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BRAIN SEROTONIN METABOLISM IN ISOLATED AGGRESSIVE MICE

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Abstract—Isolation of Albino–Swiss male mice for various periods of time induces a decrease of adrenal weight and an increase of food intake. The level of brain 5-hydroxytryptamine (5HT) was unaffected while a decrease of brain 5-hydroxyindolacetic acid (5HIAA) was evident beginning the first day of isolation. The turnover rate of brain 5HT, measured after blockade of monoaminoxidase in vivo, was decreased in isolated mice. Similar changes were observed also in discrete areas of the brain. There was no temporal correlation between the alteration of brain 5HT metabolism and the onset of an aggressive behavior. However female mice and two strains of rats which do not become aggressive after prolonged isolation did not show any change in brain 5HT turnover rate.

IT IS well known that prolonged isolation of mice results in the development of an aggressive behavior.[1–9] However only recently have these animals with abnormal behavior been utilized for pharmacological and biochemical studies.

Yen *et al.*[10] proposed suitable experimental conditions for obtaining in a reproducible way aggressive mice. The degree of aggressiveness may be scored on a quantitative basis[11] therefore permitting correlation of aggressive behavior with other physiological, pharmacological and biochemical parameters.

Previous studies have demonstrated that different animal species do not show the same tendency to become aggressive after isolation. In fact although aggressiveness has been reported to develop in rats[12] and rabbits[13] there is no doubt that mice are more suitable for these studies. However not all strains of mice develop aggressive behavior after prolonged isolation. For instance black mice seem to be less prone to become aggressive than Albino mice.[14] In a strain of mice susceptible of becoming aggressive, sex plays an essential role. Prolonged isolation failed in fact to develop aggressiveness in female mice even when the isolation lasted longer than what is necessary to make male mice aggressive.[15] Hormonal treatment or excision of sexual glands induce marked changes in the capacity to develop an aggressive behavior.[12, 16–21]

Psychologists and psychiatrists have been interested in this experimental model because in humans "sensory deprivation", a condition similar to isolation, results in a schizophrenic-like syndrome.[22–25]

Pharmacologists have been using this test to evaluate the effects of drugs with the aim of finding antiaggressive agents.[15, 26–33] It was also noticed that animals submitted to prolonged isolation change their sensitivity to drugs. In this and other

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laboratories, several studies pointed out that mice submitted to prolonged isolation become more sensitive to the effect of amphetamine and fencamfamine[34–36] and less sensitive to the action of chlorpromazine.[35]

This altered sensitivity to drugs, which for amphetamine was not due to changes in the concentrations of this drug in the brain[37] suggested the possibility of biochemical changes in the brain of aggressive mice. Obviously it was of interest to investigate changes of brain amines, such as serotonin, noradrenaline and dopamine, because of the suggested involvement of these substances in brain function.[38–45] Previous studies[46–49] indicated also that emotional changes may induce alterations in the levels of brain amines. However prolonged isolation did not induce any change in the level of brain serotonin, noradrenaline and dopamine when assayed *in toto*[14, 30] or in selected areas of brain.[11] Other authors in contrast could find at given times after isolation small changes in brain noradrenaline.[50]

As far as serotonin (5HT) is concerned it was observed that monoaminoxidase (MAO) inhibitors increase brain 5HT to a larger extent in normal than in isolated mice,[11] which suggests therefore that the rate of accumulation of brain 5HT was decreased in aggressive animals.[11]

The fact that brain 5-hydroxyindolacetic acid (5HIAA) is constantly lower in isolated than in normal mice was also similarly interpreted.[15] The advent of more refined techniques for measuring brain 5HT turnover[51] confirmed the fact that isolated aggressive mice show a decreased turnover rate and an increased turnover time of brain 5HT in comparison with grouped normal animals.[52]

The purpose of this paper is to report the studies on kinetics of brain 5HT in different experimental conditions and by using different animal species, different strains and different sex. Since it is known that isolation produces not only an aggressive behavior but also a number of other effects, including decrease of acetylcholinesterase,[53] decrease of *N*-acetyl-L-aspartic acid,[54] increase of γ-aminobutyric acid glutamic acid and glutamine in selected brain areas,[55] changes in adrenal weight[36, 53, 56, 57] decrease of thyroid weight[58] and increase of food intake,[58] other parameters have been taken into consideration and will be hereafter described.

MATERIALS AND METHODS

Albino–Swiss mice of both sexes weighing 18 $\pm$ 2 g (at the beginning of the experiment) and male Sprague–Dawley and Wistar rats weighing 180 $\pm$ 10 g (at the beginning of the experiment) have been used.

All animals were kept in a single animal room at a constant temperature of 22° and 60 per cent relative humidity and fed a normal balanced diet (ALAL 57) *ad libitum*.

The animals referred as normal were usually housed in number of 10 mice per cage (23 $\times$ 17 $\times$ 14 cm in size) or 5 rats per cage (38 $\times$ 22 $\times$ 15 cm in size) for an adaptation period of 1 week before the beginning of the experiment. All the cages were Makrolon with transparent walls. The changes of the cages for cleaning was rhythmical during all the experimental period, the last change taking place 2 days before the sacrifice of the animals.

After the adaptation time, male mice were randomized and isolated or grouped in five and twenty animals per cage; the time of isolation or grouping was 1, 5, 10, 20 and 30 days.

Rats after the adaptation period, were randomized and isolated or maintained in normal social conditions for 30 days before the sacrifice.

Food intake and increase in body weight were measured every three days; care was taken to avoid that crumbs of pellets were measured as food intake.

Aggressiveness score and latency time were determined according to the parameters described elsewhere.[11] 5HT was spectrofluorimetrically determined according to Shore and Olin[59] and Shore[60] and 5HIAA was measured according to Giacalone and Valzelli.[61]

Discrete areas of brain were dissected, pooled and immediately frozen with dry ice.

5HT turnover rate and 5HT turnover time were calculated according to Tozer *et al.*[51] Following this method, the animals were injected with a single dose of tranylcypromine 20 mg/kg i.p. and sacrificed after 60 min. always at the same time of the day (10 a.m.) to avoid the influence of the circadian rhythm.[62–66] This time was selected after previous assays to establish the linearity of 5HIAA loss. Control animals were run for every experiment. Levels of brain tranylcypromine have been previously shown to be similar in both isolated and grouped animals.[11]

Tests were carried out to determine the optimal dose of tranylcypromine according to the concepts expressed by Tozer *et al.*[51]

Heart and adrenal weight were determined immediately after sacrifice with special care to avoid important blood contamination. Plasma free fatty acids (FFA) were determined titrimetically according to Dole.[67] Triglycerides in heart, liver and plasma were determined according to Van Handel and Zilversmit.[68] Blood glucose was assayed by an enzymatic method according to Huggett and Nixon.[69, 70]

All biological specimens were frozen in dry ice and analyzed for different substances after 4 hr (brain 5HIAA), or after 24 hr (brain 5HT, heart, liver and plasma triglycerides, plasma free fatty acids and blood glucose).

The statistical significance of the changes observed was calculated with the Student's *t* test.

RESULTS

1. *Changes in some organ weight and biochemical parameters in relation to the time of isolation or grouping of mice*

Male mice were caged in numbers of 1, 5, 10 or 20 per cage for a period of time ranging from 1 to 30 days. Body weight was not substantially affected although food intake was significantly higher in isolated than in grouped animals and to a lesser extent also in animals housed 5 per cage (see Table 1). In isolated animals, the major changes were observed at the beginning and at the end of the experiment. This increase in food intake could also explain the lower levels of plasma FFA found in isolated animals compared to animals crowded 20 per cage. Similarly the decreased level of liver triglycerides in isolated animals could be a consequence of the decrease of plasma FFA. However in other experiments carried out in a different period of the year, the increase of food intake following the first day of isolation was not evident. Heart and plasma triglycerides were not changed in the various experimental groups. Also blood glucose was unaffected by the various experimental conditions.

Adrenal weight was decreased compared with normal conditions (10 mice per cage) both in isolated and crowded mice. Heart weight tended instead to increase only in isolated and sparse (5 per cage) animals.

TABLE 1. CHANGES OBSERVED BY CAGING MALE MICE IN DIFFERENT NUMBERS FOR DIFFERENT LENGTH OF TIME

(Each point is the mean of 7 determinations ± S.E.)

No. of mice /cage	Time (days)	Plasma FFA μEquiv./l	Plasma Triglyc. mg/100 ml	Heart triglyc. mg/100 g wet wt	Liver triglyc. mg/100 g wet wt	Blood glucose mg/100 ml	Adrenal wt. mg wet wt	Heart wt. mg wet wt	Body wt. g	Food intake g/day/mouse
1	1	501 ± 29	96·3 ± 10	159 ± 16	537 ± 78	146 ± 7	3·7 ± 0·19	174 ± 1·10	38 ± 1·66	8·9 ± 0·3‡
	5	570 ± 58	112·9 ± 18	149 ± 18	518 ± 49	146 ± 6	3·7 ± 0·17	165 ± 1·07	35 ± 1·28	6·6 ± 0·1‡
	10	648 ± 71	128·1 ± 5	132 ± 6	574 ± 146	138 ± 7	3·4 ± 0·17	166 ± 1·01	35 ± 1·14	5·7 ± 0·1†
	20	673 ± 97	126·4 ± 13	166 ± 2	510 ± 152	145 ± 8	*3·1 ± 0·14	158 ± 0·73	35 ± 1·33	5·8 ± 0·2†
	30	517 ± 71	119·8 ± 13	175 ± 30	578 ± 86	147 ± 5	*3·1 ± 0·11	167 ± 0·81	35 ± 1·71	7·2 ± 0·4‡
5	1	543 ± 62	142·5 ± 23	118 ± 8	428 ± 58	150 ± 5	3·6 ± 0·11	166 ± 0·70	36 ± 1·28	5·7 ± 0·3†
	5	710 ± 73	148·5 ± 14	137 ± 9	831 ± 199	164 ± 5	3·6 ± 0·11	154 ± 0·71	35 ± 1·14	5·2 ± 0·2
	10	642 ± 71	94·1 ± 4	125 ± 17	557 ± 102	156 ± 4	3·4 ± 0·11	156 ± 0·67	34 ± 1·57	5·9 ± 0·1†
	20	587 ± 51	112·6 ± 8	154 ± 20	672 ± 85	166 ± 3	3·6 ± 0·11	163 ± 1·16	36 ± 1·28	6·0 ± 0·3*
	30	560 ± 69	138·3 ± 10	147 ± 48	755 ± 244	165 ± 4	3·4 ± 0·11	165 ± 0·51	38 ± 0·86	5·8 ± 0·1†
10	30	580 ± 74	109·9 ± 27	195 ± 53	743 ± 155	154 ± 8	3·6 ± 0·11	147 ± 1·03	34 ± 1·43	4·9 ± 0·1
20	1	§665 ± 38	122·5 ± 15	173 ± 21	545 ± 128	153 ± 4	3·4 ± 0·11	145 ± 0·86	33 ± 2·00	5·0 ± 0·2
	5	685 ± 26	122·0 ± 11	141 ± 13	626 ± 128	142 ± 4	*3·2 ± 0·05	155 ± 0·74	34 ± 1·43	4·8 ± 0·1
	10	638 ± 45	132·6 ± 18	113 ± 31	758 ± 235	152 ± 6	3·5 ± 0·11	146 ± 1·00	33 ± 1·14	5·1 ± 0·1
	20	622 ± 55	104·5 ± 18	177 ± 60	774 ± 277	148 ± 6	3·6 ± 0·11	159 ± 0·70	34 ± 1·28	5·0 ± 0·4
	30	625 ± 40	111·3 ± 24	159 ± 21	786 ± 208	150 ± 6	3·4 ± 0·17	144 ± 0·78	35 ± 0·71	4·4 ± 0·1

Significance in respect to controls (10 per cage):
* $P < 0.05$,
† $P < 0.01$,
‡ $P < 0.001$.

Significance between animals kept 20 per cage vs. 1 per cage:
§ $P < 0.01$.

2. *Levels of serotonin (5HT) and 5-hydroxyindolacetic acid (5HIAA) in brain of mice*

In the same experimental conditions, as described above, brain 5HT was very stable. In fact the levels are not statistically different when measured in the brain of animals isolated or grouped in numbers of 1, 5, 10, 20 per cage. Only when the two extremes of our experimental conditions were considered, it was evident that animals isolated for 1 day had lower levels of brain 5HT than animals grouped in 20 per cage (see Table 2).

The isolation for 30 days was not effective in changing 5HT in discrete areas, as indicated in Table 3.

TABLE 2. BRAIN 5HT AND 5HIAA BASAL LEVELS, 5HT TURNOVER RATE, TURNOVER TIME AND AGGRESSIVENESS SCORE OF ALBINO–SWISS MALE MICE AT DIFFERENT TIMES OF ISOLATION OR CROWDING

(Each point is the mean of 9 determinations ± S.E.)

No. of mice/ cage	Time (days)	5HT μg/g wet wt	5HIAA μg/g wet wt	5HT turnover rate μg/g wet wt/hr	5HT turnover time min	Aggressiveness score %	Aggressiveness latency time (sec)
1	1	0.742 ± 0·024	*0·239 ± 0·021	‡0·295 ± 0·016	‡144 ± 7	15	242
	5	0·821 ± 0·061	†0·251 ± 0·011	‡0·325 ± 0·023	‡140 ± 2	0	> 300
	10	0·833 ± 0·058	†0·249 ± 0·012	0·402 ± 0·021	114 ± 4	38	171
	20	0·849 ± 0·053	†0·253 ± 0·014	‡0·387 ± 0·012	*120 ± 3	86	154
	30	0·848 ± 0·061	†0·231 ± 0·012	‡0·361 ± 0·010	‡131 ± 2	100	42
5	1	0·804 ± 0·034	0·271 ± 0·012	0·415 ± 0·019	106 ± 3	0	> 300
	5	0·887 ± 0·049	0·293 ± 0·014	0·455 ± 0·015	108 ± 2	0	> 300
	10	0·833 ± 0·041	0·269 ± 0·012	0·430 ± 0·022	107 ± 3	0	> 300
	20	0·812 ± 0·037	0·283 ± 0·021	0·466 ± 0·036	97 ± 3	0	> 300
	30	0·784 ± 0·042	0·274 ± 0·021	0·459 ± 0·030	95 ± 3	0	> 300
10	30	0·812 ± 0·054	0·291 ± 0·012	0·445 ± 0·013	100 ± 3	0	> 300
20	1	¶0·874 ± 0·041	0·301 ± 0·012	§¶0·503 ± 0·021	¶ 96 ± 3	0	> 300
	5	0·851 ± 0·072	0·333 ± 0·024	0·502 ± 0·027	§ 92 ± 3	0	> 300
	10	0·848 ± 0·045	0·304 ± 0·012	0·468 ± 0·028	102 ± 3	0	> 300
	20	0·886 ± 0·072	0·341 ± 0·031	0·513 ± 0·035	95 ± 4	0	> 300
	30	0·947 ± 0·041	0·323 ± 0·012	0·515 ± 0·037	§¶103 ± 2	0	> 300

Significance in respect to controls (10 per cage):
* $P < 0·05$,
† $P < 0·01$,
‡ $P < 0·001$.

Significance between animals kept 20 per cage vs. 5 per cage:
§ $P < 0·01$.

Significance between animals kept 20 per cage vs. 1 per cage:
¶ $P < 0·01$,
‖ $P < 0·001$.

5HIAA was decreased through the whole period of isolation. The difference was statistically significant considering as a reference normal or crowded animals. Also in the groups of 5 per cage there was a trend towards a decrease of brain 5HIAA (see Table 2).

The decrease of brain 5HIAA was evident also in diencephalon, corpora quadrigemina and mesencephalon of isolated mice (see Table 3).

The level of brain 5HT or 5HIAA did not correlate with the development of the aggressive behaviour as it can be noticed in Table 2. However the decrease of brain 5HIAA was present only in the group which developed aggressiveness.

TABLE 3. 5HT AND 5HIAA BASAL LEVELS AND 5HT TURNOVER RATE AND TURNOVER TIME IN DISCRETE BRAIN AREAS AND IN WHOLE BRAIN OF 30 DAYS ISOLATED AND GROUPED MALE MICE (10 PER CAGE)

(Each point represents the mean of 6 determinations ± S.E.)

Brain areas	% brain weight	5HT μg/g wet wt		5HIAA μg/g wet wt		5HT turnover rate μg/g wet wt/hr		5HT turnover time min	
		isolated	grouped	isolated	grouped	isolated	grouped	isolated	grouped
Whole Brain	100	0·848 ± 0·061	0·812 ± 0·054	†0·231 ± 0·014	0·294 ± 0·011	‡0·362 ± 0·013	0·453 ± 0·012	‡131 ± 2	100 ± 3
Hemispheres	65	0·554 ± 0·024	0·563 ± 0·033	0·194 ± 0·011	0·223 ± 0·011	0·254 ± 0·011	0·291 ± 0·024	*121 ± 5	107 ± 4
Diencephalon	15	1·273 ± 0·031	1·252 ± 0·054	†0·483 ± 0·021	0·591 ± 0·034 s	†0·732 ± 0·041	0·954 ± 0·081	‡96 ± 3	74 ± 3
Mesencephalon	15	0·864 ± 0·031	0·823 ± 0·031	*0·332 ± 0·021	0·413 ± 0·033	0·514 ± 0·033	0·562 ± 0·051	94 ± 4	82 ± 4
Corpora quadrigemina	5	1·661 ± 0·084	1·841 ± 0·084	*0·341 ± 0·034	0·541 ± 0·062	*0·522 ± 0·041	0·804 ± 0·124	179 ± 9	147 ± 14

Significance in respect to controls (10 per cage):
* $P < 0·05$,
† $P < 0·01$,
‡ $P < 0·001$.

3. *Turnover of brain serotonin (5HT) in isolated and grouped mice*

During the 30 days of isolation, the turnover rate of brain 5HT was lower than in animals housed in numbers of 5, 10 or 20 per cage; therefore the turnover time of brain 5HT was increased. However, the most important changes were observed at the beginning and at the end of the isolation. Again there was no correlation between the turnover of brain 5HT and the onset of the aggressiveness in isolated mice (see Table 2).

The dynamic aspects of 5HT metabolism in brain were studied also in discrete parts of the brain of both isolated and normal (10 per cage) mice.

The results (see Table 3) indicate that 5HT turnover is different according to the area of the brain. In both isolated and normal (10 per cage) mice, the turnover rate was highest in diencephalon followed by corpora quadrigemina, mesencephalon and hemispheres, the turnover time of 5HT was longest in corpora quadrigemina followed by hemispheres, diencephalon and mesencephalon. In all the areas the turnover rate and the turnover time were respectively lower and higher in isolated versus normal animals.

4. *Turnover of brain serotonin (5HT) in male and female mice*

Since female mice do not become aggressive after prolonged isolation, it was of interest to investigate the level of brain 5HT and 5HIAA as well as brain 5HT turnover in mice of both sexes submitted to short or prolonged isolation.

Table 4 shows that in male but not in female mice there is a decrease in brain 5HIAA after 1 or 30 days of isolation. As expected male, but not female mice, demonstrate a decreased turnover rate and an increased turnover time of brain 5HT. In Table 4 also the striking difference between the aggressive behaviour of female or male mice after isolation is clearly confirmed.

5. *Turnover time of brain serotonin (5HT) in male rats*

Two strains of rats, namely Sprague–Dawley and Wistar, have been selected for studies on brain 5HT level and turnover, because in our experimental conditions they did not become aggressive after 30 days of isolation. Biochemical studies, presented in Table 5, show that isolation does not change the levels of brain 5HT or 5HIAA or the 5HT turnover.

6. *Density of animals per cage and rate of loss of brain 5HIAA after MAO blockade*

In order to study possible aspecific changes responsible for the observed variation of brain 5HT turnover in isolated male mice, an experiment was designed to find out if the number of animals per cage or the number of injected (MAO inhibitor) animals per cage would have had any influence on the rate of loss of brain 5HIAA.

Therefore male mice previously kept in number of 10 per cage were divided in three groups: (a) mice injected with tranylcypromine (20 mg/kg i.p.) and immediately isolated; (b) mice injected as in *a* but kept in groups of 10 per cage; (c) mice injected as in *a* but kept with untreated mice in the ratio of 3:7.

The results reported in Fig. 1 indicate that the above mentioned experimental conditions do not significantly affect the rate of loss of 5HIAA following MAO blockade.

TABLE 4. BRAIN 5HT AND 5HIAA BASAL LEVELS, 5HT TURNOVER RATE, 5HT TURNOVER TIME AND AGGRESSIVENESS OF ALBINO–SWISS MALE AND FEMALE MICE.

(Each point represents the mean of 6 determinations ± S.E.).

Sex	Time (days)	5HT μg/g wet wt		5HIAA μg/g wet wt		5HT turnover rate μg/g wet wt/hr		5HT turnover time min		Aggressiveness			
										score %		latency time sec	
		I	G	I	G	I	G	I	G	I	G	I	G
Male	1	0·671 ± 0·034	0·701 ± 0·024	*0·224 ± 0·021	0·272 ± 0·023	‡0·341 ± 0·026	0·462 ± 0·038	†112 ± 10	84 ± 8	15	0	242	>300
	30	0·694 ± 0·021	0·714 ± 0·033	*0·222 ± 0·034	0·274 ± 0·021	‡0·348 ± 0·023	0·463 ± 0·027	†112 ± 10	84 ± 8	100	0	42	>300
Female	1	0·683 ± 0·031	0·724 ± 0·043	0·283 ± 0·051	0·251 ± 0·062	0·423 ± 0·053	0·420 ± 0·118	101 ± 12	105 ± 12	0	0	>300	>300
	30	0·692 ± 0·042	0·701 ± 0·042	0·261 ± 0·024	0·281 ± 0·034	0·427 ± 0·043	0·472 ± 0·015	94 ± 10	81 ± 3	0	0	>300	>300

I = isolated
G = grouped
Significance in respect to grouped controls (10 per cage):
* $P < 0.05$,
† $P < 0.01$,
‡ $P < 0.001$.

TABLE 5. BRAIN 5HT AND 5HIAA BASAL LEVELS, 5HT TURNOVER RATE, 5HT TURNOVER TIME AND AGGRESSIVENESS SCORE IN TWO STRAINS OF MALE RATS IN NORMAL CONDITIONS (5 PER CAGE) AND ISOLATED AT 30 DAYS

(Each point represents the mean of 6 determinations ± S.E.).

Strain	5HT μg/g wet/wt		5HIAA μg/g wet/wt		5HT turnover rate μg/g wet/wt/hr		5HT turnover time min		Aggressiveness score %	
	isolated	grouped	isolated	grouped	isolated	grouped	isolated	grouped	isolated	grouped
Wistar	0·401 ± 0·032	0·395 ± 0·021	0·253 ± 0·021	0·264 ± 0·033	0·211 ± 0·012	0·214 ± 0·012	115 ± 11	108 ± 5	0	0
Sprague–Dawley	0·415 ± 0·014	0·403 ± 0·013	0·282 ± 0·031	0·292 ± 0·014	0·273 ± 0·034	0·294 ± 0·011	88 ± 10	78 ± 5	0	0

DISCUSSION

The present study was designed in an attempt to establish if the isolation and subsequent development of aggressiveness induce any change in the metabolism of brain 5HT.

The level of 5HT in the whole brain or in discrete areas of the encephalon is not affected by changing the conditions in which the animals are caged. However, crowded animals (20 per cage) tended to increase their brain 5HT level particularly if compared with short term isolated mice.

5HIAA in the whole brain was significantly decreased even after 1 day of isolation. This change, still present 30 days after isolation, was particularly significant in the diencephalon and in corpora quadrigemina. The turnover rate of brain 5HT was

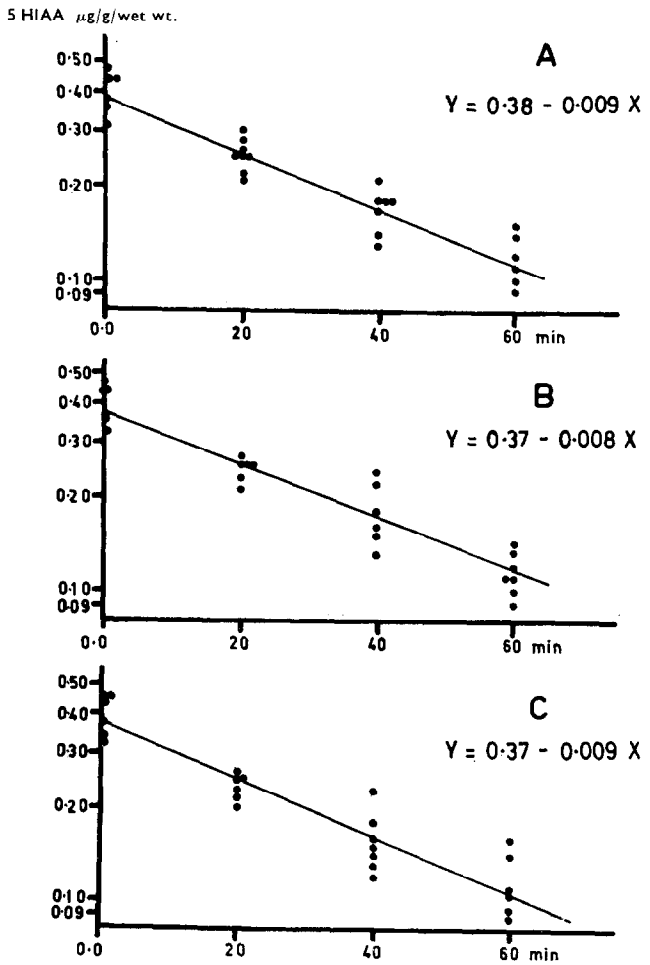

FIG. 1. Density of animals per cage and rate of loss of brain 5HIAA after MAO blockade (each point represents 1 determination; on the right equations of the curve are indicated for each type of density). (A) mice injected with tranylcypromine (20 mg/kg i.p.) and isolated immediately; (B) mice injected as in (A) but kept with untreated animals in the ratio of 3 to 7; (C) mice injected as above but kept in groups of 10 per cage.

decreased in isolated mice while the turnover time was increased when measured following the rate of loss of brain 5HIAA. The changes observed are relatively small and have probably been observed because of the care taken to match all the necessary controls in the same group of extractions.

The reason for the observed changes in brain 5HT turnover are unknown. Several explanations can be given considering the large number of factors which may interfere with the evaluation of 5HT turnover when measured by the rate of loss of 5HIAA after MAO blockade. For instance, previous studies showed that the level of brain MAO is decreased after isolation,[71] a finding recently reported also by other authors in the rat.[72] Since the synthesis of brain 5HT may depend on the availability of tryptophan as a substrate of tryptophan-5-hydroxylase[73–76] a difference in food intake may account for the observed changes. However the observations on the increased food intake during isolation were not reproducible in different experiments, while the decrease of the turnover rate of brain 5HT was quite constant.

Since the method for measuring brain 5HT turnover is based upon the use of a MAO inhibitor, differences in the rate of concentration or removal of the drug in the brain may simulate a difference in the 5HT turnover. However, previous results indicated that the concentration of tranylcypromine in the brain of normal or isolated mice is comparable.[11]

It may also be possible that the observed changes of brain 5HT turnover result from changes in other metabolites or neurohormones important for brain functions. It may be interesting to recall that changes of brain noradrenaline have been observed during isolation[77, 50] although we have been unable to confirm such changes in our experimental conditions.[11]

It may be also interesting to consider that changes in brain noradrenaline turnover may be more important than changes in noradrenaline levels as recently exemplified for various forms of stress by Pujol *et al.*[78] and Thierry *et al.*[79]

Another biochemical finding is the decrease of *N*-acetyl-L-aspartic acid in brain after prolonged isolation.[54] The relation between this effect and the decrease of brain 5HT turnover is not yet apparent, although it has been reported that 5-hydroxytryptophan administration changes the level of brain *N*-acetyl-L-aspartic acid.[80, 81]

An important problem is the possible relation between biochemical changes of brain 5HT and the development of the aggressive behavior. Female mice which do not develop aggressiveness after prolonged periods of isolation do not show any change in the level of brain 5HIAA or in 5HT turnover. Also male Sprague–Dawley rats which do not become aggressive after isolation do not show any change in brain 5HT turnover. Wistar rats have been reported to behave like mice after isolation.[12] However our results are different and brain 5HT turnover was also not affected by isolation.

However, there is no relation in time between the development of aggressiveness and the change of brain 5HT turnover in male mice. The change occurs immediately after isolation when the animals are not yet aggressive.

It may be possible to conclude that the isolation induces an early change, i.e. a decrease of brain 5HT turnover rate lasting for a long period of time and a late behavioral change consisting in the aggressive behavior. It would be very surprising if the small change present in brain 5HT turnover should be responsible for such a striking behavioral effect.

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