Pharmacology Biochemistry & Behavior, Vol. 39, pp. 183–189. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Effects of MDA on Classical Conditioning of the Rabbit Nictitating Membrane Response

KIM KIRKPATRICK-STEGER, SUSAN VANDER LINDEN AND I. GORMEZANO[1]

Department of Psychology, The University of Iowa, Iowa City, IA 52242

Received 8 November 1990

KIRKPATRICK-STEGER, K., S. VANDER LINDEN AND I. GORMEZANO. *Effects of MDA on classical conditioning of the rabbit nictitating membrane response.* PHARMACOL BIOCHEM BEHAV **39**(1) 183–189, 1991.—In Experiment 1, classical conditioning of the rabbit's nictitating membrane response (NMR) was accomplished by pairing tone and light conditioned stimuli (CSs) with a shock unconditioned stimulus (UCS). MDA impaired the acquisition of conditioned responses (CR) to a tone-CS, while significantly enhancing CR acquisition to a light-CS. Experiment 2, employing explicitly unpaired CS, UCS training, revealed no reliable effects of MDA upon nonassociative processes. Subsequent efforts determined if MDA's CR acquisition effects resulted from alterations in sensory processing of the CS, UCS, and/or UCR motor functioning. Specifically, it was determined that MDA: (a) increased the tone-CS intensity threshold for eliciting CRs (Experiment 3); (b) attenuated the tone-induced reflex modification of the unconditioned NMR (Experiment 4); and (c) enhanced UCR frequency at varying UCS intensities (Experiment 5). It was concluded that MDA's effect upon CR acquisition reflected the drug's effects upon CS and UCS/UCR processing and thereby altered the ability of these components of conditioning to enter into associative learning.

MDA Classical conditioning Nictitating membrane response Rabbit

THE present series of five investigations sought to determine the effects of (+)-3,4-methylenedioxyamphetamine HCl [(+)-MDA] on learned behavior. In particular, these studies: (a) examined the effect of MDA dosage (0, 1, 2, and 4 mg/kg) on acquisition of the rabbit's classically conditioned nictitating membrane response (NMR; Experiment 1); (b) determined whether MDA's effects on the acquisition of conditioned responses (CRs) could be attributable, in part, to the drug's effect on nonassociative processes (Experiment 2); and (c) determined if MDA's effects on CR acquisition could be localized to possible effects upon sensory processing of the CS (Experiments 3 and 4) and/or sensory (UCS)/motor (UCR) processes (Experiment 5).

As a designer drug, MDA is a substituted phenethylamine that is similar in structure to both the hallucinogen mescaline and the psychomotor stimulant methamphetamine. Like most optically active drugs, (+)-MDA is more potent than the (−) isomer (12,40). However, both isomeric forms (+, −) and the racemic form (±) are pharmacologically, physiologically, and behaviorally active substances (12, 30, 31, 40). Similar derivatives of phenylisopropylamine include the N-monomethyl (MDMA) and the N-monoethyl (MDE) forms of MDA, each of which have been substances of abuse for more than a decade (10, 28, 35). However, a substantially greater number of investigations have been completed with MDMA and MDE than with MDA. Moreover, a number of studies have revealed that MDA, as compared to its congeners, can have profound neurotoxic effects by destroying small neurons of the cerebral cortex, striatum, thalamus, and visual cortex (2, 33, 37, 46, 49).

Initially, MDA was synthesized for therapeutic use as an antitussive (4), ataractic (48), and anorexigenic agent (6) and was later used as an adjunct to psychotherapy (54,57). Abusers of MDA have reported a sense of well-being, heightened self-awareness, increased introspectiveness, general mood elevation, altered perception of time, and increased taste and visual sensations (1,54). Like many designer drugs, MDA's pronounced subjective effects (1,54) and ease of manufacture (10,22) appear to be causal factors in MDA becoming a widely abused substance in the 1960s through the 1980s (10,28). The high abuse of MDA has resulted in a number of studies on the pharmacological (3, 18, 23, 25, 29, 36, 56), physiological (30,31), chemical (32, 37, 41), neurotoxic (2, 33, 39, 46, 49), and toxic (5, 7, 9, 27, 38, 50) properties of MDA, indicating that MDA possesses both stimulant and hallucinogenic effects.

Although there have been a wide number of studies on MDA, the number of objective investigations concerned with delineating MDA's influence upon basic behavioral processes is virtually nonexistent. Of the few behavioral studies that have been conducted, none have attempted to study MDA's effects on sensory, motor, and associative processes. For example, a

[1]Requests for reprints should be addressed to I. Gormezano.

study comparing the effects of MDA and phencyclidine upon performance of a stimulus-discrimination task in monkeys (51), revealed that both substances decreased the rate of key pressing measures of discrimination, but that MDA did not alter the accuracy of discrimination learning. Furthermore, MDA has been reported to disrupt schedule-induced operant behavior and, accordingly, its underlying sensory and/or motor processes (40). Moreover, it has been observed that MDA initially decremented asymptotic shuttle-box conditioned avoidance responses with later recovery to normal levels of performance (8). However, none of these investigations attempted to study MDA's effects on acquisition processes or localize any of the observed effects to sensory or motor components of learning. Therefore, as a neurotoxic substance of continued abuse, it would appear that there is a need for more extensive study of MDA's effects on sensory, motor, and associative processes.

Recent studies have shown that the rabbit NMR preparation can serve as a model for delineating a drug's mode of action on learning (11, 15, 19, 20, 44, 45). In these studies, the strategy has been to progressively refine the localization of drug action on CR acquisition with experiments designed to distinguish between drug effects on: (a) sensory, (b) motor, and (c) associative processes (16). Accordingly, employing the NMR preparation, the present investigations sought to determine the dose-response effects of MDA upon CR acquisition and to localize the effects to these three components of conditioning by employing a well-established battery of procedures (16).

METHOD

Subjects

Each of the five experiments employed 48 New Zealand white albino rabbits for a total of 240 male and female subjects weighing approximately 2 kg on arrival from Knapp Creek Rabbitry (Amana, IA). Animals were housed individually with free access to tap water and were given 60 g of Teklad Rabbit Chow during their first week of stay and 90 g thereafter. Consistent with their rearing conditions, animals were kept in constant light.

Apparatus and General Procedure

The apparatus and procedure used in conditioning of the rabbit NMR have been described in detail (13,16). In brief, a 2-mm loop of surgical nylon (Ethilon 6-0) was sutured into the right NM, the surrounding hair was removed and two stainless-steel wound clips (Autoclip) were attached to the skin over the paraorbital region at a distance of 10 mm posterior to the canthus and 7.5 mm above and below the midline to the canthus. One day later, the rabbit was placed in a Plexiglas restrainer and fitted with a headmount that supported a phototransistor assembly for recording NMRs by attaching to the nylon loop in the NM (14). The rabbit was then positioned in a ventilated, sound-attenuated conditioning chamber containing an 11.4-cm speaker, positioned above and in front of the animal and two 6-W, 24-V DC houselights, mounted on each side of the speaker. During the course of Experiments 1–5, at least one of three stimuli were employed; 1) a 1-kHz tone delivered through the speaker by an audio-oscillator (Hewlett-Packard, model 201CR); 2) an 800-ms flickering light produced by interruption of the houselights at 10 Hz to yield a change in illumination, measured at the eye level of the animal from 32.0 lux to 8.0 lux; and 3) a 100-ms, 3-mA, 60-Hz shock delivered to the two woundclips in the paraorbital region by a constant current shock generator. A phototransistor assembly on the headmount converted nictitating membrane movements to electrical signals, which were subjected to an analog-to-digital (A/D) conversion using a 5-ms sampling rate and a resolution of 0.06 mm actual membrane extension. Experimental control of the presentation and duration of stimuli, analog-to-digital conversion, and response analysis were all accomplished by an Apple II/FIRST operating system (42).

Response Measurement

A response was defined as an NM extension of at least 0.5 mm. The onset latency and amplitude of each response was recorded. Responses occurring in the CS-UCS interval were classified as CRs, whereas with the absence of CRs, those occurring within 100 ms after shock-UCS onset were recorded as UCRs. During each trial of a session baseline responding was assessed in a prestimulus period equal in length to the CS-UCS interval.

Experimental Design

Drugs. (+)-3,4-Methylenedioxyamphetamine was dissolved in sterile, nonpyrogenic 0.9% sodium chloride saline solution. Each animal was injected with the saline vehicle or MDA (1, 2, or 4 mg/kg) on alternate days into the left- or right-marginal ear vein. The daily injections were initiated at the top of the marginal ear vein and subsequent injections were positioned at equal points along the length of the vein from the tip to the base of the ear. Injections were given with a 25-gauge butterfly needle via a Harvard infusion pump (Model No. 975) in a volume of 0.4 ml/kg at a rate of 3 ml/min approximately 30 min before each experimental session.

Adaptation. In all five experiments, rabbits were given an adaptation session which was equal in length to subsequent experimental sessions. No stimuli were presented or drug injected during the adaptation session.

Experiment 1: Paired CS-UCS training under MDA. On the day following adaptation, rabbits were randomly assigned to one of four drug conditions (n = 12) and received injections of either vehicle (0) or MDA (1, 2, and 4 mg/kg) prior to each of 9 daily (60 min) conditioning sessions. In each session, all subjects received 60 CS-UCS paired conditioning trials at a CS-UCS interval of 800 ms. The conditioning trials were composed of 30 pairings of a 75-dB, 800-ms tone and 30 light pairings with a shock-UCS presented in a randomized sequence within each 10-trial block with the restriction of no more than three consecutive presentations of the same CS. Trials occurred at intertrial intervals of 50, 60, and 70 s, with a mean of 60 s.

Experiment 2: Unpaired CS, UCS training under MDA. Experimentally naive subjects were injected with either saline or MDA (n = 12) prior to 9 daily (60 min) conditioning sessions. Each daily session consisted of 120 trials composed of 30 presentations of a 75-dB, 800-ms tone, 30 light-alone, and 60 shock-alone trials, so that the total number of tone-CS, light-CS, and shock-UCS presentations and the duration of the session was equal to that employed in the paired CS-UCS procedure. These trials were presented in a randomized sequence within 20-trial blocks with the restriction that no more than three of the same stimuli were presented consecutively. Trials occurred at restricted randomized intertrial intervals of 25, 30, and 35 s, with a mean of 30 s.

Experiment 3: Effect of MDA on CS intensity. Rabbits received 8 days of paired CS-UCS acquisition training during which only 75-dB, 800-ms tone-CSs were employed for all 60 CS-UCS pairings and no subject was injected with either drug or saline. On the day after the last acquisition session, rabbits were divided randomly into four groups and injected with either

saline (n = 11) or MDA doses of 1 (n = 12), 2 (n = 12), or 4 mg/kg (n = 12) prior to an additional (60 min) session. During this ninth session, the intensity of the tone CS was randomly varied over the 60 CS-UCS trials within each of six 10-trial blocks at 10 values of: 45, 50, 55, 60, 65, 70, 80, 85, and 90 dB (re: 20 $\mu N/m^2$).

Experiment 4: Effect of MDA on tone-induced facilitation of the NM reflex. On the day after a 56-min adaptation session, rabbits were injected with MDA or vehicle (n = 12) before each of two daily experimental sessions. In each session, all rabbits received 56 trials at randomized intertrial intervals of 50, 60, and 70 s, with a mean of 60 s. The 56 trials were composed of eight 7-trial blocks. Each block contained, in a randomly determined order, a 100-ms, 1.5-mA UCS-alone trial and six trials on which animals were exposed to an 100-ms 75-dB, tone-CS at intervals of either 0, 100, 200, 400, 800, or 1600 ms prior to UCS onset. The amplitude of the NMR for each rabbit on each UCS-alone trial provided the baseline UCR amplitude within the 7-trial block. The amplitude of UCRs at each CS-UCS interval for the six tone-shock trials within the 7-trial block were then calculated for each rabbit as a percentage of change from its baseline UCR amplitude in order to determine the extent to which the CS at each CS-UCS interval served to modify the NM reflex elicited by the UCS. Trials in which an NMR occurred during the CS-UCS interval were excluded from the analysis of reflex modification.

Experiment 5: Effect of MDA on UCS threshold. On the day following a 56-min adaptation session, rabbits were injected with vehicle (n = 12) or MDA dosages of 1 mg/kg (n = 12), 2 mg/kg (n = 12), or 4 mg/kg (n = 11) before each of two daily (56 min) sessions. In each of the daily sessions, rabbits received 56 UCS-alone trials at randomized intertrial intervals of 50, 60, and 70 s, with a mean of 60 s. The 56 trials were composed of eight 100-ms UCS presentations at each of 7 shock-UCS intensities of 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, and 4.0 mA, presented once within each of eight, randomized 7-trial blocks.

Data Analysis

A repeated measures analysis of variance was performed on the data for each experiment with follow-up analyses to localize significant sources of variation carried out by the method of Tukey (55). Levels of significance were set at the $p<0.05$ level for all analyses in the five experiments.

RESULTS

Experiment 1: Paired CS-UCS Training Under MDA

Panels (a) and (b) of Fig. 1 present the effect of MDA dosage (0, 1, 2, and 4 mg/kg) on the mean percentage of CRs to tone- and light-CSs, respectively, across the 9 days of conditioning, whereas panels (c) and (d) present MDA dosage effects on the mean number of acquisition trials required to observe the occurrence of 1 through 8 consecutive CRs to the tone- and light-CSs, respectively. A comparison of panels (a) and (b) reveals a higher level of responding to the tone- than light-CS. The dose-response functions appear to differ between the tone- and light-CS acquisition across days of training. The 4 mg/kg MDA dose impaired CRs to the tone-CS, while the 2 mg/kg dose enhanced responding to the light-CS. Panels (c) and (d) substantiate the divergent effects of MDA dosage upon the rate of CR acquisition to tone- and light-CSs. Specifically, relative to vehicle controls, MDA at the 4 mg/kg dose substantially increased the number of training trials required to attain the successive 1–8 CR criteria to tone-CSs while to light-CSs MDA's

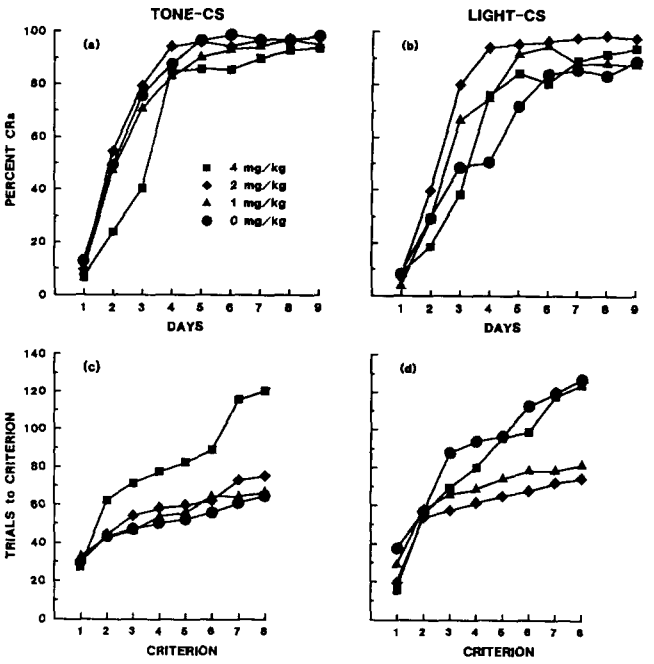

FIG. 1. Effects of MDA on acquisition of CRs during the paired CS-UCS training. In panels (a) and (b), data are expressed as the mean percentage of CRs during each of the 9 acquisition days for tone- (a) and light- (b) CSs. Panels (c) and (d) present the mean number of trials required to reach the criteria of 1 through 8 CRs in a row for tone- (c) and light- (d) CSs.

dose effect appears to have been biphasic. In particular, the 1 and 2 mg/kg doses reduced the number of trials required to attain the successive CR criteria to the light-CSs while the 4 mg/kg MDA dose had no detectable effects. Analyses of variance confirmed the descriptive aspects of the data portrayed in the panels of Fig. 1. Thus an analysis of variance on percent CRs revealed significant effects of Days, $F(8,352) = 162.5$; CS Modality, $F(1,44) = 22.2$; Dose × Days, $F(24,352) = 1.8$; and Dose × CS Modality, $F(3,44) = 6.5$. Follow-up tests localized the significant source of variation of the Dose × CS Modality interaction to the 4 mg/kg dose of MDA acting to impair to occurrence of CRs on tone-CS trials, while the 2 mg/kg dose enhanced CR occurrence on light-CS trials. Moreover, the analysis of variance on the number of trials to criterion revealed significant effects of CS Modality, $F(1,44) = 18.2$; Criterion Level, $F(9,396) = 56.9$; CS Modality × Criterion Level, $F(9,396) = 4.9$; Dose × Criterion Level, $F(27,396) = 2.4$; and Dose × CS Modality, $F(3,44) = 7.4$. Follow-up tests of the significant Dose × CS Modality interaction revealed that on tone-CS trials, animals injected with the 4 mg/kg dose of MDA took longer to initiate consecutive CRs, while on light-CS trials, the 1 and 2 mg/kg doses of MDA enhanced the rate CR acquisition.

Experiment 2: Unpaired CS, UCS Training Under MDA

Figure 2 presents the effects of MDA dosage on the percentage of NMRs occurring during the 800-ms duration tone-CS [panel (a)] and light-CS [panel (c)] and the 800-ms duration baseline measurement period preceding each presentation of tones [panel (b)] and lights [panel (d)]. An examination of these panels reveals that across the 9 days of unpaired stimulus pre-

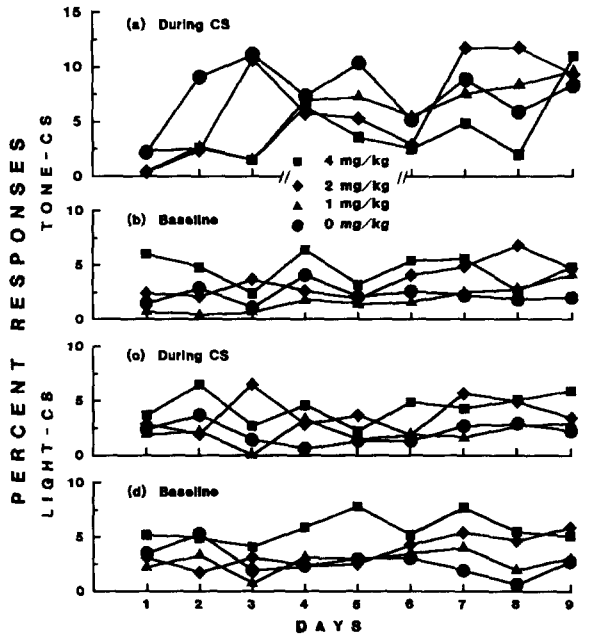

FIG. 2. Effect of MDA on responding during the 9 days of unpaired CS, UCS training. In panels (a) and (c), data are expressed as the mean percentage of responses during the tone- (a) and light- (c) CSs. Panels (b) and (d) present the mean percentage of baseline responses during an 800-ms pre-CS period occurring immediately prior to the tone- (b) and light- (d) CSs.

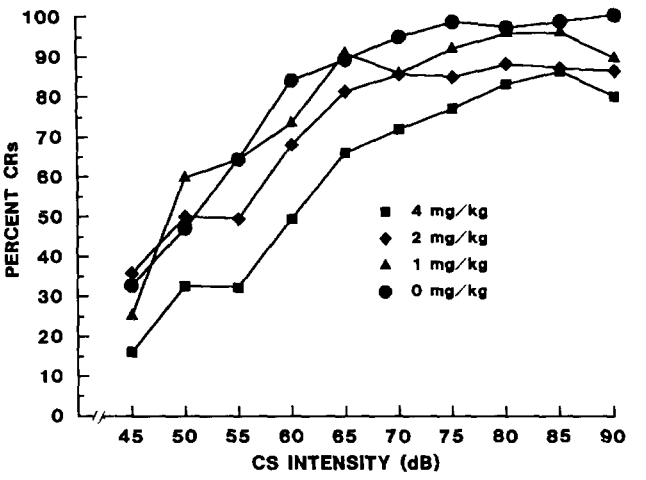

FIG. 3. Effect of MDA on the CS-intensity threshold for eliciting CRs. The data are expressed as the mean percentage of CRs as a function of CS intensity.

sentations the percentage of responses both before and during the tone and light stimuli were low, but that responding during the tone-CS appears somewhat higher than during either the light-CS or baseline periods. Consistent with these descriptive aspects of the data, an analysis of variance revealed a significant effect of CS/Baseline × CS Modality, $F(1,42) = 9.0$ and post hoc analysis localized the significant source of variation to more NMRs occurring during the tone-CS than during the light-CS or corresponding baseline periods. An examination of the frequency, latency, and amplitude of the UCRs elicited by the 3-mA shock across the 9 daily sessions (figure not shown), and an analysis of variance, revealed no systematic effects of MDA dosage, nor any other source of variation on the NM UCRs.

Experiment 3: Effect of MDA on CS Intensity

Before determinations of CS intensity-CR frequency functions, rabbits had been exposed to 8 days of CS-UCS acquisition training to a 75-dB tone-CS and a 3-mA shock-UCS in the absence of drugs. On the last day of acquisition training, these animals had achieved stable asymptotic CR levels of approximately 93%. Figure 3 presents the results obtained when these animals were then injected on the next day with either 0, 1, 2, or 4 mg/kg of MDA and tested for CR occurrence to the various intensities of the tone-CS. Inspection of the figure reveals that CR frequency increased monotonically with CS intensity, while increasing MDA dosage progressively decremented the frequency of CRs. An analysis of variance on the percent CR frequency functions portrayed in Fig. 3 revealed significant effects of CS intensity, $F(9,387) = 67.2$ and Dose, $F(3,43) = 3.2$. A follow-up analysis on the Dose effect localized the significant source of variation to the 4 mg/kg dose of MDA. Subsequently, a CS intensity threshold was calculated separately for each rabbit by interpolating the tone intensity at which CRs would have occurred on 50% of the trials (figure not shown). An analysis of variance revealed that Dosage, $F(3,43) = 3.0$, significantly elevated the CS intensity threshold, and a post hoc analysis localized the significant source of variation to the 4 mg/kg dose.

Experiment 4: Effect of MDA on Tone-Induced Facilitation of the Nictitating Membrane Reflex

Previous reflex modification studies (19,21) have indicated that the amplitude of the UCR can reliably decrement within the first 10 to 20 trials of each session, and, consequently, can influence the assessment of tone-induced reflex modification (RM). Analysis of variance of UCS-alone trials did not reveal any reliable effects of MDA dosage on the baseline UCR amplitude. The analysis did reveal that the amplitude of the UCR decremented reliably across trial blocks on the two days in all dosage conditions. A Newman-Keuls follow-up test revealed that on both days the amplitude of UCRs during the first 2 UCS-alone trials were significantly different from those occurring to subsequent UCS-alone presentations. As a result, the first two UCS-alone trials within the first two blocks, and hence, the first 12 CS-UCS presentations of each session were excluded from analysis. Accordingly, the amplitude of UCRs on the remaining UCS-alone trials served as the baseline from which percentage change in the UCR produced by the 75-dB tone were calculated.

Figure 4 presents the effects of MDA dosage on the percentage RM-CS-UCS interval function. Examination of the figure indicates that the RM was an inverted U-shape function of CS-UCS interval with a maximum at the 400-ms interval. A further inspection of the figure reveals that in the vehicle controls, the tone-CS potentiated the unconditioned reflex across the 100 to 1600-ms CS-UCS interval, whereas under MDA, facilitation of the reflex did not occur. Rather, as a direct function of MDA dosage, the 75-dB tone served to increasingly attenuate the amplitude of the UCRs, but did so with less impact as the CS-UCS interval increased. The analysis revealed significant effects of Dosage, $F(3,44) = 3.3$ and CS-UCS interval $F(5,220) = 12.0$.

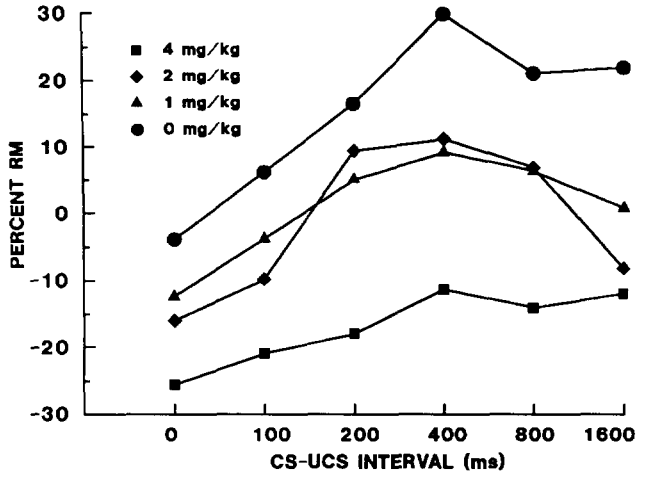

FIG. 4. Effect of MDA on the facilitation of the nictitating membrane reflex by an auditory stimulus expressed as the percentage of change in UCR amplitude from UCRs to its UCS-alone baseline.

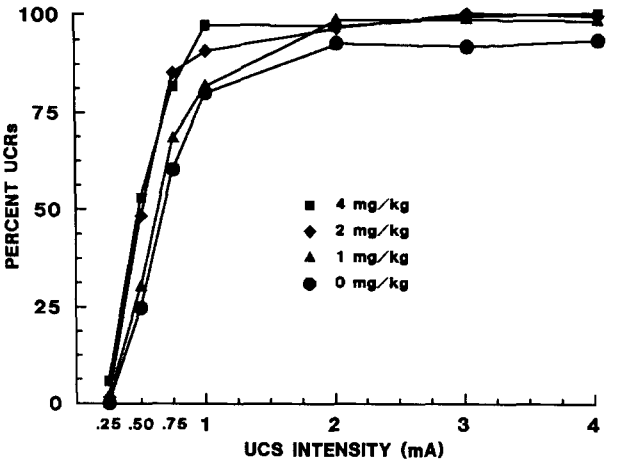

FIG. 5. Effects of MDA on the shock-UCS threshold for eliciting the UCR. The data are presented as the mean percentage of UCRs as a function of the shock-UCS intensity.

Post hoc analyses localized the significant source of variation of the Dosage effect to the tone's profound impairment of the reflex at the 4 mg/kg dose.

Experiment 5: Effect of MDA on Sensory Processing of the UCS

In determining the effect of MDA upon UCRs, measures were obtained of UCR frequency, latency and amplitude. However, no systematic effects were observed for UCR latency or amplitude (figures not shown) and, accordingly, they will receive no further treatment. Figure 5 presents the effects of MDA dosage on the percentage of UCRs across increasing UCS intensities. Examination of the figure reveals that the percentage of UCRs was an exponential increasing function of UCS intensity. Moreover, MDA appears to have enhanced the frequency of responding across the range of UCS intensities and this observation was confirmed by an analysis of variance which revealed significant effects of UCS Intensity, $F(6,258)=284.4$ and Dose, $F(3,43)=3.30$. Follow-up tests revealed that the 4 mg/kg dose of MDA significantly enhanced UCR frequency.

DISCUSSION

The principal findings of the present series of investigations were that MDA: (a) decremented the rate of CR acquisition and overall occurrence of CRs to a tone-CS while enhancing these CR measures to the light-CS (Experiment 1); (b) failed to reveal any systematic effects upon the occurrence of NMRs under unpaired CS, UCS presentations (Experiment 2); (c) increased the CS intensity threshold for eliciting CRs (Experiment 3); (d) impaired the magnitude and duration of reflex facilitation (Experiment 4); and (e) increased the frequency of UCRs to varying UCS intensities (Experiment 5).

In Experiment 1, the enhancing effect of MDA upon CRs to the light-CS at the 1 and 2 mg/kg doses may be attributed, in part, to the physiological effects of MDA. Specifically, physiological investigations (30,31) have indicated that MDA produced effects similar to amphetamine by increasing respiratory rate, salivation, body temperature, pupil dilation, motor activity and magnitude of the flexor reflex, and by suppressing appetite. For example, the observed pupil dilation may have served to augment the intensity of the light-CS and thus increased the rate of acquisition and frequency of CRs. However, the failure to observe an enhancement of CRs to the light-CS at the 4 mg/kg dose suggests that other factors, in addition to pupillary dilation effects, may be involved. In any event, in contrast to MDA's dose-dependent biphasic effects upon CRs to the light-CS, the drug impaired the frequency of CRs to the tone-CS and retarded the rate of CR acquisition as revealed by a dose-dependent increase in the number of trials required to achieve successively more stringent criteria for consecutive CRs. Moreover, the results of Experiment 2 indicate that MDA's effects upon the acquisition of CRs to tone- and light-CSs could not be attributed to the drug's effect upon possible nonassociative contributors to CR measurement. Specifically, the percentage of NMRs occurring during both the 800-ms tone- or light-CS and the corresponding 800-ms prestimulus periods were low for all MDA dosage conditions and did not differ reliably from the base rates of responding observed during adaptation. Accordingly, there was no evidence to indicate that MDA's effects could have been attributed to such nonassociative factors as base rate, pseudoconditioning, or sensitization. Thus, in Experiment 1, MDA's effects appeared to be mediated by conditioning processes affecting the occurrence of CRs.

Experiments 3 and 4 were aimed at determining whether MDA's effects upon conditioning of the rabbit's NMR to tone-CSs could be localized to the drug's effect upon the sensory processing of the CS. The results of Experiment 3 revealed that MDA produced a large elevation in the CS intensity threshold for eliciting CRs and, therefore, suggesting that the MDA, by its attenuation of the tone-CS's intensive properties, operated to impair the CS's ability to enter into the conditioning process during CS-UCS pairings (16,43). Moreover, the results of Experiment 4, in which MDA blocked the ability of the tone-CS to produce reflex facilitation of the NMR and, therefore, the tone's unconditioned excitatory effects, provided converging evidence of MDA impairing the sensory processing of the tone-CS. Together, these experiments indicated that the decrementing effects of MDA upon CRs in Experiment 1 can be attributed, at least in part, to its impairing the organism's sensory processing of the tone-CS. Moreover, MDA's effects upon CR acquisition are consistent with theoretical expectations. Briefly, it has been well-recognized in behavioral theories of conditioning that the

intensive properties of the CS may operate as a learning or performance variable or both (17, 24, 26, 34). All of these theories agree that both the rate of acquisition of CRs and the level of occurrence of CRs, once conditioning has occurred, will be proportional to the intensity of the CS. Thus, to the extent that MDA altered both the conditioned and unconditioned excitatory properties of the tone stimulus, all of these behavioral theories would predict that the drug would impair the acquisition of CRs as well as the CR performance manifested postasymptotically. These theoretical expectations are also in agreement with the results obtained in a number of previous studies on the effects of drugs on learning. These studies, also employing the rabbit NMR preparation, have revealed that scopolamine (20), haloperidol (19), and morphine (44) retarded CR acquisition and the effect was localized to their attenuating tone-CS intensity (19, 20, 45), whereas LSD enhanced CR acquisition while also potentiating tone-CS intensity (11,15). In addition, a recent rabbit NMR study employing neural recording in the interpositus nucleus, a cerebellar structure where for the NMR preparation CS and UCS information has been identified as entering (53), indicated that the disruptive effects of haloperidol upon conditioning of the NMR to a tone-CS were localized presynaptically to the CS pathway rather than to the neural substrates of CR production (47).

Experiment 5 revealed that MDA increased the frequency of UCRs across a range of UCS intensities. Furthermore, while there was an indication that MDA systematically decreased the UCS intensity threshold for eliciting UCRs, statistical analyses failed to confirm its reliability. Nevertheless, it remains to be determined if MDA's effect upon the UCR frequency-UCS intensity function could be attributed to the drug's enhancement of the sensory processing of the shock-UCS and/or motor functioning of the UCR. In physiological studies of MDA, the drug has been observed to increase motor activity and the magnitude of the flexor reflex (30,31). However, at this point, the data on MDA's effects are insufficient to conclude if the drug's enhancement of the UCS intensity-UCR frequency function is attributable to its effects upon the sensory and/or motor components of the UCR. Electrical stimulation of the brain (EBS) could be employed to help localize the effects of MDA since the neural substrates of the NMR are well known (52,53). We are, in fact, presently assessing the effects of MDA on the elicitation of UCRs to varying intensities of EBS of the sensory trigeminal nucleus (TRIG) and accessory abducens motor neurons (ACC).

ACKNOWLEDGEMENTS

This research was supported by National Institute on Drug Abuse Grant DAO-5075-02 and the University of Iowa Foundation (through the generous contributions of D. Boatman, H. Gormezano, S. L. Nusser and W. G. Nusser). The authors extend their thanks to J. Jensen for her assistance in preparation of the manuscript.

REFERENCES

1. Alles, G. A. Some relations between chemical structure and physiological action of mescaline and related compounds. In: Neuropharmacology Transactions of the Fourth Conference. Madison, NJ: Madison Printing Company; 1957:181–268.
2. Battaglia, G.; Yeh, S. Y.; O'Hearn, E.; Milliver, M. E.; Kuhar, M. H.; De Souza, E. B. 3,4-Methylenedioxymethamphatamine and 3,4-methylenedioxyamphatamine destroy serotonin terminals in rat brain: Quantification of neurodegeneration by measurement of [^{3}H] paroxetine-labeled serotonin uptake sites. J. Pharmacol. Exp. Ther. 242:911–916; 1987.
3. Brady, J. F.; Di Stefano, E. W.; Cho, A. K. Spectral and inhibitory interactions of (±)-3,4-methylenedioxyamphetamine (MDA) and (±)-3,4-methylenedioxymethamphetamine (MDMA) with rat hepatic microsomes. Life Sci. 39:1457–1464; 1986.
4. Brown, H. D. MDA as an antitussive. U.S. Patent 2820739; 1958.
5. Cimbura, G. 3,4-Methylenedioxyamphetamine (MDA): Analytical and forensic aspects of fatal poisoning. J. Forensic Sci. 17:329–333; 1972.
6. Cook, L.; Fellows, J. MDA as anorexigenic. US Patent 2984148; 1961.
7. Davis, W. M.; Catravas, J. D.; Waters, I. W. Effects of an i.v. lethal dose of 3,4-methylenedioxyamphetamine (MDA) in the dog and antagonism by chlorpromazine. Gen. Pharmacol. 17:179–183; 1986.
8. Davis, W. M.; Hatoum, H. T. Comparison of stimulants and hallucinogens on shuttle avoidance in rats. Gen. Pharmacol. 18:123–128; 1987.
9. Davis, W. M.; Hatoum, H. T.; Waters, I. W. Toxicity of MDA (3,4-methylenedioxyamphetamine) considered for relevance to hazards of MDMA (Ecstasy) abuse. Alcohol Drug Res. 7:123–134; 1987.
10. Frank, R. S. The clandestine drug laboratory situation in the United States. J. Forensic Sci. 28:18–31; 1983.
11. Gimpl, M. P.; Gormezano, I.; Harvey, J. A. Effects of LSD on learning as measured by classical conditioning of the rabbit nictitating membrane response. J. Pharmacol. Exp. Ther. 208:330–334; 1979.
12. Glennon, R. A.; Young, R. Further investigation of the discriminative stimulus properties of MDA. Pharmacol. Biochem. Behav. 20: 501–505; 1984.
13. Gormezano, I. Classical conditioning. In: Sidowski, J. B., ed. Experimental methods and instrumentation in psychology. New York: McGraw-Hill Book Company; 1966:385–420.
14. Gormezano, I.; Gibbs, C. M. Transduction of the rabbit's nictitating membrane response. Behav. Res. Methods Instrum. Comput. 20:18–21; 1988.
15. Gormezano, I.; Harvey, J. A. Sensory and associative effects of LSD in classical conditioning of the rabbit (*Oryctolagus cuniculus*) nictitating membrane response. Psychopharmacology (Berlin) 70: 137–143; 1980.
16. Gormezano, I.; Kehoe, E. J.; Marshall-Goodell, B. S. Twenty years of classical conditioning research with the rabbit. In: Sprague, J. M.; Epstein, A. N., eds. Progress in psychobiology and physiological psychology. vol. 10. New York: Academic Press; 1983:197–275.
17. Grice, G. R.; Hunter, J. J. Stimulus intensity effects depend upon the type of experimental design. Psychol. Rev. 71:246–256; 1964.
18. Harris, L. S. The stimulants and hallucinogens under consideration: a brief overview of their chemistry and pharmacology. Drug Alcohol Depend. 17:107–118; 1986.
19. Harvey, J. A.; Gormezano, I. Effects of haloperidol and pimozide on classical conditioning of the rabbit nictitating membrane response. J. Pharmacol. Exp. Ther. 218:712–719; 1981.
20. Harvey, J. A.; Gormezano, I.; Cool-Hauser, V. A. Effects of scopolamine and methylscopolamine on classical conditioning of the rabbit nictitating membrane response. J. Pharmacol. Exp. Ther. 225:42–49; 1983.
21. Harvey, J. A.; Gormezano, I.; Cool-Hauser, V. A.; Schindler, C. W. Effects of LSD on classical conditioning as a function of CS-UCS interval: Relationship to reflex facilitation. Pharmacol. Biochem. Behav. 30:443–441; 1988.
22. Henderson, G. L. Designer drugs: Past history and future prospects. J. Forensic Sci. 33:569–575; 1988.
23. Hoffman, A. J.; Maxwell, D. M.; Nichols, D. E. A simple organ bath for electrical stimulation and superfusion of brain slices. J. Pharmacol. Methods 16:185–199; 1986.
24. Hull, C. L. Stimulus intensity dynamism (V) and stimulus generalization. Psychol. Rev. 56:67–76; 1949.
25. Johnson, M. P.; Hoffman, A. J.; Nichols, D. E. Effects of the enantiomers of MDA, MDMA and related analogues on [^{3}H] serotonin and [^{3}H] dopamine release from superfused rat brain slices.

Eur. J. Pharmacol. 132:269–276; 1986.
26. Logan, F. A. A note on stimulus intensity synamism (V). Psychol. Rev. 63:63–73; 1956.
27. Lukaszewski, T. 3,4-Methylenedioxyamphetamine overdose. Clin. Toxicol. 15:405–409; 1979.
28. Mack, R. B. MDA—another stupefacente. NC Med. J. 43:777–778; 1982.
29. Marquardt, G. M.; DiStefano, V. The hapatic microsomal metabolism of b-3,4-methylenedioxyamphetamine (MDA). Life Sci. 15: 1603–1610; 1975.
30. Marquardt, G. M.; DiStefano, V.; Ling, L. L. Pharmacological effects of (±)-, (S)-, and (R)-MDA. In: Stillman, R. C.; Willette, R. E., eds. The psychopharmacology of hallucinogens. New York: Pergamon Press; 1978:84–104.
31. Martin, W. R.; Vaupel, D. B.; Sloan, J. W.; Bell, J. A. The mode of action of hallucinogens and their identification. In: Stillman, R. C.; Willette, R. E., eds. The psychopharmacology of hallucinogens. New York: Pergamon Press; 1978:118–125.
32. Nichols, D. E.; Hoffman, A. J.; Oberlender, R. A.; Jacob, P. Derivatives of 1-(1,3-benzodioxol-5-yl)-2-butane. Representatives of a novel therapeutic class. J. Med. Chem. 26:2009–2015; 1986.
33. O'Hearn, E.; Battaglia, G.; De Souza, E. B.; Kuhar, M. J.; Molliver, M. E. Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain, immunocytochemical evidence for neurotoxicity. J. Neurosci. 8:2788–2803; 1988.
34. Perkins, C. C., Jr. The relation between conditioned stimulus intensity and response strength. J. Exp. Psychol. 46:225–231; 1953.
35. Peroutka, S. J. 'Ecstasy': A human neurotoxin? Arch. Gen. Psychiatry 46:141; 1989.
36. Pierce, P. A.; Peroutka, S. J. Ring-substituted amphetamine interactions with neurotransmitter receptor binding sites in human cortex. Neurosci. Lett. 95:208–212; 1988.
37. Ramos, J. M.; Fitzgerald, R. L.; Poklis, A. MDMA and MDA cross reactivity observed with Abbott TDx amphetamine/methamphetamine reagents. Clin. Chem. 34:991; 1988.
38. Reed, D.; Cravey, R. H.; Sedgwick, P. R. A fatal case involving methylenedioxyamphetamine. Clin. Toxicol. 5:3–6; 1972.
39. Ricuarte, G. A.; Bryan, G.; Strauss, L.; Seiden, L.; Schuster, C. Hallucinogenic amphetamine selectively destroys brain serotonin nerve terminals. Science 222:986; 1985.
40. Rosecrans, J. A.; Glennon, R. A. The effect of MDA and MDMA ('Ecstasy') isomers in combination with pirenpirone on operant responding in mice. Pharmacol. Biochem. Behav. 28:39–42; 1987.
41. Ruangyuttikarn, K. W.; Moody, D. E. Comparison of three commercial amphetamine immunoassays for detection of methamphetamine, methylenedioxyamphetamine, methylenedioxymethamphetamine, and methylenedioxyethylamphetamine. J. Anal. Toxicol. 12: 229–233; 1988.
42. Scandrett, J.; Gormezano, I. Microprocessor control and A/D data acquisition in classical conditioning. Behav. Res. Methods Instrum. 12:120–125; 1980.
43. Scavio, M. J.; Gormezano, I. CS intensity effects on rabbit nictitating membrane conditioning, extinction, and generalization. Pavlov. J. Biol. Sci. 9:25–34; 1974.
44. Schindler, C. W.; Gormezano, I.; Harvey, J. A. Effect of morphine on acquisition of the classically conditioned nictitating membrane response of the rabbit. J. Pharmacol. Exp. Ther. 227:639–643; 1983.
45. Schindler, C. W.; Gormezano, I.; Harvey, J. A. Sensory and associative effect of morphine and naloxone in classical conditioning of the rabbit nictitating membrane response. Psychopharmacology (Berlin) 83:114–121; 1984.
46. Schmidt, C. J. Acute administration of methylenedioxymethamphetamine: Comparison with the neurochemical effects of its N-desmethyl and N-ethyl analogs. Eur. J. Pharmacol. 136:81–88; 1987.
47. Sears, L. L.; Steinmetz, J. E. Haloperidol impairs classically conditioned nictitating membrane responses in conditioning-related cerebellar interpositus nucleus activity in rabbits. Pharmacol. Biochem. Behav. 36:821–830; 1990.
48. Smith, Kline, and French Laboratories. MDA as an ataractic. British patent 82880; 1960.
49. Stone, D. M.; Johnson, M.; Hanson, G. R.; Gibb, J. W. A comparison of the neurotoxic potential of methylenedioxyamphetamine (MDA) and its N-methylated and N-ethylated derivatives. Eur. J. Pharmacol. 134:245–248; 1987.
50. Thiessen, P. N.; Cook, D. A. The properties of 3,4-methylenedioxyamphetamine (MDA). II. Studies of acute toxicity in the mouse and protection by various agents. Clin. Toxicol. 6:193–199; 1973.
51. Thompson, D. M.; Moerschbaecher, J. M. Differential effects of phencyclidine and MDA on complex operant behavior in monkeys. Pharmacol. Biochem. Behav. 21:453–457; 1984.
52. Thompson, R. F. The neurobiology of learning and memory. Science 233:941–947; 1986.
53. Thompson, R. F. Neural circuit for classical conditioning of the rabbit eyelid closure response. In: Byrne, J. H.; Verry, W. O., eds. Neural models of plasticity: Experimental and theoretical approaches. New York: Academic Press; 1989:160–177.
54. Turek, I. S.; Soskin, R. A.; Kurland, A. A. Methylenedioxyamphetamine (MDA) subjective effects. J. Psychedelic Drugs 6:7–14;1974.
55. Winer, B. J. Statistical principles in experimental design. New York: McGraw-Hill Book Company; 1971.
56. Woolverton, W. L. A review of the effects of repeated administration of selected phenethylamines. Drug Alcohol Depend. 17:143–150; 1986.
57. Yensen, F.; DiLeo, F.; Rhead, J.; Richards, W.; Turek, I.; Kurland, A. A. MDA-assisted psychotherapy with neurotic outpatients—a pilot study. J. Nerv. Ment. Dis. 163:233–245, 1976.