Studies on the Time Course of Entry and Subcellular Distribution of Radioactivity of (^{3}H) d-Amphetamine in the Brains of Differentially-Housed Mice[1]

From a recent study, it was concluded that injections of d-amphetamine into mice exposed to prolonged periods of differential housing decreased selectively the incorporation of carbon atoms of D-glucose into the brains of 'isolated' mice[2]. It is the purpose of this report to provide evidence for a mechanism by which d-amphetamine caused this selective change in the brains of isolated mice.

Materials and methods. Male, weanling, C-57 Black mice (Indianapolis Lab. Supply) were kept either 'isolated' or 'aggregated' in groups of 16–26 for 5–9 weeks[3]. Mice were fasted before injections which were made in the daytime during winter and spring. (^{3}H) d-amphetamine-SO_4 (New England Nuclear Corp.; 6.2 Ci/m*M*) was combined with unlabelled d-amphetamine-SO_4 (K & K Labs) to prepare a solution of 6.25 μCi/ml (2.5 mg/ml) of d-amphetamine-SO_4 in 0.154 *M* NaCl. Mice were decapitated at 2–124 min after i.p. injection of 50 mg/kg of this solution, and their brains (rostral to the inferior colliculi) were excised rapidly, weighed, and homogenized in ice-cold 80% ethanol solution (v/v). Since C-57 Black mice are very resistant to d-amphetamine[4], a high dose was used. Aliquots of homogenates were counted[5]. For studies on the subcellular distribution of (^{3}H) d-amphetamine, mice were either injected i.p. with (^{3}H) d-amphetamine (50 mg/kg) and sacrificed 30 min later, or sacrificed without injection. Brains were homogenized in 10 vols 0.32 *M* sucrose solution. For uninjected mice this contained (^{3}H) d-amphetamine (1 μCi/ml). All homogenizing fluids contained 0.2 μCi/ml U (^{14}C) sucrose (New England Nuclear Corp.; 505 mCi/m*M*). Brains were centrifuged to obtain 'crude nuclear' (P_1), 'synaptosomal, mitochondrial' (P_2) and 'microsomal, ribosomal' (S_2) fractions[6]. Particulate fractions of the brains of half of the mice in each group were prepared in isosmotic sucrose solution containing 40 m*M* NaCl. Aliquots of all fractions were counted using a double-isotope procedure, and their total protein contents were determined[7]. One isolated and one aggregated mouse were sacrificed at about 17 min after i.p. injection of (^{3}H) d-amphetamine P_2 fractions were re-suspended in 3.5 ml 0.32 *M* sucrose solution and 2.0 ml of each fraction were layered onto gradients (0.4–1.8 *M* sucrose) and centrifuged at 137,000 × *g*, 1.5 h. Small fractions of the gradients were collected, and radioactivity and total protein were estimated.

Results. Results in Figure 1 indicated that (^{3}H) d-amphetamine entered mouse brain by an initial rapid phase lasting for about 10 min and that maximal brain levels were reached at about 15–25 min. During the falling phase the (^{3}H) present in the brains of 'isolated' mice was greater than that of the 'aggregated' mice. Results shown in Table I indicated that homogenization of tissue in the presence of 40 m*M* NaCl increased markedly the amount of radioactivity due to both (^{3}H) d-amphetamine and (^{14}C) sucrose found in the P_1 fractions of brain. These differences are best explained as being due to the increases in P_1 weight caused by the 'clumping' of particles by the excess Na^+ (see ref. [6]). It was shown also that after injection of (^{3}H) d-amphetamine, the (^{3}H) contents of P_2 fractions prepared in Na^+-free media were lower for 'aggregated' than for 'isolated' mice. When the retention of d-amphetamine was expressed on a protein basis it became evident that more d-amphetamine (+ metabolites) was bound per mg protein to both the P_1 and P_2 fractions of 'isolated' mice (Table II). It was also evident that Na^+ caused an extensive release of (^{3}H) from both P_1 and P_2 particles. Using linear gradients, it was shown that more (^{3}H) was retained by the P_2 fractions from the 'isolated' mouse brain (Figure 2). The (^{3}H) in the P_2 fractions of both mice was localized in a region of low density (about 0.5–0.7 *M*) sucrose solution which has been shown to contain nerve ending membranes and synaptic vesicles[6]. All differences between 'isolated' and

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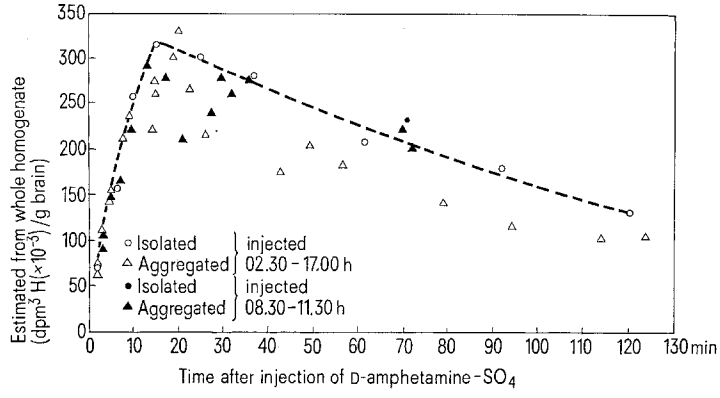

Fig. 1. Time course of (^{3}H) content of the brains of 'isolated' and 'aggregated' mice after i.p. injection of (^{3}H) d-amphetamine-SO_4 (50 mg/kg; 0.1 μCi/g). At the times designated, mice were sacrificed and their brains were excised rapidly, freed of excess blood, weighed, and homogenized in 3.0 ml of ice-cold 80% ethanol solution (v/v). Aliquots of homogenates were counted directly. Experiments were conducted in both mornings and afternoons as indicated. The estimated curve is for 'isolated' mice and lies above most of the points of 'aggregated' mice in its declining phase.

Table I. Distribution of radioactivity of (^{3}H) d-amphetamine and (^{14}C) sucrose in subcellular fractions of the brains of 'isolated' and 'aggregated' mice; effects of Na^+

Salt in homogenizing fluid	Radioactivity (dpm × 10^{-3}) In P_1 fraction		In P_2 fraction	
	^{3}H	^{14}C	^{3}H	^{14}C
	d-Amphetamine in homogenizing fluid			
	Isolated mice			
Na^+-free	468 ± 62	58 ± 6	755 ± 27	48.5 ± 3
40 m*M* NaCl	1048 ± 23[a]	126 ± 5[a]	180 ± 9[a]	22.5 ± 2[a]
	Aggregated mice			
Na^+-free	492 ± 15	60 ± 3	754 ± 18	48 ± 2
40 m*M* NaCl	1065 ± 29[a]	128 ± 5[a]	177 ± 9[a]	23.5 ± 1[a]
	Injected with d-amphetamine			
	Isolated mice			
Na^+-free	8 ± 1	59 ± 3	14.2 ± 2.2	54 ± 4
40 m*M* NaCl	15 ± 1[a]	128 ± 4[a]	3.0 ± 0.4[a]	24 ± 3[a]
	Aggregated mice			
Na^+-free	8 ± 1	70 ± 6[c]	11.8 ± 1.5[b]	54.5 ± 4
40 m*M* NaCl	15 ± 2[a]	129 ± 3[a]	2.9 ± 0.4[a]	24.5 ± 3.5[a]

Means ± standard deviations; $n = 6$ for values obtained with addition of (^{3}H) d-amphetamine-SO_4 to homogenizing fluid, and $n = 5$ for values obtained 30 min after i.p. injection of (^{3}H) d-amphetamine-SO_4; homogenizing fluids contained 0.2 μCi/ml (^{14}C) sucrose; [a] indicates $p = 0.001$ for comparisons of values for Na^+-free vs 40 m*M* NaCl; [b] and [c] indicate $p < 0.02$ and $p < 0.001$ for comparisons between these values and corresponding values for 'isolated' mice (Student's *t*-test; 2-tailed). P_1 = pellet from 1000 × *g* centrifugations of 2.0 ml of homogenate; Aliquots (1.2 ml) of S_1 fractions were centrifuged at 17,000 × *g*, 55 min to obtain P_2 (pellet) and S_2 (supernatant) fractions. Percentage recoveries were 79–110% for (^{3}H) and 81–99% for (^{14}C).

Table II. Distribution of d-amphetamine in particulate fractions of the brains of 'isolated' and 'aggregated' mice expressed on a protein basis; effects of Na^+

Salt in homogenizing fluid	d-amphetamine (*p*-mole/mg protein)	
	P_1 fraction	P_2 fraction
d-Amphetamine in homogenizing fluid		
Isolated mice		
Na^+-free	6.5 ± 0.35	6.7 ± 0.25
40 m*M* NaCl	4.6 ± 0.3[b]	6.2 ± 0.4[a]
Aggregated mice		
Na^+-free	6.4 ± 0.6	6.8 ± 0.2
40 m*M* NaCl	4.3 ± 0.15[b]	6.25 ± 0.4[a]
Injected with d-amphetamine		
Isolated mice		
Na^+-free	1.6 ± 0.25	1.65 ± 0.25
40 m*M* NaCl	1.0 ± 0.1[b]	1.45 ± 0.2
Aggregated mice		
Na^+-free	1.4 ± 0.2	1.45 ± 0.25
40 m*M* NaCl	1.0 ± 0.15[b]	1.25 ± 0.15[c]

Means ± standard deviations; $n = 6$ for values obtained with (^{3}H) d-amphetamine-SO_4 in homogenizing fluid, and $n = 5$ for values obtained with (^{3}H) d-amphetamine-SO_4 injections; [a] and [b] indicate $p < 0.05$ and $p < 0.001$ for comparisons between values for tissues homogenized in Na^+-free vs 40 m*M* NaCl; [c] indicates $p < 0.05$ between this value and the corresponding one for 'isolated' mice. Student's *t*-test; 1-tailed).

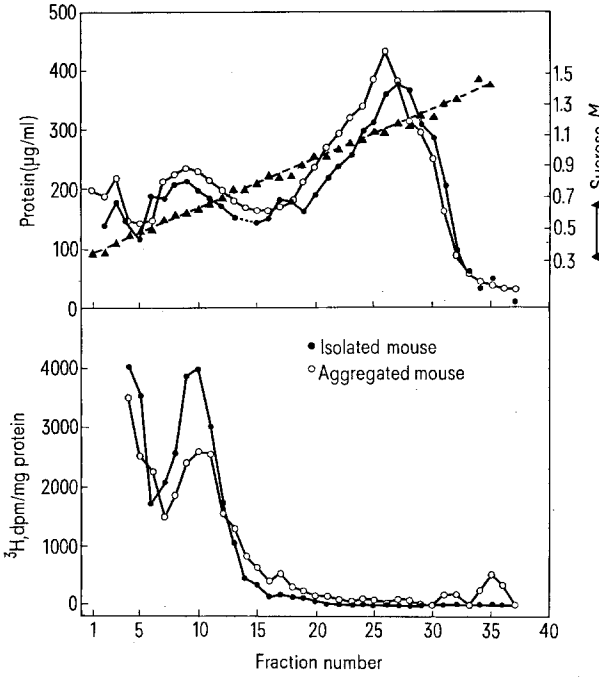

Fig. 2. Subcellular profiles of (^{3}H) and protein of P_2 fractions prepared from the brains of an 'isolated 'and an 'aggregated' mouse and resolved on linear sucrose gradients.

'aggregated' mice which have been described here were not evident unless the (^{3}H) d-amphetamine was injected into the mice; i.e., these could not be shown when brains were merely homogenized in the presence of (^{3}H) d-amphetamine.

Discussion. In this report we have presented data which indicate that the action of d-amphetamine of decreasing selectively the entry of glucose carbon atoms into the brains of 'isolated' mice[2] is correlated with an increased retention of (^{3}H) of injected (^{3}H) d-amphetamine by particles in the P_2 fractions of their brains. Perhaps the most likely explanation is that due to a more rapid rate of metabolism in 'aggregated' (hyperactive) mice (Figure 1), d-amphetamine is metabolized and excreted more rapidly and therefore does not exert such a pronounced effect on brain metabolism (see also reference [8]). It should be noted that the results presented here, as well as those in our previous reports[2,8], were obtained with mice subjected to 'prolonged' periods of differential housing and therefore are not expected to be in accord with results of 'short-term' studies[9,10]. The data obtained in this study, and in other studies involving environmental effects on metabolism and drug action, may be subject to variation due to a number of factors such as: 1. changes in body or ambient temperature[11]; 2. diurnal rhythmicity[12,13]; 3. size of test compartment[14]; 4. strain of mice[4]; 5. conditions of housing prior to experiments; 6. seasonal influences. In any case, it seems reaconable to suspect that social isolation of animals, including man, may cause changes in the central actions of addictive drugs[15].

Résumé. La rétention de (^{3}H), après l'injection de (^{3}H) d-amphétamine chez des souris, fut plus marquée dans des particules synaptiques quand les animaux avaient été soumis à un isolement prolongé.

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Pathogenesis of Gastro-Intestinal Symptomatology During Poisoning by *Amanita phalloides*

During the poisoning of humans by *Amanita phalloides* the first symptoms appear after 10–24 h – violent abdominal pains, vomiting and persistent diarrhoea.

So far it has been excluded[1] that these gastrointestinal symptoms might be due to amanitins or phalloidins, which are the cytotoxins responsible for damage to liver and kidney in *Amanita phalloides* poisoning[1–3]. This belief derives from the finding that administration of these toxins in common experimental animals (mouse, rat and guinea-pig), whether orally or by injection, does not produce gastro-intestinal symptoms. This reasoning, however, takes no account of the fact that even a total extract of *Amanita phalloides* produces no such symptoms in these animals, whereas the earlier literature[4] informs us that total extracts administered orally or by injection to dogs do produce gastro-intestinal symptoms. Therefore it is the dog, not the mouse, rat or guinea-pig, which should be chosen for investigations on the gastro-intestinal symptoms produced in Man by *Amanita phalloides*.

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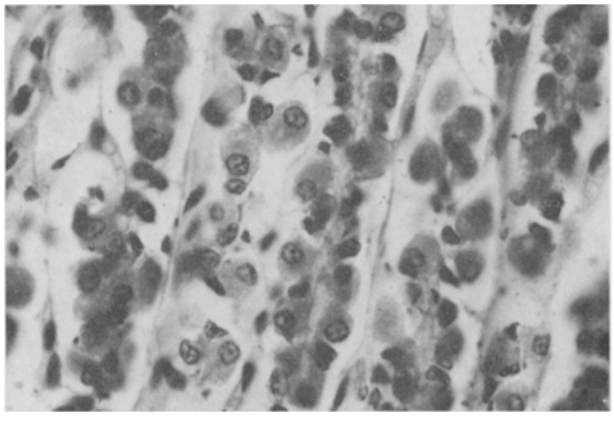

Fig. 1. Stomach of a dog killed by α-amanitin. Mucosa of the fundus. Atrophy of chief cells with picknosis of nuclei. Em. Eos. ×525.

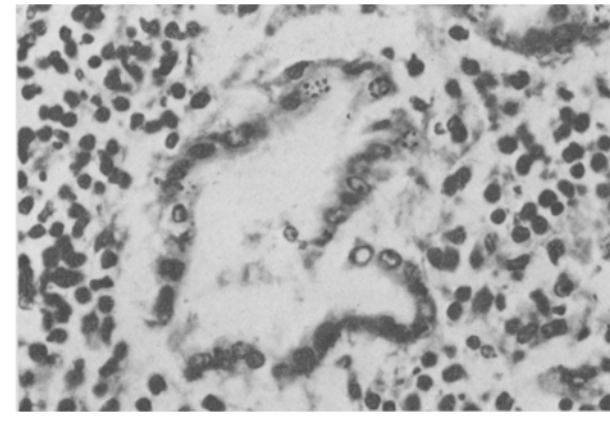

Fig. 2. Brunner's gland in the duodenum of a dog killed by α-amanitin. Marked changes of nuclei. Em. Eos. ×525.