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Persistent effects of repeated injections of D-lysergic acid diethylamide on rat brain 5-hydroxytryptamine and 5-hydroxyindoleacetic acid levels

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It is well known that D-lysergic acid diethylamide (LSD) has marked effects on central 5-hydroxytryptamine (5-HT)-containing neurons. Aghajanian *et al.* [1, 2] reported that a small dose of LSD blocks the firing of 5-HT neurons in the midbrain raphe. Similarly, the increase in 5-hydroxyindoleacetic acid (5-HIAA) in rat forebrain produced by an elevated environmental temperature and suggested to be due to an increased firing of 5-HT neurons was also prevented by pretreatment with LSD [3]. In other studies, LSD produced a short-lasting increase in the 5-HT level and a corresponding decrease in the 5-HIAA level [4], reduced 5-HT synthesis [5, 6], and blocked 5-HT release [7–11] in rat brain. Depletion of brain 5-HT by *p*-chlorophenylalanine intensified the behavioral effects of LSD [12].

Injections once a day of a small dose of LSD (20 μg/kg) for 2 weeks have been shown to result in a significantly reduced 5-HT turnover in rat midbrain when measured 24 hr after the last injection [13]. In contrast, a higher dose of LSD (100 μg/kg) for 2 weeks [14] or the same dose (20 μg/kg) given orally for 1 month [15] produced significant increases in rat brain 5-HT turnover.

Repeated LSD use in humans has been reported to result in persistent reactions including "flashbacks," i.e. a recurrence of certain drug effects long after the immediate effects of the drug have worn off (see for example Ref. 16). A frequent speculation has been that repeated LSD use may cause persistent neurochemical changes which may underlie this phenomenon. We, therefore, investigated whether changes in 5-HT and 5-HIAA levels produced by repeated LSD injections to rats persisted beyond the period of 24 hr after the final treatment previously studied.

Male Sprague–Dawley rats (85–100 g) were given injections once a day of LSD (100 μg/kg) in physiological saline (2 ml/kg) by the intraperitoneal route. All injections were given between 10:00 and 10:30 a.m. and unless otherwise stated the animals were killed by decapitation 24 hr after the final injection. In the first experiment, the animals received either LSD for 4 weeks or LSD for 2 weeks followed by saline for 2 weeks. The control group was given daily vehicle (saline) injections for 4 weeks. In the second experiment, the rats received either LSD or its injection vehicle for 2 weeks and the animals were killed 15 days later.

After decapitation the brains were quickly removed, divided sagittally into equal left and right sides and each half was dissected into cortex, cerebellum, midbrain, pons-medulla, corpus striatum, and the remainder. The regions from the right side of the brain were homogenized in 4–6 vol. of acidified butanol for the assay of 5-HT and 5-HIAA by the combined methods of Maickel *et al.* [17] and Curzon and Green [18]. The remaining brain parts were homogenized in 0.3 M trichloroacetic acid for the assay of tryptophan by the method of Denckla and Dewey [19].

Table 1 shows the effect of 4 weeks of LSD treatment on 5-HT and 5-HIAA levels in the six brain regions studied. We have previously shown that repeated LSD injections (100 μg/kg/day for 14 days) produce a marked increase in brainstem 5-HIAA level (35 per cent) without a significant change in 5-HT [14]. In the present work, in which the period of treatment was increased to 4 weeks and the brainstem sub-divided into midbrain and pons-medulla, the 5-HIAA levels were not significantly different from control values, although the midbrain 5-HT level showed a small but statistically significant increase (11 per cent, $P < 0.05$). This reduced effect of LSD on brainstem 5-HT and 5-HIAA after extended treatment may be a reflection of the tolerance that occurs after repeated LSD treatments in the rat [20]. The only brain region studied in which this long-term treatment with LSD had any marked effect was the cerebellum. The increase in 5-HIAA and the decrease in 5-HT are consistent with an increased 5-HT turnover in this region.

Table 2 shows the effect of 2 weeks of LSD treatment on 5-HT and 5-HIAA levels measured 2 weeks after the last LSD injection. The results were similar regardless of whether daily saline injections (LSD + saline group) or no further treatment (LSD only group) was given during the final 2 weeks. The 5-HT levels in cortex and midbrain were significantly increased by both treatments without significant changes in 5-HIAA levels. The lack of change in the 5-HIAA level suggests that 5-HT turnover was not

Table 1. Effect of 4 weeks of LSD treatment on 5-HT and 5-HIAA levels in various rat brain regions*

	5-HT (ng/g)		5-HIAA (ng/g)	
Brain region	Saline	LSD	Saline	LSD
Cerebellum	407 ± 19	335 ± 24†	182 ± 7	208 ± 7†
Cortex	475 ± 33	550 ± 22	261 ± 14	263 ± 21
Midbrain	1156 ± 21	1282 ± 35†	645 ± 16	612 ± 19
Pons-medulla	1203 ± 36	1111 ± 28	624 ± 22	643 ± 25
Striatum	1146 ± 43	1116 ± 84	660 ± 27	724 ± 27
Remainder	795 ± 27	730 ± 49	563 ± 9	537 ± 23

* LSD (100 μg/kg) or saline injections were given daily for 28 days and the animals killed 24 hr after the final injection. Results are given as mean ± S. E. M. for groups of ten or more rats.

† $P < 0.05$ (*t*-test).

Table 2. Effect of LSD and saline injections on 5-HT and 5-HIAA levels in various rat brain regions*

Brain region	5-HT (% control)		5-HIAA (% control)	
	LSD + saline	LSD only	LSD + saline	LSD only
Cerebellum	100 ± 5	102 ± 4	98 ± 3	109 ± 6
Cortex	120 ± 4†	138 ± 8†	103 ± 3	104 ± 6
Midbrain	109 ± 3†	116 ± 3†	91 ± 3	106 ± 3
Pons-medulla	103 ± 6	107 ± 5	103 ± 4	89 ± 6
Striatum	92 ± 5	102 ± 5	100 ± 4	87 ± 5
Remainder	104 ± 5	118 ± 9	93 ± 3	100 ± 3

* Rats received daily LSD injections (100 μg/kg) for 14 days followed by either 14 days of vehicle injections (LSD + saline group) or 14 days without further injections (LSD only group). Results are given as per cent of the corresponding control value (2 or 4 weeks saline).

† $P < 0.05$.

altered 15 days after the last LSD injection, in marked contrast to our previous reports of a marked change in 5-HIAA level and 5-HT turnover in brainstem or midbrain 24 hr after the final LSD injection [13, 14]. However, there was a consistent increase in 5-HT level after the 15-day post-LSD period which, in the absence of changes in 5-HIAA, suggests an increased 5-HT storage. It is interesting that a similar increase in cortical and midbrain 5-HT was found in the 4-week LSD experiment, although in cerebral cortex the apparent increase was not statistically significant ($P < 0.1 > 0.05$). The increased level of 5-HT in two brain regions 15 days after the last of 14 daily LSD injections suggests a persistent LSD-induced neurochemical alteration. However, the significance of this increase and its possible relationship to the persistent effects of LSD use in man are not yet clear. None of the brain regions studied showed a tryptophan level which was significantly different from the whole brain value of 5.81 ± 0.20 μg/g. The tryptophan levels were not significantly altered by LSD treatment.

In summary, two weeks after the last of 14 daily LSD injections (100 μg/kg) to rats, there was a significantly increased 5-HT level in both midbrain and cerebral cortex without a change in 5-HIAA levels. This suggests that biochemical effects of repeated LSD injections occur well after the final drug treatment.

Rats given daily LSD injections for 4 weeks and killed 24 hr after the last injection showed a much smaller decrease in midbrain 5-HIAA than previously reported 24 hr after the last of 14 daily injections of a smaller dose of LSD (20 μg/kg), suggesting tolerance to the effects of LSD with long-term treatment.

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