The Subcellular Distribution of Lysergic Acid Diethylamide in the Rat Brain

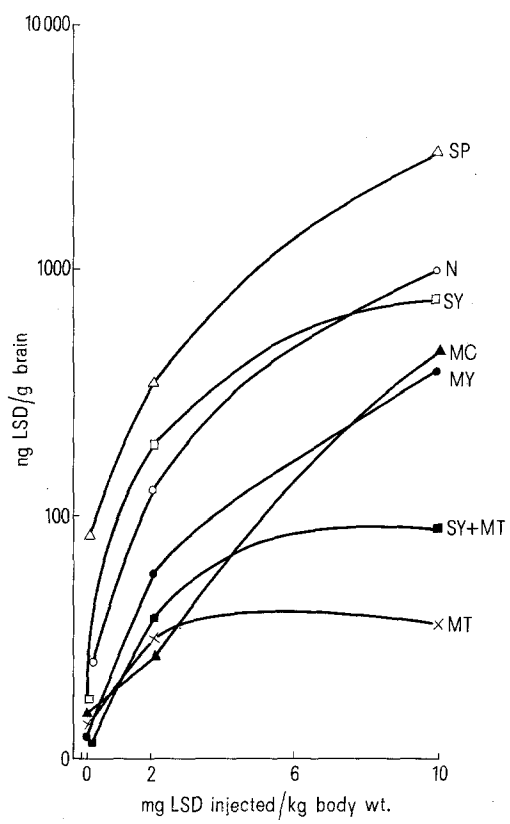
Lysergic acid diethylamide (LSD) has an extraordinary powerful and quick effect on the human psyche. It is the most potent hallucinogenic compound known, and produces a multiplicity of pharmacological actions in the central nervous system and body. With so little substance interacting on so many functions, its distribution becomes a problem of crucial importance. The distribution and localization of LSD in various organs and tissues has been investigated several times. After i.v. injection of ^{14}C LSD in mice[1], most of the organs including the brain, reached the highest level of their activity after 10–15 min, then lost it gradually. After intracerebral injection of ^{14}C LSD into rats[2] the drug left the brain rapidly, appeared in the liver, and was excreted into the intestine. 20 min after i.v. infusion of LSD in the monkey[3] the drug was found to be unequally distributed in different brains areas, with the highest concentrations appearing in the pituitary and pineal glands. In the monkey's hippocampus, frontal gray or white matter[3] the LSD was largely confined to the supernatant fraction, and to a lesser extent in the combined microsome-myelin and the combined synaptosome-mitochondrial fractions. 5 min after i.v. injection of LSD in the rat[4], 65% of the drug was found in the brain particulate fraction, and 35% in the supernatant.

In the light of the disagreement between the data of SNYDER and REIVICH[3], and FREEDMAN and COQUET[4] we thought to re-investigate the problem of the subcellular distribution of LSD in a more detailed manner than that described by these investigators. Furthermore, we also examined the LSD saturability of the brain subcellular fractions by studying LSD distribution in rats receiving various doses of the hallucinogen.

Adult male Charles River CD rats weighing 300–500 g were injected i.p. with either 0.2, 2, or 10 mg/kg body weight of LSD. The animals were sacrificed by decapitation 15 min after injection, a time at which the drug administered by the i.p. route exerts its maximum behavioral effect in the rat. Blood was collected for serum preparation. The brain was removed and cleaned from cerebral membranes, and blood capillaries, then rinsed with deionized water and blotted with Whatman paper No. 41. The whole brain was homogenized in 10 volumes of 0.25 *M* sucrose, and was fractionated into supernatant, nuclear, mitochondrial, microsomal, synaptosomal and myelin fractions as described by GRAY and WHITTAKER[5], and modified by ABDEL-LATIF[6]. The distribution of succinic dehydrogenase activity[7] in the isolated fractions was used as a subcellular marker. The LSD content in each fraction was assayed spectrophotoflourometrically[8]. As little as 0.001 μg per ml of LSD can be measured by this method. Recoveries amounted to about 90% of the LSD added to control brains which were subjected to fractionation and extraction procedures.

Of the injected LSD only 0.2–0.4% appeared in the brain. In order to attain measurable LSD concentration in the brain fractions, the LSD doses used in this study amounted to several folds the effective dose in humans. With the lower dose of 0.2 mg/kg, it became necessary to pool 3 brains for the LSD assay. The concentration of LSD in the serum of rats injected with 0.2, 2 or 10 mg/kg was respectively 89.67 ± 21.76, 1097.00 ± 72.80 and 4726.70 ± 368.70 ng/ml serum. The results shown in the Table, demonstrate that the injection of increasing dose of LSD produces an increase in the hallucinogen level in the various brain subcellular fractions. The observed total value of 140 and 825 ng LSD/g brain in rat brains receiving 0.2 and 2 mg/kg respectively are similar to the values of 80 and 690 ng LSD/g brain reported by ROSECRANS et al.[9] in rats receiving respectively 0.26 and 2.6 mg LSD/kg. The observed increase in brain LSD was not proportional to the increase in the injected dose. Plotting these data on semilog co-ordinates (Figure), indicates that the fractions containing the synaptosomes or mitochondria almost reached saturation at the 10 mg dose level whereas, the rest of the fractions did not reach such saturation.

The results shown in the Table also demonstrate that at all dose levels about 48% of LSD appearing in the brain was found in the supernatant fraction, about 20% in the nuclear fraction, about 15% in the synaptosomal fraction, about 6% in the microsomal fraction, about 5% in the myelin fraction, and about 1.7% in the mitochondrial fractions.



Distribution of LSD in brain subcellular fractions. SP, supernatant; N, nuclear; SY, synaptosomal; MC, microsomal; MY, myelin; MT, myelin; MT, mitochondrial.

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Distribution of LSD in brain subcellular fractions in rats receiving i. p. various doses of LSD

Fraction	LSD (mg/kg) 0.2		2		10	
	Brain[a] (ng/g)	% of total	Brain[b] (ng/g)	% of total	Brain[b] (ng/g)	% of total
Supernatant	79.47 ± 21.62	53.24 ± 2.23	351.20 ± 74.20	42.24 ± 2.02	3019.00 ± 327.71	51.96 ± 2.06
Nuclear	24.80 ± 3.67	19.25 ± 3.75	124.10 ± 9.90	15.71 ± 3.83	1008.00 ± 89.01	17.51 ± 1.40
Microsomal	9.73 ± 2.04	7.18 ± 0.44	26.40 ± 5.00	3.41 ± 1.27	458.00 ± 84.48	7.71 ± 0.93
Myelin	3.80 ± 0.44	2.98 ± 0.55	55.60 ± 7.00	6.78 ± 0.21	394.30 ± 37.15	7.00 ± 1.22
Synaptosome	13.83 ± 3.50	9.16 ± 1.45	199.00 ± 51.00	23.78 ± 2.22	789.70 ± 61.96	13.66 ± 0.41
Synaptosome + Mitochondria	3.67 ± 0.98	2.54 ± 0.23	37.80 ± 2.00	4.76 ± 1.06	89.30 ± 47.62	4.41 ± 0.74
Mitochondrial	11.50 ± 7.08	3.48 ± 0.39	30.30 ± 21.00	3.31 ± 2.78	35.00 ± 7.23	0.64 ± 0.18
Total	140.46 ± 34.75		825.00 ± 134.00		5797.00 ± 491.90	

[a] Mean ± S.E. of LSD in fractions obtained from 3 brain pools, each pool was prepared from 3 injected rats. [b] Mean ± S.E. of LSD in fractions obtained from 2–3 injected rats.

These results are at variance with those of FREEDMAN and COQUET[4] in the rat, but generally do agree with the data of SNYDER and REIVICH[3] which indicates that of the LSD appearing in the monkey brain as much as 73% is accounted for in the supernatant, 19% in the microsome-myelin, and 8% in the synaptosome-mitochondrial fraction. The difference between our results and those of SNYDER and REIVICH[3] concerning the percentage of LSD subcellular distribution may be due to methodological or species difference. However, the mere fact that 50% or more of LSD is found by both in the supernatant fraction may partially explain the high potency of the hallucinogenic effect of LSD, since the compound will be available to get to and exert its effect on the receptor sites particularly if one assumes that LSD in the supernatant is not completely bound. Preliminary results on this point do indicate that at least about 60% of the LSD present in the supernatant is in a free form.

The relatively high percentage of LSD in the synaptosomal fraction observed in this report is of interest in view of the in vitro studies of MARCHBANKS[10], which demonstrated that LSD is a potent inhibitor of the high affinity binding for serotonin in brain synaptosomes. However, it does not appear that the behavioral effect of LSD could be explained on this basis since ROSECRANS et al[9] have demonstrated that LSD raises the level of serotonin in the brain particulate fraction which contains among other things the synaptosomes.

Résumé. Quinze min après une injection i.p. de LSD, près de 48% de la drogue présente dans le cerveau fut trouvée dans le surnageant, 20% dans la fraction nucléaire, 15% dans la fraction synaptosomale, 6% dans la fraction microsomale, 5% dans la fraction myélinique et près de 1,7% dans la fraction mitochondriale. Les fractions synaptosomale et mitochondriale sont presque saturées en LSD lors d'une injection de 10 mg/kg de LSD.

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A Note on the Subcellular Distribution of Brain Protein in Differentially-Housed Mice[1]

Previous reports have indicated that changes in cerebral biochemistry and anatomy can be produced by exposure of rodents to different environmental conditions[2–6] and that these changes are paralleled by changes in behavior[7–9]. It has also been shown that differential housing of mice can lead to profound changes in cerebral and hepatic energy metabolism[10–12] and that prior administration of Li^+, a psychoactive agent, can affect cerebral metabolism in isolated, but not in aggregated mice[12]. Li^+ salts have also been shown to reduce agression[13,14] which can be produced by prolonged isolation of animals[2,15]. It has been claimed that animals raised in 'enriched' environments possess increased total cerebral protein and increased AChE, ChE and hexokinase activities over their 'impoverished' controls[16,17], and differences in glial proliferation have also been reported[18–20]. In a recent report it has been suggested that differential experience might actually affect synaptic ultrastructure[21], and some new evidence indicates that circadian periodicity can be demonstrated at the ultrastructural level[22]. Due to these recent reports it was considered pertinent to study the subcellular distribution of cerebral protein in the brains of 'isolated' and 'aggregated' mice.

Weanling, male, C-57 Black mice were housed either singly ('isolated') or in groups of 20–25 ('aggregated') for 5–8 weeks as previously described[10]. All animals were fasted 19–21 h before being sacrificed (between 13.30–14.00 h), and hemispheres (above the level of the inferior colliculi) were excised rapidly, weighed, and placed into homogenizing vessels (clearance 0.004–0.006) containing 10 volumes of isosmotic (320 mOs*M*) solutions of sucrose-plus-NaCl ± LiCl (4 or 40 m*M*). Aliquots (2.5 ml) of homogenates were centrifuged at 1000 × *g*, 10 min, 0°C to obtain P_1 (pellet) and S_1 (supernatant fluid) fractions, and 1.0 or 1.5 ml aliquots of S_1 fractions were recentrif-