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Learning and Memory After Neonatal Exposure to 3,4-Methylenedioxymethamphetamine (Ecstasy) in Rats: Interaction With Exposure in Adulthood

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Abstract

This study determined whether developmental and adult 3,4-methylenedioxymethamphetamine (MDMA) exposures in rats have interactive effects on body temperature, learning, other behaviors, and monoamine concentrations in the hippocampus, prefrontal cortex, and striatum. Learning was assessed in the Cincinnati water maze (CWM), Morris water maze (MWM), and novel object recognition (NOR). On acquisition trials in the MWM, significant differences from developmental MDMA exposure were found on latency, cumulative distance, path length, and angle of first bearing to the goal, but the early and adult MDMA exposure group performed no worse than the developmental-only MDMA group. In the reversal trials, however, an interaction was seen: latency to the goal, cumulative distance, and angle of first bearing were increased in animals treated both developmentally and in adulthood with MDMA compared with those treated only developmentally. Other tests (elevated zero maze, CWM, NOR, and open-field activity) did not show an interaction, nor did hippocampal concentrations of serotonin or dopamine. However, several behavioral tests showed neonatal MDMA effects, including increased errors in the CWM, reduced time spent with a new object in the NOR test, and reduced locomotor activity in the open-field. By contrast, adult MDMA decreased the number of entries into open quadrants of the elevated zero maze. Litter effects were controlled by treating litter as the experimental unit and using mixed models repeated measures analyses. Correlational analyses suggested that the MWM reversal interaction involves multiple monoamine changes. The results indicate that developmental MDMA exposure can interact with adult exposure to interfere with some aspects of learning.

Keywords

MDMA; development; amphetamines; serotonin; dopamine; spatial learning; preclinical

INTRODUCTION

Chronic abuse of 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) among adult users is associated with persistent memory impairments (Morgan, 1999; Parrott, 2000; Rodgers, 2000), heightened behavioral impulsivity (Halpern et al., 2004; Morgan, 1998; Parrott,

2000), other cognitive deficits, such as strategic, planning, and attentional abilities (McCardle et al., 2004; Morgan, 2000; Parrott, 2000), and compromised processing speed (Halpern et al., 2004). Psychological effects persist even in abstinent users (McCardle et al., 2004; Morgan, 2000; Verkes et al., 2001), and the extent of previous drug use affects the degree of cognitive deficit observed (Dafters et al., 2004; Halpern et al., 2004; Morgan et al., 2002). For example, reduced memory performance is associated with heavier MDMA use (Turner and Parrott, 2000). Acute effects in humans (such as hyperthermia, prepulse inhibition of the startle response, and serotonergic correlates) parallel those observed for animals, thus validating preclinical animal studies of the effects of MDMA (Green et al., 2003; Morford et al., 2004; Turner and Parrott, 2000). Indeed, some developmental toxicological landmarks overlap for humans and animals (Morford et al., 2004). Because lasting changes in brain serotonin innervation in developmentally and adult MDMA-treated animals have been reported (Broening et al., 2001; Fischer et al., 1995), the neuronal effects of developmental MDMA may interact with adult exposure. Thus, the purpose of the present study was to determine if differences occur in hyperthermic response, learning and memory, other behaviors, and in monoamine concentrations in rats treated neonatally with MDMA (during a period corresponding to the third trimester of human pregnancy; Bayer et al., 1993) followed by exposure to the drug in adulthood at doses sufficient to cause reductions in brain serotonin (5-HT) (Green et al., 2003). Dosing from P1–10 has shown almost no effects on adult learning and memory, whereas dose-related impairments occurred in relation to P11–20 drug administration (Broening et al., 2001). The P11–20 exposure period correlates with human third trimester dentate granule cell neurogenesis (Bayer et al., 1993). In addition, it has been shown that third trimester MDMA exposure is associated with adverse effects on the infants. Although MDMA exposure during the first trimester occurs more frequently than does third trimester exposure, McElhatton et al.'s (1999) data indicate that ~4% of MDMA exposures occurred during the third trimester in a group of 136 prenatal MDMA cases.

Most behavioral studies in animals have focused on either the neonatal effects of MDMA (Broening et al., 2001; Koprach et al., 2003; Sprague et al., 2003; Vorhees et al., 2004; Williams et al., 2003b) or adult effects (Bull et al., 2004; Cole and Sumnall, 2003; Green et al., 2003; Meyer et al., 2004), rather than on a combination of neonatal and adult exposure. The lack of data on this topic exists despite the fact that it has been shown that prior adult exposure to MDMA produces changes in subsequent MDMA self-administration (Cole and Sumnall, 2003). Specifically, prior exposure to MDMA enhances the rewarding actions of cocaine (Horan et al., 2000) and reduces the rewarding properties of ethanol (Cole et al., 2003). However, no studies exist on whether early MDMA exposure changes adult responses to MDMA itself.

The present experiment builds upon previously identified, neonatally-acquired MDMA-induced deficits in path integration and spatial learning and memory (Broening et al., 2001; Vorhees et al., 2004; Williams et al., 2003b) and compares these effects with animals receiving MDMA only as adults or receiving MDMA both neonatally and as adults. Relationships between adult MDMA-induced body temperature changes, serotonergic and dopaminergic monoamines, and behavior were analyzed so as to determine if the behavioral effects could be related to monoamine markers or monoamine markers could be related to body temperature changes. In addition, a multiple correlational approach was taken as it is known that single neurotransmitter changes seldom explain behavioral effects because behavior is multifactorially determined.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley CD IGS rats bred from stock obtained from Charles River, Raleigh, NC, were randomly assigned to neonatal treatment groups (MDMA or saline) and later subdivided for adult exposure to MDMA or saline. Before breeding, dams were acclimated in the colony room for one or more weeks and then placed with male breeders. Embryonic day 0 (E0) was assigned when a sperm plug was detected. After two weeks of housing with a male, each dam was removed from the breeding cage and transferred to an individual polycarbonate cage. Beginning on E20, dams were checked for litters with the date of birth designated as postnatal day 0 (P0). Litters were culled to eight males on P1 using a random assignment system. Food and water were provided ad libitum, and temperature in the vivarium was maintained at $21 \pm 1^\circ\text{C}$. Only males were used in the present study, since we have seen only minor (if any) differences between males and females following neonatal MDMA exposure. The experimental protocol was approved by the Cincinnati Children's Research Foundation Laboratory Animal Care and Use Committee, and the vivarium is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Drugs and treatments

Pairs of males within a litter ($n = 15$ litters) received either 20 mg/kg ($\pm$)-MDMA HCl (expressed as the free base, MDMA was obtained from the National Institute on Drug Abuse through its provider Research Triangle Institute, Research Triangle Park, NC) in saline or saline alone, s.c., 2 times/day (8 h apart) on P11–20. Animals were weighed prior to each dose and injection sites were varied to minimize dermal irritation. On P28, animals were weaned and separated into pairs composed of one neonatally saline-treated and one neonatally MDMA-treated rat. Between P80 and P98, temperature transponders (IPTT-200, BioMedic Data Systems, Seaford, DE) were injected s.c. in the dorsum of animals under isoflurane anesthesia as previously reported (Williams et al., 2002). A baseline temperature was obtained at that time. Subsequently, rats were administered 15 mg/kg ($\pm$)-MDMA HCl (expressed as the free base) in saline or saline alone, s.c., four times at 2-h intervals between P82–100 in cohorts balanced for neonatal treatment. Treatment groups were randomized for neonatal and adult treatment into four groups as follows: (1) MM = MDMA (P11–20) + MDMA (Adult); (2) MS = MDMA (P11–20) + Saline (Adult); (3) SM = Saline (P11–20) + MDMA (Adult); and (4) SS = Saline (P11–20) + Saline (Adult). Temperatures were monitored every 30 min after the first injection until 4 h after the last injection. Animals were placed in a cage with shallow water to provide cooling if body temperature exceeded 40°C or rose 3° above baseline. During and following the adult injection period, rats were maintained in individual housing.

Behavioral procedures

Zero maze—Elevated zero maze (Shepherd et al., 1994) assessment commenced 1 week after the adult injections. This test measures anxiety using an elevated zero-shaped apparatus (105 cm diameter and 10 cm path width) with two closed areas separating two open areas spaced evenly apart, as detailed previously (Williams et al., 2003a). The test was conducted under dim halogen lighting. In a 5-min videotaped trial, behavior was scored as follows: entries and time in the open (front paws outside of the closed area); head dips in open areas; and stretch-attend postures (stretching into an open area with torso and forepaws remaining in a closed area). The maze was cleaned with 70% ethanol between animals.

Straight channel—Straight channel swims, as described previously (Broening et al., 2001; Williams et al., 2003a), commenced 1 day after the elevated zero maze test. A trial was started with the rat facing the end wall of the channel at the opposite end from an escape ladder. Rats swam the 15×244 cm channel and escaped from the water by climbing on the ladder. A total

of four trials were conducted (2 min limit/trial). Latency to reach the goal was measured for each trial with a stopwatch.

Morris water maze acquisition—Morris water maze (MWM) testing commenced the week following straight channel testing. The MWM apparatus, used herein for assessing spatial learning, was a 210-cm diameter black, circular tank. Within the tank, a 10 × 10 cm platform was submerged ~2 cm beneath the water. Distal room cues were made available to the animals and included (in addition to features already present in the room) a set of white curtains (bundled and tied) and several added geometric shapes mounted on the walls. The animals were given 4 trials/day (2 min maximum/trial) separated by an intertrial interval of 15 s. For acquisition, the platform was located in the southwest quadrant, with north defined as the furthest position from the experimenter. The animal was started at distal positions (with head facing the wall) that did not include locations immediately adjacent to the platform quadrant, as defined previously (Williams et al., 2003a). These were NW, N, E, SE, but not W or S because the latter are close to the platform. Each trial was recorded by a camera located over the tank and attached to a computer and monitor. The performance of each rat was tracked automatically using a video tracking system (Polytrack System, San Diego Instruments, San Diego, CA). Animals were tested for acquisition on five consecutive days (for a total of 20 trials) and then, on the subsequent day, given one, 30-s probe (memory) trial in which the platform was removed. For the probe trial, the rat was started from the NE position, a unique start point that it had not experienced previously. For acquisition learning trials, latency to the goal, path length, cumulative distance, and angle of first bearing (measured when the animal had moved 13 cm from the start) were recorded. Animals that did not find the platform in a trial were placed on the platform for the duration of the intertrial interval. For the probe trial, first bearing, average distance from the platform's former position, and percent time spent in the target quadrant were analyzed.

MWM reversal—Reversal training in the MWM commenced the week following acquisition. The dependent variables were the same as for acquisition. During reversal, the platform was reduced in size to 5 × 5 cm and situated in the opposite (NE) quadrant and the start positions adjusted accordingly, however, all other aspects remained the same as in acquisition. The day after the 5 days of reversal learning, a probe trial was conducted with the platform removed, and the dependent measures were the same as in acquisition.

Cincinnati maze—Cincinnati water maze (CWM) testing commenced the week following MWM reversal testing. The maze is a nine-unit multiple T maze placed in water as described previously (Vorhees, 1987). Path integration is required for the animal to solve the maze and reach an escape ladder.

Animals were given two 5-min trials/day spaced at least 5 min apart. The number of dead-end arms entered (errors) and latency to escape were measured for six consecutive days. Animals that did not solve the maze in 5 minutes were assigned a latency of 5 minutes and removed. An additional set of two trials was conducted 2 weeks after the initial learning to measure retention of the task.

Novel object recognition—Novel object recognition (NOR) and locomotor activity were conducted after the tests described earlier were completed because we previously tested for P11–20 MDMA effects without these two tests, and we did not want experience from these tests to alter the out-come on the maze procedures, which were central to the hypothesis under investigation.

NOR, based on the method introduced previously (Clark et al., 2000), commenced the week following the CWM. Animals were placed in 91-cm circular chambers and habituated for four

consecutive days for 10 min each day. Day 5 consisted of two phases, familiarization and retention. During familiarization, two identical objects, equidistant from the sides, were placed within the chamber and the animal was then placed in the center and allowed to freely explore. For this phase to be complete, the animal had to accumulate 30 s of exploration of both objects combined. Object exploration, scored when the animal was within 1 cm of the object and attending to it, was scored using a computer program kindly provided by Robert Clark, Ph.D. One hour later, a retention phase was administered in which two new objects were placed in the arena: a novel object replaced one of the familiar objects, and an identical copy of the other served to control for residual olfactory cues. During this phase, exploration was again scored until 30 s of cumulative attention had accrued. For each object, the number of explorations and the time spent exploring the object was computed. Test chambers and objects were cleaned with 70% ethanol between animals.

Open-field activity—Open-field/locomotor activity testing commenced the week following NOR testing. Animals were placed in a 41 × 41 cm activity chamber for 1 h, undisturbed (AccuScan, Columbus, OH). Horizontal activity (a count of the total number of beam interruptions in the horizontal sensor), total distance traversed (path length), rears (number of vertical movements separated by 1 s), distance traveled in the margin or corners, time spent within 1 cm of the walls, distance traveled in the center, and time spent away (greater than 1 cm) from the walls were measured.

Tissue collection and biochemical analysis

Two days after the last behavioral test (6 weeks after adult drug administration), animals were decapitated and brains removed. The hippocampus, prefrontal cortex, and neostriatum were dissected from the brain and stored at −80°C for later analysis of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA), and 3,4-dihydroxyphenylacetic acid (DOPAC). The neostriatum was inadvertently retained from only 8 out of the 15 litters. Monoamines were analyzed by high-pressure liquid chromatography with electrochemical detection (Nair and Gudelsky, 2004). In the hippocampus, 5-HT and 5-HIAA were measured, and in the prefrontal cortex and striatum, 5-HT, 5-HIAA, DA, and DOPAC were measured. Ratios of 5-HIAA/5-HT and DOPAC/DA were calculated. The thymus and adrenal weights were also obtained for each animal.

Statistics

Data were analyzed using SAS[®] mixed models repeated measures methodology (Littell et al., 1996; Littell et al., 2002) with litter as the experimental unit to control for litter effects (Holson and Pearce, 1992). Estimates of main-effect differences, namely, adult MDMA (M) vs. saline (S) and neonatal MDMA (M) vs. saline (S), and the simple-effect differences, MM vs. MS and MM vs. SM were computed for mixed model analyses of all behaviors, tissue weights, and monoamines. For the simple-effect differences, significance levels were reported only in instances in which both differences were significant. The same estimate procedure was followed for the body temperature data with the exception that polynomial regression coefficient differences were not tested for main-effects. For NOR and open-field activity, simple-effect difference estimates for SS vs. MS were computed and significant findings reported. This comparison was added to NOR and open-field assessments because neither behaviors have previously been assessed in neonatal, MDMA-treated animals. Where applicable, data were transformed to correct for heterogeneous variances, although nontransformed data are presented graphically.

Interrelationships between the hippocampal and prefrontal cortex monoamine measures and behavior, irrespective of treatment, were assessed by canonical correlation. In this procedure, linear combinations of either serotonergic or dopaminergic variables are computed and each

combination is tested in relation to a linear combination of behavioral performance variables (SAS Institute Inc., 1999). Individual correlations were examined for the limited data set obtained for the neostriatum. Significance was set at $P \leq 0.05$.

RESULTS

Temperatures

Temperatures were not monitored during the neonatal period, since others have demonstrated that neonatal animals do not display hyperthermia (Broening et al., 1995; Meyer et al., 2004); however, adult temperatures were monitored during MDMA exposure because of its known hyperthermic effect. A total of 25 animals were placed in water baths for cooling. Two of the 25 died despite placement in water. As expected, adult MDMA treatment resulted in significantly higher body temperatures [$F(1,33.9) = 9.24$, $P < 0.01$]. Polynomial analyses of group temperature increases from time 0 (just before the onset of dosing) are shown in Figure 1. For linear, quadratic and cubic regression coefficients, there were significant differences for MM vs. MS [$F(1,278) = 22.14$, $F(1,280) = 18.71$, and $F(1,281) = 15.20$, respectively; $P \leq 0.0001$] and MM vs. SM [$F(1,276) = 5.67$, $F(1,276) = 5.74$, and $F(1,276) = 4.65$, respectively; $P \leq 0.05$] and no differences between the treatment groups at time 0. These results indicate that the MM-group underwent a complex pattern of temperature change that went beyond either simple neonatal or adult MDMA exposure.

The effect of the cooling intervention for those groups that received adult MDMA (groups SM and MM) was examined by combining and sorting these groups into those animals that were cooled and those that were not. These subgroups were then compared on all monoamine markers and for MWM acquisition and reversal latencies. Even using multiple *t*-tests without correction for multiple comparisons, there were no statistically significant differences between the cooled and uncooled adult MDMA-treated animals on any monoamine marker or on MWM latency measures.

Behavioral tests

Elevated zero maze—For the zero maze, there was a significant effect of adult MDMA treatment on entries into the open [$F(1,14) = 8.35$, $P < 0.05$]; however, there was no significant effect of neonatal MDMA treatment. Rats treated with MDMA in adulthood had fewer entries into the open than saline-treated rats, irrespective of neonatal treatment condition (Fig. 2A). No differences were observed for the percent time in the open, number of stretch-attend movements or head dips between the treatment groups (see Fig. 2B).

Straight channel swim—There were no significant treatment effects for straight channel swimming times. There was a significant effect of trial [$F(3,144) = 26.56$, $P < 0.0001$]. Latencies decreased to an asymptotic, minimum level over the four trials for all treatment groups (not shown).

MWM-acquisition—As shown in Figure 3, in the MWM, acquisition trials resulted in a significant effect of neonatal treatment on latency [$F(1,152) = 19.45$, $P < 0.0001$, Fig. 3A], path length [$F(1,164) = 15.68$, $P = 0.0001$, data not shown], cumulative distance [$F(1,158) = 23.62$, $P < 0.0001$; Fig. 3C], and first bearing [$F(1,189) = 24.80$, $P < 0.0001$; Fig. 3E]. Animals treated with neonatal MDMA had deficits on each of these measures compared with animals treated neonatally with saline, irrespective of adult treatment. There were no significant differences resulting from adult administration or for the simple-effect comparisons (see Figs. 3B, 3D, and 3F). There were no significant differences obtained on the probe trial on any measure.

MWM-reversal—In the reversal trials, there again was a significant neonatal treatment effect on latency [$F(1,158) = 6.50, P = 0.01$], cumulative distance [$F(1,152) = 10.76, P = 0.001$], and first bearing [$F(1,40.4) = 9.67, P < 0.01$; not shown]. A significant adult effect was observed for latency [$F(1,172) = 5.90, P < 0.05$] and cumulative distance [$F(1,164) = 7.45, P = 0.01$; not shown]; however, simple-effect differences were obtained between MM and MS, and MM and SM for latency [$F(1,173) = 4.75, P < 0.05$; $F(1,177) = 6.30, P < 0.05$, respectively; Fig. 4A], cumulative distance [$F(1,166) = 8.01, P < 0.01$; $F(1,169) = 12.39, P = 0.001$, respectively; Fig. 4B], and first bearing [$F(1,50.7) = 5.29, P < 0.05$; $F(1,47.8) = 13.03, P = 0.001$; respectively; Fig. 4C]. The magnitude of the combination can be summarized as follows. In comparison with Group MS, Group MM performed 11.2%, 26.0%, and 28.0% worse on first bearing, cumulative distance, and latency, respectively. In comparison with Group SM, Group MM performed 20.8%, 21.9%, and 24.0% worse on first bearing, cumulative distance, and latency, respectively. In the reversal probe trial (data not shown), adult MDMA treatment resulted in a larger angle of first bearing ($68.9^\circ \pm 5.37^\circ$) than did saline treatment ($52.3^\circ \pm 5.03^\circ$) [$F(1,14) = 7.62, P < 0.05$].

Cincinnati water maze—The Cincinnati Maze revealed an effect of neonatal MDMA treatment on errors [$F(1,40.2) = 4.84, P < 0.05$; Fig. 5A]. No adult MDMA treatment or simple-effect differences were observed (Fig. 5B). No differences were noted during the retention phase.

Novel object recognition—Neonatally MDMA-treated rats spent less time investigating a novel object during the NOR task relative to neonatally saline-treated rats [$F(1,19.8) = 9.61, P = 0.01$; Fig. 6A]. No adult treatment or simple-effect differences were observed [the MS vs. SS comparison missed significance at $P = 0.08, F(1,24.5) = 3.42$; Fig. 6B].

Open-field—There were significant neonatal treatment effects on locomotor activity (Fig. 7). Animals treated with neonatal MDMA traversed less total distance [$F(1,14) = 10.69, P = 0.01$; Fig. 7A], distance in the center [$F(1,42) = 12.32, P = 0.01$; Fig. 7A], and spent less time away (>1 cm) from the walls of the chamber [$F(1,14) = 22.28, P < 0.001$; Fig. 7C] compared with those of neonatally saline-treated animals. Neonatal MDMA treatment also increased the time spent within 1 cm of the walls (thigmotaxis) relative to animals treated with saline during the neonatal period [$F(1,42) = 10.69, P = 0.01$; Fig. 7C]. Adult administration of MDMA also significantly decreased the time spent away from the walls [924.1 ± 141.47 (S) vs. 732.2 ± 122.08 (M); $F(1,14) = 5.11, P < 0.05$]. Differences between the MS vs. SS groups were noted for total distance [$F(1,14) = 11.49, P < 0.01$], distance in the center [$F(1,14) = 7.34, P < 0.05$], time away from the walls [$F(1,14) = 9.00, P = 0.01$], and thigmotaxis [$F(1,14) = 5.48, P < 0.05$].

Tissue weights and biochemical analyses

No differences in adrenal or thymus weights were obtained and there were no simple-effect differences between neonatal and adult MDMA exposure with regard to the monoamine measures.

In the hippocampus (Table I), adult MDMA was associated with significant reductions in 5-HT [$F(1,14) = 74.13, P < 0.0001$] and 5-HIAA [$F(1,14) = 115.78, P < 0.0001$]. Neonatal MDMA was also associated with a significant reduction in 5-HIAA [$F(1,14) = 9.67, P = 0.01$], but not in 5-HT or the ratio of 5-HIAA:5-HT.

In the prefrontal cortex (Table I), adult MDMA was associated with significant reductions in 5-HT [$F(1,14) = 32.95, P < 0.0001$] and 5-HIAA [$F(1,14) = 56.40, P < 0.0001$] and an increase in DOPAC [$F(1,14) = 6.00, P < 0.05$] with no significant changes in DA. No differences in

the ratios of metabolite and parent were observed. There were no significant effects of neonatal MDMA treatment.

In the striatum (Table I), adult MDMA was associated with significant reductions in 5-HT [$F(1,7) = 5.71, P < 0.05$] and 5-HIAA [$F(1,7) = 17.89, P < 0.01$] with no significant change in the 5-HIAA:5-HT ratio. Furthermore, these animals had a significant reduction in DA [$F(1,7) = 87.61, P < 0.0001$] but not in DOPAC and an increased DOPAC:DA ratio [$F(1,7) = 14.44, P = 0.01$]. No differences resulted from neonatal exposure.

In summary, the magnitude of the adult MDMA-induced depletions was hippocampus (5-HT, 46%; 5-HIAA, 52%), prefrontal cortex (5-HT, 44%; 5-HIAA, 37%), and striatum (5-HT, 39%; 5-HIAA, 37%; DA, 33%). DOPAC doubled in the prefrontal cortex, and the utilization of DA in the striatum of adult MDMA-treated animals was enhanced 75%. A small reduction (13%) in 5-HIAA in the hippocampus was associated with neonatal MDMA treatment.

Correlations of monoamines and behavior

For an examination of whether the behavioral variables were related to the hippocampal, prefrontal cortex, or striatal monoamine levels, we generated the analyses presented in Table II, which shows the following. (1) Hippocampal serotonergic markers were significant for MWM acquisition summed over trials and days, CWM latencies, and NOR. For MWM acquisition, the correlation over trials for first bearing was also significant. (2) Prefrontal cortex serotonergic markers were significantly correlated to MWM reversal probe, CWM errors, and NOR, and (3) Prefrontal cortex dopaminergic markers were significantly correlated with MWM acquisition for angle of first bearing over trials and reversal cumulative distance over trials.

Striatal monoamine correlations ($|r| \geq 0.39, P \leq 0.05$; not shown) showed more serotonergic (21/32) rather than dopaminergic markers for MWM behaviors and the zero maze (2/3), whereas open-field activity and NOR showed more correlations to the DA system (5/7 and 2/2, respectively).

DISCUSSION

As a result of preclinical studies on the effects of early developmental MDMA exposure, it has been suggested that MDMA use during pregnancy may be increasing the risk for abnormal neural and behavioral development in the offspring (Broening et al., 2001; Koprich et al., 2003; Meyer et al., 2004; Vorhees et al., 2004; Williams et al., 2003b). Clinical data are limited. One small clinical study reports an increased risk of selected congenital malformations in humans (McElhatton et al., 1999), and one study shows that pregnant MDMA users who call a teratogen information center tend to reduce their use later in pregnancy (Ho et al., 2001), but there are no studies of infant outcome on CNS development and function after prenatal MDMA exposure.

The results of the present study in animals extend earlier preclinical findings and suggest that a combination of in utero and adult exposure to MDMA may interact to increase the effects on hyperthermic responses and some behaviors. These additional behavioral consequences were demonstrated in the MWM when animals were required to learn the position of a reduced-size platform located in a different quadrant after previously learning the platform's position in another quadrant during acquisition. These results are consistent with the previous finding that cognitive effects of adult MDMA in humans can persist long after cessation of use (Green et al., 2003). Thus, the concept that deficits are largely attributable to the extent of previous exposure (Morgan et al., 2002) now calls for the inclusion of a developmental influence interacting with later MDMA use so as to understand the full impact of the drug's long-term

effects. Future research in this area should be conducted to elucidate whether the exclusive effect on reversal learning (and not acquisition) is likely to be reflected in specific human outcomes relating to executive functions.

In the present study, the combination of neonatal and adult MDMA was not associated with additive changes in hippocampal, prefrontal, or striatal 5-HT or DA tissue concentrations, although there were monoamine depletions associated with adult MDMA treatment. Monoamine depletions are part of the adult pharmacologic profile following MDMA use (Turner and Parrott, 2000). Consequently, our finding of adult depletions of 5-HT and 5-HIAA concurs with many previous studies of adult-only MDMA treatment in animals (Marston et al., 1999; Sprague et al., 2003). The DA changes do not support the hypothesis that MDMA is entirely selective for 5-HT terminal fields (Marston et al., 1999), but these previous studies did not include animals that had been trained in learning tasks. The small hippocampal 5-HIAA effect is commensurate with previous findings attributable to neonatal MDMA treatment (Broening et al., 2001).

The effects associated with neonatal MDMA exposure on NOR and locomotor behavior also constitute new findings. For NOR, as shown in Figure 6A, neonatally MDMA-treated rats spent less of the 30-s object attending period investigating the new object. Further, the division of time spent between investigations of the new vs. the old object was closer to 50:50. This suggests that neonatally MDMA-treated rats remembered the old object less efficiently than controls (Gaskin et al., 2003). Previous work on memory has shown that the rat hippocampus is essential for normal object recognition (Clark et al., 2000). Thus our NOR data, combined with the neonatal MDMA-effect observed for MWM performance, argue for MDMA-induced hippocampal impairment due to early MDMA exposure. For locomotor activity, neonatal MDMA diminished overall locomotor activity in favor of increased thigmotaxis. In previous research, adult MDMA-treatment has been found to increase the activity in the periphery of the test chamber (Green et al., 2003); likewise, we found increased time in thigmotaxis for both the adult-treated and neonatally-treated animals. Thigmotaxic hyperactivity has been attributed to both 5-HT and DA systems (McCreary et al., 1999).

Serotonergic and dopaminergic markers were correlated with MWM acquisition performance but only dopaminergic markers were correlated with MWM reversal learning (except for reversal probe). Canonical correlation analysis suggested modest predictive power ($|r| \geq 0.26$, $P \leq 0.05$) for markers of hippocampal 5-HT and prefrontal DA on spatial acquisition performance. Summed over trials and days, hippocampal serotonergic markers partially predicted first bearing performance, and, spread over trials, partially predicted performance on some trials (6/20 trials), primarily Trials 1 or 2 on each day. Conversely, prefrontal 5-HT markers had no predictive power for first bearing, and DA markers predicted performance in only 2/20 trials. Previous studies, either using simple correlation between monoamine levels and behavior (Broening et al., 2001) or dietary tryptophan depletion and behavior (Lieben et al., 2004), found no quantitative relationship between hippocampal or prefrontal monoamines and acquisition learning; however, the present results suggest that the relationship between monoamines and spatial acquisition may involve a combination effect rather than a single neurotransmitter. In addition, neither Broening et al., (2001) nor Lieben et al. (2004) reported first bearing performance in the MWM, whereas the present results suggest that for MWM acquisition learning, serotonergic markers predict first bearing somewhat better.

Prefrontal cortex serotonergic markers predicted percent time spent in the target quadrant and average distance from the previous target in the reversal probe trial. Thus, the memory component of reversal testing (as opposed to acquisition probe) may reflect serotonergic involvement. Acquisition probe testing has not been associated with 5-HT (Lieben et al.,

2004). Also, it is notable that lowering 5-HT via dietary tryptophan deficiency has been shown to impair declarative memory consolidation in humans (Harrison et al., 2004).

The spatial working memory version of the MWM has not been found to be significantly affected by neonatal MDMA (Vorhees et al., 2004). However, Ennaceur and Meliani (1992) have argued that multiple forms of working memory may be involved in object recognition. Our finding that early MDMA exposure compromises NOR performance, when considered in light of Vorhees et al.'s finding that MDMA has no effect on the working memory in the MWM (Vorhees et al., 2004), suggests that the effect on working memory is not detectable in the Morris Maze. Although prefrontal cortex DA is important to the regulation of working memory and other cognitive functions (Durstewitz et al., 2004; Henze et al., 2000), in the present study, we did not find prefrontal cortex DA depletions in either neonatal or adult MDMA-treated animals; however, DOPAC was increased in adults exposed to MDMA. Whether the correlations of the prefrontal dopaminergic markers and cumulative distance in reversal testing reflects the involvement of DA in goal-directed behavior (Durstewitz et al., 2004) remains to be determined. Indeed, in humans, spatial working memory is impaired when the amino acid precursors of DA are depleted from the diet (Harrison et al., 2004).

With regard to the CWM, serotonergic markers in the hippocampus were found to partially predict latencies on some days (3/6 days), and, in the prefrontal cortex, errors on all six days of testing. These findings confirm the hypothesis of Williams et al. (2003b) that the prefrontal cortex is important for learning the CWM and extend the hypothesis to include the hippocampus. The finding of a neonatal MDMA effect on errors in the CWM in the present study replicates Broening et al. (2001) and Williams et al. (2003b); the lack of an effect on latencies in the present study could be due to the reversed order of sequential/spatial task testing in comparison with that in the previous studies. For example, it has been demonstrated that handling affects maze performance (Holscher, 1999) and so too does test order (Vorhees et al., 2004). Alternatively, latency measures may be less sensitive than other measures obtained in learning tasks as has been suggested for MWM learning (Gallagher et al., 1993; Lindner, 1997).

Hippocampal and prefrontal serotonergic markers were not predictive of elevated zero maze performance. However, there were significant serotonergic and dopaminergic striatal correlations associated with the elevated zero maze. These findings are in partial agreement with previous findings on rats (Schwartz et al., 1998) wherein an increased index of anxiety was found to be associated with striatal 5-HT markers but not DA markers. However, work on humans suggests a role for the dopaminergic system in the regulation of anxiety (Laakso et al., 2003).

Our results demonstrated a combination trend for neonatal and adult exposure on temperature changes over the adult dosing period. Further research is necessary to determine whether these trends are indeed related to the severity of the long-term effects of MDMA on the central nervous system, as has been shown to be true for the absolute values of the temperatures themselves after adult dosing. Green et al. (2004) reviewed the acute hyperthermic response to MDMA that has been known to lead to fatality. Because we were interested in behavioral outcomes, preventing death by cooling was a prerequisite to the successful completion of the present experiment. Hence, some rats were subjected to water-bath cooling to attenuate severe hyperthermia as used previously (Williams et al., 2002). An analysis of correlations between maximum body temperature and monoamine values in MDMA-treated animals resulted in statistical significance for only the metabolite, 5-HIAA, and only in the prefrontal cortex ($r = -0.52$). Therefore, the range of temperatures that MDMA-treated animals obtained did not directly influence the reduction in neurotransmitters. In most studies of adult substituted amphetamine effects, no intervention is made to prevent increased mortality in the drug-treated

group. Hence, the reported results are only on those that survive; potentially, leading to survivor effects that are not representative of the full effect of the drug. This is especially problematic when a drug-treated group shows mortality rates of 20, 30, or 40%. Under these circumstances, it is difficult to know whether the effects reported on the remaining animals represents an accurate, under- or over-estimate of the true effect. We sought to prevent this problem by cooling animals that reached body temperatures that place the animal at risk of dying. By doing so, we reduced mortality to a very low level. A question arises, however, as to whether the cooling intervention itself alters the effect of the drug. By rescuing these animals from probable death, however, the present design allows us to directly compare the effects on monoamines and behavior in the subsets of adult MDMA-treated rats that were cooled versus those that were not. To maximize the power to detect even subtle differences, we compared these subgroups using *t*-tests uncorrected for multiple comparisons. We found no statistically significant differences between cooled and uncooled animals on any monoamine or metabolite in any of the regions analyzed in this experiment or in MWM acquisition or reversal learning latency. These findings suggest that the cooling intervention prevents drug-induced mortality without altering the typical markers of neurotransmitter changes used in adult studies of MDMA and increases the power of the study by allowing almost all the treated animals to complete the experiment and have tissue available for assessment. Whether this finding may be generalized to all neurochemical changes in all brain regions or with other substituted amphetamines remains to be determined.

In summary, neonatal MDMA has long-term effects on spatial acquisition and reversal learning, and neonatal and adult MDMA-exposure interact to increase the effect of neonatal MDMA on spatial reversal learning in the MWM. The effect appears to be specific to the enhanced demand of spatial reversal learning combined with a smaller platform to increase the spatial accuracy required to find the goal. The results suggest that the underlying physiology is likely to involve multiple monoaminergic interactions. The data have ramifications for understanding the history of MDMA use when there may be both early and later exposure. Women of child-bearing age who may become pregnant and who used MDMA during this period may have children at increased risk for later learning problems, and this effect may be compounded if their children later become MDMA users themselves. In addition, late third trimester exposure to MDMA has ramifications for exploratory and hyperactivity behaviors as assessed herein in the NOR and open-field tests. Drug exposure at other developmental time points besides the third trimester could elicit different patterns of effects.

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REFERENCES

- Bayer SA, Altman J, Russo RJ, Zhang X. Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. *Neurotoxicology* 1993;14:83–144. [PubMed: 8361683]
- Broening HW, Bowyer JF, Slikker W Jr. Age-dependent sensitivity of rats to the long-term effects of the serotonergic neurotoxicant (+/-)-3,4-methylenedioxymethamphetamine (MDMA) correlates with the magnitude of the MDMA-induced thermal response. *J Pharmacol Exp Ther* 1995;275:325–333. [PubMed: 7562567]
- Broening HW, Morford LL, Inman-Wood SL, Fukumura M, Vorhees CV. 3,4-Methylenedioxymethamphetamine (Ecstasy)-induced learning and memory impairments depend on the age of exposure during early development. *J Neurosci* 2001;21:3228–3235. [PubMed: 11312307]

- Bull EJ, Hutson PH, Fone KCF. Decreased social behaviour following 3,4-methylenedioxymethamphetamine (MDMA) is accompanied by changes in 5-HT_{2A} receptor responsivity. *Neuropharmacology* 2004;46:202–210. [PubMed: 14680758]
- Clark RE, Zola SM, Squire LR. Impaired recognition memory in rats after damage to the hippocampus. *J Neurosci* 2000;20:8853–8860. [PubMed: 11102494]
- Cole JC, Sumnall HR. The pre-clinical behavioural pharmacology of 3,4-methylenedioxymethamphetamine (MDMA). *Neurosci Biobehav Rev* 2003;27:199–217. [PubMed: 12788333]
- Cole JC, Sumnall HR, O'Shea E, Marsden CA. Effects of MDMA exposure on the conditioned place preference produced by other drugs of abuse. *Psychopharmacology* 2003;166:383–390. [PubMed: 12601504]
- Dafters RI, Hoshi R, Talbot AC. Contribution of cannabis and MDMA (“ecstasy”) to cognitive changes in long-term polydrug users. *Psychopharmacology* 2004;173:405–410. [PubMed: 12955295]
- Durstewitz D, Seamans JK, Sejnowski TJ. Dopamine-mediated stabilization of delay-period activity in a network model of prefrontal cortex. *J Neurophysiol* 2004;83:1733–1750. [PubMed: 10712493]
- Ennaceur A, Meliani K. A new one-trial test for neurobiological studies of memory in rats. III. Spatial vs. non-spatial working memory. *Behav Brain Res* 1992;51:83–92. [PubMed: 1482548]
- Fischer C, Hatzidimitriou G, Wlos J, Katz J, Ricaurte G. Reorganization of ascending 5-HT axon projections in animals previously exposed to the recreational drug (+/-)3,4-methylenedioxymethamphetamine (MDMA, “Ecstasy”). *J Neurosci* 1995;15:5476–5485. [PubMed: 7643196]
- Gallagher M, Burwell R, Burchinal M. Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. *Behav Neurosci* 1993;107:618–626. [PubMed: 8397866]
- Gaskin S, Tremblay A, Mumby DG. Retrograde and anterograde object recognition in rats with hippocampal lesions. *Hippocampus* 2003;13:962–969. [PubMed: 14750658]
- Green AR, Mehan AO, Elliott JM, O'Shea E, Colado MI. The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, “Ecstasy”). *Pharmacol Rev* 2003;55:463–508. [PubMed: 12869661]
- Green AR, O'Shea EO, Colado MI. A review of the mechanisms involved in the acute MDMA (ecstasy)-induced hyperthermic response. *Eur J Pharmacol* 2004;500:3–13. [PubMed: 15464016]
- Halpern JH, Pope HG Jr, Sherwood AR, Barry S, Hudson JI, Yurgelun-Todd D. Residual neuropsychological effects of illicit 3,4-methylenedioxymethamphetamine (MDMA) in individuals with minimal exposure to other drugs. *Drug Alcohol Depend* 2004;75:135–147. [PubMed: 15276218]
- Harrison BJ, Olver JS, Norman TR, Burrows GD, Wesnes KA, Nathan PJ. Selective effects of acute serotonin and catecholamine depletion on memory in healthy women. *J Psychopharmacol* 2004;18:32–40. [PubMed: 15107182]
- Henze DA, Gonzalez-Burgos GR, Urban NN, Lewis DA, Barrionuevo G. Dopamine increases excitability of pyramidal neurons in primate prefrontal cortex. *J Neurophysiol* 2000;84:2799–2809. [PubMed: 11110810]
- Ho E, Karimi-Tabesh L, Koren G. Characteristics of pregnant women who use ecstasy (3,4-methylenedioxymethamphetamine). *Neurotoxicol Teratol* 2001;23:561–567. [PubMed: 11792525]
- Holscher C. Stress impairs performance in spatial water maze learning tasks. *Behav Brain Res* 1999;100:225–235. [PubMed: 10212070]
- Holson RR, Pearce B. Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. *Neurotoxicol Teratol* 1992;14:221–228. [PubMed: 1635542]
- Horan B, Gardner EL, Ashby CR Jr. Enhancement of conditioned place preference response to cocaine in rats following subchronic administration of 3,4-methylenedioxymethamphetamine (MDMA). *Synapse* 2000;35:160–162. [PubMed: 10611642]
- Koprach JB, Chen E-Y, Kanaan NM, Campbell NG, Kordower JH, Lipton JW. Prenatal 3,4-methylenedioxymethamphetamine (ecstasy) alters exploratory behavior, reduces monoamine metabolism, and increases forebrain tyrosine hydroxylase fiber density of juvenile rats. *Neurotoxicol Teratol* 2003;25:509–517. [PubMed: 12972064]

- Laakso A, Wallius E, Kajander J, Bergman J, Eskola O, Solin O, Ilonen T, Salokangas RK, Syvalahti E, Hietala J. Personality traits and striatal dopamine synthesis capacity in healthy subjects. *Am J Psychiatry* 2003;160:904–910. [PubMed: 12727694]
- Lieben CKJ, van Oorsouw K, Deutz NEP, Blokland A. Acute tryptophan depletion induced by a gelatin-based mixture impairs object memory but not affective behavior and spatial learning in the rat. *Behav Brain Res* 2004;151:53–64. [PubMed: 15084421]
- Lindner MD. Reliability, distribution, and validity of age-related cognitive deficits in the Morris water maze. *Neurobiol Learn Mem* 1997;68:203–220. [PubMed: 9398584]
- Littell, RC.; Milliken, GA.; Stroup, WW.; Wolfinger, RD. SAS System for Mixed Models. Cary, NC: SAS Institute Inc.; 1996. Analysis of repeated measures data; p. 87-134.
- Littell, RC.; Stroup, WW.; Freund, RJ. SAS for Linear Models. Fourth Edition. Cary, NC: SAS Institute Inc.; 2002. Repeated-measures analysis; p. 265-303.
- Marston HM, Reid ME, Lawrence JA, Olverman HJ, Butcher SP. Behavioural analysis of the acute and chronic effects of MDMA treatment in the rat. *Psychopharmacology* 1999;144:67–76. [PubMed: 10379626]
- McCardle K, Luebbers S, Carter JD, Croft RJ, Stough C. Chronic MDMA (ecstasy) use, cognition and mood. *Psychopharmacology* 2004;173:434–439. [PubMed: 15088077]
- McCreary AC, Bankson MG, Cunningham KA. Pharmacological studies of the acute and chronic effects of (+)-3,4-methylene-dioxymethamphetamine on locomotor activity: role of 5-hydroxytryptamine 1A and 6-hydroxytryptamine 1b/1d receptors. *J Pharmacol Exp Ther* 1999;290:965–973. [PubMed: 10454466]
- McElhatton PR, Bateman DN, Evans C, Pughe KR, Thomas SH. Congenital anomalies after prenatal ecstasy exposure. *Lancet* 1999;354:1441–1442. [PubMed: 10543673]
- Meyer JS, Grande M, Johnson K, Ali SF. Neurotoxic effects of MDMA (“ecstasy”) administration to neonatal rats. *Int J Dev Neurosci* 2004;22:261–271. [PubMed: 15380826]
- Morford LL, Wheck JW, Breslin WJ, DeSesso JM. Hazard identification and predictability of children’s health risk from animal data. *Environ Health Perspect* 2004;112:266–271. [PubMed: 14754582]
- Morgan MJ. Recreational use of “ecstasy” (MDMA) is associated with elevated impulsivity. *Neuropsychopharmacology* 1998;19:252–264. [PubMed: 9718589]
- Morgan MJ. Memory deficits associated with recreational use of “ecstasy” (MDMA). *Psychopharmacology* 1999;141:30–36. [PubMed: 9952062]
- Morgan MJ. Ecstasy (MDMA): a review of its possible persistent psychological effects. *Psychopharmacology* 2000;152:230–248. [PubMed: 11105933]
- Morgan MJ, McFie L, Fleetwood LH, Robinson JA. Ecstasy (MDMA): are the psychological problems associated with its use reversed by prolonged abstinence? *Psychopharmacology* 2002;159:294–303. [PubMed: 11862362]
- Nair SG, Gudelsky GA. Protein kinase C inhibition differentially affects 3,4-methylenedioxymethamphetamine-induced dopamine release in the striatum and prefrontal cortex of the rat. *Brain Res* 2004;1013:168–173. [PubMed: 15193525]
- Parrott AC. Human research on MDMA (3,4-methylene-dioxymethamphetamine) neurotoxicity: cognitive and behavioural indices of change. *Neuropsychobiology* 2000;42:17–24. [PubMed: 10867552]
- Rodgers J. Cognitive performance amongst recreational users of “ecstasy”. *Psychopharmacology* 2000;151:19–24. [PubMed: 10958112]
- SAS Institute Inc.. SAS/STAT User’s Guide, Version 8. Cary, NC: SAS Institute Inc.; 1999. The CANCELL Procedure; p. 635-663.
- Schwartz RK, Thiel CM, Muller CP, Huston JP. Relationship between anxiety and serotonin in the ventral striatum. *Neuroreport* 1998;9:1025–1029. [PubMed: 9601661]
- Shepherd JK, Grewal SS, Fletcher A, Bill DJ, Dourish CT. Behavioural and pharmacological characterisation of the elevated “zero-maze” as an animal model of anxiety. *Psychopharmacology* 1994;116:56–64. [PubMed: 7862931]
- Sprague JE, Preston AS, Leifheit M, Woodside B. Hippocampal serotonergic damage induced by MDMA (ecstasy): effects on spatial learning. *Physiol Behav* 2003;79:281–287. [PubMed: 12834800]

- Turner JJ, Parrott AC. "Is MDMA a human neurotoxin?": diverse views from the discussants. *Neuropsychobiology* 2000;42:42–48. [PubMed: 10867555]
- Verkes RJ, Gijsman HJ, Pieters MSM, Schoemaker RC, de Visser S, Kuijpers M, Pennings EJM, de Bruin D, van de Wijngaart G, van Gerven JMA, Cohen AF. Cognitive performance and serotonergic function in users of ecstasy. *Psychopharmacology* 2001;153:196–202. [PubMed: 11205419]
- Vorhees CV. Maze learning in rats: a comparison of performance in two water mazes in progeny prenatally exposed to different doses of pentylenetetrazol. *Neurotoxicol Teratol* 1987;9:235–241. [PubMed: 3627087]
- Vorhees CV, Reed TM, Skelton MR, Williams MT. Exposure to 3,4-methylenedioxymethamphetamine (MDMA) on postnatal days 11–20 induces reference but not working memory deficits in the Morris water maze in rats: implications of prior learning. *Int J Dev Neurosci* 2004;22:247–259. [PubMed: 15380825]
- Williams MT, Morford LL, McCrea AE, Wood SL, Vorhees CV. Administration of D, L-fenfluramine to rats produces learning deficits in Cincinnati water maze but not the Morris water maze: relationship to adrenal cortical output. *Neurotoxicol Teratol* 2002;24:783–796. [PubMed: 12460661]
- Williams MT, Moran MS, Vorhees CV. Refining the critical period for methamphetamine-induced spatial deficits in the Morris water maze. *Psychopharmacology* 2003a;168:329–338. [PubMed: 12684734]
- Williams MT, Morford LL, Wood SL, Rock SL, McCrea AE, Fukumura M, Wallace TL, Broening HW, Moran MS, Vorhees CV. Developmental 3,4-methylenedioxymethamphetamine (MDMA) impairs sequential and spatial but not cued learning independent of growth, litter effects or injection stress. *Brain Res* 2003b;968:89–101. [PubMed: 12644267]

Fig. 1. Simple-effect means $\pm$ SEM for the increases in body temperature at time points after administration of the first dose of MDMA. **** $P \leq 0.0001$ for linear, quadratic, and cubic regressions, MM vs. MS. # $P < 0.05$ for linear, quadratic and cubic regressions, MM vs. SM.

Fig. 2. Mean $\pm$ SEM for behaviors measured in the Zero maze during a 5-min trial. **A:** Main-effect means. **B:** Simple-effect means (adult). HD, head dips; SA, stretch attends; Ent, entries into the open; and %TIO, time in the open. * $P < 0.05$.

Fig. 3. Mean $\pm$ SEM for behaviors measured in learning acquisition trials using the Morris maze in four consecutive trials conducted on each of five consecutive days. **A**, **C**, and **E**: Main-effect means (neonatal). **B**, **D**, and **F**: Simple-effect means. **** $P < 0.0001$.

Fig. 4.

Mean $\pm$ SEM for behaviors measured in learning reversal trials using the Morris maze in four consecutive trials conducted on each of five consecutive days, MM vs. MS, $*P < 0.05$, $**P < 0.01$ and MM vs. SM, $\#P < 0.05$, $P < 0.001$.

Fig. 5. Mean $\pm$ SEM errors counted in learning trials using the Cincinnati maze in two consecutive trials spaced 30 min apart for 6 days. **A:** Main-effect means (neonatal). **B:** Simple-effect means. * $P < 0.05$.

Fig. 6. Mean $\pm$ SEM for time spent in novel object investigation during a NOR protocol. **A:** Main-effect means (neonatal). **B:** Simple-effect means. ** $P = 0.01$.

Fig. 7.

Mean $\pm$ SEM for locomotor activity during a 1-h open-field test interval. **A** and **C**: Main-effect means (neonatal). **B** and **D**: Simple-effect means (symbols apply to MS vs. SS). TD, total distance; DC, distance in the center; T1, time within 1 cm of the walls; TA, time >1 cm away from the walls. * $P < 0.01$, ** $P < 0.01$, *** $P < 0.001$.

TABLE I

Monoamine levels^a

	Group				Neonatal			Adult		
	SS	SM	MS	MM	S	M	M	S	M	M
Hippocampus										
5-HT	0.50 ± 0.02	0.25 ± 0.03	0.42 ± 0.03	0.24 ± 0.03	0.37 ± 0.03	0.33 ± 0.03		0.46 ± 0.02		0.25 ± 0.02 ^{***}
5-HIAA	0.31 ± 0.02	0.14 ± 0.01	0.26 ± 0.01	0.14 ± 0.02	0.23 ± 0.02	0.20 ± 0.01 ^{**}		0.29 ± 0.01		0.14 ± 0.01 ^{***}
Ratio	0.64 ± 0.03	0.64 ± 0.04	0.67 ± 0.06	0.62 ± 0.04	0.64 ± 0.03	0.64 ± 0.04		0.66 ± 0.04		0.63 ± 0.03
Prefrontal cortex										
5-HT	0.47 ± 0.05	0.25 ± 0.03	0.44 ± 0.03	0.25 ± 0.02	0.36 ± 0.03	0.34 ± 0.03		0.45 ± 0.03		0.25 ± 0.02 ^{***}
5-HIAA	0.19 ± 0.02	0.11 ± 0.01	0.18 ± 0.02	0.12 ± 0.01	0.15 ± 0.01	0.15 ± 0.01		0.19 ± 0.01		0.12 ± 0.01 ^{***}
Ratio	0.44 ± 0.04	0.51 ± 0.07	0.44 ± 0.06	0.53 ± 0.07	0.48 ± 0.04	0.49 ± 0.05		0.44 ± 0.04		0.52 ± 0.05
DA	0.11 ± 0.03	0.22 ± 0.08	0.19 ± 0.05	0.13 ± 0.04	0.16 ± 0.04	0.16 ± 0.03		0.15 ± 0.03		0.18 ± 0.04
DOPAC	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00		0.01 ± 0.00		0.02 ± 0.00 [*]
Ratio	0.18 ± 0.05	0.16 ± 0.03	0.16 ± 0.04	0.24 ± 0.04	0.17 ± 0.03	0.20 ± 0.03		0.17 ± 0.03		0.20 ± 0.03
Striatum										
5-HT	0.48 ± 0.07	0.25 ± 0.05	0.44 ± 0.07	0.31 ± 0.04	0.37 ± 0.05	0.38 ± 0.04		0.46 ± 0.05		0.28 ± 0.03 [*]
5-HIAA	0.40 ± 0.04	0.24 ± 0.04	0.42 ± 0.03	0.29 ± 0.03	0.32 ± 0.03	0.36 ± 0.03		0.41 ± 0.02		0.26 ± 0.03 ^{**}
Ratio	0.88 ± 0.10	1.07 ± 0.13	1.04 ± 0.09	0.99 ± 0.11	0.98 ± 0.08	1.02 ± 0.07		0.96 ± 0.07		1.03 ± 0.08
DA	10.56 ± 0.75	7.18 ± 1.02	12.20 ± 0.41	8.02 ± 0.74	8.87 ± 0.75	10.11 ± 0.68		11.38 ± 0.46		7.60 ± 0.62 ^{***}
DOPAC	0.91 ± 0.19	0.97 ± 0.15	1.03 ± 0.13	1.14 ± 0.14	0.94 ± 0.12	1.08 ± 0.09		0.97 ± 0.11		1.06 ± 0.10
Ratio	0.08 ± 0.02	0.14 ± 0.02	0.08 ± 0.01	0.14 ± 0.02	0.11 ± 0.01	0.11 ± 0.01		0.08 ± 0.01		0.14 ± 0.01 ^{**}

^aValues given are mean ± SEM (ng/mg tissue) by Group or main-effect.^{*}Significantly different from control, $P < 0.05$.^{**}Significantly different from control, $P \leq 0.01$.^{***}Significantly different from control, $P < 0.0001$.

TABLE II

Significant correlations of monoamines and behavior

Tissue	System	Procedure	r^a	Statistic	P
Hippocampus	Serotonergic	MWM acquisition ^b	0.57	$F(12,140.52) = 2.40$	<0.01
		CWM latencies	0.60	$F(18,144.74) = 2.13$	<0.01
		Novel object	0.56	$F(12,140.52) = 2.34$	<0.01
		MWM acquisition, br ^c	0.83	$F(60,111.22) = 1.76$	<0.01
Prefrontal cortex	Serotonergic	MWM reversal probe	0.49	$F(9,131.57) = 2.52$	0.01
		CWM errors			
		1st canonical variate	0.60	$F(18,144.74) = 2.93$	<0.0001
		2nd canonical variate	0.58	$F(10,104) = 2.46$	0.01
		Novel object	0.62	$F(12,140.52) = 3.04$	<0.001
	Dopaminergic	MWM acquisition, br ^c	0.76	$F(60,111.22) = 1.49$	<0.05
		MWM reversal, cd ^c	0.76	$F(60,111.22) = 1.44$	0.05

^aFor the 1st canonical variate, except where noted.^bSummed over trials and days.^cbr, first bearing; cd, cumulative distance.