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BRIEF COMMUNICATION

Enhanced Learning Following a Single, Acute Dose of MDA

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

Division of Behavioral Neurobiology, Department of Pharmacology, Medical College of Pennsylvania at Eastern Pennsylvania Psychiatric Institute, 3200 Henry Avenue, Philadelphia, PA 19129

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ROMANO, A. G. AND J. A. HARVEY. *Enhanced learning following a single, acute dose of MDA*. PHARMACOL BIOCHEM BEHAV 44(4) 965-969, 1993.—The behavioral effects of a single, acute dose of methylenedioxyamphetamine (MDA) were assessed using the rabbit nictitating membrane (NM) preparation. Acquisition of the classically conditioned NM response was carried out in a single session under MDA at doses of 10 and 20 μmol/kg (1.79 and 3.58 mg/kg, respectively) and retention was assessed during a drug-free state 48 h later. In agreement with previous reports, MDA at a dose of 10 μmol/kg enhanced acquisition of the conditioned NM response and learning was retained in the nondrug state. However, MDA at a dose of 20 μmol/kg failed to affect either acquisition or retention. In further agreement with previous reports, MDA at 10 μmol/kg facilitated the amplitude of the unconditioned response during unpaired stimulus presentations. It is suggested that this enhanced motor response may be due to enhanced neural transmission in the afferent limb of the unconditioned reflex arc.

Rabbit Nictitating membrane Classical conditioning Pavlovian conditioning Nonassociative learning Sensitization Unconditioned reflex Hallucinogens MDA

THE psychotomimetic agent methylenedioxyamphetamine (MDA) produces several novel effects. Human volunteers report that racemic MDA produces both hallucinogenic- and stimulant-like effects (10). Similarly, animals trained to discriminate racemic MDA from saline show generalization to the hallucinogens, d-lysergic acid diethylamide (LSD) and *d,l*-2,5-dimethoxy-4-methylamphetamine (DOM), and generalization to the central stimulants, amphetamine and cocaine (3,4).

Our own results with MDA suggest additional novel effects. In particular, racemic MDA is the only hallucinogen we have examined that can enhance acquisition of the rabbit's classically conditioned nictitating membrane (NM) response without also affecting nonassociative responding or sensory processing of the conditioned or unconditioned stimuli (1,8). A lack of effect on processing of the unconditioned stimulus is surprising given that MDA appears to sensitize the unconditioned response during unpaired stimulus presentations. The classic hallucinogen, LSD, also enhances acquisition of the rabbit's classically conditioned NM response (2). However, unlike MDA, LSD also affects sensory processing of a tone-conditioned stimulus (5). Further, LSD's effect on learning is state dependent; enhanced learning under LSD is no longer evident when learning is assessed during a subsequent, drug-free state (9). By contrast, enhanced learning under MDA is retained during the drug-free state, 72 h after the last drug treatment and conditioning session (1,8).

The present experiments examined MDA's effects on NM conditioning and unpaired stimulus presentations in a single training session. In our previous experiments, enhanced learning under MDA was not evident until the second of four training sessions. By contrast, evidence for a sensitized unconditioned response appeared during the first session of unpaired stimulus presentations. Further, animals undergoing paired stimulus presentations were essentially overtrained under MDA before the drug treatment was discontinued and retention was assessed. The purpose of the present experiments was to see if MDA would enhance learning in a single training session if the training trials were massed and determine if the relatively weak learning that resulted would be retained in the nondrug state. Additional evidence for a sensitized unconditioned response under MDA was also sought. Finally, the use of a single acute dose of drug would allow us to determine if drug-induced sensitization contributed to the enhanced rate of acquisition we found when using multiple sessions.

The experiments to be reported used either a 10- or 20-μmol/kg dose of MDA. The 10-μmol/kg dose (1.79 mg/kg as

[1] To whom requests for reprints should be addressed.

the base) was the most effective in enhancing acquisition of the rabbit's classically conditioned NM response (1,8). A pilot experiment had established that a dose of 30 μmol/kg (5.37 mg/kg) was near the LD_{100} for rabbits. Thus, an intermediate dose, 20 μmol/kg (3.58 mg/kg), was chosen as the maximum nonlethal dose for assessing the drug's effects on rabbit NM conditioning.

METHOD

Subjects

New Zealand White rabbits of both sexes and weighing 2.0 kg were obtained from Hazleton Research Animals (Denver, PA). Rabbits were individually housed and maintained on a daily diet of 150 g Aqway Prolab High Fiber with water available at all times. The colony room was illuminated according to a 12 L : 12 D cycle.

Apparatus and General Procedure

The conditioning apparatus and data acquisition system are described in detail elsewhere (8). Briefly, each animal was placed in a Plexiglas restrainer and fitted with a headmount that supported a potentiometer 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 A/D 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 an air puff unconditioned stimulus (US). Tailor hooks were used to hold the eyelids open. Animals were trained in illuminated, sound-attenuated chambers with a stimulus and interconnection panel mounted above and in front of the animal. The conditioned stimulus (CS) was a 300-ms, 90-db, 1-kHz tone. The US was a 100-ms puff of compressed air measuring 200 g/cm^2 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.

Drugs

d,l-Methylenedioxyamphetamine HCl (MDA, MW = 215.68) was provided by the National Institute on Drug Abuse. MDA, 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.

Paired CS–US Procedure: Experiment 1a

Rabbits were injected with either MDA at a dose of 10 μmol/kg (n = 16) or saline vehicle (n = 16) prior to a single acquisition session. The acquisition session consisted of 120 CS–US pairings presented at an average intertrial interval of 30 s (range = 25–35 s). The interstimulus interval was 275 ms. Retention testing was conducted 48 h after acquisition. The retention session consisted of 120 paired CS–US trials using the parameters outlined above. No injections were given for the retention session.

Paired CS–US Procedure: Experiment 1b

Rabbits were injected with MDA at a dose of 20 μmol/kg (n = 8) prior to a single acquisition session. A second, naive group of rabbits (n = 8) served as uninjected controls. The acquisition and retention sessions were carried out exactly as described for Experiment 1a.

Unpaired CS/US Procedure: Experiment 2

Rabbits were given explicitly unpaired presentations of the CS and US in one session. A total of 120 CSs and 120 USs were presented in a quasirandom order. The intertrial interval averaged 15 s; all other parameters were the same as in the paired procedure. Rabbits were injected with either vehicle (n = 7) or MDA at a dose of 10 μmol/kg (n = 7). The frequency of baseline responses, responses to the tone CS, and the amplitude of the unconditioned response (UR) were recorded. Forty-eight hours later, animals were returned to the conditioning chambers and given 120 paired CS–US trials using the paired CS–US procedure. No injections were administered for this testing session.

Data Analysis

The data were analyzed with repeated-measures analyses of variance using the SYSTAT statistical package, version 5.0 (12). For the paired procedure, the data were analyzed in 10 blocks of 12 CS–US trials each. Separate analyses were conducted for Experiments 1a and 1b due to the procedural differences in the control groups. For the unpaired procedure, the data were analyzed in 10 blocks of 12 trials of each type, CS or US. The significance level for all tests was 0.05.

RESULTS

Paired CS–US Procedure: Experiment 1a

The percentages of conditioned responding during acquisition are shown in Fig. 1A. Both MDA- and vehicle-injected groups showed reliable increases in conditioned responding during the session, $F(9, 270) = 26.99$, $p < 0.001$. However, the performance of the two groups began to diverge after the fourth trial block, with MDA-treated animals showing the faster rate of acquisition. The enhanced rate of acquisition under MDA produced both a significant group main effect, $F(1, 30) = 4.97$, $p < 0.05$, and a significant group × trial block interaction, $F(9, 270) = 2.06$, $p < 0.05$. The increase in the percentage of conditioned responses was accompanied by a significant decrease in NM response latency, $F(9, 270) = 38.92$, $p < 0.001$. The decrease in response latency occurred at a faster rate in MDA animals and was reflected by a significant group × trial block interaction, $F(9, 270) = 1.97$, $p < 0.05$.

The superiority of MDA-treated animals was also evident during retention testing, when no drug or vehicle injections were given. As shown in Fig. 1B, both groups showed reliable retention of conditioned responses during the first trial block and continued to show increases in conditioned responding over the remaining trial blocks, $F(9, 270) = 9.79$, $p < 0.001$. The superior performance of MDA animals in conjunction with their near asymptotic performance throughout retention led to a significant group × trial block interaction, $F(9, 270) = 2.19$, $p < 0.025$. A concomitant decrease in onset latencies was also evident during retention but only the trial block main effect was significant, $F(9, 270) = 16.54$, $p < 0.001$.

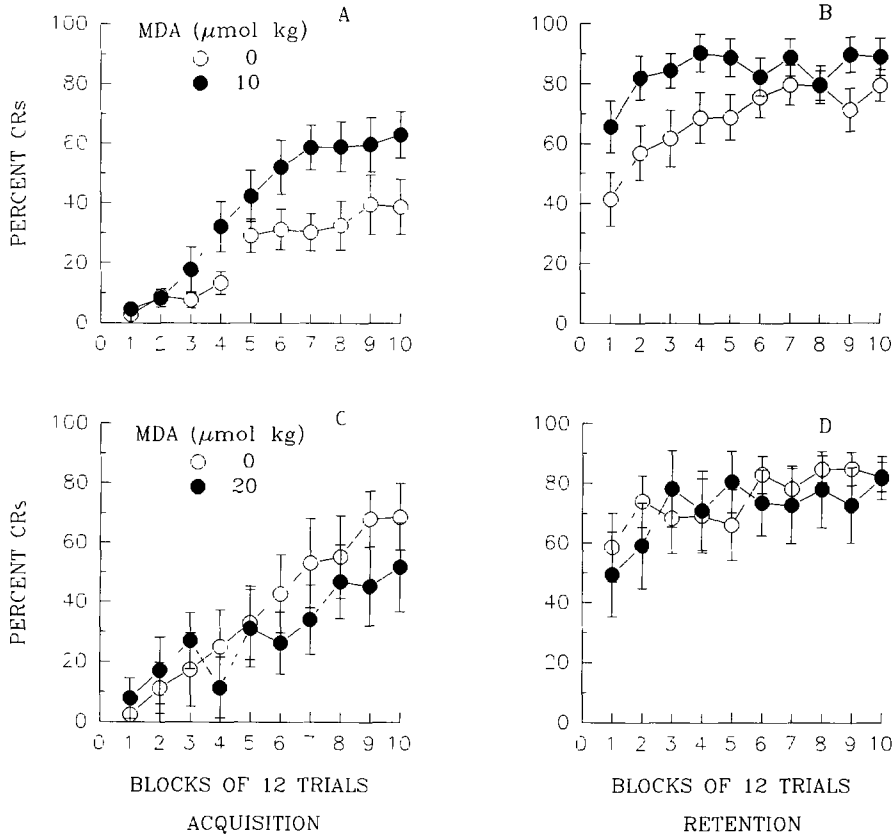

FIG. 1. Effect of MDA on acquisition and retention of conditioned responses during paired CS-US presentations. Top: Experiment 1a (0 vs. 10 μmol/kg), mean percentages of conditioned responses obtained during acquisition (A) and retention (B). Bottom: Experiment 1b (0 vs. 20 μmol/kg), mean percentages of conditioned responses obtained during acquisition (C) and retention (D).

Paired CS-US Procedure: Experiment 1b

The percentages of conditioned responding during acquisition are shown in Fig. 1C. Uninjected controls and animals given the 20-μmol/kg dose of MDA showed significant increases in the percentage of conditioned responses during acquisition, $F(9, 126) = 13.45$, $p < 0.001$. However, the 20-μmol/kg dose of MDA failed to either enhance or retard acquisition and this lack of effect was reflected by a nonsignificant group main effect, $F(1, 14) < 1$, and nonsignificant group × trial block interaction, $F(9, 126) = 1.31$. MDA at this dose also had no effect on response latencies; neither the group main effect, $F(1, 14) < 1$, nor the group × trial block interaction, $F(9, 126) = 1.37$, were significant. However, response latencies showed a reliable decrease over trial blocks, $F(9, 126) = 20.5$, $p < 0.001$.

Performance during retention, when no drug was administered, is shown in Fig. 1D. The only significant effects were an increase in the percentage of conditioned responses over trial blocks, $F(9, 126) = 3.50$, $p < 0.001$, and a concomitant decrease in response latencies, $F(9, 126) = 10.74$, $p < 0.001$.

Unpaired CS/US Procedure: Experiment 2

Baseline responding averaged 3% or less in both saline- and MDA (10 μmol/kg)-treated groups and responding to the tone CS averaged 6.5% or less. Neither of these measures yielded significant group main or interaction effects. However, a 10% increase in the frequency of responses to the tone CS led to a significant trial block main effect, $F(9, 108) = 2.43$, $p < 0.025$.

Figure 2 shows UR amplitudes plotted over blocks of 12 US-alone trials. During the first half of the session, saline

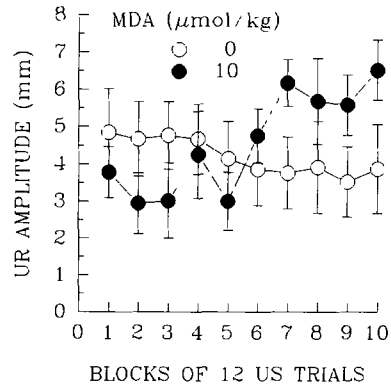

FIG. 2. Mean UR amplitudes exhibited by MDA- vs. saline-injected animals during a session of unpaired CS-US presentations.

animals showed slightly higher UR amplitudes than MDA animals. This trend was reversed during the second half of the session. Between trial blocks 5 and 7, UR amplitudes more than doubled in MDA animals. By contrast, saline animals showed a small but consistent decrease in UR amplitudes throughout the session. Analysis of the data revealed a significant group × trial block interaction, $F(9, 108) = 4.11$, $p < 0.001$. To isolate the source of this interaction, separate analyses were conducted for each group. There was no evidence of a change in UR amplitudes for saline animals, $F(9, 54) < 1$. By contrast, the increase in UR amplitudes in MDA animals was significant, $F(9, 54) = 3.61$, $p < 0.005$.

When animals were subsequently switched to paired CS–US presentations and injections discontinued, the two groups responded at equivalent rates. Only the trial block main effect was significant, $F(9, 108) = 9.50$, $p < 0.001$. Thus, prior administration of MDA during unpaired presentations of the CS and US had no effect on the subsequent rate of acquisition in the nondrug state.

DISCUSSION

The two experiments replicate and extend our previous findings with MDA (1,8). In Experiment 1a, a 10-μmol/kg dose of MDA enhanced the rate of conditioned response acquisition and that enhanced learning was still evident in the nondrug state 48 h later, suggesting the absence of any state-dependent learning effects. We previously reported a similar enhancement in acquisition and lack of state-dependent learning following multiple injections of the 10-μmol/kg dose of MDA in conjunction with multiple training sessions. The results of the present study indicate that a single acute dose of MDA is sufficient to enhance learning within one training session and suggest that MDA's effects on learning are not due to the development of drug-induced sensitization. By contrast, Experiment 1b established that a 20-μmol/kg dose of MDA had no effect on either acquisition of conditioned responding or on retention during the subsequent nondrug state 48 h later.

Experiment 2 showed that MDA at 10 μmol/kg had no influence on the frequency of either baseline responses or nonassociative responses to the CS. Thus, the superior performance of animals given paired stimulus presentations in Experiment 1a satisfies our operational definition of associative learning. In further agreement with our previous report (8), unpaired CS/US presentations under MDA led to a significant increase in the amplitude of the UR. Because this increase occurred over successive US presentations, it appears to be a nonassociative learning effect. Specifically, MDA appears to have produced sensitization of the UR. This effect is somewhat unusual given that drug effects on nonassociative learning in the rabbit NM preparation in general appear as an increase in the frequency of responses to the CS during unpaired stimulus presentations (6). However, the frequency of nonassociative responding to the tone CS during Experiment 2 was not differentially affected by MDA. The development of UR sensitization in the form of an enhanced motor response to the US may be indicative of enhanced neural transmission somewhere within the unconditioned reflex arc. It is possible that alterations in the spinal trigeminal nucleus, the afferent limb of the reflex, are responsible for the enhanced motor response. We recently reported that rats given a regimen of MDA known to produce neurotoxicity in forebrain structures show signs of neurotoxicity throughout the trigeminal sensory complex (7). Although the acute dose used in the present experiments is well below that required for neurotoxicity, our behavioral results suggest that a subtle alteration in the functioning of the trigeminal sensory complex, most notably the spinal trigeminal nucleus, pars oralis, took place following MDA treatment.

The present results with MDA and those reported previously (1,8) are similar to those obtained with the hallucinogens, LSD and DOM (2,6). All three drugs—LSD, DOM, and MDA—enhance acquisition of the rabbit's classically conditioned NM response at doses comparable to the human effective doses. Thus, enhanced acquisition occurs with LSD at doses of 0.001–0.1 μmol/kg, with DOM at doses of 0.3–3 μmol/kg, and with MDA at doses of 3–10 μmol/kg. At higher doses, both LSD and MDA are ineffective in enhancing acquisition. Thus, LSD loses its enhancing effects at a dose of 0.3 μmol/kg and MDA loses its enhancing effects at a dose of 20 μmol/kg. Both LSD and MDA differ from DOM in that the two former hallucinogens have no effect on nonassociative responding to the CS whereas DOM, at a dose of 3 μmol/kg, increases the frequency of nonassociative responses to the CS. Given that all three hallucinogens appear to affect the serotonergic system via a common action on 5-HT_2 receptors (11), other functional similarities among these compounds are likely to be obtained.

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