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## Comparison of monoamine and corticosterone levels 24 h following (+)methamphetamine, (+/-)3,4-methylenedioxymethamphetamine, cocaine, (+)fenfluramine or (+/-)methylphenidate administration in the neonatal rat

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### Abstract

We have previously shown that neonatal administration of (+/-)3,4-methylenedioxymethamphetamine and (+)fenfluramine produce deficits in spatial and path integration learning, whereas (+)methamphetamine causes deficits in spatial learning. Conversely, cocaine and (+/-)methylphenidate have no effect on either form of learning following neonatal administration. The purpose of the present study was to determine whether corticosterone and/or monoamine levels were changed following subcutaneous administration of 10 mg/kg (+)methamphetamine, (+/-)3,4-methylenedioxymethamphetamine, (+)fenfluramine, (+/-)methylphenidate or cocaine every 2 h (total of four injections) on postnatal day 11. Twenty-four hours after the first dose, plasma, striatum and hippocampus were collected. Corticosterone levels were increased in methamphetamine-, fenfluramine-, methylenedioxymethamphetamine- and methylphenidate-treated rats relative to levels in saline-treated rats, whereas cocaine-treated rats were unaffected. In the striatum and hippocampus, serotonin and 5-hydroxyindolacetic acid were reduced in animals treated with methylenedioxymethamphetamine or fenfluramine, compared with levels in saline controls. Dopamine levels were not changed by any of the drugs, although 3,4-dihydroxyphenylacetic acid was decreased following methylenedioxymethamphetamine or methamphetamine. Minimal effects were seen in neurotransmitter levels following injection of cocaine or methylphenidate. These data suggest that drugs that affect corticosterone and hippocampal serotonin are associated with both spatial learning and path integration deficits, and those that affect corticosterone and 3,4-dihydroxyphenylacetic acid are associated with spatial learning deficits only.

### Keywords

cocaine; corticosterone; ecstasy; fenfluramine; methamphetamine; methylphenidate

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The abuse of substituted amphetamines has been reported in women of childbearing age (EMCDDA 2004; Johnston *et al.* 2004). (+/-)3,4-Methylenedioxymethamphetamine (MDMA), (+)methamphetamine (MA), (+/-)methylphenidate (MPH) and cocaine (COC) can sometimes increase the likelihood of sexual activity (Buffum 1988) and are often abused in social gatherings. However, the anorectic properties of these drugs and others such as (+)fenfluramine (FEN) (even though it has been associated with valvulopathy; Khan *et al.* 1998) may be appealing because it has been suggested that some women who become pregnant still strive to be thin (Franko and Walton 1993). These factors increase the likelihood of fetal exposure and it is known that MDMA (Ho *et al.* 2001), MA (Smith *et al.* 2003; Chomchai *et al.* 2004), MPH (Debooy *et al.* 1993), COC (Richardson *et al.* 1999) and FEN (Jones *et al.* 2002) have been used during pregnancy, including the third trimester. Despite this, the ramifications of late fetal exposure are largely unknown.

Developmental psychostimulant exposure in humans is suspected to alter normal brain function that can last into adulthood (Billing *et al.* 1985; Cernerud *et al.* 1996; Plessinger 1998; Chang *et al.* 2004; Noland *et al.* 2005), but the hormonal and biochemical changes that occur following exposure to these drugs remain to be elucidated. In rats, we have shown that developmental exposure to MA (Vorhees *et al.* 2000a; Williams *et al.* 2002, 2003b,d), MDMA (Broening *et al.* 2001; Williams *et al.* 2003c; Vorhees *et al.* 2004) or FEN (Morford *et al.* 2002) produces deficits in latency, path length and cumulative distance as well as probe trial performance in the Morris water maze, a hippocampally mediated spatial learning task (Morris *et al.* 1982). Neonatal administration of MDMA (Broening *et al.* 2001; Williams *et al.* 2003c) and FEN (Morford *et al.* 2002) also produces an increased number of errors of commission and latency to locate an escape platform in the Cincinnati water maze which assesses path integration learning. Conversely, COC (Vorhees *et al.* 2000b) or MPH (Vorhees C. V., Moran M. S., Williams M. T., unpublished results) administration does not affect learning assessed by the Morris or Cincinnati water mazes (only COC-treated animals were tested in the Cincinnati water maze). Few studies have detailed the immediate changes induced by neonatal drug exposure during the sensitive period when long-term learning and memory changes originate, i.e. from postnatal day (P)11–20 (Broening *et al.* 1994; Williams *et al.* 2000, 2005; Koprach *et al.* 2003). The limited existing studies suggest there are changes in adrenal hormone production as well as monoamine levels in the brain following exposure to some of these drugs. We previously showed that administration of MDMA or MA to P11 rats caused an increase in corticosterone (CORT) levels that remained significantly increased for 24 h following the first dose of MDMA and at least 1 h after MA exposure (Williams *et al.* 2000; 2005). We and others have shown that administration of a divided daily dose of 40 mg/kg MDMA on P10 or P11 or P11–20 also produces decreases in serotonin (5-HT) in the hippocampus and striatum the day after the last administration (Broening *et al.* 1995; Koprach *et al.* 2003; Williams *et al.* 2005).

It has been proposed that alterations in glucocorticoids and monoamine levels during early developmental stages may disrupt normal brain development and alter later learning and memory. For example, fluctuations in CORT interfere with the proliferation of neurons during development, especially in the hippocampus (Woolley *et al.* 1990; Gould *et al.* 1991; Liu *et al.* 2003). The hippocampus is known to be involved in learning and memory functions, and is still going through neurogenesis in the late gestational period in humans and comparably in early postnatal weeks in the rat (Bayer *et al.* 1993). 5-HT is abundant in the brain during neurogenesis and is known to be important in neuronal development. 5-HT is important for the differentiation of neurons and receptive target cells, and is involved in organizing the developing brain (Lauder and Krebs 1978; Lauder 1993). Furthermore, disruptions to 5-HT in rats during early development produce learning and memory deficits when the animals are tested as adults (Mazer *et al.* 1997).

The main purpose of this study was to compare the effect of the drugs noted above following a single day of administration on plasma CORT and on monoamine content in the striatum and hippocampus. This is the first study to directly compare the changes that occur following administration of MDMA, MA, MPH, COC or FEN on P11, the first day of the critical period for the induction of later cognitive deficits following substituted amphetamine exposure.

## Materials and methods

### Animals and housing

Nulliparous female Sprague–Dawley CD (International Genetic Strain) rats (Charles River Laboratories, Raleigh, NC, USA) were mated with males of the same strain and supplier. Rats were housed in a  $22 \pm 1^\circ\text{C}$  environment at 50% humidity with a 14/10 h light/dark cycle (lights on at 06.00 hours). Before mating, animals were allowed at least 2 weeks to acclimate to the vivarium. Each female spent 2 weeks housed with one male in hanging wire cages before being housed singly in a polycarbonate cage ( $45.7 \times 23.8 \times 20.3$  cm) containing wood chip bedding and *ad libitum* food and water. Pups from the matings were used as subjects. The Cincinnati Children's Research Foundation's Institutional Animal Care and Use Committee approved all procedures, and the vivarium was fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

The day of birth was considered P0, and on P1 litters were culled to eight pups with six males and two females. Only males were used for this study because we have previously shown no or minor effects of sex and neonatal drug treatment on the spatial learning abilities of these animals in adulthood. Furthermore, no significant differences between males and females in adrenal–pituitary hormone levels were noted following P11 methamphetamine (Williams *et al.* 2000) or MDMA administration (Williams *et al.* 2005). On P7, pups were weighed and individually identified by ear punch.

### Dosing procedures

(+)-Methamphetamine HCl (National Institutes of Health, NIH, National Institute on Drug Abuse, NIDA), (+/-)-3,4-methylenedioxymethamphetamine HCl (NIH, NIDA), (+)-fenfluramine HCl (Sigma, St Louis, MO), cocaine HCl (Sigma, St. Louis, MO, USA) and MPH (Mallinckrodt, St. Louis, MO) were dissolved in isotonic saline at a concentration of 10 mg/3 mL (each expressed as the free base; purity > 95%). All drugs were administered at a dose of 10 mg/kg. This was a within-litter design such that the six treatments were represented within each of the eight litters,  $n = 8$ , and therefore treatment was considered a repeated factor (Kirk 1995; Winer 1978). Therefore, one male from each litter was randomly assigned to one of the following treatment groups: MA, MDMA, FEN, MPH, COC or saline (SAL). Each dose was administered through a subcutaneous injection in the dorsum. On P11, each animal received one injection every 2 h for a total of four injections. Each animal's weight was recorded just before drug administration and injection sites were varied to prevent irritation to the dermis.

### Blood and tissue collection

Each animal was decapitated 24 h after the first injection (i.e. 18 h following the last injection) because in a previous study we demonstrated that the greatest reduction in neurotransmitter content was induced with MDMA at this time point (Williams *et al.* 2005). All animals were decapitated between 0.900 and 11.00 hours in order to control for ultradian and circadian rhythm. Trunk blood was collected in tubes containing 2% EDTA (0.05 mL) and then centrifuged (1399 rotational centrifugal force) for 25 min at  $4^\circ\text{C}$ . Plasma was collected and stored at  $-80^\circ\text{C}$  until assayed for CORT.

The brain was removed and a chilled brain block (Zivic-Miller, Pittsburgh, PA, USA) was used to aid in dissection of the neostriatum and hippocampus. For the neostriatum, a coronal cut was made at the optic chiasm and then another cut made 2 mm rostral to the first. The neostriatum was dissected from this 2-mm slab. Hippocampi were removed from the remaining tissue. All dissections were done on a chilled dissection plate and tissues frozen immediately on dry ice. Tissue weights were determined by placing the tissues in preweighed tubes and then re-weighing the tubes. All tissues were stored at  $-80^{\circ}\text{C}$  until monoamines were quantified by HPLC with electrochemical detection.

### Plasma CORT assessment

Plasma was diluted 3 : 1 for CORT in a kit-specific assay buffer before determination of hormone. CORT was assayed in duplicate using a commercially available enzymatic immunoassay (ALPCO Diagnostics, Windham, NH, USA). This assay has little cross-reactivity with other hormones or precursors ( $< 0.05\%$ ).

### Monoamine determination

The tissue concentrations of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-HT and 5-hydroxyindolacetic acid (5-HIAA) in the neostriatum, and 5-HT and 5-HIAA in the hippocampus, were quantified using HPLC with electrochemical detection. Tissues were homogenized in 50 volumes of 0.2 M perchloric acid and centrifuged for 6 min at 10 000 g. Aliquots of 20  $\mu\text{L}$  were injected on to a C18 column (MD-150,  $3 \times 150\text{mm}$ ; ESA, Chelmsford, MA, USA) connected to either a LC-4B amperometric detector (Bioanalytical Systems, West Lafayette, IN, USA) or a Coulochem (ESA, Chelmsford, MA, USA) detector and an integrator recorded the peak heights that followed each injection. The potential for the LC-4B was 0.6 V and the potentials of E1 and E2 on the analytical cell (model 5014B) of the Coulochem were  $-150$  and  $160$  mV respectively. The mobile phases consisted of 35 mM citric acid, 54 mM sodium acetate, 50 mg/L disodium ethylenediamine tetraacetate, 70 mg/L octanesulfonic acid sodium salt, 6% (v/v) methanol and 6% (v/v) acetonitrile, pH 4.0, and pumped at a flow rate of 0.4 mL/min. Quantities of the analytes were calculated on the basis of known standards. Retention times for DOPAC, DA, 5-HIAA and 5-HT were approximately 6, 8, 11, and 17 min respectively.

### Statistical analysis

Data were analyzed with repeated measures ANOVA using the general linear modeling procedure (Proc GLM; SAS, Cary, NC, USA). The within-subject factors were treatment and, for body weights, time of day. Litter membership was treated as a matching factor and so treatment was handled in the ANOVA as a repeated measures factor, in accordance with both Kirk (1995) and Winer (1978). The experimental unit was therefore the litter ( $n = 8$ ) (Holson and Pearce 1992). The Greenhouse–Geisser correction was used in instances in which symmetry of the variance–co-variance matrices were significantly non-spherical. Significant main effects were analyzed further to determine differences using the step-down  $F$ -test procedure (Kirk 1995). Simple effect  $F$ -tests were used to analyze significant interactions followed by step-down  $F$ -tests for individual group comparisons. In a few cases, where previous data from this laboratory on the same dependent variable were available, pre-planned comparisons between specific groups were made where indicated. Significance was set at  $P = 0.05$  and trends at  $P = 0.10$ .

## Results

### Body weight

No main effect of time or treatment was observed for the analysis of body weight during P11 dosing, but there was a time–treatment interaction ( $F_{5,35} = 4.88, p < 0.04$ ). Simple effect analyses revealed no significant differences between treatments across time points. The interaction was apparently the result of a reduction in body weight gain during dosing in the FEN- and MDMA-treated animals that caused the growth curves to be non-parallel even though these groups did not differ from any of the other groups at any single time point (Table 1).

### CORT

CORT samples obtained 24 h after the first dose demonstrated a significant treatment effect ( $F_{5,35} = 6.43, p < 0.01$ ). Step-down analysis demonstrated increases in CORT in the animals treated with MA, FEN and MPH compared with SAL-treated animals. Because we previously demonstrated an increase in CORT following MDMA on P11 (Williams *et al.* 2005), a planned comparison was used and the CORT level was significantly higher than that in SAL-treated animals ( $p < 0.05$ ) (Fig. 1). COC-treated animals did not differ from SAL-treated animals. MA treatment produced the greatest increase and was significantly different from all other treatments except FEN. The second largest increase was found in the FEN group, whereas the MDMA and MPH increases were the smallest and were similar to one another. Other than the MA group, none of the other drug-treated groups differed among themselves.

### Monoamines

**Striatum**—5-HT in the striatum showed a significant effect of treatment ( $F_{5,35} = 7.86, p < 0.004$ ). *Post hoc* analysis demonstrated that FEN, MDMA and MPH administration significantly decreased 5-HT compared with SAL (Fig. 2a), whereas no differences were noted following MA or COC administration. FEN produced the greatest 5-HT decrease. The rank order for magnitude of effect on 5-HT was  $FEN \geq MDMA \geq MPH \geq COC = MA = SAL$ .

As with 5-HT, 5-HIAA in the striatum was altered by treatment ( $F_{5,35} = 11.86, p < 0.0004$ ). *Post hoc* analysis showed that only FEN and MDMA administration produced significant decreases in 5-HIAA compared with SAL administration (Fig. 2b). For 5-HIAA the rank order of drug effect was  $MDMA \geq FEN > MA = MPH = COC = SAL$ .

There was a significant treatment effect on striatal turnover, i.e. the 5-HIAA/5-HT ratio ( $F_{5,35} = 3.89, p < 0.02$ ). Only FEN-treated animals demonstrated a significant increase in the 5-HIAA/5-HT ratio compared with SAL-treated animals (Fig. 2c). The turnover of FEN-treated animals was also significantly different from that of MA-treated animals, which produced the lowest turnover ratio. The 5-HIAA/5-HT ratio observed in MA-treated animals was significantly lower than that of MPH- and COC-treated animals, although it did not differ from that in SAL-treated animals.

No significant treatment effects were observed on DA levels in the striatum (Fig. 3a). Treatment effects were exhibited for DOPAC ( $F_{5,35} = 13.45, p < 0.0001$ ) (Fig. 3b). *Post hoc* analysis revealed that animals treated with MDMA or MA had decreased levels of DOPAC compared with SAL-treated animals, and there was a trend for an increase in the MPH-treated animals relative to levels after SAL treatment.

Although there were no differences observed in DA concentrations in the striatum, the DOPAC/DA ratio demonstrated a significant treatment effect ( $F_{5,35} = 27.81, p < 0.0001$ ). MDMA and MA treatment produced a decrease in the DOPAC/DA ratio, whereas MPH treatment significantly increased and COC tended to increase the DOPAC/DA ratio compared with SAL



treatment (Fig. 3c). The rank order for magnitude of decreased DA turnover was  $MA \geq MDMA > FEN > SAL$ ; the rank order for increased turnover was  $MPH > COC > SAL$ .

**Hippocampus**—In the hippocampus, both 5-HT ( $F_{5,35} = 18.60, p < 0.0001$ ) and 5-HIAA ( $F_{5,35} = 21.53, p < 0.0001$ ) were significantly affected by treatment (Figs 4a and b respectively). FEN- and MDMA-treated animals showed significantly decreased 5-HT and 5-HIAA levels compared with SAL-treated animals. There was also a trend for 5-HIAA to be decreased in the MA-treated animals and increased in the MPH-treated animals relative to SAL-treated animals. The rank order effect of the drugs at reducing 5-HT was  $FEN > MDMA > MA = MPH = COC = SAL$ . For 5-HIAA the rank order effect was similar and was  $FEN = MDMA > MA > MPH = COC = SAL$ .

The 5-HIAA/5-HT ratio in the hippocampus showed a significant treatment effect on 5-HT turnover ( $F_{5,35} = 11.81, p < 0.0008$ ). FEN-treated animals had a higher ratio compared with all other treatments (Fig. 4c). No other drug-treated animals differed from SAL-treated animals. MA and COC treatments were significantly different from one another.

## Discussion

This study is the first to directly compare the developmental effects of several psychostimulants and fenfluramine on early hormonal and monoamine changes 24 h after administration on P11. There were several major findings. First, MA and FEN, and to a lesser extent MDMA and MPH, administration produced prolonged adrenal activation as demonstrated by increases in plasma CORT long after the last treatment. The extent of this increase varied, with MA producing a greater than 3-fold increase, FEN a greater than 2-fold increase, and MDMA and MPH about a 50% increase in CORT. Second, FEN and MDMA treatment produced dramatic decreases in 5-HT and 5-HIAA in both the striatum and hippocampus, whereas MA administration produced only a trend toward decreased 5-HIAA in the hippocampus. Third, unlike adult administration of several of these drugs (especially MA), DA levels were not affected. Because it has been established previously that treatment for 10 days with FEN (20–60 mg/kg/day) (Morford *et al.* 2002), MA (20–60 mg/kg/day) (Williams *et al.* 2003a,d) or MDMA (10–40 mg/kg/day) (Broening *et al.* 2001; Williams *et al.* 2003c) beginning on P11 results in long-term spatial learning and memory deficits, whereas treatment with MPH (20–120 mg/kg/day) (Vorhees C. V., Moran M. S., Williams M. T., unpublished observation) or COC (60 mg/kg/day) (Vorhees *et al.* 2000b) does not, and the substituted amphetamines when administered for 1 day dramatically affect 5-HT and/or CORT, but little or no effect of MPH or COC is observed, it appears that these early drug-induced perturbations may be antecedent to and possibly associated with later cognitive outcomes. However, it must remain clear that behavioral deficits were seen after a 10-day dosing regimen of FEN, MA or MDMA (40 mg/kg/day for each drug) and the monoamine and CORT data in this study were obtained following only a single day of drug administration, although the fact that there is a pattern of associations with later learning and memory deficits raises the possibility that some or all may be causally linked.

In previous studies, we have examined the effect of neonatal drug exposure on learning and memory ability in the offspring as adults. We have found that administration of FEN (Morford *et al.* 2002), MDMA (Broening *et al.* 2001; Williams *et al.* 2003c; Vorhees *et al.* 2004), and MA (Vorhees *et al.* 2000a; Williams *et al.* 2002, 2003d) at similar doses when given on P11–20 all cause deficits in Morris water maze performance. Moreover, FEN (Morford *et al.* 2002) and MDMA (Broening *et al.* 2001; Williams *et al.* 2003c; Cohen *et al.* 2005) also produce path integration deficits in the Cincinnati water maze. Neither COC (Vorhees *et al.* 2000b) nor MPH (Vorhees C. V., Moran M. S., Williams M. T., unpublished observations) administered on these same days, even at higher doses, produced similar deficits. The efficacious drugs are

all similar in that they all are substituted amphetamines whereas the ineffective drugs are not. It is likely that the long-term effects of the substituted amphetamines originate from changes induced by these drugs during the period of exposure. For example, the behavioral differences we observed might involve changes in CORT and/or monoamine levels in the neonate that lead to altered stress responsiveness or miswiring of critical learning circuits. This is supported by the finding that increases in CORT or depletions of 5-HT during the neonatal period produce cognitive deficits (Olton *et al.* 1975; Mazer *et al.* 1997).

In adult animals, MDMA, MA, COC, MPH and FEN are known to affect monoaminergic systems, but reports describing the ramifications of fetal or neonatal exposure are virtually non-existent, with the exception of COC. In adult subjects these drugs produce effects on serotonergic and/or dopaminergic neurons. For example, FEN primarily affects 5-HT (Rothman *et al.* 2001; Rowland and Carlton 1986) and MDMA has also been reported to preferentially affect the serotonergic system, but to some extent it also affects the dopaminergic system (Lyles and Cadet 2003). In addition to decreasing monoamine levels, MDMA, MA and FEN have been shown to cause short-term release of various neurotransmitters from axon terminals in adults (Rothman *et al.* 2001). However, we felt that the protracted changes in neurotransmitter content would be more likely to affect brain development and possibly alter function later in life than the immediate release of neurotransmitters. Similar to effects in adults, in developing animals we found decreases in striatal and hippocampal 5-HT levels that were reduced by 72 and 66% respectively following FEN administration, and 50 and 49% respectively following MDMA administration. Adult MA exposure produces depletion in both dopaminergic and serotonergic neurons (Cappon *et al.* 1997; Frost and Cadet 2000). However, in this study, four doses of 10 mg/kg MA did not produce any reductions in either 5-HT or DA at the 24-h time point. Therefore, there appears to be a different action for MA on monoaminergic pathways during brain development relative to that seen in the mature brain. This is evident because MA has been shown to produce a 40–60% decrease in DA and 5-HT even 3 days after drug administration in adult animals (Wallace *et al.* 1999; Cappon *et al.* 2000; O’Callaghan and Miller 2002), whereas no effect was observed in the present study after developmental exposure. Cocaine, like MPH, primarily blocks the DA transporter (Kollins *et al.* 2001; Muller *et al.* 2003), but does not affect levels of brain DA. Although we found no effects of COC in this study, MPH produced a slight decrease in 5-HT in the striatum, but without changes in 5-HIAA, and trends for increased DOPAC in the striatum and 5-HIAA in the hippocampus. The dissimilarities between COC and MPH are difficult to reconcile, but these small monoamine changes might imply that MPH may produce modest alterations in brain development that the Morris water maze, as used previously, could not detect.

In the developing brain, 5-HT not only functions as a neurotransmitter but is also thought to influence neuronal differentiation and synaptogenesis (Whitaker-Azmitia *et al.* 1996). Depletion of 5-HT is thought to cause disruptions in brain development (i.e. reductions in neuronal number and disruption of neuronal differentiation and synaptogenesis) that may manifest later in life as cognitive deficits. For example, transient depletion of 5-HT using *p*-chloroamphetamine or permanent reduction by 5,7-dihydroxytryptamine has been shown to produce fewer spines on the dendrites of neurons in the dentate gyrus without affecting dendritic length (Yan *et al.* 1997). We have found a similar effect in spine densities following MA administration from P11–20 (Williams *et al.* 2004). Although we did not observe a decrease in 5-HT levels at the 24-h time point following only 1 day of MA administration, it is possible that multiple days of treatment may affect brain 5-HT concentrations. It has also been found that the depletion of 5-HT from P10–20 using the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine causes spatial learning and memory deficits (Mazer *et al.* 1997). Taken together, it is evident that 5-HT is important in the development of circuits involved in cognitive ability, and that FEN and MDMA can alter this developmental pattern and lead to later cognitive dysfunction.

Not only is 5-HT important in development, but adrenal hormones also play a role in neuronal differentiation and survival. Rodents exhibit an endogenously generated decreased responsiveness to stressors during the neonatal period that is hypothesized to ensure optimal glucocorticoid levels for normal brain development (Sapolsky and Meaney 1986). This period is termed the stress hypo-responsive period (SHRP) and lasts for approximately the first 2 weeks of life. The decreased hypothalamic-pituitary-adrenal axis responsiveness during the SHRP is thought to prevent stress-induced increases in glucocorticoids from interfering with neuronal proliferation, especially in regions that express abundant glucocorticoid receptors such as the dentate gyrus (Sapolsky and Meaney 1986; Vazquez 1998). During the sensitive period of drug administration for substituted amphetamine-induced cognitive deficits, which begins on P11, the hippocampus is growing and differentiating rapidly (Bayer *et al.* 1993; Liu *et al.* 2003) and is an essential mediator involved in spatial learning and memory (Morris *et al.* 1982). In the present study, we found that just 1 day of FEN, MDMA or MA treatment produced increases in CORT for up to 18 h following the last dose (24 h following the first dose), much longer than we previously documented using MA (e.g. 60 min after the last dose) (Williams *et al.* 2000). Moreover, MA induced a >3-fold increase in CORT that persisted over this time span during the SHRP. This effect has an early onset and can be seen by 30 min following the first dose of MA (Williams M. T., Furay A. F., Ehrman L. A., Schaefer T. L., Vorhees C. V. unpublished data). This is unlike any other chemical or stressor effect reported in the literature at this age while pups are with their mother, although it is known that maternal separation is a potent stressor during the SHRP. Maternal separations of  $\leq 2$  h can induce small, but significant, increases in CORT (Pihoker *et al.* 1993; Huot *et al.* 2002); however, it takes at least 8 h of separation before a substantial increase in CORT is achieved (Levine *et al.* 1991). MPH produced a small increase in CORT, but not of the magnitude observed following MA or FEN administration, and COC had no effect. Consistent with the CORT levels found for MPH and COC, no spatial learning deficits were found after exposure to these drugs. Hence, MA treatment appears unique among the psychostimulant/monoamine releasers tested in terms of the magnitude of adrenal output it induces at this age.

The central aim of this study was to identify neurochemical changes important in neurodevelopment, such as in levels of CORT and 5-HT that are perturbed by psychostimulants. In this study, we examined a single time point, 24 h after the first exposure, which was 18 h after the last drug exposure. This time point was selected based on previous time-course data following MDMA, because this was when the largest decrease in 5-HT occurred (Williams *et al.* 2005). Nevertheless, we do not know whether the other drugs administered in this study have a temporal profile of effects similar to those of MDMA, so there are limitations to the present results. For example, there might be transient changes in monoamines following administration of the other drugs studied here that were not detected 24 h after the first dose. However, we were most interested in lasting, rather than transient changes in this study. The age chosen (P11) was selected because this is the first day of the critical period of induction of later cognitive deficits after exposure to substituted amphetamines. As described previously, we found that FEN, MA and MDMA cause cognitive deficits following drug administration from P11–20, although evidence suggests that shorter exposure periods are likely to have the same effects. For example, MA administration from P11–15, but not P16–20, is sufficient to cause deficits in spatial learning (Williams *et al.* 2003b). In addition, a single exposure of MA on P14 in gerbils has been shown to cause alterations in behavior, neuroanatomical structure and neurotransmitter content (Dawirs *et al.* 1996; Blaesing *et al.* 2001; Busche *et al.* 2002; Neddens *et al.* 2002), suggesting that even a single day of exposure is enough to perturb some aspects of neurodevelopment.

If we compare studies in which MA, MDMA, FEN, MPH or COC were administered to rats from P11–20 and tested during adulthood in the Morris water maze acquisition phase, and express the data as a percentage of the control value, there is a clear rank ordering of the



behavioral effects. It should be noted that each of the behavioral studies presented also used a within-litter design. In future studies it would be valuable to compare the behavioral effects of the drugs within the same study. As illustrated in Fig. 5, FEN administration (40 mg/kg/day) produced the greatest deficits in the Morris water maze (Morford *et al.* 2002) followed by MDMA (40 mg/kg/day) (Williams *et al.* 2003c) and MA (40 mg/kg/day) (Williams *et al.* 2002), whereas MPH (40 mg/kg/day) (Vorhees C. V., Moran M. S., Williams M. T. unpublished results) and COC (60 mg/kg/day) (Vorhees *et al.* 2000b) had no effects. In the present study, we have shown that FEN produces the second largest increase in CORT and the most dramatic decrease in striatal and hippocampal 5-HT levels. MDMA produced a decrease in 5-HT in both the striatum and hippocampus, and a modest increase in CORT. Therefore, both FEN and MDMA produce extended changes in early signaling molecules that are important for normal brain development and are likely to be related to later learning ability. MA administration caused significant deficits in the Morris water maze (Williams *et al.* 2002), but they were not as severe as after FEN or MDMA administration. However, MA produced the largest increases in CORT, but no changes in 5-HT, at least when measured at 24 h. This suggests that the CORT increases produce damage that is less severe than the combination of increased CORT and reduced 5-HT. COC (Vorhees *et al.* 2000b) or MPH (Vorhees C. V., Moran M. S., Williams M. T. unpublished results) treatment produced no learning or memory deficits after P11–20 exposure (Fig. 5) and COC did not affect CORT or 5-HT. MPH, on the other hand, produced small changes in CORT and 5-HT levels in the striatum but not enough to affect cognition. Although we did not see learning deficits, MPH during this sensitive period does cause developmental problems such as growth retardation and may produce other as yet uncharacterized behavioral effects (Gauron and Rowley 1974). It should be noted that in the behavioral studies reported, drug was administered for 10 days instead of a single day as in the present study. In addition, there is a possibility that some of the drugs have pharmacological profiles that are different from those of MDMA with respect to early neurochemical changes and the prospect of these differences needs to be considered when evaluating the neurochemical/behavioral comparison. Although we used similar mg/kg doses in this study, it should be noted that these doses were not meant to be equivalent in relative potency but they represented doses of the substituted amphetamines known to produce long-term learning and memory differences when administered for 10 days. Nonetheless, higher levels of MPH (up to 120 mg/kg/day) or COC (60 mg/kg/day) did not produce learning deficits. Therefore, we used similar dosing concentrations of each drug for this comparison. Data from the present study, considered together with the degree of impairment observed following FEN, MDMA, MA, MPH or COC in the Morris water maze, suggest that developmental disruptions in CORT and 5-HT are important in the etiology of substituted amphetamine-induced learning and memory deficits. Therefore, closer examination of these effects on long-term learning and memory deficits warrants further investigation.

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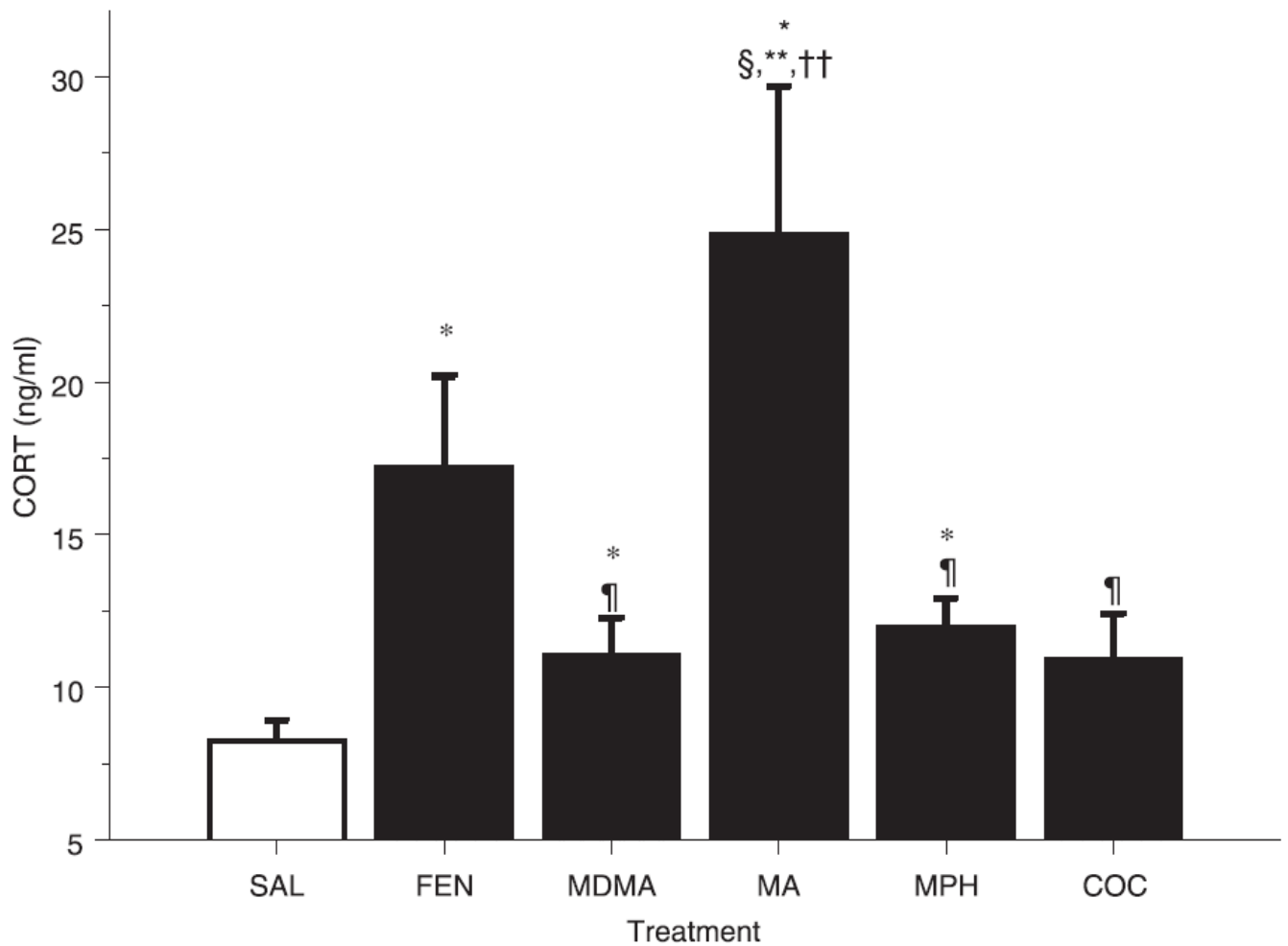
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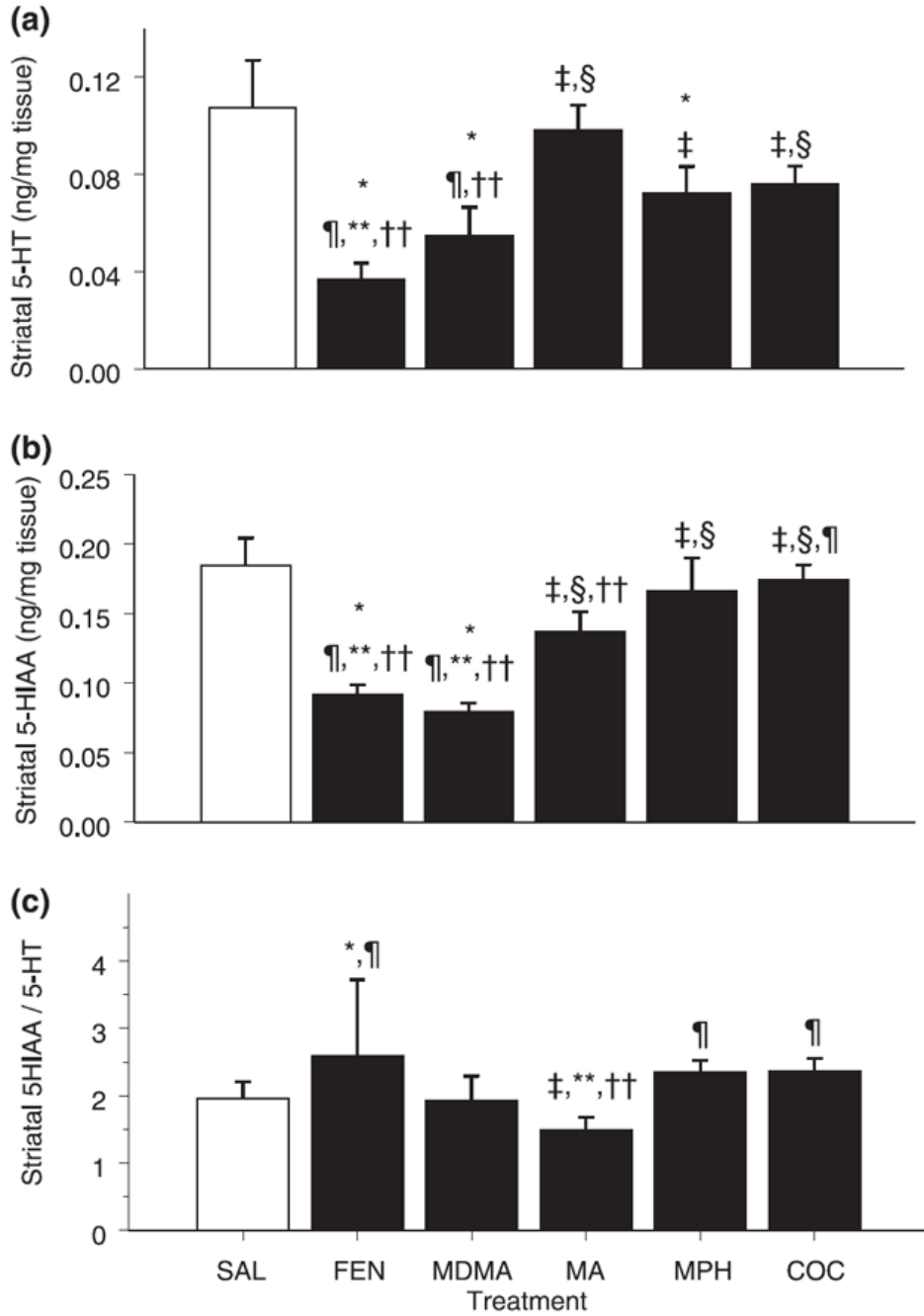
## Abbreviations used

<b>COC</b>	cocaine
<b>CORT</b>	corticosterone
<b>DOPAC</b>	3,4-dihydroxyphenylacetic acid
<b>DA</b>	dopamine
<b>FEN</b>	(+)fenfluramine
<b>5-HIAA</b>	5-hydroxyindolacetic acid
<b>5-HT</b>	serotonin
<b>MA</b>	(+)methamphetamine
<b>MDMA</b>	(+/-)3,4-methylenedioxymethamphetamine
<b>MPH</b>	(+/-)methylphenidate
<b>P</b>	postnatal day
<b>SAL</b>	saline
<b>SHRP</b>	stress hyporesponsive period

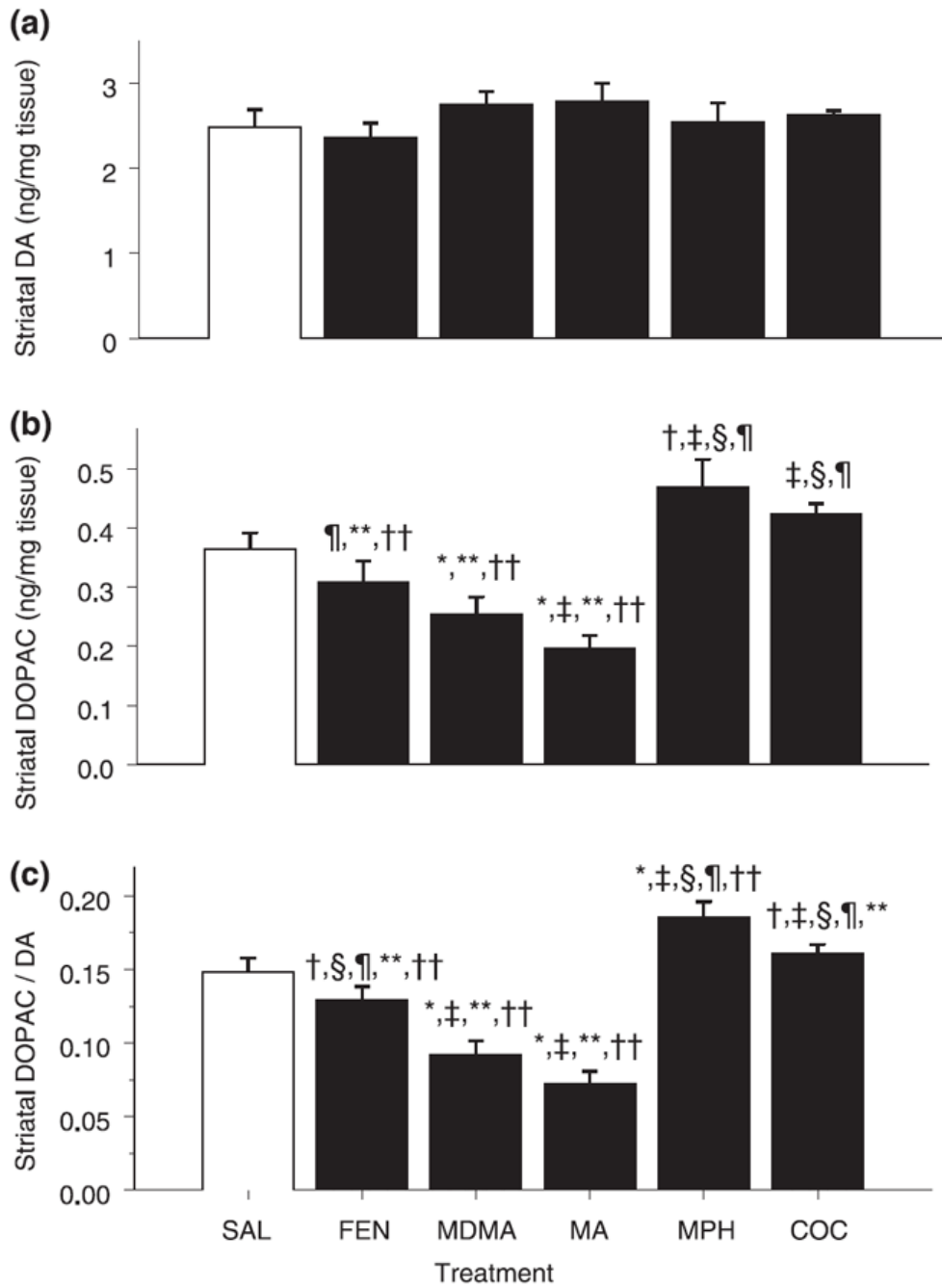




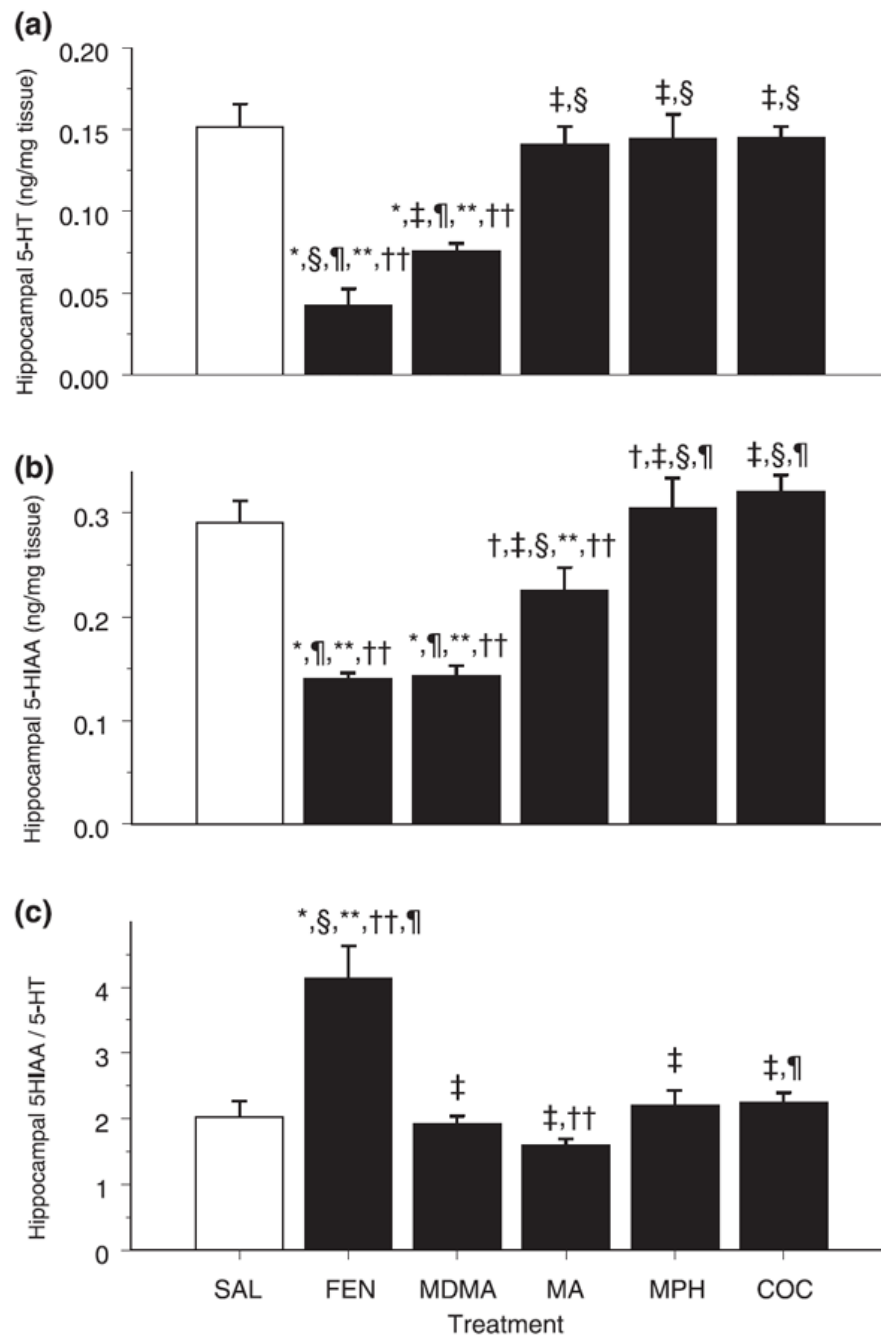
**Fig. 1.** Corticosterone concentrations in plasma 24 h after the first of four doses (every 2 h) of FEN, MDMA, MA MPH and COC (10 mg/kg) that began on P11. Values are mean  $\pm$  SEM. MA produced the greatest increase in CORT, giving significantly different levels from those in animals treated with SAL, MDMA, MPH and COC. FEN also produced an increase in these P12 animals. MDMA and MPH produced similar increases compared with SAL. \* $p$  < 0.05 versus SAL control; § $p$  < 0.05 versus MDMA; ¶ $p$  < 0.05 versus MA; \*\* $p$  < 0.05 versus MPH; †† $p$  < 0.05 versus COC. ANOVA with post-hoc step-down F-tests.



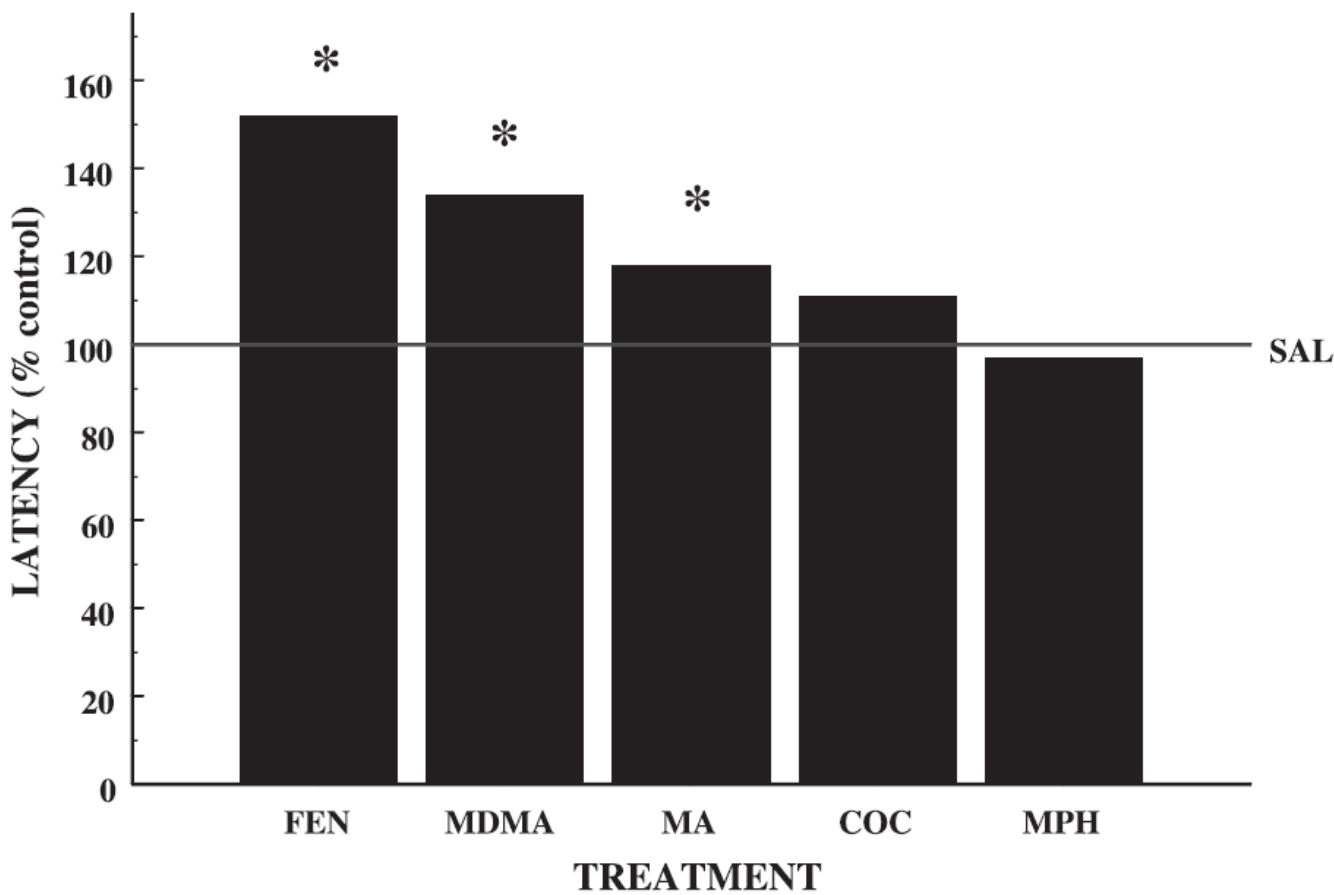
**Fig. 2.** Serotonergic markers in striatum. Values are mean  $\pm$  SEM ( $n = 8$ /group). Concentrations of (a) 5-HT, (b) 5-HIAA and (c) 5-HIAA/5-HT ratio in the striatum of animals exposed to four doses of FEN, MDMA, MA, MPH or COC on P11 and examined on P12. Decreases in 5-HT were observed in FEN-, MDMA- and MPH-treated animals, and FEN and MDMA also decreased 5-HIAA. FEN produced a significant increase in the 5-HIAA/5-HT ratio compared with the SAL group. \* $p < 0.05$  versus SAL control; ‡ $p < 0.05$  versus FEN; § $p < 0.05$  versus MDMA; ¶ $p < 0.05$  versus MA; \*\* $p < 0.05$  versus MPH; †† $p < 0.05$  versus COC. ANOVA with post-hoc step-down F-tests.



**Fig. 3.** Dopaminergic markers in striatum. Values are mean  $\pm$  SEM ( $n = 8/\text{group}$ ). Concentrations of (a) DA, (b) DOPAC and (c) DOPAC/DA ratio in the striatum on P12 following administration of FEN, MDMA, MA, MPH or COC on P11. No changes were observed in DA. MA and MDMA produced a significant decrease in DOPAC. The ratio of DOPAC/DA was significantly decreased following MDMA and MA treatments, and increased in the MPH group. There was also a trend for the DOPAC/DA ratio to be decreased following FEN and increased following COC. \* $p < 0.05$ , † $p < 0.10$  versus SAL control; ‡ $p < 0.05$  versus FEN; § $p < 0.05$  versus MDMA; ¶ $p < 0.05$  versus MA; \*\* $p < 0.05$  versus MPH; †† $p < 0.05$  versus COC.



**Fig. 4.** Serotonergic markers in the hippocampus. Values are mean  $\pm$  SEM ( $n = 8$ /group). Concentrations of (a) 5-HT, (b) 5-HIAA and (c) 5-HIAA/5-HT ratio in the hippocampus of animals on P12 after four doses of FEN, MDMA, MA, MPH or COC on P11. Decreases in 5-HT were observed in FEN- and MDMA-treated animals. FEN and MDMA treatment also produced a significant decrease in 5-HIAA. There was a trend for a decrease in 5-HIAA in the MA group and an increase following MPH. Only FEN produced a significant increase in the 5-HIAA/5-HT ratio compared with the SAL group. \* $p < 0.05$ , † $p < 0.10$  versus SAL control; ‡ $p < 0.05$  versus FEN; § $p < 0.05$  versus MDMA; ¶ $p < 0.05$  versus MA; \*\* $p < 0.05$  versus MPH; †† $p < 0.05$  versus COC.



**Fig. 5.**

Comparison of FEN, MDMA, MA, COC and MPH effects on Morris water maze spatial learning from previous experiments. Each of the drugs was administered at a dose of 40 mg/kg/day from P11–20 with the exception of COC that was administered at 60 mg/kg/day. There is a clear rank order of effects (expressed as percent of SAL from each study) in the acquisition phase of the Morris water maze. FEN produced the longest latencies in finding the hidden platform (Morford *et al.* 2002). In the present study, FEN-treated animals showed the most dramatic decreases in both striatal (Fig. 2a) and hippocampal (Fig. 4a) 5-HT, and a significant increase in CORT (Fig. 1). MDMA produced the next greatest deficit in the Morris maze (Williams *et al.* 2003c), and next largest decreases in striatal (Fig. 2a) and hippocampal (Fig. 4a) 5-HT levels. MA produced significant increases in latency (Williams *et al.* 2002), but not to the degree of FEN or MDMA, and it did not produce any changes in 5-HT (Figs 2a and 4a), but produced the largest increase in CORT (Fig. 1). Neither MPH (Vorhees C. V., Moran M. S., Williams M. T. unpublished results) nor COC (Vorhees *et al.* 2000b) produced any changes in latency and only MPH slightly affected 5-HT in the striatum (Fig. 2a). \* $p < 0.05$  versus SAL controls (100% reference line).



**Table 1**

Body weights measured during the first and last dose

Treatment	<i>n</i>	Weight at dose 1 (g)	Weight at dose 4 (g)
SAL	8	25.59 ± 0.95	25.75 ± 1.02
FEN	8	26.14 ± 0.91	25.64 ± 1.01
MDMA	8	26.89 ± 1.26	26.10 ± 1.35
MA	8	26.89 ± 0.94	25.96 ± 0.92
MPH	8	26.15 ± 1.09	26.25 ± 1.10
COC	8	26.81 ± 1.04	26.73 ± 1.14

Values are mean ± SEM.