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Effects of Morphine and LSD on the Classically Conditioned Nictitating Membrane Response

C. W. SCHINDLER, I. GORMEZANO AND J. A. HARVEY[1]

Departments of Psychology and Pharmacology, The University of Iowa, Iowa City, IA 52242

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SCHINDLER, C W., I GORMEZANO AND J A HARVEY *Effects of morphine and LSD on the classically conditioned nictitating membrane response* PHARMACOL BIOCHEM BEHAV 22(1) 41–46, 1985 —Two experiments were carried out to determine the effects of LSD and morphine on the unconditioned nictitating membrane response of the rabbit elicited by 5 intensities of a 100 msec puff of air directed at the cornea, and on the acquisition of conditioned responses to a tone and light conditioned stimulus using the air-puff as an unconditioned stimulus In Experiment 1, LSD tartrate (0 013 mg/kg) had no effect of the frequency, amplitude, magnitude or latency of the unconditioned response. However, LSD significantly enhanced the rate of acquisition of conditioned responses to both tone and light conditioned stimuli In Experiment 2, morphine sulfate (5 mg/kg) had no effect on the frequency, amplitude, magnitude or latency of the unconditioned response, but significantly retarded the acquisition of conditioned responses to both tone and light conditioned stimuli The results indicated that the enhancement of acquisition produced by LSD and the retardation of acquisition produced by morphine were not due to effects of the drugs on either the sensory processing of the air-puff unconditioned stimulus or on the motoric expression of the unconditioned response.

LSD Morphine Classical conditioning Nictitating membrane response Rabbit

THE results of recent studies, employing the classically conditioned nictitating membrane response (NMR) of the rabbit, suggest that drugs alter the rate of acquisition of conditioned responses (CRs) to a tone conditioned stimulus (CS), by altering the sensory processing of the tone CS in a manner analogous to an increase or decrease in its nominal intensity [18] For example, *d*-lysergic acid diethylamide (LSD) enhanced the rate of CR acquisition to a tone CS and, in previously trained animals, lowered the intensity threshold of a tone CS for elicitation of CRs [6]. Atropine [2], haloperidol [7], morphine [18] and scopolamine [9,14] retarded the rate of CR acquisition and raised the intensity threshold of a tone CS for elicitation of CRs. Moreover, the degree of enhancement or retardation of CR acquisition was highly correlated with the degree of change in the CS intensity threshold [18]. Since atropine [2], haloperidol [7] and scopolamine [9] had no effect on the intensity threshold of the shock unconditioned stimulus (UCS) for elicitation of unconditioned responses (UCRs) or on the amplitude and latency of the elicited UCR, one can conclude that these drugs retarded CR acquisition by their ability to block the sensory processing of the CS.

Attempts to localize the behavioral actions of morphine and LSD have not been as successful. Although, LSD tartrate (0.013 mg/kg) had no effect on latencies of the UCR elicited by a shock UCS, it did decrease the UCS intensity threshold for elicitation of UCRs, increased the magnitude of UCRs and increased the amplitude of elicited UCRs (J. A Harvey, I Gormezano and V A. Cool-Hauser, unpublished data). Morphine (0.2 to 10 mg/kg) had no effect on the amplitude or latency of UCRs elicited by a fixed intensity (3 mA) shock UCS during the unpaired presentations of tones, lights and shock UCSs [17]. Using a range of shock intensities, morphine (5 mg/kg) again had no effect on either UCR latency or on the UCS intensity threshold for elicitation of UCRs, but did produce a significant decrease in both the magnitude and amplitude of the UCR elicited by a shock UCS [18]. These results suggest that the effects of LSD and morphine on CR acquisition may have been due to their effects on the sensory processing of the tone CS and/or on the unconditioned nictitating membrane reflex, but leaves unclear which of these effects was primarily responsible for the observed changes in CR acquisition.

The rabbit NMR consists of the passive extension of the nictitating membrane across the cornea, however, this response can be produced by two separate reflex pathways depending on the UCS employed. Nictitating membrane responses elicited by tactual stimulation of the cornea by means of an air puff are primarily mediated by retractor bulbi motoneurons in the accessory abducens nucleus via the VIth nerve [10,12]. The contraction of the retractor bulbi muscle pulls the eye back into the orbit and the force of this action squeezes the nictitating membrane across the globe [1,15]. In contrast, NMRs elicited by electric shock delivered to the skin over the paraorbital region of the head are produced to an approximately equal extent by contraction of the retractor

[1]Requests for reprints should be addressed to John A Harvey, Department of Psychology, The University of Iowa, Iowa City, IA 52242.

bulbi muscle via the VIth nerve and by contraction of the orbicularis oculi muscle via the VIIth nerve which then squeezes the nictitating membrane over the cornea [12]

The differences in the effector systems involved in the UCRs elicited by electric shock vs air puff allows for the possibility of differential drug actions on the NMR depending on the UCS employed We have, therefore, examined the effects of both morphine and LSD on the unconditioned NMR elicited by a wide range of air puff intensities in order to obtain a measure of possible drug effects on the UCS intensity threshold for elicitation of UCRs as well as on the latency, magnitude and amplitude of the elicited UCRs In addition, we have determined whether morphine and LSD would affect the acquisition of CRs to tone and light CSs when the UCS was an air puff as was done in previous studies using electric shock as the UCS [6,18].

EXPERIMENT 1

The purpose of this experiment was to compare the effects of LSD on unconditioned responding and on acquisition of conditioned responding to tone and light CSs when the UCS was a puff of air directed at the cornea The dose of LSD tartrate used in this experiment, 0 013 mg/kg (30 nmol/kg), has been repeatedly shown to produce maximal enhancement of CR acquisition to tone and light CSs paired with a shock UCS [3, 6, 8]

METHOD

Subjects

Forty-eight experimentally naive rabbits (New Zealand white albino) were obtained from local suppliers. The rabbits weighed approximately 2 2 kg on arrival and were housed individually with free access to food and water

Apparatus and General Procedure

The apparatus and procedures have been described in detail [4,5] In brief a small loop of surgical nylon (Ethicon 4-0) was sutured into the right nictitating membrane, and the surrounding hair was removed. One day later, the rabbit was placed in a Plexiglas restrainer and an external eyelid retractor was applied to the right eye The rabbit was then fitted with a headmount that supported a photoresistive assembly for recording the NMR by physically coupling the arm of the assembly to the loop in the right nictitating membrane. The transducer assembly converted nictitating membrane movements to electrical signals, which were subjected to an analog-to-digital conversion using a 5-msec sampling rate and a resolution of 0.06 mm actual membrane movement A 16-gage, blunt hypodermic needle through which the air-puff UCS was presented was also connected to the headmount and positioned so that the end of the needle was directed at the center of the cornea of the rabbit's right eye and at a distance of 6 mm from its surface The rabbit was then positioned in a ventilated, sound-attentuated chamber facing a stimulus panel containing an 11.4-cm speaker and two 6-w, 24-V, d.c houselights, one mounted on each side of the speaker Experimental control, analog-to-digital conversion of nictitating membrane movement and data processing were all accomplished by an Apple II/FIRST operating system [16]

Drugs

LSD (*d*-lysergic acid diethylamide tartrate, NIDA) was dissolved in sterile, nonpyrogenic, distilled water LSD or its vehicle were injected into the marginal ear vein via a Harvard infusion pump (Model No 975) in a volume of 0 4 ml/kg at a rate of 3 ml/min The dose of LSD (0 013 mg/kg) is expressed as the salt form

Procedure for UCS-UCR Psychophysical Functions

Twenty-four experimentally naive rabbits received one 50-min adaption session during which no stimuli were presented or drug injected However, in order to obtain a measure of baseline responding, NMRs were recorded at observation intervals employed during testing One day after adaptation, rabbits were randomly assigned to two groups and injected with either LSD (0 013 mg/kg) or vehicle 20–30 min prior to each of the following three daily sessions Each daily (50-min) session consisted of 50 air puff-alone trials presented in 10, 5-trial blocks One of five air-puff intensities of 2, 10, 18, 26 and 34 lbs/in^2 was presented 5 times within each block The order of presentation of the five intensities of air puff was randomly generated Air-puff intensities refer to the gage reading of air pressure in the line The intertrial interval was randomly generated with a mean of 60 sec (range 50–70 sec) A UCR was defined as at least 0 5 mm of membrane extension occurring within 400 msec after onset of the 100-msec air-puff UCS The frequency, onset latency and peak amplitude of the UCRs were recorded

Procedure for Paired CS-UCS Training

Twenty-four experimentally naive rabbits received one 60-min adaptation session identical to that described above One day after adaptation, the rabbits were randomly assigned to two groups and given either an injection of LSD (0 013 mg/kg) or vehicle 20–30 min prior to each of the following 10 daily sessions Each daily session consisted of 60 trials composed of 30 tone-air puff and 30 light-air puff trials presented in a randomized sequence within 10-trial blocks with the restriction that not more than three tone- or light-CS trials could occur consecutively The 800-msec, 1 kHz, 75-dB tone CS (0 0002 dynes/cm^2 reference) was delivered through the speaker by an audio-oscillator (Hewlett-Packard, Model 201CR) The 800-msec flashing light CS was produced by interruption of the houselights at 10 Hz to yield a change in illumination, measured at the eye level of the rabbit, from 32 0 lx to 8.0 lx The 100-msec, 30 lbs/in^2 air-puff UCS was delivered to the cornea through the blunt hypodermic needle On each conditioning trial the offset of the 800-msec tone or light CS occurred simultaneously with the onset, at the cornea, of the 100-msec air-puff UCS The intertrial interval was randomly generated with a mean of 60 sec (range 50–70 sec) A response was defined as at least a 0 5 mm extension of the nictitating membrane, and was recorded as a CR if it occurred during the 800-msec CS and as a UCR if it occurred after UCS onset The onset latency of the NMR was also recorded

Data Analysis

A repeated measures analysis of variance was performed on the data of each experiment with follow-up analyses to localize significant sources of variation carried out by the method of Dunnett [11] Significance level was set at $p<0.05$, two tailed

RESULTS

As shown in Fig 1, the vehicle and LSD injected groups

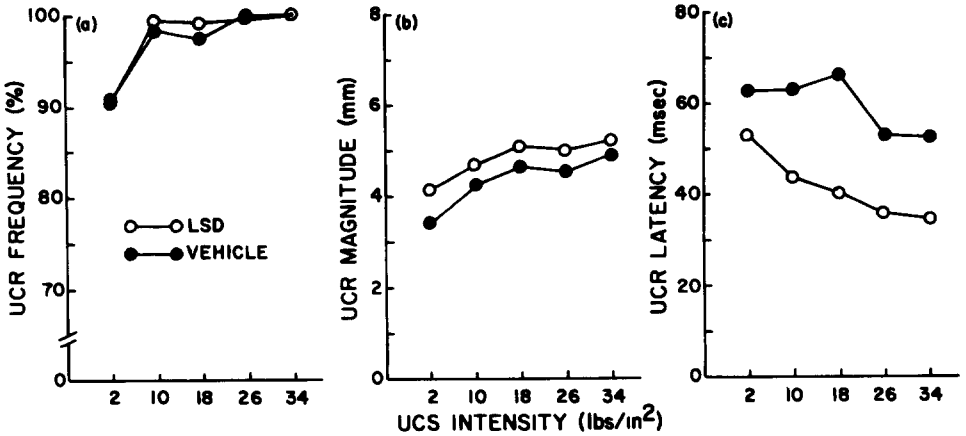

FIG 1 Effect of LSD tartrate (0 013 mg/kg) on UCR frequency (panel a), UCR magnitude (panel b) and UCR latency (panel c) as a function of UCS intensity Each point is the mean of 12 rabbits

demonstrated significant ($p<0.001$) increases in UCR frequency (panel a), increases in UCR magnitude (panel b) and decreases in UCR latency (panel c) as a function of increasing intensities of the air-puff UCS. However, LSD had no significant effect on the frequency, magnitude or latency of UCRs as compared with vehicle injected controls. A separate analysis of UCR amplitudes also failed to reveal any significant differences between LSD and vehicle treated controls (data not shown). In addition, there were no significant two-way interactions between drug treatment and either UCS intensity or daily session.

LSD (0 013 mg/kg) significantly ($p<0.001$) enhanced the overall level of CRs as well as the rate of acquisition of CRs, to both tone and light CSs combined, during paired CS-UCS training (Fig. 2, panel a). Rabbits receiving LSD had reached a level of 94.0% CRs by the 10th conditioning session as compared with 69.5% CRs for the vehicle controls. The enhancement of CR acquisition produced by LSD was also reflected in the significantly ($p<0.001$) shorter NMR onset latencies during training. By the 10th conditioning day the average NMR onset latency for the LSD group was 227 msec as compared to 440 msec for vehicle controls The statistical analyses based on either percent CRs or NMR onset latency revealed that CS Modality was not a significant source of variation nor did Modality enter into any significant interactions with Days or Drug Treatment. In order to obtain a measure of the rate of CR acquisition we calculated, for each animal, the number of trials required to reach a criterion of 10 consecutive CRs, irrespective of CS modality Rabbits injected with LSD reached this stringent criterion of CR acquisition in 188 trials which was significantly ($p<0.001$) faster than the 402 trials required by controls

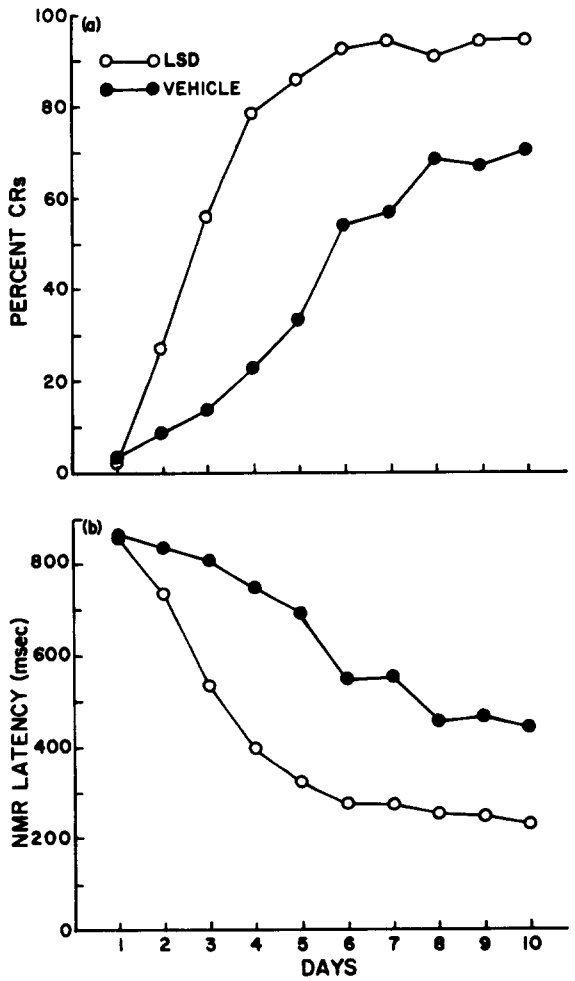

FIG 2 Effect of LSD tartrate (0 013 mg/kg) on acquisition of CRs to tone and light CSs combined across the 10 days of paired CS-UCS training Each point is the mean of 12 rabbits

EXPERIMENT 2

The purpose of Experiment 2 was to determine whether morphine would also have a differential effect on CR acquisition and UCR elicitation when the UCS was tactual stimulation of the cornea by means of a puff of air. The dose of morphine sulfate used in this experiment (5 mg/kg) had been shown in a number of other studies to produce a reliable

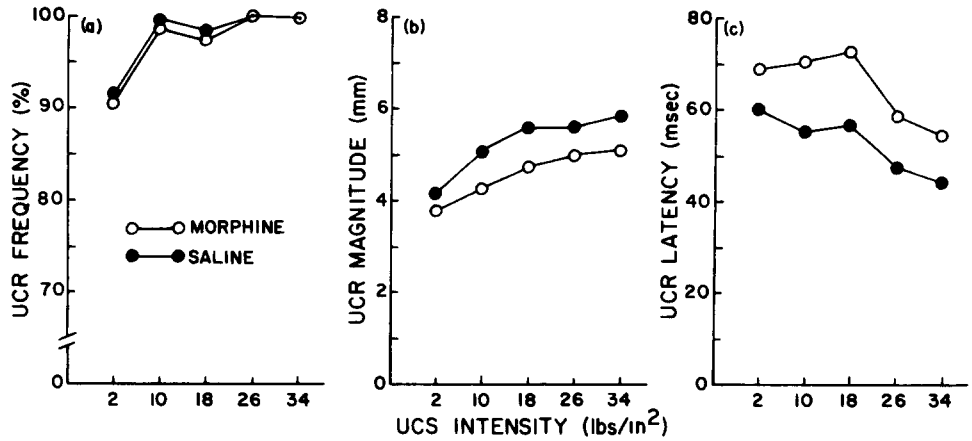

FIG 3 Effect of morphine sulfate (5 mg/kg) on UCR frequency (panel a), UCR magnitude (panel b) and UCR latency (panel c) as a function of UCS intensity Each point is the mean of 12 rabbits

retardation in CR acquisition and in UCR magnitudes when the UCS was electric shock [17, 18, 19]

METHOD

Subjects

The subjects were 48 experimentally naive rabbits (New Zealand white albino) obtained and housed as described in Experiment 1

Procedure

The procedures for obtaining the UCS-UCR psychophysical functions and for paired CS-UCS training were identical to those of Experiment 1 except that the intensity of the tone CS was increased from 75- to 84-dB. Morphine sulfate (Mallinckrodt) was dissolved in sterile, nonpyrogenic, saline to give a final dose of 5 mg/kg as the salt Injections were carried out as described for Experiment 1

RESULTS

As shown in Fig 3, both the morphine and vehicle injected rabbits demonstrated an increase in UCR frequency (panel a), increase in UCR magnitude (panel b) and decrease in UCR latency which were a significant function of increasing intensities of the air-puff UCS ($p<0.001$, for all comparisons) However, morphine (5 mg/kg) had no significant effect on the frequency, magnitude or latency of the UCR Morphine also had no significant effect on UCR amplitude (data not shown)

Morphine (5 mg/kg) significantly ($p<0$ 001) retarded acquisition of percent CRs to both tone and light CSs combined during paired CS-UCS training (Fig 4, panel a). Rabbits receiving morphine had only reached a level of 25 8% CRs by the 10th conditioning session as compared with 73 1% CRs for the saline controls. None of the rabbits receiving morphine reached a criterion of 10 consecutive CRs by the last (600th trial) while 10 of the 12 rabbits receiving saline reached this criterion. Thus the average number of trials to reach criterion was saline controls, 419, morphine, > 600 The retardant effect of morphine on CR acquisition was also reflected in significantly ($p<0$ 001) longer NMR onset laten-

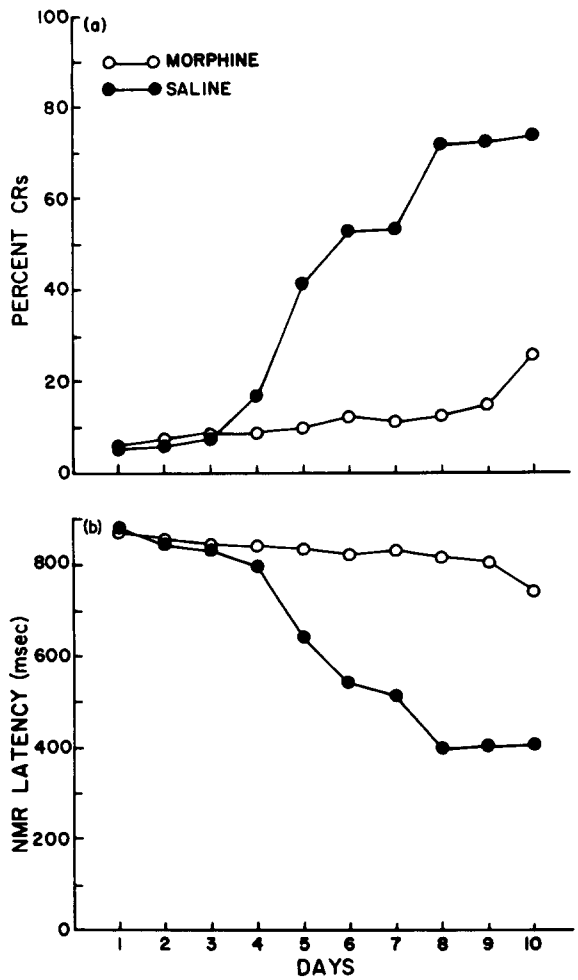

FIG 4 Effect of morphine sulfate (5 mg/kg) on acquisition of CRs to tone and light CSs combined across 10 days of paired CS-UCS training Each point represents the mean of 12 rabbits

cies during the 10 days of conditioning (Fig. 4, panel b). By the last, 10th, day of conditioning, the average NMR onset latency for the morphine group (741 msec) was still significantly ($p<0.001$) longer than that of controls (406 msec). The statistical analyses indicated the absence of any significant effect of CS Modality or its interactions with Days or Drug Treatment for either percent CRs or NMR onset latency

GENERAL DISCUSSION

Previous studies had demonstrated that LSD tartrate (0.013 mg/kg) enhanced [3, 6, 8] while morphine sulfate (5 mg/kg) retarded [17, 18, 19] the rate of CR acquisition to both tone and light CSs when they were paired with a UCS consisting of electric shock delivered to the skin just lateral to the eye. Using previously trained animals, LSD was found to decrease [6] and morphine to increase [18] the intensity threshold of a tone CS for elicitation of CRs. In addition, using a wide range of shock intensities, LSD was found to decrease the intensity threshold of the shock UCS for elicitation of UCRs and increase the magnitude as well as the amplitude of the elicited UCR (J. A Harvey, I Gormezano and V. A Cool-Hauser, unpublished data), while morphine decreased UCR magnitude and amplitude without affecting either UCR latency or the intensity threshold of the shock UCS for elicitation of UCRs [18]. These findings suggested that the changes in the rate of CR acquisition produced by LSD and morphine might be due to changes in the sensory processing of both the CS and UCS Moreover, to the extent that LSD increased and morphine decreased the amplitude of the shock-elicited UCR, it was also possible that changes in CR acquisition might be related to changes in the motoric expression of the NMR

In contrast with the outcome of previous studies that employed electric shock as the UCS, the results of Experiments 1 and 2 indicated that LSD tartrate (0.013 mg/kg) and morphine sulfate (5 mg/kg) had no significant effect on either the threshold of the air-puff UCS for elicitation of UCRs or on the amplitude, magnitude and latency of the UCR. Nevertheless, LSD still produced a significant enhancement and morphine a significant retardation of CR acquisition to both tone and light CSs that had been paired with the air-puff UCS. Thus, changes in CR acquisition produced by LSD and morphine do not appear to be due to any effects of these drugs on the sensory processing of the air-puff UCS or on the motoric expression of the UCR. Since, with the use of an air-puff UCS, both the UCR and CR are mediated by the same final common pathway via the VIth nerve [10,12], these results also suggest that LSD and morphine are not affecting the motoric expression of the CR. Using the rabbit NMR preparation, Mauk *et al.* [13] also found, in previously trained rabbits, that morphine could block the occurrence of CRs to a tone CS without an effect on the amplitude of the UCR elicited by the air-puff UCS These results, therefore, support previous suggestions that the changes in the rate of CR acquisition produced by both LSD and morphine are due to their effects on the sensory processing of the CS [18].

Although this study did not directly compare the effects of LSD and morphine on the UCR elicited by an air puff vs. a shock UCS, the results do suggest a differential drug action on these two UCSs. Since the unconditioned NMR elicited by tactual stimulation of the cornea is primarily mediated by the accessory abducens nucleus via a VIth nerve reflex [10,12], the results of the present study suggest that LSD and morphine have little effect on this VIth nerve reflex However, NMRs elicited by electric shock are mediated to an equal extent by both a retraction of the eyeball via the VIth nerve and a contraction of the orbicularis oculi muscle via the VIIth, facial, nerve [12]. Thus, the previous findings that morphine and LSD have significant effects on the NMR elicited by an electric shock UCS suggests that these drug effects may be primarily due to an action on the VIIth nerve reflex

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