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Neonatal 3,4-methylenedioxymethamphetamine (MDMA) exposure alters neuronal protein kinase A activity, serotonin and dopamine content, and [³⁵S]GTPγS binding in adult rats

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Abstract

Recreational use of methylenedioxymethamphetamine (MDMA) has dramatically increased among juveniles and young adults of child-bearing age, and the potential for fetal exposure has increased. For this reason, it is surprising that comparatively few studies have assessed the long-term impact of early MDMA exposure on serotonin (5-HT) and dopamine (DA) neurotransmitter systems. The purpose of this study was to determine whether repeated exposure to MDMA during the preweaning period would cause long-term changes in 5-HT and DA functioning. Rats were treated with saline or 20 mg/kg MDMA (two injections per day) from postnatal day (PD) 11–20. At PD 90, rats were killed, and their dorsal striatum, prefrontal cortex, and hippocampus were removed. 5-HT and DA content, as well as their metabolites, were measured using HPLC. In addition, cAMP-dependent protein kinase A (PKA) activity and agonist-stimulated [³⁵S]GTPγS binding was assayed using tissue homogenates from each brain region. Results indicated that early MDMA exposure caused a decrease in PKA activity and 5-HT content in the prefrontal cortex and hippocampus while increasing the efficacy of 5-HT_{1A} receptors as measured by agonist-stimulated [³⁵S]GTPγS binding. Additionally, DA content was reduced in the dorsal striatum and prefrontal cortex. These data indicate that early MDMA exposure has long-term effects on the 5-HT and DA neurotransmitter systems that may be mediated, at least partially, by changes in 5-HT_{1A} receptor sensitivity.

Keywords

MDMA; Ecstasy; Protein kinase A; Ontogeny; Serotonin; 5-HT_{1A} receptors

1. Introduction

Determining the consequences of 3,4-methylenedioxymethamphetamine (MDMA) exposure on brain development has become more important with the increased use of MDMA by juveniles and young adults of child-bearing age (Banken, 2004; Green et al., 2003; Landry, 2002). Although illicit drug use in the United States declined from 1991 to 2000, MDMA use among high school seniors nearly quadrupled during the same period (Landry, 2002). Smaller but significant increases in MDMA use occurred in 8th and 10th grade students as well as

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college students (Banken, 2004; Landry, 2002; NIDA, 2002). Because of these demographics, the rate of prenatal MDMA exposure is on the increase in the United States. That younger age groups are using MDMA is of special concern, since significant brain development, especially in forebrain structures, continues through the second decade (Bayer et al., 1993; Fuster, 2002). At present, however, there is limited information on the long-term effects of early MDMA exposure on later CNS functioning in humans. Those studies that are available suggest that human MDMA users exhibit a variety of cognitive impairments, including deficits in divided and selective attention, abnormal hippocampal responses during working memory tasks, and declines in both executive functioning and syllogistic reasoning (Jacobsen et al., 2004; Montgomery et al., 2005; von Geusau et al., 2004).

In adult rodents and nonhuman primates, MDMA has potent and long-lasting effects on serotonin (5-HT) neurons. In adult rats, MDMA causes persistent reductions in 5-HT content, 5-hydroxyindoleacetic acid (5-HIAA) levels, 5-HT transporters, and tryptophan hydroxylase activity (for reviews, see Green et al., 2003; McCann and Ricaurte, 2004; Simantov, 2004). Nonhuman primates show similar changes in 5-HT markers because 5-HIAA and 5-HT transporters are reduced after repeated MDMA exposure (for a review, see Lyles and Cadet, 2003). Importantly, these MDMA-induced changes in 5-HT neurochemistry are correlated with long-term declines in cognitive functioning in both rats and nonhuman primates, including impaired spatial memory, executive functioning, and attention (Cohen et al., 2005; McCann et al., 1999; Sprague et al., 2003; Vorhees et al., 2004; Williams et al., 2003). In addition to the well-documented effects of MDMA on 5-HT systems, it has also been reported that MDMA alters dopamine (DA) system functioning (Cohen et al., 2005; Koprich et al., 2003a,b; Miller and O'Callaghan, 1995); however, declines in DA content and DA metabolite levels are typically found in mice and not in rats or nonhuman primates (Colado et al., 2004; Green et al., 2003; Logan et al., 1988; but see Cohen et al., 2005; McGregor et al., 2003).

A very different pattern of effects occurs when MDMA exposure occurs during early ontogeny. Specifically, early MDMA exposure causes long-term impairments on a variety of cognitive tasks (Broening et al., 2001; Cohen et al., 2005; Vorhees et al., 2004; Williams et al., 2003) while not causing adult-like declines in 5-HT levels or 5-HT transporters (Broening et al., 1994, 2001; Cohen et al., 2005). Because of this apparent dissociation between the behavioral impact of MDMA and changes in the 5-HT system (e.g., reductions in 5-HT levels), it is possible that the behavioral changes occurring after early MDMA exposure are not due to the loss of monoamine containing terminals or neurons. Instead, early exposure to MDMA may affect learning and memory processes by causing long-term alterations in cyclic adenosine monophosphate (cAMP) signal transduction mechanisms. This hypothesis is supported by two sets of findings: (i) early exposure to other amphetamine analogs (D-amphetamine and D-methamphetamine) causes long-term declines in dorsal striatal and accumbal cAMP-dependent protein kinase A (PKA) activity (Crawford et al., 2000a,b, 2003); and (ii) decreased PKA activity is associated with hippocampal memory impairment (Abel et al., 1997; Nagakura et al., 2002; Wu et al., 2002). MDMA-induced changes in PKA activity are most likely due to altered receptor/G protein coupling because many monoamine receptors are coupled to the cAMP transduction system. Consistent with this suggestion, both adult and developmental studies have shown that repeated exposure to psychostimulants causes persistent alterations in receptor-mediated cAMP system functioning (Barnett et al., 1987; Roseboom et al., 1990; Unterwald et al., 2003).

The purpose of the present study, therefore, was to determine whether exposing rats to MDMA during the preweanling period would have long-term effects on 5-HT and DA functioning in adulthood. Specifically, rats were treated with saline or 20 mg/kg MDMA (two injections per day) from postnatal days (PD) 11–20. This injection period was chosen for two reasons: (i) exposing rats to MDMA from PD 11–20 disrupts performance on hippocampus-dependent

memory tasks when assessed in adulthood (Broening et al., 2001; Cohen et al., 2005; Vorhees et al., 2004; Williams et al., 2003); and (ii) dramatic changes in CNS maturation, especially in forebrain structures, occur between PD 11 and PD 20: a period analogous to late third trimester human brain development (Altman et al., 1973; Bayer et al., 1993; Dobbing and Sands, 1979). At PD 90, rats were killed, and their dorsal striatum, prefrontal cortex, and hippocampus were removed. cAMP-dependent PKA activity, monoamine (5-HT and DA) and metabolite content, and agonist-stimulated [^{35}S]GTP γ S binding assays were performed on tissue homogenates from each brain region. It was hypothesized that early MDMA exposure would cause persistent declines in PKA activity and increases in agonist-stimulated [^{35}S]GTP γ S binding (a measure of receptor/G protein coupling) that would be detectable in adulthood.

2. Results

2.1. 5-HT and 5-HIAA content

Adult rats that had been exposed to MDMA on PD 11–20 showed long-term reductions of 5-HT in the prefrontal cortex [drug main effect: $F_{1,18} = 12.22$, $P < 0.01$] and hippocampus [drug main effect: $F_{1,18} = 87.06$, $P < 0.001$], but not in the dorsal striatum (Table 1). Although there was a trend towards a drug-induced decline in 5-HT metabolite levels, MDMA exposure did not significantly reduce 5-HIAA content in any of the brain areas tested. MDMA treatment did increase 5-HT turnover (5-HIAA/5-HT) in the hippocampus [drug main effect: $F_{1,18} = 8.49$, $P < 0.01$] but not in the prefrontal cortex or dorsal striatum. There was little evidence that 5-HT functioning differed according to sex, with the one exception being that hippocampal 5-HIAA levels were greater in females rats (107.57 ± 10.17 pg/mg wet weight tissue) than male rats (82.89 ± 7.44 pg/mg wet weight tissue) [sex main effect: $F_{1,18} = 7.53$, $P < 0.05$].

2.2. DA and DOPAC content

MDMA exposure during the neonatal period caused a long-term decrease in DA levels in the prefrontal cortex (Table 1) [drug main effect: $F_{1,18} = 5.83$, $P < 0.05$]. Likewise, the dorsal striatal DA levels of MDMA-treated adult male rats ($20\,586 \pm 3097$ pg/mg wet weight tissue) were reduced relative to male saline controls ($26\,496 \pm 2644$ pg/mg wet weight tissue) [drug $\times$ sex interaction: $F_{1,18} = 5.78$, $P < 0.05$]. DOPAC levels were unaffected by MDMA treatment, although MDMA did increase DA turnover in the prefrontal cortex [drug main effect: $F_{1,18} = 8.25$, $P < 0.05$]. The only sex main effect involved DOPAC levels, as female rats (9.64 ± 0.70 pg/mg wet weight tissue) had greater amounts of DOPAC in the prefrontal cortex than male rats (8.36 ± 0.42 pg/mg wet weight tissue) [sex main effect: $F_{1,18} = 4.70$, $P < 0.05$].

2.3. PKA activity

Exposing rats to MDMA on PD 11–20 significantly reduced PKA activity in the prefrontal cortex [drug main effect: $F_{1,18} = 10.85$, $P < 0.01$] and hippocampus [drug main effect: $F_{1,18} = 6.66$, $P < 0.05$] of adult rats (Table 2). In contrast, PKA activity in the dorsal striatum was not significantly affected by MDMA exposure (Table 2). Dorsal striatal PKA activity did vary according to sex, however, as mean striatal PKA activity of female rats (0.860 ± 0.05 nmol/min/mg protein) was greater than male rats (0.728 ± 0.07 nmol/min/mg protein) [sex main effect: $F_{1,18} = 5.33$, $P < 0.05$].

2.4. [^{35}S]GTP γ S binding

2.4.1. Basal [^{35}S]GTP γ S binding—Basal [^{35}S]GTP γ S specific binding was unaffected by early MDMA exposure and did not differ according to sex. Mean basal [^{35}S]GTP γ S specific binding was measured in the prefrontal cortex (90.72 ± 8.5 fmol/mg protein), hippocampus (152.46 ± 11.9 fmol/mg protein), and dorsal striatum (230.59 ± 23.2 fmol/mg protein).

2.4.2. NPA- and 5-HT-stimulated [³⁵S]GTPγS binding in the prefrontal cortex—

Neonatal MDMA exposure did not affect either the efficacy (i.e., $E_{\max}$) or potency (i.e., pEC_{50}) of NPA-stimulated [³⁵S]GTPγS specific binding in the prefrontal cortex (Table 3). The efficacy of NPA-stimulated binding differed according to sex because $E_{\max}$ values in female rat prefrontal cortex (72.68%, ± 10.1) were 27% smaller than in male rat prefrontal cortex (100.0%, ± 6.4) [sex main effect: $F_{1,12} = 7.03$, $P < 0.05$].

In the prefrontal cortex, 5-HT-stimulated [³⁵S]GTPγS binding was enhanced in rats previously exposed to MDMA (Table 3) [drug main effect: $F_{1,18} = 4.84$, $P < 0.05$]. Specifically, 5-HT had a greater maximal effect in MDMA-treated rats than controls, indicating that MDMA enhanced the efficacy of 5-HT stimulation (see Fig. 1 for a representative dose–response curve). MDMA did not affect the potency of 5-HT-stimulated [³⁵S]GTPγS binding nor did the $E_{\max}$ or pEC_{50} values differ according to sex.

2.4.3. NPA- and 5-HT-stimulated [³⁵S]GTPγS binding in the dorsal striatum—

In the dorsal striatum, MDMA exposure did not alter the efficacy or potency of NPA-stimulated [³⁵S]GTPγS binding (Table 4). In contrast, MDMA treatment decreased the potency (i.e., increased pEC_{50} values) of 5-HT-stimulated [³⁵S]GTPγS specific binding in the dorsal striatum [drug main effect: $F_{1,15} = 4.87$, $P < 0.05$] while not altering 5-HT-stimulated $E_{\max}$ values. This pattern of results suggests that MDMA desensitized the ability of 5-HT to stimulate [³⁵S]GTPγS binding without altering G protein coupling at 5-HT receptors. Dorsal striatal [³⁵S]GTPγS binding did not differ according to sex.

2.4.4. R(+)-8-OH-DPAT- and 5-HT-stimulated [³⁵S]GTPγS binding in the hippocampus—

Neonatal MDMA exposure caused an increase in the efficacy of R(+)-8-OH-DPAT-stimulated [³⁵S]GTPγS binding in the hippocampus (Table 5) [drug main effect: $F_{1,15} = 14.91$, $P < 0.01$]. The effects of MDMA treatment on R(+)-8-OH-DPAT-stimulated [³⁵S]GTPγS binding were more prominent in male rats than female rats (see Fig. 2 for a representative dose–response curve) [drug $\times$ sex interaction: $F_{1,15} = 5.40$, $P < 0.05$]. MDMA exposure did not affect the potency (i.e., pEC_{50} values) of R(+)-8-OH-DPAT-stimulated [³⁵S]GTPγS binding.

Similar to the R(+)-8-OH-DPAT data, the efficacy of 5-HT-stimulated [³⁵S]GTPγS binding in the hippocampus was enhanced after MDMA exposure (Table 5 and Fig. 3) [drug main effect: $F_{1,15} = 4.58$, $P < 0.05$], whereas the potency of 5-HT-stimulated [³⁵S]GTPγS binding was not affected by MDMA. 5-HT-stimulated [³⁵S]GTPγS binding did not differ according to sex.

3. Discussion

In the present study, exposing rats to MDMA during the preweanling period caused long-term neurochemical alterations that were detectable in adult rats. Specifically, rats treated with MDMA from PD 11–20 exhibited long-term declines in DA content in the prefrontal cortex and dorsal striatum (male rats only) and reduced 5-HT levels in the hippocampus and prefrontal cortex. Early MDMA treatment also increased the utilization of dorsal striatal DA and hippocampal 5-HT as measured by metabolite/neurotransmitter ratios. It is notable that MDMA caused persistent declines in dorsal striatal and prefrontal DA content because many researchers believe that MDMA acts as a selective 5-HT neurotoxin in adult rats (Colado et al., 2004; Lyles and Cadet, 2003; McCann and Ricaurte, 2004; but see Cohen et al., 2005; McGregor et al., 2003). The effects of MDMA may be less selective in younger rats than adults, however, because prenatal or neonatal exposure to MDMA has been reported to alter DA turnover (Koprich et al., 2003a,b). In the present study, MDMA reduced prefrontal DA levels by 18.4% at PD 90, whereas Broening et al. (2001) reported that early MDMA exposure caused only a nonsignificant 8.3% decline in prefrontal DA levels at PD 105. These discrepant results

probably reflect that early MDMA exposure has only a weak impact on DA levels. Even so, the ability of MDMA to cause long-term alterations in DA turnover (Koprich et al., 2003a,b) and reduce DA levels in the prefrontal cortex and dorsal striatum (Table 1) indicates that early MDMA exposure does affect the functioning of DA systems.

5-HT systems were also affected by early MDMA exposure because MDMA increased 5-HT utilization in the hippocampus and reduced 5-HT content in the hippocampus and prefrontal cortex. Although there is considerable evidence showing that MDMA treatment induces substantial declines in 5-HT content in adult rats (Green et al., 2003), results from developmental studies are mixed, with some studies reporting that prenatal or neonatal MDMA exposure decreases 5-HT content (Broening et al., 2001; Cohen et al., 2005; Koprich et al., 2003a; Williams et al., 2005), while others report no differences (Koprich et al., 2003b; Meyer et al., 2004). For example, in two previous experiments employing the same exposure period and dose as used here, it was found that treating rats with MDMA on PD 11–20 induced small but significant reductions (6% and 16%) in hippocampal 5-HT content (Broening et al., 2001; Cohen et al., 2005). Although the latter effect was not indicated as significant in the report (Table 1, Cohen et al., 2005) because the statistical approach was limited to only those hypotheses being tested, the hippocampal 5-HT reduction was significant. Even so, the magnitude of the MDMA-induced declines in prefrontal (32%) and hippocampal (41%) 5-HT levels (see Table 1) was unexpected, especially considering that there was not a corresponding significant decline in 5-HIAA levels (see Broening et al., 1994; Colado et al., 2004). Although speculative, the absence of the expected decline in 5-HIAA levels could be indicative of a homeostatic adjustment to maintain normal levels of 5-HT activity. While this effect is not typically observed in adult MDMA exposure paradigms, it is possible that early MDMA treatment could lead to adult-atypical neuronal responses. More generally, it is also possible that the MDMA-induced reductions in 5-HT may have impacted the maturation of synaptic connections and circuitry formation in the prefrontal cortex and hippocampus (see Herlenius and Lagercrantz, 2004). Whether the amount of 5-HT depletion observed in the present study is sufficient to affect neurogenesis and synapse formation is uncertain, however, postnatal rats are known to be susceptible to the deleterious effects of 5-HT depletion (Brezun and Daszuta, 1999; Gaspar et al., 2003).

The ability of MDMA to induce long-term declines in PKA activity has not been previously reported; however, this result is consistent with studies showing that PKA activity is reduced after early exposure to amphetamine or methamphetamine (Crawford et al., 2000a,b, 2003). We previously hypothesized that psychostimulant-induced decreases in dorsal striatal PKA activity were due to either a down-regulation of DA D₁ receptors (which are coupled to G_s G proteins) or an up-regulation of DA D₂ receptors (which are coupled to G_i G proteins). This explanation is probably incorrect because PKA activity shows robust drug-induced declines despite inconsistent changes in dopamine receptor density (Crawford et al., 2000b, 2003). Instead, we hypothesize that psychostimulant-induced decreases in PKA activity are due to altered receptor sensitivity caused by changes in receptor/G protein coupling. In the present study, MDMA-induced reductions in PKA activity were apparent in the hippocampus and prefrontal cortex. Because of the large number of 5-HT receptors in these brain regions, and the ability of MDMA to robustly reduce 5-HT release (Green et al., 2003; Hoyer et al., 2002; Lyles and Cadet, 2003; Meltzer et al., 2003), it is likely that changes in 5-HT receptor sensitivity are responsible for the decreased PKA activity. In particular, changes in the sensitivity of 5-HT₁ receptor subtypes (i.e., 5-HT_{1A} and 5-HT_{1B}) are suspected, since these receptors are coupled to G_i G proteins and, thus, depress cAMP formation (Hoyer et al., 2002; Meltzer et al., 2003).

To further examine the hypothesis that changes in receptor sensitivity are responsible for the MDMA-induced declines in PKA activity, we measured agonist-stimulated [³⁵S] GTPγS

binding. When receptor proteins are stimulated by agonists, guanosine diphosphate (GDP) is released from the receptor complex and guanosine triphosphate (GTP) binds in its place. In vivo GTP is then hydrolyzed back into GDP, and the receptor returns to its resting state (Happe et al., 2001). In the [35 S]GTP γ S binding assay, GTP γ S is resistant to hydrolysis and is not converted into GDP, thus allowing the measurement of [35 S]GTP γ S labeled G protein alpha subunits. This measurement provides a direct estimation of the functional coupling between G protein and receptor (Harrison and Traynor, 2003; Milligan, 2003). In the present study, 5-HT-stimulated [35 S] GTP γ S binding in the prefrontal cortex and hippocampus was enhanced after early MDMA exposure. The enhanced ability of 5-HT to stimulate [35 S]GTP γ S binding suggests that MDMA increased the sensitivity of 5-HT receptors. Although 5-HT is a nonselective agonist, it is likely that the increased sensitivity involves 5-HT $_1$ receptors. This assumption is based on evidence that (i) the [35 S]GTP γ S binding assay primarily measures changes in the G $_i$ family of G proteins (Milligan, 2003), and (ii) 5-HT $_1$ receptors are coupled to G $_i$ proteins. That 5-HT $_1$ receptors are responsible for changes in 5-HT-stimulated [35 S] GTP γ S binding is supported by agonist stimulation assays in the hippocampus using the selective 5-HT $_{1A}$ receptor agonist, *R*(+)-8-OH-DPAT. These assays provided very similar data to the 5-HT stimulation experiment, suggesting that 5-HT and *R*(+)-8-OH-DPAT were affecting the same 5-HT receptor subtype.

MDMA-induced changes in the sensitivity of 5-HT $_{1A}$ receptors could have profound effects on both 5-HT and DA neuronal activity. 5-HT $_{1A}$ receptors function as somatodendritic autoreceptors in the raphe nuclei and as postsynaptic receptors in forebrain areas, including the hippocampus, prefrontal cortex, and hypothalamus (Hoyer et al., 2002; Meltzer et al., 2003). Because of their role as autoreceptors in the raphe, a persistent increase in the sensitivity of 5-HT $_{1A}$ receptors should decrease 5-HT neurotransmission (Hoyer et al., 2002; Meltzer et al., 2003) and reduce 5-HT levels. Activation of 5-HT $_{1A}$ receptors also stimulates the release of other neurotransmitters, including DA (Ichikawa et al., 2001; Meltzer et al., 2003). Although speculative, it is possible that an MDMA-induced increase in the sensitivity of 5-HT $_{1A}$ receptors enhances DA neuronal activity which, in turn, decreases DA synthesis. The MDMA-induced increase in prefrontal DA turnover is consistent with this idea. Therefore, while a loss of DA terminals or cells may be responsible for the MDMA-induced reductions in dorsal striatal and prefrontal DA levels, an alternative possibility is that the decline in 5-HT and DA levels was mediated by an MDMA-induced increase in the sensitivity of 5-HT $_{1A}$ receptors.

Adult rats exposed to MDMA as neonates show robust impairments in spatial learning and memory, while typically exhibiting only mild declines in 5-HT content or 5-HT transporters (Broening et al., 2001; Cohen et al., 2005; Williams et al., 2003). Examination of 5-HT content during the period of neonatal MDMA exposure, however, demonstrates that there are initial reductions in 5-HT that show significant recovery over time (Williams et al., 2005). Therefore, it seems likely that the learning and memory impairments caused by early MDMA exposure are not due to the loss of monoamine terminals or neurons alone but may be the result of alterations in other neural mechanisms. In this case, enhanced sensitivity of 5-HT $_{1A}$ receptors, caused by early MDMA exposure, could be responsible for these long-term deficits in learning and memory. For example, just as performance on spatial memory and avoidance learning tasks is impaired by stimulating 5-HT $_{1A}$ receptors (Bertrand et al., 2000; Bevilacqua et al., 1997; Lee et al., 1992; Pitsikas et al., 2005), it is possible that an MDMA-induced increase in the sensitivity of 5-HT $_{1A}$ receptors also impairs memory performance.

Of the sex differences occurring in the present study, the most interesting finding was that the efficacy of NPA-stimulated [35 S]GTP γ S binding in the prefrontal cortex of female rats was 27% less than in males. This finding may represent a sex-specific difference in either the density or sensitivity of dopamine receptors in the prefrontal cortex. The former explanation seems unlikely, however, because DA receptor density in prefrontal cortex is similar in male and

female rats (Andersen and Teicher, 2000; Harrod et al., 2004). A more likely possibility is that D₂ receptors of male rats are more sensitive than those of female rats, since the D₂ agonist quinpirole causes greater behavioral responsiveness in males than females (Schindler and Carmona, 2002).

In conclusion, the present study adds to the current body of work showing that MDMA exposure during early ontogeny produces long-term adverse effects. We found that early MDMA exposure caused persistent declines in PKA activity, 5-HT and DA content, as well as increased sensitivity of 5-HT receptors. Moreover, these data suggest a potential mechanism (i.e., increased 5-HT_{1A} receptor sensitivity) that may partially explain why early MDMA exposure causes long-term impairments in spatial learning and memory. The present results may also have implications for certain therapeutic drugs because pharmacotherapies that increase 5-HT levels during development, such as selective serotonin reuptake inhibitors (SSRIs), could potentially have long-term negative consequences. Specifically, administration of an SSRI may cause the same increased sensitivity to 5-HT_{1A} receptors and decreased 5-HT levels that were observed after MDMA exposure.

4. Experimental procedures

4.1. Animals and rearing conditions

Nulliparous female (151–175 g) Sprague–Dawley CD IGS rats were obtained from Charles River Laboratories (Raleigh, NC). Rats were allowed to acclimate to housing conditions in the vivarium at the Cincinnati Children's Research Foundation for at least 2 weeks prior to breeding. Rats were pair housed in polycarbonate cages with lights on from 6:00 to 20:00. Food and water were freely available. On the day of breeding, one female was placed with one male rat in a hanging wire cage until a sperm plug was detected [embryonic day (ED) 0] or until the female had been with the male for 2 weeks. Two weeks after being placed with the male, the females were transferred back to polycarbonate cages and singly housed. The presence of a litter was checked twice daily starting on ED 21, and the day of birth was considered PD 0. Litters remained undisturbed until PD 1, at which time litters were culled to ten pups (six males and four females). Subjects were treated according to the National Institute of Health guidelines for the care and use of laboratory animals (*Principles of Laboratory Animal Care*, NIH Publication #85-23) under research protocols approved by the Institutional Animal Care and Use Committees of the Cincinnati Children's Research Foundation and California State University, San Bernardino.

4.2. MDMA administration

Each animal in the litter was randomly assigned to receive either MDMA or saline. $\pm$ 3,4-MDMA HCl (20 mg/kg, expressed as the freebase and greater than 95% pure, NIDA) or saline vehicle was administered twice daily, 8 h apart from PD 11–20. MDMA and saline were injected subcutaneously in the back at a volume of 3 ml/kg. Injection sites were varied to ensure that the surrounding dermis did not become aggravated. Animals were weighed prior to each injection and thereafter at weekly intervals. On approximately PD 60, the animals were shipped to the vivarium at California State University, San Bernardino. Rats were left undisturbed until PD 90, when they were killed by rapid decapitation. Brains were quickly removed, and dorsal striata (i.e., caudate-putamen), hippocampus, and prefrontal cortex were dissected on dry ice and stored at -80°C until time of assay.

4.3. Monoamine content assays

Frozen brain sections were sonicated in 10 volumes of 0.1 N HClO₄ and centrifuged at $20\,000 \times g$ for 30 min at 4°C . The supernatant was then filtered through a 0.22- μm centrifugation unit at $2000 \times g$ for 5 min at 4°C . Twenty microliters of the resulting extracts was then assayed for

5-HT, 5-HIAA, DA, and DOPAC using high performance liquid chromatography (582 pump and an MD-150 column; ESA, Chelmsford, MA) with electrochemical detection (Coulochem II; ESA). The mobile phase consisted of 75 mM NaH₂PO₄, 1.4 mM 1-octane sulfonic acid, 10 mM EDTA, and 10% acetonitrile [(pH 3.1) MD-TM Mobile Phase; ESA] and was pumped at a rate of 0.5 ml/min.

4.4. PKA assay

PKA assays were performed using the Protein Kinase A (cAMP-dependent protein kinase) Assay System protocol (Life Technologies, Grand Island, NY) with slight modifications. Briefly, frozen sections from the dorsal striatum, prefrontal cortex, and hippocampus were placed in homogenization buffer [50 mM Tris (pH 7.4), 100 ng/ml leupeptin, 100 ng/ml aprotinin, and 5 mM EDTA] and homogenized using a hand-held Teflon homogenizer. Protein concentrations were determined using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA) based on the method of Bradford (1976), using bovine serum albumin (BSA) as a standard.

Duplicate homogenates containing 4 µg of protein for each subject were incubated for 5 min at 30 °C in phosphorylation buffer [50 mM Tris (pH 7.4), 10 mM MgCl₂, and 0.25 mg/ml BSA], containing 50 µg of kemptide and 100 µM [γ -³²P]ATP (ICN, Costa Mesa, CA). In addition, the buffer contained either cAMP (10 µM) or PKI (6–22) amide (1 µM/reaction). Following incubation, the phosphorylation mixture was blotted on phosphocellulose filter paper. The filter paper was washed twice with 1% phosphoric acid for 5 min, followed by two 5-min washes with distilled water. Filters were then placed in scintillation fluid and quantified by liquid scintillation spectrophotometry. cAMP-dependent PKA activity was defined as the difference between PKA activity in the presence of cAMP and that measured in the presence of PKI.

4.5. Homogenate [³⁵S]GTPγS binding assay

On the day of assay, tissue was thawed on ice, and crude membrane homogenates were homogenized in 100 volumes of 50 mM Tris–HCl buffer (pH 7.4) for approximately 20 s using a Brinkman Polytron. Homogenates were centrifuged at 20 000 × *g* for 30 min. The pellet was resuspended in 100 volumes of the same buffer and centrifuged again at 20 000 × *g* for 30 min. The final pellet was suspended in approximately 20 volumes of buffer (pH 7.4) and incubated for 30 min at 30 °C to remove endogenous transmitter. Protein concentrations for the final pellet were determined using the Bio-Rad Protein Assay with BSA as the standard.

Agonist–effect curves of [³⁵S]GTPγS binding were performed in assay buffer (50 mM Tris–HCl, 120 mM NaCl) containing 30 µM GDP, 10–20 µg protein, and 5-HT (0.1 nM to 0.1 mM), *R*(+)-8-OH-DPAT (0.1 nM to 0.1 mM), NPA (0.1 pM to 10 µM), or equivalent volumes of water. Nonspecific binding was determined in the presence of 30 µM cold GTPγS. The tubes were preincubated for 15 min at 30 °C, and then 0.1 nM [³⁵S]GTPγS was added. Following the addition of [³⁵S]GTPγS, tubes were incubated for an additional 30 min at 30 °C. The incubation period was ended by filtering the contents of the tubes using glass fiber filters. Net agonist-stimulated [³⁵S]GTPγS binding values were calculated by subtracting basal binding values (without agonist) from agonist-stimulated values (with agonist) and dividing by basal values. Agonist potency (pEC₅₀) and agonist efficacy (*E*_{max}) were determined by iterative nonlinear regression fitting using Prism (Graph Pad Software).

4.6. Data analysis

Separate 2 × 2 (drug × sex) ANOVAs for each brain area were used to analyze PKA and HPLC data, as well as pEC₅₀ and *E*_{max} data from the GTPγS binding assays. For clarity, data are presented collapsed over the sex variable because few main effects or interactions involving

sex reached statistical significance. Those sex main effects and interactions reaching statistical significance are presented and discussed in the text. For the GTP γ S binding experiments, a logarithmic transformation of the $E_{\max}$ data was conducted ($Y' = \log_{10} Y$), and results were presented as percent of controls (calculation of the pEC₅₀ also involves a logarithmic transformation). Litter effects were controlled by treating litter as a random factor in all analyses (Hughes, 1979). When appropriate, post hoc analysis of data was done using Tukey tests ($P < 0.05$).

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References

- Abel T, Nguyen PV, Barad M, Deuel TA, Kandel ER, Bourtchouladze R. Genetic demonstration of a role for PKA in the late phase of LTP in hippocampus-based long-term memory. *Cell* 1997;88:615–626. [PubMed: 9054501]
- Altman J, Brunner RL, Bayer SA. The hippocampus and behavioral maturation. *Behav Biol* 1973;8:557–596. [PubMed: 4196186]
- Andersen SL, Teicher MH. Sex differences in dopamine receptors and their relevance to ADHD. *Neurosci Biobehav Rev* 2000;24:137–141. [PubMed: 10654670]
- Banken JA. Drug abuse trends among youth in the United States. *Ann N Y Acad Sci* 2004;1025:465–471. [PubMed: 15542750]
- Barnett JV, Segal DS, Kuczenski R. Repeated amphetamine pretreatment alters the responsiveness of striatal dopamine-stimulated adenylate cyclase to amphetamine-induced desensitization. *J Pharmacol Exp Ther* 1987;242:40–47. [PubMed: 2956412]
- 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]
- Bertrand F, Lehmann O, Lazarus C, Jeltsch H, Cassel JC. Intraseptal infusions of 8-OH-DPAT in the rat impairs water-maze performances: effects on memory or anxiety. *Neurosci Lett* 2000;279:45–48. [PubMed: 10670784]
- Bevilaqua L, Ardenghi P, Schröder N, Bromberg E, Schmitz PK, Schaeffer E, Quevedo J, Bianchin M, Walz R, Medina JH, Izquierdo I. Drugs acting upon the cyclic adenosine monophosphate/protein kinase A signaling pathway modulate memory consolidation when given late after training into rat hippocampus but not amygdala. *Behav Pharmacol* 1997;8:331–338. [PubMed: 9832992]
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248–254. [PubMed: 942051]
- Brezun JM, Daszuta A. Depletion of serotonin decreases neurogenesis in the dentate gyrus and the subventricular zone of adult rats. *Neuroscience* 1999;89:999–1002. [PubMed: 10362289]
- Broening HW, Bacon L, Slikker W Jr. Age modulates the long-term but not the acute effects of the serotonergic neurotoxicant 3,4-methylenedioxymethamphetamine. *J Pharmacol Exp Ther* 1994;271:285–293. [PubMed: 7965726]
- 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]
- Cohen MA, Skelton MR, Schaefer TL, Gudelsky GA, Vorhees CV, Williams MT. Learning and memory after neonatal exposure to 3,4-methylenedioxymethamphetamine (Ecstasy) in rats: interaction with exposure in adulthood. *Synapse* 2005;57:148–159. [PubMed: 15945064]
- Colado MI, O'Shea E, Green AR. Acute and long-term effects of MDMA on cerebral dopamine biochemistry and function. *Psychopharmacology* 2004;173:249–263. [PubMed: 15083264]

- Crawford CA, Zavala AR, Karper PE, Collins RL, Loring-Meier TL, Watson JB, McDougall SA. Amphetamine treatment during the preweanling period produces long-term changes in striatal protein kinase A activity. *Pharmacol Biochem Behav* 2000a;66:835–840. [PubMed: 10973523]
- Crawford CA, Zavala AR, Karper PE, McDougall SA. Long-term effects of postnatal amphetamine treatment on striatal protein kinase A activity, dopamine D₁-like and D₂-like binding sites, and dopamine content. *Neurotoxicol Teratol* 2000b;22:799–804. [PubMed: 11120384]
- Crawford CA, Williams MT, Newman ER, McDougall SA, Vorhees CV. Methamphetamine exposure during the preweanling period causes prolonged changes in dorsal striatal protein kinase A activity, dopamine D₂-like binding sites, and dopamine content. *Synapse* 2003;48:131–137. [PubMed: 12645038]
- Dobbing J, Sands J. Comparative aspects of the brain growth spurt. *Early Hum Dev* 1979;3:79–83. [PubMed: 118862]
- Fuster JM. Frontal lobe and cognitive development. *J Neurocytol* 2002;31:373–385. [PubMed: 12815254]
- Gaspar P, Cases O, Maroteaux L. The developmental role of serotonin: news from mouse molecular genetics. *Nat Rev, Neurosci* 2003;4:1002–1011. [PubMed: 14618156]
- 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]
- Happe HK, Bylund DB, Murrin LC. Agonist-stimulated [³⁵S]GTPγS autoradiography: optimization for high sensitivity. *Eur J Pharmacol* 2001;422:1–13. [PubMed: 11430907]
- Harrison C, Traynor JR. The [³⁵S]GTPγS binding assay: approaches and applications in pharmacology. *Life Sci* 2003;74:489–508. [PubMed: 14609727]
- Harrod SB, Mactutus CF, Bennett K, Hasselrot U, Wu G, Welch M, Booze RM. Sex differences and repeated intravenous nicotine: behavioral sensitization and dopamine receptors. *Pharmacol Biochem Behav* 2004;78:581–592. [PubMed: 15251267]
- Herlenius E, Lagercrantz H. Development of neurotransmitter systems during critical periods. *Exp Neurol* 2004;190:S8–S21. [PubMed: 15498537]
- Hoyer D, Hannon JP, Martin GR. Molecular, pharmacological, and functional diversity of 5-HT receptors. *Pharmacol Biochem Behav* 2002;71:533–554. [PubMed: 11888546]
- Hughes CW. Outcome of early experience studies as affected by between-litters variance. *J Nutr* 1979;109:642–645. [PubMed: 430265]
- Ichikawa J, Ishii H, Bonaccorso S, Fowler WL, O'Laughlin IA, Meltzer JJ. 5-HT_{2A} and D₂ receptor blockade increases cortical DA release via 5-HT_{1A} receptor activation: a possible mechanism of atypical antipsychotic-induced cortical dopamine release. *J Neurochem* 2001;76:1521–1531. [PubMed: 11238736]
- Jacobsen LK, Mendl WE, Pugh KR, Skudlarski P, Krystal JH. Preliminary evidence of hippocampal dysfunction in adolescent MDMA ("ecstasy") users: possible relationship to neurotoxic effects. *Psychopharmacology* 2004;173:383–390. [PubMed: 14647960]
- Koprich JB, Campbell NG, Lipton JW. Neonatal 3,4-methylenedioxymethamphetamine (ecstasy) alters dopamine and serotonin neurochemistry and increases brain-derived neurotrophic factor in the forebrain and brainstem of the rat. *Dev Brain Res* 2003a;147:177–182. [PubMed: 14741762]
- Koprich JB, Chen EY, 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* 2003b;25:509–517. [PubMed: 12972064]
- Landry MJ. MDMA: a review of epidemiologic data. *J Psychoact Drugs* 2002;34:163–169.
- Lee EHY, Lin WR, Chen HY, Shiu WH, Liang KC. Fluoxetine and 8-OH-DPAT in the lateral septum enhances and impairs retention of an inhibitory avoidance response in rats. *Physiol Behav* 1992;51:681–688. [PubMed: 1534416]
- Logan BJ, Lavery R, Sanderson WD, Yee YB. Differences between rats and mice in MDMA (methylenedioxymethylmethamphetamine) neurotoxicity. *Eur J Pharmacol* 1988;152:227–234. [PubMed: 2906000]

- Lyles J, Cadet JL. Methylenedioxymethamphetamine (MDMA, Ecstasy) neurotoxicity: cellular and molecular mechanisms. *Brain Res Rev* 2003;42:155–168. [PubMed: 12738056]
- McCann UD, Ricaurte GA. Amphetamine neurotoxicity: accomplishments and remaining challenges. *Neurosci Biobehav Rev* 2004;27:821–826. [PubMed: 15019431]
- McCann UD, Mertl M, Eligulashvili V, Ricaurte GA. Cognitive performance in ($\pm$) 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) users: a controlled study. *Psychopharmacology* 1999;143:417–425. [PubMed: 10367560]
- McGregor IS, Gurtman CG, Morley KC, Clemens KJ, Blokland A, Li KM, Cornish JL, Hunt GE. Increased anxiety and “depressive” symptoms months after MDMA (“ecstasy”) in rats: drug-induced hyperthermia does not predict long-term outcomes. *Psychopharmacology* 2003;168:465–474. [PubMed: 12700882]
- Meltzer HY, Li Z, Kaneda Y, Ichikawa J. Serotonin receptors: their key role in drugs to treat schizophrenia. *Prog Neuro-Psychopharmacol Biol Psychiatry* 2003;27:1159–1172.
- 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]
- Miller DB, O’Callaghan JP. The role of temperature, stress, and other factors in the neurotoxicity of the substituted amphetamines 3,4-methylenedioxymethamphetamine and fenfluramine. *Mol Neurobiol* 1995;11:177–192. [PubMed: 8561961]
- Milligan G. Principles: extending the utility of [35 S]GTP γ S binding assays. *Trends Pharmacol Sci* 2003;24:87–90. [PubMed: 12559773]
- Montgomery C, Fisk JE, Newcombe R, Wareing M, Murphy PN. Syllogistic reasoning performance in MDMA (Ecstasy) users. *Exp Clin Psychopharmacol* 2005;13:137–145. [PubMed: 15943546]
- Nagakura A, Takagi N, Takeo S. Impairment of cerebral cAMP-mediated signal transduction system and of spatial memory function after microsphere embolism in rats. *Neuroscience* 2002;113:519–528. [PubMed: 12150772]
- National Institute on Drug Abuse. Secondary School Students, NIH Publication 02-5106. Vol. I. Bethesda, MD: US Department of Health and Human Services, National Institutes of Health; 2002. Monitoring the Future. National Survey Results on Drug Use, 1975–2001.
- Pitsikas N, Tsitsirigou S, Zisopoulou S, Sakellariadis N. The 5-HT $_1A$ receptor and recognition memory possible modulation of its behavioral effects by the nitrenergic system. *Behav Brain Res* 2005;159:287–293. [PubMed: 15817191]
- Roseboom PH, Hewlett GHK, Gnegy ME. Repeated amphetamine administration alters the interaction between D1-stimulated adenylyl cyclase activity and calmodulin in rat striatum. *J Pharmacol Exp Ther* 1990;255:197–203. [PubMed: 2145422]
- Schindler CW, Carmona GN. Effects of dopamine agonists and antagonists on locomotor activity in male and female rats. *Pharmacol Biochem Behav* 2002;72:857–863. [PubMed: 12062575]
- Simantov R. Multiple molecular and neuropharmacological effects of MDMA (Ecstasy). *Life Sci* 2004;74:803–814. [PubMed: 14659969]
- 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]
- Unterwald EM, Ivkovic S, Cuntapay M, Stroppolo A, Guinea B, Ehrlich ME. Prenatal exposure to cocaine decreases adenylyl cyclase activity in embryonic mouse striatum. *Dev Brain Res* 2003;147:67–75. [PubMed: 14741752]
- von Geusau NA, Stalenhoef P, Huizinga M, Snel J, Ridderinkhof KR. Impaired executive function in male MDMA (“ecstasy”) users. *Psychopharmacology* 2004;175:331–341. [PubMed: 15034712]
- 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, 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* 2003;968:89–101. [PubMed: 12644267]

- Williams MT, Schaefer TL, Ehrman LA, Able JA, Gudelsky GA, Sah R, Vorhees CV. 3,4-Methylenedioxymethamphetamine administration on postnatal day 11 in rats increases pituitary–adrenal output and reduces striatal and hippocampal serotonin without altering SERT activity. *Brain Res* 2005;1039:97–107. [PubMed: 15781051]
- Wu J, Li J, Huang KP, Huang FL. Attenuation of protein kinase C and c-AMP-dependent protein kinase signal transduction in the neurogranin knockout mouse. *J Biol Chem* 2002;277:19498–19505. [PubMed: 11912190]

Fig. 1.

Representative sigmoid dose–response curve of 5-HT-stimulated GTP γ S binding in membranes from the prefrontal cortex of female rats exposed to saline or MDMA (20 mg/kg $\times$ 2 injections per day) from PD 11–20. Results showed rats exposed to MDMA had greater levels of 5-HT-stimulated GTP γ S binding as compared to saline controls. $\bullet$ = 0 mg/kg MDMA; $\blacksquare$ = 20 mg/kg MDMA.

Fig. 2.

Representative sigmoid dose–response curve of *R*(+) 8-OH-DPAT-stimulated GTP γ S binding in hippocampal membranes of male rats exposed to saline or MDMA (20 mg/kg $\times$ 2 injections per day) from PD 11–20. Results showed rats exposed to MDMA had greater levels of 5-HT-stimulated GTP γ S binding as compared to saline controls. $\bullet$ = 0 mg/kg MDMA; $\blacksquare$ = 20 mg/kg MDMA.

Fig. 3.

Representative sigmoid dose–response curve of 5-HT-stimulated GTP γ S binding in hippocampal membranes of male rats exposed to saline or MDMA (20 mg/kg $\times$ 2 injections per day) from PD 11–20. Results showed rats exposed to MDMA had greater levels of 5-HT-stimulated GTP γ S binding as compared to saline controls. $\bullet$ = 0 mg/kg MDMA; $\blacksquare$ = 20 mg/kg MDMA.

Table 1

Effects of MDMA treatment on monoamine levels (pg/mg wet weight tissue) in various brain regions

	5-HT	5-HIAA	5-HIAA/5-HT	DA	DOPAC	DOPAC/DA
<i>Prefrontal cortex</i>						
Saline	457.5 (±34.1)	145.8 (±10.5)	0.33 (±0.03)	58.2 (±5.80)	8.9 (±0.48)	0.16 (±0.01)
MDMA	311.5 (±40.1) ^a	112.4 (±18.2)	0.36 (±0.03)	47.5 (±4.51) ^a	9.1 (±0.70)	0.20 (±0.01) ^a
<i>Hippocampus</i>						
Saline	396.8 (±7.0)	102.2 (±8.7)	0.25 (±0.02)			
MDMA	234.9 (±20.0) ^a	88.3 (±9.9)	0.39 (±0.05) ^a			
<i>Dorsal striatum</i>						
Saline	148.8 (±16.1)	121.3 (±10.5)	0.86 (±0.05)	22,577 (±1768)	1,623 (±96)	0.07 (±0.01)
MDMA	135.7 (±13.0)	106.4 (±8.1)	0.81 (±0.03)	21,750 (±1624) ^b	1,597 (±90)	0.08 (±0.02)

Rats were treated with saline or MDMA (20 mg/kg × 2 injections per day) from PD 11–20. Monoamine levels (mean ± SEM) were assayed at PD 90.

^aSignificantly different from saline-treated rats.^bSignificantly different from saline-treated male rats (see text for fuller discussion).

Table 2

Effects of MDMA treatment on protein kinase A (PKA) activity (nmol/min/mg protein) in various brain regions

Brain area	Drug treatment		
	Saline	MDMA	Percent of saline
Prefrontal Cortex	0.92 (± 0.07)	0.66 (± 0.04) ^a	71.19
Hippocampus	0.52 (± 0.06)	0.38 (± 0.04) ^a	73.47
Dorsal striatum	0.83 (± 0.04)	0.75 (± 0.07)	90.92

Rats were treated with saline or MDMA (20 mg/kg $\times$ 2 injections per day) from PD 11–20. PKA activity (mean $\pm$ SEM) was assayed at PD 90.

^aSignificantly different from saline-treated rats.

Table 3Effects of MDMA treatment on agonist-induced [³⁵S]GTPγS specific binding in prefrontal cortex

Drug treatment	NPA-stimulated		5-HT-Stimulated	
	<i>E</i> _{max}	pEC ₅₀	<i>E</i> _{max}	pEC ₅₀
Saline	100.0% (±12.7)	100.0% (±5.6)	100.0% (±5.4)	100.0% (±5.0)
MDMA	95.1% (±7.8)	100.6% (±6.6)	111.6% (±3.1) ^a	96.03% (±3.0)

Data are presented as percent of saline controls. Rats were treated with saline or MDMA (20 mg/kg × 2 injections per day) from PD 11–20. Agonist-induced [³⁵S]GTPγS specific binding was assayed at PD 90.

^aSignificantly different from saline-treated rats.

Table 4Effects of MDMA treatment on agonist-induced [³⁵S]GTPγS specific binding in dorsal striatum

Drug treatment	NPA-Stimulated		5-HT-Stimulated	
	<i>E</i> _{max}	pEC ₅₀	<i>E</i> _{max}	pEC ₅₀
Saline	100.0% (±10.7)	100.0% (±5.6)	100.0% (±11.3)	100.0% (±3.2)
MDMA	113.3% (±10.4)	95.5% (±3.0)	101.2% (±19.0)	138.6% (±15.4) ^a

Data are presented as percent of saline controls. Rats were treated with saline or MDMA (20 mg/kg × 2 injections per day) from PD 11–20. Agonist-induced [³⁵S]GTPγS specific binding was assayed at PD 90.

^aSignificantly different from saline-treated rats.

Table 5Effects of MDMA treatment on agonist-induced [³⁵S]GTPγS specific binding in hippocampus

Drug treatment	<i>R(+)</i> 8-OH-DPAT- stimulated		5-HT-stimulated	
	<i>E</i> _{max}	pEC ₅₀	<i>E</i> _{max}	pEC ₅₀
Saline	100.0% (±1.3)	100.0% (±6.9)	100.0% (±3.0)	100.0% (±2.0)
MDMA	109.1% (±2.4) ^a	101.5% (±8.1)	107.6% (±2.6) ^a	98.7% (±3.1)

Data are presented as percent of saline controls. Rats were treated with saline or MDMA (20 mg/kg × 2 injections per day) from PD 11–20. Agonist-induced [³⁵S]GTPγS specific binding was assayed at PD 90.

^aSignificantly different from saline-treated rats.