Pharmacology Biochemistry & Behavior, Vol. 30, pp. 427–431. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Effects of LSD-25 on Classical Trace Conditioning

SHEPARD SIEGEL

Department of Psychology, McMaster University, Hamilton, Ontario

AND

DANIEL X. FREEDMAN[1]

Department of Psychiatry & Biobehavioural Sciences, Neuropsychiatric Institute UCLA School of Medicine, Los Angeles, CA

Received 4 December 1987

SIEGEL, S. AND D. X. FREEDMAN. *Effects of LSD-25 on classical trace conditioning.* PHARMACOL BIOCHEM BEHAV **30**(2) 427–431, 1988.—This study examined the effects of LSD-25 on the excitatory properties of auditory conditioned stimuli as a function of the interstimulus interval. The rabbit's eyeblink response was conditioned using a discriminative trace procedure by the pairing of a 500-msec auditory conditioned stimulus with a 100-msec shock unconditioned stimulus at intervals of 1000, 2000, 4000 and 8000 msec. Animals were able to acquire conditioned responses across all intervals. They then received doses of 35 or 85 μg/kg of LSD-25 prior to additional conditioning sessions. LSD-25 produced an increase in the magnitude of conditioned responses to both the positive and negative conditioned stimuli at all interstimulus intervals. It was concluded that LSD did not alter discriminative conditioning but rather enhanced the excitatory properties of both positive and negative conditioned stimuli.

LSD-25 Classical trace conditioning

THIS investigation was a preliminary attempt to determine the effects of d-lysergic acid diethylamine (LSD-25) on conditioned responses. This study was undertaken at Yale in 1965 and the report prepared in 1966, at a time when each of the authors moved to other locales—a distraction that preempted the steps necessary for final publication. Because the data are deemed relevant to ongoing inquiries, we now repair that lapse.

A classical trace conditioning paradigm [15] was employed, in which the conditioned stimulus (CS) terminates before the onset of the unconditioned stimulus (US). This procedure seems to be especially appropriate for systematically investigating reports of drug-induced heightened perceptual sensitivity (e.g., [1,14]). Specifically, the use of an auditory trace CS might enable evaluation of introspective reports of drug-induced hyperacusia (e.g., [18]). Inasmuch as the anticipatory conditioned response (CR) is based on the *trace* of the CS (rather than its actual coincidental application with the US), it might be expected that with LSD a subject should demonstrate an ability to condition effectively with *longer* traces, i.e., a longer CS-US interval.

One problem inherent in using conditioning techniques to investigate drug effects on the reception of stimuli is that of motor side effects such as hind limb ataxia with LSD (e.g., [11]). This can be obviated in the trace conditioning paradigm by utilizing a response measure not directly affected by drugs. The usual autonomic responses (e.g., heart rate, GSR) studied in the classical conditioning situation also seem to be affected by LSD-25 [3,19]. Although many of these previously reported drug-induced reactions were duplicated with a wide range of dosage levels in our laboratory, no evidence was obtained indicating that the drug affects unconditioned eyelid closure. Eyelid closure can be elicited by a small electric shock delivered to *S*'s cheek, and this serves as an effective US in the classical conditioning situation [5]. Preliminary investigations indicated that the topography of this unconditioned eyelid response and unaffected by the range of dosage of LSD-25 employed in this experiment.

METHOD

Subjects

The *S*s were seven, male, New Zealand White rabbits, weighing 2.7 to 4.5 kg.

Apparatus

Conditioning was conducted in a sound-attenuated, temperature-regulated chamber. The eyeblink response was graphically recorded by modified version of the system employed by Thomas and Wagner [20]. Briefly, movement of

[1]Requests for reprints should be addressed to Dr. Daniel X. Freedman, Department of Psychiatry, NPI-UCLA, 760 Westwood Plaza, Los Angeles, CA 90024.

S's right eyelid was transmitted, via a small string and pulley arrangement, to the shaft of a microtorque potentiometer. This potentiometer, which was attached to the top of *S*'s head before each daily conditioning session, was held in place by means of two stainless steel screws chronically implanted in *S*'s skull. Voltage changes through this potentiometer provided a measure of unconditioned and conditioned eyelid activity. The US, a shock to the right cheek, was delivered through a pair of chronically implanted stainless steel electrodes, mounted approximately one cm apart and one cm below the right eye. The auditory conditioned stimuli were provided by LeHigh Valley Stimulus Generator. The visual stroboscopic stimulus was provided by a Grass Instrument Company strobe light. A tape programmer enabled presentation of trials at appropriate intervals, and automated stimulus programming equipment enabled presentation of conditioned and unconditioned stimuli at appropriate times during a trial.

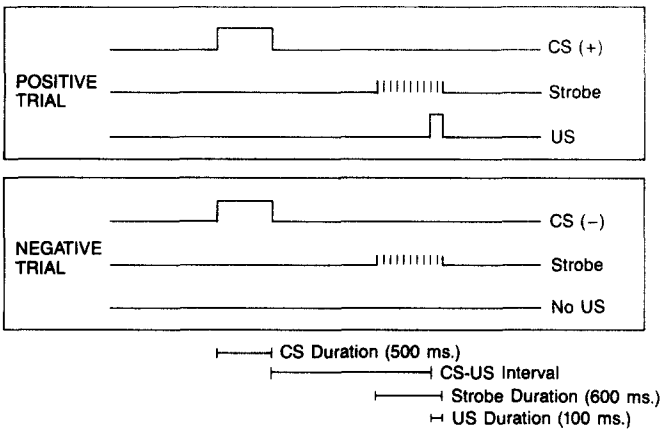

FIG. 1. Schematic representation of temporal parameters of the conditioning paradigm.

Procedure

The stainless steel screws for the mounting of the microtorque potentiometer and the stainless steel electrodes through which the shock US was delivered were implanted while *S*s were anaesthetized. Following recovery, *S*s were habituated for two daily 2-hr sessions by being restrained in the experimental apparatus.

Following habituation, all *S*s received discriminative trace conditioning training. Each *S* received 64 trials per day, half of them consisting of a positive auditory CS and shock US, and the other half consisting of a negative auditory CS, which was not followed by a US. One of the auditory stimuli was a train of clicks (8/sec), and the other was a moderately loud 1000 cps tone. For some *S*s, the tone was the positive stimulus, and the clicks the negative stimulus, while this relationship was reversed for other *S*s. This CS was presented for 500 msec. At some interval following the termination of the positive CS (the CS-US interval), the US was presented. The US consisted of a 4.0 mA shock of 100 msec duration delivered to *S*'s right cheek. No US was presented following the negative CS. A conditioned response (CR) would be an eyelid closure which occurred during the CS-US interval. Since this interval was varied during the investigation, it was necessary to adjust the situation such that the frequency of CRs was not spuriously large on trials when there was a relatively long CS-US interval; on such trials *S* would have a greater period of time in which to evidence a CR. Hence, on every trial, at a predetermined period after the positive or negative CS was terminated, a strobe light (20 flashes/sec) was turned on. This strobe lasted for 600 msec, during the last 100 msec of which the US was presented on positive trials (no US being presented on negative trials). This signal served to accentuate the inhibition of delay gradient [15] such that, for all *S*s, virtually all CRs occurred during that 500 msec segment of the CS-US interval in which the strobe was on, but before the US was delivered on positive trials. On negative trials, all eyelid closures which occurred during the first 500 msec of the isolated action of the strobe were scored as conditioned responses. Short latency reflex blinks to the CS, or "alpha responses" [12] were not evidenced by any *S*s.

The sequential relationship of the presentation of stimuli is schematically illustrated in Fig. 1. On positive trials, the interval between the offset of the positive CS and the onset of the US will be referred to as the CS-US interval. On negative trials, this term will be used when referring to the interval between the offset of the negative CS and that period starting during the last 100 msec of the isolated action of the strobe, i.e., the interval between the offset of the negative CS and the start of the period during which the US would be presented were the trial a positive one. As may be seen in Fig. 1, the other temporal parameters of the paradigm were held constant throughout the investigation.

All *S*s were initially trained with a CS-US interval of 1000 msec. The schedule of positive and negative trials was such that the first order sequential probabilities of type of trial (positive or negative) were counterbalanced. The intertrial intervals were varied between 1, 2, and 3 min and averaged 2 min. All *S*s were trained in this manner until they had reached a level of performance such that CRs were evidenced on more than 80% of the 32 daily positive trials, and less than 20% of the 32 daily negative trials. Starting with the next daily session, the CS-US interval was varied from trial to trial. On each of the positive and negative trials, this interval was equally likely to be 1000, 2000, 4000, or 8000 msec. That is, within a daily session, eight types of trials were presented. These are schematically represented in Fig. 2. First order probabilities of CS-US interval (1000, 2000, 4000 or 8000 msec), as well as type of trial (positive or negative), were counterbalanced. With the exception of this constraint, trials were presented in a predetermined random order.

Subjects were trained in this manner until analysis of their daily records indicated that they had reached a stable level of discrimination performance. For each *S*, a measure of conditioning was computed daily for both positive and negative trials at each of the four CS-US intervals. Following a period of stabilization of the magnitude of discriminative conditioning, the effects of LSD-25 were tested. The drug, in ampules containing 100 μg/ml, were supplied by Sandoz Pharmaceuticals. It was administered intraperitoneally immediately before the animal was placed in the conditioning apparatus. Each *S* was run under the effects of the drug for two successive days, and was run without the drug for several days following the last day of drug testing. For each *S*, data from the two drug days were combined, and were compared to that *S*'s performance on the day preceding the first day of drug administration and the day following the last day of drug administration. Three of the seven *S*s received a dosage

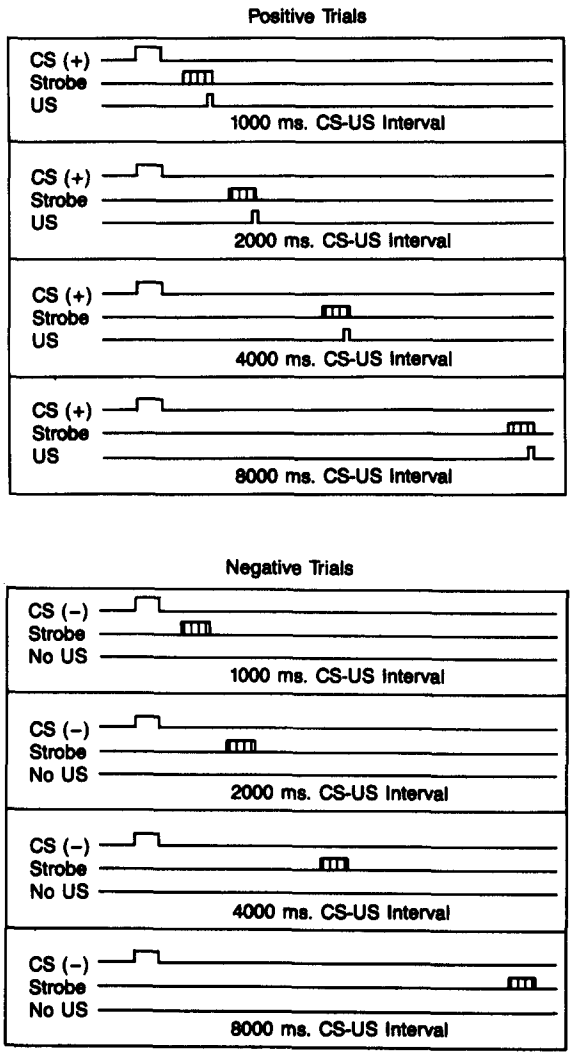

FIG. 2. Schematic representation of the different types of trials presented. Each day, each *S* received eight presentations of each of the eight types of trials.

of 85 μg/kg of LSD-25 on each of the two drug test days. The dosage for the remaining four *S*s was 35 μg/kg.

RESULTS

Conditioned response magnitude was determined by Pennypacker's [16] technique. This measure is defined as the intergral of the amplitude of the conditioned eyelid closure over the time course of the blink, and may be approximated by measuring the area (in square mm) of the graphic record of *S*'s CR. This magnitude measure provides a description of the physical properties (amplitude and latency) of the CR which is statistically superior to the more commonly used measures, and apparently is more sensitive to the physiological determinants of the eyelid closure [16].

The data from the *S*s injected with 85 μg/kg of LSD-25 showed a good deal of within- and between-*S*s variability not found with the *S*s injected with the lower dosage of 35 μg/kg. The overall effect of the higher dosage level, however, was to increase the magnitude of the CRs at all CS-US intervals, for both the positive and negative conditioned stimuli. This

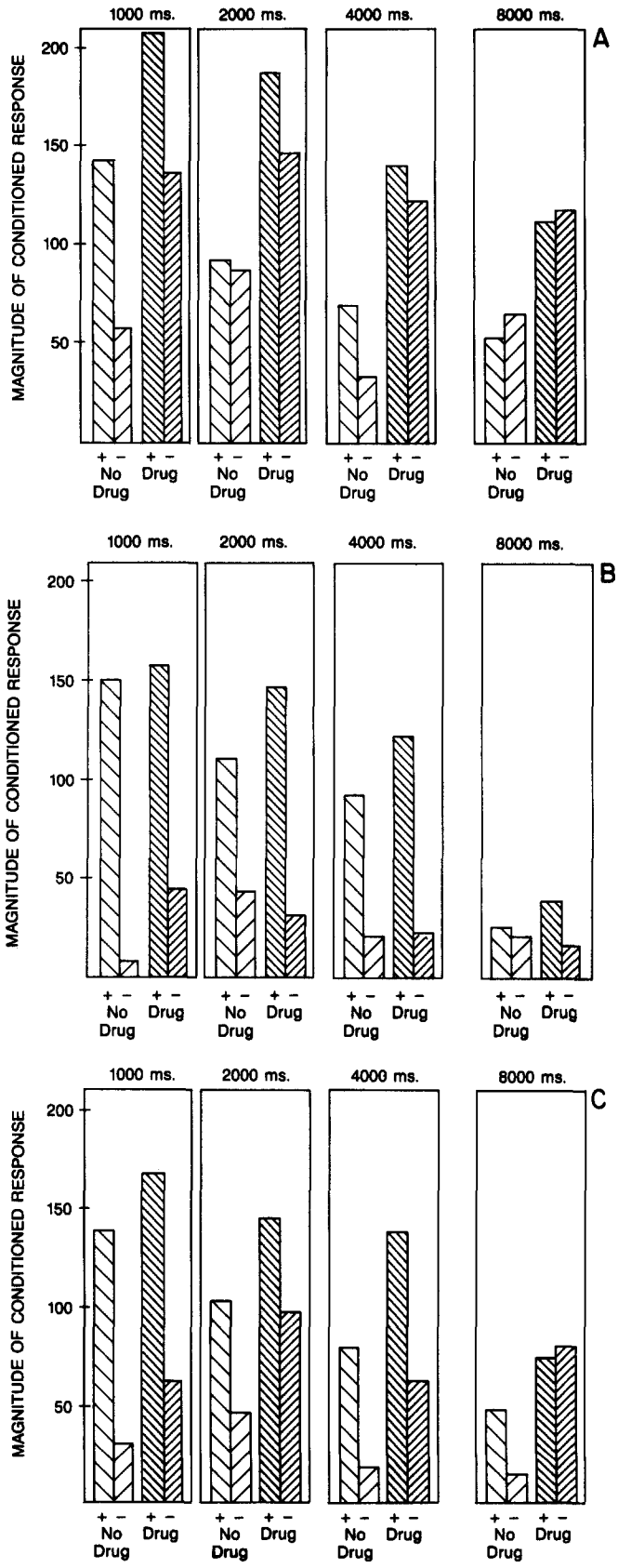

FIG. 3. Mean CR magnitude at all four CS-US intervals for three *S*s (A,B,C) injected with 35μg/kg LSD-25.

same general finding was evidenced, albeit considerably more clearly, at the lower dosage levels.

Examination of the data from the two successive days of drug testing did not indicate a systematic cumulative effect of the drug.

Figure 3 presents the mean CR magnitude at all CS-US intervals for each of three *S*s injected with 35 μg/kg of LSD-25. The results from the fourth *S* were similar, but not directly comparable because of its anomalously low baseline level of conditioned discrimination learning. The "no drug" values presented in Fig. 3 were determined by taking the mean of *S*'s last days session prior to the first administration of the drug, and its first session subsequent to the second administration of the drug. The "drug" values were obtained by averaging performance over the two successive daily sessions during which *S* was run immediately after injection of LSD-25.

As may be seen from examination of Fig. 3, all *S*s maintained a conditioned discrimination while under the effects of the drug, as they did while not under the effects of the drug. That is, they tended (except perhaps as the longest CS-US interval) to consistently respond to a greater extent to the positive stimulus than to the negative stimulus. The absolute magnitude of responding, however, was strikingly different. Response magnitude to the traces of both the positive and negative stimuli were considerably larger at all CS-US intervals for each *S* when it was run under the effects of the drug than when it was run in the undrugged state.

COMMENT

The present findings may be interpreted in a number of ways. It is possible that the drug somehow acts on the learning process (as suggested by Ivonova *et al.* [11]), therefore increasing conditioned response magnitude. However, the fact that CR magnitude increased to both positive and negative conditioned stimuli would indicate that the drug primarily produced an increase in stimulus dynamism which would be expected to increase both learning and performance variables [10].

Thus, the effect of LSD on response magnitude would be similar to the occurrences of larger responses when conditioned stimuli are increased in intensity. This phenomenon has been amply demonstrated in the classical conditioning situation (e.g., [9,17]).

It might further be proposed that the relevant effect of the drug was to increase the noxiousness of the shock US. However, analysis of the graphic record of each *S*'s URs under drug and no drug conditions did not indicate any systematic changes in the parameters of their topography. The amplitude (i.e., maximum degree of unconditioned eyelid closure), latency (i.e., speed of initiating blink response following presentation of the US) and slope (i.e., speed of completing unconditioned closure once initiated) did not appear to be affected by the drug. Actually increasing the intensity of the shock US a slight amount led to a sharp increase in amplitude and slope of the UR. Thus, the fact that such changes in UR topography did not occur under LSD-25 cannot be attributed to a "ceiling effect." Tolerance to LSD-25 (evident in food reinforced behavior in operant paradigms [4] but not in shock-induced escape behaviour [6]) was not systematically tested.

The present results indicate that LSD-25 affects CR magnitude in the classical trace conditioning situation by enhancing the excitatory properties of the CS. Since a trace procedure was employed, one can also conclude that the increased excitation produced by the CS persisted for at least 6900 msec after its offset. These conclusions are in agreement with previous studies in our laboratories. For example, in a study of flicker-fusion using operant conditioning with pigeons, the effects of LSD were concluded to be due to an enhanced sensory impact of the stimuli [1]. Similar conclusions have been reached in studies examining the effects of LSD on EEG arousal (e.g., [2,13]). The formulations of stimulus intensity dynamism by Hull [10] and experimental demonstrations [9,17] predict that the increased excitatory properties of a CS would not only increase the magnitude of an evoked CR, but also would enhance the rate at which the CS enters into associative learning.

Subsequent to the completion of these studies with trace conditioning, there have been a number of reports which have in general replicated and extended these findings using delay conditioning procedures [7]. Most recently, these trace conditioning findings with intervals from 1,000 to 8,000 milliseconds have also been replicated by Harvey *et al.* [8] at intervals from simultaneity to 800 milliseconds.

REFERENCES

1. Becker, D. I., J. B. Appel and D. X. Freedman. Some effects of lysergic acid diethylamide on visual discrimination in pigeons. *Psychopharmacologia* **11**: 354–364, 1967.
2. Bradley, P. B. and B. J. Key. The effects of drugs on arousal responses produced by electrical stimulation of the reticular formation of brain stem. *Electroencephalogr Clin Neurophysiol* **10**: 97–110, 1958.
3. Freedman, D. X., G. K. Aghajanian, E. M. Ornitz and B. S. Rosner. Patterns of tolerance to lysergic acid diethylamide and mescaline in rats. *Science* **127**: 1173–1174, 1958.
4. Freedman, D. X., J. B. Appel, F. R. Hartman and M. E. Molliver. Tolerance to behavioral effects of LSD-25 in rat. *J Pharmacol Exp Ther* **143**: 309–313, 1964.
5. Gormezano, I. Classical conditioning. In: *Experimental Methods and Instrumentation in Psychology*, edited by J. B. Sidowski. New York: McGraw-Hill, 1966, pp. 385–420.
6. Hamilton, C. L. Effects of LSD-25 and amphetamine on a running response in the rat. *Arch Gen Psychiatry* **2**: 104–109, 1960.
7. Harvey, J. A. Effects of drugs on associative learning. In: *Psychopharmacology: The Third Generation of Progress*, edited by H. Y. Meltzer. New York: Raven Press, 1987, pp. 1485–1491.
8. Harvey, J. A., I. Gormezano, V. A. Cool-Houser 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**: 433–441, 1988.
9. Hovland, C. I. The generalization of conditioned responses. II. The sensory generalization of conditioned responses with varying intensities of tone. *J Genet Psychol* **51**: 279–291, 1937.
10. Hull, C. L. Stimulus intensity dynamism (V) and stimulus generalization. *Psychol Rev* **56**: 67–76, 1949.
11. Ivanova, R. A., K. A. Laricheva and G. I. Mil'shtein. Features of experimental neurosis produced by lysergic acid diethylamine. *Zh Nevropatol Psikniatr* **62**: 1359–1362, 1962.
12. Kimble, G. A. *Hilgard and Marquis' Conditioning and Learning*. New York: Appleton-Century-Crofts, 1961.

13. Killam, K. F. and E. K. Killam. Drug action on pathways involving the retucular formation. In: *Reticular Formation of the Brain*, edited by H. H. Jasper, L. D. Proctor, S. S. Knighton, W. C. Noshay and R. T. Costello. Boston: Little, Brown, 1958, pp. 111–222.
14. Ling, T. M. and J. Buckman. *Lysergic Acid (LSD-25) and Ritalin in the Treatment of Neurosis*. London: Lambarde Press, 1963.
15. Pavlov, I. P. *Conditioned Reflexes*, translated by G. V. Anrep. London: Oxford University Press, 1927.
16. Pennypacker, H. S. Measurement of the conditioned eyelid reflex. *Science* **144**: 1248–1249, 1964.
17. Razran, G. Stimulus generalization of conditioned responses. *Psychol Bull* **46**: 337–365, 1949.
18. Rothlin, E. Lysergic acid diethylamide and related substances. *Ann NY Acad Sci* **66**: 668–676, 1957.
19. Rothlin, E. and A. Cerletti. Pharmacology in LSD-25. In: *Lysergic Acid Diethylamide and Mescaline in Experimental Psychiatry*, edited by L. Cholden. New York: Grune & Stratton, 1956, pp. 1–7.
20. Thomas, E. and A. R. Wagner. Partial reinforcement of the classically conditioned eyelid response in the rabbit. *J Comp Physiol Psychol* **58**: 157–158, 1964.