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-

Distribution of protein in P_1 and S_1 fractions of the brains of isolated and aggregated mice; effects of Li^+

Salts in homogenizing fluid	Protein (mg)				
	per g brain	in P_1/g brain	in S_1/g brain	% in P_1	% Recovery
	Isolated mice				
None	145 ± 20 (11)	30 ± 3 (11)	100 ± 5 (11)	22 ± 2 (11)	90 ± 9 (11)
4 m*M* NaCl	128 ± 5 (5)	29 ± 3 (5)	94 ± 6 (5)	24 ± 3 (5)	96 ± 4 (5)
40 m*M* NaCl	138 ± 11 (16)	87 ± 7 (16)	45 ± 6 (16)	66 ± 4 (16)	101 ± 14 (16)
4 m*M* NaCl + 4 m*M* LiCl	134 ± 6 (5)	43 ± 6 (5)	89 ± 7 (5)	33 ± 4 (5)	98 ± 6 (5)
40 m*M* NaCl ± 40 m*M* LiCl	135 ± 5 (5)	79 ± 10 (5)	44 ± 2 (5)	64 ± 3 (5)	91 ± 9 (5)
	Aggregated mice				
None	144 ± 13 (11)	32 ± 4 (11)	100 ± 6 (11)	24 ± 3 (11)	92 ± 9 (11)
4 m*M* NaCl	134 ± 18 (5)	33 ± 2 (5)[a]	94 ± 5 (5)	26 ± 2 (5)	96 ± 11 (5)
40 m*M* NaCl	139 ± 11 (16)	93 ± 9 (16)	46 ± 4 (16)	67 ± 2 (16)	104 ± 11 (16)
4 m*M* NaCl + 4 m*M* LiCl	122 ± 5 (5)	42 ± 3 (5)	90 ± 4 (5)	32 ± 2 (5)	109 ± 6 (5)
40 m*M* NaCl + 40 m*M* LiCl	146 ± 11 (5)	98 ± 7 (5)[b]	43 ± 2 (5)	70 ± 2 (5)[b]	96 ± 6 (5)

Means ± standard deviations; numbers of mice in parentheses; [a] and [b] indicate $p < 0.05$ and $p < 0.01$, respectively, for comparisons between fractions from isolated and aggregated mice (Student's *t*-test; two-tailed).

$$\% \text{ Recovery} = \frac{\text{protein in } P_1 + S_1}{\text{protein in homogenate}} (100).$$

uged at 17,500 × *g*, 55 min, 0°C to obtain P_2 and S_2 fractions[23]. Wet weights of pellets were taken using tared centrifuged tubes and a micro-balance. The protein contents of subcellular fractions were determined in 5–25 µl aliquots by the method of LOWRY, ROSEBROUGH, FARR and RANDALL[24].

No difference in total protein content of hemispheres was found between the 2 groups of mice; 'isolated' mouse brain contained 137.7 ± 11.28 (s.d., $n = 42$) and 'aggregated' brain contained 138.7 ± 11.51 (s.d., $n = 42$) mg/g protein. In the Table it is shown that homogenization of mouse brain in the presence of salt increased the protein content of the P_1 fraction but these increases coincided with increases in pellet weight (data not shown). However, when 40 m*M* LiCl was used in addition to 40 m*M* NaCl the protein content of the P_1 fraction was significantly lower in the brains of isolated mice and pellet weights were not changed. Also, in the presence of 4 m*M* NaCl the P_1 fractions of isolated mice contained less protein than those of aggregated mice. Though the protein contents of the P_2 fractions were decreased upon addition of salts, no differences were evident between the brains of isolated and aggregated mice. Other differences caused by Li^+ were not specific to either isolated or aggregated mice (Table).

The results presented herein provide further evidence that neurochemical changes can be caused by subjecting animals to differential housing. It should be noted that with the increases in ionic strength of the homogenizing fluids produced by addition of NaCl and LiCl, more of the nerve ending particles would sediment in the P_1 fraction. But, this 'clumping' effect[23] provided the essential tool with which the difference in distribution of cerebral protein between isolated and aggregated mice could be shown. It is suggested that the difference in protein distribution which occurs upon exposure of mice to different environments may well reflect a difference in synaptic morphology.

Résumé. Utilisant une technique subcellulaire pour étudier la distribution de la protéine dans des cerveaux de souris, on a constaté certaines différences.

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