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Behavioral impairments of the aging rat

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Abstract

Several disturbances occurring during aging of humans and rodents alike stem from changes in sensory and motor functions. Using a battery of behavioral tests we have studied alterations in performance with advancing age in female and male rats of some frequently used strains. In parallel, we collected survival and body weight data. The median survival age was similar for female and male Sprague–Dawley rats, inbred female Lewis and outbred male Wistar rats (29–30 months). In contrast, male Fisher 344 had a significantly shorter median life span. During aging there is a gradual decline in locomotor activity and explorative behavior while disturbances of coordination and balance first became evident at more advanced age. In old age, also weight carrying capacity, limb movement and temperature threshold were impaired. While whole body weight continues to increase over the better part of a rats' life span, the behavioral changes in old age associated with a decrease in both total body weight and muscle mass. Dietary restriction increases median life span expectancy; retards the pace of behavioral aging and impedes sarcopenia. Housing in enriched environment did not improve the scoring in the behavioral tests but tended to increase median life span. Finally, there was an agreement between behavioral data collected from longitudinal age-cohorts and those obtained from multiple age-cohorts.

Keywords: Survival; Motor system; Sensory system; Sarcopenia; Caloric restriction

1. Introduction

Depreciation of sensations, postural and locomotor disturbances and senile tissue atrophy are typical phenotypic alterations in normal human aging. Aging-dependent changes in body composition and behaviors are evident in rodents suggesting that rats, in particular, may serve as a useful model for sensorimotor disturbances in aged humans [reviewed in [1,2–5]]. Among the prerequisites on an animal model of aging is that the aging-related changes can be consistently read-out in behavioral tests and, moreover, that life span characteristics are established to allow selection of appropriate controls. For example, a behavioral disturbance may be the result of a process across the entire life span or a process confined to old age. A multi age-point approach is often recommended but may in fact not always be needed if the model is well characterized.

This report serves to give an account on changes in explorative and sensorimotor-dependent behaviors with advancing age in otherwise healthy rats. To this end, we have used a battery of well-established behavioral tests (cf. [2]SHIRPA, [3]EMPrESS), and records of median survival age and body weight change with advancing age. Most of the data derive from groups of outbred female Sprague–Dawley (SD) rats but groups of outbred male SD and outbred male Wistar (Wi), inbred male Fisher 344 (Fi) and female Lewis rats were included for comparison. We also address the issues of gender differences, husbandry (dietary restriction and environmental enrichment) and consistency in results obtained with a longitudinal cohort and a multiple age-cohort study design, respectively.

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[3] http://empress.har.mrc.ac.uk/.

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2. Materials

2.1. Strains and stocks

All animals used were purchased, or bred in-house, during 1995–2004 and used in experiments until 2006. The main strain used was outbred Sprague–Dawley (SD) rats (colonies originating from Harlan Sprague–Dawley, Houston, Texas, US), either delivered at an age of 2 months by a commercial breeder (ALAB:SD, ALAB, Sollentuna, Sweden (1993–1995); Bkl:SD and ALAB:SD, B&K Universal, Sollentuna, Sweden (1996–2002); or Bkl:SD, Scanbur, Stockholm, Sweden (2002–2004); Crl:CD(SD); Charles River, Germany (2004)) or bred in-house with founders (ALAB:SD; Crl:CD(SD) (2001–2006)) from one of these stocks, see Table 1. Male SD rats were invariably virgins and of the ALAB:SD stock, while females were from all 3 stocks of SD and either virgins or retired breeders (4 deliveries). Records from a total of 726 female and 296 male SD rats, form the basis of our compilation (Table 1). In addition, smaller groups of single-point delivered Lewis (female, $n=40$; inbred stock LEW/Crl; Charles River, Germany), Wistar, Wi, (male, $n=30$; out bred Bkl: WISTAR, B&K Universal, Sollentuna, Sweden) and Fischer 344, Fi, (male, $n=30$; inbred F-344/DuCrl, Charles River, Germany) rats were used for comparison, Table 1. The Lewis (1999), Fi (1995) and Wi (1995) rats were single deliveries from respective breeder, Table 1.

2.2. Housing conditions and health inventories

All animals were purchased as SPF (specified pathogen free) animals. After arrival, they were kept under conventional conditions in the animal facility at the Department of Neuroscience, Karolinska Institutet. Health inventories, according to the FELASA recommendations (http://www.felasa.org/recommendations.htm), showed that the animals were free of pathogens with the exceptions of *Pasteurellaceae* (occasionally found) and *Helicobacter* spp. species.

Animals were kept 5 to a cage up to a body weight of about 500 g, then re-housed 3 to a cage in type Makrolon™, M4, cages (Techniplast, Buguggiate (Va) Italy), provided with woodchip standard bedding material (Tapvei, Kortteinen, Finland). With advancing age, animals died from natural courses or euthanasia and the survivors were re-housed to avoid single housing. Cages were cleaned once a week and the animals were inspected on a daily basis. Room temperature was kept at 21±0.2 °C and relative humidity at 50±5% (variance estimates based on continuous records covering one month). A 12/12 h light/dark cycle was used with a dawn and dusk system of 0.5 h each. A radio was used as background noise.

Groups of female SD rats were also housed in an enriched environment, EE, consisting of large cages with a standardized set of play materials: running wheel, transparent and non-transparent tubes, and an inclined mesh leading to an elevated platform. The volume of the cage is approximately 4× that of a standard M4 cage. The number of animals per cage was 9 or 10.

Food and water were served ad libitum, and changed twice a week at about 10 AM. All animals were fed commercially available food-pellets (Lactamin R70, Lantmannen, Stockholm, Sweden). Follow-up on levels of trace elements and vitamins (Selene and vitamin E) revealed no decrease in old age (supplementary Table S1). Animals on dietary restriction (DR) were fed with 70% of the daily consumption recorded for ad libitum fed aged-matched controls (same strain and stock) and this ration was served at about 10 AM on a daily basis. DR was imposed either post-weaning or at 1 year of age, and maintained until the animals died from natural courses or euthanasia.

Animals were weighted with interval varying between 1 and 4 months. When animals were euthanized, tissues were rapidly recovered for further analysis. The soleus muscle was used to evaluate the adaptation of hind limb muscle to body weight bearing demands. The rational for using soleus is that this muscle is postural and steadily active in all types of locomotion [6]. Based on this we assumed that this muscle would adapt to everyday body weight bearing demands also in the very restricted environment of standard M4 cages. A ratio, muscle weight (mg) to whole body weight (g) was created (MBR).

2.3. Data sets

This study is based on animal behavioral tests and surveillance data collected over an extended period of time and, in addition, female SD rats were of several stocks delivered

Table 1
Compilation of rat strains, stocks and vendors used in this study, including data contributed by each set of animals

				Ad libitum						
Sex	Strain	Vendor	Total	Survival	Weight	Behav.[§]	Stage	MBR[§]	DR**	AC**
Female	Alab:SD*	B&K Universal	178	69	40	95	115	31		10
	Bkl:SD	B&K Universal	424	207		230	270	78	51	34
	Bkl:SD	Scanbur	60			30	30	40		
	Crl:CD(SD)	Charles River	64		20	7[#]		15	37[#]	
	LEW/Crl	Charles River	40	40	40		20			
Male	Alab: SD*	B&K Universal	296	49	34	138	208		15	
	Bkl:WISTAR	B&K Universal	30	30	23	23	16			
	F-344/DuCrl	Charles River	30	30	6	6				

*This stock was originally supplied by ALAB but later maintained by B&K Universal when they took over the operation in Sollentuna, Sweden. [#] Tested in LABORA system, ad libitum $n=7$ and DR $n=9$. **Animals on DR and housed in EE were also subjected to behavioral testing. [§] Soleus muscle-to-whole body weight ratio. [§] Represent the number of animals used in the behavioral test from respective strain and stock. In "Stage" column is number of animals subjected to stage ranking.

from 3 different commercial suppliers (Table 1). The behavioral test protocols was highly standardized and maintained over time. Housing conditions were perturbed as little as possible, however, re-housing was needed among other things to avoid single-housing. Several of the strains and stocks used here are point deliveries (LEWIS/Crl, F-344/DuCrl and Bkl:Wistar rats) and adult, middle aged and aged SD males were all from the same stock (ALAB:SD), housed and tested during a restricted period of time. The SD females were derived from different vendors and stocks (Table 1). All-in-all, 20 age-cohorts of SD female rats were tested (Table 2). In order to examine consistency over time and possible differences among stocks and vendors, we compared test results from four cohorts of adult female rats (3–4 months old) with complete records from the behavioral tests (supplementary Table S2). It should be noted that in the analysis of effects of dietary restriction and environmental enrichment, the experimental animals were compared with age-matched controls from the same batch of the stock. Furthermore, testing of aged male strains (ALAB:SD; F-344/DuCr and Bkl: WISTAR) was accomplished in one series of experiments.

The animals used here were house under supervision of a laboratory animal veterinarian and animals with clinical signs of sickness were omitted from the behavioral testing and tissue analysis.

2.4. Behavioral testing

All animals had at least one week of acclimatization following arrival or re-housing prior to behavioral testing. Test sessions started at 2 PM and continued until about 5 PM; two observers were present throughout each session. A more detailed description of the test protocols is given in Supplementary data. Briefly, the test battery is included:

Table 2
Compilation of all SD female age-cohorts subjected to behavioral testing

Cohort ID	Cohort size	Age (months)
Cohort 1	20	3
Cohort 2	20	3
Cohort 3	9	3
Cohort 4	10	4
Cohort 5	10	4
Cohort 6	14	12
Cohort 7	29	14
Cohort 8	12	15
Cohort 9	18	19
Cohort 10	25	21
Cohort 11	33	22
Cohort 12	20	24
Cohort 13	21	27
Cohort 14	7	27
Cohort 15	11	28
Cohort 16	20	29
Cohort 17	22	30
Cohort 18	22	30
Cohort 19	16	30
Cohort 20	16	30

Indicated are cohort number, number of animals and age at which the animals were tested.

2.4.1. Open field activity

Explorative behavior was examined with the open field test as described in [7–9], using a square area with walls (70 × 70 × 30 cm) in gray colored plastic. Explorative behavior was recorded for 180 s.

2.4.2. Crossing a wire mesh screen

Using 70 cm long, 2.5 cm-wire mesh screen the time-to-pass and hind limb performance was evaluated [10].

2.4.3. Beam balance

A 2.5 cm-wide wooden beam was suspended 0.5 m above a soft surface. The rat was placed on the beam for a maximum of 60 s, and the performance was ranked according to [11]. Each animal was subjected to three consecutive trials, and the mean score of these trials was calculated.

2.4.4. Walking track analysis

For this test, the animals feet were stained with non-toxic acrylic paint (fore paws with red and hind paws with black color) and they then had to walk through an 8.5 × 42 cm transparent Plexiglas tunnel with the "home cage" at the other end. The following records were made from the walking tracks: a) stride length (distance between fore paw-fore paw and hind paw-hind paw); b) gait width (distance between left and right hind paws), c) placement of hind paw relative to fore paw (distance between hind paw-fore paw in each step cycle) (supplementary Fig. S1).

2.4.5. Placing reaction

Tactile placing was evaluated according to [10,12]: while supporting the animals trunk, the dorsal and plantar surface of each foot were gently touched. A score of 1 was given for normal, immediate placing; a score of 0.5 was given if the placing was delayed or incomplete; a score of 0 indicated absent placing.

2.4.6. Righting response

The rat was held in the examiner's hand approximately 30 cm above a soft surface, and the righting reflex was elicited by turning the rat over on its back upon release. A score of 2 was given if the animal showed a normal righting response, i.e. counter to the roll direction; a score of 1 was given if the righting response was weak, delayed or in the direction of the roll; a score of 0 indicated no righting attempt [12,13].

2.4.7. Nociceptive hot plate test

The animal was placed on the heated surface until it licked paws, jumped or vocalized [14,15]. The cut-off time was set at 30 s to avoid tissue damage.

2.4.8. Automated recording of rodent behavior

The *LABORAS system* for automated recording of rodent behaviors in plastic cages was used to compare basic motor activities of middle-aged female rats (Crl:CD(SD)) fed ad libitum or maintained on DR. The animals were placed individually, with the same bedding material and access to food and water as in their standard home cages. In our set up, a group four cages were run in parallel and the recording time was

set to 23 h for practical reasons (1 h for to allow for cleaning between animal groups).

2.5. Patterns of aging and stage ranking

Gait cycle disturbance and weight bearing incapacitation (for example showing up as a decline in rearing behavior and impaired support of body weight) are characteristics of the old age phenotype. However, not all senescent rats disclose these stigmata. This is particularly evident in outbred rat strains. We therefore developed a simple stage-ranking protocol based on two parameters of hind limb function [reviewed in [3,16]]:

(a) Is a limb weight bearing? This was determined by the animals ability to extend the limb and use only the (sole of the) paw as supportive surface during a gait cycle. Infrequent errors to extend the leg were scored 1, while more frequent errors scored as 2. Complete failure to extend the limb during gait, leg and trunk resting on the supportive surface, was scored as 3.

(b) Does a limb show a complete gait cycle coordinated with the other limb(s)? A score of 0 was given if a limb showed all 4 phases (stance, paw-off, swing, paw-on) coordinated with the movement of the other limbs. Infrequent error disrupting the stride rhythm was given a score of 1, while frequent errors resulting in a limping stride pattern was scored as 2. Severe limping and partial immobility (dragging the limb along) were scored as 3.

According to this scheme low symptom animals belong to stage 0 (no symptoms; score 0) or 1 (minor signs or infrequent errors; score 1) and high symptom animals belong to stage 2 (clear gait cycle aberrations, and decreased body weight support power in at least one limb, score 2) and stage 3 (advanced gait aberrations with signs of partial immobility of at least one limb, score 3). The stage ranking protocol was applied by two observers on unrestrained animals shortly before euthanasia. The ranking was done by observers not knowing the performance of the rats in other behavioral tests.

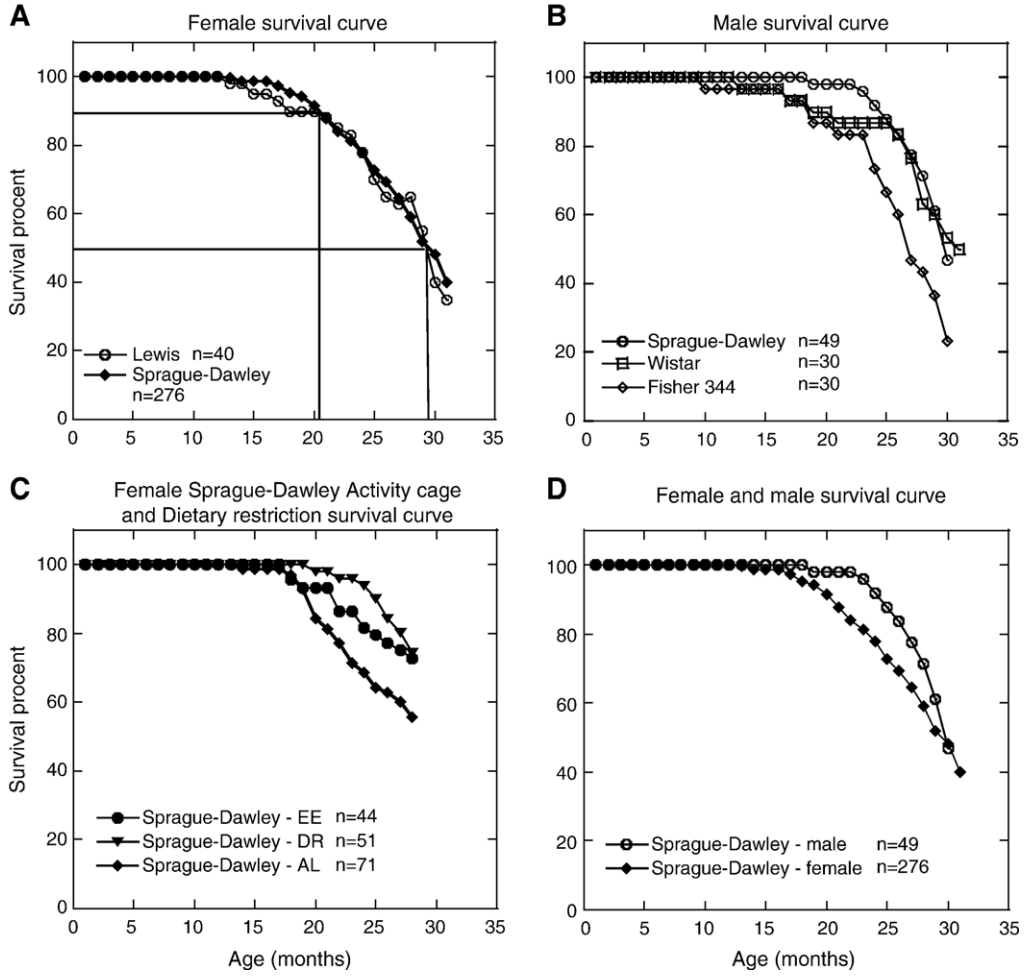

Fig. 1. Survival data for rats. (A) female Sprague–Dawley and Lewis rats; (B) male Sprague–Dawley, Wistar and Fischer 344 rats. (C) Survival data for female Sprague–Dawley rats housed under standard conditions (AL), dietary restriction (DR) and in an enriched environment (EE). Note, in (C) AL are the age-matched controls for this particular study, while in (A) accumulated survival data from multiple cohorts have been lumped. Survival curve for males and female SD rats is shown in panel (D). In (A) solid lines indicate the 90% and 50% survival ages, respectively. The only statistically significant deviations in survival were recorded for Fisher 344 rats vs. Wistar and SD rats ($p<0.05$); and among female SD rats, animals on DR showed an improved survival ($p<0.01$).

2.6. Plots and statistical analysis

Box plots have the following definitions: box limits represent upper and lower quartile values, and are separated by the median (crossbar within box). The interquartile distance thus contains 50% of the data. Maximum and minimum values, which are not defined as outliers, are illustrated using error bars. Outliers (circles) are defined as values deviating from the quartile borders by more than 1.5 times the interquartile distance. In all other plots, the median and the standard error of the mean (SEM) have been indicated. All statistics were performed using Statistica 6.1 (Statsoft, Tulsa, USA). Comparisons of experimental groups were mainly carried out with nonparametric analysis of variance (Kruskal–Wallis test) and a post-hoc test for pair wise comparisons (multiple comparison of mean rank for all groups; two-tailed test). Comparison of two dependent samples was accomplished using Wilcoxon matched-pairs test. Record series of repeated measurement were analyzed by ANOVA for repeated measurements and Bonferoni's post-hoc test. Kaplan–Meier plots were used to analyze survival records and the log-rank test was used to compare samples [17]. Variance in body weight among litter mates fed ad libitum and on dietary restriction, respectively, were compared by calculating the coefficient of variance (CV, standard deviation divided by mean weight per cage) followed by student *t*-test. Correlation of two parameters (interval scale) was accomplished using the Spearman Rank correlation test with significance testing of the rank correlation coefficient (r_s). Statistical significance levels were set to: $*p<0.05$; $**p<0.01$; $***p<0.001$.

3. Results

3.1. Survival data

Survival data for male and female SD rats, female Lewis, male Wistar and male Fisher 344 rats are shown in Fig. 1. A 90% survival is expected at 20–21 months while the median survival age is 29–30 months for all strains except the Fisher 344 rats. The median life expectancy was similar for female and male SD rats (Fig. 1).

3.2. Body weight

Ad libitum fed rats continue to grow and gain weight over a considerable part of their life span [18,19]. There is an initial fast increase covering about 24 weeks (Fig. 2C), after which growth continues but at a slower pace until weight peaks at 18–24 months (Fig. 2A and D). Beyond two years, the animals maintain their weight or disclose a modest decrease; this loss in whole body mass accelerates in old age. The weight loss in old age, compared to peak weight, was significant for female SD

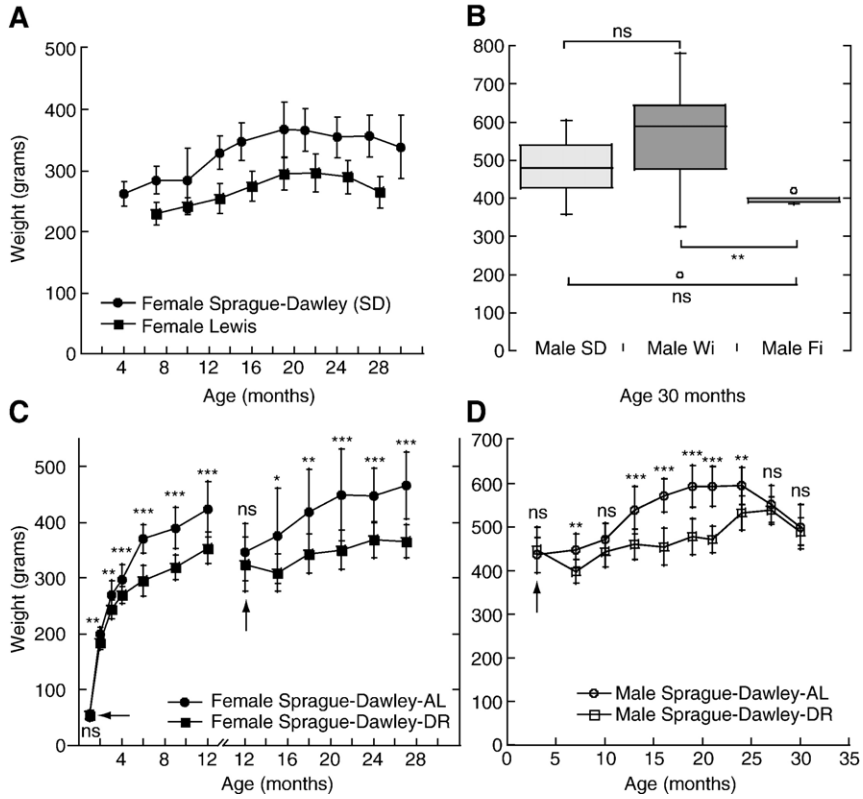

Fig. 2. Weight data for (A) female Sprague–Dawley and Lewis rats; (B) single point measurement at the 30 month-age of male SD, Wistar and Fischer 344 cohorts. (C) Body weight gain for female Sprague–Dawley rats under DR are shown, introduced in the post-weaning period (horizontal arrow; virgins) or at one year of age (vertical arrow; ex-breeders) along with there respective control groups. (D) Male SD rat's response to DR introduced at 3 months of age (vertical arrow). Statistical significant differences between DR and AL fed animals have been indicated. ns=non significant; *, ** and *** correspond to $p<0.05$, 0.01 and 0.001, respectively.

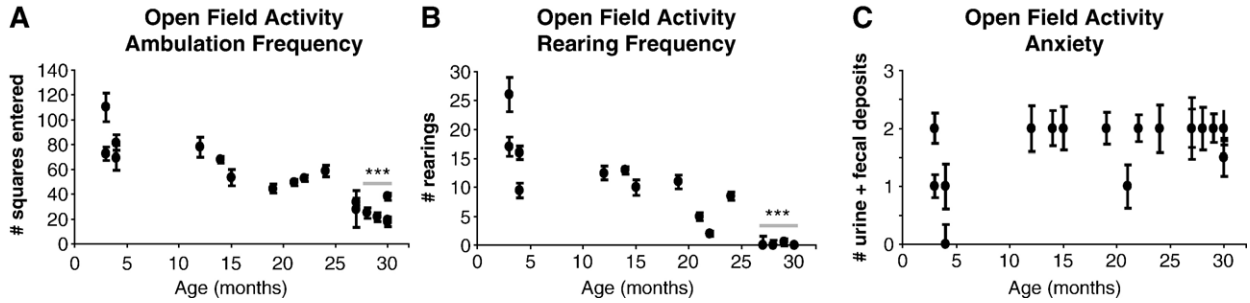

Fig. 3. Plots showing change in activity (median value and SEM have been indicated) with age in the open field-test. Locomotion (A), rearing events (B) but not anxiety (C) level decreases with advancing age in SD female rats (age-cohorts listed in Table 2). The aged cohorts (>27 month old, horizontal bar) were all significantly different from the adults (3–4 month old) in ambulation frequency (A). (B) All rats older than 26 months (horizontal bar) were significantly different from adults (3–4 month old) in rearing frequency. The middle aged rats formed an intermediate group in A and B. Level of anxiety did not vary with age. Key to symbols in Fig. 2.

($p<0.05$), Lewis ($p<0.01$), Fisher 344 ($p<0.01$, data not shown) and Wistar ($p<0.01$, data not shown) rats (Fig. 2). The drop in weight in old age of male SD rats fed ad libitum ameliorated the difference to the age-matched SD males on DR (Fig. 2D).

Out bred strains are usually larger than inbred strain, cf. SD females vs. Lewis females (Fig. 2A) or Wistar and Fisher 344 males (Fig. 2B). Different stocks of out bred SD strain (Fig. 2A and C) vary in absolute weight gain and peak weight but these variations do not alter the shape of the weight curve across life span. Among female SD rats, ex-breeders weigh less than virgins (Fig. 2C).

Dietary restriction introduced in the post-weaning period, or at one year of age, decrease body weight gain and thus body growth (Fig. 2C–D) but does not alter the shape of the weight curve. Body weight variability among cage-mates was less for rats on DR (CV: 0.08±0.04; 9 cages, 5 animals per cage) than among those fed ad libitum (CV: 0.13±0.09; 11 cages, 5 animals per cage); however, this difference did not reach statistical significance ($p=0.081$).

3.3. Analysis of variance among female SD stocks

Variance analysis of adult female SD rats (supplementary Table S2) showed that the scoring in the sensorimotor tests was quite similar between stocks (Bkl:SD and ALAB:SD; B&K Universal and Scanbur) and over time (1999–2004). The open field arena, disclosed larger differences among the cohorts of

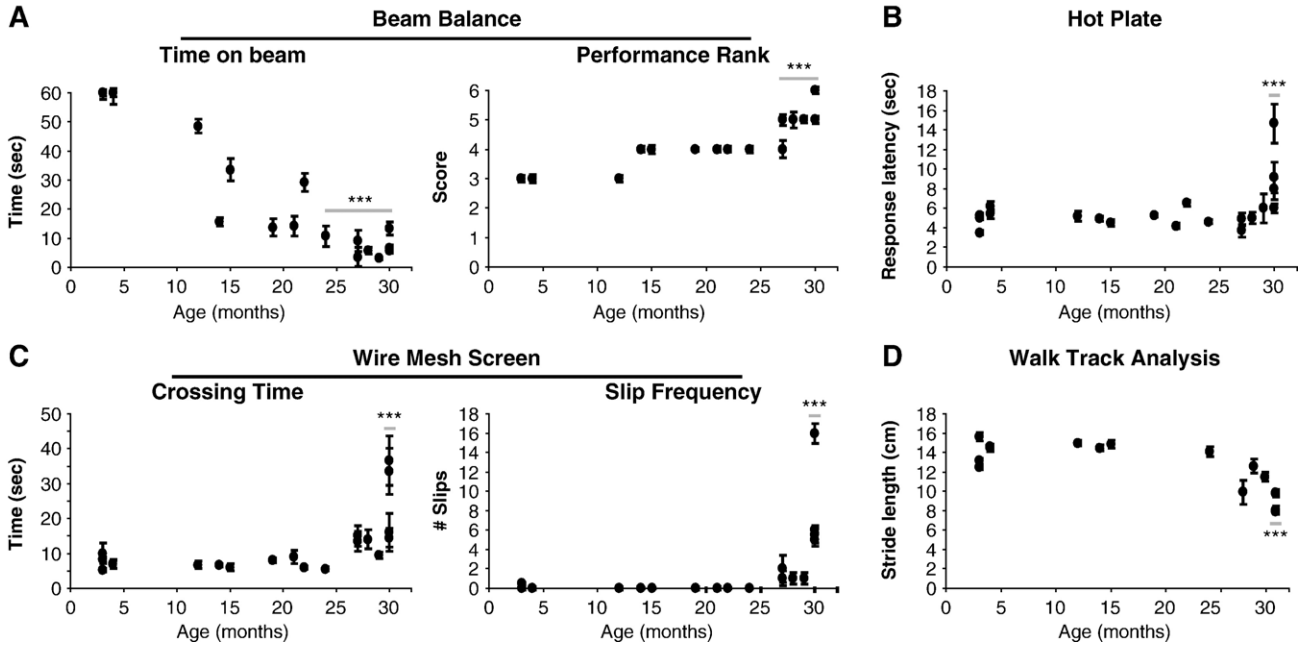

Fig. 4. Pots showing performances (median value and SEM have been indicated) of female SD rats at different ages (age-cohorts listed in Table 2) in (A) the Beam balance test; (B) the nociceptive hot-plate test; (C) wire mesh crossing test; and (D) stride length. Time on beam (A) was significantly reduced in aged (>22 month old; horizontal bar) compared to adult rats (3–4 month old), and performance score was significantly worse in rats older than 24 months compared to adults. The middle aged rats formed an intermediate group in-between these extremes. In B–D, the middle-ages (12–21 moths) and early-age group (22–25 months) were not different while adult (3–4 month old) showed a significantly better performance than old age rats (30 month old; horizontal bar). Key to symbols in Fig. 2.

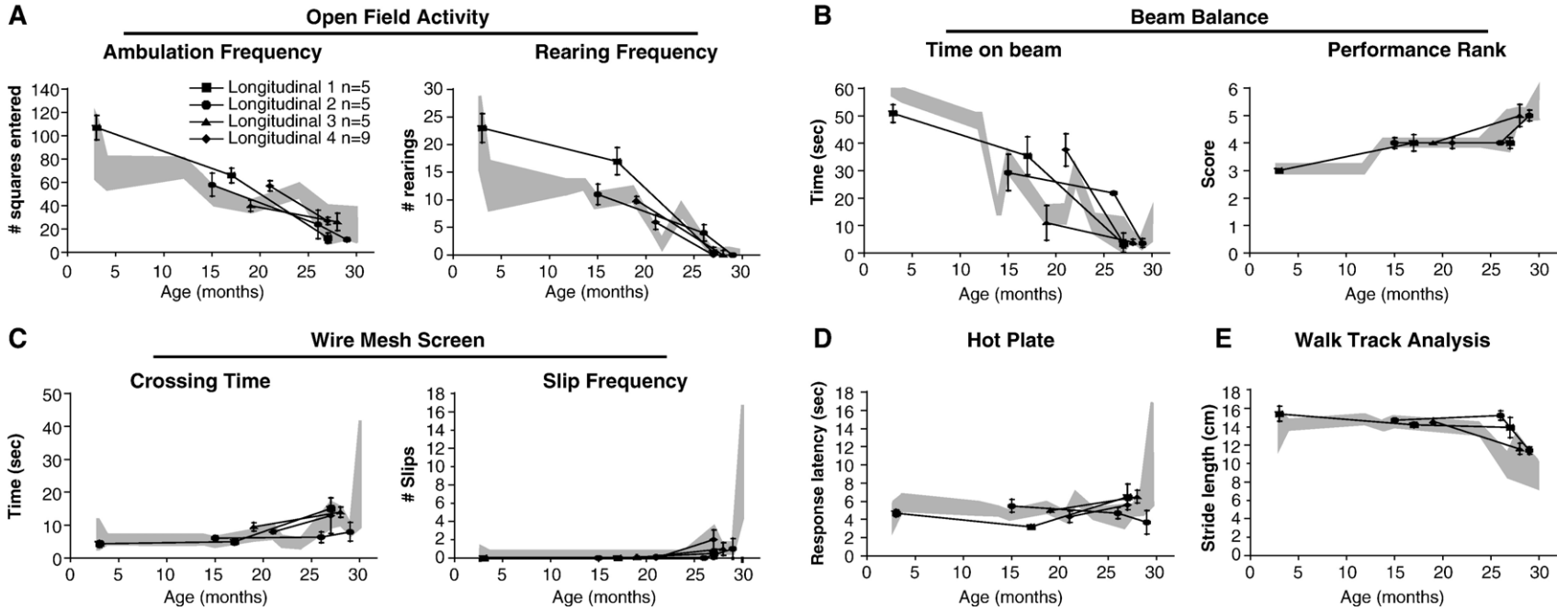

Fig. 5. Comparison of behavioral data obtained with a longitudinal (groups indicated in A) and multiple age-cohort study design (age-cohorts listed in Table 2), respectively. (A) Explorative behavior; (B) the Beam balance test; (C) Wire-mesh crossing; (D) the Hot-plate test and (E) Stride length. The medians and standard errors of the means (SEM) have been indicated for the longitudinal age-cohorts, while the shaded fields correspond to the variation in SEM of the SD female's cohorts also shown in Figs. 3 and 4. Interconnected points (carrying symbols to reflect that they represent different longitudinal age-cohorts; see key to symbols) indicate data from repeated recordings of single age-cohorts with advancing age. The longitudinal data sets include only records from surviving animals. Furthermore, the longitudinal and multiple data sets are unique; thus, the longitudinal data was not used in the multiple age group plot and visa versa.

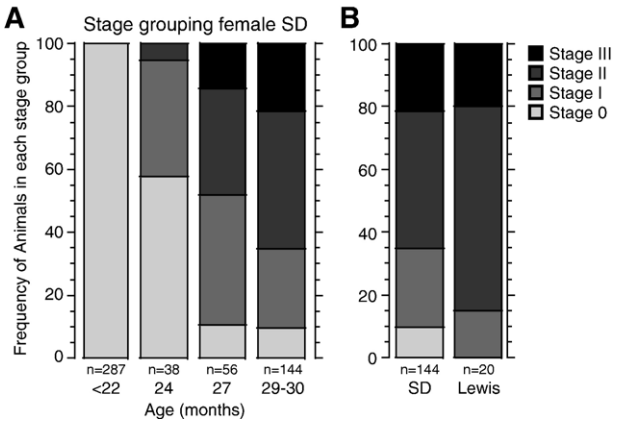

Fig. 6. (A) Histogram showing the distribution of stages in female SD rats of different ages: adults and middle-age rats (21 months and younger) were lumped since all rats in these age groups were stage 0; early aged (24 month old), aged (27 month old) and old (29–30 months old). (B) Stage distribution in old (29–30 months old) inbred Lewis and out bred SD female rats. For further details see text.

which some were significant (Table S2), yet these differences are small in comparison with the changes recorded during aging (see below).

3.4. Explorative behavior

The reaction to a novel environment was recorded with the open-field test. With advancing age there is a gradual decrease in locomotion and rearing (Fig. 3). Loss of muscle power in the hind limbs probably contributed to absence of rearing in the old. A similar pattern of changes in activity-level was evident in male SD rats (supplementary Fig. S2) and, moreover, old SD males' behavior was comparable to old age Wistar and Fisher 344 male rats (supplementary Fig. S2).

3.5. Sensorimotor behavior

We used a battery of tests to characterize alterations in sensorimotor dependent behavior (see Materials section). Results from 20 cohorts of female SD rats (Table 2) have been summarized in Fig. 4 (corresponding data from male rats is shown in supplementary Fig. S3). In the beam balance test the performance gradually declined with advancing age (Fig. 4A; for details on test and scoring see Materials section, and Supplementary data). In contrast, both the nociceptive hot-plate test (Fig. 4B) and the wire mesh screen test (Fig. 4C), disclosed a different pattern with no, or only minor, alteration in behavior across adulthood and middle ages; however, in old age there was a significant change in behavior. A similar pattern of alterations was evident in the gait analysis. For example, stride length tended to increase from adulthood to the middle ages along with increase of body size (Fig. 4D) but dropped significantly in old age. Also the Righting response ($p<0.001$; data not shown) and the Placing reaction ($p<0.001$; data not shown) were preserved until old age.

In conclusion, a number of the tests showed no alterations until old age while other tests disclose a pattern of a gradual change with advancing age with an accelerated incapacitation in senescence. Although we have less data on males, the changes were close to those seen in females (supplementary Fig. S3).

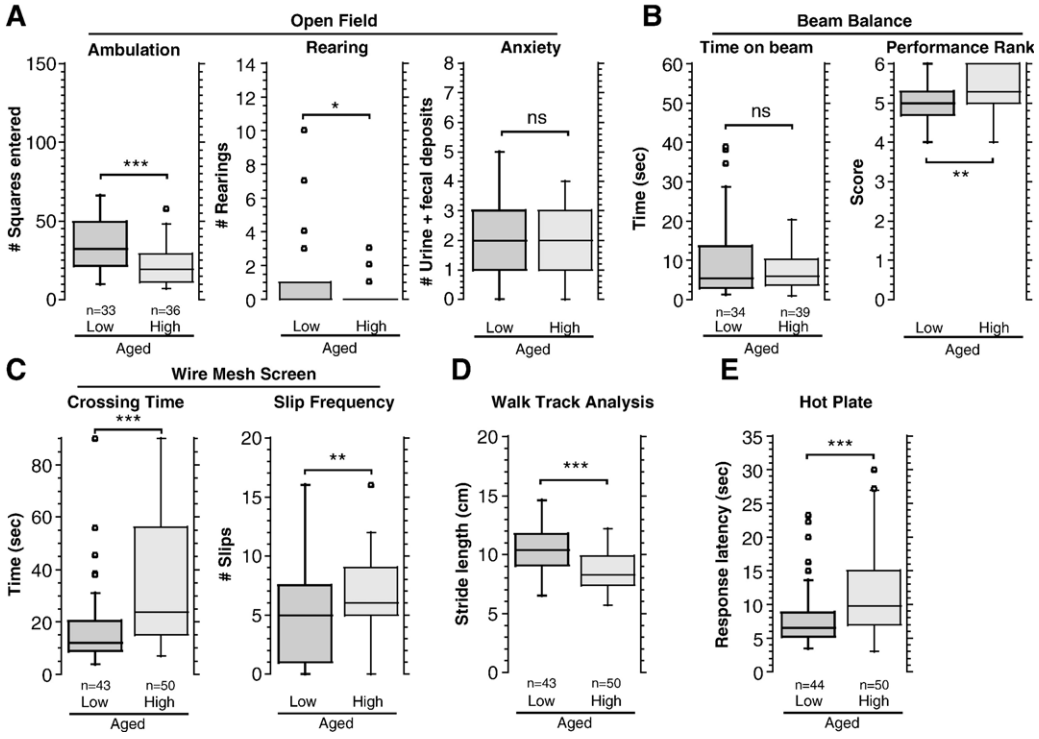

Fig. 7. Box plots showing the test results of the old age-cohorts divided into a high (stage 2–3) and a low (stage 0–1) symptom group, respectively, using the stage-ranking protocol described in the text. Key to symbols in Fig. 2.

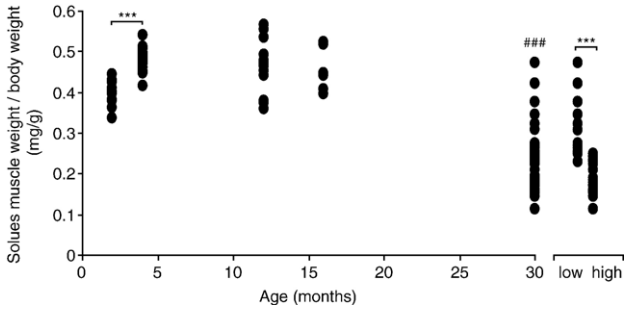

Fig. 8. Scatter plot showing the distribution of m. soleus weight (mg) divided by total body weight (g) in different age-groups of female SD rats. On a separate abscissa, the old group with a complete set of behavioral data has been partitioned into one group with low (stage 0–1) and one group with high (stage 2–3) symptoms; according to the stage-ranking protocol. Relative muscle weight increase during adolescence (***) and remains thereafter stable across adulthood including the middle ages. In the old, relative muscle weight decrease significantly (###) and this drop correlated with stage of behavioral deficit symptoms (***). Key to symbol: $^{***/\#\#\#}p<0.001$. $n=10$ at 2 m (months); $n=20$ at 4 m; $n=14$ at 12 m; $n=6$ at 16 m; $n=38$ at 30 m; $n=15$ in low and $n=23$ in high.

3.6. Longitudinal cohorts vs. multiple age groups

An important issue in studies of aging is whether a multiple age-groups design will show results consistent with the preferred, but more laborious, longitudinal age-cohort design. We analyzed this by comparing longitudinal data recorded from smaller groups of female SD rats with those obtained with the multiple age group design (Fig. 5). Although this comparison is only descriptive, the results show that there is a fair degree of consistency in the results obtained with the two study designs.

3.7. Patterns of aging and stage ranking

By applying the stage-ranking protocol (see Materials section) to the SD female shown in Fig. 4, we found animals with symptoms in the early aged, aged and the old age groups (Fig. 6A). In comparison with female SD rats, both male SD and male Wistar rats had more cases in the advanced stages in the old age-cohort (supplementary Fig. S4), while inbred Lewis females showed less variability in the stage ranking with no low-symptom cases in old age (Fig. 6B).

Next, we explored if stage could predict the outcome in the different behavioral tests by partitioning the 29–30 month old rats (cohorts 16–20, Table 2) in one group holding animals with low symptoms (stages 0–1) and one group holding animals with a high ranking score (stages 2–3). The outcome of this meta-analysis is shown in Fig. 7 and indicates that the stage-ranking predicts the outcome in several but not all of the tests. We therefore conclude that using stage ranking alone, or in combination with behavioral tests, old age-cohorts can be partitioned into subgroups of successful and less successful patterns of aging.

3.8. Loss of body weight and muscle mass in the old rat

We weighed the soleus muscle, immediately after euthanasia and calculated a ratio of muscle weight over body weight

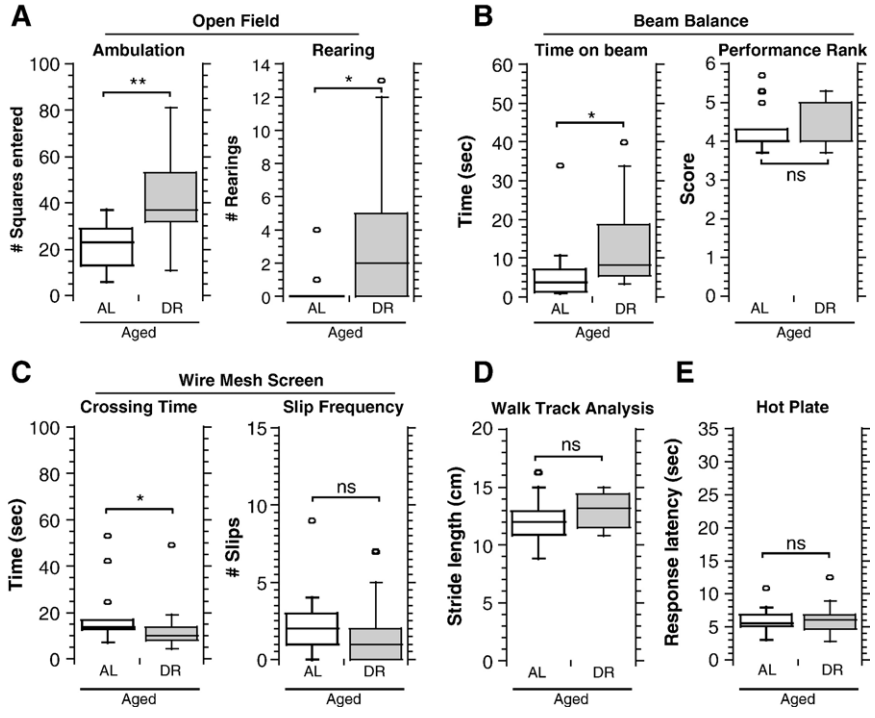

Fig. 9. Box plots of behavioral test results among 28-month-old female SD rats housed under dietary restriction (DR; $n=13$)) and aged-matched controls housed under standard conditions (AL; $n=13$). The tests are the same as those shown in Figs. 3 and 4. Key to symbols in Fig. 2.

(Fig. 8). A mature ratio is reached at about 4 month of age and maintained throughout the middle ages. This adaptation of soleus mass to whole body weight seemingly fails in senescence and the degree muscle wasting associates with the animal's pattern of aging (according to the stage-ranking protocol). A correlation analysis of the loss of muscle mass and stage ranking (0–3; supplementary Fig. S5) among female SD rats disclosed a significant covariation (r_s=0.726; p<0.001; n=38).

3.9. Environmental influence on the pattern of aging

Animals allowed to age in an enriched environment did not accomplish significantly better than aged-matched controls housed under standard conditions in the behavioral tests used here (supplementary Fig. S6). In contrast to rats housed in enriched environment, animals on DR performed better than their aged-matched AL fed controls in several but not all of the behavioral tests (Fig. 9).

When comparing middle aged rats housed under DR with their AL fed aged-matched controls, using the LABORAS™ automated behavioral analysis system, the most striking difference was the over-all increased locomotor activity (Fig. 10), showing that DR affects the basal activity pattern of the animals not only in old-age.

Finally, DR reduces the loss of muscle mass relative to total body mass (Fig. 11) in old age [see also [20]]. Housing in an enriched environment improved the muscle-to-whole body weight ratio somewhat in senescence (Fig. 10); however, this difference was not statistically significant (p=0.07). Many of the senescent DR animals showed relative muscle weights typical for normal middle aged rats and DR improved the muscle weight-to-whole body weight ratio not just in old age but already in the middle ages (Fig. 11).

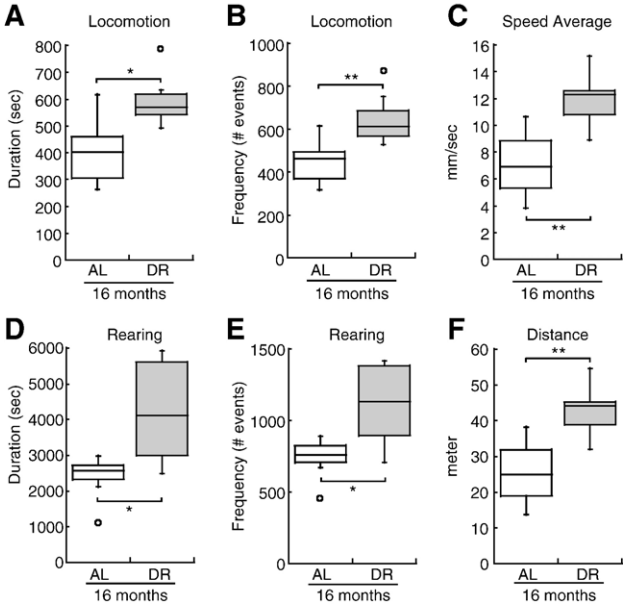

Fig. 10. Box plots depicting (A–B) locomotion duration (s per 23 h) and frequency (# events per 23 h); (D–E) rearing duration (s per 23 h) and frequency (# events per 23 h); (C, F) average speed (mm/s) and total distance (m) sampled over 23 consecutive time-windows for 16 month old female SD rats fed ad libitum (AL; n=7) or on restricted diet (DR; n=9) female SD rats. Key to symbols in Fig. 2.

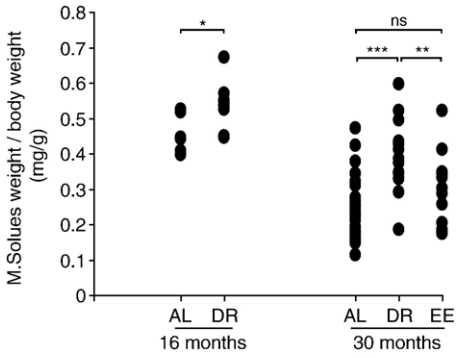

Fig. 11. Scatter plot showing the distribution of soleus muscle-to-whole body weight in 16 month-old and 30 month-old SD females housed in standard cage with free access (AL; n=7 and n=38, respectively) or restricted access to food (DR; n=9 and n=13, respectively). Thirty month-old female SD rats housed in an enriched environment with free access to food (EE; n=14). Key to symbols in Fig. 2.

4. Discussion

4.1. Life span characteristics

In aging-studies of rodents, the chronological age is rarely set in a life span perspective of the specific strain and stock used. This hampers both critical reviews as well as comparison between studies. As remarked by Burek and Hollander [21], a minimum of information should hold the median survival age for the strain or stock in use. While variation in reported life span expectancy (in a historical perspective) to a large extent may be attributed to quality of husbandry and exposure to pathogenic microorganisms, life span does vary among strains also in high quality pathogen-free housing conditions [5,22]. For several of the strains and stocks studied here, however, the median survival age was similar, i.e. around 30 months [this study, [18,19,21,23–25]]. A stock-to-stock variability of 1–2 months in median survival age was reported previously for female SD rats [3]. Considering the short life span recorded for rodents in wild life [26], a protected environment is a major modulator of life span. We saw no distinct effect of gender or, among females, a difference between virgins and breeders, on median life span expectancy in a protected environment. We suggest, based on our large body of data on SD rats, that life span may be divided into segments to aid in the selection of appropriate control group(s). We will not discuss the prevalent pathologies among the rat strains used in this study since there are a number of excellent reviews covering this topic [19,24,27–30], which stresses the importance of considering strain-common morbidities when selecting a rat strain for aging research [compare with [5,22]].

4.2. Behavioral analysis

To understand the consequences of an altered cell function on the organism level behavioral tests serve as a high-end read-

out. A great advantage with using rats is that many of the established behavioral tests were developed for this species and can be employed without modifications. Corresponding protocols validated for mouse are now available at the EMPrESS web site (see Introduction). Of obvious importance is that the testing protocol does not change over time and that the operators are well-trained. As pointed by others, test protocols should be validated over time, since subtle changes in the testing conditions may occurs and, also, there is usually a rotation of laboratory staff over time.

In aging research appropriate controls poses a particular problem and, as mentioned above, may demand a study design with more than two age groups. A multiple age-groups design is less laborious and therefore usually favored over a longitudinal study design. The latter design has several advantages, since it eliminates the risk of drift in the genetic background as well as subtle differences in husbandry and will also decreases the number of animals needed. A shortcoming is the very limited possibility to correlate alterations in behavior to cell biological changes at the tissue level (to blood, urine and possibly also skin samples) and inherited to the longitudinal design is that many tests are not well-suited for repetitive measurements. The comparison made here between study-designs is only descriptive and includes a limited number of animals but indicates that there is a fair consistency in the results obtained with the two study-designs.

It is evident from our results that some behavioral changes develop gradually during aging (from maturity to death), as alteration in explorative behavior, whereas other behaviors, like reaction to noxious stimuli, are maintained until old age. Thus, some behavioral modifications is the results of a continuous process starting after development has finished, while other alterations are specific for old age. In common for most of the test is that functional decline accelerates towards the end of the expected life span. Furthermore, our data indicates that the pattern of changes, in the behaviors analyzed, is quite similar across strains and stocks and between genders. The sensorimotor tests-battery records quite basic phenotype characteristics and this may be one contributing factor to the observed small variance in behaviors between stocks and strains. The open field test was previously shown to be able to differentiate more subtle differences among strains and stocks [31], and in this test the 3 strains of aged males tended to show more, possibly strain-related, differences than in the sensorimotor tests (supplementary Figs. S2–S3).

Selection of test is important, by inference from the test design it may be obvious that a test will favor a small body size over a large, like the beam balance test. The poor performance during the rat's middle ages may at least in part be explained by the continued body growth, i.e. their larger size. In favor of this explanation is the observation that the righting reflex was intact until old age (response time was sufficient to enable correction of body position). However, also increased body stiffness with advancing age may have contributed to the poorer performance in the beam balance test. In old age, the beam balance behavior deteriorates further, despite the decrease in body weight, probably reflecting the compounded effect of aging-related loss of skeletal muscle mass and sensorimotor impairments [3,32,33]. It is also important to recognize that changes in behaviors during aging reflect a read-out of the functionality of multiple systems (sensory pathways, central integration and processing, motor output, as well as the peripheral target tissues like skeletal muscle). Thus, systematic correlative work using behavior as the high-end read-out and system/cell analysis with physiological, histological and molecular approaches may, combined, reveal the sequence of events in the emergence of aging-related impairments.

4.3. Patterns of aging

An important observation is that the pattern of aging varies between animals in the same age-cohort. Some show early symptoms and developed extensive aberrations in old age while others are clearly less affected also in senescence. Thus similar to humans, we may talk about successful and less successful patterns of aging in the rat. Given that the living conditions are strictly standardized, the variability should mainly reflect differences in the genetic make up among cohort members. In support of this, the inbred strains Lewis and Fisher 344 used here showed less variability than the outbred SD and Wistar rats. An outbred rat strain may more closely model the human variability in pattern of (successful/unsuccessful) aging and therefore worth exploitation to pin-down factors contributing to the pace and extent of aging-induced impairments. The fact that also inbred rats show variability indicates that epigenetic modifications influence the pattern of aging. Thus, combining inbred and out bred stocks and strains may aid in dissecting the contributions made by genetic background, epigenetic modifications and other environmental influences, respectively [see [31]].

4.4. Environmental influence — the issue of husbandry

The boredom of animal cage-life has been debated extensively and is the focus of numerous animal welfare studies [reviewed in [34]]; it is claimed that the standard housing lacking environmental enrichment promotes depression, physical inactivity and over-eating (as such an interesting model for some of our concurrent medical problems in humans). Environmental enrichment came into focus in aging research when it showed to improve animals' scoring in a range of mainly cognitive tests and possibly also extended life span expectancy [[35,36], reviewed in [37]]. Moreover, experiments show that social enrichment is important for the animal's normal behavior [38,39]. To test if enrichment also affects the pattern of aging, we housed animals in enriched environment (see Materials section). Even though the number of observations still is limited, this housing regime may increase the expected life span (see Fig. 1C) [see also [35]] but had little, if any, effect on body weight (control group: 413 g±82; AC: 438 g±86; $p=0.27$). Furthermore, these enrichments did not improve the scoring in the behavioral tests used here. This outcome may be the admixture of beneficial and nonbeneficial effects by the housing group size, the enlarged and enriched environment, and the free access to food. Available data on

effects of social (housing) conditions on health and longevity is conflicting; while this and other studies (see references above) indicates that life span expectancy may increase, there are reports evidencing that single housing may improve health and increase longevity in rodents [40,41]. The beneficial effects of increased regular physical activity have also been explored, often by using a prompting paradigm like putting the animals on a treadmill or in a running wheel [42]. In rodents' increased, prompted, or unprompted exercise reduce growth and body weight gain in ad libitum fed animals but appears not to increase life span expectancy [43]. In our study, animals housed in enriched environment showed a somewhat improved body composition (smaller loss of muscle mass in old age) in senescence but the effect was weaker than in animals on DR (Fig. 11).

Restricted access to food is the only general paradigm known to improve both health and life span expectancy. Standard housing of laboratory rodents includes free access to food – ad libitum regime – and may result in obesity when the animals grow old. To test the effect of food restriction we imposed a modest dietary restriction at 70% of the ad libitum intake; and irrespective if this regime was introduced in the post-weaning period or at one year of age, it affected both whole body weight, degree of senile muscle wasting and expected median survival age (Figs. 1, 2 and 11). DR improves behavioral performance in explorative tasks as well as sensorimotor dependent behaviors probably by slowing the aging process. We found that DR animals already in the middle ages are different from aged-matched controls, in the significant increase of unprompted physical activity (locomotion and rearing, Fig. 10) [44]. The recent finding that ablation of the *sirt1* (mammalian homologue to *sir2*) gene in the mouse blunts the increase in locomotor activity seen in wild types on DR [45] may provide a first direct link between gene, metabolism, and behavior.

4.5. Concluding remarks

Our data show that the difference in median life span expectancy is small between Lewis, Wistar and Sprague–Dawley rats and that gender is not a significant modulator of life span in a protected environment. The growth pattern is also similar among the strains albeit inbred strains are smaller than out bred and, importantly, in senescence all strains showed a drop in body weight. This senile loss of body mass affects striated muscle conspicuously as evidenced by the decrease in muscle weight relative to whole body weight. Aging is a continuous process from completion of development until death. Changes that come with advancing age modulate certain aspects of the basic behavior in a continuous manner, like locomotor activity and explorative behavior. In old age, behavioral impairments accelerate and become evident in most of the sensorimotor test used here. Dietary restriction (DR) probably slows down the pace of aging by influencing basic cellular mechanism of metabolism and is reflected in the modulation of motor activity on the organism level. Enriched environment with larger social groups and less restriction on unprompted physical activity did not improve behavior compared with controls but may increase life span expectancy and improve body composition. Finally, there is a good agreement between (this type of) behavioral data collected from longitudinal age-cohorts and those obtained from multiple age-cohorts.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.physbeh.2007.06.017.

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