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Effect of LSD on Acquisition, Maintenance, Extinction and Differentiation of Conditioned Responses

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SCHINDLER, C. W., I. GORMEZANO AND J. A. HARVEY. *Effect of LSD on acquisition, maintenance, extinction and differentiation of conditioned responses.* PHARMACOL BIOCHEM BEHAV **24**(5) 1293–1300, 1986.—Three experiments were conducted to compare the effects of LSD (30 nmol/kg) on the acquisition, maintenance, extinction and differentiation of the rabbit's classically conditioned nictitating membrane response. LSD significantly enhanced the acquisition of conditioned responses to tone and light conditioned stimuli as compared with vehicle injected controls (Experiments 1 and 2), but had no detectable effect on differential conditioning in Experiment 3. The conditioned responses acquired under LSD in Experiments 1 and 2 exhibited some unusual features in that: (1) they were more rapidly extinguished under continued injections of LSD; (2) they demonstrated a significant decrement when animals were switched from LSD to vehicle during maintenance; and (3) they were virtually eliminated when animals were switched from LSD to vehicle during extinction. In contrast, conditioned responses acquired under saline injections in Experiments 1 and 2 were not affected when animals were switched to LSD injections during either maintenance or extinction. These results of Experiments 1 and 2 were interpreted as indicating that LSD produces an asymmetrical state-dependent learning.

LSD Classical conditioning Rabbit Nictitating membrane Extinction Differentiation State-dependent learning

LSD (*d*-lysergic acid diethylamide) increases the rate of acquisition of classically conditioned responses (CRs) in the rabbit to both tone and light conditioned stimuli (CSs) at doses ranging from 1–100 nmol/kg, with maximal effects occurring at 30 nmol/kg [7]. The enhancement of CR acquisition produced by LSD (30 nmol/kg) has been demonstrated to be due to an effect of the drug on associative processes in both classical defense conditioning of the rabbit's nictitating membrane response (NMR) [7,9] and classical appetitive conditioning of the rabbit's jaw movement response (JMR) [10]. The enhanced acquisition of both conditioned NMRs and JMRs occurs with little or no effect on the ability of the unconditioned stimuli (UCSs) to elicit unconditioned responses (UCRs) but with a significant decrease in the intensity threshold of a tone CS for eliciting CRs [9, 10, 22].

The purpose of the present experiments was to extend our understanding of the effects of LSD (30 nmol/kg) on classical conditioning by comparing its effects on the acquisition of conditioned NMRs with its effects on maintenance, extinction and differentiation of conditioned responses. Three experiments were carried out. The first two experiments examined the effects of LSD on the occurrence of CRs under: (1) maintenance conditions, during which animals continued to receive CS-UCS pairings; and (2) extinction conditions, during which only the CS was presented. In both Experiments 1 and 2, controls for state-dependent learning were used. Evidence for state-dependent learning would be an indication that performance of those CRs acquired under one drug state (i.e., LSD or vehicle), depended on the reinstatement of that same drug state. Thus, in Experiments 1 and 2, drug state was either maintained or switched between the acquisition and maintenance or extinction training conditions. In Experiment 3 we examined the effects of LSD on differential conditioning during which one stimulus (the CS+) is paired with the UCS while another stimulus (the CS−) is not.

EXPERIMENT 1

The purpose of this experiment was to examine the ef-

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fects of LSD (30 nmol/kg) on the maintenance of conditioned responding. Rabbits were given classical conditioning training for 10 days such that conditioned responding had reached asymptotic levels. These 10 days were the acquisition phase. Immediately following acquisition the rabbits were trained for an additional 4 days which were the maintenance phase. To control for state-dependent effects, the standard 2×2 design described by Overton [17] was used. Four groups were thus obtained such that the acquisition-maintenance drug conditions were: Control-Control, LSD-Control, Control-LSD, and LSD-LSD.

METHOD

Subjects

Twenty-four experimentally naive male and female rabbits (New Zealand albino) obtained from local suppliers, weighing approximately 2.2 kg on arrival, were housed individually with free access to food and water.

Apparatus and General Procedure

The apparatus and procedures used in conditioning the rabbit NMR have been described in detail [8, 11, 12]. In brief, each rabbit was placed in a Plexiglas restrainer and fitted with a headmount that supported a transducer for recording the NMR by physically coupling with a length of thread to a loop of nylon sutured into the right nictitating membrane. The rabbits were then positioned in ventilated, sound-attenuated chambers containing an 11.4-cm speaker positioned above and in front of the rabbit for delivery of tone CSs, and two 6-W, 24-V DC houselights, one mounted on each side of the speaker, for delivery of the light CS. The unconditioned stimulus (UCS) was electric shock delivered to the skin through two stainless-steel clips (Autoclip), located 10 mm posterior and 7.5 mm above and below the canthus of the right eye. 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. Analog-to-digital conversion, response analysis and experimental control were all accomplished by an Apple II/FIRST operating system [19].

Drug

LSD (*d*-lysergic acid diethylamide tartrate, MW 430.5), obtained from NIDA, was dissolved in sterile, distilled, water. The drug solution or water vehicle were injected into the marginal ear vein of the rabbit by means of 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 was 30 nmol/kg (12.9 μg/kg as the salt).

Procedure

Rabbits received one 60-min adaptation session during which no stimuli were presented, however, in order to obtain a measure of baseline responding, NMRs were recorded at observation intervals employed during training. At no time did percent responding during adaptation exceed 5%. One day after adaptation all rabbits entered the acquisition phase consisting of 10 daily conditioning sessions. Each daily session consisted of 60 trials composed of 30 pairings of a tone CS with a shock UCS and 30 pairings of a light CS with the shock UCS. The offset of the 800-msec tone CS (1000 Hz, 75 dB, SPL) or 800-msec light CS (10-Hz flash of the houselights) occurred simultaneously with the onset of the 100-msec shock UCS (60-Hz, 3-mA). Trials were presented with an average intertrial interval of 60 sec (range 50–70 sec) with the restriction that not more than 3 tone or light trials could be presented consecutively. 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 period and a UCR if it occurred after shock onset. During the first 10 days of training, one group of rabbits (n=12) was injected with LSD (30 nmol/kg) and a second group of rabbits (n=12) was injected with the sterile water vehicle 20–30 min prior to each session. On the day after the 10th acquisition session, all rabbits entered the maintenance phase of this experiment. During the four daily sessions of the maintenance phase the two groups of rabbits were further divided such that 6 rabbits injected with LSD during acquisition continued to be injected with LSD during maintenance (group LSD-LSD) and 6 were injected with vehicle (LSD-Control). Six of the rabbits injected with vehicle during acquisition continued to be injected with vehicle during maintenance (Control-Control) and 6 were injected with LSD (Control-LSD). All rabbits then received four days of continued training identical to that of the first 10 days.

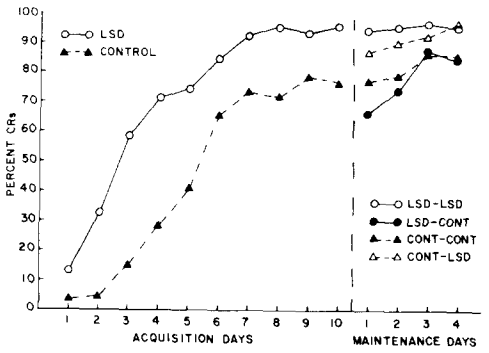

FIG. 1. Effects of LSD (30 nmol/kg) on acquisition and maintenance of CRs to tone and light CSs in Experiment 1. Results are presented as percent CRs, irrespective of CS modality, on each of the 10 days of the acquisition phase (left panel) and on each of the 4 days of the maintenance phase (right panel). Each point during the acquisition phase represents the mean of 12 rabbits receiving LSD and 12 control rabbits receiving vehicle. Each point during the maintenance phase represents the mean of 6 rabbits receiving either LSD or its vehicle (CONT) as indicated.

Data Analysis

A repeated measures analysis of variance was performed on the data with follow-up analysis to localize significant sources of variation carried out by the method of Dunnett [16]. The significance level was set at $p<0.05$, two tailed.

RESULTS AND DISCUSSION

During the 10 days of the acquisition phase, rabbits receiving LSD (30 nmol/kg) demonstrated an enhanced acquisition of CRs to both tone and light CSs combined as compared with vehicle injected controls (Fig. 1, left panel). This enhanced acquisition was reflected in significant effects of LSD on percent CRs, $F(1,22)=18.8$, $p<0.001$. Rabbits did not demonstrate differences in CR acquisition as a function of CS modality nor did LSD produce differential enhance-

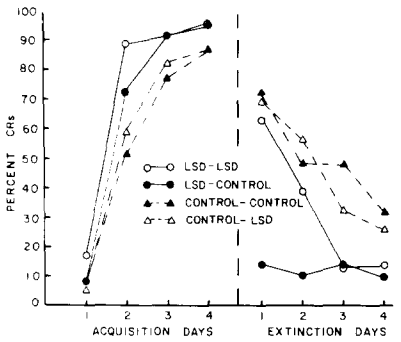

FIG. 2. Effects of LSD on acquisition (left panel) and extinction (right panel) of CRs to a tone CS in Experiment 2. Each point represents the mean of 12 rabbits. Drug dose and conditions were identical to Fig. 1.

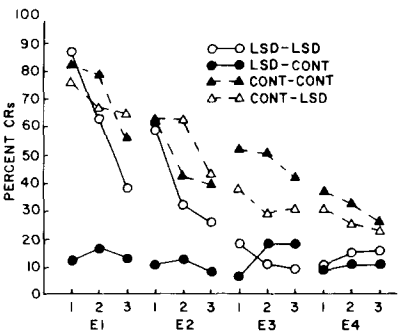

FIG. 3. Effects of LSD on Extinction of CRs in Experiment 2. The data from the right panel of Fig. 2 are presented as a function of 3, 20 trial blocks per day.

ment of CR acquisition to the tone and light CSs as reflected by the absence of any significant effect of modality or of a modality × drug interaction. To further assess the effects of LSD on 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. LSD significantly decreased the number of trials required to reach this criterion of CR acquisition, $F(1,22)=11.5$, $p<0.01$.

Only group LSD-Control showed any dramatic or significant change in percent CRs from the acquisition to the maintenance phase (Fig. 1, right panel). Percent CRs for group LSD-Control dropped 25.2% from day 10 under LSD to day 1 under vehicle, $F(3,20)=5.6$, $p<0.01$. Percent CRs for the other 3 groups showed only small changes from day 10 of acquisition to day 1 of maintenance, with the actual values in terms of day 10 to day 1 differences being: Control-Control, +5.5%; Control-LSD, +6.1%; and LSD-LSD, −4.6%. The decrease in responding occurring when rabbits were switched from LSD during acquisition to vehicle during maintenance (the LSD-Control group) was evident during the first few trials of day 1. For example, percent CRs for group LSD-Control were 92.8%, 95.0% and 87.5% for each of the three blocks of 20 trials on day 10 of acquisition. In contrast, during day 1 of maintenance the percent CRs for these three 20-trial blocks were 68.0%, 62.6% and 69.2%. During days 1–4 of maintenance, groups Control-Control and LSD-LSD maintained a level of responding similar to that achieved by day 10 of acquisition, while percent CRs for group Control-LSD increased to the level of group LSD-LSD and group LSD-Control recovered to a level comparable to group Control-Control.

The results of Experiment 1 failed to indicate any effect of LSD on maintenance since the critical group, Control-LSD, did not demonstrate a large or significant change in percent CRs when switched from injections of vehicle during acquisition to injections of LSD during maintenance. However, the large and immediate decrement in percent CRs demonstrated by rabbits in the LSD-Control group did suggest a possible state-dependent learning under LSD that did not transfer to the vehicle state. The absence of an equivalent effect in the Control-LSD group suggests that the state-dependent effect was asymmetric as defined by Overton [17].

EXPERIMENT 2

The purpose of Experiment 2 was to determine the effects of LSD (30 nmol/kg) on the occurrence of CRs during extinction conditions. Rabbits were given acquisition training under a shorter CS-UCS interval and with only one CS modality (auditory) in order to increase the rate of acquisition and final asymptotic performance of CRs. Therefore, acquisition was carried out over 4 daily conditioning sessions and this was followed by 4 days of extinction during which only the CS was presented. To again control for state dependent effects, four groups representing the drug conditions identical to those in Experiment 1 were used.

METHOD

Subjects

Forty-eight experimentally naive rabbits of either sex were obtained and housed as described for Experiment 1.

Procedure

Except as noted below, all apparatus and methods were as described for Experiment 1. One day after a 66-min adaptation session, carried out as described in Experiment 1, all rabbits were exposed to four daily conditioning sessions. Each 66-min session consisted of 66 trials divided into 6 blocks of 11 trials each. The first 10 trials within each block consisted of the pairing of a tone CS with a shock UCS while the 11th trial was always the test trial during which only the tone CS was presented. On paired trials the offset of the 200-msec tone CS (1000 Hz; 84 dB, SPL) occurred simultaneously with the onset of the 100-msec shock UCS (60-Hz, 3-mA). 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 200-msec tone CS on the 60 daily paired trials and as a UCR if it occured after shock onset. On the 6 daily test trials, responses were scored as CRs if they occurred within 800 msec of CS onset.

On the day after the last (4th) acquisition day animals were exposed to 4 daily sessions of extinction. Each daily, 60-min, extinction session consisted of 60 tone-alone trials. The 60 trials were divided into 6 blocks of 10 trials each. Within each block of 10 trials the first 9 trials were scored differently from the tenth. Thus, a response was defined as a CR if it occurred within 200 msec of tone onset for the first 9 trials of each block (to be comparable to scoring procedures employed during CS-UCS pairing in acquisition) or within 800 msec of tone onset on the 10th trial of each block (to be

comparable to scoring procedures employed during CS alone, test, trials during acquisition). For both acquisition and extinction the intertrial interval was randomly generated with a mean of 60 sec (range 50–70 sec). During acquisition, 24 rabbits were injected with LSD (30 nmol/kg) 20–30 min prior to each session and 24 rabbits were injected with sterile water. In extinction, 12 of those rabbits injected with LSD during acquisition continued to be injected with LSD during extinction (group LSD-LSD) and 12 were injected with vehicle (LSD-Control). Twelve of those rabbits injected with vehicle during acquisition continued to be injected with vehicle during extinction (Control-Control) and 12 were injected with LSD (Control-LSD). Rabbits in the LSD-LSD and LSD-Control groups were matched on the bases of percent CRs during acquisition prior to extinction as were rabbits in the Control-Control and Control-LSD groups.

RESULTS AND DISCUSSION

During acquisition rabbits injected with 30 nmol/kg LSD acquired CRs earlier and reached a higher asymptotic level of responding than rabbits injected with vehicle (Fig. 2, left panel). This enhanced acquisition of CRs was reflected in significant effects of LSD on percent CRs, $F(1,43)=7.2$, $p<0.01$, latency of the NMR, $F(1,43)=6.8$, $p<0.01$, and trials to reach a criterion of 10 consecutive CRs, $F(1,43)=7.0$, $p<0.02$. An analysis of test trials also revealed significant effects of LSD on percent CRs, $F(1,43)=4.8$, $p<0.05$, and NMR latency, $F(1,43)=7.0$, $p<0.05$. In contrast, the analysis revealed no significant effects of the extinction drug conditions on acquisition ($ps>0.24$), indicating the successful matching of groups prior to the beginning of extinction.

Rabbits in the Control-LSD group that had received vehicle injections during acquisition and were then switched to LSD injections during extinction did not differ significantly in percent CRs across the 4 days of extinction as compared with the Control-Control group (Fig. 2, right panel). Responding for both groups remained at a high level of approximately 70% on day 1 of extinction and decreased to a level of approximately 30% by day 4. In contrast, animals trained under LSD during acquisition and then either continued on LSD or switched to vehicle during extinction demonstrated significantly fewer CRs across the four days of extinction as compared with the Control-Control group. For example, group LSD-LSD demonstrated a more rapid rate of extinction than group Control-Control, despite the fact that percent CRs for group LSD-LSD were higher at the end of acquisition. Further, when rabbits injected with LSD in acquisition were injected with vehicle during extinction (group LSD-Control), extinction responding dropped dramatically to only 13.7% on day 1 and remained at approximately that level for all four extinction days. The low level of responding during extinction for group LSD-Control does not appear to reflect a rapid rate of extinction as percent responding in the first 20-trial block of extinction was 12.0% (Fig. 3) and did not decrease for the final 2 blocks of day 1 (16.8% and 13.3% respectively). A modest amount of spontaneous recovery was observed for group LSD-LSD on day 2 of extinction and on days 2 and 3 for group Control-Control (Fig. 3). Group Control-LSD did not demonstrate spontaneous recovery on any of the extinction days and group LSD-Control remained at a constant low level of responding through each of the 20-trial blocks on all four days of extinction. These differences were confirmed statistically, $F(3,129)=3.7$, $p<0.05$, with follow-up tests ($p<0.05$) revealing that percent CRs for group LSD-Control were lower than group Control-Control on days 1, 2 and 3 of extinction while group LSD-LSD was lower than group Control-Control on day 3. Group Control-LSD did not differ from group Control-Control on any of the four days. The analysis by 20-trial blocks revealed similar patterns of results, $F(18,258)=2.5$, $p<0.001$, with group LSD-Control differing from group Control-Control on all but the third block of Extinction day 4. Group LSD-LSD differed from group Control-Control for all three blocks of day 3 and for the first block of day 4. Finally, group Control-LSD never differed from group Control-Control on any block. An analysis based on test trial data from extinction revealed an identical pattern of results.

These results confirm the conclusions reached for Experiment 1 that LSD produces an asymmetric state-dependent learning in which acquisition of CRs under LSD does not transfer to the non-drug state. These results also confirm the findings of Experiment 1 that LSD does not produce a significant change in the occurrence of CRs since animals switched from saline to LSD (group Control-LSD) demonstrated no significant or consistent difference in percent CRs during extinction as compared with group Control-Control. The large and significant enhancement in CR acquisition produced by LSD in Experiments 1 and 2 in the absence of any detectable effect on the occurrence of CRs during maintenance or extinction led us to examine the effects of LSD on differential conditioning.

EXPERIMENT 3

The purpose of the third experiment was to determine the effects of LSD on the acquisition of differentiation. Three different differentiation procedures were explored. For Experiments 3a and 3b a two-stage procedure was used. During stage 1 both the stimuli to be used as CS+ and CS− in stage 2 were paired with the shock UCS. In stage 2, CS+ continued to be paired with the UCS, while CS− was presented alone. For Experiment 3a a tone-light differentiation procedure was used and in Experiment 3b differentiation was established to tones of different frequencies. For Experiment 3c a one-stage procedure was used in which differentiation was established to tones of different frequencies without any prior training.

EXPERIMENT 3a

Subjects

Forty-four experimentally naive rabbits of either sex were obtained and housed as described for Experiment 1.

Procedure

This experiment was carried out in two stages. For stage 1, rabbits were exposed to a 60-min adaptation session followed by 10 daily acquisition sessions consisting of tone and light CSs paired with a shock UCS exactly as described for Experiment 1, except that there were no injections of drug or vehicle during this time. The day after the 10th acquisition session, all rabbits were exposed to stage 2, consisting of 10 days of differentiation training. During differentiation each daily session continued to consist of 60 trials, however for 30 of these trials one CS (the CS+) continued to be paired with the shock UCS, while for the other 30 trials the other CS (the CS−) was presented alone, i.e., not paired with the shock UCS. The CS+ and CS− were counterbalanced so that for

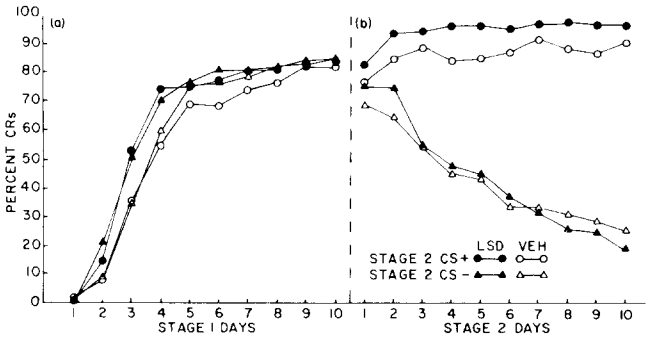

FIG. 4. Effects of LSD (30 nmol/kg) on tone-light differentiation. During stage 1 (panel a), both the tone and light CS were followed by the shock UCS and there were no drug injections prior to the 10 daily sessions. In stage 2 (panel b) rabbits were injected with either LSD or its vehicle prior to each of the 10 daily sessions during which one CS continued to be paired with shock (CS+) while the other CS was not (CS−). The modality of the CS+ and CS− was counterbalanced. Each point is the mean of 12 rabbits.

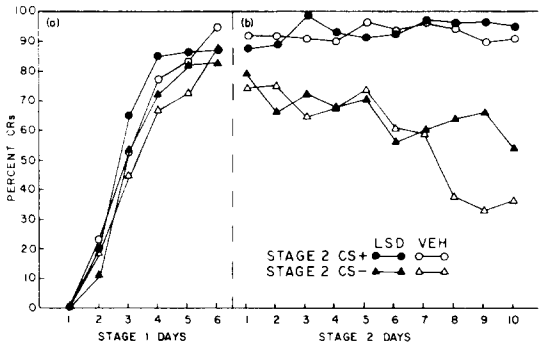

FIG. 5. Effects of LSD (30 nmol/kg) on tone-tone differentiation. During stage 1, both the 1000- and 5000-Hz tone CS were followed by the shock UCS. There were no injections of drug or its vehicle prior to the 10 conditioning sessions of stage 1. In stage 2, rabbits were injected with LSD or vehicle prior to each of the 10 daily sessions during which the 1000-Hz tone continued to be followed by shock (CS+), while the 5000-Hz tone was not (CS−). Each point is the mean of 9 rabbits.

two groups (n=12 per group) the tone was CS+ while for two other groups (n=10 per group) the light was CS+. One group of rabbits in each training condition was injected with LSD (30 nmol/kg) and the other group was injected with vehicle. Injection of drug or vehicle occurred 20–30 min prior to each of the 10 daily differentiation sessions. For both CS+ and CS− a CR was defined as a response occurring during the 800-msec CS.

RESULTS AND DISCUSSION

During stage 1 acquisition (Fig. 4, panel a) percent CRs increased comparably for both the tone and light stimuli. Furthermore, an analysis using stage 2 groups (light CS+ and tone CS+, stage 2 stimuli (CS+ and CS−) and stage 2 drug condition (LSD and vehicle) as dummy variables failed to indicate any significant effect of these variables ($ps>0.025$).

During stage 2 differentiation (Fig. 4, panel b), responding to CS− decreased steadily over training for both the LSD and vehicle injected groups, $F(9,360)=52.6$, $p<0.001$, reaching a level of approximately 20% by day 10. There were no consistent or significant effects of LSD on percent responding to the CS− across the 10 days of differentiation as compared with the vehicle controls. Responding to CS+ remained high and showed little change across the 10 days of differentiation training for both the LSD and vehicle control groups. Although, the mean percent responding to the CS+ by the LSD group was consistently higher than that of controls, this difference failed to reach significance, $F(9,360)=0.8$, $p>0.05$.

The analysis in terms of percent CRs gives an indication of the effect of LSD on CS+ and CS− alone, but does not give a clear indication of the rabbit's ability to differentiate the two stimuli. As an index of the rabbit's ability to differentiate CS+ from CS− a difference score based on percent CRs to CS+ and CS− was calculated for each rabbit across the 10 days of differentiation. There was no significant effect of LSD on this measure of differentiation.

EXPERIMENT 3b

Subjects

Eighteen experimentally naive rabbits of either sex were obtained and housed as described for Experiment 1.

Procedure

Experiment 3b employed the same methods and procedures as Experiment 3a with only the following exceptions. Rabbits received only 6 days of acquisition training. Each daily session consisted of 30 pairing of a 1000-Hz tone-CS (75 dB, SPL, 600 msec) and 30 pairings of a 5000-Hz tone-CS (75 dB, SPL, 600 msec) with a 100-msec shock UCS (60-Hz, 3-mA). No drug or vehicle was injected prior to these 6 acquisition sessions. Preliminary studies (data not presented) indicated that differentiation would not develop with the 5000-Hz tone as CS+, therefore differentiation training consisted of 30 1000-Hz tone CS+ and 30 5000-Hz tone CS− trials per day. Differentiation training began immediately following acquisition training, and 20–30 min prior to each session half the rabbits were injected with 30 nmol/kg LSD (n=9) and half were injected with vehicle (n=9). Differentiation training lasted 10 days. Restrictions on stimulus presentation order and the intertrial interval were identical to Experiment 3a. A CR was defined as a response occurring during the 600-msec CS.

RESULTS AND DISCUSSION

During stage 1 acquisition (Fig. 5, panel a) percent CRs increased consistently more rapidly for the 1000-Hz tone (Stage 2 CS+) than for the 5000-Hz tone, $F(5,80)=54.8$, $p<0.01$. However, this effect of tone frequency on acquisition did not interact with the dummy variable of stage 2 drug condition ($p>0.5$). During stage 2 differentiation (Fig. 5, panel b), responding to CS+ (1000 Hz) remained high over the 10 days with no apparent effect of LSD on responding. Responding to CS− decreased more slowly than for the tone-light differentiation procedure (Experiment 3a); how-

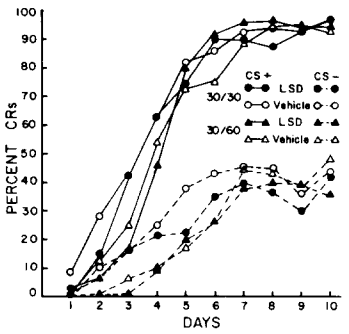

FIG. 6. Effect of LSD (30 nmol/kg) on one-stage tone-tone differentiation. The CS+ (a 1000-Hz tone) was paired with the shock UCS while the CS− (a 5000-Hz tone) was not. For half the rabbits there were 30 CS+ trials and 30 CS− trials per day (30/30) while for the other half there were 30 CS+ trials per day and 60 CS− trial per day (30/60). Rabbits were injected with either LSD or its vehicle prior to each session. Each point is the mean of 12 rabbits.

ever, LSD did appear to slow the development of differentiation in the last 3 days of stage 2. A significant effect of drug condition was observed on averaged responding to CS+ and CS−, $F(9,144)=2.0$, $p<0.05$, with the control group responding at a lower level on days 8 and 9 of stage 2 ($p<0.05$). Again the difference between percent responding to CS+ and CS− was calculated for each rabbit to determine if LSD was affecting differentiation in a way not reflected in percent CRs. The result of this analysis failed to reveal any significant effect of LSD for any of the 10 days of stage 2 ($p=0.09$).

EXPERIMENT 3c

Subjects

Forty-eight experimentally naive rabbits of either sex were obtained and housed as described for Experiment 1.

Procedure

Following adaptation all the rabbits were given 10 days of differentiation training. For two groups of rabbits (ns=12) differentiation training consisted of 30 1000-Hz tone-CS+ (75 dB, SPL; 800 msec) and 30 5000-Hz tone-CS− (75 dB, SPL; 800 msec) trials per day for 10 days (groups 30/30). For two other groups of rabbits (ns=12) there were 30 CS+ and 60 CS− trials per day for 10 days (groups 30/60). In all cases the offset of the 800 msec CS+ was paired with the onset of the 100-msec shock UCS (60-Hz, 3-mA). Restrictions on trial presentation order were identical to Experiment 3a. For groups 30/30 the average intertrial interval was 60 sec (range 50–70 sec). To maintain a constant session time, for groups 30/60 the average intertrial interval was 40 sec (range 30–50 sec). Response definition was identical to Experiment 3a. One group of rabbits in each training condition was injected with 30 nmol/kg LSD 20–30 min prior to each session and one group was injected with vehicle.

RESULTS AND DISCUSSION

Percent CRs to CS+ increased rapidly for both groups 30/30 and 30/60, with percent CRs increasing slightly more rapidly for groups 30/30 (Fig. 6). Percent CRs to CS− also increased over days for both the 30/30 and 30/60 groups, and again percent CRs for groups 30/30 increased slightly more rapidly than for groups 30/60. Overall, percent CRs for groups 30/30 were higher than for groups 30/60, $F(9,396)=1.9$, $p<0.05$, on days 2, 3, 4 and 6 ($p<0.05$). For neither group 30/30 nor group 30/60 did LSD have a significant effect on responding to either CS+ or CS− ($p>0.5$). The analysis of CS+/CS− difference also failed to reveal any significant effect of LSD. In addition, this analysis also indicated that differentiation was not affected by the number of CS− trials as the analysis failed to reveal any significant effect of number of CS− trials ($p>0.5$).

GENERAL DISCUSSION

The results of Experiments 1 and 2 were in agreement with those of previous studies demonstrating that LSD (30 nmol/kg) significantly enhanced the acquisition of conditioned NMRs as compared with vehicle injected controls [7, 9, 13, 22]. Although the same dose of LSD had no effect on the occurrence of CRs during the first day of maintenance (Experiment 1), the gradual increase in percent CRs across the last 3 days of the maintenance condition demonstrated by the Control-LSD group as compared with the Control-Control group suggests that LSD was still able to produce a detectable enhancement of CR acquisition. Experiment 2 indicated that LSD also had no effect on the occurrence of CRs during extinction as revealed by the absence of any difference between group Control-LSD and group Control-Control in percent CRs during either the first day of extinction or on the subsequent rate of extinction.

The finding that LSD can enhance CR acquisition without affecting the rate of extinction might be viewed as contradictory by those who consider extinction to be a form of learning. It should be noted, therefore, that extinction of the classically conditioned NMR would not appear to involve any new learning, since the CS undergoing extinction does not acquire any conditioned inhibitory properties [11]. The results of Experiments 1 and 2 are, therefore, consistent with the observations of a number of investigators who have noted that drugs have a greater effect on the acquisition of new responses than on the occurrence of established responses and that increased training decreases the ability of a drug to disrupt established behavior [1, 15, 27].

In Experiment 3, LSD had no effect on the acquisition of differentiation. Unfortunately, there are no previous reports of the effects of LSD on differentiation of classically conditioned CRs with which these results could be compared, and although there are a number of studies dealing with the effects of LSD on the performance of previously established discriminations using operant methodology, the results have been contradictory. For example, some investigators have observed improved discriminations [2,3], while others report either no effect or disruption of performance [5, 6, 24, 25, 26].

A possible reason for our inability to detect any effects of LSD on the acquisition of differentiation may lie in the difficulty of the discrimination and the dose of drug employed. As noted by Dews [4], doses of drugs that have little effect on the performance of simple discriminations can produce profound deficits in more complex (i.e., conditional) discriminations. For example, Sharpe *et al.* [24] found that LSD (10–40 μg/kg) had no effect on what they described as an easy size discrimination in monkeys while hindering a difficult discrimination. A similar finding has been reported for the rabbit NMR where the acquisition of differentiation was measured under conditions similar to those of Experiment 3c

[18]. Using what was described as an easy (700 vs. 1900 Hz tones) and a hard (700 vs. 1300 Hz tones) discrimination, Peel and Yehle [18] reported that *d*-amphetamine had no effect on acquisition of the easy discrimination while enhancing acquisition of the difficult discrimination.

The findings that LSD had no significant or detectable effect on the elicitation of conditioned NMRs by tone and light CSs during maintenance or extinction in Experiments 1 and 2 and no effect on the acquisition of differentiation in Experiment 3 were not in complete agreement with previous suggestions that LSD enhances CR acquisition by enhancing the excitatory properties of the CS in a manner analogous to an increase in its nominal intensity [9,23]. For the NMR of control rabbits, increases in the nominal intensity of a CS have been demonstrated to produce an immediate increase in percent CRs under maintenance or extinction procedures [20]. The failure to observe such an immediate effect of LSD in Experiments 1 and 2 suggests that while LSD may increase the excitatory properties of the CS it does not do so in a manner analogous to simply increasing the intensive properties of a stimulus in control rabbits.

Rabbits switched from LSD to vehicle in Experiments 1 and 2 demonstrated a significant decrease in the occurrence of CRs during the first day of the maintenance condition and a virtual elimination of CRs across all days of the extinction condition. This finding indicates that what was acquired under LSD was not retained in the non-drug state, i.e., that learning under LSD was state dependent [17]. However, if state-dependent learning did occur in Experiments 1 and 2, it was asymmetrical since groups Control-LSD showed no evidence of disrupted responding on the first day of the maintenance condition and no difference from group Control-Control during extinction. The precise basis for asymmetrical state-dependent learning is not understood but has been suggested to possibly reflect multiple drug effects [17]. For example, animals in the LSD-LSD condition also demonstrated a more rapid rate of extinction than did the Control-LSD or Control-Control groups, suggesting that what is learned under LSD is less resistant to extinction than what is learned under placebo. This effect might be expected to be additive with any state-dependent effects of LSD. Other investigators have reported that LSD can function as a discriminative stimulus [14,21] but there have been no previous reports of state-dependent learning under LSD. Indeed, state-dependent learning has not been observed during classical conditioning studies with other drugs, including scopolamine, haloperidol and morphine [23].

In summary, although LSD enhances the acquisition of CRs during classical conditioning, these CRs are less resistant to extinction and not retained in the non-drug state. In addition, LSD has little effect on maintenance or extinction of CRs and no detectable effect on the acquisition of a simple discrimination.

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