Olfactory and auditory stress on mice

(*Mus musculus*)

KRISTA M. SATTLER*

Zoology Department, Duke University, Durham, N.C. 27706

The importance of olfactory and auditory stimuli in causing adrenal hypertrophy and a decreased birthrate in albino mice was investigated. The stimuli (scents and sounds) were derived from a crowded group of mice. They caused an enlargement of the adrenal glands, but failed to reduce reproductive activities. This leads one to doubt the direct simple influence of the adrenal hypertrophy on fecundity; it rather suggests that the social reinforcement and the size of a group determine to what extent behavioral changes, particularly reproductive failure, develop.

A population of mice (*Mus musculus*), reared under crowded conditions, shows distinct changes in behavioral as well as physiological aspects. One example is excessive aggregation, which causes a frequent interruption of normal behavior patterns (Calhoun 1962, 1963). This coincides with a reduction of litter size and weight of individuals and the enlargement of the adrenal cortex (Christian, 1963; Christian & Davis, 1964).

Although these effects have been investigated, knowledge of the external factors that initiate changes of the animals' means of sensing them, and of the relative importance of different sensory modalities is not adequate. However, the role of olfaction in the effects of crowding has been extensively studied by Ropartz (1968).

METHODS

Adult nulliparous albino mice of the Dublin RCS strain, 100 days (± 5) old, were used for the experiments. The animals of each sex were of the same age and similar in weight. From these animals, three groups were formed: (I) the crowded, (II) the pseudocrowded, and (III) the controls.

(I) The crowded group, which provided a source of sounds and scents, consisted of 12 males and 12 females that were kept together in an aluminum pan 33 x 18 x 13 cm.

(II) The pseudocrowded group consisted of five isolated pairs that received olfactory and auditory stimuli from the crowded group. The aluminum pans housing them were 23 x 15 x 13 cm.

(III) The control group of five pairs was raised under conditions identical

to those of the pseudocrowded mice (II), but without receiving the additional olfactory and auditory stimulation.

The pans of all three groups of animals were kept in individual isolation boxes made of double-walled 2-cm plywood with 15-cm Fiberglas insulation between the walls. The inner wall was lined with pile fabric (Fig. 1). Sound attenuation was about

40 dB between $\frac{1}{2}$ and 6 kHz.

Air was forced under pressure through the crowded cage and thence to the pseudocrowded pairs. In addition to this source of scents, excrement from the crowded cage was put beside the pans of the pseudocrowded group every fifth day.

Daily checks for litters were made. If babies occurred, they were removed immediately and their number and weight were recorded.

The above experiments were replicated; as the results were statistically similar¹ (Tables 1 and 2), the data were combined in Fig. 2, which is based upon 24 crowded, 10 pseudocrowded, and 9 control male animals.²

RESULTS

The results are shown in Fig. 2 and Table 3. The pseudocrowded and control groups differed significantly from one another in their adrenal ratios ($p = .0012$) (Siegel, 1956). The crowded group showed the expected high variance in adrenal ratios. They did not differ significantly from the control group ($p = .18$) (Fig. 2).

Fig. 1. Scheme of the experimental setup: I—Crowded cage; II—pseudocrowded cage; III—control cage; A—air conditioning system; AM—amplifier (Knight, 30 W); C—animal cages (crowded: 33 cm long, 18 cm wide, 12.5 cm high; others: 23 cm long, 15 cm wide, 12.5 cm high); E—excrement of crowded cage; HACO₁₋₅—hose air conditioning system to control (Cage 1-5); HACR—hose air conditioning system, crowded cage; HC-PC₁₋₅—hose crowded to pseudocrowded cage; I—fiber glass isolation, 15 cm thick; L—light (fluorescent bulbs, 42 cm, 15 W); M—microphone; T—table; and S—speaker (8 ohm).

*I would like to thank Professor Peter H. Klopfer for his help throughout the experiment, Dr. Richard B. Hemmes for his help with statistics, and Professor David E. Davis for critical discussion of the data. Support came from Grant MH 04453 of the NIMH (to P. H. Klopfer).

+Present address: Institut für Zoophysiologie, Universität Innsbruck, A 6020 Innsbruck, Austria.

Table 1
Results of Data Derived from Male Animals in Experiment I, Which Was Started November 19, 1969 and Terminated January 22, 1970

	1	2	3
	Crowded Group	Pseudo-crowded Group	Control Group
1a: Weight of Paired Adrenal Glands Fresh Weight (Milligrams)			
	3.6	5.1	3.2
	4.0	5.2	3.8
	4.2	5.6	3.7
	4.3	6.5	4.0
	4.4	6.6	4.9
	4.4		
	4.5		
	4.5		
	5.2		
	5.8		
	6.2		
	6.6		
Mean	4.8	5.8	3.9
SD	.93	.71	.62
1b: Terminal Body Weight (Grams)			
	23.1	22.7	27.4
	24.6	27.0	28.3
	26.2	27.1	28.4
	26.4	30.2	35.2
	27.6	32.4	
	28.0		
	29.5		
	30.0		
	30.3		
	31.3		
	31.9		
	33.6		
Mean	28.5	27.9	29.9
SD	3.12	3.68	3.76
1c: Ratios Microgram Adrenal Glands Per Gram Body Weight			
	122.0	173.2	113.1
	133.9	200.6	133.8
	134.8	207.4	139.2
	138.6	224.7	146.0
	140.6	243.5	
	160.7		
	162.6		
	166.6		
	173.6		
	210.2		
	251.9		
	268.4		
Mean	172.0	209.7	133.0
SD	47.5	26.6	14.2
Mann-Whitney U Test	1/2	1/3	2/3
Probability	> .05	> .1	.008

Table 2
Results of Data Derived from Male Animals in Experiment II, Which Was Started January 22, 1970 and Terminated March 24, 1970

	Group								
	I	II	III	I	II	III	I	II	III
2a: Weight of Paired Adrenal Glands Fresh Weight (Milligrams)			2b: Terminal Body Weight (Grams)			2c: Ratios Microgram Adrenal Glands Per Gram Body Weight			
	3.5	5.9	4.2	29.5	29.5	26	86.4	182.8	139.8
	3.8	6.4	4.2	30.5	30.5	27.5	128.8	190.3	142.8
	4.4	6.9	4.5	31.3	31.0	29.0	138.8	191.6	144.8
	5.3	6.9	5.1	33.5	34.9	29.4	140.6	234.0	185.5
	5.5	7.7	5.4	33.8	35.9	32.2	140.8	252.5	207.7
	5.6			35.5			154.1		
	5.9			35.7			158.8		
	5.9			35.8			165.7		
	6.1			40.5			170.4		
	6.5			41.9			190.6		
	6.9			42.5			194.4		
	8.1			42.5			213.1		
Mean	5.6	6.7	4.7	36.1	32.4	28.8	156.8	210.3	164.1
SD	1.29	.66	.54	4.7	2.9	2.3	33.8	30.5	30.7
Mann-Whitney U Test Probability							1/2	1/3	2/3
							.025	.1	= .028

Note I = crowded group, II = pseudocrowded group, III = control group.

cages seemed very weak; 6 were stillborn. Many females in the crowded cage showed various degrees of embryo resorption on dissection.

DISCUSSION

A behavioral-physiological feedback mechanism has been postulated as a control in rodent populations. The enlargement of the adrenal cortex, and thereby the presumed increase in

output of hormones, which is constant per gram of gland tissue (Christian & Davis, 1963). In this experiment, the crowded group did show the usual high variance in adrenal weights (Fig. 1), coincidental with a distinct suppression of reproduction. This would be expected, since in the crowded population the dominant animals are supposed to experience no

Fig. 2. Graph of the ratios microgram adrenal gland per gram body weight (R) of the male animals of the crowded (I), pseudocrowded (II), and control (III) group.

Table 3
Means, Standard Deviations, and Statistical Comparisons of the Three Groups

Group	Mean	SD	Mann-Whitney U Test	
			Groups	Probability
I Crowded	164.5	41.02	II/III	.0012
II Pseudocrowded	210.1	27.16	I/III	.18
III Control	150.3	28.57	I/II	> .05

stress and, therefore, they should have small adrenals (Welch & Klopfer, 1961). In the pseudocrowded cage, however, although the conditions were sufficient to cause an enlargement of the adrenal glands, effects on reproduction could not be detected.

The data suggest that there is not necessarily a causal connection between the two phenomena, suppression of reproduction and adrenal hypertrophy, as was previously postulated (Christian, 1963; Christian & Davis, 1964).

REFERENCES

- CALHOUN, J. B. A "behavioral sink." In E. R. Bliss (Ed.), *Roots of behavior*. New York: Hoeber, 1962.
- CALHOUN, J. B. Population density and social pathology. *Scientific American*, 1963, 206, 139-148.
- CHRISTIAN, J. J. Endocrine adaptive mechanisms and the physiologic regulation of population growth. In W. V. Mayer and R. G. Van Geldern (Eds.), *Physiological mammalogy*. New York: Academic Press, 1963.
- CHRISTIAN, J. J., & DAVIS, D. E. Endocrines, behavior and population. *Science*, 1964, 146, 1550-1560.

ROPARTZ, P. Olfaction et comportement social chez les rongeurs. *Mammalia*, 1968, 32, 550-569.

SIEGEL, S. *Nonparametric statistics for the behavioral sciences*. New York: McGraw-Hill, 1956.

WELCH, B. L., & KLOPFER, P. H. Endocrine variability as a factor in the regulation of population density. *The American Naturalist*, 1961, XCV, 256-260.

NOTES

1. Due to a mistake in sample collecting, the datum for body weight of one animal is missing.

2. Due to some unknown reason the crowded animals of the second set of experiments were very fat (Table 2b). This obesity accounts for the significant difference of the ratios of microgram adrenal weight per gram body weight between the crowded and pseudocrowded groups (Table 2c). This obesity has occurred sometimes with groups of animals in similar experiments, also without apparent reason (D. E. Davis, personal communication).