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Effects of MDA upon Differential Serial Compound Conditioning and Reflex Modification of the Rabbit's Nictitating Membrane Response

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KIRKPATRICK-STEGER, K., S. VANDER LINDEN AND I. GORMEZANO. *Effects of MDA upon differential serial compound conditioning and reflex modification of the rabbit's nictitating membrane response.* PHARMACOL BIOCHEM BEHAV **41**(2) 333-342, 1992.—The present investigations sought to determine the effects of 3,4-methylenedioxyamphetamine (MDA) on: 1) differential conditioning of the rabbit's nictitating membrane response to the serial compounds A-X-US (tone-light-reinforced compound) and B-X (white noise-light-unreinforced compound) by examining differential responding to A and B and their conditional control over responding to X within the compounds (Experiment 1); and 2) the ability of the compound stimuli and their components to modify the amplitude of the unconditioned nictitating membrane response (Experiment 2). Experiment 1 revealed that MDA decremented differential responding to the serial compounds and their A and B components, while enhancing conditioned responding to the X component. In addition, Experiment 2 indicated that MDA attenuated reflex modification to the compounds and their A and B components, but facilitated reflex modification to X alone. The results of these experiments indicated that MDA operated to alter the intensity, distinctiveness, and persistence (short-term memory) of stimulus representations.

MDA Differential conditioning Reflex modification Nictitating membrane response Rabbit

THE present investigations sought to determine the effects of 3,4-methylenedioxyamphetamine (MDA) upon: 1) differential conditioning of the rabbit's nictitating membrane response (NMR) to two serial compounds, namely, a reinforced tone-light (A-X+) and a nonreinforced noise-light (B-X-) sequence, their different elements (A and B), and their shared element (X; Experiment 1); and 2) the unconditioned excitatory properties of the compound stimuli and their components, measured by their ability to produce heterosynaptic reflex modification of the NMR (Experiment 2). At a more theoretical level, these studies were concerned with examining whether MDA would affect: 1) the distinctiveness of conditioned stimuli (CS's); 2) the intensity of the compound CS's and their components; and 3) short-term memory.

The phenethylamine hallucinogens, including MDA and its *N*-methylated (MDMA) and *N*-ethylated (MDE) relatives, constitute a class of designer drugs structurally similar to both the stimulant amphetamine and the hallucinogen mescaline. As a synthetic substance, MDA has been a drug of abuse for nearly 30 years (12,34) and its abusive potential is verified by studies in which MDA supported self-administration in animals (20). Pharmacologically, MDA has demonstrated a high degree of activity at serotonin receptors (28,35) that appears to be due to a methyoxy substitution on the fourth carbon of the benzene ring (39,40). Moreover, recent studies have indicated that MDA injections produced initial decreases in the levels of serotonin and its metabolite 5-hydroxyindoleacetic acid [e.g., (49)] and later resulted in profound decreases in serotonin neuronal populations in the cerebral cortex, striatum, thalamus, and visual cortex (2,42,45,49,50). In addition, MDA is a toxic substance that has resulted in a number of deaths due to overdose (6,8,33,44), but its toxic effects have been reversed by chlorpromazine (7), methysergide (32), and phenobarbitol (51), indicating both dopamine (stimulant) serotonin (hallucinogen) activity. Since MDA is both a toxic and neurotoxic drug that continues to be abused, there has been a particular need to gain an understanding of its basic behavioral effects. However, until recently, there had been no systematic investigation of MDA's effects upon behavioral processes such as sensory processing, production of motor responses, and acquisition (learning) processes.

In a recent series of investigations aimed at assessing

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MDA's effects upon sensory, motor, associative, and nonassociative processes (30), it was discovered that MDA-impaired conditioned response (CR) acquisition to a tone-CS, while enhancing CR acquisition to a light-CS. Subsequent efforts determined that MDA: 1) had no measurable effect upon base rate, sensitization, and pseudoconditioning; 2) increased the tone-CS intensity threshold for eliciting CR's; 3) attenuated tone-induced reflex modification (RM) of the unconditioned NMR; and 4) enhanced unconditioned response (UR) frequency across a range of unconditioned stimulus (US) intensities. It was concluded that MDA's effect upon CR acquisition reflected the drug's effect upon CS and US/UR processing and thereby altered the ability of the CS and US to enter into learning. A second study (31) determined the effects of MDA, CS, and US intensity on NM CR's and revealed that MDA dosage interacted with both CS and US intensity by enhancing CR's at high US intensities and decrementing CR's at high tone-CS intensities.

The experimental results obtained with MDA are consistent with previous work in this laboratory that has resulted in the observation that cocaine (36), scopolamine (23), haloperidol (21), and morphine (47) retarded CR acquisition and the effect was localized to their attenuation of CS intensity (21,23,48), whereas LSD (13) and DOM (22) enhanced CR acquisition and tone-CS intensity thresholds (17). In contrast to the other substances, amphetamine produces no effect upon responding to a tone-CS, but enhanced CR's to a light-CS (22). Thus, the previous investigations have revealed, in general, that stimulants either decremented conditioning or had no detectable effect when the CS was a tone and had mixed results with light-CS's, while hallucinogens enhanced CR acquisition to both CS's. Thus, our research with MDA indicates that both stimulant (decrease to tone) and hallucinogenic (increase to light) components may be present in its effects upon single-CS conditioning. Although the effects of several drugs have been assessed with single-CS conditioning procedures, only one substance has been used with a more complex design involving differential conditioning with compound CS's. Specifically, a recent study (38) assessed morphine's effects upon differential serial compound conditioning and reflex modification of the rabbit NMR, finding that morphine, in a dose-dependent manner, operated to profoundly attenuate the distinctiveness and persistence (short-term memory) of stimuli as measured by its reduction of CR's to the compounds A–X and B–X and their components A, B, and X. The results with MDA, morphine, and the other pharmacological agents are consistent with stimulus trace formulations (1,15,18,25,26) in which CS intensity is postulated to affect the CS's rate of entry into associative learning by altering: 1) the intensity of an underlying neural trace at the point of US occurrence and 2) its ability to elicit CR's after conditioning has occurred. Stimulus trace formulations predict that to the degree a drug impairs or potentiates CS intensity it would operate to impair or facilitate both CR acquisition and CR elicitation.

EXPERIMENT 1

The present study sought to: 1) expand the understanding of MDA's effects on sensory processing of auditory and visual stimuli in single-stimulus conditioning to include its effects on these CS's in two-element serial compound conditioning; and 2) determine MDA's effects upon the discriminability, intensity, and short-term retention (memory) of stimuli. Thus, our experimental objectives were to ascertain whether MDA (0, 2, and 4 mg/kg) would affect: 1) the ability of serial compounds A–X+ and B–X− and their components to enter into conditioning; 2) differential responding to the distinctive components (A and B) and common element (X) of the serial compounds; and 3) the persistence of representations A and B.

The concept of the stimulus trace in conditioning was originated by Pavlov (43), who invoked the idea to explain why the CS in a trace conditioning paradigm does not have to be explicitly contiguous with the US for the CR to develop. Later theories have extended the notion to hypothesize that CS onset initiates a trace that changes in strength over time, with the rate of learning being proportional to the strength of the trace at the time of US onset (15,25,26).

Stimulus trace formulations (1,15,18,25,26) can be employed to predict the effects of MDA upon the intensity, distinctiveness, and persistence of stimuli in differential serial compound conditioning. By examining the acquisition of CR's to the compounds and their components, it is possible to separate out MDA's effects upon the three aspects of the CS trace. As for CS intensity effects of MDA, stimulus trace theories [e.g., (15,25,26)] would predict that the rate of acquisition depends on the intensity of the CS trace at the time of US occurrence. As a result, stimulus trace formulations would predict that to the extent that MDA alters CS intensity (and the underlying neural stimulus trace), and thereby CR acquisition and CR elicitation in single-CS conditioning, it would also operate to attenuate (or enhance) the intensity of the CS components in serial compounds. Thus, any effect of MDA (increase or decrease) upon the intensity of the CS trace should reveal itself in the form of an increase or decrease in responding to the compound stimuli and their components. As a consequence, MDA would be expected to impair CR acquisition and CR elicitation to A–X+ (and A) and generalized CR's to B–X− (and B) while having no detectable effects on the amount of differential responding. By the same token, MDA should enhance responding to X (the visual component CS).

The effects of MDA upon the distinctiveness of stimuli would be detected by an alteration in the degree of differential responding between the compounds and their A and B components. If MDA produces decrements in the distinctiveness of stimuli, then it might be revealed by: 1) a decrease in differential responding to A–X (or A) and B–X (or B); and 2) decrements in conditional control over responding to X following A and B. Specifically, it has been demonstrated (29) that CR's to X following the occurrence of A are much higher than responding to X after B, indicating that the A and B stimuli exerted conditional control over responding to X. Finally, the effect of MDA upon the persistence (short-term memory) of stimuli can be assessed by examining conditional control by A and B over responding to X at different A–X and B–X component interstimulus intervals (ISI's). Therefore, as a means of assessing MDA's effect upon the persistence of A and B's representation, the present investigation employed A–X+ and B–X− component ISI's of 900 and 1900 ms. In addition, examination of responding during the A–X and B–X trace interval should reveal any effects of MDA upon the persistence of A and B stimuli.

METHOD

Subjects

Subjects were 71 male and female New Zealand white albino rabbits weighing approximately 2 kg on arrival from Knapp Creek Rabbitry (Amana, IA). animals were housed individually with free access to tapwater and given 60 g Teklad Rabbit Chow during their first week of stay and 90 g there-

after. Consistent with their rearing conditions, animals were kept in constant light.

Apparatus

The apparatus and procedure used in conditioning the rabbit NMR have been described in detail (14,19). In brief, each subject was trained individually in 1 of 12 sound-attenuating, ventilated conditioning chambers constructed from legal-sized, fireproof filing cabinets. Unimpeded recording of NM movement was accomplished by holding the subject's right external eyelids open by two No. 3 tailor hooks attached to velcro straps that wrapped around the animal's head. Gormezano and Gibbs (16) detailed modifications in transducing NM movements from previous descripts (14). Specifically, a small hook was attached to a 2-mm diameter nylon loop (Ethilon 6-0) sutured 1.5 mm from the posterior edge of the NM of the rabbit's right eye. The hook was housed within a 22-g hypodermic tube that was coupled by a ball-bearing joint at a right-angle to a 19-g hypodermic tube that served as the armature for the transducer. Inside the transducer housing, the armature was attached to a polarized plate positioned between a 5-V constant intensity LED (Industrial Devises, Model 4302H1) and a photoresistive cell (Vatec, Model BT212-L). A second polarized plate was permanently fixed to the opaque housing in front of the photocell, such that the movement of the membrane, transmitted by the lever arm, rotated the first polarized plate and altered the amount of illumination reaching the photocell. The analog NMR signal was converted to digital output by an Apple II/FIRST system at a sampling rate of 2 ms and a resolution of 62.5 μm actual membrane movement. The on-line analysis of the digitized signal on each trial allowed the determination of NMR onset latency, amplitude, and peak latency of the response. During the experimental session, an Apple II/FIRST microprocessor system and associated interfacing (46) controlled the sequence and timing of stimuli, collected analog/digital (A/D) data, extracted dependent variable measures, and processed data for statistical analysis. The auditory CS's consisted of either an 800-ms, 75-dB, 1-kHz tone or an 800-ms, 75-dB white noise superimposed on an ambient noise level of 55 dB provided by an exhaust fan. A computer-controlled interface card containing digital-to-analog converters and a voltage-controlled oscillator regulated the amplitude and frequency of the tone delivered to an audio amplifier. A visual CS was provided by two houselights located 15 cm anterior and 20 cm above the animals head that were flashed at a rate of 10 Hz for 400 ms to yield a decrease in illumination, measured at the eye level of the rabbit, from 32 to 8 lx. The US was a 100-ms, 3-mA, 60-Hz AC stimulus delivered to the paraorbital region of the right eye via stainless steel Autoclip wound clips positioned 10 mm apart and 15 mm posterior to the dorsal canthus.

Response Definition

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

Drugs

(+)-3,4-Methylenedioxyamphetamine HCl was dissolved in sterile nonpyrogenic, 0.9% sodium chloride saline solution. Injections were given into either the left- or right-marginal ear vein on alternate days with a 25-g × ½-in winged infusion needle via a Harvard infusion pump (Model no. 975) in volume of 0.4 ml/kg at a rate of 3 ml/min. Over the eight consecutive days of training, drug solutions (2 and 4 mg/kg) or saline vehicle were initiated near the top of the marginal ear vein and subsequent injections were positioned along the length of the vein from the tip to the base of the ear. The doses of MDA or saline were given daily at approximately 30 min prior to the onset of the experimental session.

Experimental Procedure

Preparation. On the preparation day, hair surrounding the rabbit's right eye was removed, a loop of surgical nylon was sutured into the NM, and the woundclips, serving as US electrodes, were applied to the paraorbital region of the eye.

Adaptation. Prior to the onset of experimental training, rabbits were given an adaptation session in which they were placed in the conditioning apparatus for 70 min. During adaptation, no stimuli were presented and no drug or vehicle injected.

Experimental training. Following adaptation, rabbits were assigned randomly to one of six conditions derived from the cells of a 3 × 2 factorial design involving MDA of 0 (n = 24), 2 (n = 24), and 4 mg/kg (n = 23) and component ISI of 900 and 1900 ms between the onset of A (or B) and X. Each session consisted of differential serial compound training of two serial compounds, namely, a tone-light sequence and a white noise-light sequence, at component interstimulus intervals of 900 and 1900 ms. One compound was paired with the US and was designated A-X+, while the other compound, designated B-X−, was never paired with the US. Within the 900- and 1900-ms ISI's, the US was presented at the offset of X and the assignment of the tone-light and white noise-light compounds to the roles of A-X+ and B-X− were counterbalanced.

Figure 1 displays the A-X+ and B-X− compounds at the 900- and 1900-ms component ISI's, providing the identifica-

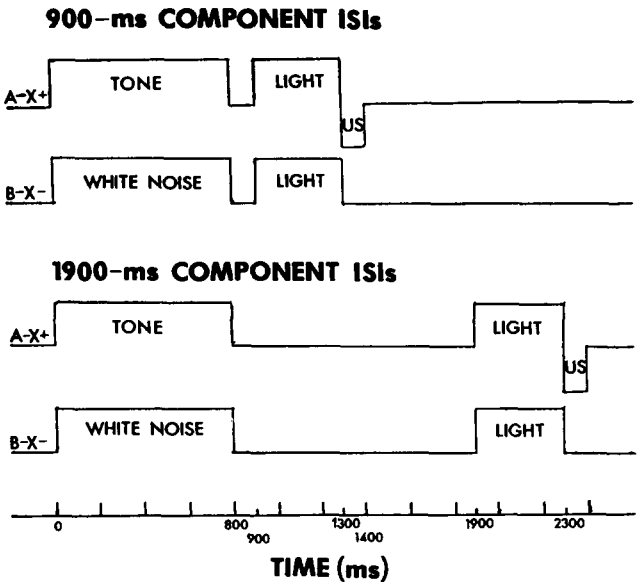

FIG. 1. Schema of the durations and temporal sequence of stimuli in A-X+ and B-X− serial compounds at the 900- and 1900-ms component ISIs.

tion, duration, and temporal position of each component in the compounds. Each day of training contained 30 A-X+ trials, 30 B-X− trials, and 10 nonreinforced test trials that included 2 trials each of A-X, B-X, A, B, and X each presented at an intertrial interval (ITI) of 60 ± 10 s. A test trial was presented on every sixth trial such that each type of test trial was given only once within a block of 30 training trials.

Data analysis. A repeated-measures analysis of variance (ANOVA) was performed on the data for both experiments with follow-up analyses to localize significant sources of variation carried out by the method of Tukey (54).

RESULTS

Responding to Compounds A-X and B-X

Panels (a) and (b) of Fig. 2 present the effect of MDA dosage (0, 2, and 4 mg/kg) on the mean percentage of CR's to compound A-X on A-X+ training trials at the 900- and 1900-ms component ISI's, respectively, across the 8 days of experimental training, whereas panels (c) and (d) present MDA dosage effects on the percentage of CR's to compound B-X on B-X− training trials at the 900- and 1900-ms ISI's, respectively. An inspection of panels (a) and (c) of the figure reveals: 1) higher levels of responding to A-X than to B-X; 2) increased responding over days of training; and 3) that MDA operated to substantially decrease responding to A-X while having a much smaller effect upon responding to B-X. Thus, the overall mean percentage of CR's to A-X were 72.7, 73.6, and 48.4% while responding to B-X was 28.2, 35.2, and 24.1% for the 0-, 2-, and 4-mg/kg dosages of MDA, respectively. Panels (b) and (d) of the figure (1900-ms ISI) also reveal that percent CR's increased over days and were higher to A-X than to B-X compounds. In addition, MDA dosage operated to impair responding to A-X, but had no effect upon generalized CR's to B-X, with responding to A-X of 68.7, 63.4, and 58.5% and to B-X of 19.8, 24.6, and 27.7% for the 0-, 2-, and 4-mg/kg dosages, respectively.

An ANOVA on percent CR's to A-X and B-X revealed significant main effects of CS type, $F(1,65) = 341.66$, $p < 0.001$, and days, $F(7,455) = 104.78$, $p < 0.001$. In addition, the analysis yielded the following significant interactions: days × CS type, $F(7,455) = 49.63$, $p < 0.001$; dosage × CS type, $F(2,65) = 7.49$, $p < 0.01$; dose × days, $F(14,455) = 2.13$, $p < 0.01$; ISI × days, $F(7,455) = 3.56$, $p < 0.01$; and ISI × days × CS type, $F(7,455) = 2.49$, $p < 0.05$. A follow-up test of the significant dosage × CS type interaction revealed that the 4-mg/kg dose of MDA impaired responding to A-X, but there were no significant dosage effects on responding to B-X [critical difference (6,65) = 10.6% CR's, $p < 0.01$].

Responding to A and B

Figure 3 presents the effect of MDA dosage upon the mean percentage of CR's occurring during the 800-ms duration of A and B across the 8 consecutive days of training. Panels (a) and (b) feature percent CR's to stimulus A on A-X+ training trials at the 900- and 1900-ms ISI's, respectively, while panels (c) and (d) present responding to stimulus B on B-X− trials at the 900- and 1900-ms component ISI's, respectively. An examination of the figure (and an ANOVA of the data) reveals that across all experimental conditions there was a higher level of responding to A than B [CS type, $F(1,65) = 127.92$, $p < 0.001$] and an increase in responding across days of training [days, $F(7,455) = 53.63$, $p < 0.001$]. In addition, the analy-

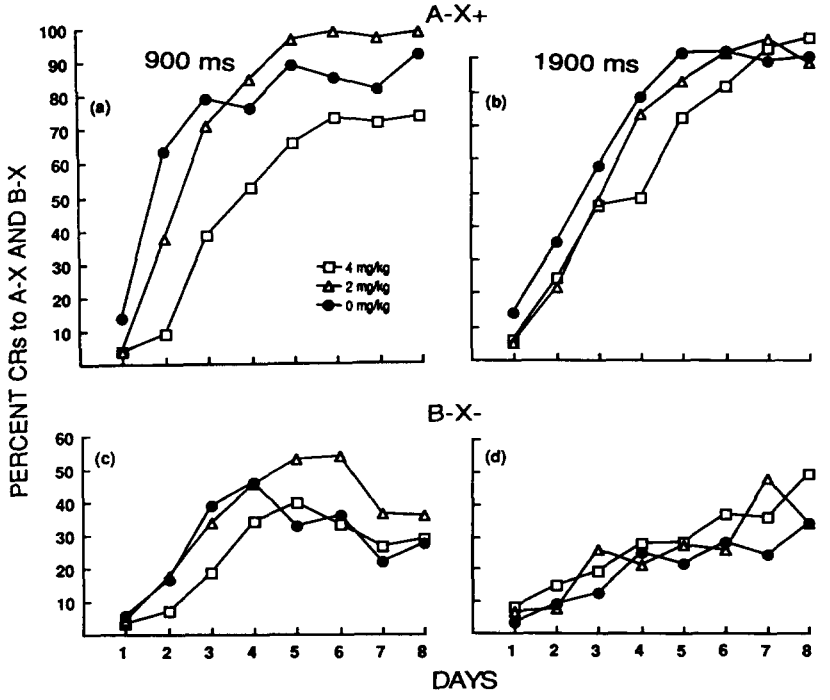

FIG. 2. Effect of MDA dosage (0, 2, and 4 mg/kg) upon the mean percentage of CR's to A-X+ [panels (a) and (b)] and B-X− [panels (c) and (d)] at the 900- and 1900-ms component ISI's across the 8 days of training.

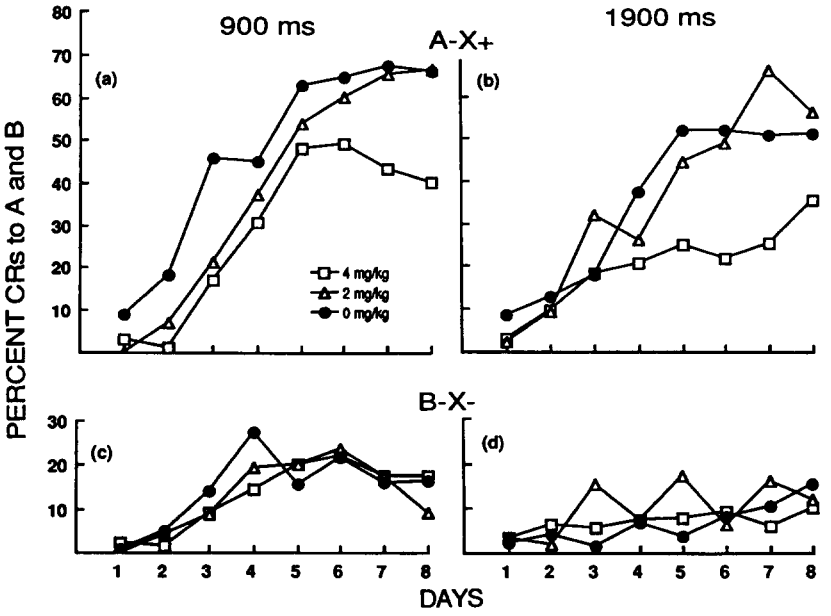

FIG. 3. Mean percentage of CR's to the initial elements A and B of the A-X+ and B-X− compounds across the 8 days of training as a function of MDA dosage and component ISI.

sis revealed that as MDA dosage increased responding to A decreased but that responding to B was not significantly affected [dose × CS type, $F(2,65) = 53.5$, $p < 0.01$]. Specifically, the left-hand panels (900-ms ISI) reveal that over the 8 days of training MDA dosages of 0, 2, and 4 mg/kg yielded decreasing percent CR's to A of 47.5, 39.1, and 29.1%, respectively. However, responding to B was 14.5% for the 0-mg/kg dose, 12.9% for the 2-mg/kg dose, and 12.9% for the 4-mg/kg dose of MDA. Moreover, an examination of the right-hand panels (1900-ms ISI) reveals that the overall mean levels of responding to A were 35.1, 35.5, and 19.9%, while percent CR's to B were 6.6, 10.0, and 7.0% for MDA dosages of 0, 2, and 4 mg/kg, respectively. A follow-up analysis on the significant dose × CS type effect indicated that the 4-mg/kg dose of MDA decremented CR's to A, but that MDA dosage had no detectable effects upon responding to B [critical difference (6,65) = 10.9% CR's, $p < 0.01$). The ANOVA also revealed significant effects of: 1) days × CS type, $F(7,455) = 42.7$, $p < 0.001$, confirming a differentially higher rate of acquisition and terminal level of responding to A vs. B over training; 2) ISI × day, $F(7,455) = 3.5$, $p < 0.01$, validating the higher rate and overall level of CR acquisition across days at the 900- vs. 1900-ms component ISI's; and 3) dosage × day × CS, $F(14,455) = 2.4$, $p < 0.01$, reflecting MDA's differential effects upon the rate and terminal level of responding to A and B.

Responding to X

The panels of Figure 4 show the effects of MDA dosage on the mean percentage of CR's to X on A-X+ [panels (a) and (b)] and B-X− [panels (c) and (d)] training trials and on X-alone test trials [panels (e) and (f)] at the 900- and 1900-ms ISI's across the 8 days of training. The measure of CR likelihood on a given trial was conditional upon the absence of responding during the entire interval preceding the onset of the 400-ms duration of X. An examination of the left-hand (900-ms ISI) panels of Figure 4 reveals: 1) substantially higher levels of differential CR's to X conditional upon the prior absence of responding to A (X/A') than absence of responding to B (X/B'); 2) little evidence of CR's on X-alone test trials; and 3) the 2-mg/kg dose of MDA slightly enhanced, while the 4-mg/kg dose decremented, the occurrence of conditional differential CR's to X. Thus, on the terminal day of conditioning the level of responding to X on A-X+ trials was 76.9, 79.0, and 67.2%, and B-X− trials was 14.6, 29.2, and 19.1% for MDA dosages of 0, 2, and 4 mg/kg, respectively. The right-hand (1900-ms ISI) panels indicate that: 1) conditional responding to X on A-X+ trials was higher than on B-X− trials; 2) there were low levels of responding on X-alone trials that were not significantly affected by MDA dosages; and 3) increasing MDA dosage resulted in higher levels of conditional differential responding to X. Specifically, on the terminal day of training, responding to X on A-X+ trials was 50.9, 60.1, and 82.4%, and on B-X− trials was 11.7, 11.5, and 31.9% for MDA dosages of 0, 2, and 4 mg/kg, respectively.

An ANOVA on percent CR's to X conditional upon no prior response on A-X+, B-X−, and X-alone trials confirmed the descriptive aspects of the data portrayed in Figure 4. The analysis revealed significant effects of CS type, $F(2,130) = 195.0$, $p < 0.001$; days, $F(7,455) = 28.77$, $p < 0.001$; component ISI, $F(1,65) = 12.38$, $p < 0.01$; days × CS type, $F(14,910) = 41.32$, $p < 0.001$; dosage × days, $F(14,455) = 2.10$, $p < 0.05$; ISI × CS type, $F(2,130) = 18.43$, $p < 0.05$; ISI × days, $F(7,455) = 3.51$, $p < 0.01$; ISI × dosage, $F(2,65) = 4.54$, $p < 0.05$; and dosage × days × CS type, $F(28,910) = 3.00$, $p < 0.001$. Posthoc analysis on the component ISI × dosage interaction revealed that at the 900-ms ISI the 4-mg/kg dose significantly decremented CR's to X, but within the 1900-ms ISI the 4-mg/kg dose significantly enhanced CR's [critical difference (6,65) = 11.9% CR's, $p < 0.05$]. In addition, follow-up analysis

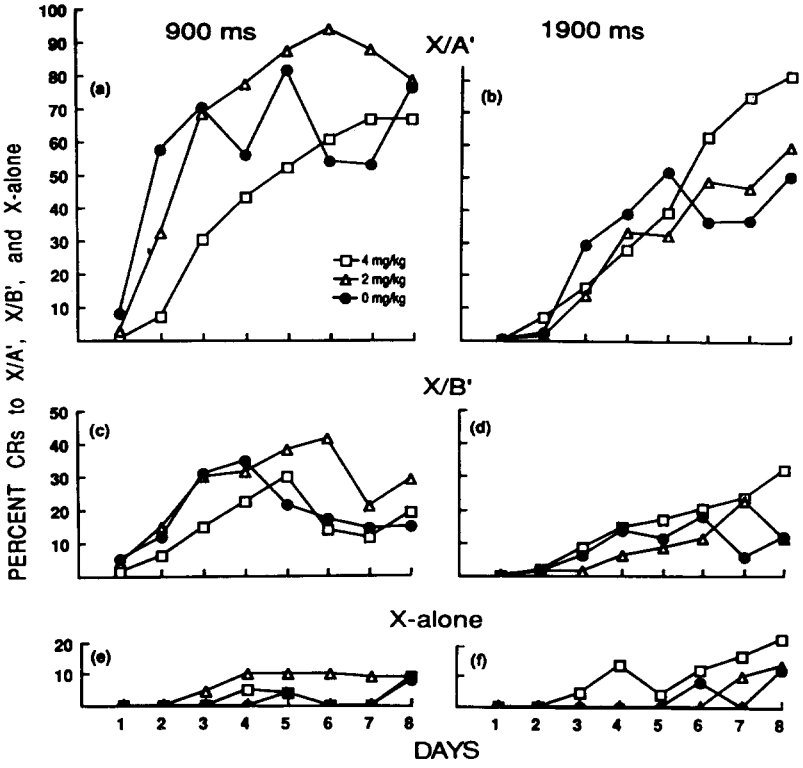

FIG. 4. Mean percentage of CR's across the 8 days of conditioning to the common, second element X, on X/A′ and X/B′ training trials and on X-alone trials as a function of MDA dosage and component ISI.

indicated that the component ISI × CS type interaction could be localized to: 1) more conditional responding to X on A-X+ trials than on B-X− or X-alone trials; 2) fewer responses occurring to X on X-alone trials than on either A-X+ or B-X− trials; and 3) more CR's occurring to X on both A-X+ and B-X− trials at the 900 ms than the 1900-ms component ISI [critical difference (6,130) = 7.6% CR's, $p < 0.05$].

Responding during the A-X and B-X Trace Interval

As an additional measure of MDA's effects upon the persistence of A and B's representations, the levels of responding during the A-X and B-X trace intervals at the 900- and 1900-ms component ISI's were assessed (figure not shown). Specifically, an examination of the data (and an analysis of variance) revealed significant effects of CS type, $F(1,65) = 165.4$, $p < 0.001$; days, $F(7,455) = 39.5$, $p < 0.001$; days × CS type, $F(7,455) = 47.9$, $p < 0.001$; ISI, $F(1,65) = 86.7$, $p < 0.001$; ISI × CS type, $F(1,65) = 33.1$, $p < 0.001$; and ISI × days, $F(7,455) = 8.49$, $p < 0.001$. In addition, an examination of the data revealed that the 4 mg/kg produced CR's during the A-X trace of only 27.9% as compared to 34.0 and 35.3% for the 2- and 0-mg/kg doses, respectively, but that trace responding during B-X trials was undifferentiated by MDA dosage with levels of 8.2, 7.4, and 4.8% for the 4-, 2- and 0-mg/kg doses, respectively. However, the described dose × CS type interaction failed to reach statistical significance, $F(2,65) = 2.8$, $p = 0.068$.

Responding on Test Trials

The nonreinforced test trials for A-X, B-X, A, and B stimuli were assessed to further identify MDA's effects upon the stimulus trace. An examination of test-trail responding revealed patterns of CR's that closely approximated the data presented for the A-X+ and B-X− and thus will not be presented here. However, the nonreinforced A and B test trials were analyzed for responding after A and B offset on A and B test trials to yield an indicant of the strength of the CS trace at the time of US onset. The analysis revealed fairly high levels of responding after A and B (overall mean = 18.6% CR's) and that there was evidence of differential responding after A and B offset with 27.0% CR's to A and only 10.2% to B. As for MDA dosage effects, the 4-mg/kg dose decremented CR's after A (21.4%) relative to the 2- (28.1%) and 0-mg/kg (31.6%) MDA dosages. Responding after B revealed that the 4-, 2-, and 0-mg/kg doses produced CR's of 7.5, 15.6, and 7.9%, respectively. Although a description of the data indicated a significant dose × CS type interaction, the source of variance failed to meet statistical significance, $F(2,65) = 2.64$, $p = 0.077$. As for responding to X's representation, an analysis of A-X and B-X test trials revealed that responding after X's termination was low (< 10%) and was not differentiated by MDA dosage.

DISCUSSION

The principal finding of Experiment 1 was evidence of: 1) differential responding to the compounds A-X and B-X with more responding to the reinforced compound; 2) higher responding to component A than to B; and 3) differential responding to the common second element (X), following the A and B elements of the two compounds. In addition, the magnitude of responding to X following A and B (but not to A, B, or the compounds A-X and B-X) was affected by

component interstimulus interval with more responding to X at the 900-ms ISI than at the 1900-ms interval. Finally, MDA acted to: 1) impair responding to the compound A–X, but not to B–X, at both ISI's; 2) impair responding to A at both component ISI's; and 3) decrement CR's to X following A and B at the 900-ms ISI, but facilitate responding to X at the 1900-ms component ISI.

The pattern of responding to the compounds and their initial elements (A and B) suggests that increasing MDA dosage produced decreased in the intensity of the stimuli. This was evidenced by smaller levels of responding to A–X (and A) and, to a lesser extent, a decrease in generalized CR's to B–X (and B). In addition, the presence of a dose × CS type interaction, but not a dose main effect, indicated that MDA altered the distinctiveness of the compounds and their initial elements. Specifically, MDA operated to profoundly decrease responding to the reinforced compound while producing only modest effects upon the nonreinforced B–X compound, revealing an overall decrease in the degree of differential responding with increasing MDA dosage. However, the failure to observe decreased responding to B–X of a comparable magnitude to A–X could have been due to a floor effect. In particular, responding to B–X was quite low in the saline condition (24%) and thus restricted the degree of change in the downward direction. In any event, animals still responded differentially to the compounds and their A and B components, indicating that any effects upon distinctiveness of stimuli were insufficient to abolish differential conditioning. Finally, the results of the present experiment indicate that MDA affected the persistence of A and B's representations. Specifically, MDA: 1) appeared to decrease responding during the trace interval, although this effect failed to achieve statistical significance; 2) decreased responding after A and B offset on nonreinforced A and B test trials (not statistically significant); 3) decremented responding to X after A and B at 900-ms ISI, but enhanced responding to X at the 1900-ms ISI. Specifically, at the short ISI the A and B stimuli appeared to exhibit strong conditional control over responding to X, but did so to a lesser degree in MDA-treated animals. Moreover, at the longer (1900 ms) ISI there was still evidence of conditional control over responding to X, but the dose–response function shifted such that MDA increased responding to X/A′ and X/B′. This result indicates that although A and B still exerted conditional control over responding to X the initial stimuli must have decayed sufficiently so as to no longer dominate responding to X and thus responding to X more closely approximated the generation of CR's when X (a visual CS) was given alone in single-CS conditioning.

The results obtained with MDA are relevant to studies of the neural substrates of the NMR. Specifically, neural recording studies have revealed a change in neural firing that closely approximates the time course of the CR. The areas that display altered firing patterns during the CR include the abducens nucleus (5,55), accessory abducens nucleus (11), hippocampus (3,53), and cerebellum (4,37). Although the evidence is correlational, it has suggested that the CR production (and the CS trace) may be represented in neural firing patterns and has led to the development of neural network models of CR processing and CR production (9,10). These models have postulated that the CS initiates activity in the nervous system that spreads by the successive activation of synapses. The strength of the trace (and the likelihood of CR production) is determined by the number of synapses eligible for modification when the US occurs. Thus, since MDA alters the availability (or activity) of serotonin and dopamine at the synapse, this model would predict altered rates of conditioning. Therefore, it is possible that MDA's synaptic effects could be a determining factor in its interference with the intensity, distinctiveness, and persistence of the neural stimulus trace.

EXPERIMENT 2

The purpose of Experiment 2 was to further delineate the effects of MDA dosage (0, 2, and 4 mg/kg) upon the serial compounds A–X and B–X and their components. Thus, the study sought to determine MDA's effects upon the time course and distinctiveness of the unconditioned excitatory effects of the A–X and B–X serial compounds, their initial-different elements, A and B, and their second-common element, X. To determine the effects of MDA on the unconditioned excitatory properties of the stimuli, assessments were made as to the extent and time course over which these stimuli would operate to produce heterosynaptic reflex modification of the rabbit's unconditioned NMR (27,52,55). It has been well documented that prior to conditioning of the rabbit's NMR auditory stimuli produce detectable neural activity within the accessory abducens (52) and the VIth nerve (4), but not an overt NMR (14,15,19). In addition, although a tone-CS does not elicit overt responses it does provide sufficient excitation to produce facilitation of this reflex. For example, the amplitude of the NMR elicited by a paraorbital shock-US, an airpuff to the cornea, or by direct electrical stimulation of the abducens nucleus is significantly increased if preceded by an auditory stimulus (55). Moreover, an auditory CS will affect the amplitude of the unconditioned NMR as a function of the CS–US interval elicited by corneal stimulation (52) or electrical stimulation of the abducens nucleus (41) and thus provide a measure of the time course of excitation of the auditory stimulus within the reflex arc. Previous investigations [i.e., (21,24, 30,38)] indicated that drugs that alter conditioning processes to a tone-CS also block the occurrence of RM and thus the unconditioned excitatory effects of the tone stimulus. For example, research with morphine and serial compounds (38) revealed that morphine attenuated stimulus processing in differential serial conditioning and also blocked RM with serial compounds, indicating that the drug had interfered with the unconditioned excitatory effects of the tone- and light-CS's.

METHOD

Subjects

Subjects were 46 naive male and female albino rabbits (*Oryctolagus cuniculus*) that on arrival were 80–100 days old and weighed approximately 2 kg. All rabbits were maintained in the same manner as in Experiment 1.

Apparatus and Procedure

Unless otherwise indicated, the apparatus and general procedure were the same as those used in Experiment 1. All subjects received 1 day of preparation, 1 day of adaptation, and 4 days of RM training. During the adaptation session, animals were placed into the experimental chambers for a period of time equal to the length of the daily RM session (100 min). Subsequently, all subjects were randomly assigned to one of three groups consisting of MDA dosages of 0 (n = 16), 2 (n = 16), and 4 mg/kg (n = 14) at the 900-ms component ISI employed in Experiment 1. During each of the four daily RM sessions, animals received a total of 80 trials at a mean intertrial interval of 75 ± 10 s. The 80 trials were composed of: 1) 10 US-alone presentations given on every eighth trial and

2) 14 presentations each of A-X-US, B-X-US, A-US, B-US, and X-US at each of 14 CS-US intervals of 0, 100, 200, 400, 800, 900, 1000, 1100, 1300, 1900, 2000, 2100, 2300, and 2700 ms. The 14 CS-US intervals were selected to assess RM effects at: 1) 100, 200, 400, and 800 ms (CS-US intervals) after the onset of the 800-ms first element (A or B) of the compounds; and 2) 0, 100, 200, and 400 ms after the onset of the 400-ms second element (X) in the 900-ms component ISI employed in the present experiment and the 1900-ms ISI corresponding to Experiment 1. Accordingly, the US presentations were received at CS-US intervals of 900, 1000, 1100, and 1300 ms and 1900, 2000, 2100, and 2300 ms. Moreover, to assess RM to any residual trace of X in the 1900-ms compound employed in Experiment 1, US presentations were given at a CS-US interval of 2700 ms. Because both A-X and B-X were reinforced, it was not necessary to counterbalance the assignments of tone and white noise to A and B. One of the A-X-US, B-X-US, A-US, B-US, and X-US trials at each ISI were randomly selected and assigned to random position within 1 of 5 16-trial blocks consisting of 1 of each of the 14 CS-US ISI's and 2 US-alone presentations. At all CS-US intervals, each trial terminated with US offset. Reflex modification was computed as the algebraic difference between the average UR amplitude observed at each ISI on A-X-US, B-X-US, A-US, B-US, and X-US trials and the average UR amplitude on the US-alone trial within that block.

Follow-Up Tests

Many of the follow-up tests employed in Experiment 2 were used to localize higher-order interactions of dose, days, ISI, and CS type. Higher-order interactions are of a complexity that normally precludes a clear interpretation of findings and thus limits the report of follow-up tests to basic trends in the results. To characterize the nature of the higher-order interaction without reporting all the significant comparisons, the results are presented by indicating the ISI's and CS types significantly affected by MDA dosage on at least one of the 4 days.

RESULTS

Overall Amplitude Change

Figure 5 displays the effect of MDA dosage upon the mean amplitude change at CS-US intervals ranging from 0-2700 ms. An examination of the figure reveals that across the CS-US intervals the RM functions were an inverted U-shape with the maximum amplitude change occurring at 400 ms after the onset of the stimuli. It is apparent from the panels of the figure that the MDA dosages revealed an inverted dose-response relationship, with the 2-mg/kg dose facilitating and the 4-mg/kg dose decrementing the amplitude change across the 14 ISI's. An ANOVA on the RM data revealed significant effects of: 1) CS-US interval, $F(13,559) = 27.44$, $p < 0.001$, indicating that the highest amount of amplitude change occurred at the 400-ms CS-US interval, with less amounts of RM at shorter and longer ISI's; days × CS-US interval, $F(39,1677) = 1.77$, $p < 0.01$, revealing that on days 1, 3, and 4 the RM function had a maximum of 400 ms, but on day 2 the function was shifted to the right with maximum reflex modification at 800 ms; and dosage × days × CS-US interval, $F(78,1677) = 1.46$, $p < 0.01$. Posthoc analysis of the dosage × days × CS-US interval interaction revealed that: 1) the 2-mg/kg dosage enhanced amplitude change on days 1-4 combined at the 100-, 200-, 400-, 800-, 900-, 1000-, 1900-, and 2300-ms ISI's; and 2) the 4-mg/kg dose of MDA decremented RM on days 1-4 combined at the 100-, 200-, 400-, 800-, 900-, 1000-, 1100-, 1300-, 2000-, 2100-, and 2300-ms CS-US intervals.

Amplitude Change to A-X and B-X

An analysis of amplitude change in the presence of A-X and B-X at the 14 CS-US intervals across the 4 days of RM training (figure not shown) revealed significant main effects of: 1) ISI, $F(13,559) = 26.53$, $p < 0.001$, indicating two peaks in responding, with one at 400 ms after A or B onset and the other at X onset (900 ms ISI); and 2) CS type, $F(1,43) = 26.53$, $p < 0.001$, yielding slightly more responding to B-X than A-X. Moreover, the analysis yielded the significant interactions of CS type × ISI, $F(13,559) = 2.65$, $p < 0.01$; days × ISI, $F(39,1677) = 6.27$, $p < 0.001$; days × CS type, $F(3,129) = 4.80$, $p < 0.01$; and dosage × CS type, $F(2,43) = 3.70$, $p < 0.05$. Follow-up analysis of the significant dosage × CS type interaction revealed that there were no dosage effects on RM during A-X, but that the 4-mg/kg dose significantly decremented responding to B-X relative to both the 2-mg/kg dose and saline vehicle [critical difference (6,43) = 0.176 mm, $p < 0.05$].

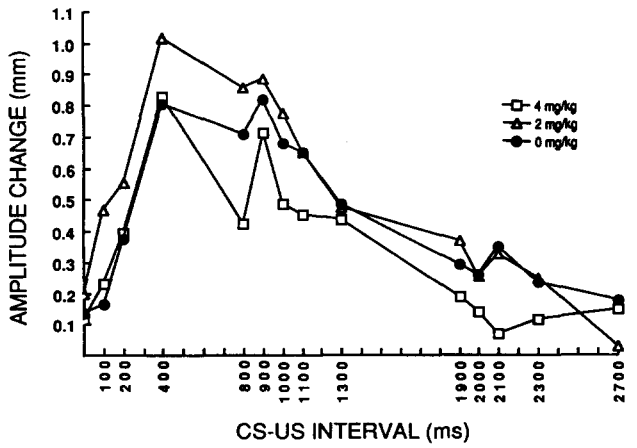

FIG. 5. Effect of MDA dosage on the mean amplitude change collapsed across CS type (A-X+, B-X−, A, B, and X) occurring prior to US offset for 14 CS-US intervals.

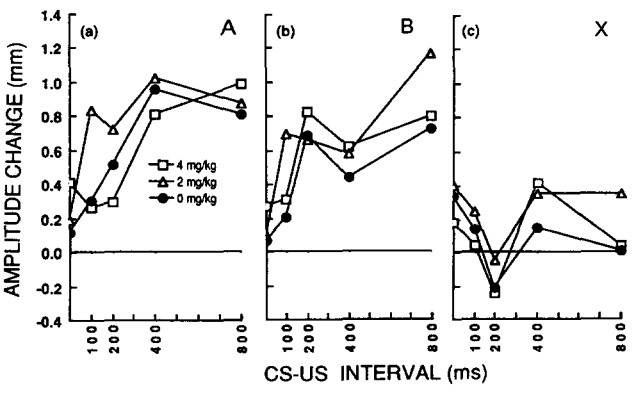

FIG. 6. Effect of MDA dosage on the mean amplitude change to A, B, and X on A-US, B-US, and X-US trials occurring prior to US offset for the 0-, 100-, 200-, 400- and 800-ms CS-US intervals.

Amplitude Change to A, B, and X

Panels (a), (b), and (c) of Fig. 6 present the effect of MDA dosage on the mean amplitude change at the CS-US intervals of 0, 100, 200, 400, and 800 ms for A, B, and X stimuli. An examination of the panels of the figure indicates that the RM effects of A (tone) and B (white noise) were both increasing functions of CS-US interval and were greater than RM to X (flickering light). An ANOVA revealed significant effects of CS-US interval, $F(4,172) = 6.75$, $p < 0.001$; CS type (A, B, and X), $F(2,86) = 15.68$, $p < 0.001$; CS type × CS-US interval, $F(8,344) = 5.70$, $p < 0.001$; days × ISI, $F(12,516) = 9.27$, $p < 0.001$; days × CS type, $F(6,258) = 18.69$, $p < 0.001$; dosage × days × CS-US interval, $F(24,516) = 1.54$, $p < 0.05$; and dosage × days × CS type × CS-US interval, $F(48,1032) = 4.99$, $p < 0.001$. Follow-up analysis of the main effects revealed that: 1) RM at the 400- and 800-ms CS-US intervals was greater than that of the 0-, 100-, and 200-ms intervals; and 2) amplitude change was not significantly different between A and B, but both stimuli produced higher RM than X. In addition, posthoc analysis of the significant dosage × days × CS type × CS-US interval interaction revealed that: 1) the 4-mg/kg dosage significantly decremented RM across days 1-4 to stimulus A at the 100- and 200-ms ISI's, to B at the 100- and 800-ms ISI's, and enhanced RM to X at the 0-, 100-, 200-, 400-, and 800-ms ISI's; and (b) the 2-mg/kg dose enhanced RM across days 1-4 to A at the 100-, 200-, 400-, and 800-ms ISI's, to B at the 0-, 100-, and 800-ms ISI's, and to X at the 0-, 100-, 200-, 400-, and 800-ms CS-US intervals.

DISCUSSION

Experiment 2 revealed that: 1) the overall RM function varied directly with CS-US interval with a maximum at 400 ms; 2) amplitude change to the compounds produced two peaks, one at 400 ms after A and B onset and one at X onset (900 ms); and 3) the amplitude change was greater for both A and B as compared to X. Moreover, the RM effects revealed that: 1) the 4-mg/kg dose of MDA decremented RM to the compounds and to A and B, while enhancing responding to X; and 2) the 2-mg/kg dose enhanced RM to A, B, and X, but had no effect on RM to A-X and B-X.

The effects of MDA upon reflex modification served to determine the drug's effects upon the unconditioned excitatory properties of the compounds and their components. In stimulus trace accounts of conditioning (15,18,25,26), these unconditioned excitatory effects are postulated to reflect the intensity of the Cs's and, thus, the CS's rate of entry into conditioning and the CS's ability to elicit CR's once conditioning has occurred. Accordingly, MDA's attenuation of the intensity and duration of the unconditioned excitatory properties of both the compounds and their A and B components served to impair CR's to both A-X and A and generalized CR's to B-X and B. In addition, MDA's enhancement of the amplitude change to X served to facilitate CR's to X conditional upon no prior response to A and B at the 1900-ms component ISI in Experiment 1. The present findings and theoretical account are consistent with earlier NMR findings (30) that MDA's impairment of CR's to a tone-CS in single-stimulus conditioning appeared to be mediated by its ability to attenuate auditory-CS intensity.

Finally, the results of Experiment 2 indicate that MDA's modulation of the stimulus trace may reside in the neural structures involved in reflex modification. Thus, the evidence suggests that MDA may affect the stimulus trace by altering synaptic action in structures such as the accessory abducens (52) and abducens (41) nuclei, which have been demonstrated to participate in reflex modification, whereas structures such as the hippocampus and cerebellum, which have not been shown to support RM, are less likely candidates for the site of MDA's modulation of the stimulus trace.

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