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Effects of LSD-25 on Performance of a Visual Discrimination Task in Brain-Damaged Rats[1]

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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(3) 421–424, 1975.– Rats subjected to either a frontal cortex lesion or to a sham operation were trained to discriminate between a lighted and unlit alley in order to escape shock. Following intubation with either placebo or LSD-25 (1.0 mg/kg), they were given discrimination trials 24 hr, 1 week, 2 weeks, 3 weeks, 4 weeks, and 5 weeks later. During the last 3 sets of trials, the discrimination task was increased in difficulty each week. A relatively long-term single-dose drug effect was observed in decreased accuracy of performance, with a drug-lesion interaction reflected in slower running time upon initial increase in level of task difficulty.

LSD Discrimination performance Brain-damaged animals Drug synergism

SEVERAL reports [3,4] of an association between intracranial organic pathology and the ingestion of lysergic acid diethylamide (LSD-25) in humans raise the possibility of a synergistic effect of a single dose of LSD-25 and pre-existing, rather than consequential, brain damage. From one standpoint this may support the rather common, but not systematically investigated, clinical observation of a history suggestive of pre-existing, at least minimal, organic brain impairment in those cases presenting severe psychotic reaction, with long-lasting cognitive deficits, following ingestion of a single dose of LSD-25. Holden and Itil [3] have reported that following minimal single doses (1–2 μg/kg) schizophrenic patients lobotomized 10–20 years earlier responded with increased psychopathology when compared to non-lobotomized schizophrenics. Korein and Musacchio [4] have observed that LSD-induced aberrant behavioral phenomena could be related to neurological deficit so that 6 of 14 patients with focal cerebral lesions responded with psychosis to an IV injection of 100 μg of LSD, and 12 showed significant EEG changes.

In general, investigations with LSD-25 have not been reported in human subjects with established pre-existing intracranial organic pathology. More typically, effects of LSD have been studied in a variety of clinically normal subjects or patients with diagnoses of functional psychiatric disorders. Thus, McWilliam and Tuttle [6] have provided an extensive review of longterm psychological effects of LSD and although concluding that the research is "very poor" stated that "when the drug was administered to psychologically normal subjects under secure circumstances, lasting adverse reactions did not occur." The investigations reviewed involved, as a rule, multiple drug doses with the rare single dose studies showing no prolonged adverse reactions. Yet there are occasional reports raising the question of subsequent brain damage, as, for example, that of McGlothlin *et al.* [5] who found evidence for moderate impairment of abstract abilities suggestive of minimal brain damage in LSD users, though repeated dosages were involved in all cases.

The present study is an initial investigation into the effects of a single dose administration of LSD-25 upon the visual discrimination behavior of minimally brain-damaged rats. As such, it represents an analogue experiment to provide clues as to possible synergistic effects of LSD-25 and pre-existing intracranial organic pathology upon human behavior as well as some indication of adverse effects of LSD-25 lasting beyond the acute stage.

METHOD

Animals

A total of 48 experimentally naive Sprague-Dawley rats, approximately 100 days old, were used. Sixteen of the animals were used for a preliminary screening in order to arrive at an effective drug dosage level, and 32 were used for the main body of the investigation. 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 daily as a check on well-being. Mean weight of the animals in the main body of the investigation was 441.2 g (σ = 67.4) and there was no difference between the mean weights of the 4

[1] LSD tartrate solution and placebo for LSD were supplied by the FDA-NIMH Drug Abuse Advisory Committee.

treatment groups into which they were subdivided ($F < 1.0$).

Apparatus

The discrimination box, 135 cm long by 25 cm wide overall, had walls of black Plexiglas and, to permit observation of the animals, a top of clear Plexiglas. The start box, 39 cm long by 25 cm wide, was separated from the choice chamber by an opaque hand-operated guillotine door. The choice chamber, 62 cm long by 95 cm wide, was divided equally into two alleys by a black Plexiglas partition. The goal box, 34 cm long by 25 cm, was constructed entirely from black Plexiglas. Each choice alley was separated from the goal box by an opaque 11 cm square door which could be rotated upward to allow access to the goal box. Release of the doors was accomplished by a remotely controlled system of pushbutton solenoids and spring returns. The floor of the discrimination box consisted of a shock grid from the start box to the Plexiglas floor of the goal box. Foot shock was delivered by a Lehigh Valley Electronics Model 1531 Constant Current Shocker. The cue for correct choice was a light bulb of 281 millilamberts luminance inside the exterior wall of each alley.

Procedure

Surgery and histology. Animals were divided into 2 groups, equated on the basis of weight, and assigned randomly to either a lesion group or to a sham operation group. Surgery was aseptic and performed under deep ether anaesthesia. The lesion group animals received bilateral frontal cortex lesions made with a temperature controlled RF lesion maker (Radionics, Inc.) at the following DeGroot coordinates: anterior 3.0, lateral 1.5, 2.0 mm below dura. A Kopf stereotaxic instrument was used to position the thermister electrode in the frontal cortex. Since there were no guides in the literature as to specific cortical areas for significant drug–lesion relationship, the coordinates were arbitrarily selected simply to provide minimal, easily verifiable, and consistently localized brain damage. Current was adjusted to achieve a temperature at the electrode tip of 55° C which was maintained for 60 sec. The sham operation group was treated identically but surgery consisted only of exposing the skull without insertion of the electrode. Postoperatively each rat was given an IM injection of 200,000 units of procaine penicillin and allowed 2 weeks of recovery. At the conclusion of all testing the animals were sacrificed and conventional histologic procedures were employed to obtain cresyl violet stained brain sections. These were then examined microscopically to verify the lesions. Fig. 1 demonstrates typical lesions.

Prediscrimination training. Two weeks after surgery each animal received the following: (a) One no-shock 15 min adaptation period to permit exploration and adaptation to the novelty of the discrimination box; (b) Then 10 shock escape trials in which a 1 mA scrambled grid shock was given in the discrimination box. The escape training was conducted to insure that all rats learned rapidly to escape the foot shock by running from the start box to the goal box. During this training both cue lights remained on and both goal box doors were open.

Discrimination training. (a) In the darkened testing room, each animal was placed in the start chamber and one alley light was turned on as a correct choice while the non-

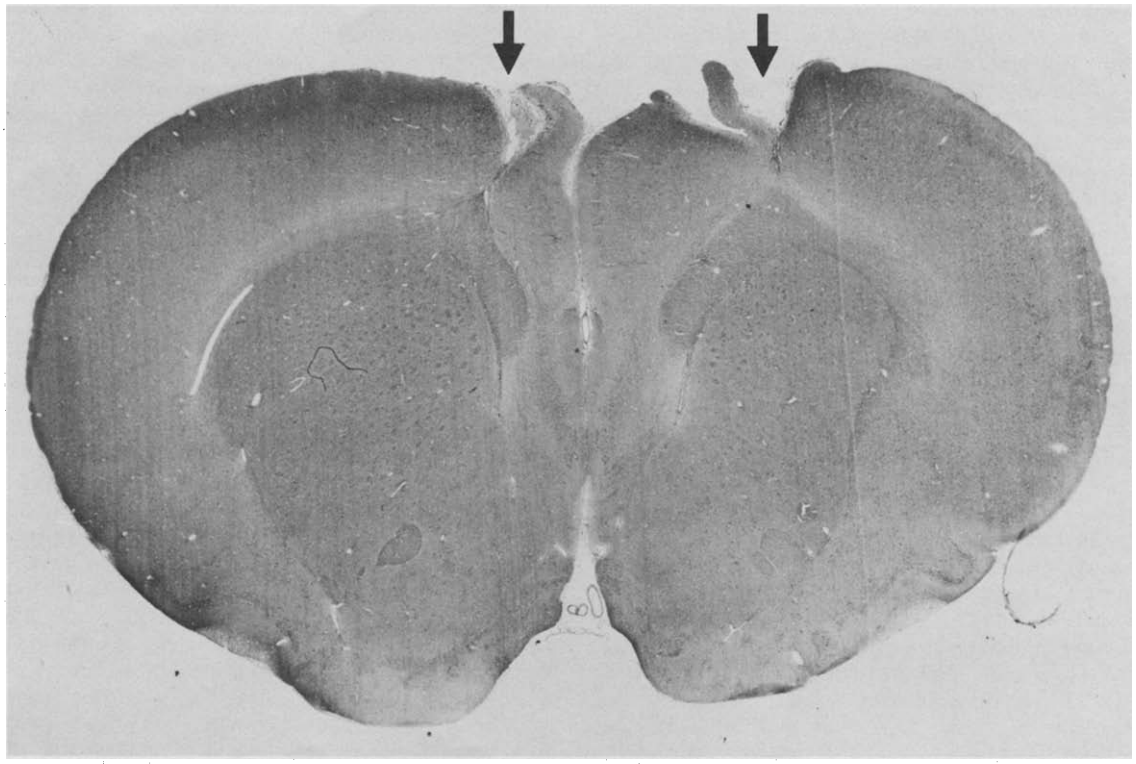

FIG. 1. Photograph of a cresyl violet stained 6 μ Formalin-fixed rat brain section showing typical lesions.

lit alley indicated incorrect; (b) the start chamber door was raised which signalled onset of the shock to the grid floor after 10 sec delay; (c) If the correct alley was entered, the start chamber door was lowered to prevent retracing, the goal box door raised, and shock terminated upon entering the goal box; (d) If the incorrect alley was chosen, the start chamber door was lowered and the animals received 15 sec of shock while being blocked in the incorrect alley; (e) After each trial the testing room was lit, the animal returned to the start chamber, and the next trial started after a 30 sec interval.

In each daily session all animals were given 2 blocks of 10 trials each with random presentation of an equal number of left and right light cues. Daily sessions were run until the animal reached the criterion of 9 correct choices out of 10 trials for each of the 2 blocks of 10 trials on a given day. Upon reaching criterion each animal was returned to the home cage to await the criterion point for the remainder of its squad. All animals of every squad reached criterion within one week. The mean number of trials to criterion was 67.8 (σ = 32.0) with no significant differences in means of the treatment groups described below ($F < 1.0$).

Testing procedure. On the first day after the week of discrimination training, each animal was given 20 retest trials to assure adequate retention. There were no significant differences between groups in mean number of days ($\bar{x}$ = 5.3, σ = 1.5) from end of discrimination training to this point ($F < 1.0$). The mean number of errors during the retest trials was 0.9 (σ = 1.1) with no significant differences in the means of the 4 treatment groups. Immediately following the retest trials the animals, under light ether anaesthesia, underwent intragastric intubation with either LSD-25 or placebo at the dosage level of 1.0 mg/kg body weight and at the concentration of 0.1 mg/ml. This dosage level, for the main body of the experiment, was arrived at following preliminary screenings with concentrations of 0.1, 0.2, 0.4, an 0.8 mg/kg with a squad of 4 animals at each level. For these preliminary trials only the easiest discrimination, i.e., the same luminance differential as in the discrimination training period, was used. Not until the 1.0 mg/kg level was reached was there any indication of a possible drug effect, and at this level there was, throughout the investigation, no evidence of toxic effects as determined by food and water intake and daily observation of behavior. (Intubation rather than injection was used in order to approximate more closely the oral type of administration commonly reported with human subjects as well as the route used almost exclusively by drug-dependent patients.) The main body of the experiment, then, contained 8 squads of 4 animals each for a run, with each of the 4 animals under a different set of conditions: drug–lesion (D-L), drug–sham (D-S), placebo–lesion (P-L), or placebo-sham (P-S). Each animal was then tested for visual discrimination performance in the same manner described in the Discrimination training above with test trial periods given 24 hr, 1 week, 2 weeks, 3 weeks, 4 weeks, and 5 weeks postintubation. During the first three trial periods the light–dark discrimination was identical with that used in training. During the next 3 periods, progressively more difficult discriminations were introduced. Thus, on the third week postintubation the alley lights were 281 and 50 millilamberts (Discrimination 2), on the fourth week 281 and 110 millilamberts (Discrimination 3), and on the fifth week 281 and 174 millilamberts luminance (Discrimination 4). Order of running for all animals was random since the animal handler had no knowledge of treatments given.

RESULTS

Eight squads of 4 animals each were available for intubation at the 1.0 mg/kg dosage and were run through the 3 weekly postintubation trials at Discrimination Level 1, then subsequently at Discrimination Levels 2, 3, and 4 at weekly intervals. Error scores were arrived at for each animal by taking the differences between the number of errors made in the 20 trials of a test day and the animal's baseline which was the number of errors made on the 20 retention trials given immediately prior to intubation. At Discrimination Level 4 (fifth week postintubation) the task became so difficult for most animals that the mean number of correct choices for each group decreased to chance levels. Therefore, this set of trials was excluded from further analysis. The error scores were subjected to a 3-factor analysis of variance with 2 between animals variables (drug, lesion) and 1 within animals variable (discrimination level) as described in Winer [10]. As expected, performance for all groups varied significantly overall as a function of trails $F(4,112) = 63.50$, $p<0.001$, which simply reflects the increasing levels of discrimination difficulty. More to the point is the finding that although no significant drug–lesion interaction was found, a significant drug effect was observed which extended through the sets of trials, $F(1,28) = 5.40$, $p<0.05$. There were no other significant findings with error scores. Figure 2 plots the error scores of the combined drug groups and combined placebo groups by sets of trials.

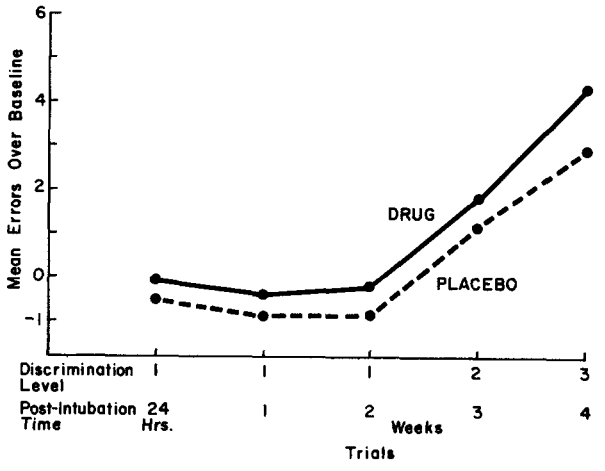

FIG. 2. Mean number of errors over baseline for all drug and all placebo animals at each postintubation time and discrimination level.

Time scores were obtained by taking the differences between the median number of sec required to run from start chamber to goal box in the trials of a test day and the animal's baseline, which was the median time measure for retention trials given immediately prior to intubation. The same type of analysis of variance as used with error scores was applied. Again the over-all significant variance of groups with trials was observed, $F(4,112) = 6.20$, $p<0.001$, but with time changes probably reflecting the increasing familiarity of most animals with the apparatus. More perti-

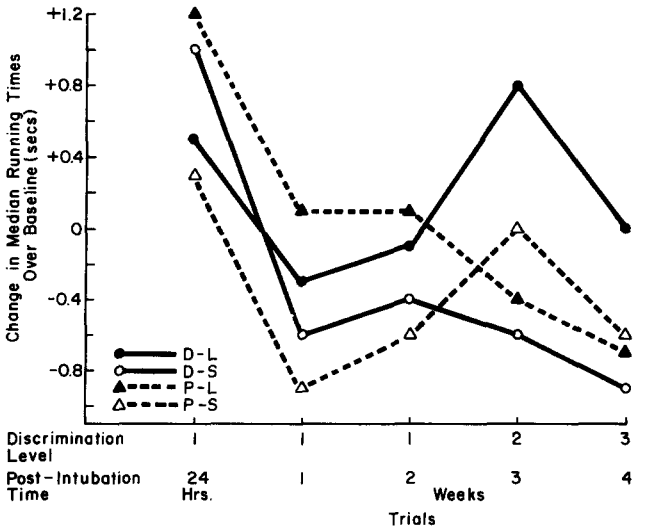

FIG. 3. Means of median running times over baseline for all groups at each postintubation time and discrimination level.

nently, a trend toward an overall signficant drug–lesions–trials interaction appeared, $F(4,112) = 2.40$, $p<0.06$. Figure 3 is a plot of the time scores by sets of trials and suggests, from inspection, that the interaction was due to a drug–lesion effect which appeared with the more difficult discrimination levels on Weeks 3 and 4. A further analysis of these sets of trials showed that with the first increase in discrimination difficulty to level 2 there was a statistically significant drug–lesion interaction, $F(1,28) = 5.14$, $p<0.05$.

DISCUSSION

The results clearly indicate that a single dose of LSD-25 at the level of 1.0 mg/kg of body weight has an overall significant effect upon brightness discrimination performance insofar as errors are concerned, and persists over a period of at least 4 weeks after drug ingestion.

Unlike the error scores, running time scores showed no overall significant effect of drug alone. However, a trend toward significant drug–lesion interaction over trials did appear. With the first increase in discrimination difficulty 3 weeks after intubation, the drug–lesion interaction was statistically significant. In terms, therefore, of the main interest of this investigation, there are some suggestions that over time, drug and lesion effects combine synergistically to result in slower running times. Apparently the effect is most clearly observed by increasing the difficulty of the learned task so that finer discrimination for task success is required. Thus, when animals are required to perform the identical training task three times over a period of 15 days, the changes in running time scores between groups are not statistically significant. The addition, however, of several repetitions with more difficult discriminations does point to a drug effect. This method of revealing drug effects which might otherwise be obscured is in keeping with findings of other investigators. Sharpe *et al.* [8] observed that a "difficult" size discrimination was disrupted in squirrel monkeys following injections of LSD-25, whereas an "easy" discrimination was relatively unaffected. Uyeno [9] found that although running time of rats injected with LSD-25 was not significantly different from a control group, there was a significant increase in time when the novel stimulus of a hurdle was placed in the runway. Becker *et al.* [1] investigated the response of pigeons to various dosage levels of LSD and several levels of visual discrimination difficulty. Although a major finding was a significant increase in accuracy of performance at the low dosage level of 20 μg/kg with the suggestion of a decrement at the higher level of 80 μg/kg, inspection of the curves presented further suggests that the drug effect may be more apparent at the more difficult discrimination levels.

The changes in error scores in drug animals and the elevations of running times in the drug–lesion group extending over several weeks point to single dose LSD-25 long-term effects. This is consistent with the findings of Rosen and Buga [7]. In an investigation of the effects of LSD-25 on instrumental conditioning, extinction, and discrimination learning in the rat, they concluded that behavioral consequences outlasted the drugged state; a conclusion that they felt was at variance with the considerable literature pointing to the largely transient nature of LSD effects in animals, but in keeping with clinical observations of LSD effects in psychotherapy [2].

It is recognized that the use of both consecutive time intervals with increasing levels of discrimination difficulty may lead to some confounding of the results. Nevertheless, the direction of the findings, i.e., increased errors in the combined drug group over the combined placebo group, and heightened running time scores over trials in the drug-lesion group, permits some overall conclusions. A relatively long-term single-dose drug effect is observed in decreased accuracy of performance, with a drug–lesion interaction reflected in slower running time upon initial increase in level of task difficulty.

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. Chandler, A. L. and M. A. Hartman. Lysergic acid diethylamide (LSD-25) as a facilitating agent in psychotherapy. *Archs gen. Psychiat.* Chicago **2**: 286–299, 1960.
3. Holden, J. M. and T. M. Itil. Lobotomized patients show increased response to LSD. *Roche Rep.: Frontiers of Psychiatry* **1**: 1–2, 1971.
4. Korein, J. and J. M. Musacchio. LSD and focal cerebral lesions. *Neurology* **18**: 147–152, 1968.
5. McGlothlin, W. H., D. O. Arnold and D. X. Freedman. Organicity measures following repeated LSD ingestion. *Archs gen. Psychiat.* **21**: 704–709, 1969.
6. McWilliam, S. A. and R. J. Tuttle. Long-term psychological effects of LSD. *Psychol. Bull.* **79**: 341–351, 1973.
7. Rosen, A. J. and J. A. Buga. Effects of lysergic acid diethylamide on simple instrumental conditioning, extinction and discrimination learning in the rat. *Pharmac. Biochem. Behav.* **1**: 619–627, 1973.
8. 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.
9. Uyeno, E. T. Lysergic acid diethylamide and novel stimulus. *Psychonom. Sci.* **18**: 52, 1970.
10. Winer, B. J. *Statistical Principles in Experimental Design.* New York: McGraw-Hill, 1962.