females (D). The overall analysis showed interactions between gender and conditioning, and between conditioning and conditioning day. Further analyses showed that animals in the LiCl groups, regardless of gender, showed decreases in saccharin consumption as a function of conditioning day with more saccharin solution drank on the first conditioning day prior to the first LiCl injection (p 's < 0.05; as indicated by "*"). Such a decrease in saccharin consumption was not observed in SAL-treated subjects, regardless of gender. The male and female data were presented separated in this figure due to gender-related differences observed. Vertical bars indicate standard errors of the mean (SEMs).

Figure 5.

Two-bottle choice test results in the conditioned taste aversion. Volume of saccharin solution consumed (ml) by animals that received either LiCl or saline injections after 30 min (A) or 24 hr (B). The LiCl-treated animals, regardless of gender, drank significantly less saccharin solution compared with the saline-treated animals (p 's < 0.05 ; as indicated by “*”). There was no difference in saccharin consumed at either 30 min or 24 hr, thus, the data between male and female rats were combined in this figure. Vertical bars indicate standard errors of the mean (SEMs).

Figure 6.

Gender-related differences in total fluid consumption. Total volume (both water and saccharin solution; ml) consumed by animals during the 24 hr two-bottle choice test. There was no difference in total solution consumed as a function of amphetamine treatment. Nonetheless, the female rats, across all treatment groups, showed significantly less volume of solution consumed within 24-hr testing period. Vertical bars indicate standard errors of the mean (SEMs).

Table 1

Modified water maze probe trial results. Mean daily values for the different parameters measured in the modified water maze. Numbers in parentheses indicate standard error of the means (SEMs).

Variable	Probe Trial 1	Probe Trial 2
Mean latency (sec)		
NC	10.5 (1.5)	20.3(7.8)
IC	13.4 (4.3)	20.0(6.7)
AMPH 5	12.1 (3.6)	14.1(7.4)
AMPH 15	16.3 (3.3)	13.0(1.7)
AMPH 25	11.8 (2.6)	11.2(2.1)
Mean pathlength (cm)		
NC	206.0 (26.0)	444.5(199.0)
IC	283.2 (79.0)	358.1(101.0)
AMPH 5	260.6 (63.6)	331.2(158.8)
AMPH 15	348.1 (67.2)	290.0(43.8)
AMPH 25	277.3 (58.9)	264.6(44.4)
Time in target quadrant (%)		
NC	41.1 (2.9)	26.5(2.2)
IC	37.7 (3.2)	27.8(1.6)
AMPH 5	41.5 (3.5)	31.7(2.8)
AMPH 15	39.2 (2.0)	30.1(1.7)
AMPH 25	45.7 (2.6)	30.7(2.4)
Mean distance to target (cm)		
NC	55.9 (5.1)	64.3(4.9)
IC	62.7 (4.9)	59.1(2.8)
AMPH 5	54.6 (4.4)	59.7(3.6)
AMPH 15	52.5 (3.4)	54.0(2.1)
AMPH 25	61.9 (3.7)	61.7(2.9)