Pharmacology Biochemistry & Behavior, Vol. 9, pp. 809–812. Printed in the U.S.A.

Long-Term Effects of LSD-25 on Easy and Hard Visual Discrimination in Rats[1]

HOWARD FRIEDMAN AND ROBERT J. CAREY

Veterans Administration Hospital and State University of New York, Upstate Medical Center Syracuse, NY 13210

(Received 17 August 1978)

FRIEDMAN, H. AND R. J. CAREY. *Long term effects of LSD-25 on easy and hard visual discrimination in rats.* PHARMAC. BIOCHEM. BEHAV. 9(6) 809–812, 1978.—Rats subjected either to unilateral frontal cortex lesion or sham operation were trained to discriminate between a lighted and unlit alley to escape shock. After reaching criterion, the rats were intubated with either placebo or LSD-25 (1.0 mg/kg). One week or 1 month later they were given discrimination trials with either an increased (hard) level of task difficulty, or with the same (easy) level used in pre-intubation training. A single-dose drug effect, as long as 1 month post-intubation, was observed both in error scores and running time, but only with hard discriminations. The study emphasizes the sensitivity of the experimental paradigm which reduces the risk of Type II error.

LSD-25 Discrimination performance Single-dose effects Long-term effects

IN TWO previous investigations [3,4] the possibility of synergistic or combinatory effects of lysergic acid diethylamide (LSD-25) and preexisting intracranial organic pathology was evaluated. This interest stemmed not only from clinical observations in humans, but also by reports [5,8] of significant single-dose drug effects in patients with preexisting brain damage. Both analogue studies dealt with the visual discrimination performance of rats subjected either to a frontal cortex lesion or to a sham operation followed by intubation with a single dose of LSD-25 (1.0 mg/kg) or placebo.

Although these studies [3,4] provided suggestive evidence of a drug-lesion interaction, two other issues meriting further investigation were raised. First, indications of single-dose chronic drug effects, rarely reported in the literature, occurred. Second, the technique of increasing task difficulty after drug administration seemed to provide a paradigm for uncovering otherwise undetected drug effects, an observation infrequently reported, but not emphasized, by other investigators [2, 11, 12].

The previous studies [3,4], oriented primarily toward elucidating either a synergistic or combinatory effect of drug and lesion, involved the same groups of animals for both acute and chronic single-dose effects, and thus a possible differential learning effect may have entered to confound the results. The present study uses a somewhat different design to avoid this difficulty and thus attempts to clarify the single-dose chronic drug effect, as well as to establish most clearly the sensitivity of the paradigm with increased post-treatment task difficulty. Further, at the same DeGroot stereotaxic coordinates, unilateral, rather than bilateral, lesions are used in order to try to de-emphasize possible overriding lesion effects, as well as to bring the intracranial pathology somewhat closer to the concept of "minimal brain damage" referred to in observations with humans.

METHOD

Animals

A total of 174 experimentally naive Sprague-Dawley rats, approximately 100 days old, were used. Upon arrival each was housed separately and allowed one week of ad lib food and water prior to assignment to surgery. Following surgery, and throughout testing, water intake was monitored as a check on well-being.

Apparatus and Procedure

The animals were subjected either to unilateral frontal cortex lesion or to sham operation, with a post-operative recovery period of 2 weeks. After the recovery period, each animal received 11 prediscrimination training trials followed by discrimination training consisting of two successive blocks of 10 trials each daily to reach a criterion of 9 correct choices in each 10-trial block. The discrimination box, with a shock grid floor and two alleys in the choice chamber, used a light bulb of 281 millilamberts luminance as a cue for correct choice.

Apparatus, surgery and histology, prediscrimination and discrimination training were all identical with that reported in the previous studies [3,4] and will therefore not be repeated in further detail here.

[1]This study was performed as part of VA Research Project No. 029-12-0694-02. LSD tartrate solution was supplied by the Biomedical Research Branch-NIDA.

Testing Procedure

On the first day after the week of discrimination training, each animal received 20 retest trials to assure adequate retention. As previously, following retest trials, the animals, under light ether anesthesia, underwent intragastric intubation with either LSD-25 or placebo at the dosage level of 1.0 mg/kg and concentration of 0.1 mg/ml. (Again, from the standpoint of devising an analogue study most comparable to human ingestion of LSD-25, as well as the relatively large volume of solution required, intragastric intubation rather than injection was used as the more appropriate route of administration.) Throughout the investigation there was no evidence of toxic effects as determined by water intake and observation of behavior.

Animals were assigned to one of the four treatment groups of 44 rats each: drug-lesion (D-L), drug-sham (D-S), placebo-lesion (P-L), and placebo-sham (P-S). Each group was subdivided into 4 subgroups of 11 each which were then subjected to one of 4 post-intubation testing conditions. These four conditions involved differences in post-intubation testing times and in discrimination difficulty. Thus, although each animal was tested for visual discrimination in the same manner described in the discrimination training, test periods were given 1-week or 1-month post-intubation. Furthermore, half of the test periods were of the same discrimination difficulty as in the pre-intubation retention trials (unlit alley vs 281 millilamberts luminance), characterized as an "easy" discrimination. The other half utilized a more difficult discrimination (50 millilamberts luminance vs 281 millilamberts) and was characterized as "hard." There were then 44 animals in the hard discrimination group and 44 in the easy discrimination group, each consisting of 11 D-L, 11 D-S, 11 P-L, and 11 P-S, which were tested 1-week post-intubation. The other 44 animals in the hard discrimination group and 44 in the easy discrimination group were tested 1-month post-intubation. Although the same distribution was planned, i.e., 44 per group, for the 1-week post-intubation and the 1-month post-intubation easy discrimination groups, 2 animals, 1 L-D and 1 S-P, were lost between retention and test trials from the 1-month easy groups. Thus results were obtained from a grand total of 174 animals.

RESULTS

Two types of measures, as in the previous studies, were used. The error score, reflecting performance accuracy, was arrived at for each animal by taking the difference between the number of errors made in the 20 test-day trials and the animal's baseline which was the number of errors made in the 20 retention trials immediately preceding intubation. Time scores, reflecting locomotor speed, were obtained by taking the differences between the median number of seconds required to run from start chamber to the appropriate goal box in the 20 trials of a test day and the animal's baseline, i.e., the median time measure for the 20 retention trials.

Each set of data was subjected to a 4-way analysis of variance (ANOVA) using the "default" (classic) option as described in Nie, Hull, Jenkins, Steinbrenner and Bent [10], with the "between subjects" variables of drug, lesion, post-intubation time, and test task discrimination difficulty.

ANOVA for error scores revealed significant main effects for drug, $F(1,158)=7.92$, $p=0.006$, and for test task discrimination difficulty, $F(1,158)=70.16$, $p<0.001$. The only significant interaction effect, drug by test task difficulty, $F(1,158)=4.77$, $p=0.030$, is plotted in Fig. 1. A breakdown of the interaction into its simple main effects indicates significant increases in errors in both drug and placebo groups for hard discrimination: drug, $F(1,158)=55.7$, $p<0.001$; placebo, $F(1,158)=19.4$, $p<0.001$, but with the drug groups showing much larger increases than the placebo groups. Thus, there was no difference between drug and placebo when easy discriminations were involved, $F(1,158)=<1.0$, but a highly significant change when hard discriminations were undertaken, $F(1,158)=12.5$, $p<0.001$.

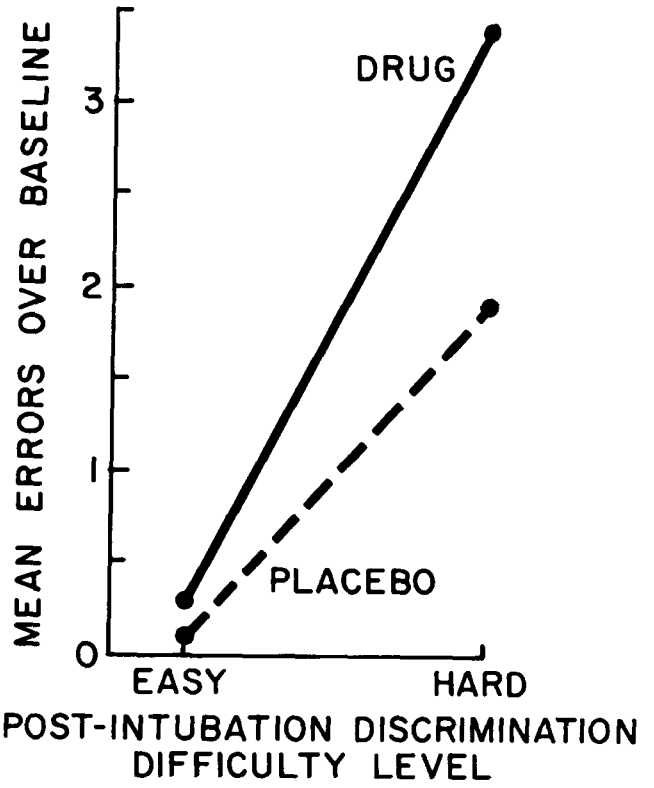

FIG. 1. Mean number of errors over baseline for all drug and all placebo groups at post-intubation discrimination difficulty levels.

Time scores showed a significant main effect for post-intubation time, $F(1,158)=4.36$, $p=0.038$. A significant interaction effect, $F(1,158)=6.97$, $p=0.009$, between drug and test task difficulty was observed, and is reflected in Fig. 2.

The simple main effects breakdown of the significant interaction indicated that although the drug group was not significantly different on easy and hard discriminations, the placebo group did show significantly faster running time on the hard vs. easy discriminations, $F(1,158)=6.12$, $p<0.05$. The difference between the two groups was significant at the hard level, $F(1,158)=4.73$, $p<0.05$, but not at the easy level, $F(1,158)=2.38$, $p>0.05$.

No other time score effects were significant, although a lesion-test task difficulty interaction approached, but did not reach, the conventional level of statistical significance: $F(1,158)=3.84$, $p=0.052$.

DISCUSSION

The results derived from the drug-task difficulty interactions point clearly to a single-dose drug effect elucidated

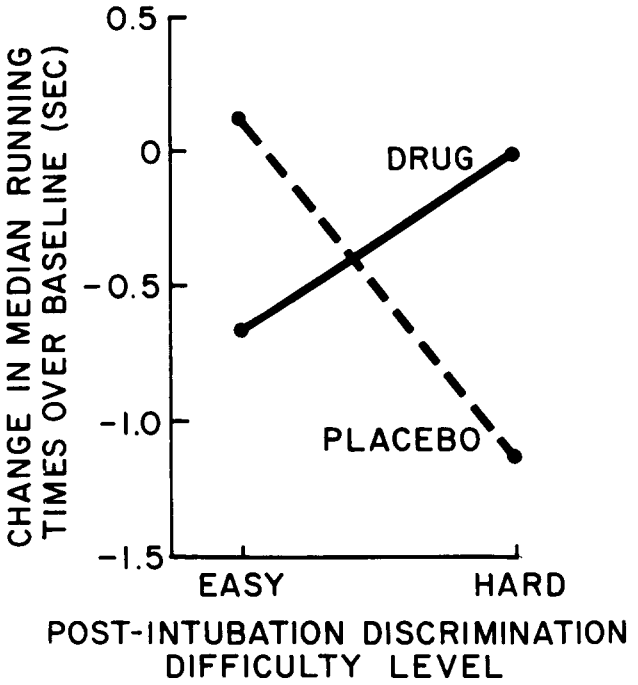

FIG. 2. Mean of median running times over baseline for all drug and all placebo groups at post-intubation discrimination difficulty levels.

only when the level of task difficulty is increased somewhat over that upon which the animals were trained to criterion. This effect, found as long as one-month post-intubation, is reflected in significant differences between the combined drug groups and the combined placebo groups wherein the former had significantly more errors in performance and the latter displayed significantly faster running time. No significant effects are observed when the pre- and post-intubation levels of discrimination task difficulty are identical, a finding highly consistent with the previous [3,4] studies.

The shape of the drug-task difficulty interaction is somewhat more complex in the case of running time than in performance accuracy. In the latter case, both drug and placebo groups revealed an increased number of errors with the more difficult discrimination, with the drug group displaying a significantly greater amount than the placebo. In the former case, running times for the drug group, though in the direction of an increase with hard discriminations, were not significantly different from the easy discrimination animals. The placebo animals, however, were significantly faster in hard discriminations than in easy, and this provided the interaction effect which accounted for a significant difference between drug and placebo groups at the hard discrimination level. The simplest explanation may lie in the likelihood that error trials provide more grid shock than correct trials. Therefore, the placebo animals respond to this additional reinforcement during the hard discrimination tests with greater speed, while drug treated animals are less affected by the additional stimulation. To check on this point, the time scores of all placebo animals exhibiting the same or fewer errors than obtained on baseline trials were compared with all placebo animals making more errors. By t-test a significant difference appeared in the expected direction: $t=3.04$, $df=85$, $p<0.01$. The drug animals, compared in a similar fashion, showed no significant difference: $t=1.06$, $df=85$, $p>0.10$. This time score finding in the placebo animals is quite consistent with the running time changes in the previous studies, although it was then felt that with designs using the same animals for different repeated post-intubation trial times, the changes could be attributed to increased task familiarity. Apparently more work is required to evaluate whether LSD treated animals are less responsive to grid shock or more difficult to train under these conditions. Related to this point are some observations of a drug effect which results in increased running time [4, 6, 9].

Although the initial interest of the previous studies centered about evaluation of the behavioral effects of a single dose of LSD-25 in the presence of pre-existing intracranial organic pathology, it has proved difficult to find other than suggestive evidence of this type of interaction. The first study [3], with several levels of post-intubation task difficulty, elucidated a significant drug-lesion interaction reflected in slower running time scores upon initial increase in task difficulty. The second study [4] was able to contribute to this issue only an ordering of the sub-groups, both in error and time scores, in the expected direction. The present study indicates an ordering in time scores of the subgroups, both at one week and one month, in the direction appropriate for the interaction, but again without statistical significance.

More definitive has been the evidence for a chronic single-dose drug effect, a finding rarely, if ever, observed in view of the multiple drug dosages with human subjects. The first study [3] pointed to this effect in decreased accuracy of performance, the second [4] in running time, and the current study now displays it most clearly in both measures.

Perhaps the most important observation from these studies resides in the emphasis on the type of experimental paradigm which could yield consistent evidence of a single-dose drug effect as long as one-month post-intubation. It is apparent that the more common paradigm of identical pre- and post-treatment task, used widely in studies of the effects of drugs [1] or brain damage [7] upon memory and learning, runs the risk of a Type II error in any type of investigation in which the effects are subtle rather than blatant. Thus the present study re-affirms the sensitivity of the strategy of providing a slightly more difficult level of task difficulty subsequent to the intervening variable in order to tease out even the most elusive effects upon the dependent variable.

REFERENCES

1. Abel, E. L. Marihuana, learning, and memory. *Int. Rev. Neurobiol.* **18:** 329–356, 1975.
2. 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.
3. Friedman, H. and R. J. Carey. Effects of LSD-25 on performance of a visual discrimination task in brain-damaged rats. *Pharmac. Biochem. Behav.* **3:** 421–424, 1975.
4. Friedman, H. and R. J. Carey. Acute and chronic single dose effects of LSD-25 on visual discrimination in rats. *Pharmac. Biochem. Behav.* **5:** 223–226, 1976.

5. Holden, J. M. and T. M. Itil. Lobotomized patients show increased response to LSD. *Roche Rep: Front. Psychiat.* **1:** 1–2, 1971.
6. Hughes, R. N. Effects of LSD on exploratory behavior and locomotion in rats. *Behav. Biol.* **9:** 357–365, 1973.
7. Isaacson, R. L. Experimental brain lesions and memory. In: *Neural Mechanisms of Learning and Memory*, edited by M. R. Rosenzweig and E. L. Bennett. Cambridge, Mass: MIT Press, 1976, pp. 521–543.
8. Korein, J. and J. M. Musacchio. LSD and focal cerebral lesions. *Neurology* **18:** 147–152, 1968.
9. Lowe, G. and D. I. Williams. The effect of LSD-25 on light-reinforced behavior in the rat. *Psychopharmacologia* **27:** 255–263, 1972.
10. Nie, N. H., C. H. Hull, J. G. Jenkins, K. Steinbrenner and D. H. Bent. *Statistical Package for the Social Sciences*. 2nd ed. New York: McGraw-Hill, 1970.
11. Sharpe, L. G., L. S. Otis and R. J. Schusterman. Disruption of size discrimination in squirrel monkeys (saimiri Sciureus) by LSD-25. *Psychonom. Sci.* **7:** 103–104, 1967.
12. Uyeno, E. T. Lysergic acid diethylamide and novel stimulus. *Psychonom. Sci.* **18:** 52, 1970.