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# MDMA Enhances Associative and Nonassociative Learning in the Rabbit

ANTHONY G. ROMANO[1] AND JOHN A. HARVEY

*Division of Behavioral Neurobiology, Department of Pharmacology,*
*Medical College of Pennsylvania at Eastern Pennsylvania Psychiatric Institute, Philadelphia, PA 19129*

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ROMANO, A. G. AND J. A. HARVEY. *MDMA enhances associative and nonassociative learning in the rabbit.* PHARMACOL BIOCHEM BEHAV 47(2) 289–293, 1994.—The rate of associative learning was assessed in the presence of saline versus methylenedioxymethamphetamine (MDMA) at doses of 0.95, 1.9, and 3.8 mg/kg. The conditioned stimuli (CSs) were lights and tones and the unconditioned stimulus (US) was a corneal air puff. Learning was enhanced by all but the highest dose of drug tested, and the enhancement was most pronounced when light was used as the conditioned stimulus. Nonassociative responding was assessed using unpaired presentations of the lights, tones, and air puffs. MDMA (1.9 mg/kg) produced a slight increase in the percentage of baseline responses but failed to produce an increase in the frequency of nonassociative responding in the presence of the lights or tones. MDMA produced a significant increase in the amplitude of the unconditioned response to the corneal air puff across the 10 sessions. This increase was taken as evidence for sensitization of the unconditioned response, a nonassociative learning phenomenon. In summary, MDMA, like the parent compound methylenedioxyamphetamine (MDA), enhances both conditioned and unconditioned responding. Because this dual effect has not been seen with related psychedelic compounds, the effect appears to be unique to this class of phenylethylamine drugs.

Rabbit Nictitating membrane Classical conditioning Associative learning Nonassociative learning Sensitization Unconditioned reflex Hallucinogens Psychedelics MDMA

THE classic hallucinogen *d*-lysergic acid diethylamide (LSD) and the phenylethylamine hallucinogens *d,l*-2,5-dimethoxy-4-methylamphetamine (DOM) and methylenedioxyamphetamine (MDA) have a common effect on classical conditioning of the rabbit's nictitating membrane (NM) response. All three hallucinogens enhance the rate of conditioned response (CR) acquisition, albeit by apparently different underlying mechanisms. Thus, LSD enhances CR acquisition but has no effect on nonassociative determinants of responding (1,6–8,15–17). By contrast, DOM enhances both the acquisition of CRs and the frequency of nonassociative responding by an as yet unspecified mechanism (7). The phenylethylamine hallucinogen MDA enhances CR acquisition, has negligible effects on nonassociative responding, and enhances the amplitude of the unconditioned response (UR) during unpaired presentations of the conditioned (CSs) and unconditioned stimuli (USs) (12,13).

Drug discrimination studies in rats indicate that MDA produces multiple stimulus effects; animals trained to discriminate racemic MDA from saline show generalization to the hallucinogens LSD and DOM and generalization to the central stimulants amphetamine and cocaine (4,5). *N*-methylation of MDA yields methylenedioxymethamphetamine (MDMA). Not surprisingly, MDA will substitute for MDMA and vice versa (3,11). Despite this ability to cross-substitute for each other, MDA and MDMA apparently have different discriminative stimulus properties when assessed by other drugs in the drug substitution paradigm. Thus, whereas both LSD and DOM substitute for MDA, LSD shows only partial substitution for MDMA, and DOM shows none (11). By contrast, amphetamine will substitute for either MDA (5) or MDMA (11). The differential substitution for MDA versus MDMA by the hallucinogens LSD and DOM coupled with the finding that amphetamine substitutes for either MDA or MDMA has led to the suggestion that *N*-methylation of MDA attenuates its hallucinogenic properties but has little or no effect on its stimulant properties (3).

The results of binding studies are in agreement with the preceding behavioral results in suggesting a common mode of action of LSD, DOM, MDA, and MDMA. All four hallucinogens bind with high affinity at 5-$HT_{1C}$ and/or 5-$HT_2$ receptor sites and appear to act as agonists at these sites (2,14,18). Thus, there is some suggestion that the enhanced rate of rabbit NM conditioning following treatment with LSD, DOM, or MDA may be due to activation of 5-$HT_{1C}$ and/or 5-$HT_2$ receptors.

[1] Requests for reprints should be addressed to Anthony G. Romano, Ph.D., Department of Pharmacology, Medical College of Pennsylvania, 3200 Henry Avenue, Philadelphia, PA 19129.

The experiments reported here assessed the effects of MDMA on rabbit NM conditioning and on nonassociative responding during unpaired stimulus presentations. Given the similarities among MDA, MDMA, and amphetamine in the drug substitution paradigm and our own reports of enhanced rates of NM CR acquisition following either MDA (12,13) or amphetamine treatment (7), we expected to observe an enhanced rate of acquisition following treatment with MDMA. In addition, given that MDA and MDMA cross-substitute for each other (3,11) and that MDA but not LSD, DOM, or amphetamine sensitize the rabbit's unconditioned NM response (7,12,13), we expected to observe an MDA-like sensitization of the rabbit's unconditioned NM response in the presence of MDMA during unpaired stimulus presentations.

## METHODS

### *Subjects*

New Zealand White rabbits of both sexes and weighing between 1.75 and 2.25 kg were obtained from Ace Animals, Inc. (Boyertown, PA). Rabbits were individually housed and had free access to food and water. The colony room was illuminated according to a 12/12-h light/dark cycle.

### *Apparatus and General Procedure*

The conditioning apparatus and data acquisition system are described in detail elsewhere (12). Briefly, each animal was placed in a Plexiglas restrainer and fitted with a headmount that supported a potentiometer which was directly coupled to a suture placed in the right NM. Movements of the NM were transduced to DC voltages and digitized every 5 ms with a resolution of 0.03 mm of NM movement per analog-to-digital count. A response was defined as a 0.5-mm or greater extension of the NM, and its onset latency was calculated from the time at which the response first deviated from baseline by at least 0.03 mm. The headmount also supported a 2-mm-diameter metal tube positioned 6 ± 1 mm from the center of the right cornea for delivery of the air puff US. Tailor hooks were used to hold the eyelids open. The animals were trained in illuminated, sound-attenuated chambers with a stimulus and interconnection panel mounted above and in front of the animal. Two conditioned stimuli were employed: an 800-ms, 90-dB (20 $\mu N/m^2$ reference), 1-kHz tone and an 800-ms flashing light produced by interruption of the houselights at a frequency of 10 Hz. The US was a 100-ms corneal air puff exerting a pressure of 210 $g/cm^2$ measured at the end of the delivery tube. Two behavioral training procedures were employed, as described below. One day prior to each of these procedures animals were given one 60-min adaptation session during which no stimuli were presented or drugs administered. However, to obtain a baseline measure of the frequency of NM responding, responses were recorded at the intervals to be used during the experimental sessions.

### *Drug*

*d,l*-Methylenedioxymethamphetamine hydrochloride (MDMA, mol wt = 229.71) was provided by the National Institute on Drug Abuse. MDMA, dissolved in 0.9% sterile saline, or saline vehicle injections were given SC between the shoulder blades in a volume of 1.0 ml/kg, 20–30 min prior to behavioral testing. The doses of MDMA are expressed as the base with 0.95, 1.9, and 3.8 mg/kg corresponding to doses of 5, 10, and 20 $\mu$mol/kg.

### *Experiment 1: Paired CS-US Training*

Forty-five experimentally naive rabbits were given five days of acquisition training followed by a two-day rest period and then five more days of acquisition training. Separate groups of rabbits were injected with saline ($n$ = 12) or MDMA at doses of 0.95, 1.9, or 3.8 mg/kg ($n$s = 11, 12, and 10, respectively). Each acquisition session consisted of 60 trials composed of 30 pairings of the tone CS and air puff US and 30 pairings of the light CS and airpuff US. The offset of the CS, either light or tone, was coincident with the onset of the US. Trials were presented at an average intertrial interval of 60 s (range: 55–65 s) with the restriction that no more than three tone or light trials could be presented consecutively. A response was scored as a CR if it occurred within 800 ms of CS onset.

### *Experiment 2: Unpaired CS/US Training*

Sixteen rabbits were given explicitly unpaired presentations of the CSs and US for a total of 10 sessions. In each session, 30 tone CSs, thirty light CSs, and 60 USs were presented in a randomized order with the restriction that no more than three trials of the same type could occur consecutively. The intertrial interval averaged 30 s (range: 25–35 s); all other parameters were the same as in experiment 1. Rabbits were injected with either vehicle ($n$ = 8) or MDMA (1.9 mg/kg, $n$ = 7). Baseline responses, responses to the CSs, and the amplitude of the UR were recorded.

### *Data Analysis*

The data were analyzed with repeated-measures analyses of variance (ANOVAs) using the SYSTAT statistical package, version 5.0 (19). For the paired procedure, multiple group comparisons were made using Dunnett's $t$ test (20). The alpha level for all tests was .05.

## RESULTS

### *Experiment 1: Paired CS-US Training*

The percentages of CRs and average response onset latencies are shown in Fig. 1. Reliable increases in conditioned responding were evident in each group with the overall percentages for the 0-, 0.95-, 1.9-, and 3.8-mg/kg doses averaging 34.88%, 55.93%, 57.90%, and 50.52%, respectively. The rate of acquisition was significantly enhanced by MDMA, as evidenced by a significant dose main effect, $F(3, 41) = 4.64$, and a significant Dose × Days interaction, $F(27, 369) = 2.00$. Dunnett's $t$ test indicated that both the 0.95- and 1.9-mg/kg (5 and 10 $\mu$mol/kg) doses produced greater percentages of conditioned responding than saline. Thus, all but the highest dose of MDMA enhanced the overall rate of acquisition. As shown in Fig. 2, all four groups responded more frequently on tone CS versus light CS trials, and this difference in responding produced a significant CS modality effect, $F(1, 41) = 38.54$, and a significant Dose × CS Modality interaction, $F(3, 41) = 4.53$. Reference to Fig. 2 suggests that this interaction was primarily due to the inferior performance of saline-treated animals on light CS versus tone CS trials. Conse-

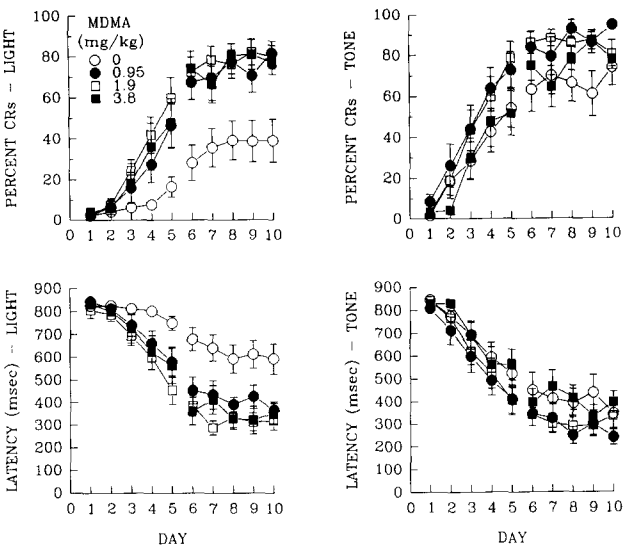


FIG. 1. Percentages of conditioned responding (CRs) (top panels) and nictitating membrane (NM) response latencies (bottom panels) obtained during 10 sessions of paired conditioned stimulus-unconditioned stimulus (CS-US) trials. Means and standard errors are plotted separately for light CS-US pairings (left panels) and tone CS-US pairings (right panels).

quently, separate analyses of percent CRs were conducted for each CS modality. Significant group differences were obtained only on light CS trials, $F(3, 41) = 8.39$, and Dunnett's $t$ test indicated that all three doses of MDMA were significantly different from saline.

Response onset latencies showed a reliable decrease (see Fig. 1) in all four groups across the 10 days of training, $F(9, 369) = 131.62$. MDMA facilitated the decrease in onset latencies and produced both a significant dose main effect, $F(3, 41) = 3.40$, and a significant Dose × Days interaction, $F(27, 369) = 1.71$. As shown in Fig. 2, a significant Dose × CS Modality interaction was also obtained, $F(3, 41) = 6.18$. Separate analyses of response latencies for each CS modality yielded a significant drug effect only for light CS trials, $F(3, 41) = 8.74$.

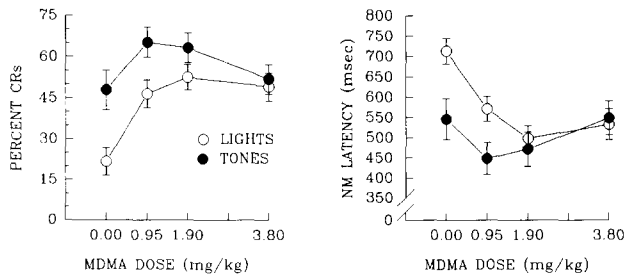


FIG. 2. Percentages of conditioned responses (CRs) and response latencies as a function of dose and conditioned stimulus (CS) modality. The data are the means (± SE) collapsed over the 10 conditioning sessions.

*Experiment 2: Unpaired CS/US Training*

Because the 1.9-mg/kg (10 μmol/kg) dose of MDMA appeared to be the most effective in enhancing acquisition, this dose was used to assess the drug's effects on nonassociative responding. The results are summarized in Fig. 3. Baseline responding during the 800-ms pre-US period averaged 6.69% in MDMA-treated animals and 2.62% in saline-treated animals. This difference in the frequency of baseline responding produced a significant dose main effect, $F(1, 13) = 11.05$. However, baseline responding was fairly invariant throughout training, and thus neither the days main effect, $F(9, 117) < 1$, nor the Dose × Days interaction, $F(9, 117) < 1$, was significant.

Nonassociative responses to the light averaged 5.93% and 8.13% for saline- and MDMA-treated groups, respectively. Neither the interaction effect nor the two main effects were significant. Nonassociative responses to the tone averaged 16.97% for saline-treated animals and 7.48% for MDMA-treated animals. The greater frequency of responding on the part of saline-treated animals produced a significant dose main effect, $F(1, 13) = 5.0$, but no significant days main effect, $F(9, 117) < 1$, or Dose × Days interaction, $F(9, 117) = 1.59$.

The frequency of URs and measures of UR topography are summarized in Fig. 4. The frequency of URs increased significantly from 66.52% on day 1 to 85.91% on day 10, $F(9, 117) = 7.65$. However, neither the dose main effect, $F(1, 13) < 1$, nor the Dose × Days interaction, $F(9, 117) < 1$, was significant. Latency to peak UR amplitude also showed a significant change across days, $F(9, 117) = 2.40$, but no significant Dose × Days interaction, $F(9, 117) < 1$. Furthermore,

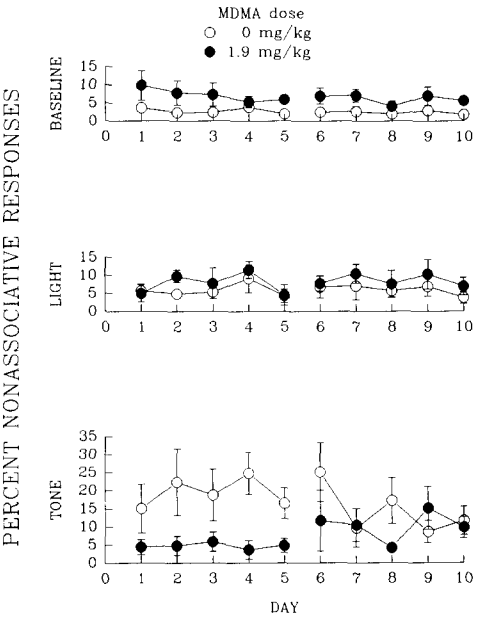


FIG. 3. Means and standard errors of the percentages of baseline responding and nonassociative responding to the light and tone conditioned stimuli (CSs) during 10 sessions of unpaired conditioned stimulus/unconditioned stimulus (CS/US) presentations. The 1.9-mg/kg dose of MDMA was the most effective in enhancing conditioned response (CR) acquisition in experiment 1.

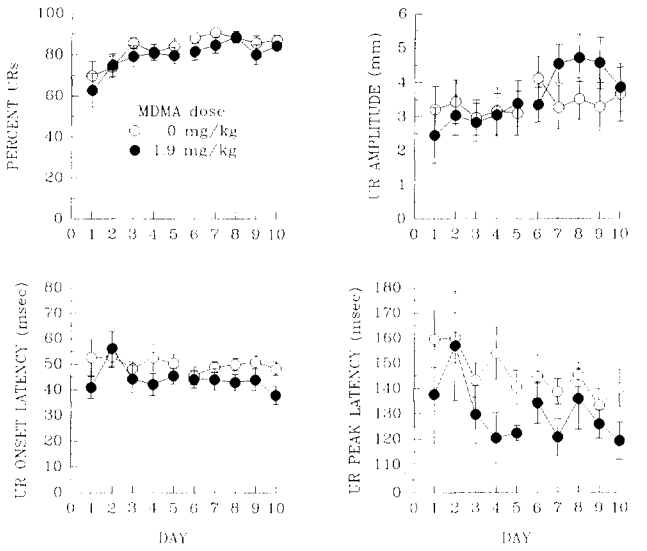


FIG. 4. Means and standard errors of the percentages of unconditioned responses (URs) and measures of UR topography obtained on unconditioned stimulus (US)-alone trials during unpaired conditioned stimulus/unconditioned stimulus (CS/US) presentations. Only UR amplitudes were significantly altered by MDMA.

although saline-treated animals responded with a peak latency somewhat longer than MDMA-treated animals, 146 ms versus 130 ms, the dose main effect was not significant, $F(1, 13) = 3.76$. Onset latency of the UR was also slightly longer in saline-treated animals (50 ms) versus MDMA-treated animals (44 ms), but neither the interaction effect nor the two main effects were significant.

Analysis of UR amplitudes yielded a significant days effect, $F(9, 117) = 2.81$, and a significant Dose × Days interaction, $F(9, 117) = 2.08$. Reference to Fig. 4 suggests that both effects were primarily due to an increase in UR amplitudes in the MDMA-treated group. On average, UR amplitudes in the MDMA-treated group increased from 2.45 mm on day 1 to 3.86 mm on day 10. By contrast, saline-treated animals exhibited a more modest increase in UR amplitudes, from 3.2 mm on day 1 to 3.66 mm on day 10. Thus, separate analyses were conducted for each group to further isolate the source of the Dose × Days interaction. The change in UR amplitudes across sessions was significant for MDMA-treated animals, $F(9, 54) = 4.19$, but not for saline-treated animals, $F(9, 63) < 1$.

## DISCUSSION

MDMA produced a dose-dependent enhancement in the rate of CR acquisition which was reflected by a differential increase in the frequency of CRs in conjunction with a differential decrease in the latency of the NM response. In addition, MDMA produced a dose-dependent enhancement in the overall percentage of CRs across conditioning sessions but was ineffective at the highest dose tested (3.8 mg/kg), thus producing an inverted, U-shaped function. The facilitating effect of MDMA on acquisition also appeared to be modality-specific in that only the light air puff pairings produced significant differences in conditioned responding between MDMA- and saline-treated animals. However, the inferior performance of saline animals on light CS versus tone CS trials suggests that CS modality and CS salience were confounded, thus making the interaction between dose and CS modality difficult to attribute to a qualitative difference between the two stimuli. Several of our previous studies have used a less intense tone than that employed here, 75 dB versus 90 dB (7). Under those conditions, control animals showed essentially equal rates of acquisition to the tone and light CSs. Thus, the present results suggest that the use of a more intense tone produced a ceiling effect which masked any enhancing effect of MDMA on acquisition to the tone.

The enhanced rate of acquisition following MDMA treatment is probably not due to an increase in nonassociative responding. Although MDMA-treated animals showed a slight increase in the frequency of baseline responding during unpaired presentations of the CSs and US, MDMA tended to suppress nonassociative responding in the presence of the tone while having no effect on nonassociative responding in the presence of the light. The frequency, latency, and latency to peak amplitude of the UR were also unaffected by MDMA. However, peak UR amplitudes increased over sessions in MDMA-treated animals, suggesting that the drug produced a long-term sensitization of the UR.

The preceding results with MDMA are similar to those we reported for the parent compound, MDA (12,13). Both drugs enhance acquisition of the rabbit's classically conditioned NM response while having negligible effects on nonassociative responding. An enhanced rate of acquisition appears to be a common behavioral effect of a number of psychedelics. Thus, amphetamine, DOM, and LSD produce an enhanced rate of acquisition, although DOM also produces a marked increase in the frequency of nonassociative responding (7), unlike amphetamine, LSD, MDA, and MDMA. At the receptor level, these last three compounds also show high binding affinities and agonist actions at 5-$HT_{1C}$ and/or 5-$HT_2$ sites (2,14,18). Because MDMA appears to be a weak hallucinogen (3), our behavioral results with LSD, DOM, MDA, and MDMA coupled with the similarities in binding profiles for these compounds suggests that learning, and perhaps other cognitive processes, may be modulated by activation of 5-$HT_{1C}$ and/or 5-$HT_2$ receptors and that this modulation may be independent of hallucinogenic activity.

MDMA shares a further behavioral characteristic with MDA and LSD; all three drugs increase the amplitude of the UR during unpaired stimulus presentations (8,12,13). However, in the case of LSD, the increase in UR amplitudes appears to be an unlearned phenomenon as it is evident during the first block of trials. By contrast, MDA and MDMA increase UR amplitudes by what appears to be a process of response sensitization; the effect does not appear until after repeated presentations of the US. This facilitating effect on a reflex is not unique to the rabbit NM preparation. Facilitation of the startle response of the rat has also been reported following treatment with MDMA. It was initially reported that MDMA at doses ranging from 0.3 to 10 mg/kg had no effect on acoustic-elicited startle (10). However, a second laboratory subsequently reported that the amplitude of acoustic-elicited startle was increased following a relatively high dose of MDMA, 20 mg/kg, but that tactile-elicited startle was much more sensitive to the effects of the drug and was increased in amplitude at a dose as low as 5 mg/kg (9). It is interesting to note that the increase in startle amplitudes did not occur un-

til after repeated presentations of the startle-eliciting stimulus. Although the authors attributed the increase in startle amplitudes to a time-dependent effect of MDMA, it seems difficult to rule out response sensitization as a contributing factor.

In summary, MDMA, like the parent compound MDA, enhances both associative learning, as reflected by a faster rate of CR acquisition, and nonassociative learning, as reflected by sensitization of the UR. Because this dual effect has not been seen with the related psychedelic compounds amphetamine, DOM, and LSD (1,6–8,15–17), the effect appears to be unique to this class of phenylethylamine drugs.

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