

THE INFLUENCE OF PERSISTENT CROWDING ON THE AGE CHANGES OF BEHAVIORAL PARAMETERS AND SURVIVAL CHARACTERISTICS OF RATS*

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SUMMARY

One hundred fifty-six male Sprague–Dawley rats were submitted to crowding (12 rats/Makrolon[®]-IV cage) from an age of 5 months onwards. An equal number from the same age cohort served as a control (6 rats/Makrolon[®]-IV cage). As part of an age-test program, behavioral parameters (spontaneous motor activity, reactive motor activity and maze-learning ability) were measured at various ages between 8 and 30 months. The rats were sacrificed for additional measurements after the behavioral tests. Survival curves and age-specific mortality rates were calculated for those rats which died spontaneously in the course of the study. Control rats showed a significant decrease in spontaneous motor activity after an age of 18 months. Reactive motor activity of the controls revealed a fall in the number of large movements between 9 and 15 months, whereas the number of small movements increased up to an age of 30 months. Crowding conditions increased significantly both spontaneous and reactive activity. Maze-learning ability declined significantly with age in the controls whereas crowded rats revealed a tendency to better performance which seemed to be submitted to a seasonal rhythm. Crowded rats showed an improved survival characteristic, beginning at an age of 700 days. Mortality curves turned out to be distinct and parallel by straight line regression. It has been concluded that the positive effects of crowding on behavioral parameters and survival could be attributed to a decrease in vulnerability rather than to a lowered rate of aging.

Key words: Crowding; Motor activity; Maze-learning; Mortality rates; Rat

INTRODUCTION

Crowding may act as a potent stressor in animals and man, inducing typical manifesta-

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tions of the general adaptation syndrome (for review see Selye [1]). In a long-term cohort study on stress and aging in the rat [2] we used persistent crowding as a presumably deleterious influence which might accelerate biological aging. However, in the course of our study, it turned out that behavioral parameters of our age-test program indicated beneficial effects of crowding rather than deleterious ones. This paper describes the effects of crowding on the spontaneous motor activity, reactive motor activity and maze-learning ability of the rat.

MATERIALS AND METHODS

Animals

An age cohort of 468 male Sprague–Dawley rats (OFA-17, Institut für Versuchstierzucht der Universität Wien) was taken into our rat room at an age of 3 weeks and housed in groups of six in Makrolon[®]-IV cages under standard conditions (25°C, light from 6 a.m. to 6 p.m., Altromin[®]-R rat diet and tap water *ad libitum*). At an age of 5 months the cohort was divided at random into three groups of equal size. Two groups were submitted to influences presumed to be stressors (either “noise” or “crowding”), the third one served as a control. In this paper only the group “crowding” (C) will be considered as compared with the “normally aging controls” (A).

Starting at the age of 5 months, crowding was imposed on the rats by placing them in groups of twelve in Makrolon[®]-IV cages. Animals which died spontaneously in the course of the study were replaced by rats from other cages in such a way as to maintain fully crowded conditions in as many cages as possible. Beginning at an age of 8 months, usually every week two animals from each group were submitted to behavioral tests and sacrificed afterwards in order to assess a number of additional parameters [3]. For technical reasons testing was restricted to several periods as shown in Fig. 1. For a first statistical evaluation four age groups were formed, the mean age of which is shown in Table I.

Behavioral tests

Spontaneous motor activity [4] was assessed by an electronic recording system (Animex Activity Meter[®] DSEP with three external coils and a time-sharing system). The rats were registered individually in Makrolon[®]-III cages in complete darkness from 6 p.m.

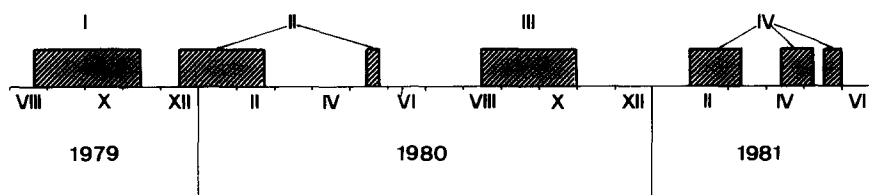


Fig. 1. Testing periods in the long-term cohort study (hatched fields). The animals were born within the 1st week of January 1979. Age groups for statistical evaluation (I–IV) are indicated on top of the hatched fields.

TABLE I

AGE RANGE, MEAN AGE AND NUMBER OF ANIMALS TESTED IN AGE GROUPS I-IV

Age group	Age range (months)	\bar{x} of the age	No. of animals in the age groups	
			A	C
I	8-11	10	24	24
II	11-17	14	20	19
III	20-23	21	23	24
IV	25-30	27	19	21

to 4 a.m. During the registration the animals were deprived of food and water. By means of two sensitivity settings it was possible to distinguish between *total movements* ($45 \mu A$) and *large/fast movements* ($5 \mu A$). Every 10-h registration period, three animals (one from each group) were recorded. Movements were counted over 1-h intervals and summed up for the total registration period. Only the 10-h totals are used in the present paper.

Reactive motor activity [4] was assessed by a cinematographic method. The rats were placed individually in adjacent boxes (base area 600 cm^2 , 15 cm high) closed at the top with thin steel wire mesh. Two animals of each group were recorded simultaneously by an automatic camera at low light from 6 p.m. to 4 a.m. A photograph was taken every 5 min with an exposure time of 3 sec. The activity was graded 0 (rest or sleep), 1 (partial movements) and 2 (locomotion) [4]. The frequencies of movements (1 + 2) and of locomotion (2) were calculated for each hour and for the total registration period. In this paper the 10-h totals are presented. The movements assessed by this method were regarded mainly as reactions to the unfamiliar environment and the noise of the camera action.

Maze learning ability was tested in a semi-automatic T-maze with 7 choice point [5]. Each animal's passage through any choice point was recorded by means of two photo cells; the primary decision was electronically registered, and those points at which a correct decision was made were displayed on a panel. In accordance with the operational definition of learning as a change in probability, given by Hofstätter [6], the performance of the rats in each maze run was expressed in values of probability (P): we calculated the probabilities of the occurrence of *at least* 0 to 7 correct decisions at random during a single run through the maze (Table II) and used these values as a rating scale [5]. Consequently, a better performance is expressed by lower values of P . To evaluate the learning capacity, each rat was trained on 5 successive days, 3 times a day. An appropriate motivation was obtained by depriving the animals of water for 60 h before the start of learning tests. As a reinforcement, the rats were allowed to drink water for 1 min after their arrival in the goal box. In this paper learning ability will be represented by the mean values of 15 runs performed by each animal.

TABLE II

PROBABILITY SCALE (P) FOR EVALUATION OF LEARNING PERFORMANCE IN THE T-MAZE(n = number of correct decisions in a single maze run).

n	P
0	1.000
1	0.992
2	0.938
3	0.773
4	0.500
5	0.227
6	0.063
7	0.008

RESULTS

Means and standard errors were calculated for the various age groups of both the crowded (C) and the control (A) animals. Differences between successive age groups as well as between the corresponding age groups of the A- and C-animals were checked by means of the t -test.

Spontaneous motor activity. Figure 2a depicts the changes with age in *total movements* ($45 \mu A$). Control group A, which represents the "normal aging", shows a slightly rising tendency between the age groups I and II ($P < 0.2$), followed by a significant fall in activity towards age group III ($P < 0.001$) by approximately 27%. From III to IV no further change can be observed. The animals from crowded cages reveal a slight depression of activity in age group I ($P < 0.1$). In age group II activity increases to the values of group A and, in contrast to group A, remains at the elevated level. This results in significantly higher values of activity in III and IV as compared with the age-matched controls (III: $P < 0.01$, IV: $P < 0.05$).

Changes with age in *large/fast movements* ($5 \mu A$) (Fig. 2b) of the control group A are different from those of total movements: the means of activity decline steadily between the age groups I and III by 25%, the difference being significant between II and III with $P < 0.05$. The curve of the crowded rats (C) declines along with that of the controls from I to II, however, then increases to a level significantly higher than that of the controls (III: $P < 0.001$, IV: $P < 0.05$).

Reactive motor activity. The patterns of reactive activity (Figs. 3a and b) differ basically from those found in spontaneous activity. The *total movements* ($1 + 2$) of the control group A show an initial slight decline ($P < 0.1$), which is followed by a significant incline ($P < 0.05$) towards a peak in age group III. The frequency of *locomotion* (2), however, decreases between I and II ($P < 0.05$) with no detectable changes afterwards.

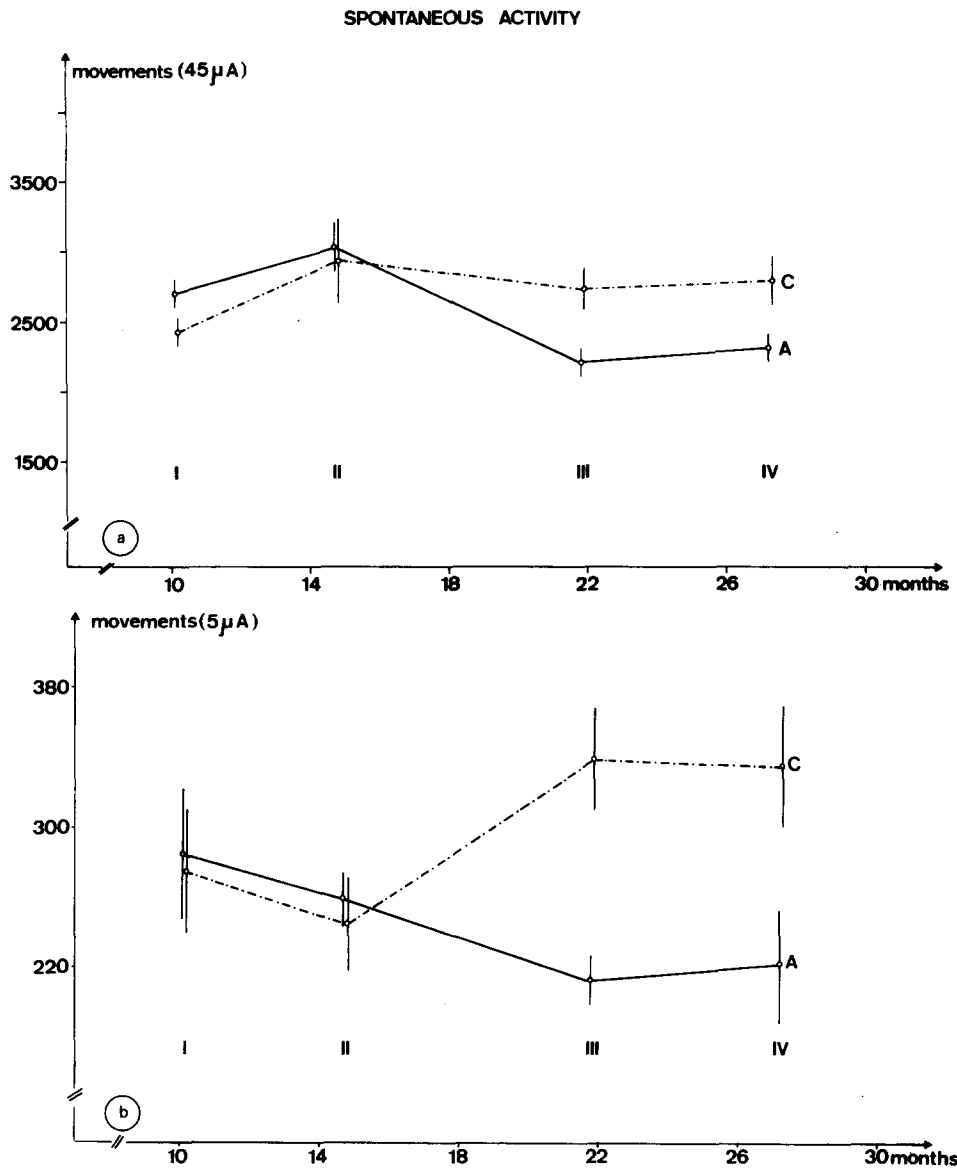


Fig. 2. Spontaneous motor activity (means \pm S.E.M.) of crowded (C) and control rats (A): (a) total movements (45 μ A); and (b) large/fast movements (5 μ A).

The rats from crowded cages (C) showed no difference from the controls in the first testing period (I). However, with increasing age and duration of crowding, the curves of both the total movements (1 + 2) and locomotion (2) deviate progressively from the values of group A towards a higher frequency. In age group IV, the difference becomes

REACTIVE ACTIVITY

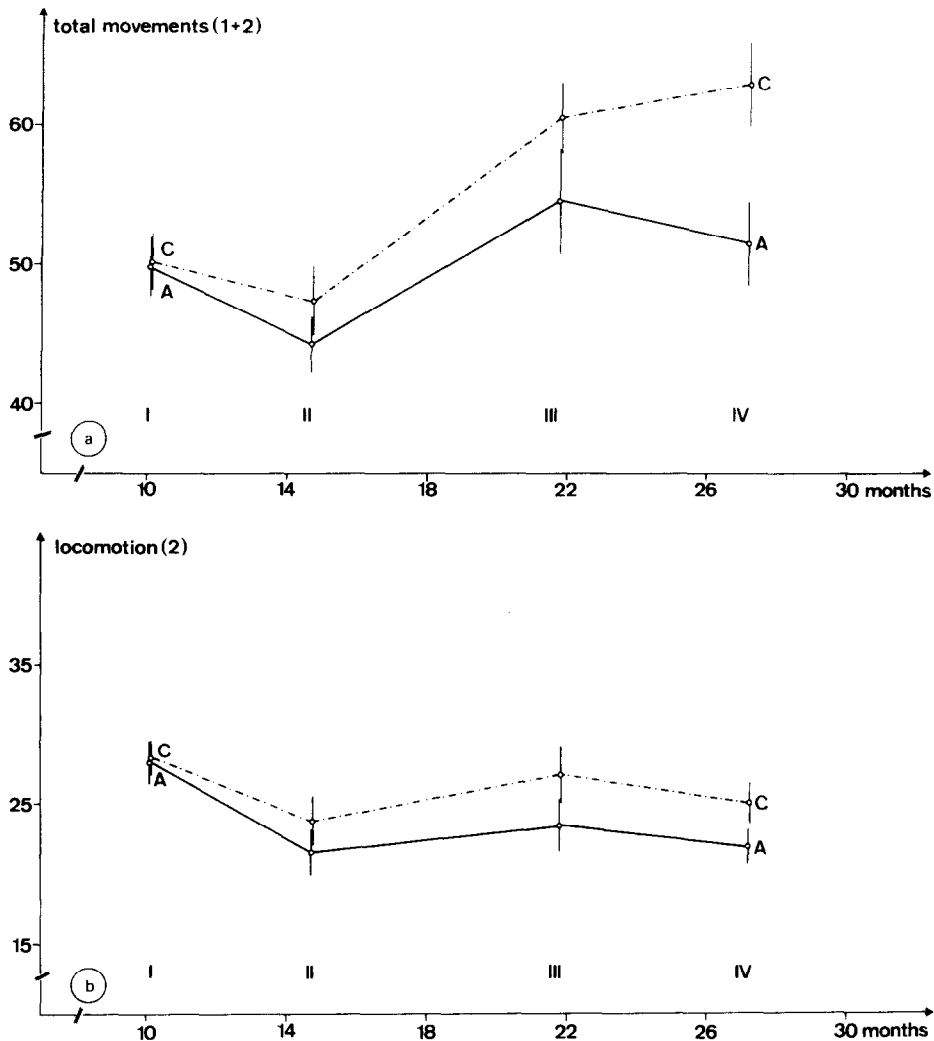


Fig. 3. Reactive motor activity (means \pm S.E.M.) of crowded (C) and control rats (A): (a) total movements (1 + 2); and (b) locomotion (2).

significant for total movements ($P < 0.001$), while it does not exceed the level of $P < 0.2$ for locomotion.

Maze learning ability, as assessed by the probability scale (Fig. 4), decreases steadily with age (increasing values of P) in the control group A, the difference being significant between the age groups I and IV ($P < 0.05$). This age-dependent change disappeared in group C and was replaced by a rhythmic pattern in which the values of the age groups I

MAZE - LEARNING ABILITY

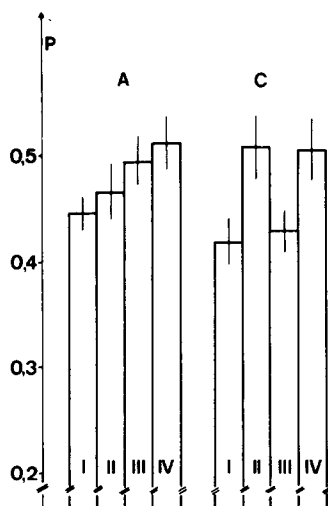


Fig. 4. Maze-learning ability evaluated by the probability (P) scale of crowded (C) and control rats (A) (means \pm S.E.M.).

and III were significantly lower than those of groups II and IV ($P < 0.05$). In comparing corresponding age groups, the crowded rats performed significantly better in the age group III ($P < 0.05$).

Survival characteristics. In order to judge the relevance of changes in biological parameters to the aging of the individual, it is desirable to know the predictive value of the parameter for survival. This requires a correlation of the parameter values of each animal

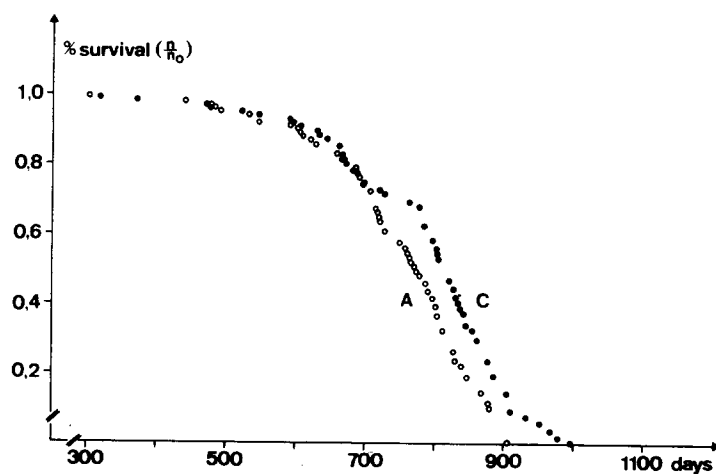


Fig. 5. Survival curve of those animals which died spontaneously in the course of the cohort study.

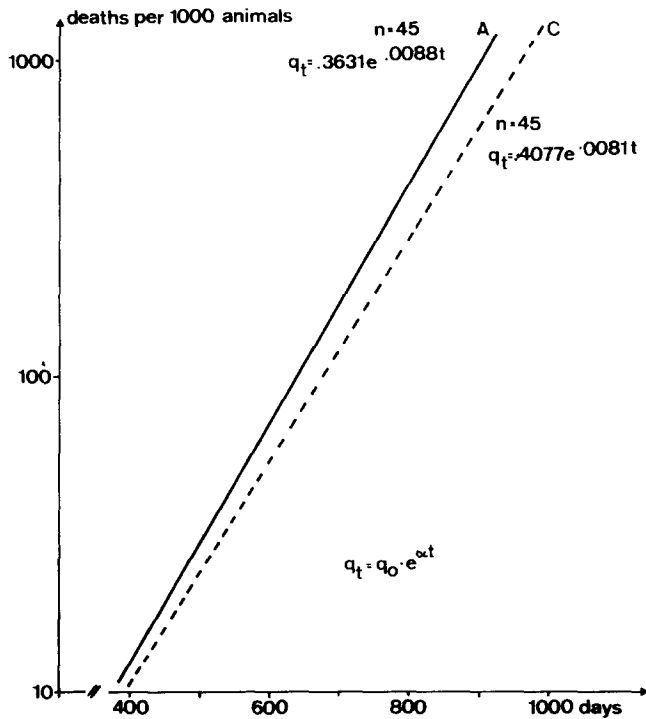


Fig. 6. Age-specific mortality rates calculated from overlapping 100-day periods, starting at an age of 400 days.

with the survival period. However, this was impossible in our long-term study since the rats were sacrificed for the assessment of additional parameters. As a surrogate for the required correlation, we established the survival curves of those animals from both experimental groups which died spontaneously in the course of our investigation. The curves were calculated with the n_0 -values newly adjusted each time that rats were sacrificed for the investigation.

The results are shown in Fig. 5. At the beginning, the curves of A and C are identical. Starting approximately at an age of 700 days, the survival curve of the rats living under crowded conditions shifted towards a higher age, extending the mean and maximum life span. In spite of the relatively small number of animals, we tried to obtain a rough estimate of the Gompertz function by calculating mortality rates from a number of overlapping 100-day periods starting from an age of 400 days. As can be seen in Fig. 6, group C shows lower age-specific rates than the controls. A "straight line regression" proved the lines to be distinct and parallel.

DISCUSSION

The behavior of an animal reflects the top level of integration of biological functions.

With regard to the measurement of biological age, behavioral parameters reflect the overall functional capability of an organism, in particular its capability to integrate its system and to adapt them to environmental challenges. However, the value of behavioral parameters to aging research is limited by the fact that a great number of endogenous and exogenous non-aging influences may cause much larger variations of the parameters than aging does. Nevertheless, behavioral parameters should be employed in age-test programs because they can be expected to reflect the changes in vitality more validly than parameters at a low level of organization [7].

A number of rodent studies have indicated age-related deficits in behavioral functions, e.g. in general motor activity [4,8,9] or in a number of learning tasks including maze learning [10–13]. The deficit in behavioral performance with advancing age seems to be dependent on the type of test and the complexity of the task [11]. Therefore, the parameters of the present study were chosen as to cover distinct realms of behavior and increasing levels of complexity.

Spontaneous motor activity is considered as a base-level behavior which makes little demand on the vitality of the organism [14]. In a previous cross-sectional study with male rats aged 9, 15 and 29 months [4] we showed by the same method that this type of activity, which consists mainly of grooming and appetitive behavior, is maintained without any remarkable age-related change throughout early adult life and only starts to decrease significantly within the second year. Large/fast movements (presumably searching behavior), which make up approximately 10–15% of the total spontaneous activity, start to be reduced earlier, at least before an age of 9 months. Our present study confirms these findings. Crowding conditions caused a distinct change in this pattern for young adult and senescent rats: whereas in younger rats activity appeared to be suppressed, the opposite was found to be the case in senescent animals with regard to both total and large/fast movements. We think that this may be due to the age-related changes in the animal's need for motion. The higher need in young animals obviously makes the immobilization, which results from severe crowding, a considerable stressor that may weaken motivation. Decreasing need for activity with advancing age seems to reduce this negative effect, allowing the positive effects of crowding to come into action. Since the rat, like most other rodents, is a "contact animal" [15], the intimate social contact may be a considerable benefit obtained from crowding conditions. In addition, a methodological aspect may be important: the animals in crowded cages will get habituated to the intimate social contacts and, therefore, will show an increased social appetite when isolated in the registration cages. The remarkable increase in large/fast movements beyond initial levels support this view.

Reactive motor activity, as assessed by our cinematographic method, consists of various types of behavior. From the registration photographs and from direct observations we conclude that the majority of movements are reactions to the environmental stimulation (e.g. orientation movements evoked by the noise of the automatic camera, exploration, or attempts to escape through the wire mesh at the top of the boxes). In our previous cross-sectional study cited above [4], reactive activity was assessed as well.

It showed a steep decline already present between 9 and 15 months, which lowered the activity by some 35%. This clear-cut change has not been found in our present long-term cohort study. With regard to locomotion, a much less dramatic fall is indicated in the early phase of senescence, which levels out in advanced age. However, as is indicated by the curve of total movements, the frequency of partial movements increased in the second year to form a peak in age group III. The inconsistency with our earlier results may be explained by two assumptions: the main age-related decline in reactive motor activity may have already begun at an earlier age than that covered by our study. So we assume that what is shown by our curves is the end of this decline after which the activity is maintained at a low level without any further age-related changes until approaching death. If this is the case, the observed changes in the total movements should reflect influences other than aging. We have good reason to assume that a seasonal rhythm may be superimposed on the smaller age-related changes. This is suggested by several other parameters, such as blood cell counts [16] or age parameters of the tail tendon (unpublished results). In reactive motor activity, the tests which yielded higher values (age groups I and III) were both performed from August to November. Crowded conditions elevated progressively the level of activity compared with the controls and, in addition, seem to have accentuated the presumed circannual rhythm.

Maze learning ability showed an increasing deficit with age in the controls. In crowded animals a kind of rhythmic variation replaced the normal aging pattern. The change of periods of high and weak performance suggests the presence of a seasonal rhythm, which modifies the effects of crowding on maze learning ability. As is indicated by the only significant difference between the crowded rats and the controls (age group III), crowding tends to improve learning ability. This may, in part, be due to the hormone changes under stress since it was found by File and Clark [17] that increased levels of ACTH are apt to improve performance in learning tasks. In addition, we observed a similar improvement in the "noise-group" which is not considered in this paper.

The positive effects of crowding, shown to various degrees in all of our behavioral parameters, exclude the possibility that these are due merely to a specific condition of the testing method, as discussed in connection with spontaneous motor activity. Furthermore, survival curves of crowded and control rats lead to the conclusion that crowding must have caused a marked improvement in those bodily conditions which are particularly relevant to biological aging. In this context it should be noted that some parameters of connective tissue (tail-tendon stability, soluble collagen and hexosamin content of dermis) pointed to an accelerated aging process [18]. Therefore, we feel that it is necessary to reconsider the present concepts used for the assessment of biological age.

The analysis of age-specific mortality rates, according to the model proposed by Sacher [19], can provide some insight into the action of beneficial influences on aging and survival. Sacher outlines the two basic possibilities of changes in the Gompertz function: (1) lowering the initial vulnerability, leading to a parallel shift of the mortality curve; and (2) reducing the rate of aging, causing a divergence of the Gompertz curves. In our study, the curves of crowded animals and controls appear to be parallel rather than

divergent, pointing to the first type of life extension. This type has been attributed by Sacher [20] to a so-called hormetic action of environmental influences, which ameliorates a preexisting unfavorable condition. Robert *et al.* [21] have shown that rats immobilized in isolation developed gastric ulcers, while this effect was prevented by keeping the animals under crowded conditions. The development of ulcers was also avoided by increasing the environmental temperature or by the application of prednisolon. However, it is improbable that the behavioral effects we obtained by crowding are due only to metabolic changes. It rather seems appropriate to assume that the primary events took place within the central nervous system. Since laboratory animals normally live in an (unfavourable) impoverished environment, the intense social contact in the crowded cages may have acted as an enrichment of the environment. Enriched conditions have been proved to promote the development of the nervous system in young animals [22]. More recently it has been shown in senescent rats that the neuronal connectivity is maintained at a higher level in a complex environment than is the case in isolation. We therefore feel that, in investigating possibilities of influencing aging, the integrative mechanisms of the central nervous system deserve increasing attention, in accordance with the view of Shock [23] that "aging, as we see it in the total animal, may be more a function of the breakdown in integrative mechanisms than of changes in individual cells, tissues, or organs".

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