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Actions of Repeated Injections of LSD and Apomorphine on the Copulatory Response of Female Rats[1,2,3]

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ELIASSON, M. *Actions of repeated injections of LSD and apomorphine on the copulatory response of female rats.* PHARMAC. BIOCHEM. BEHAV. 5(6) 621–625, 1976. – LSD, a serotonin receptor stimulating agent, inhibits copulatory behavior (lordosis response) in the ovariectomized and estrogen + progesterone treated female rat. The same effect is obtained by apomorphine, a dopamine receptor stimulating compounds. The lordosis has been shown to be dependent on serotonin, but also dopamine has been implicated in its mediation. Tolerance develops to certain responses after repeated injections of LSD and in the present study the influence of apomorphine and LSD was compared, when given in repeated doses. Possible cross-tolerance between the two compounds was also tested on the frequency of lordosis responding in ovariectomized and hormone treated female rats. Tolerance to LSD develops over seven days, while the suppressing influence of apomorphine on lordosis in seven repeated doses is not significantly altered from that of a single dose. No cross-tolerance was observed on the lordosis response with either order of treatments. Repeated doses of LSD did not influence locomotor activity differently from a single dose, while repeated doses of apomorphine enhanced this response in comparison with the effect of an acute dose. These results indicate differential sensitivity to the repeated treatments and further support an interpretation of the LSD effects on lordosis responding to be primarily on serotonergic rather than dopaminergic receptors.

LSD Apomorphine Lordosis response Tolerance

TOLERANCE appears in certain behavioral responses to lysergic acid diethylamide (LSD). This has been found in animal as well as human studies, and is seen in the clinic as well as in the laboratory [1, 16, 17, 24].

When tested in laboratory rats, tolerance to LSD develops for such diverse behaviors as rope-climbing [12,18] and bar pressing for food reinforcement [5,13]. On the other hand, in a behavioral performance maintained by means of escape from electric foot shock, tolerance to LSD in comparable doses does apparently not emerge, but extinction of the response is delayed [15].

The behavioral modifications appearing as a consequence of repeated LSD injections are accompanied by certain biochemical alterations that are related to brain monoamines. Repeated doses of a magnitude comparable to those used in behavioral studies give an unchanged level of serotonin, while turnover is significantly increased [23]. Higher doses seem to affect the catecholamine levels in addition to those of serotonin [6, 14, 26].

LSD is considered to be a serotonin receptor stimulating compound [2,4] and in accordance with this, functions mediated by serotonin have been found to be influenced by single doses of LSD. One such behavior component is the copulatory response of the female rat (lordotic response). This response, which consists of an arching of the back, head and perineum elevation and tail deviation, is dependent on ovarian hormones [31] and is readily elicited in receptive female rats by a mounting male. Much evidence is available demonstrating inhibition of the lordosis response to be mediated by activation of serotonin and also dopaminergic neurons in ovariectomized female rats treated with estrogen and progesterone [19, 20, 22]. This also seems to be the case when the lordosis response is studied in ovariectomized female rats treated with repeated doses of estrogen and no progesterone [11, 28, 32]. LSD in a rather small dose (0.05 μg/kg) lacking significant effects on e.g. locomotor behavior, inhibits lordosing in female rats receiving estrogen + progesterone [7,8]. Apomorphine, a compound with dopamine receptor stimulating properties [3,9] also has an inhibitory action on the lordosis behavior activated by estrogen and progesterone [8,22]. Another dopamine agonist (ET 495) is reported to have similar effects, when lordosis is induced by estrogen only [10]. The influence of apomorphine was found to be completely

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[3] The hormones were generous gifts supplied by N.V. Organon through Erco Ltd., Stockholm.

counteracted by pretreatment with a dopamine receptor blocker (pimozide), while the LSD induced inhibition of lordosis was only decreased in its duration. This was taken as an indication of the differential mechanisms of action behind the effects of the two drugs on lordosis responding [8].

The purpose of the present investigation was to further analyze the extent of dopaminergic relative to serotonergic involvement in the LSD induced inhibition of the lordosis response by comparing it to apomorphine, when both drugs are given in repeated doses. The development of tolerance and possible cross-tolerance, might further elucidate the relationship of these two drugs and their effects on lordosis responding. Lordosis has the advantage, in addition to its known relation to serotonin and dopamine, of not requiring any training before its manifestation, making it particularly suitable for a study of tolerance.

METHOD

Animals

Ovariectomized female rats of the Sprague-Dawley strain (Purchased as Specific Pathogen Free, Anticimex, Stockholm) were used. They were housed 5–6 to a cage in stainless steel cages, where tap water and commercial rat pellets (Anticimex 210 or R 3) were available at all times. The animal room held a constant temperature (21 ± 1°C), had forced ventilation and controlled humidity. The day-night cycle was reversed (lights on from 9 p.m. to 9 a.m.). Wistar male rats with considerable sexual experience were used for testing of the females. The males were housed in individual cages (40 × 40 × 30 cm), which also served as test cages, in a different section of the animal room.

Materials injected. The hormones, estradiol benzoate (EB) and progesterone (both from Organon), were dissolved in olive oil and injected subcutaneously. Apomorphine and lysergic acid diethylamide (LSD) tartrate (both from Sandoz) were dissolved in saline. LSD was injected intraperitoneally, while apomorphine was given subcutaneously. Fresh solutions were made up of the two latter compounds for every drug test. The doses which appear in the text and tables refer to the form of the compounds given above.

Behavioral observations. Before being used in a drug test each group of 10–12 animals was subjected to a standard test with the regular doses of hormones used (10 μg/kg EB and 0.4 mg progesterone 48 hr later) to asses the level of lordosis responding in relation to what is the standard of the laboratory. This is about 60–80% response in a group during the height of the hormone effect, 6–7 hr after progesterone. The values before each test are indicated in the tables. The hormones were injected at 8 a.m. and testing was performed between noon and 5 p.m. at certain intervals, when the hormone influence is relatively stable. Animals given repeated injections of drug were always injected at the same time of the day, i.e. 24 hr apart, with lordosis testing beginning 10 min after the last injection.

The females were tested individually in the home cages of the males. Only absence or presence of lordosis was scored for each mount and a maximum of six mounts by two different males was allowed. To be rated as positive by the observer the female had to show two consecutive lordoses with head and perineum raised and the tail deviated each time. Thus, the measure for each treatment was the percentage females showing lordosis according to our criterion on a particular test. (For further details regarding methods see Eliasson and Meyerson [7]).

Locomotor exploratory activity. Another indication of drug effects was the amount of locomotor exploratory activity exhibited during a 10 min test. The animals were placed one at a time in a testing box located on top of an Animex meter (Farad Inc., Hägersten, Sweden), where their locomotor activity was recorded automatically and accumulated for the whole testing period. The sensitivity of the apparatus was adjusted so that fine motor activity like grooming and sniffing was not recorded. Testing was performed in a dark and quiet room with no observer present. This took place after repeated and single injections of LSD or apomorphine in independent groups. The animals were the same as those participating in the lordosis study and those receiving multiple injections had received also EB the morning of the day before testing and were scheduled to be tested on lordosis on the following day. (Svensson and Thieme [27] give the details regarding the testing apparatus).

Statistics. Differences between groups of independent animals were analyzed statistically using the Chi square test corrected for continuity [25]. For certain treatments these groups have been combined in the tables for a easier presentation of results. Differences between locomotor scores in groups receiving single and groups receiving multiple injections were analyzed through the use of the *t*-test [29].

RESULTS

As is shown by Table 1, treatment with repeated injections of LSD for four days and with testing commencing 10 min after the last injection does not give any altered response on the lordosis inhibition test at a dose of 0.10 mg/kg. This is a dose that in a single injection significantly decreases the percentage lordosis responding females, enhances startle responding and gives some decrease of locomotor activity [7,8]. Still after repeated injections during four days the general appearance of the animals is more normal, there is less piloerection, fewer flight responses to sudden stimuli, but ataxia of the hindlegs is still present. The larger doses of 0.25 and 0.50 mg/kg, when administered for four days, also do not attenuate the lordosis inhibitory effect of LSD in comparison with single injections.

A longer period of daily LSD injections does give an altered response as is evident from Table 2. When LSD, 0.10 mg/kg is given daily for a period of seven days, lordosis inhibition tested on the last day of injection with the appropriate hormone treatment, is smaller than after a single injection. Significantly more females respond with lordosis after multiple injections than after a single one, when tested at the time of a maximal effect, 10 min after injection. The duration of the LSD influence is altered also, as a significantly lower frequency of lordosis inhibition is obtained 40 min after the LSD injection, when there is a multiple treatment regimen in comparison with a single treatment ($\chi^2 = 4.88$, $df\ 1$; $p<0.05$).

Repeated treatments with apomorphine in a dose that significantly inhibits lordosis responding after a single injection does not appear to have any effect comparable to that of LSD, when tested under the same conditions. The pattern of lordosis responding after seven daily injections of

TABLE 1

EFFECTS OF SINGLE DOSE AND FOUR REPEATED DOSES OF LSD ON DISPLAY OF LORDOSIS IN THE FEMALE RAT

Treatment mg/kg	Percentage Females Responding preinj.	10 min	40 min	90 min	180 min	N
Saline	71	75	79	82	71	28 (3)†
LSD 0.10 x 1	67	25	48	92	92	24 (2)
LSD 0.10 x 4	73	45	73	91	100	11 (1)
LSD 0.25 x 1*	68	0	23	73	73	22 (4)
LSD 0.25 x 4	52	0	24	82	94	17 (2)
LSD 0.50 x 1*	84	0	0	58	90	31 (3)
LSD 0.50 x 4	50	0	11	50	89	18 (2)

*Data taken from Eliasson and Meyerson (7).
†Numbers within parentheses indicate number of replications tested.

apomorphine at 0.5 mg/kg does not differ significantly from the influence of a single injection at this dose.

After a series of six daily apomorphine injections preceding one dose of LSD, there is an effect of LSD not different from the acute influence of the same dose as shown by Table 3 A. The reverse order of treatments does not give any significant results either (Table 3 B):

There was an obvious qualitative difference, however, between females receiving a single dose of LSD and those getting LSD after a series of apomorphine injections. The latter groups resisting and rejecting the mounting attempts by the males to an extent not observed after other treatments.

Repeated treatments with LSD do not seem to affect locomotor behavior in any way different from a single treatment with an equal dose, as indicated by the results in Table 4 ($t = 0.87$, df 9; NS). Apomorphine, on the other hand, acts differently under the two treatment regimens, when tested on locomotor exploration. A single dose of apomorphine suppresses the behavior, while six daily

TABLE 2

EFFECTS OF SINGLE AND REPEATED INJECTIONS OF LSD AND APOMORPHINE ON DISPLAY OF LORDOSIS IN THE FEMALE RAT

Treatment mg/kg	Percentage Females Responding Preinj.	10	40/60*	90/120	180 min	N
Saline	71	68	76	81	71	21 (4)†
LSD 0.10 x 1	63	38	54	79	83	24 (2)
LSD 0.10 x 7	83	72‡	81‡	88	94	36 (3)
Apomorphine 0.5 x 1	83	33	89	89	—	18 (2)
Apomorphine 0.5 x 7	100	50	92	100	—	18 (2)

*LSD was tested 10, 40, 90 and 180 min after the last injection. Apomorphine was tested 10, 60 and 120 min after the last injection. Saline controls have been pooled, as there were no differences between groups tested according to either schedule.
†Same as Table 1.
‡Significantly different from LSD 0.10 x 1 at $p<0.05$.

TABLE 3

EFFECTS OF REPEATED INJECTIONS OF APOMORPHINE+SINGLE INJECTION OF LSD AND OF REPEATED INJECTIONS OF LSD+SINGLE INJECTION OF APOMORPHINE ON THE FREQUENCY OF LORDOSIS RESPONDING

	Treatment mg/kg	Percentage Females Responding Preinj.	10	40/60*	90/120	180 min	N
A.	Apomorphine 0.2 x 6+ LSD 0.1	75	17	58	83	100	12 (1)†
	Apomorphine 0.5 x 6+ LSD 0.1	61	14	64	90	90	28 (3)
	LSD 0.1	82	32	50	86	86	22 (2)
B.	LSD 0.1 x 6+ Apomorphine 0.2	63	50	69	88	—	16 (2)
	LSD 0.1 x 6+ Apomorphine 0.5	79	21	83	83	—	24 (2)
	Apomorphine 0.2	83	42	92	92	—	12 (1)
	Apomorphine 0.5	85	40	60	95	—	20 (2)

*†See Tables 1 and 2.

TABLE 4

EFFECTS OF SINGLE AND REPEATED DOSES OF LSD AND APOMORPHINE ON LOCOMOTOR EXPLORATORY ACTIVITY (N = 10)

Treatment mg/kg	Average Score ± SE	
Saline	775.5 ± 32.61	
LSD 0.10 x 1	635.4 ± 47.03	
LSD 0.10 x 6	577.9 ± 19.63†	
Apomorphine 0.5 x 1	447.4 ± 36.05†	
Apomorphine 0.5 x 6	1001.2 ± 43.18*	
Apomorphine 0.5 x 5 + NaCl	723.3 ± 77.98	(n = 6)

*Significantly different from saline controls at $p<0.05$.
†Significantly different from saline controls at $p<0.01$, as tested by Students t-test (Two-tailed).

injections preceding testing seem to stimulate it. There is a highly significant difference between these two treatment schedules ($t = 6.78$, $df\ 9$; $p<0.01$). This effect is enhanced by the results obtained in the group treated with repeated doses of apomorphine and tested after an injection of saline. Stereotype sniffing was observed under both conditions. Locomotor behavior in both LSD and apomorphine treated animals differs from the saline group, when the drugs have been given repeatedly ($t = 3.74$, $df\ 9$; $p<0.01$ and $t = 2.98$, $df\ 9$; $p<0.05$, respectively) however, the direction of this deviation is different for each compound.

DISCUSSION

With repeated doses of LSD tolerance to the inhibitory influence of this drug on lordotic responding in the ovariectomized, estrogen and progesterone treated female rat, does appear to develop. There is an attenuation of the lordosis inhibition and a clear abbreviation of the duration of the LSD effect. A certain length of time seems to be a prerequisite for this action to develop, since tolerance is obtained after seven or more daily injections, but not after only four at the same dose. Larger doses of LSD, which in single injections have a longer duration and a greater effect, are not effective in inducing tolerance over a shorter period. Apomorphine, when compared under identical conditions, does not show any evidence of tolerance, there is no modification of the effect of a single treatment, with the presently used doses and number of injections.

Tolerance to repeated doses of LSD has previously been shown on more complex kinds of conditioned behavior [5, 12, 13, 18, 30], where the response typically is interrupted by LSD for varying periods of time. Daily injections and daily testing show this period to be gradually shortened over 3–4 or 7–8 days with doses comparable to the presently used one. The results obtained on lordosis responding are in agreement with these studies in that the maximal disrupting effect is attenuated and the duration of the effect is decreased by repeated injections of LSD. However, four days do not appear to be enough for tolerance to LSD to develop, when tested on the unlearned lordosis response. In previous investigations pretreatment with a serotonin synthesisinhibitor, PCPA, was found to have an enhancing influence on the LSD suppression of lordosis. Interference with the biosynthesis of catecholamines by pretreatment with α-methyl-para-tyrosine in comparable tests if anything shortened the duration of the LSD effect [7]. In addition, the prevention of lordosis inhibition, when induced by apomorphine, by pimozide, a dopamine receptor blocking agent, did not take place when lordosis responding was inhibited by LSD. Also here was there an abbreviated effect [8]. Both of these findings indicate differential involvements of dopamine and serotonin receptors in the effects on lordosis inhibition by apomorphine and LSD, respectively. The influence of pimozide on the apomorphine effect was interpreted as a direct influence on dopamine receptors, however, this does not seem to be the case for the action of LSD. The significant lordosis inhibitory effects of this compound can be obtained also in small doses without any significant effects on e.g. locomotor behavior, further indicating that the action of LSD on lordosis is most likely due to an effect on serotonergic receptors, mainly. These interpretations could be extended to include the present results.

When receptors have been stimulated repeatedly by LSD there is a difference in sensitivity to the last injection in the series compared to that of a single one, as tested on the lordosis. There is tolerance as the original effect is decreased. That this tolerance to LSD is probably not due to a change in metabolism is suggested by the findings of Winter [30]. In his study a dose, equivalent to the one inducing tolerance in the present investigation, did give tolerance on a barpressing test. There was also no difference in its effects on brain and liver concentrations of LSD between groups receiving repeated treatments and groups getting a single injection, nor were these concentrations of different durations in respective groups. Freedman and Boggan [14] reported similar observations.

When apomorphine is tested under the same conditions the influence after seven injections on lordosis is not significantly different from that of a single one. That it is possible to modify a response by repeated injections of apomorphine over a similar time period is indicated by the test of locomotor behavior, which was facilitated by repeated apomorphine treatments.

Present data taken together with previous work [7,8] strengthens the view that lordosis inhibition by LSD is more likely mediated by an action primarily on serotonergic than on dopaminergic mechanisms.

REFERENCES

1. Abramson, H. A. Tolerance to LSD-25 and a theory of psychoses. In: *Neuropharmacology*, edited by H. A. Abramson. New York: Josiah Macy, Jr. Foundation, 1956.
2. Aghajanian, G. K., W. E. Foote and M. H. Sheard. Lysergic acid diethylamide: Sensitive neuronal units in the midbrain raphe. *Science* **161**: 706-708, 1968.
3. Andén, N. -E., A. Rubenson, K. Fuxe and T. Hökfelt. Evidence for dopaminergic receptor stimulation by apomorphine. *J. Pharm. Pharmac.* **19**: 627-629, 1967.
4. Andén, N. -E., H. Corrodi, K. Fuxe and T. Hökfelt. Evidence for a central 5-hydroxytryptamine receptor stimulation by lysergic acid diethylamide. *Br. J. Pharmac.* **34**: 1-7, 1968.
5. Appel, J. B. and D. X. Freedman. Tolerance and cross-tolerance among psychotomimetic drugs. *Psychopharmacologia* **13**: 267-274, 1968.
6. Boggan, W. O. and D. X. Freedman. LSD tolerance and serotonin metabolism. *Fedn Proc.* **32**: 694, Abstract. 1973.

7. Eliasson, M. and B. J. Meyerson. The effects of lysergic acid diethylamide on copulatory behavior in the female rat. *Neuropharmacology,* in press, 1976.
8. Eliasson, M. and B. J. Meyerson. Comparison of the actions of LSD and apomorphine on the copulatory response in the female rat. *Psychopharmacologia,* in press, 1976.
9. Ernst, A. M. Mode of action of apomorphine and dexamphetamine on gnawing compulsion in rats. *Psychopharmacologia* **10:** 316-323, 1967.
10. Everitt, B. J., K. Fuxe and T. Hökfelt. Inhibitory role of dopamine and 5-hydroxytryptamine in the sexual behavior of female rats. *Eur. J. Pharmac.* **29:** 187-191, 1974.
11. Everitt, B. J., K. Fuxe, T. Hökfelt and G. Jonsson. Role of monoamines in the control by hormones of sexual receptivity in the female rat. *J. comp. physiol. Psychol.* **89:** 556-572, 1975.
12. Freedman, D. X., G. K. Aghajanian, B. Rosner and E. M. Ornitz. Patterns of tolerance to lysergic acid diethylamide and mescaline in rats. *Science* **127:** 1173-1174, 1958.
13. Freedman, D. X., J. B. Appel, F. R. Hartman and M. E. Molliver. Tolerance to behavioral effects of LSD-25 in rat. *J. Pharmac. exp. Ther.* **143:** 309-313, 1964.
14. Freedman, D. X. and W. O. Boggan. Brain serotonin metabolism after tolerance dosage of LSD. *Adv. Biochem. Psychopharmac.* **10:** 151-157, 1974.
15. Hamilton, C. L. Effects of LSD-25 and amphetamine on a running response in the rat. *Archs gen. Psychiat.* **2:** 104-109, 1960.
16. Isbell, H., H. F. Fraser. A. Wikler and R. E. Belleville. Tolerance to diethylamide of lysergic acid (LSD-25). *Fedn Proc.* **14:** 354, 1955.
17. Isbell, H., R. E. Belleville, H. F. Fraser, A. Wikler and C. R. Logan. Studies on lysergic acid diethylamide (LSD-25). I. Effects in former morphine addicts and development of tolerance during chronic intoxication. *A.M.A. Archs Neurol. Psychiat.* **76:** 468-478, 1956.
18. Mahler, D. J. and F. L. Humoller, Effect of lysergic acid diethylamide and bufotenine on performance of trained rats. *Proc. Soc. exp. Biol. Med.* **102:** 697-701, 1959.
19. Meyerson, B. J. Central nervous monoamines and hormone induced estrous behavior in the spayed rat. *Acta physiol. scand.* **63:** Suppl. 241, 1964.
20. Meyerson, B. J. The effect of neuropharmacological agents on hormone-activated estrous behaviour in ovariectomized rats. *Archs Int. Pharmacodyn.* **150:** 4-33, 1964.
21. Meyerson, B. J., M. Eliasson, L. Lindström, A. Michanek and A. Ch. Söderlund. Monoamines and female sexual behavior. In: *Psychopharmacology, Sexual Disorders and Drug Abuse,* edited by T. A. Ban *et al.* Amsterdam: North-Holland Publishing Company, 1973, pp. 463-473.
22. Meyerson, B. J., H. Carrer and M. Eliasson. 5-Hydroxytryptamine and sexual behavior in the female rat. *Adv. Biochem. Psychopharmac.* **11:** 229-242, 1974.
23. Peters, D.A.V. Comparison of the chronic and acute effects of D-lysergic acid diethylamide (LSD) treatment on rat brain serotonin and norepinephrine. *Biochem. Pharmac.* **23:** 231-237, 1974.
24. Rosenberg, D. E., A. B. Wolbach, Jr., E. J. Miner and H. Isbell. Observations on direct and cross tolerance with LSD and D-amphetamine in man. *Psychopharmacologia* **5:** 1-15, 1963.
25. Siegel, S. *Nonparametric Statistics for the Behavioral Sciences.* New York: McGraw-Hill, 1956.
26. Smith, R. C., W. O. Boggan and D. X. Freedman. Effects of single and multiple doses of LSD on endogenous levels of brain tyrosine and catecholamines. *Psychopharmacologia* **42:** 271-276, 1975.
27. Svensson, T. H. and G. Thieme. An investigation of a new instrument to measure motor activity of small animals. *Psychopharmacologia* **14:** 157-163, 1969.
28. Ward, I. L., W. R. Crowley, F. P. Zemlan and D. L. Margules. Monoaminergic mediation of female sexual behavior. *J. comp. physiol. Psychol.* **88:** 53-61, 1975.
29. Winer, B. J. *Statistical Principles in Experimental Design.* 2nd Ed. New York: McGraw-Hill, 1971.
30. Winter, J. C. Tolerance to a behavioral effect of lysergic acid diethylamide and crosstolerance to mescaline in the rat: Absence of a metabolic component. *J. Pharmac. exp. Ther.* **178:** 625-630, 1971.
31. Young, W. C. The hormones and mating behavior. In: *Sex and Internal secretions,* edited by W. C. Young, Vol. 2. Baltimore, Maryland: Williams and Wilkins Co., 1961.
32. Zemlan, F. P., I. L. Ward, W. R. Crowley and D. L. Margules. Activation of lordotic responding in female rats by suppression of serotonergic activity. *Science* **179:** 1010-1011, 1973.