EFFECT OF GROUPING OF MICE ON THEIR TOLERANCE TO AMPHETAMINE IN RELATION TO SEX, GROUP POPULATION, AND LIVING SPACE

(UDC 615.785-092.259:599.323.4)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 58, No. 11, pp. 66-70, November, 1964
Original article submitted August 4, 1963

The action of amphetamine in stimulating motor activity is known to be stronger in mice placed together in small groups in a small area than in mice kept in isolation [9]. Grouping also causes a considerable increase in the mortality rate among mice poisoned with amphetamine [6]. After it had been shown that phenothiazines and reserpine prevent death of grouped mice poisoned with amphetamine [5, 11], the phenomenon of the "group toxicity of amphetamine," including the two factors—the higher mortality of grouped mice than isolated, and the motor excitation of the grouped mice with manifestations of rage, squeaking, and biting ("emotional excitation" in the terminology of non-Soviet workers)—became widely used, at the suggestion of Burn and Hobbs [5], as a test for the evaluation of tranquilizers. The increase in excitation and the rise in the mortality rate among the grouped mice, poisoned with amphetamine, have been used to determine the activity of the antidepressant imipramine [2, 3], and the abolition of the protective effect of reserpine, to determine the activity of antidepressants of the monoamine-oxidase group [10]. Experience of the use of the "group toxicity of amphetamine" has shown that the degree of excitation and the mortality rate vary considerably, depending on differences in method; in particular, the experimenter has to pay maximal attention to factors such as the environmental temperature, the sex of the animals, the number in the groups, and the area in which they are confined.

Whereas in regard to the environmental temperature, our previous experience [2] fully confirmed the data in the literature [6], stating that with a rise in temperature the mortality rate among the grouped mice poisoned with amphetamine is increased, in regard to the other factors our observations and the data in the literature were not so definite. For instance, we have repeatedly observed that, when grouped together, females are less sensitive and more tolerant to amphetamine than males. Chance [7] found no sex differences in tolerance to amphetamine by isolated mice, while Fink and Larson [8] found none in grouped mice. It is often considered that one of the essential factors of the "group toxicity of amphetamine" is the constancy of the numerical composition of the group, for which reason the experiment is carried out in such a way that reserved mice are added to the groups to make good the loss due to the dying mice [4]. We know of no experimental results which would indicate that it is necessary to maintain a constant numerical strength of the groups. The area of the space in which the animals are confined, determining the degree of "closeness," is of great importance to the experimental results. With an increase in the area of living space, the mortality rate among the grouped mice falls [6], although this also depends on the population of the group: for groups of 5 mice, doubling of an area of 237.5 cm^2 lowers the mortality, but for groups of 10 mice it leaves the mortality unchanged [8].

The indefinite and conflicting nature of the results cited led us to investigate the influence of sex, the group population, and the area of living space on the tolerance of mice to amphetamine, paying particular attention to sex differences which, as demonstrated in our laboratory, are the cause of the inconsistent results regarding the role of hyperthermia in the phenomenon of the "group toxicity of amphetamine."

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TABLE 1. Sex Differences in Tolerance of Isolated and Grouped Mice to Amphetamine

Dose of amphetamine (in mg/kg)	Mortality			
	In groups of 10 mice		In isolation	
	Males	Females	Males	Females
5	0/10			
7,5	2/10	0/10		
10	30/40	4/30		
12,5	—	7/10		
15	9/10	8/10		
25			—	6/15
50			4/15	11/15
75			16/30	—
100			16/25	15/20
125			9/15	8/10
LD_{50}	8,8 (7,85—9,86)	12 (10,9—13,2)	80 (61,5—104)	42 (28,9—60,9)
Ratio LD_{50} of isolated / LD_{50} of grouped	Males		Females	
	$9^1/_2$		$3^1/_2$	

Note. Numerator—number of dying mice; denominator—total number of mice. This applies also to Tables 2 and 3.

TABLE 2. Mortality of Grouped Mice Poisoned with Amphetamine Depending on Maintenance of Constancy of Group Population

Sex	Experimental conditions	Dose (in mg/kg)							P
		15	15	15	12.5	10			
		Air temperature (in degrees)							
		19-20	19-20	19-20	20	21-22			
		Mortality							
		In individual experiments				Over-all		%	
♂	Without replacement	10/10	6/10	4/10	10/10	8/10	38/50	76	> 0.1
	With replacement	9/10	6/10	4/10	9/10	7/10	35/50	70	
		Air temperature (in degrees)							
				22-23	23-24	23-24			
♀	Without replacement			10/10	7/10	4/10	21/30	70	> 0.1
	With replacement			10/10	7/10	3/10	20/30	67	

Note. The experiments were conducted in compartments with an area of 180.5 cm^2.

METHOD

Experiments were carried out in the spring and summer months on 800 sexually mature mice of both sexes, weighing 17-20 g. Before the experiment the animals were kept in the vivarium in cages containing 50-100 mice each. During the 40-60 min after removal of the mice from the vivarium and before the start of the experiment (weighing, preparation) the animals were kept in glass jars, 23 cm in diameter, each containing 10 mice. During the experiment (immediately after the injection of amphetamine) the mice were kept in compartments of a large, plywood box. The dimensions of the floor of the compartments for the single mice were 9.5 × 9.5 cm (area S = 90.25 cm^2), and for the groups of 10 mice 19 × 9.5 cm (area S = 180.5 cm^2), 38 × 9.5 cm (S = 361 cm^2), and 40 × 19 cm (S = 760 cm^2). The height of the compartments was 19 cm. In the normal sitting position, one mouse occupies an

TABLE 3. Mortality of Grouped Mice Poisoned with Amphetamine, in Relation to Living Space

Series of experiment	Sex	Area of compartment (in cm^2)	Mortality			
			In individual experiments	Over-all	%	P
I	♂	180,5	7/10+9/10+6/10	22/30	73,3	$P_{1-2}>0,1$
		361	4/10+8/10+8/10	20/30	66,6	$P_{1-3}<0,001$
	♀	180,5	0/10+3/10+1/10	4/30	13,3	$P_{2-4}<0,001$
		361	0/10+3/10+0/10	3/30	10	$P_{3-4}>0,1$
II	♂	180,5	10/10+10/10+5/10+4/10	29/40	72,5	$P_{5-6}>0,05$
		760	9/10+7/10+2/10+2/10	20/40	50	
	♀	180,5	7/10+5/10+4/10+8/10+7/10+7/10	38/60	63,3	$P_{7-8}<0,001$
		760	0/10+0/10+3/10+1/10+2/10+2/10	8/60	13,3	

area of 22-24 cm^2. In a control series intact mice (in groups of 10 animals) were put in a chamber with an area of 180.5 cm^2 close together, but climbing up on each other, so that they did not occupy all the floor space. The air temperature above the box, which was measured in all the experiments, was 20-24°. During the experiment the mice were kept without food and water.

Amphetamine, in aqueous solution, was injected subcutaneously in doses of 0.01 or 0.005 ml/g body weight. Observations were made of the animals' behavior, the time of death was recorded during the first 4 h after the injection, and the mortality over a period of 20 h was determined. All the experiments in which a comparison was made were carried out on the same day, at the same time, and in identical conditions. Estimation of the value of LD_{50} of amphetamine for isolated mice were made repeatedly, for the absolute values of the lethal doses varied considerably on different days, although the qualitative relationship between the tolerance of the males and females remained constant. On one day of the experiment each dose of amphetamine was injected into 5 isolated mice. During determination of the mortality rates the constancy of the group populations was maintained by replacing each mouse dying successively from amphetamine poisoning by an intact mouse of the same sex.

The significance of the differences between the mortality rates was calculated by the X^2 method, and the values of LD_{50} with their confidence limits by the method of Litchfield and Wilcoxon [1].

RESULTS

It was found that males are more tolerant to amphetamine than females in isolated conditions, and less tolerant when in groups (Table 1). Grouping of the mice had a much more marked influence on the tolerance of the males than of the females (the value of LD_{50} was reduced by 89.5% and 61.4% respectively). The higher tolerance of the grouped females may also be deduced from the data shown in Table 3.

The results of the experiments of series I showed that the mortality from poisoning with equal doses of amphetamine (10 mg/kg) was independent of the area of the space available for the male mice.

Qualitative differences were present between the phenomenon of the "group toxicity of amphetamine" in the males and females: 1) motor excitation and, in particular, the squeaking and aggressiveness were more marked in the males; 2) the decrease of excitation and onset of death took place more rapidly in the males, and with smaller differences in time between individual animals. Among the qualitative differences may also be included the fact, previously established in our laboratory, that hyperthermia (increasing the rectal temperature to 40.5-41°) is one of the causes of death of females, but not of males. It is possible that this divergence of our findings regarding the sex differences of tolerance to amphetamine from those given in the literature [7, 8] may be attributed to differences between the lines of the mice.

Maintenance of the constancy of the group population by replacing the dying mice with intact animals in the experimental compartment had no effect on the mortality (Table 2). This was evidently because, at the time when death of the least tolerant mice of the group began to take place, the rest of the mice were in a serious condition,

in which their reflex excitability was considerably depressed, so that the presence of other animals in the compartment had no effect on the outcome of poisoning. On the basis of the results we consider that the procedure of replacing the dying mice in the course of the experiment in order to maintain a constant group population is unjustifiable and unnecessary.

A living space with an area of 760 cm^2 (possible slightly larger) was found to be small enough to exhibit the "group toxicity of amphetamine" in groups of 10 mice, especially in males (Table 3). A further decrease in the area caused a significant decrease in the mortality among the females, but the decrease in the male mortality was not significant. A clear exhibition of the phenomenon of the "group toxicity of amphetamine" in females requires closer packing of the animals than in males.

The results of three experiments, in each of which there were 4 groups of 10 mice (2 groups of males and 2 groups of females), are given in series I of Table 3. All the animals received the same dose of amphetamine – 10 mg/kg. In series II only the experiments on mice of the same sex are compared, for they were carried out on the same day and with the same doses of amphetamine (the course and outcome of poisoning were compared in compartments of different floor area). Experiments on mice of the other sex were carried out on other days and with different doses of amphetamine. The doses of amphetamine in the experiments of series II, which varied with the room temperature, were 7.5, 10, and 12.5 mg/kg for the males and 12.5, 15, and 20 mg/kg for the females.

In face of the results of these experiments it is necessary to take strictly into account the technical conditions that have been investigated in order to obtain stable and comparable data concerning the "group toxicity of amphetamine." Social activity of animals is known to be a factor which essentially modifies the central effects, not only of amphetamine, but also of many other drugs, and the data described above may be used if these substances are investigated in groups of mice.

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