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Serotonin Release is Responsible for the Locomotor Hyperactivity in Rats Induced by Derivatives of Amphetamine Related to MDMA

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Summary. A structural derivative of amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), is a potent serotonin (5-HT) releasing drug. When administered to humans, MDMA produces psychological effects that are distinct from the effects of classical psychostimulants and hallucinogens. In rats, MDMA produces locomotor hyperactivity, but the spatial pattern of locomotion and the suppression of investigatory behaviors in MDMA-treated rats differs qualitatively from the pattern of exploration produced by other psychostimulants. Previous experiments have indicated that the motor activating effects of MDMA in rats depend upon 5-HT release. Antagonism of MDMA-induced norepinephrine release by pretreatment with nisoxetine was less effective than serotonergic manipulations at reducing the behavioral effects of MDMA, suggesting a minor contribution of norepinephrine release to the behavioral effects of MDMA. A derivative of MDMA with greater selectivity for serotonergic systems over catecholaminergic systems, 5,6-(methylenedioxy)-2-aminoindan (MDAI), produced a biphasic effect in rats consisting of an early suppression of exploratory activity and a later motor activation. Only the motor activation by MDAI is antagonized by treatments designed to prevent drug-induced 5-HT release, consistent with the importance of 5-HT release for the activating effects of MDMA. Finally, the locomotor activation, suppression of investigatory responding and detailed spatial patterning of activity produced by MDMA was not diminished in a familiar environment. Similar effects produced by hallucinogens are attenuated in a familiar testing environment, suggesting that these effects of MDMA do not reflect hallucinogenic properties of this drug. These results demonstrate the potential utility of 5-HT releasing drugs in future studies of the influence of endogenous 5-HT on unconditioned exploratory activity.

Introduction

Many behavioral effects have been attributed to stimulation of central serotonin (5-HT) receptors (Glennon and Lucki 1988). This diversity of responses is not surprising considering the large number of central 5-HT receptor subtypes and the anatomical heterogeneity of central 5-HT systems. However, one conceptual difficulty in the interpretation of the behavioral effects of selective 5-HT ligands is extrapolating from the actions of these drugs to the physiological actions of the endogeneous neurotransmitter. One approach to unraveling this difficulty is to assess

the behavioral actions of drugs that are 5-HT releasing agents. Presumably, the behavioral effects of 5-HT releasing drugs will result from stimulation of only those 5-HT receptors that are innervated by serotonergic terminals and from coordinated activation of various 5-HT receptor subtypes in the same proportions that they are normally stimulated by the endogenous transmitter.

Structural derivatives of amphetamine have varying potencies as 5-HT releasing agents. One such drug, 3,4-methylenedioxymethamphetamine (MDMA), releases 5-HT from synaptosomes and striatal slices (Nichols et al. 1982; Schmidt et al. 1987). *In vivo*, MDMA produces an acute decrease in tissue 5-HT levels that is consistent with depletion of 5-HT stores by release (Schmidt et al. 1987; Johnson and Nichols 1989). Both the 5-HT release *in vitro* and the decrease in 5-HT levels *in vivo* are blocked by 5-HT uptake inhibitors (Schmidt et al. 1987; Hekmatpanah and Peroutka 1990). These data suggest that the MDMA-induced release of 5-HT is dependent upon the functional integrity of the 5-HT uptake carrier, and that MDMA releases 5-HT via a mechanism similar to that whereby amphetamine releases dopamine (Fischer and Cho 1979; Raiteri et al. 1979).

Both biochemical and electrophysiological studies reveal that, like amphetamine, MDMA affects dopamine (DA) neurotransmission. For example, MDMA inhibits the uptake of DA into synaptosomes (Steele et al. 1987). Similarly, MDMA releases DA from striatal slices with the *S*-isomer being more potent (Johnson et al. 1986). *In vivo*, MDMA increases tissue levels of DA and decreases levels of the primary DA metabolite DOPAC (Matthews et al. 1989). This pattern of biochemical changes was interpreted as reflecting decreased DA utilization ("turnover"), but is also consistent with inhibition of DA reuptake. *In vivo* voltammetry with a stearate electrode reveals MDMA-induced increases in DA levels in both the nucleus accumbens and caudate of conscious rats (Yamamoto and Spanos 1988). In contrast, a study using graphite electrodes in chloral hydrate anesthetized rats revealed decreased DA levels after MDMA (Gazzara et al. 1989). The different results obtained in these two studies may result from the use of anesthetic, because a separate study also found that MDMA increased caudate DA levels using *in vivo* dialysis in conscious rats (Hiramatsu and Cho 1990). The differential effect of anesthesia should also be considered in reports that MDMA decreases DA neuronal firing in the substantia nigra of anesthetized rats (Kelland et al. 1989; Matthews et al. 1989). Most interestingly, the effects of MDMA on indices of DA neurotransmission are attenuated by prior depletion of central 5-HT, suggesting that drug-induced release of 5-HT indirectly influences dopaminergic neurotransmission (Gazzara et al. 1989; Kelland et al. 1989).

In humans, MDMA produces novel mood-altering effects that have promoted its recreational use by some individuals. Originally the struc-

tural similarity of MDMA to the hallucinogenic amphetamine derivatives 3,4-methylenedioxyamphetamine (MDA) and 2,5 dimethoxy-4-methyl-amphetamine (DOM), prompted the hypothesis that the unique subjective effects of MDMA resulted from a combination of hallucinogenic and stimulant-like stimulus cues (Anderson et al. 1978). However, the subjective effects of MDMA that are associated with its recreational use consist of alterations in the perception of emotions and in the ability to communicate with other people that are qualitatively different from the euphoria produced by amphetamine and other psychostimulants, while perceptual distortions associated with hallucinogenic amphetamine derivatives are reported infrequently (Peroutka et al. 1988). Animals also discriminate MDMA from both psychostimulants and hallucinogens. For example, MDMA is not recognized by rats trained to discriminate either psychostimulants or hallucinogens from saline (Glennon et al. 1982; Oberlender and Nichols 1988; Broadbent et al. 1989). Thus, subjective reports and drug-discrimination data reveal that the acute effects of MDMA differ from psychostimulants and hallucinogens, prompting some to consider MDMA as a representative of a new drug class, separate from both psychostimulants and hallucinogens (Nichols et al. 1986).

MDMA resembles classical psychostimulants by virtue of producing behavioral hyperactivity during tests of unconditioned exploratory behavior in rats (Spanos and Yamamoto 1989). However, multivariate behavioral studies reveal that the detailed pattern of MDMA-induced behavioral activation is markedly different from that produced by psychostimulants (Gold et al. 1988). One hypothesis to explain these observations and the drug-discrimination data is that the release of DA by MDMA produces amphetamine-like behavioral stimulation. The concurrent release of 5-HT modifies particular components of this behavioral activation and accounts for the unique stimulus cues of MDMA. An approach to testing this hypothesis would be to selectively block DA or 5-HT release by MDMA, thereby unmasking the effects of 5-HT or DA release respectively. The following experiments were designed, therefore, to identify the respective contributions of 5-HT and DA activation to the effects of MDMA on exploratory behavior.

Materials and Methods

In the present studies, the neurochemical basis for the behavioral effects of MDMA in rats was investigated using a Behavioral Pattern Monitor (BPM) that records both locomotor activity and investigatory behaviors (Flicker and Geyer 1982). Briefly, the BPM consists of a 30.5 × 61.0 cm Plexiglas box. Three 2.5 cm holes are placed in each long wall and in the long axis of the floor, and a single hole is placed in one short wall.

Infrared photobeams detect the X-Y position of the animal with a resolution of 3.5 cm and separate photobeams detect nosepokes into the holes. A metal touchplate 15.2 cm above the floor detects rearings. A computer continuously monitors and records the status of all photobeams and the touchplate. Transitions between eight equal 15.25 cm × 15.25 cm regions in the BPM are calculated as an index of motor activity (crossings). The geometrical pattern of the locomotor path followed by an individual rat can be reconstructed from the raw data for subsequent analysis. Moreover, the number and duration of entries into the center of the BPM are calculated, along with the number and durations of all holepokes and rearings.

In addition to recording the time spent in various regions of the BPM, the raw data are used to calculate measures that describe the spatial character of the rat locomotor path. One such measure, the coefficient of variation (CV), is influenced by the variety or predictability of the locomotor path (Geyer et al. 1986). Briefly, the number of each of 40 possible transitions between any of 9 regions in the BPM are tabulated over the entire 60 min test session for an individual animal. The CV for that animal is then calculated as the standard deviation of the 40 transition frequencies divided by the mean of these frequencies. Values for the CV increase when certain transitions are preferentially repeated and decrease when the locomotor path becomes more evenly distributed throughout the BPM. A second measure to describe the spatial character of the locomotor path is the "spatial d" or "d" (Paulus and Geyer 1991). This measure is based conceptually on fractal geometry and describes the relative smoothness or roughness of the locomotor path. Briefly, the length of the entire locomotor path taken by an individual rat through the BPM is calculated from the raw data using several different spatial resolutions. Using scaling arguments, the rate with which the calculated path length decreases as a function of decreasing spatial resolution is fitted to an exponential curve, and the fitted coefficient for the exponent of this function is taken as d. Values for d increase when the locomotor path is rougher (many direction changes per path length) and decrease when the path is smoother (fewer direction changes).

Results

Acute Behavioral Effects of MDMA

In the BPM, MDMA produces behavioral stimulation consisting primarily of increased forward locomotion (Gold et al. 1988). The behaviorally activating effects of MDMA have been confirmed in other studies (Spanos and Yamamoto 1989), yet several measures distinguish

MDMA from amphetamine. (1) MDMA-treated rats frequently circle the periphery and avoid the center of the testing chamber (Gold et al. 1988), whereas amphetamine-treated rats exhibit more varied spatial patterns of locomotion and explore the entire chamber (Geyer et al. 1987; Paulus and Geyer 1991). (2) Investigatory behaviors (holepokes and rearings) are suppressed by the same doses of MDMA that increase locomotion (Gold et al. 1988). In contrast, amphetamine increases the absolute number of holepokes and rearings (Geyer et al. 1987). (3) As the dose of MDMA is increased, focussed stereotypy is not observed (Gold et al. 1988; Spanos and Yamamoto 1989), although focussed stereotyped behaviors become the primary activity at high doses of amphetamine. Thus, MDMA and amphetamine produce qualitatively different behavioral effects in rats across a range of doses, supporting the hypothesis that different neurochemical mechanisms mediate the effects of MDMA and amphetamine.

The spatial character of MDMA-induced locomotor hyperactivity has been characterized more closely using both CV and d measures. Both racemic MDMA and its *S*-isomer produce dose-dependent increases in CV (Gold et al. 1988; Callaway et al. 1990). Because changes in CV occurred at the same doses that produced the repetitive circling of the periphery of the BPM, it was hypothesized that the high predictability of this rotational locomotor pattern accounted for the changes in CV. The d statistic, however, is markedly reduced by MDMA and its isomers, even at doses that are too low to evoke rotational locomotor paths or changes in CV (Paulus et al. unpublished). Thus, MDMA appears to stimulate straighter locomotor paths without necessarily altering the spatial distribution of these paths.

Neurochemical Basis for Behavioral Effects of MDMA

We attempted to identify the contribution of 5-HT release to the acute behavioral effects of MDMA by examining the response to MDMA after pretreatment with selective 5-HT uptake inhibitors (Callaway et al. 1990). Biochemical studies *in vitro* indicated that these pretreatments will selectively prevent release of 5-HT by MDMA without affecting the direct actions of MDMA on catecholamine release (Schmidt et al. 1987; Hekmatpanah and Peroutka 1990). Surprisingly, pretreatment with selective 5-HT uptake inhibitors prevents the locomotor hyperactivity induced by *S*-MDMA, suggesting that 5-HT release rather than DA release mediates the behavioral activating effects of MDMA. Providing converging evidence for the role of 5-HT release, depletion of central 5-HT with *p*-chlorophenylalanine also attenuates the response to MDMA, while depletion of catecholamines with alpha-methyl-*p*-tyrosine is ineffective (Callaway et al. 1990). The effects of MDMA on the

spatial pattern of locomotor hyperactivity is also sensitive to fluoxetine. The conclusion that MDMA produces its stimulant-like behavioral effects via release of 5-HT rather than DA is consistent with the previous observations that MDMA-induced hyperactivity is qualitatively different from the stimulation produced by amphetamine.

While data indicate that 5-HT release mediates the behavioral stimulation by MDMA, this evidence does not preclude a contribution of catecholamine release to the MDMA-induced behavioral syndrome. Preliminary studies have been undertaken to identify the contribution of DA release to the behavioral effects of MDMA using pretreatment with selective DA uptake inhibitors. This pretreatment was designed to prevent the drug-induced release of DA (Fischer and Cho 1979; Raiteri et al. 1979), just as 5-HT uptake inhibitors were employed to prevent the release of 5-HT (Callaway et al. 1990). However, DA uptake inhibitors including nomifensine, methylphenidate and GBR12909 produce significant behavioral stimulation by themselves (unpublished data), thus obscuring any interaction between these drugs and MDMA. Biochemical data suggest that structural relatives of MDMA also can alter the release of norepinephrine (NE) (Marquardt et al. 1978). Therefore, the following experiment was designed to assess the contribution of NE release to the behavioral effects of MDMA, using pretreatment with the norepinephrine uptake inhibitor nisoxetine.

Nisoxetine (10 mg/kg, i.p.) or saline was administered to rats 50 min prior to the administration of *S*-MDMA (3 mg/kg, s.c.) or saline. At 10 min after the second injection, rats were placed into the BPM and behavior was monitored for 60 min. Nisoxetine produced no change in

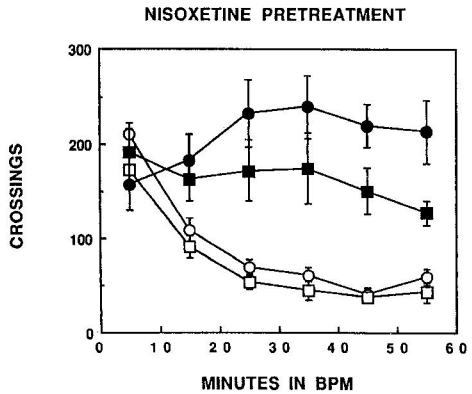

Figure 1. Effect of nisoxetine pretreatment on MDMA-induced locomotor hyperactivity. Rats were pretreated with saline (circles) or 10 mg/kg nisoxetine (squares) i.p. 50 min prior to receiving saline (open symbols) or 3 mg/kg of *S*-MDMA s.c. (closed symbols). After 10 min, animals were placed into the BPM. Crossings per 10 min (mean ± SEM) are indicated at times after being placed into the BPM ($n = 8$ per group).

the number of crossings recorded for control animals (Figure 1). Furthermore, no significant interaction between pretreatment and treatment was observed. A significant interaction between pretreatment, treatment and time ($F = 2.86$; df = 5, 140; $p < 0.05$) reflected a decrease in the mean number of crossings observed for the nisoxetine + MDMA group relative to the saline + MDMA group during the last 20 min of the session. Nisoxetine did not affect holepokes or rearings, and did not alter the reductions of these measures by *S*-MDMA (data not shown). Furthermore, the decrease in the d statistic produced by MDMA (saline: 1.630 ± 0.011; MDMA: 1.467 ± 0.020) was not attenuated by nisoxetine (nisoxetine: 1.649 ± 0.011; nisoxetine $\pm$ MDMA: 1.524 ± 0.020). The antagonism of MDMA by nisoxetine is much smaller in magnitude than the decrease observed after fluoxetine pretreatment (Callaway et al. 1990). Thus, these data may indicate that NE release contributes to MDMA-induced hyperactivity, but does not account for as large a component of the behavioral stimulation as does 5-HT release. Release of NE probably does not contribute to the primary effects of MDMA on investigatory behavior or on the spatial pattern of locomotor activity.

Behavioral Effects of More Selective 5-HT Releasing Drugs

The behavioral and biochemical profiles of MDMA congeners provide an alternative approach to determine the relative contributions of 5-HT and DA release to the acute effects of MDMA. Particular structural features of phenethylamines can increase or decrease the selectivity of these drugs for DA versus 5-HT systems. For example, derivatives of MDMA with elongated alpha-side chains are less potent at releasing DA from synaptosomes, but have undiminished potency as 5-HT releasers (Johnson et al. 1986). Thus, a spectrum of phenethylamines with varying selectivities for 5-HT and DA release are available as tools for identifying the relative contributions of DA and 5-HT release to behavior. In particular, the indan derivative of MDMA, 5,6-(methylenedioxy)-2-aminoindan (MDAI) retains the 5-HT releasing properties of MDMA, but produces no direct effects on DA release *in vitro* (Nichols et al. 1990). The following experiments characterized the acute behavioral effects of MDAI in the BPM to determine what components of the MDMA behavioral syndrome were also produced by this selective 5-HT releasing agent.

Rats were injected with saline or MDAI (2.5, 5 or 10 mg/kg, s.c.). After 10 min, each rat was placed into the BPM and behavior monitored for 60 min. Figure 2A depicts the resulting data. At every dose, MDAI reduced the amount of locomotor activity during the first 20 min in the testing apparatus. During the second half of the testing session,

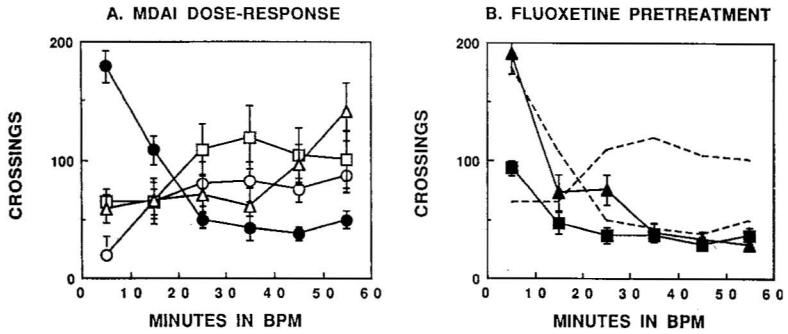

Figure 2. Effect of MDAI on exploratory behavior. Crossings per 10 min are indicated at times after being placed into the BPM. (A) Rats were treated with saline, 2.5, 5 or 10 mg/kg MDAI (closed circles, open circles, squares or triangles, respectively) and were placed into the BPM 10 min later. (B) Rats were treated with 10 mg/kg fluoxetine 50 min prior to receiving saline (triangles) or 5 mg/kg MDAI (squares), and were placed into the BPM 10 min later. Saline and 5 mg/kg MDAI groups from (A) are shown as dotted lines in (B). ($n = 7-10$ per group).

however, MDAI-treated animals maintained or slightly increased their modest initial levels of locomotor activity and were thus more active than the saline-treated animals that had habituated to the BPM (time × drug interaction for crossings: $F = 9.33$; df = 15, 165; $p < 0.0001$). Holepokes were reduced relative to saline controls (114.9 ± 26.2 holepokes per 60 min) after 2.5, 5.0 and 10.0 mg/kg MDAI (56.5 ± 7.0; 42.4 ± 9.2 and 55.0 ± 9.6 respectively) ($F = 11.39$; df = 3, 33; $p < 0.0001$). Similarly, normal levels of rearing (48.4 ± 17.9 rearings per 60 min) were reduced after administration of these doses of MDAI (24.1 ± 8.2; 2.9 ± 1.5 and 0.0 ± 0.0 respectively) ($F = 7.33$; df = 3, 33; $p < 0.001$). Thus, MDAI produced weaker locomotor activation than MDMA but resembled MDMA by decreasing investigatory responding.

In order to test the hypothesis that these behavioral effects of MDAI were mediated via carrier-dependent release of 5-HT, the behavioral response to MDAI was examined in animals pretreated with fluoxetine (10 mg/kg, i.p., 60 min). As shown in Figure 2B, fluoxetine pretreatment attenuated the MDAI-induced locomotor hyperactivity observed during the second half of the test session but did not reverse the initial suppression of locomotor activity by MDAI (time × pretreatment × treatment interaction for crossings: $F = 6.71$; df = 5, 150; $p < 0.0001$). As was the case for MDMA, fluoxetine pretreatment did not alter the MDAI-induced suppression of holepokes and rearings. Thus, it appears that the response to MDAI is multiphasic, consisting of intervals of both decreased and increased locomotor activity, but only the period of increased activity can be attributed to fluoxetine-sensitive 5-HT release.

The behavioral effects of other 5-HT releasing drugs also resemble MDMA in the BPM. Within a particular dose range, MDA and *p*-chloroamphetamine, as well as the alpha-ethyl derivative of MDMA, N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine, produce locomotor hyperactivity with decreased investigatory behaviors (Callaway et al., 1991). Furthermore, the spatial pattern of locomotor hyperactivity consisted of straight paths with fewer direction changes than exhibited by control rats, resulting in a decrease in the d statistics. In some cases, the straight paths were restricted to the periphery of the chamber ("rotation" about the periphery). As with MDMA and MDAI, the behavioral hyperactivity induced by these drugs was attenuated by fluoxetine pretreatment, suggesting that these agents also affect behavior via serotonergic mechanisms. These data confirm the importance of 5-HT release in generating the locomotor hyperactivity produced by MDMA and its congeners.

Comparison of MDMA Effects with Hallucinogens

The unique behavioral properties of MDMA were originally hypothesized to reflect combined hallucinogen-like and stimulant-like actions. The data so far indicate that the behavioral stimulant effects of MDMA and its congeners result from 5-HT release rather than DA release and therefore differ from classical psychostimulants like amphetamine. However, it is possible that the release of endogeneous 5-HT also produces effects that resemble the hallucinogenic effects of many direct 5-HT agonists. In particular, avoidance of the center of the chamber and the decrease in investigatory behaviors which distinguish MDMA from amphetamine are also produced by lysergic acid diethylamide and other hallucinogens that are more selective 5-HT_2 receptor agonists (Adams and Geyer 1985; Wing et al. 1990). Notably, the behavioral effects of hallucinogenic drugs are affected by the novelty of the testing environment, and both the decrements in investigatory responding and the avoidance of the center are less pronounced when the animal has been familiarized with the testing chamber previously (Wing et al. 1990). In order to assess whether the behavioral effects of MDMA resemble those of these direct 5-HT receptor agonists, the following experiments determined the influence of prior exposure to the BPM on the behavioral response to MDMA.

Rats were assigned to one of three treatment groups. Each rat was injected with saline or MDMA (5 mg/kg, s.c.) on three occasions at 48 h intervals. The first treatment group received saline for all three injections (SSS). The second group received saline for the first two injections, and MDMA for the final injection (SSM). The third group received MDMA on all three occasions (MMM). Each rat was placed

Table 1. Effect of 5.0 mg/kg MDMA on locomotor activity and investigatory behaviors in novel and familiar environments ($n = 13–14$ per group)

	Day 1	Day 2	Day 3
Crossings			
SSS*	452.2 ± 16.8	375.8 ± 28.9	311.8 ± 26.4
SSM*	517.0 ± 25.6	397.1 ± 24.5	881.9 ± 83.2[a,c]
MMM*	1221.2 ± 71.6[a]	1024.9 ± 53.6[a]	911.2 ± 68.4[a]
Holepokes			
SSS	156.5 ± 12.0	111.4 ± 12.2	93.9 ± 8.9
SSM	143.5 ± 10.9	111.3 ± 12.6	42.5 ± 4.3[b]
MMM	75.3 ± 15.8[a]	49.1 ± 12.6[a]	48.5 ± 8.7
Rearings			
SSS	137.4 ± 12.4	104.8 ± 11.1	82.2 ± 9.1
SSM	155.2 ± 9.5	119.2 ± 6.8	43.3 ± 9.4
MMM	20.8 ± 6.4[a]	39.4 ± 10.0[a]	55.5 ± 17.3

[a]Significantly different from SSS, $p < 0.01$
[b]Significantly different from SSS, $p < 0.05$
[c]Significantly different from MMM, day 1, $p < 0.05$
*See text for explanation of abbreviations

into the BPM 10 min after being injected with saline or MDMA, and behavior was monitored for 60 min.

Results from this study are depicted in Table 1. First, the locomotor activating effects of MDMA persisted in the familiar environment, as revealed by the significant elevation of crossings in the SSM group on the third day. However, the level of activity in rats first exposed to MDMA in a familiar environment (SSM, day three) was significantly less than the level of activity in rats first exposed to MDMA in a novel environment (MMM, day one). This decrease was proportional to the similar decrease observed in the activity of control animals (group SSS). Thus, the influence of between-sessions habituation to the testing environment on this component of the behavioral response to MDMA cannot be discounted.

The MDMA-induced suppression of investigatory responding was not diminished in a familiar environment. Holepokes were significantly reduced in the SSM group relative to the SSS group on day three, and were also decreased in rats receiving MDMA on all three testing days (group MMM). Thus, the decrease in this component of investigatory responding does not exhibit tolerance. Rearings, however, were reduced significantly only after acute administration of MDMA in a novel environment (group MMM, day one). The failure to observe a significant reduction in rearings by MDMA on day three is consistent with the development of tolerance to this effect of MDMA, although the habituation of control animals across testing days may have obscured any drug effect. In contrast, the suppression of investigatory responding by 5-HT_2 receptor agonists does not persist in a familiar environment and

exhibits rapid tolerance (Adams and Geyer 1985; Wing et al. 1990). Interestingly, rearings and holepokes also are regulated differentially by hallucinogens, with tolerance developing to the suppression of rearings but not to the suppression of holepokes. Conversely, 5-HT_{1A} receptor agonists diminish investigatory responding in both novel and familiar environments (Mittman and Geyer 1989). These data indicate that the effects of MDMA on investigatory behavior more closely resemble the actions of 5-HT_{1A} receptor agonists.

The different measures employed to describe the spatial pattern of MDMA-induced locomotor hyperactivity were differentially affected by the familiarity of the testing environment and the repeated drug administration (Table 2). Confirming the tendency of MDMA to decrease the number of entries into the center of the BPM relative to the amount of locomotor activity, the ratio of center entries to crossings was decreased by MDMA in both novel (group MMM, day one) and familiar (group SSM, day three) environments. However, the decrease in this ratio was not evident upon the third administration of MDMA (group MMM, day three), consistent with the development of tolerance to this action of MDMA. Thigmotaxis (maintaining contact with the walls) is part of the behavioral response to hallucinogenic drugs that also results in a relative decrease of center entries in the BPM (Adams and Geyer 1985). As with the MDMA-induced thigmotaxis observed here, hallucinogen-induced thigmotaxis persists in a familiar environment and rapidly develops tolerance. Thus, the thigmotaxic component of the MDMA response may actually resemble behavioral actions similar to those of classical hallucinogens.

Table 2. Effect of 5.0 mg/kg MDMA on spatial patterning of locomotor activity in novel and familiar environments ($n = 13-14$ per group)

	Day 1	Day 2	Day 3
Center Entries/Crossings			
SSS*	0.32 ± 0.03	0.36 ± 0.02	0.39 ± 0.02
SSM*	0.32 ± 0.02	0.32 ± 0.02	0.25 ± 0.02[a]
MMM*	0.17 ± 0.03[a]	0.19 ± 0.03[a]	0.30 ± 0.04
Coefficient of Variation ("CV")			
SSS	1.05 ± 0.03	1.02 ± 0.02	1.10 ± 0.04
SSM	0.96 ± 0.02	1.05 ± 0.03	1.06 ± 0.03
MMM	1.15 ± 0.07	1.18 ± 0.07	1.25 ± 0.05[b]
Spatial d ("d")			
SSS	1.54 ± 0.02	1.58 ± 0.01	1.59 ± 0.01
SSM	1.54 ± 0.01	1.55 ± 0.01	1.43 ± 0.02[a]
MMM	1.40 ± 0.02[a]	1.41 ± 0.02[a]	1.47 ± 0.02[a]

[a]Significantly different from SSS, $p < 0.01$
[b]Significantly different from SSS, $p < 0.05$
*See text for explanation of abbreviations

In contrast to the center entry ratio, the increased predictability (increased CV) of the locomotor path exhibited by MDMA-treated rats was only marginally evident following this dose of MDMA. The CV was not increased significantly by MDMA in a familiar environment (group SSM, day three) or in a novel environment (group MMM, day one). Moreover, the significant increase of CV on the third administration of MDMA (group MMM, day three) certainly indicates the absence of tolerance to the effects of MDMA on this measure. Hallucinogens produce almost the opposite effect: a decrease in CV for which tolerance rapidly develops (Adams and Geyer 1985). This dissociation between CV and center entry ratio suggests that the MDMA-induced increases in the predictability of the rat locomotor paths are not merely a function the rotational (thigmotaxic) locomotor paths produced by some animals.

The most robust change in the spatial pattern of locomotion induced by MDMA was captured by the d statistic. MDMA-treated animals exhibited significantly lower values for d reflecting a tendency to make straighter locomotor paths (fewer direction changes). This decrease in d was evident in both novel (group MMM, day one) and familiar (group SSM, day three) environments, and did not exhibit any tolerance (group MMM, day three). One interpretation of the decrease in d is that the rotational pattern of MDMA-induced locomotion produces a relatively straighter locomotor path because it is constrained by the walls of the BPM. The fact that the decreased center entries/crossings ratio was attenuated after multiple administrations of MDMA, when the drug still effectively decreased d, indicates a dissociation between the d statistic and the rotational pattern of locomotion. Thus, each of the measures examined in this study capture a different aspect of the spatial pattern of MDMA-induced locomotor activity, and these various patterns are differentially regulated. Future studies may reveal the neurochemical mechanisms for these effects.

Other Mechanisms of MDMA Action: Future Directions

The present data do not identify the 5-HT receptor subtype(s) that mediate the various behavioral effects of 5-HT releasing agents. In the same paradigm, direct 5-HT_{1A} and 5-HT_2 receptor agonists decrease activity (Mittman and Geyer 1989; Wing et al. 1990), indicating that activation of these two receptors probably does not account for the behavioral activation observed following MDMA. Increased locomotor activity has been reported following administration of a direct agonist with affinity for 5-HT_{1B} receptors (Oberlander et al. 1986). Future experiments with selective antagonists at the various 5-HT receptor subtypes may identify the receptor subtypes that mediate the behavioral activation by MDMA.

Although 5-HT release is necessary for these behavioral effects of MDMA, other observations point to a role for the mesolimbic DA system in the locomotor stimulant effects of MDMA. Lesions of the mesolimbic DA system with 6-hydroxydopamine, for example, reduce the locomotor simulation produced by MDMA (Gold et al. 1989). In recent biochemical studies, infusion of 5-HT into the region of the midbrain DA nuclei increased release of DA in the nucleus accumbens (Guan and McBride 1989). Taken together, these data prompt the hypothesis that the behavioral activating effects of MDMA-induced 5-HT release are mediated indirectly via serotonergic stimulation of DA release rather than via direct release of DA by MDMA, as originally postulated. Alternatively, DA neurotransmission may facilitate the expression of hyperactivity even if the effects of MDMA on motor activity are mediated through nondopaminergic systems. Future studies with specific neurochemical lesions and selective receptor antagonists aim to explore the respective contributions of 5-HT and DA systems to the acute behavioral effects of MDMA and its more selective congeners.

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