Psychopharmacology 62, 145 – 149 (1979)

Psychopharmacology

# Effects of LSD-25 on Avoidance Behavior and Locomotor Activity in Mice

Claudio Castellano

Laboratorio di Psicobiologia e Psicofarmacologia del C. N. R., Via Reno 1, I-00198 Roma, Italy

**Abstract.** C57BL/6 (C57) mice were tested in a shuttle box under the influence of LSD-25 in two sets of experiments.

In the first set, the pretrial administrations of LSD (0.05, 0.1, and 0.2 mg/kg) were followed by dose-related performance improvements. In the second set, the posttrial administration of LSD (0.2 mg/kg) immediately after each experimental session improved the performances of the mice. No effect was evident when the hallucinogen was injected 2 h after training, thus showing that LSD improved the memory processes of the C57 mice in these experimental conditions.

Dose-related activity depressant effects were evident in a third set of experiments, carried out in a toggle-floor box.

**Key words:** LSD-25 – Avoidance – Activity – Inbred mice

The effects of lysergide (LSD-25) on animal behavior have been studied in a number of investigations, with contrasting results. Performance impairments have been described, for example, in rats injected with LSD-25 during training in a pole-jumping situation (Domino et al., 1965; Banerjee, 1971), while performance improvements following administration of LSD have been found in rats trained in the shuttle box (Bignami, 1972). Facilitation of brightness discrimination reversal learning has, moreover, been observed in the rat following LSD administration (King et al., 1974). Some researchers have considered the effects of LSD on poorly performing animals. It has thus been demonstrated that it has an excitatory effect on the performances of pretrained rats that are poor performers of shuttle box escape/avoidance (Bignami et al., 1975), and of naive hamsters trained in the same experimental conditions (Sansone et al., 1974). Waser et al. (1976) have recently hypothesized, working with rats trained in a Y maze, that LSD might not have the same effect on 'stupid' and 'intelligent' animals, the former reaching a 'possibly higher level of performance' following the administration of the hallucinogen.

Contrasting results have also been obtained when the effects of LSD on memory processes have been considered. Time-dependent disruption of memory in rats trained in a passive avoidance test, and no effect in rats trained to bar-press for water in a two-way avoidance condition have been described (Roberts et al., 1965; Weissman, 1967; Izquierdo, 1975). Stoff et al., (1974) have observed, moreover, that acute administration of LSD produced an excitatory effect (faster avoidance response latencies) in rats trained in the shuttle box. This effect was still present on the following saline test day, thus suggesting a possible effect of the hallucinogen on learning rather than on performance variables.

Finally, contrasting results, i.e., depressant or stimulating effects depending on the experimental conditions, have been obtained when the influence of LSD on activity has been investigated (Taeschler et al., 1960; Hamilton, 1960; Dandiya et al., 1969; Fontenay et al., 1970).

It must be underlined that some studies have suggested a possible involvement of serotonin and the adrenergic system in the behavioral effects of LSD (Dixon, 1968; Sugrue, 1969; King et al., 1974), even if its mechanisms of action are not yet well clarified (see review by Brawley and Duffield, 1972).

The C57BL/6 (C57) strain of mice, classified by Meier et al., (1963) among the 'high arousal' strains, is well-known both as concerns behavior and the levels and turnovers of mediators in the brain.

As concerns behavior, the C57 mice have shown to be poor performers in several experimental conditions

(Bovet-Nitti, 1969; Bovet et al., 1969; Elias, 1970; Sprott, 1971). They exhibit, moreover, high levels of locomotor activity in the toggle-floor box (Oliverio and Messeri, 1973; Oliverio et al., 1973). As concerns brain chemicals, the levels and turnovers of mediators in brains of C57 mice have been analyzed by a number of researchers (Eleftheriou, 1971; Kempf et al., 1974, 1976). The investigations have demonstrated high levels of noradrenergic and low levels of serotonergic turnovers at the brain stem level of this strain (see also review by Oliverio et al., 1979).

In the present research, the effects of LSD-25 on avoidance behavior, measured in the shuttle box (Bovet et al., 1969), and on locomotor activity, measured in the toggle-floor box (Oliverio and Messeri, 1973), were investigated in C57 mice. In the shuttle box, in particular, the effect of the hallucinogen on the consolidation processes were studied by injecting the drug after each training session. It is in fact known (McGaugh, 1966) that the consolidation processes may be influenced by the posttrial administration of drugs: drugs that improve performance may be regarded as enhancing memory, while drugs that impair performance may be regarded as disrupting memory.

Finally, the results of the present research are compared with those previously obtained with other hallucinogens, in the same strain tested in different experimental conditions (Castellano, 1978).

## Materials and Methods

*Subjects.* Male C57BL/6 mice (Charles River, Italy), weighing approximately 25 g at the beginning of the experiments, were used.

### *Avoidance Behavior*

*Apparatus and Testing Procedure.* Two batteries, each consisting of eight automated shuttle boxes, were used. The apparatus consisted of a rectangular Plexiglas box divided into two equal compartments by a small opening through a black partition (see Bovet et al., 1969). The CS was a 10-W light preceding the US by 5 s and overlapping it for 25 s. The level of illumination when the light was on was 2.0 fc at the floor level. The animals could avoid the US (continuous shock through the grid floor, 1.5 mA, delivered through a selenium rectifier) by running into the adjacent compartment within 5 s after the onset of the CS. If animals failed to avoid the shock they could escape it by crossing during the US. The intertrial interval was 30 s. Intertrial crossings were punished with shock. The shuttle boxes were located in sound-isolated cubicles.

Each group of mice was given a daily session of 100 trials (over 50 min) for five consecutive days. Intertrial responses, at a level lower than 3% for all groups, were evident only during the first session. The mean percentage avoidance responses constituted the score of each mouse.

### *Activity*

*Apparatus and Testing Procedure.* Two batteries, each consisting of eight toggle-floor cages, were used (Oliverio and Messeri, 1973). Each cage consisted of a rectangular white 40 × 10 cm Plexiglas box divided into two equal compartments by a 3 × 3 cm opening through a black partition. The number of crossings from one side of the box to the other was recorded automatically by means of a microswitch connected to the tilting floor of the box and constituted the score of the mouse. Circuitry was arranged so that whenever the mouse crossed the cage a cumulative counter was advanced. The cages were located in a sound-isolated cubicle. A light located 1.5 m above the top of the boxes was the source of illumination (0.25 fc at the cage floor level). Each group of mice was given a 50-min daily session for five consecutive days.

In both the activity and the avoidance-pretrial experiments, three different groups of eight mice were injected with 0.05, 0.1, and 0.2 mg/kg LSD, respectively, 15 min before testing. In the avoidance-posttrial experiments LSD (0.2 mg/kg) was injected to two different groups of eight mice, immediately or 2 h after each training session. The performances of the drugged mice were always compared with those of groups of eight saline-injected controls.

In all the experiments, LSD-25 (substance) was dissolved in saline and injected i.p.

The effects of the treatments on both avoidance behavior and activity were statistically evaluated by a trend analysis (Edwards, 1960). Additional analyses were carried out, when necessary, in order to obtain individual between-treatment comparisons (Lison, 1961).

## Results

### *Avoidance*

*Pretrial Treatments* (Fig. 1). In agreement with previous results (Bovet et al., 1969) the saline-injected C57 mice performed poorly in the shuttle box situation. Performance improvements were evident following LSD administration. The trend analysis for avoidance responses showed, in particular, significant differences between treatments ($df = 3,28$; $F = 580.9$; $P < 0.001$) between sessions ($df = 4,112$; $F = 694.2$; $P < 0.001$), and also a significant treatments × sessions interaction ($df = 12,112$; $F = 52.4$; $P < 0.001$).

In the additional analysis of individual between-treatment comparisons the significance of $F$ values was tested on the basis of 1,28 $df$.

All doses of LSD (0.05, 0.1, and 0.2 mg/kg) improved significantly the performances of the mice, as compared with those of the saline-injected controls ($F = 10.7$; $P < 0.01$; $F = 202.8$ and 1419.5, $P < 0.001$, respectively).

*Posttrial Treatments* (Fig. 2). Significant avoidance improvements were evident in the mice injected with LSD (0.2 mg/kg) immediately after each training session, compared with the saline-injected controls.

The trend analysis showed, in particular, significant differences between treatments ($df = 1,14$; $F = 1236.4$; $P < 0.001$), between sessions ($df = 4,56$; $F = 638.8$; $P < 0.001$) and also a significant treatments × sessions interaction ($df = 4,56$; $F = 166.9$; $P < 0.001$).

As concerns the mice injected 2 h following each training session, the trend analysis showed no signif-

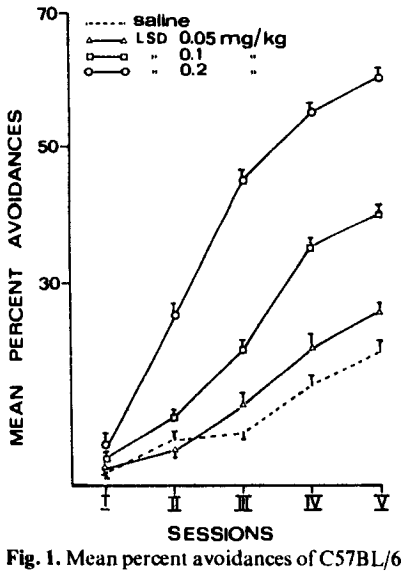


**Fig. 1.** Mean percent avoidances of C57BL/6 mice injected with saline or LSD-25 (0.05, 0.1, 0.2 mg/kg) 15 min before each training session in the shuttle box. Groups of eight animals. Vertical bars represent the standard errors

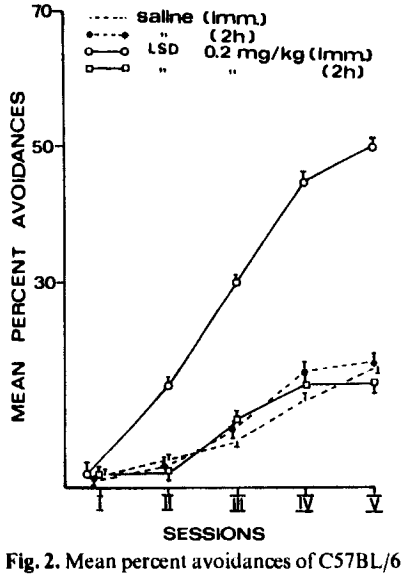


**Fig. 2.** Mean percent avoidances of C57BL/6 mice injected with saline or LSD-25 (0.2 mg/kg) immediately (imm.) or 2 h after each training session in the shuttle box. Groups of eight animals. Vertical bars represent the standard errors

icant differences between treatments ($df = 1,14$; $F = 0.1$; $P > 0.05$), significant differences between sessions ($df = 4,56$; $F = 145.2$; $P < 0.001$), and a significant treatments × session interaction ($df = 4,56$; $F = 5.66$; $P < 0.001$).

*Activity* (Table 1)

In agreement with previous experiments (Oliverio and Messeri, 1973; Oliverio et al., 1973) the saline-injected

**Table 1.** Effect of LSD-25 on the locomotor activity of C57BL/6 mice

| | |
|---|---|
| Saline | 112.0 ± 1.0 |
| LSD 0.05 mg/kg | 75.8 ± 1.1 |
| LSD 0.1 mg/kg | 47.1 ± 0.6 |
| LSD 0.2 mg/kg | 28.0 ± 1.2 |

Each value represents the mean number of crossings (± SE) (sessions I – V) of a group of eight mice from one side of the toggle-floor box to the other. Each session lasted 50 min

C57 mice displayed high levels of locomotor activity in the toggle-floor box. Activity decrements were evident following LSD administration. The trend analysis showed, in particular, significant differences between treatments ($df = 3,28$; $F = 1938.4$; $P < 0.001$), between sessions ($df = 4,112$; $F = 548.7$; $P < 0.001$) and a significant treatments × sessions interaction ($df = 12,112$; $F = 19.9$; $P < 0.001$).

In the additional analysis of individual between-treatment comparisons the significance of $F$ values was tested on the basis of 1,28 *df*.

All doses of LSD (0.05, 0.1, and 0.2 mg/kg) significantly decreased the activity levels of the mice, as compared with those of the saline-injected controls ($F = 951.6$, 3067.3, and 5138.5, respectively; $P < 0.001$).

## Discussion

As far as the saline-injected mice are concerned, the above reported results show, in agreement with previous data (Bovet et al., 1969), that the C57 strain performs poorly in the shuttle box.

From the first set of experiments, in which LSD-25 was injected to the mice before each experimental session, it is clearly evident that dose-related performance improvements followed its administration. Related to this point, it must be considered that performance impairments following pretrial LSD administrations have been observed by some investigators (Domino et al., 1965; Banerjee, 1971), while others (Bignami, 1972; King et al., 1974) have demonstrated performance improvements. As far as the effects of LSD on poorly performing animals are concerned, in particular, performance improvements after LSD injection have been described by Bignami et al. (1965) in pretrained rats that were poor performers of shuttle box escape/avoidance, and by Sansone et al. (1974) in naive hamsters injected with LSD during training in the same experimental conditions.

The results of the second set of experiments, in which LSD was injected immediately, or 2 h, after each

training session, clarify those of the pretrial experiments. It is in fact evident that, following the administration of LSD immediately after each experimental session, the performances of the mice were improved, while no effect was evident when it was injected 2 h after training.

The parallelism between the results of the pre- and posttrial administrations suggests that consolidation effects might account for the acquisition effects observed in the C57 strain. The existence of consolidation effects following the administration of the hallucinogen is clearly demonstrated by the fact that the LSD treatment 2 h after training was ineffective.

It must be, moreover, considered that the effect of the pretrial application could also be due to an influence on consolidation, because consolidation begins also during a multitrial session, and the effect of LSD applied before training probably lasts some time longer than the training session.

As concerns the influence of LSD on memory processes, it must be underlined that positive, negative or no effects have been found by investigators (Roberts et al., 1965; Weissman, 1967; Stoff et al., 1974; Izquierdo, 1975). However, experimental procedure, animal species, dose, and task seem to play important roles in modulating the effects of the hallucinogenic agents on the memory processes (Kahn et al., 1974). It must be pointed out, in particular, that high doses of LSD were necessary to affect the behavior of the mice in our experimental conditions, in comparison with its hallucinogenic effect.

As concerns the mechanism of action of LSD, one of the possible hypotheses could be that of an amphetamine-like effect on the behavior of the mice tested in the present research. This hypothesis would be in agreement with the results of previous researches carried out on different animal species tested in a variety of experimental conditions (Taeschler, 1967; Sansone et al., 1974). It must be pointed out, in particular, that the effects of LSD on performance have been correlated with a number of neurotransmitters; possible involvements of serotonergic and noradrenergic mechanisms, as well as effects of this hallucinogen on the dopamine receptors have in fact been suggested by some investigators (Dixon, 1968; Sugrue, 1969; King et al., 1974; Menon et al., 1977).

It seems interesting, at this point, to stress that analogies may be found between the results of the present research and previous results obtained with the same strain of mice following the administration of other hallucinogens. Recent researches (Castellano, 1978; Castellano, 1979) have in fact demonstrated that mescaline and psilocin, similarly to LSD in the present conditions, can improve the consolidation processes of C57 mice trained in light-dark or pattern discrimination tests. It must be underlined that researches carried out in man and in animals have shown that these three hallucinogens probably share common mechanisms of action (Wolbach et al., 1962; Hirshorn and Winter, 1971; Schechter and Rosencrans, 1972).

From the third set of experiments, it is clearly evident that LSD administration was followed by dose-related decrements of locomotor activity in C57 mice as compared with saline-injected controls. It must be observed, on this point, that both activity-stimulating and activity-depressing effects have been demonstrated following LSD administration (Taeschler et al., 1960; Hamilton, 1960; Dandyia et al., 1969; Fontenay et al., 1970). One possible explanation of the contrasting results might be, according to Fontenay et al. (1970), differences in the experimental conditions. Moreover, it must be emphasized that some authors have considered the behavioral depression or excitement evident in the LSD-injected animals to be two different aspects of the same central stimulation (see Boissier and Simon, 1969).

In conclusion, from the present research it is clearly evident that LSD-25 improves the acquisition and memory processes while, at the same doses, it depresses the locomotor activity of the mice belonging to the C57BL/6 strain.

Further researches to be carried out with high-performing strains of mice and nonhallucinogenic LSD derivatives, together with wider neurochemical investigations, will clarify the mechanisms through which LSD influences the behavioral measures considered.

*Acknowledgment.* Thanks are due to Mr. Giuseppe Olimpieri for his technical assistance.

## References

Banerjee, U.: Acquisition of conditioned avoidance responses in rats under the influence of addicting drugs. Psychopharmacologia **22**, 133–143 (1971)

Bignami, G., Robustelli, F., Yankü, I., Bovet, D.: Action de l'amphetamine et de quelques agents psychotropes sur l'acquisition d'un conditionnement de fuite et d'évitement chez des rats selectionnés en fonction du niveau particulièrement bas de leurs performances. C. R. Acad. Sci. **260**, 4273–4278 (1965)

Bignami, G.: Facilitation of avoidance acquisition by LSD-25. Possible effects on drive modulating systems. Psychopharmacologia **25**, 146–151 (1972)

Boissier, J. R., Simon, P.: Evaluation of experimental techniques in the psychopharmacology of emotion. Ann. N. Y. Acad. Sci., **159**, 898–914 (1969)

Bovet, D., Bovet-Nitti, F., Oliverio, A.: Genetic aspects of learning memory in mice. Science **163**, 139–149 (1969)

Bovet-Nitti, F.: Facilitation of simultaneous visual discrimination in four inbred strains of mice. Psychopharmacologia **14**, 193–199 (1969)

Brawley, P., Duffield, J. C.: The pharmacology of hallucinogens. Pharmacol. Rev. **24**, 31–66 (1972)

Castellano, C.: Effects of mescaline and psilocin on acquisition, consolidation and performance of light-dark discrimination in two inbred strains of mice. Psychopharmacology **59**, 129–137 (1978)

Castellano, C.: Effects of mescaline and amphetamine on simultaneous visual discrimination in two inbred strains of mice. Psychopharmacology (in press 1979)

Dandyia, P. C., Gupta, B. D., Gupta, M. L., Patni, S. K.: Effects of LSD on open field performance in rats. Psychopharmacologia **15**, 333–340 (1969)

Dixon, A. K.: Evidence of catecholamine mediation in the 'aberrant' behaviour induced by lysergic acid diethylamide (LSD) in the rat. Experientia **24**, 743–747 (1968)

Domino, E. F., Caldwell, D. F., Henke, R.: Effects of psychoactive agents on acquisition of conditioned pole jumping in rats. Psychopharmacologia **8**, 285–289 (1965)

Edwards, A. L.: Experimental design in psychological research. New York: Holt, Rinehart and Winston 1960

Eleftheriou, B. E.: Regional brain norepinephrine turnover rates in four strains of mice. Neuroendocrinology **7**, 329–336 (1971)

Elias, M. F.: Differences in reversal learning between two inbred mouse strains. Psychon. Sci. **20**, 179–180 (1970)

Fontenay, M., Le Cornex, J., Zaczinska, M., Debarle, M. C., Simon, P., Boissier, J. R.: Problèmes posés par l'utilisation de trois tests de comportement du rat pour l'étude des médicaments psychotropes. J. Pharmacol. **2**, 243–254 (1970)

Hamilton, C. L.: Effects of LSD-25 and amphetamine on a running response in the rat. Arch. Gen. Psychiatry **2**, 104–109 (1960)

Hirshorn, I. D., Winter, J. C.: Mescaline and lysergic acid diethylamide (LSD) as discriminative stimuli. Psychopharmacologia **22**, 64–71 (1971)

Izquierdo, I.: Relations between orienting, pseudoconditioned and conditioned responses in the shuttle box: A pharmacological analysis by means of LSD and dibenamine. Behav. Biol. **15**, 193–205 (1975)

Kahn, J., Gorelick, D. A., Bridger, W. H.: Mescaline facilitates retention of passive avoidance in rats. Physiol. Psychol. **2**, 120–122 (1974)

Kempf, E., Greilsamer, J., Mack, G., Mandel, P.: Correlation of behavioral difference in three strains of mice with differences in brain amines. Nature New Biol. **287**, 483–485 (1974)

Kempf, E., Gill, M., Mack, G., Mandel, P.: Effects of acute morphine administration on the catecholamine metabolism of three strains of mice. Psychopharmacol. Comm. **2**, 241–250 (1976)

King, A. R., Martin, I. L., Melville, A.: Reversal learning enhanced by lysergic acid diethylamide (LSD): concomitant rise in brain 5-Hydroxytryptamine levels. Br. J. Pharmacol. **52**, 419–426 (1974)

Lison, L.: Statistica applicata alla biologia sperimentale. Milano: Casa Editrice Ambrosiana 1961

McGaugh, J. L.: Time dependent processes in memory storage. Science **134**, 328–342 (1966)

Meier, G. W., Hatfield, J. L., Foshee, D. P.: Genetic and behavioral aspects of pharmacologically induced arousal. Psychopharmacologia **4**, 81–90 (1963)

Menon, M. K., Clark, W. G., Masuoka, D. T.: Possible involvement of the central dopaminergic system in the antireserpine effect of LSD. Psychopharmacology **52**, 291–297 (1977)

Oliverio, A., Castellano, C., Puglisi Allegra, S.: A genetic approach to behavioral plasticity and rigidity. In: Theoretical advances in behavioral genetics, J. R. Royce, ed., NATO Adv. Study Inst. New York: Plenum 1979 (in press)

Oliverio, A., Eleftheriou, B. E., Bailey, D. W.: Exploratory activity: genetic analysis of its modification by scopolamine and amphetamine. Physiol. Behav. **10**, 893–899 (1973)

Oliverio, A., Messeri, P.: An analysis of single-gene effects on avoidance, maze, wheel running and exploratory behavior in the mouse. Behav. Biol. **8**, 771–783 (1973)

Roberts, M. H. T., Darlington, D., Bradley, P. B.: The effect of chlorpromazine or *d*-lysergic acid diethylamide on one-trial passive avoidance. In: Neuropsychopharmacology, Vol. 4. Proc. of the 4th Meeting Coll. Int. Neuro-Psychopharmacologicum, D. Bente and P. B. Bradley, eds. New York: Elsevier 1965

Sansone, M., Castellano, C., Messeri, P.: Facilitation of avoidance behaviour by LSD-25 and mescaline in hamsters. Pharmacol. Res. Commun. **6**, 369–375 (1974)

Schechter, M. D., Rosencrans, J. A.: Lysergic acid diethylamide (LSD) as a discriminative cue: drugs with similar stimulus properties. Psychopharmacologia **26**, 313–316 (1972)

Sprott, R. L.: Inheritance of avoidance learning. The Jackson Laboratory's Ann. Rep. **42**, 78 (1971)

Stoff, D. M., Mandel, I. J., Gorelick, D. A., Bridger, W. H.: Acute and chronic effects of LSD and 3,4-Dimetoxyphenylethylamine on shuttle-box escape/avoidance in rats. Psychopharmacologia **36**, 301–312 (1974)

Sugrue, M. F.: A study of the role of noradrenaline in behavioral changes produced in the rat by psychotomimetic drugs. Br. J. Pharmacol. **35**, 243–252 (1969)

Taeschler, M.: Pharmacology of psychotomimetic agents. In: Neuropsychopharmacology, H. Brill, J. O. Cole, P. Deniker, H. Hippius, P. B. Bradley, eds., pp. 393–397. Amsterdam: Excerpta Medica 1967

Taeschler, M., Weidmann, H., Cerletti, A.: The effect of LSD on reaction time in a conditioned avoidance reaction and in the analgesia test. Helv. Physiol. Pharmacol. Acta **18**, 43–49 (1960)

Waser, P. G., Martin, A., Heer-Carcano, L.: The effect of $\Delta^9$-Tetrahydrocannabinol and LSD on the acquisition of an active avoidance response in the rat. Psychopharmacologia **46**, 249–254 (1976)

Weissman, A.: Drugs and retrograde amnesia. Int. Rev. Neurobiol. **10**, 167–198 (1967)

Wolbach, A. B., Miner, E., Isbell, H.: Comparison of psilocin with psilocybin, mescaline and LSD-25. Psychopharmacologia **3**, 219–223 (1962)

Received August 7, 1978; Final Version November 12, 1978