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Effects of LSD on Classical Conditioning as a Function of CS-UCS Interval: Relationship to Reflex Facilitation

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HARVEY, J. A., I. GORMEZANO, V. A. COOL-HAUSER AND C. W. SCHINDLER. *Effects of LSD on classical conditioning as a function of CS-UCS interval: Relationship to reflex facilitation.* PHARMACOL BIOCHEM BEHAV 30(2) 433–441, 1988.—Classical conditioning of the rabbit nictitating membrane response was accomplished by presenting a 100-msec tone CS at intervals 0, 100, 200, 400 and 800 msec before the presentation of a 100-msec shock UCS. In addition, tone-alone trials were used to monitor CR acquisition and shock-alone trials to measure facilitation of the nictitating membrane reflex by the tone CS at the various CS-UCS intervals. LSD at a dose of 13 μg/kg (30 nmol/kg) increased the excitatory effects of the shock UCS as measured by a greater frequency and amplitude of UCRs elicited across a wide range of UCS intensities and by the ability of a low intensity shock to produce reflex facilitation. Consequently, LSD produced a higher amplitude of UCRs on UCS-alone trials and on paired trials across all CS-UCS intervals during measurement of tone-induced reflex facilitation. LSD also enhanced CR acquisition across all CS-UCS intervals. Because LSD produced larger amplitude reference UCRs on the UCS-alone trials as compared with controls, calculations of reflex facilitation as a percentage change from these reference amplitudes led to an artifactually smaller effect for the LSD group as compared with controls. Nevertheless, both reflex facilitation as measured prior to CR acquisition on the first day of conditioning and CR acquisition across 10 conditioning sessions were a function of CS-UCS intervals and these two measures were highly correlated in the LSD (+0.94) and vehicle control (+0.85) groups. It was concluded that LSD enhances CR acquisition by enhancing the excitatory effects of both the CS and UCS and thus increasing their ability to enter into associative learning.

LSD Rabbit Reflex facilitation Learning

STUDIES employing classical conditioning of the rabbit's nictitating membrane response have reported that the ability of a drug to alter the rate at which an animal acquires conditioned responses (CRs) to a tone-conditioned stimulus (CS) can be attributed to change in the conditioned and unconditioned excitatory properties of the tone [11,23]. For example, haloperidol [12] and scopolamine [15] retarded the acquisition of CRs and reduced the conditioned excitatory properties of the tone CS as measured by an elevation in the CS-intensity threshold for eliciting CRs once learning has occurred. Both drugs also reduced the unconditioned excitatory properties of the tone stimulus [1] prior to any occurrence of learning as measured by a decrease in the magnitude of heterosynaptic reflex facilitation, i.e., by a decreased ability of the tone to increase the amplitude of the nictitating membrane response when presented just prior to a shock-unconditioned stimulus (UCS) [12,15].

A number of investigators have suggested that heterosynaptic reflex facilitation represents the basis for the plastic changes that lead to learning [12, 15, 17, 27]. In agreement with this view, the amount of reflex facilitation produced by a tone at various tone-shock intervals and the amount of CR acquisition demonstrated at those intervals was found to be highly correlated in control rabbits [15]. In

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addition, the decrease in CR acquisition produced by scopolamine at various CS-UCS intervals was highly correlated (+0.95) with the decreases it produced in reflex facilitation [15].

The results described above suggest that scopolamine, haloperidol and possibly other drugs reduce CR acquisition by decreasing the unconditioned excitatory properties of the tone, thus reducing the duration and intensity of heterosynaptic reflex facilitation upon which subsequent learning is dependent. We have chosen to extend the generality of these views by examining whether a drug that enhances CR acquisition through an effect on associative processes would also increase heterosynaptic reflex facilitation. The present study used d-lysergic acid diethylamide (LSD) to examine this possibility at a dose (12.9 μg/kg) that had been shown to enhance the rate of CR acquisition [5] and, in trained animals, to increase the conditioned excitatory properties of a tone CS [7]. For example, in a previous study [5] LSD enhanced CR acquisition to both tone and light CSs at doses of 0.43, 4.3, 12.9 and 43 μg/kg (as the salt form), whereas 129 μg/kg retarded acquisition as compared with vehicle controls. Peak enhancement of CR acquisition occurred at a dose of 12.9 μg/kg (30 nmol/kg) and the dose producing half-maximal enhancement of CR acquisition was 1.4 μg/kg (3.3 nmol/kg). These findings have been replicated in several studies [7, 14, 24], and the enhancement of CR acquisition produced by LSD (12.9 μg/kg) was demonstrated to be due to an effect on associative processes [5, 7, 14]. CR acquisition to both tone and light CSs was also enhanced by the phenethylamine hallucinogen dl-2,5-dimethoxy-4-methylamphetamine (DOM) but this effect was accompanied by increases in nonassociative responding [14]. The effects of LSD and DOM on CR acquisition were not mimicked by CNS stimulants. Thus, d-amphetamine produced a modality specific effect characterized by an increase in CR acquisition to a light but not to a tone CS [14], while caffeine and theophylline had no significant effect on CR acquisition to tone or light CSs or on the excitatory properties of the tone CS [29].

Four separate experiments were carried out using LSD at the dose (12.9 μg/kg) previously shown to have maximal effects on CR acquisition. First, we employed several CS-UCS intervals to compare the effects of LSD on heterosynapitc facilitation of the nictitating membrane reflex by a tone CS during the first day of conditioning, when no learning had yet occurred, with the effects of LSD on the subsequent rate of CR acquisition to the tone CS at the same CS-UCS intervals. Second, we used explicitly unpaired presentations of CSs and UCSs to control for possible nonassociative contributors to our measures of reflex facilitation and CR acquisition. Third, based on the outcomes of the first two experiments, we examined the effects of LSD on the frequency and amplitude of the unconditioned response (UCR) elicited across a range of UCS intensities. Finally, using the data of Experiment 3, we examined the effects of LSD on monosynaptic reflex facilitation produced by a low intensity UCS.

METHOD

Subjects

Experimentally naive rabbits (New Zealand white albino) of either sex were obtained from local suppliers. The rabbits weighed approximately 2 kg on arrival and were housed singly with free access to Purina Rabbit Chow and water.

Apparatus and General Procedure

The apparatus and procedure, including the positioning of the rabbit in the experimental chamber, delivery of stimuli, coupling of the phototransistor assembly for measurement of nictitating membrane extension as well as the Apple II/FIRST operating system for experimental control, analog-to-digital conversion of membrane movement and data processing, have been described in detail [6, 10, 22] and were identical with those of a previous study of drug effects as a function of CS-UCS interval [15]. For all experiments of this study, the CS was a 100-msec, 1-kHz, 84-dB tone (2×10^{-4} dynes/cm^2 reference) and was delivered by an audio-oscillator (Hewlett-Packard, model 201CR) through an 11.4 cm speaker positioned above and in front of the animal. The UCS was a 100-msec, 60-Hz shock delivered through two woundclips attached to the skin over the paraorbital region of the head at a distance 100 mm posterior to the canthus and 15 mm apart in the vertical direction. The intensity of the UCS employed in the various experiments is described below. For all experiments of this study, a response was defined as at least a 0.5 mm extension of the nictitating membrane.

Drugs

LSD (d-lysergic acid diethylamide tartrate, NIDA) was dissolved in sterile, nonpyrogenic, distilled water. LSD or its water 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, 20–30 min prior to behavioral testing. The dose of LSD used in all of the experiments was 13 μg/kg as the salt form (30 nmol/kg).

Experiment 1: Paired CS-UCS Training

Prior to behavioral training rabbits received a 72-min adaptation session during which no stimuli were presented or drug injected, but responses occurring during the observational intervals to be employed during conditioning were recorded to obtain a measure of baseline responding. On the next day, rabbits were injected with LSD or vehicle prior to a 72-min conditioning session consisting of 72 trials spaced an average of 60 sec apart (range 50–70 sec). The 72 trials were divided into six 12-trial blocks. Within each 12-trial block, the 6th trial was always a tone-alone (test) trial and the 12th trial was always a shock-alone trial. The remaining 10 trials within each block consisted of CS-UCS pairings at a designated CS-UCS interval. Separate groups of animals received 10 such daily conditioning sessions at CS-UCS intervals of either 0, 100, 200, 400 or 800 msec. A CS-UCS interval was defined as the time between onset of the 100-msec, 84-dB tone CS and the 100-msec, 2-mA shock UCS. A response was recorded as a CR if it occurred after CS onset but prior to UCS onset and as a UCR if it occurred after UCS onset. It should be noted that CRs could not be recorded on paired CS-UCS trials for the group of animals receiving simultaneous onset of CS and UCS (i.e., animals at the CS-UCS interval of 0 msec). However, CRs could be recorded for all groups during the CS-alone (test) trials. The amplitude and latency of each CR and UCR were also recorded.

Experiment 2: Unpaired CS and UCS Training

Animals received a 66-min adaptation session carried out

as described above. One day later, animals were injected with LSD or vehicle prior to a 66-min session consisting of 132 trials composed of 66 tone-alone and 66 shock-alone trials. The intertrial interval averaged 30 sec (range 25 to 35 sec). The tone and shock stimuli were identical to those used during paired CS-UCS training. The 132 trials were divided into six 22-trial blocks. Within each 22-trial block equal numbers of tone and shock stimuli were presented in a random fashion with the restriction that the 11th and 22nd trial always consisted of a tone and shock stimulus, respectively. Responses occurring to the tone on the 11th trial of each block served as a control for the test (CS-alone) trials of the paired CS-UCS procedure. Similarly, the amplitude of the UCR to the shock UCS on the 22nd trial of each block served as a control for the amplitude of the UCR on the shock-alone trial of the paired CS-UCS procedure. Responses were recorded if they occurred within 800 msec of tone or shock onset. In addition, responses occurring during the 800 msec prior to shock onset provided a measure of baseline responding.

Experiment 3: UCS-UCR Psychophysical Functions

Animals received a 35-min adaptation session as described above. On the next day they were injected with LSD or vehicle prior to a 35-min session. There were two such sessions spaced 24 hours apart. Each session consisted of 35 shock-alone trials divided into 5 blocks of 7 trials each. Seven shock intensities of 0.25, 0.50, 0.75, 1.0, 2.0, 3.0 and 4.0 mA were randomly presented within each 7-trial block. The 60-Hz shocks were 100 msec in duration. The intertrial interval averaged 60 sec (range 50–70 sec). Responses were recorded if they occurred within 800 msec of shock onset. The latency and amplitude of each response were also recorded.

Experiment 4: Shock-UCS-Induced Facilitation of the Nictitating Membrane Reflex

Animals received one 60-min adaptation session carried out as described above and on the next day they were injected with LSD or vehicle prior to a single 60-min testing session consisting of 60 trials presented, on the average, every 60 sec (range 50–70 sec). Two shock-UCSs were employed, each 100 msec in duration: UCS^1, a 0.25 mA shock; and UCS^2, a 1.50 mA shock. The 60 trials were divided into 6 blocks of 10 trials each. Each block contained, in randomly determined order, one UCS^1-alone trial, one UCS^2-alone trial and eight trials on which onset of UCS^1 was followed within 0, 25, 50, 100, 200, 400, 800 or 1600 msec by onset of UCS^2. Thus, each animal was exposed to 6 repetitions of eight UCS^1–UCS^2 intervals. A response was recorded if it occurred within 800 msec after a UCS^1-alone or UCS^2-alone trial and after UCS^2 on paired trials.

Calculation of Reflex Facilitation

Tone-induced reflex facilitation. The amplitudes of UCRs obtained on the first day of paired CS-UCS training were used to assess reflex facilitation of the UCR by the tone CS, in a manner previously described [15]. Several problems were faced in carrying out these calculations because LSD produced a large and significant enhancement in the amplitude of UCRs as measured on the UCS-alone trials ($p<0.005$) and UCR amplitudes of both LSD and vehicle-injected animals underwent significant habituation ($p<0.001$) across the 6 blocks of trials (see Fig. 1). Consequently we were faced with the methodological problems inherent in comparing drug effects from two different baselines both of which were varying as a function of blocks of trials. Therefore, two calculations were carried out. Because UCR amplitudes underwent the greatest amount of habituation across the first two blocks of trials (see Fig. 1), these were excluded from our calculations. To further reduce any influence of habituation, we took the conservative approach of estimating reflex facilitation from the paired CS-UCS trial immediately preceding (e.g., trial 35) and the trial immediately following (e.g., trial 37) the UCS-alone trial (e.g., trial 36 or the third block). This procedure was carried out around the UCS-alone trial in blocks 3, 4 and 5. Because separate groups of animals were examined at each CS-UCS interval thus requiring a between groups comparison, an attempt was made to normalize the data by expressing reflex facilitation for each animal as a percentage change in UCR amplitude on a paired trial from its amplitude on a UCS-alone trial in blocks 3, 4 and 5. Since these percentage changes were being calculated from different baselines we also calculated the actual amplitudes of the UCRs on paired trials in blocks 3, 4 and 5. Trials on which a CR occurred during the CS-UCS interval were excluded from these analyses. The occurrence of the CRs in the 0-msec interval could not, of course, be determined. However at the remaining CS-UCS intervals only 9 of the 576 trials had to be eliminated. The frequency of these CRs (1.6%) was comparable to the baseline rate of responding (0.7%) during the unpaired CS and UCS procedure on Day 1, indicating that CR acquisition had not occurred during the first day of training to any extent that would substantially affect the measure of reflex facilitation. As a control for the measurement of reflex facilitation in the paired CS-UCS condition, comparable calculations were employed for the unpaired CS and UCS condition in blocks 3, 4 and 5. Thus, in each of these blocks the equivalent UCS along trial (e.g., trial 66, in block 3) was used as the reference trial from which the amplitude of the UCR on the immediately preceding and following trials (e.g., trials 65 and 67, respectively) were calculated as a percentage change.

Shock-UCS-induced reflex facilitation. Reflex facilitation produced by the pairing of two UCSs was calculated in the same manner as above. However, since UCRs elicited by the two UCSs did not undergo significant habituation, the data of all 6 blocks of trials were employed. Because LSD again produced a significant ($p<0.05$) increase in the amplitude of the UCR to the more intense UCS (UCS^2, see Fig. 7A), two calculations were again carried out. Thus, reflex facilitation was expressed both as the actual amplitude of the UCR on paired trials at each of the eight UCS^1–UCS^2 intervals and as a percentage change in these amplitudes from the amplitude of the UCR on the UCS^2-alone trial.

Statistical 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 [28].

RESULTS

Experiments 1 and 2: Effects of LSD on Tone-Induced Facilitation of the Nictitating Membrane Reflex

Figure 1 presents the amplitudes of the UCR elicited on

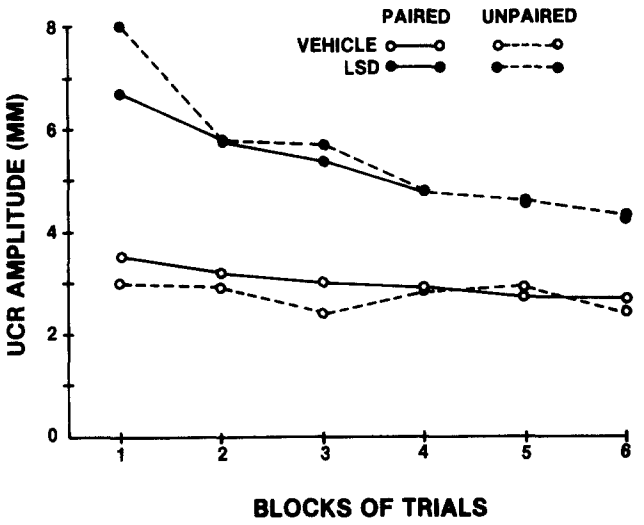

FIG. 1. Habituation of the UCR during the first day of training across blocks of trials. Data are presented as mean amplitudes of the UCR, in millimeters of actual membrane extension, elicited on each of the six shock-alone trials during the first day of paired CS-UCS training, irrespective of CS-UCS interval (PAIRED) and on each of the comparable shock-alone trials during the first day of the unpaired CS and UCS procedure (UNPAIRED).

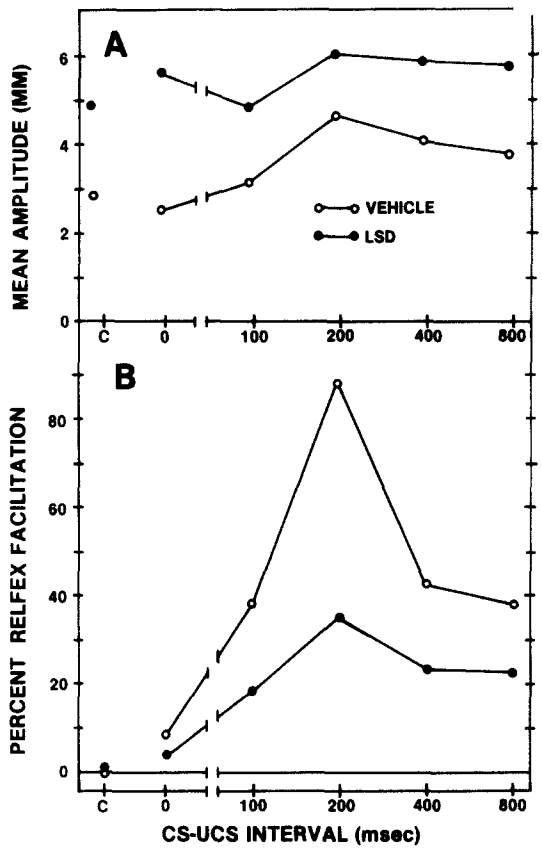

FIG. 2. Tone-induced facilitation of the nictitating membrane reflex during the first day of training as a function of CS-UCS interval. Panel A presents the mean amplitude of the UCR on UCS-alone trials (points above C) and on paired trials at the indicated CS-UCS intervals. Panel B presents the mean percentage change in the amplitude on the UCR on paired CS-UCS trials from the amplitude of the UCR on the UCS-alone trial. The points above C represent comparable calculations for animals in the unpaired CS and UCS procedure (see the Method section for exact details on data calculations).

the UCS-alone trials of the paired CS-UCS procedure (Experiment 1) and on the comparable UCS-alone trials of the unpaired procedure (Experiment 2) across each of the 6 blocks of trials during the first day of training. LSD significantly increased UCR amplitudes for both the unpaired and paired CS-UCS procedures as compared with vehicle controls ($p<0.005$). UCR amplitudes underwent significant habituation across blocks of trials ($p<0.001$). Habituation was more rapid for the LSD-injected animals than for controls as indicated by a significant drug × blocks interaction ($p<0.001$) for both the paired and unpaired procedures. For example, the UCR amplitude of vehicle controls in the paired CS-UCS procedure declined by 0.83 ± 0.43 mm from Block 1 to Block 6 while for LSD-injected animals the decline was 2.42 ± 0.41 mm.

UCR amplitudes of the LSD and vehicle groups during UCS-alone presentation in blocks 3, 4 and 5, from which reflex facilitation was calculated on the first day of conditioning, were also significantly different ($p<0.01$) from each other (see points above C in Fig. 2A). Moreover, the actual amplitudes of UCRs on the paired CS-UCS trials of blocks 3, 4 and 5 were consistently and significantly ($p<0.001$) greater for the LSD as compared with the vehicle groups across all of the CS-UCS intervals (Fig. 2A). However, this method of calculating the data failed to reveal a significant change in UCR amplitudes as a function of CS-UCS interval or an interval by drug interaction. Therefore, to obtain a better estimate of the shape of the reflex facilitation curve, the data were normalized by expressing the UCR amplitudes on paired trials as a percentage change from the UCR amplitude on the reference, UCS-alone trials (see the Method section). These calculations revealed orderly changes in reflex facilitation (Fig. 2B) that were a significant function of the CS-UCS interval ($p<0.001$). The points above the symbol C in Fig. 2B represent the equivalent calculations of percentage change in UCR amplitude for the unpaired CS and UCS procedure (see the Method section), the actual values being 0.1 and 0.7% for vehicle and LSD-injected animals, respectively. These low values confirm that the reflex facilitation obtained in the paired CS-UCS procedure was due to the temporal proximity of the CS to the UCS. The shape of the reflex facilitation curves were quite similar for vehicle and LSD-injected animals. Thus, at the 0-msec CS-UCS interval reflex facilitation in the vehicle and LSD groups was not significantly different from the values obtained during the unpaired procedure. For both groups reflex facilitation was present at the 100-msec interval, reached a maximum at the 200-msec interval and then declined at longer intervals. The analysis of variance also indicated that reflex facilitation was significantly less for the LSD as compared with the vehicle-injected animals ($p<0.01$) and that this difference was not a function of the CS-UCS interval as indicated by the absence of a significant drug × interval interaction. Thus, although animals injected with LSD demonstrated a larger UCR amplitude on paired trials (Fig. 2A), the percentage increase in UCR amplitude produced by the tone CS was significantly less than that of vehicle controls (Fig. 2B).

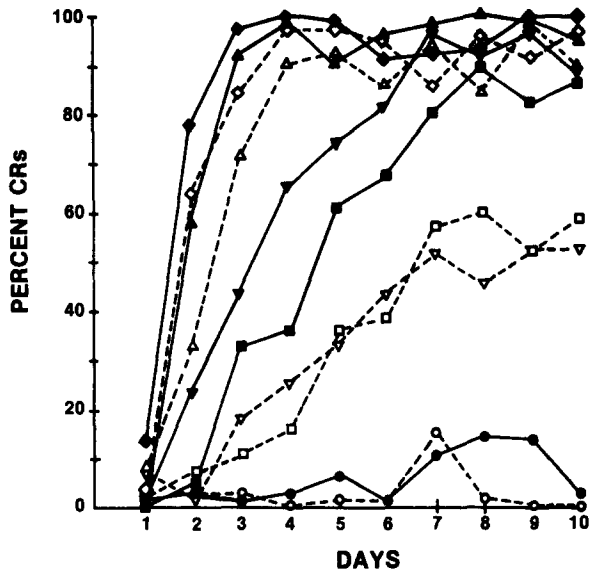

FIG. 3. Acquisition of CRs to the tone CS across 10 days of paired CS-UCS conditioning for animals injected with vehicle (open symbols connected by dashed lines) and LSD, 13 μg/kg (solid symbols connected by solid lines). Data are expressed as mean percentage CRs calculated for the six daily tone-alone test trials. CS-UCS intervals (in msec) are represented by the following symbols: 0, circle; 100, square; 200, diamond; 400, triangle; and 800, upside down triangle.

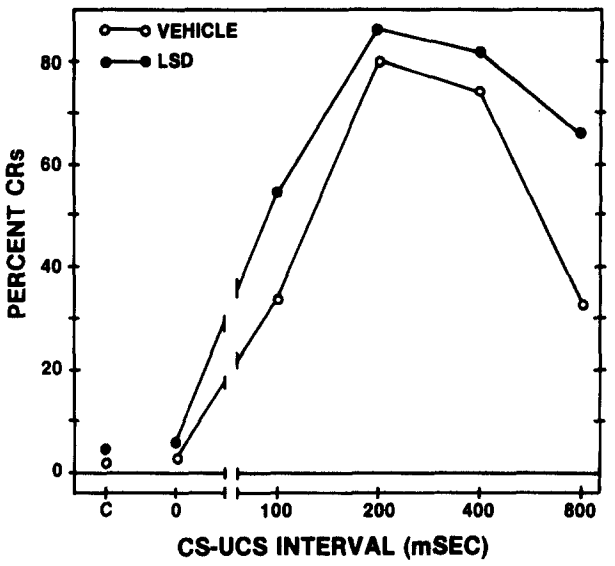

FIG. 4. Mean percentage of CRs, calculated across all 10 days of paired CS-UCS conditioning, as a function of CS-UCS interval for vehicle and LSD-injected animals. Data are based on tone-alone test trials. The points above C are the mean percentage responding during the comparable tone-alone trials of the unpaired CS and UCS procedure.

Experiments 1 and 2: Effect of LSD on CR Acquisition

The percent occurrence of CRs on the tone-alone (test) trials is presented in Fig. 3 for each of the 10 days of paired CS-UCS training of Experiment 1. There was a significant acquisition of CRs to the tone CS ($p<0.001$) across the 10 days of conditioning which was a function of CS-UCS interval ($p<0.001$). LSD significantly increased CR acquisition as compared with vehicle-injected controls ($p<0.001$) and this effect was also a function of CS-UCS interval ($p<0.05$). These relationships are more clearly seen in Fig. 4 which plots the mean percentage of CRs, occurring across all 10 days of conditioning, as a function of CS-UCS interval. Vehicle and LSD-injected animals trained at the 0-msec CS-UCS interval failed to demonstrate a significant acquisition of CRs across the 10 days of training. Also, the overall percentage of CRs for the vehicle (2.6%) and LSD (5.7%) groups of Experiment 1 was not significantly different from that of vehicle (1.7%) and LSD (4.7%) groups of Experiment 2 that received explicitly unpaired presentations of CS and UCS (Fig. 4, points above C). Acquisition of CRs by vehicle and LSD-injected animals was significant at all other CS-UCS intervals with maximum acquisition occurring at the 200-msec interval. The enhancement of CR acquisition produced by LSD occurred at each of these intervals but was most pronounced at the CS-UCS intervals of 100 and 800 msec (Fig. 4).

There was a high degree of similarity between the shape of the curves relating CS-UCS intervals to reflex facilitation (Fig. 2B) and to CR acquisition (Fig. 4). This is most clearly seen in Fig. 5 which plots the percent CRs of Fig. 4 as a function of the percent reflex facilitation of Fig. 2B. The Pearson product moment coefficient of correlation for these two variables was +0.86 for vehicle controls and +0.94 for LSD-injected animals ($p<0.01$ for each correlation). Since the 0-msec CS-UCS interval might have contributed disproportionately to these correlations (see Fig. 5), we examined the effects of eliminating that interval from our calculations. The resulting coefficients of correlation were reduced but still highly significant ($p<0.01$), the actual values for vehicle and LSD-injected animals being +0.72 and +0.79, respectively.

Experiment 3: Effects of LSD on UCS Theshold for Elicitation of UCRs

Figure 6 presents the percentage of UCRs (Panel A) and their amplitudes (Panel B) as a function of UCS intensity. Both the frequency and amplitudes of UCRs increased in a systematic manner as a function of increased intensity of the UCS ($p<0.001$ for each function). LSD significantly increased the percentage of UCRs ($p<0.005$) and the amplitude of the elicited UCRs ($p<0.01$) as compared with vehicle controls. Follow-up analyses of significant drug × UCS intensity interactions of UCR frequency ($p<0.001$) and amplitude ($p<0.01$) revealed that LSD-injected animals demonstrated a significantly higher percentage of UCRs at shock intensities of 0.5 and 0.75 mA ($p<0.01$; Fig. 6A) and higher UCR amplitudes from 0.5 to 2.0 mA. It should be noted that the low frequency of UCRs elicited by the 0.25 mA UCS (Fig. 6A) resulted in a more variable estimate of UCR amplitudes at this shock intensity (Fig. 6B). Since a response was defined as a 0.5 mm or greater extension of the nictitating membrane, UCR amplitudes could not fall below that value for vehicle controls even though the frequency of UCR occurrence was only 2%. Similarly, the low frequency of UCRs exhibited by the LSD group reduced the reliability of the amplitude measure at the 0.25 mA UCS. Consequently, the differences in UCR amplitudes between the LSD and vehicle groups obtained at 0.25 mA were not significant.

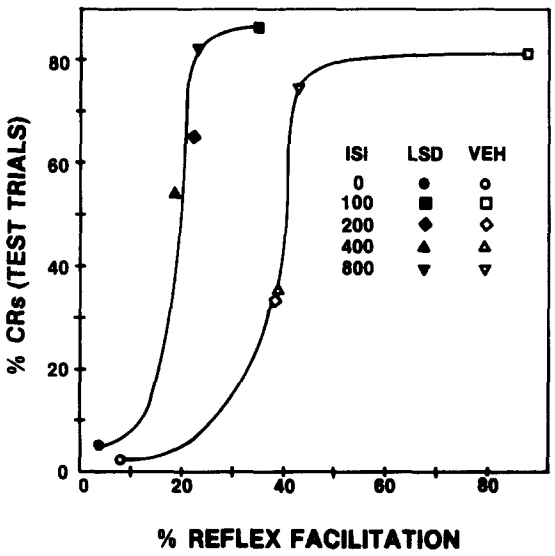

FIG. 5. Relationship between reflex facilitation and CR acquisition in animals injected with vehicle and LSD at the indicated CS-UCS intervals. Data are taken from Figs. 2B and 4.

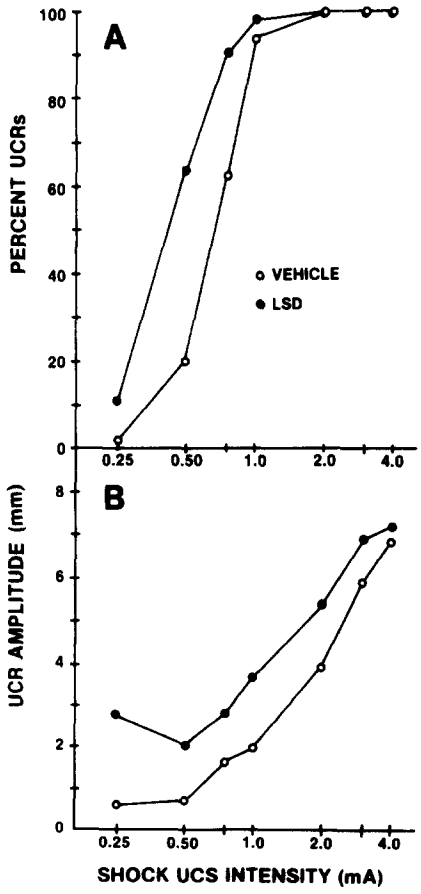

FIG. 6. Percentage UCRs (Panel A) and UCR amplitudes (Panel B) as a function of UCS intensities for vehicle and LSD-injected rabbits.

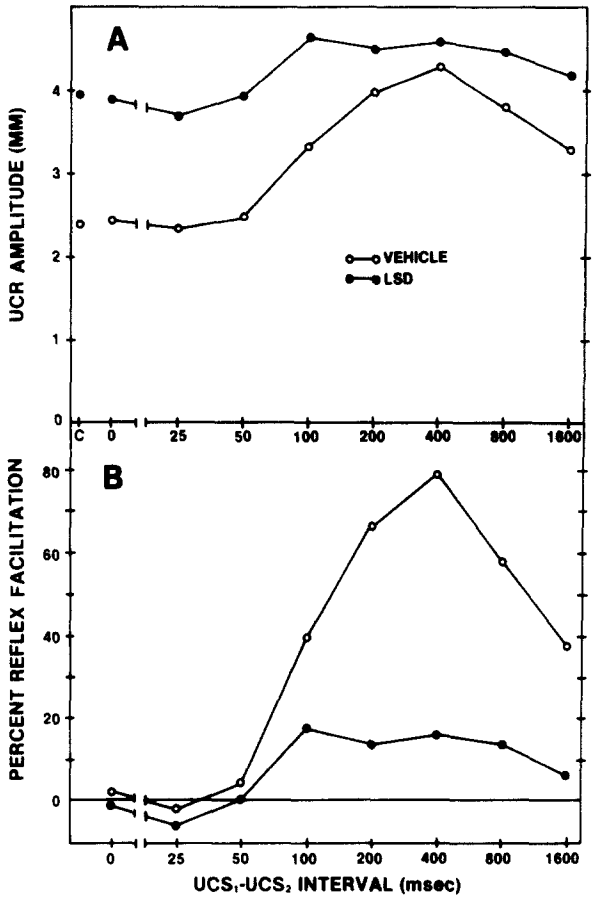

FIG. 7. Shock-induced facilitation of the nictitating membrane reflex during one day of testing. The UCS^1 (a 100-msec, 0.25 mA shock) preceded by the UCS^2 (a 100-msec, 1.5 mA shock) by the indicated UCS^1–UCS^2 intervals. Panel A presents the mean amplitude of the UCR on UCS^2-alone trials (points above C) and on the indicated paired UCS^1–UCS^2 intervals. Panel B presents the mean percentage change in the amplitude of the UCR on paired UCS^1–UCS^2 trials from the amplitude of the UCR on the UCS^2-alone trial.

Effect of LSD on Shock-Induced Facilitation of the Nictitating Membrane Reflex

Figure 7 presents the reflex facilitation produced during the pairing of a 0.25 mA shock (UCS^1) with a 1.50 mA shock (UCS^2) as a function of the UCS^1–UCS^2 interval. These data are presented as either the actual amplitudes of the UCR elicited by UCS^2 on the paired trials (Panel A) or as a percentage change in these UCR amplitudes from the amplitude of the UCR on UCS^2-alone trials (Panel B). In agreement with the data of Fig. 6B, LSD produced a significant increase in UCR amplitudes to the 1.50 mA shock on the UCS^2-alone trials (see points above UCS^2 in Fig. 7A). Also in agreement with the data of Fig. 6B, there were no significant differences between the UCR amplitudes of the LSD and vehicle groups on the 0.25 mA UCS^1-alone trials, the actual values being 1.8 and 1.4 mm, respectively. The amplitudes of the UCR on the paired UCS^1–UCS^2 trials were a significant function of interval ($p<0.05$) and drug condition ($p<0.01$). Follow-up analyses of a significant drug × interval interaction ($p<0.05$) indicated that the significant effects of LSD on UCR amplitude occurred at UCS^1–UCS^2 intervals of 0 to 100

msec. The shape of the reflex facilitation curve is more clearly revealed when data are expressed as a percentage change (Fig. 7B). Again there was a significant effect of interval ($p<0.001$). There was no significant reflex facilitation by the vehicle or LSD groups at the UCS^1–UCS^2 intervals of 0, 25 and 50 msec. For vehicle controls, reflex facilitation first occurs at 100 msec, increases to a maximum at 400 msec and then declines at longer intervals. Reflex facilitation for the LSD group is also first evident at the 100-msec interval but then remains flat across the remaining intervals. The magnitude of the reflex facilitation was significantly greater in vehicle controls ($p<0.05$). Follow-up analyses of a significant interval × drug interaction ($p<0.01$) indicated that the vehicle controls were significantly different from the LSD group at intervals of 200, 400 and 800 msec ($p<0.05$).

DISCUSSION

Control animals demonstrated an orderly change in reflex facilitation as a function of CS-UCS intervals that was quite similar to previous reports in the rabbit using delay and trace intervals in normal rabbits or vehicle controls [2, 12, 15, 16, 27, 30]. The relationship between CS-UCS interval and CR acquisition was also orderly and essentially identical with previous reports using normal rabbits [26] or vehicle controls [15]. In addition, there was a high correlation of +0.85 between the ability of a tone to produce facilitation of the nictitating membrane reflex at the different CS-UCS intervals and its ability as a CS to enter into associative learning at those intervals. The results obtained in control rabbits were in complete agreement with a previous study using the same experimental design that obtained a correlation of +0.86 between identical measures of reflex facilitation and CR acquisition [15]. These data support the view that reflex facilitation may be important in initiating the changes underlying learning [12, 15, 17, 27]. To further explore these relationships, we have also examined whether drugs that alter the rate of CR acquisition might do so by affecting reflex facilitation. In a previous study, the reduction in reflex facilitation produced by scopolamine (0.4 mg/kg) was found to be highly correlated (+0.95) with its ability to retard CR acquisition [15]. Since scopolamine had no effect on the amplitude of the UCR elicited by the shock-UCS, it could be concluded that the decrease in heterosynaptic reflex facilitation produced by scopolamine was solely due to a decrease in the unconditioned excitatory properties of the tone CS. The data obtained with LSD provide additional support for the view that the level of excitation produced within the reflex arc of the target response by the contiguous occurrence of the CS and UCS determines the rate of associative learning [8].

Previous studies employing the rabbit's nictitating membrane response have demonstrated that LSD (13 μg/kg) enhanced the acquisition of CRs to tone and light CSs under delay conditioning procedures employing an 800 msec CS whose offset coincided with the onset of a 100 msec air-puff or shock UCS [5, 7, 14, 24]. The present study demonstrated that LSD enhanced CR acquisition to a tone CS across the range of CS-UCS intervals but did so without altering the general shape of the function obtained for vehicle controls. For example, both LSD and vehicle-injected animals failed to acquire CRs at the 0-msec CS-UCS interval and demonstrated maximum CR acquisition at the 200-msec interval. However, analysis of the relationship between the enhancement of CR acquisition produced by LSD and its effects on reflex facilitation was complicated by the finding that LSD also increased the excitatory properties of the UCS on the nictitating membrane reflex.

The effect of LSD on the unconditioned reflex consisted of a decrease in the UCS threshold for eliciting UCRs and an increased amplitude of the elicited UCR. Thus, during both the paired CS-UCS and the unpaired stimulus procedures LSD enhanced the amplitude of the UCR on the UCS-alone trials from which the percent changes in UCR amplitude were used to calculate reflex facilitation. Consequently, the analysis of reflex facilitation faced the methodological problems inherent in any attempt to compare drug effects between groups of animals having unequal baselines [3,18]. For example, the actual UCR amplitudes on paired trials were significantly larger for animals injected with LSD across all of the CS-UCS intervals, suggesting that the LSD group was exhibiting a higher degree of excitability within the reflex arc as compared with vehicle controls. Although this outcome is consistent with the ability of LSD to enhance CR acquisition across those CS-UCS intervals the locus of the effect remains unclear. The larger UCR amplitudes demonstrated by the LSD group on paired trials were undoubtedly due to the increased excitatory effects of the shock-UCS but it was not possible to establish whether or not this effect was occurring along with an increase in the excitatory effects of the tone CS. Expressing the data on reflex facilitation as a percentage change in UCR amplitudes produced by the CS-UCS pairing did produce an orderly curve for reflex facilitation which had a high degree of correlation (+0.94) with CR acquisition. However, as might be expected from the difference in baseline UCRs, the percent reflex facilitation for the LSD group was less than that of vehicle controls. This outcome could have been due to a number of factors which are conventionally referred to as the law of initial values. For example, the nictitating membrane is attached to both the globe of the eye and the external eyelids by connective tissue that sets a physical limit to membrane extension [4]. This physical upper limit would have served as a ceiling effect so that the percentage increase in UCR amplitude that could be possibly obtained in the LSD group would be far less than that obtained from the lower baseline amplitudes of controls. We conclude, therefore, that the heterosynaptic reflex facilitation in animals injected with LSD indicated a higher degree of excitability within the reflex arc as compared with control animals but we cannot tell whether this increased excitability was due solely to the increased excitatory properties of the UCS or to both the UCS and CS. We attempted to gain further insight into the effects of LSD on the nictitating membrane reflex by examining monosynaptic reflex facilitation resulting from the pairing of two shock UCSs. Again, the consistently higher amplitudes of UCRs exhibited by the LSD-injected rabbits during UCS pairings suggested a greater excitability within the reflex arc as compared with controls. The conversion of these data to percentage changes again produced reliable evidence of monosynaptic reflex facilitation in control animals that had an onset and duration quite similar to that observed for tone-induced facilitation. Animals injected with LSD also demonstrated evidence of UCS-induced reflex facilitation but the percentage changes were again smaller than that of controls.

The data of the present study, in conjunction with previous findings, suggest that LSD enhances both the excitatory properties of the shock UCS and of a tone being used as the CS prior to the occurrence of learning. There are two lines

of evidence that suggest that LSD enhances CR acquisition by affecting the excitability of the tone CS as well as the shock UCS. In previously trained animals, LSD enhances the conditioned excitatory properties of the tone CS as measured by a 8 dB decrease in the intensity threshold of the CS for eliciting CRs [7,23]. This effect on the conditioned excitatory properties of the tone CS has also been demonstrated using Pavlovian conditioning of an appetitive CR, the rabbit jaw-movement response [9]. Secondly, LSD can enhance the acquisition of CRs without affecting the unconditioned reflex depending on the UCS employed. For example, LSD had no significant effect on the frequency or amplitude of UCRs elicited by an air-puff UCS but did significantly increase the rate of CR acquisition during paired presentations of a tone-CS and air-puff UCS [24]. Previous research indicates that the final common pathways for the unconditioned extension of the nictitating membrane are different for a shock and air-puff UCS. Thus, UCRs elicited by an air-puff UCS result primarily from eyeball retraction mediated by the VIth (abducens) nerve while those elicited by electric shock are mediated equally by the VIth nerve and by external eyelid closure via the VIIth (facial) nerve [20]. The potent effects of LSD on UCRs elicited by shock in the present study and the absence of effects on UCRs elicited by air-puff may be related to the fact that serotonin receptors, upon which LSD acts [19], are found in the facial but not in the abducens motor nucleus [21]. An action of LSD to ehance a defensive reflex to a painful stimulus through an action on serotonergic function would be consistent with what is known concerning serotonin and pain sensitivity [13].

In summary, the findings of this and previous studies suggest that increased CR acquisition to a shock UCS results from the increased excitatory effects of the tone and shock stimuli. The effects of this would be to considerably broaden the duration of reflex facilitation and, therefore, also extend the temporal span between CS and UCS onset across which LSD could produce greater rates of CR acquisition as compared with vehicle-injected controls. These conclusions have been supported by the recently published study of Siegel and Freedman [25] who examined the effects of LSD (35 or 85 μg/kg) on classical conditioning of the rabbit's eyeblink response. Using a discriminative trace conditioning procedure, they also found that LSD enhanced the excitatory properties of a tone CS and were able to demonstrate that this effect could be detected at CS-UCS intervals as long as 8000 msec.

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