APPENDIX[#1]

(1994-1999)

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Introduction

The rat is the most commonly used animal in aging research. It has some clear advantages including a reasonably short life-span; it is neither too small or too large; data on expected age-associated diseases are available; there is no single dominant disease; outbred as well as inbred strains are available; it is a social animal that readily can be used in behavioral analysis; a vast body of data on normal young adult cell- and system-biology exists. The main drawback with using the rat, is the problems associated with generating transgenic models.

Through initiatives of the National Institute of Aging (US) and National Cancer Institute (US), a number of reports on the incidence /prevalence of aging-related lesions in the rat have been made available (Burek et al., 1976; Cohen and Anver, 1976; Coleman et al., 1977; Burek, 1978; Cohen et al., 1978; Hoffman, 1979; Burek and Hollander, 1980; Sher, 1982; see also Ross and Bras, 1975; Masoro, 1980). However, it is clear from scanning literature on aging research, that “aged/old” are often poorly defined hampering both critical examination of published data as well as comparison between studies/laboratories. As claimed by (Burek and Hollander, 1980), a minimum of information should hold the median survival age for the strain, or stock, of the rats used. Even better would be survival curves (or equivalent plots) and a detailed description of breeding conditions.

We have over the past decade used rats to study, in particular, aging-related sensorimotor disturbances. This *Appendix* serves to give a detailed description of the animal cohorts, including strain/stock, gender, breeding conditions and the behavioral tests employed over the epoch 1994-1999. In retrospective, it should be mentioned that this line of research was initiated 1989. During the period preceding 1994, most studies were based on male Sprague-Dawley or male Wistar rats, the median survival age was then determined to be about 30 months and a “staging” protocol was set up (for details see Johnson et al., 1995). However, behavioral testing as well as life-time curves were not sampled systematically. Thus, this *Appendix* contains only records from the epoch when systematic behavioral and breeding data are available.

Strain and stock

The main strain used here is outbred Sprague-Dawley (SD) rats (Bkl, Harlan Sprague-Dawley, Houston, Texas, US), usually delivered post weaning at an age of 2 months by a local breeder (B&K, Stockholm, Sweden). The SD rats are derived from two stocks, i.e. stock #20 (essentially animals used prior to 1995) and stock #54. Males are invariably virgins, while females are either virgins or retired breeders. The breeder made the latter category of SD rats available after four deliveries, corresponding to an age of approximately 12 months. Complete records from in total 300 female and 119 male SD rats form the basis for our compilation (see Table 1). In addition, smaller groups of Wistar (inbred Wistar/Bkl, Wistar Institute, Philadelphia, US) and Fischer 344 (inbred CDF/CrlBR, Charles River, Germany) rats have been included (Table 1).

There are good arguments for the use of inbred as well as outbred strains in aging research. In studies of the nervous system in rats, we hope to obtain data that are also of relevance for man. The use of out bred strains

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STRAIN	GENDER	AGE	# RATS	WEIGHT (g)
Sprague-Dawley	female	3	20	210
Sprague-Dawley	female	3	20	225
Sprague-Dawley	female	14	30	450
Sprague-Dawley	female	15	30	467
Sprague-Dawley	female	18	18	527
Sprague-Dawley	female	22	33	399
Sprague-Dawley	female	27	21	516
Sprague-Dawley	female (1)	28	11	526
Sprague-Dawley	female (2)	29	19	454
Sprague-Dawley	female (3)	29	25	498
Sprague-Dawley	female	30	22	301
Sprague-Dawley	female (4)	30	16	329
Sprague-Dawley	female (5)	30	22	338
Sprague-Dawley	female (6)	30	13	355
Sprague-Dawley	male	3	25	260
Sprague-Dawley	male	4	18	388
Sprague-Dawley	male	7	16	604
Sprague-Dawley	male	22	16	681
Sprague-Dawley	male	24	30	635
Sprague-Dawley	male	30	14	637
Wistar	male	30	23	551
Fischer 344	male	30	6	396

Table 1. *Summary of cohort characteristics, including strain, gender as well as age, number of animals and their weight when subjected to behavioral testing. Cumulative survival data, presented in Figure 1, are derived from the female cohorts indicated (1-6).*

would then provide more information considering the interplay between epigenetic and genetic factors in the process of aging. However, mutations, random genetic drift and genetic admixtures may impede comparison with historical data. An inbred strain may in this context provide a more stable genetic background. It is also highly relevant to include both females and males, since gender differences are significant. We have chosen to use out bred female SD rats as our standard. The quality control includes comparison of cohort data from the different out bred stocks and to store tissue samples for future genetic fingerprinting. Out bred males are used more infrequently, and will mainly serve as controls for gender differences. In addition, from time to time, inbred strains (Wistar and Fischer 344) have been, and will be, included, mainly to serve as controls for strain specificity and experimental replication in a genetically stable animal model.

Breeding and living quarters

All delivered animals were so called "high quality pathogen free animals". After arrival, they are kept in-house under barrier conditions in the animal quarters. Although this implies that the living conditions for the rats are "clean" throughout life, it does not mean that the animals live in a pathogen-free environment. Minor signs of "infection" are accepted, while animals with severe infections (usually respiratory, more rarely GI-related) are taken out from the cohorts (see also below). All clinically overt cases of infection in the animal quarters are subjected to investigation. Guard-post animals are used routinely for inventory of pathogens. Historically, the most common infectious agents have been Corona and Parvoviruses. In-house epidemics occur perhaps every other, or every third year, and are caused by admittance of infected animals to the animal quarters.

As a rule, animals are kept 3 by 3 in standard macrolon cages. Cages are cleaned once a week. The animals are inspected on a daily basis the year around. A 12/12 hr day/night cycle is used. A background of "white noise" is accepted. Efforts are made to keep the local environment as constant as possible. This includes control of temperature and humidity; that a limited number of persons handle the animals; that the cages are not "moved around". In this way, the amount of environmental stressors can not only be reduced but also kept at a reasonably constant level. In fact, this is a prerequisite to allow records to be compared over time. For the same reason, all behavioral testing is done in the local environment and by a limited number of trained investigators, with whom the animals have become acquainted.

Food and water are served ad libitum, and changed at least twice a week. All animals are feed with commercially available food-pellets having a reduced protein content (Lactamin R70, Vadstena, Sweden; or Beekay's Low protein Rodent Diet, B&K, Sweden). Caloric restriction is considered an experimental paradigm and is not relevant for the data presented here.

Growth

Ad libitum fed rats continue to grow (increase in weight) over a considerable part of their life-span (Masoro, 1980; see also Ryle et al., 1995). There is an initial fast increase over 10-to-20 weeks, after which growth continue at a slower pace until weight peak around 18-24 months. Beyond 2 years, there is usually a slow decrease in body weight, which becomes more accentuated around 30 months (or 1-2 months prior to death). Food composition and also housing conditions appears to play a role in the difference seen among ad libitum fed animals. It is interesting to note that differences in weight are as large within strains as between strains (see references cited above). This general description fits well with our own observations. As can be concluded from the single-point measurements presented in Table 1, female SD rats peak at 400-550 gram b.w. and decline to 300-400 gram b.w. at 30 months. Males, including Wistar, are 100-200 grams heavier. Notably, our Fischer 344 rats were smaller.

Survival and mortality characteristics

Records from the period preceding 1994 yielded reproducible median lifetime records of 30 months (±2 months across cohorts). With a few exceptions cohort size is either 30 or 40 animals post weaning. Life-span data from 6 female SD (n=240) cohorts, one Wistar (n=40) and one Fischer 344 (n=30) cohort, all from the epoch 1994-1999, is shown in Figure 1. It can be seen that the 90% survival is 20-21 months, with a spread of about 2 months across cohorts and strains. The median survival for this sample of cohorts was 29.3 months with a deviation of 2-3 months across cohorts and strains. For SD rats we have preliminary data indicating a 10% survival at 36-38 months. From these data it could be inferred by comparing with the literature that the maximum survival age should be in the range of 40-45 months (Burek and Hollander, 1980). We cannot detect any significant difference in longevity between virgins and retired breeders. Nor can we see any clear effect of gender and, at large, the expected life-span appears stable over time and among the employed stocks of SD rats. The survival data presented here agrees closely with reports from others (Berg, 1967; Coleman et al., 1977; Burek, 1978; Burek and Hollander, 1980; Masoro, 1980; Ryle et al., 1995). However, in the literature at large, there is a much greater variance in average life-span (see reviews cited above). The reason for this "spread" in life-time

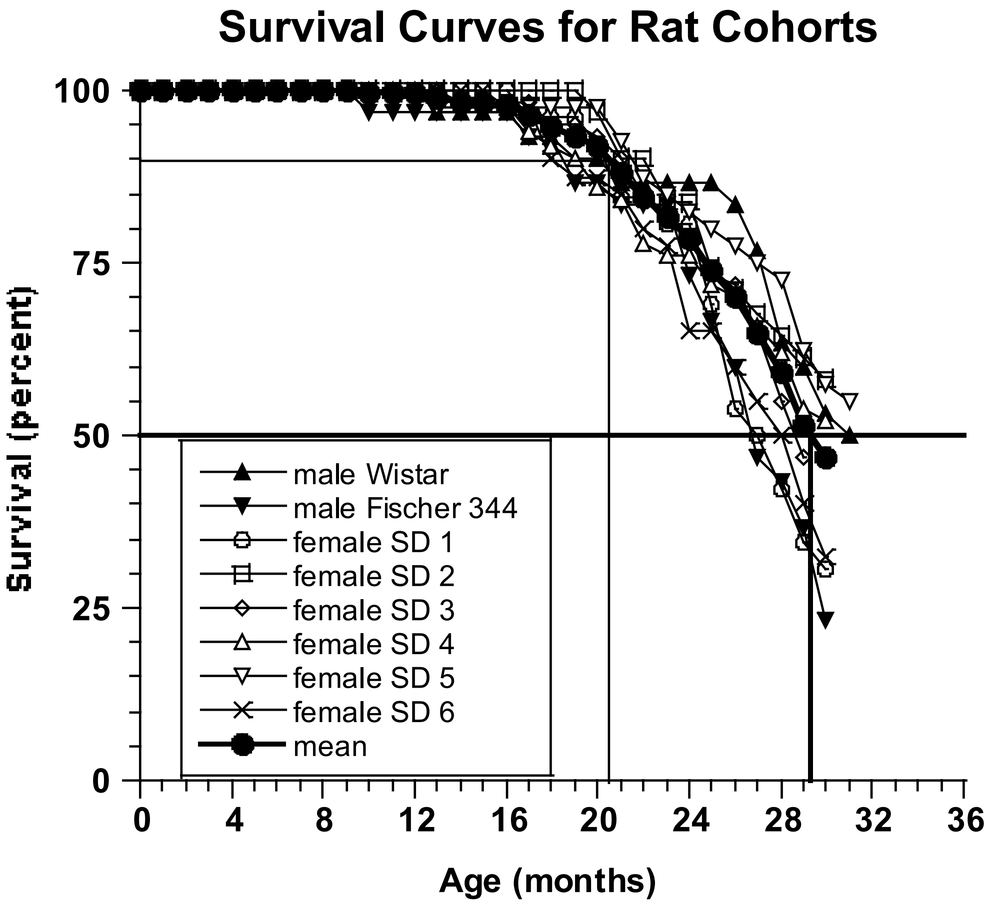

Figure 1. *Cumulative survival data for female Sprague-Dawley cohorts (SD 1-6) and male Wistar and Fischer 344 cohorts. See Table 1 for a reference to the different female cohorts included. In the plot, the 90% and 50% survival has been indicated with thin and thick lines, respectively.*

expectancy is probably owing to variability in the quality of the breeders; if the animals are barrier protected or not; as well as differences in other rearing conditions discussed above. However, it is important to conclude that if appropriate measures are taken concerning breeding, housing and feeding, the expected life-span is in the range of 27-33 months (maximum life-span: 40-48 months) regardless of strain, gender, or if the animal is virgin or ex-breeder.

Our line of research is not primarily concerned with the natural causes of death among the animals. A number of excellent compilations are available dealing with the occurrence of lesions during aging (Coleman et al., 1977; Cohen et al., 1978; Goodman et al., 1979; Sher, 1982; Ryle et al., 1995). Apart from infections, which are more rare in barrier-protected animals, tumors are very frequent (60 to 100%). Fischer 344 rats have predisposition for interstitial testicular tumors, fibrosis of the bile ductule and advanced glomerulonephropathy. Female SD rats have a high incidence of mammary tumors (40 to 65%) and chronic myocarditis. Also Wistar rats have a high incidence of tumors. Fischer 344, Wistar and SD rats, all have a fairly high incidence of pituitary adenoma (10-30%), perhaps with a somewhat higher frequency among females than males. In our material, soft tissue tumors, and in females also mammary tumors are frequent. About 50-75% of the females in a cohort will develop soft tissue or mammary tumors. We are now routinely removing these subcutaneous tumors.

We also see pituitary tumors at a frequency of about 25% in females and in a somewhat lower percentage of the males.

Cases of unexpected death are submitted to autopsy on a regular basis. The most common cause of death among these animals is respiratory inflammation and signs of cardiac failure. More rarely, hyperemia of the GI tract has been observed and quite infrequently bladder stones together with hydronephrosis.

Of relevance for our research is also the virtual absence of disc hernia and osteosclerosis of the vertebrate column in SD rats (<1%). However, such lesions were not uncommon among male Wistar rats (40%).

Behavioral Testing

A number of different test procedures intended to measure several behavioral aspects have been used. In general 20-30 animals were tested in each session, lasting for a period of 3 days. It should be noted that all tests were performed in most but not all cohorts.

Open field activity

Explorative behavior was examined with the open field test (Dorce and Palermo-Neto, 1994; Peng et al., 1994; Drago et al., 1996), using a square area with walls (70 × 70 × 30 cm) in gray colored plastic. The floor was subdivided into 25 equally large squares. In dim light the animal was placed in the center of the arena and allowed to freely explore the field for 180 sec. During this period the following behavioral characteristics were evaluated: a) ambulation frequency (number of squares entered with all four feet); b) rearing frequency (number of times animals stood on hind limbs); c) immobility frequency (number of episodes of more than 3 sec without movement); d) the frequency of urination and defecation. In between animals, the open field was carefully washed with a water-ethanol solution to eliminate possible bias due to odors left by previous subjects.

The effects of aging on locomotion, rearing and immobility frequencies and on urine and fecal deposits are for female rats summarized in Figure 2. As can be seen, aging is associated with a decline in ambulatory behavior and rearing, while the immobility frequency increase. In contrast, no effect of age on presumed stress responses, such as number of urine and fecal deposits, could be detected.

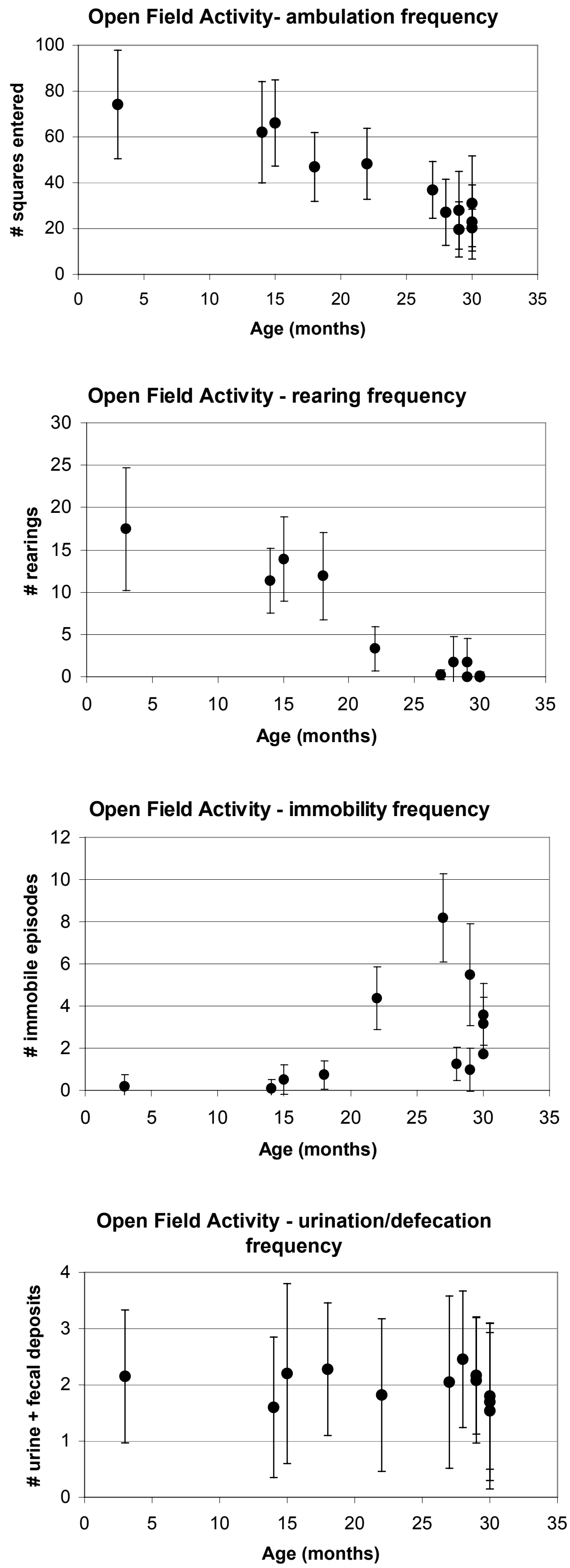

Figure 2. *Summary of female rat performance in the open field activity test. Aging is associated with a decrease in locomotor activity and rearing, while the number of immobile episodes increase. No effect of aging on stress parameters, i.e. number of fecal and urine deposits, was found. All data are presented as cohort mean +/- standard deviation.*

Crossing a wire mesh screen

A 70 cm long, 2.5 cm-wire mesh screen was used. In dim light, the animal was placed at one end of the path and a 60W light source was directed to this spot. At the other end of the path, the "home cage" with littermates was placed. Each animal was given 90 sec to cross the path. Records included distance, time, and number of errors, i.e. instances of misplaced hind paws (slips). Hind limb performance was evaluated since this test, like the beam walking test (Alexis et al., 1995), presumably examines hind limb sensorimotor function.

All animals, except a few 30-month-old subjects, completed the 70 cm wire mesh within 90 sec. Among female rats between 3 to 22 months of age the time required to complete the task was on average 9 sec, with no major difference observed between the different age groups. However, aged rats (27-30 months old) needed on average 19 sec to cross the mesh. As shown in Figure 3, a similar pattern was also present with regard to the number of errors recorded, where aged rats had a substantially increased incidence of hind paw slips.

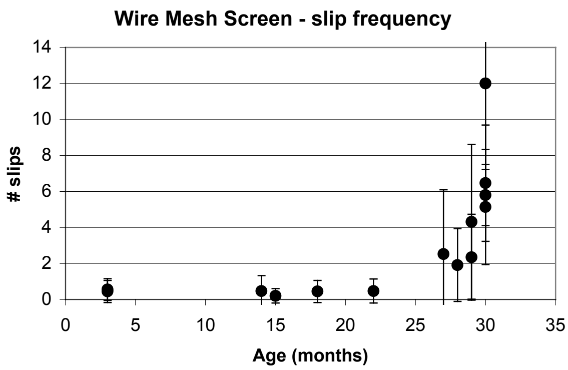

Figure 3. *Results from the wire mesh screen test showing the number of errors, i.e. misplaced hind paws, for different female cohorts. Note the increased incidence of slips among the oldest rats. All data are presented as cohort mean +/- standard deviation.*

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 sec, and the performance was ranked according to the following scale (adopted from Clifton et al., 1991):

1. Balances with steady posture; paws on top of the beam;
2. Grasps sides of beam and/or has shaky movement;
3. One ore more paw(s) slip off beam;
4. Attempts to balance on beam but falls;
5. Drapes over beam and/or hangs on beam and falls off;
6. Fall off beam with no attempt to balance or hang on.

Each animal was subjected to three consecutive trials, and the mean score of these trials was calculated. As shown in Figure 4, three-month-old rats almost without exception manage to remain on the beam for 60 sec, while this ability rapidly declines with advancing age. Among the oldest rats, the average time on the beam was approximately 10 sec, and only quite few animals attempted to balance.

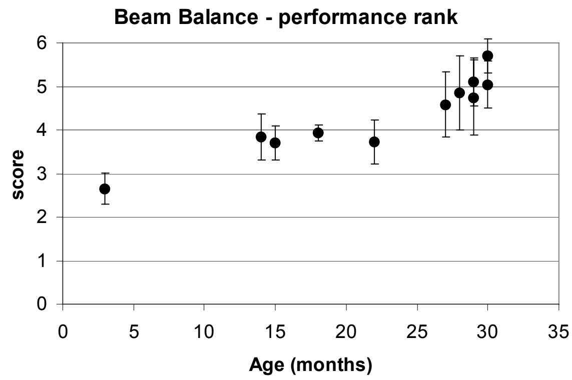

Figure 4. *Female rat performance on the beam balance task. While young adults in most instances managed to remain on the beam for 60 sec (as indicated by a score of ≤ 3), aging female rats revealed a substantially decreased ability. Among the oldest rats, only a few individuals even attempted to balance. All data are presented as cohort mean +/- standard deviation.*

Walking track analysis

For this test, the animals feet were immersed in non-toxic acrylic color (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. A stripe of high-quality paper was placed on the runway floor and taken out for analysis after the animal had crossed the path. 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).

As can be seen in Figure 5, the stride length of female rats increases somewhat from 3 to 15 months of age, possibly relating to the growth of the animals, but then shows a substantial decline among the oldest rats. With regard to gait width and differences in the placement of hind paw relative to fore paw, both parameters show an increase with advancing age (Fig. 5).

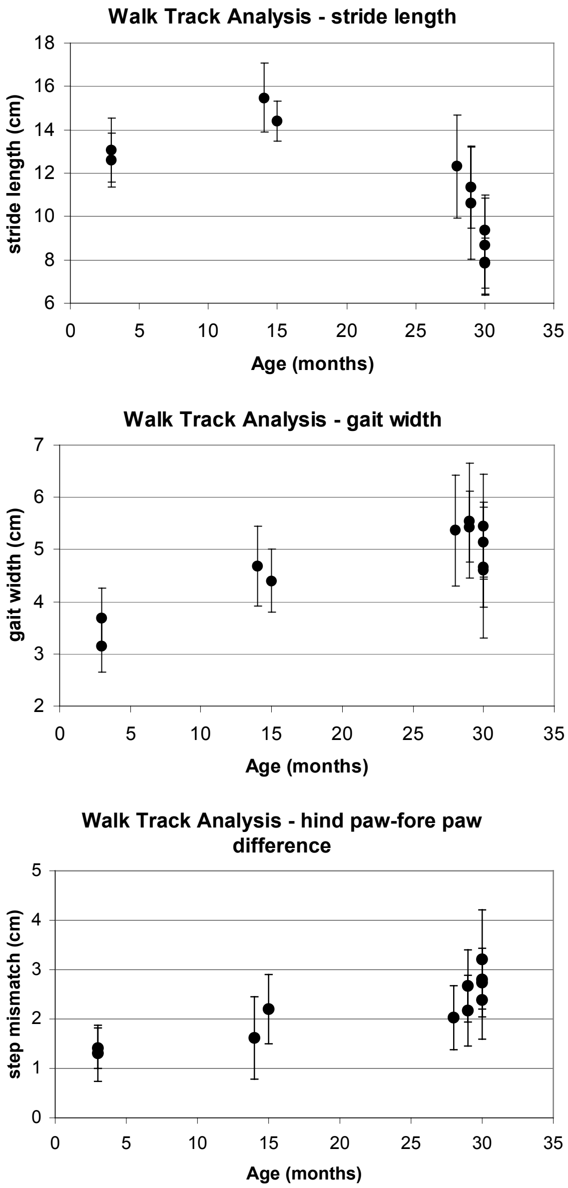

Figure 5. *Summary of the results from the walk track analysis. Female rats show a decrease in stride length, paralleled by an increased gait width with advancing age. Moreover, elderly female rats disclose an increase in the distance between hind paw-fore paw in each step cycle. All data are presented as cohort mean +/- standard deviation.*

Placing reaction

Tactile placing was evaluated by supporting the animals trunk while allowing the limbs to hang freely. The animal was then brought towards a table edge, and the dorsal and plantar surface of each foot were gently touched. For each test 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 (Gale et al., 1985; Alexis et al., 1995).

All female rats, irrespective of age, had a normal placing reaction in their fore limbs. However, as shown in Figure 6, a decline in the hind limb placing reaction was observed among the oldest female animals.

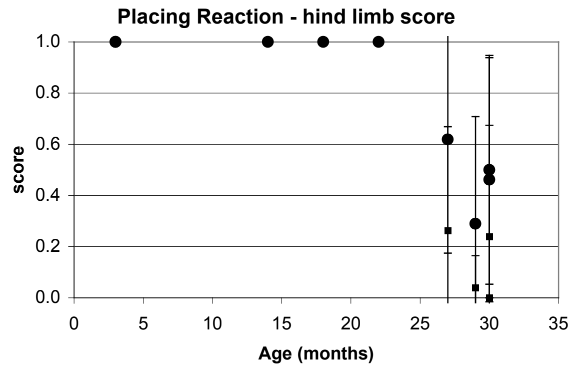

Figure 6. *Effect of age on hind limb placing reaction in female rats. In the oldest cohorts the placing reaction was either delayed or absent. Circles and squares indicate score following dorsal and plantar hind limb stimulation, respectively. All data are presented as cohort mean +/- standard deviation.*

Righting response

The rat was held in the examiner's hand approximately 20 cm above a soft surface, and the righting reflex was elicited by turning the rat over on its back upon release. The rat's attempt to right itself was studied and 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 (Gale et al., 1985; von Euler et al., 1996).

The results are summarized in Figure 7, where it can be seen that although the oldest female rats show abnormal righting responses, most of them exhibit attempts to right.

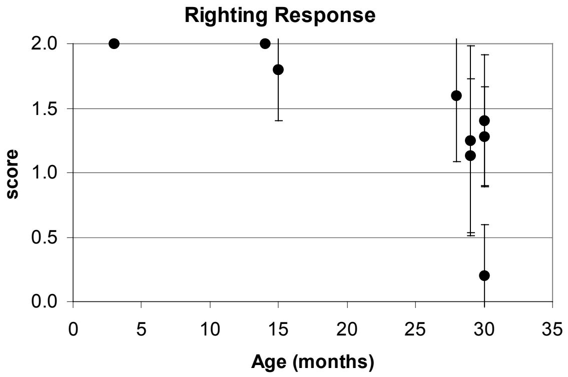

Figure 7. *Summary of righting responses in female rat cohorts. Although senescent rats exhibit impaired righting responses, the majority still attempt to right themselves. All data are presented as cohort mean +/- standard deviation.*

Prehensile reflex

A taut horizontal steel wire was elevated approximately 50 cm above a soft surface. Individual rats were raised to this wire, which they grasped with their fore paws. The time each rat managed to hold onto the wire in three consecutive trials was recorded. The prehensile reflex refers to an animal's ability to grasp a horizontal bar or wire with its fore paws and to remain suspended without dropping off, and has been reported to be a measure of muscle strength (Dean III et al., 1981).

On the first trial, three-month old female rats were able to hold onto the wire for an average of 7 sec, while the corresponding figure for their 27-30 month-old counterparts was 4 sec. Moreover, while the third trial time was virtually unchanged for young adult rats, aged rats appeared more fatigable and only managed to remain suspended for an average of 1.5 sec.

Nociceptive hot plate test

A plexiglas cylinder with a diameter of 20 cm was placed on a plate (30cm x 40cm), set to maintain a temperature of 52 ± 0.5°C. The animal was placed on the heated surface until it licked paws, jumped or vocalized. The time lapse between placement and reaction was recorded as response latency (Espejo and Mir, 1993; Langerman et al., 1995). The cut-off time was set at 30 sec to avoid tissue damage.

A shown in Figure 8, nociceptive response latencies do not change notably in female rats from 3 to 22 months of age. Although there is a considerable variability among the oldest rats, there is a clear tendency for a substantial increase in hot plate response latencies.

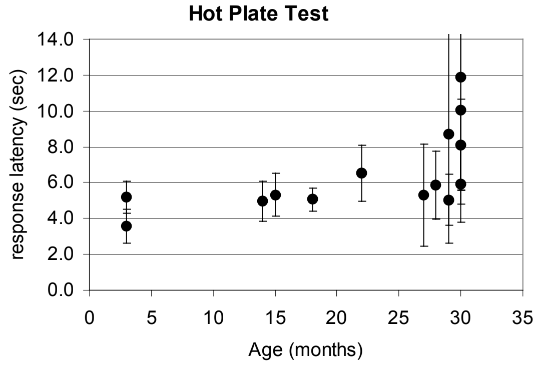

Figure 8. *Results from the nociceptive hot plate test in female rats. Note the tendency for increased response latencies the oldest cohorts. All data are presented as cohort mean +/- standard deviation.*

von Frey test

Animals were put in a plexiglass container with a very fine mesh floor, and allowed to habituate for at least 10 min. von Frey hairs (a series of nylon monofilaments with logarithmically increasing stiffness that exert defined levels of force as they are pressed to the point where thy bend), were then applied perpendicular to the paw skin and depressed slowly until they bent. The hairs were tested in ascending order of stiffness, with each applied 5 times, and the threshold was defined as the first hair in a series evoking at least one response. The response criterion was a reflexive withdrawal that was clearly separate from stepping (Tal and Bennett, 1994). Thresholds were determined separately for hind limbs (mid-plantar region) and fore limbs (mid-palmar region).

In 3-month-old female rats the tactile threshold was found to be higher in hind limbs (filament #15) than fore limbs (filament #14). This pattern was maintained in aged (30 months old) female rats that, moreover, disclosed a substantial increase in von Frey thresholds (hind limbs- filament #17; fore limbs- filament #16).

Behavioral data from male Sprague-Dawley, Wistar and Fisher-344 rats

The data presented above all adhere to the female cohorts examined. Male rats, irrespective of age or strain, disclose a behavior that in most respects closely agrees with that observed for age-matched female SD rats. Thus, the open field test generated similar results in the male cohorts, however, aged Wistar rats seemed to have a somewhat decreased stress tolerance, as revealed by an increase in the number of fecal and urine deposits. The beam balance test showed no differences among young male cohorts, while elderly male rats, possibly relating to their larger size, consistently managed the test worse than their age-matched female counterparts. Despite the difference in size, no difference was found between male and female rats considering stride length or gait width. However, aged male rats had a somewhat more pronounced difference in the distance between hind paw and fore paw in each step cycle.

Staging

In the initial phase of this project a staging protocol was set up allowing us to rank-order the aged animals according to their behavioral motor impairments. This was described in some detail in the early publications: "*…All aged rats used in this study disclosed clinical signs of neuromuscular dysfunction. Mild cases (stage I) involved moderate muscular wasting of the hind limb, an adduction insufficiency causing the rats to stride with their hind limbs abducted compared to young adults, and with the lower part of the trunk closer to the supportive surface. Such rats were able to rise on their hind limbs -a common posture while a rat investigates e.g. a new environment-, but did so more infrequently and for shorter periods as compared to young adult rats. Aged rats, in general, also stride at a slower pace. With increasing symptoms (stage II) a clear clumsiness and a poorer co-ordination (ataxia-like) of the hind limbs became evident. Such cases showed a more pronounced atrophy of the hind limb musculature. At this stage there was often an asymmetry with respect to the severity of the symptoms between the two hind limbs. Still, rats at this stage managed to climb across a 1.5-inch wall but seemed less eager to search new environments and often reacted more slowly to audio-visual stimuli. Stage III involved a more or less complete paralysis of the hind limbs, again often engaging only one of the hind limbs first. These rats dragged themselves around using their fore limbs. The hind limbs were held in a flexed retracted position below the pelvis. When such rats were lifted from the supportive surface and then placed back, they did not extend their hind limbs on replacement. Furthermore, stage III rats usually did not manage to climb walls of one inch height and disclosed a severe atrophy of the hind limb muscles and sometimes also of the hip and low-back muscles. In the most severe cases, a tendency for both urinary and bowel emptying dysfunction began to show. At this stage the rats usually began losing weight rapidly and often appeared quite indifferent to the surrounding environment. Death usually followed from one-to-several weeks later.*

Clinical symptoms begin to develop in some rats at an age of 24-26 months, and progressive deterioration then occurred over a 4-8 month period. In the 30-month cohorts, about 90% of the surviving rats had some symptoms (stage I-III). About 40-70% were either stage II or stage III. In an earlier study we found that the median survival age of this rat strain under the standard condition in our department was 30 months, and this was also the rationale for using this group as "aged" rats (see Johnson et al., 1993)…." (from Johnson et al., 1995). This is essentially valid also for male and female SD rats used 1994-1999. In Figure 9, a compilation of stage-frequencies among female SD rats is shown at different survival times. Wistar rats show a similar frequency distribution, however, the number of observations is still quite small. We do not have experience enough to comment on stage-frequencies among Fischer 344 rats.

Over-time the staging protocol has yielded consistent results, probably owing to its simplicity. In essence, two parameters guide the examinator to select a stage, namely:

(a) Is a limb weight bearing?
(b) Does a limb show a complete gait cycle coordinated with the other limb(s)?

Stage 0 animals show no signs of insufficiency concerning these two variables;
Stage 1 animals show some signs, including a lowering of the trunk towards the supportive surface and minor signs of gait cycle aberrations;
Stage 2 animals show clear gait cycle aberrations, often involving a poorer coordination between limbs. Body weight bearing is often clearly incapacitated in at least one limb;
Stage 3 animals disclose incomplete or complete paralysis of one or both hind limbs. Only minor efforts to initiate a gait cycle may

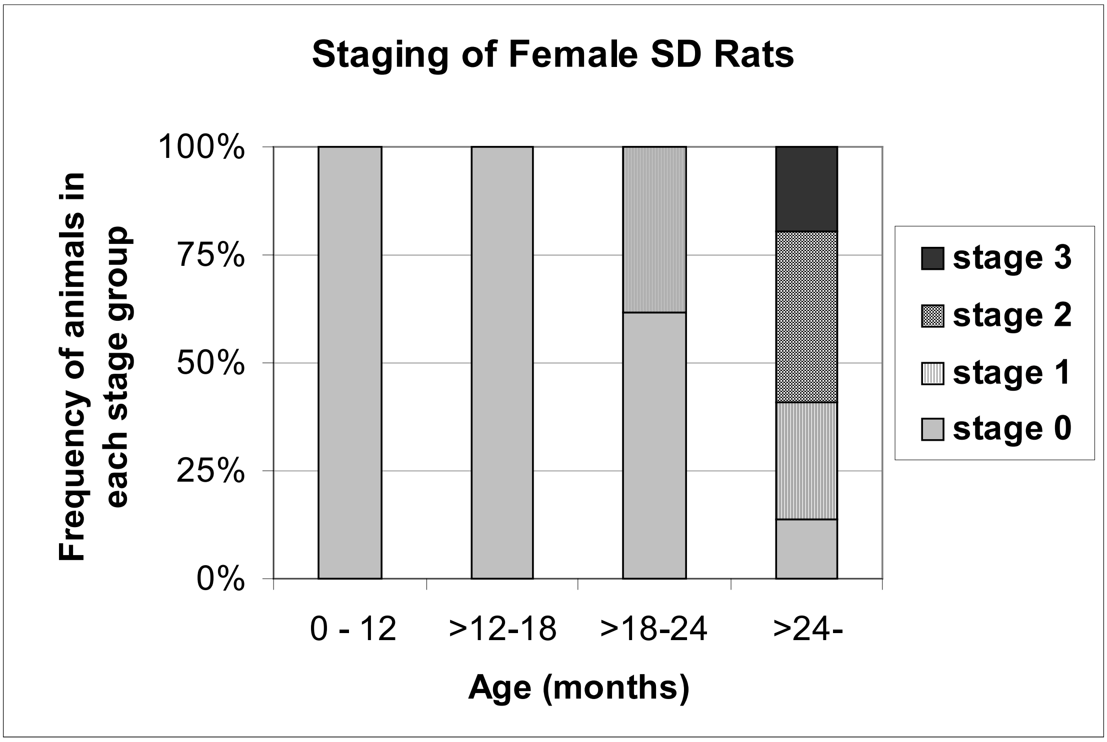

Figure 9. *Stage distribution of different age groups of female Sprague-Dawley (SD) rats.*

be evident and the limb(s) cannot support any body weight.

In addition there are minor parameters such as the degree of muscle atrophy-speed-alertness that may finally decide if an animal should be ranked e.g. stage 1 or stage 2.

Stage ranking and behavioral test

The simple stage ranking of aged rats is also used to divide members of an aged cohort (see e.g. Bergman and Ulfhake, 1998; Johnson et al., 1999). The rational is that correlation analysis shows that stage co-varies with results recorded in most of the behavioral tests. The strength of correlation between stage and a particular record of a test may not be very high (typically “r” is within the range of 0.4-to-0.6). However, there is a strong consistency of significant correlation between on one hand, stage rank and on the other, the different behavioral tests (Fig. 10). For example, stage is inversely related to the activity variables recorded in the Open field test (Fig. 10A). Stage correlates with the different measures of the walking track analysis, except gait width (Fig. 10B-C). Moreover, rats in stage 2 and 3 perform less well than stage 0 and 1 rats in the mesh-crossing test (Fig. 10D). Finally, rats in stage 2-3 showed increased thresholds in the hot plate test (Fig. 10E).

The co-variation described above is not surprising since successful performance in many of the tests depend on the integrity of the neuro-muscular system, appropriate sensory feedback, intact vestibular function and supraspinal control. Thus, there are fairly strong cross-correlations among the records obtained in mesh crossing, gait analysis and beam balance among aged (28-30 months old) animals (Fig. 11A-B). In the latter test one would expect that body weight should influence the results, however, as shown in Figure 11C this is not the case. The hot plate test showed no, or very weak, correlation with the motor behavior tests among aged rats, indicating that changes in nociceptive circuitries are not closely linked with sensory pathways of direct importance for normal motor behavior (Fig. 11D).

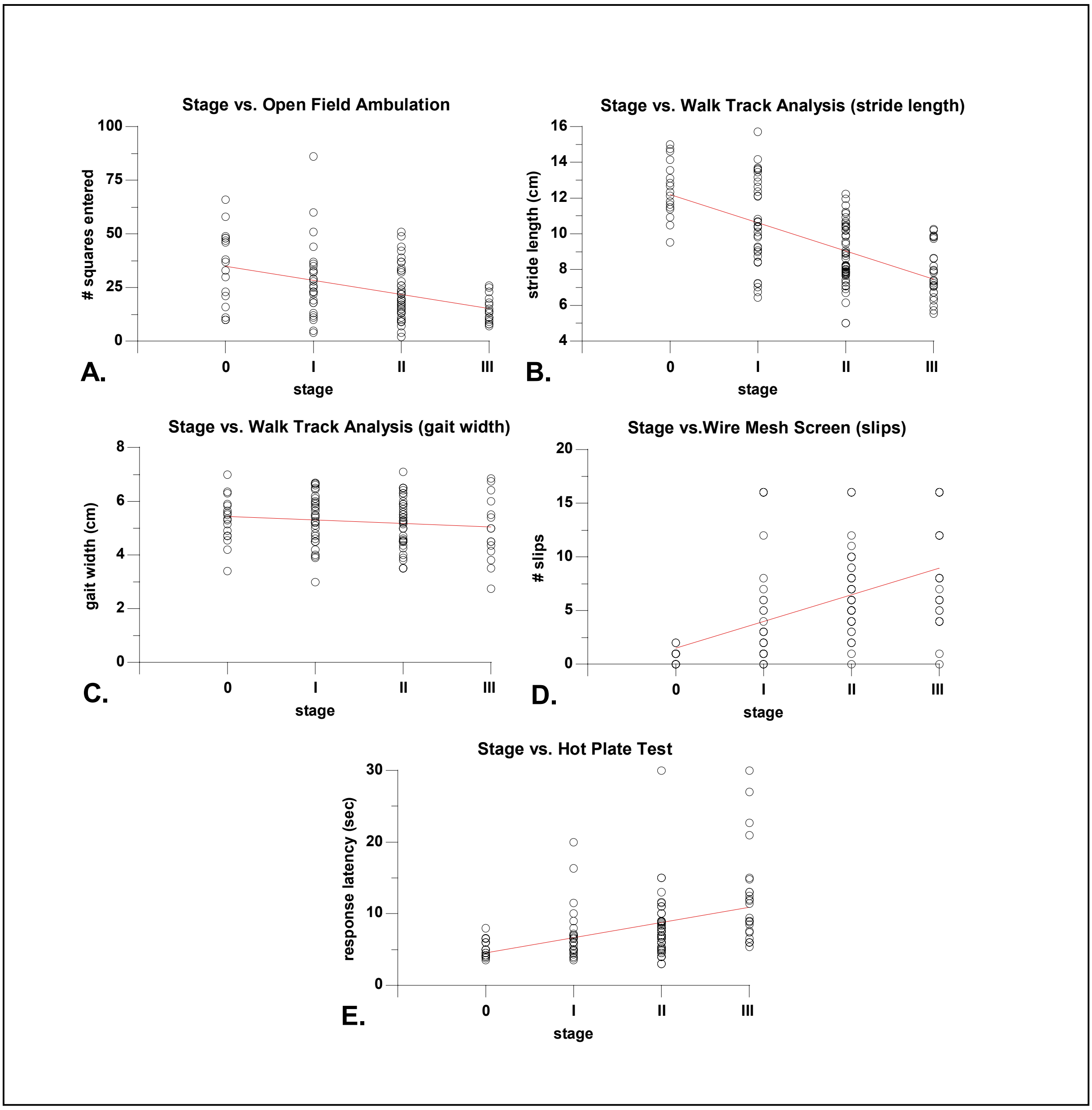

Figure 10. *Analysis of correlation between stage and different behavioral test parameters in individual aged (28-30 months old) female Sprague-Dawley rats. Stage was found to inversely correlate to locomotor activity in the open field activity test (A; r=0.42) and to stride length in the walking track analysis (B; r=0.63). However, in the latter test no correlation was found between stage and gait width (C; r=0.13). Moreover, stage correlated to number of hind limb slips in the wire mesh screen test (D; r=0.5) and to response latency in the hot plate test (E; r= 0.42).*

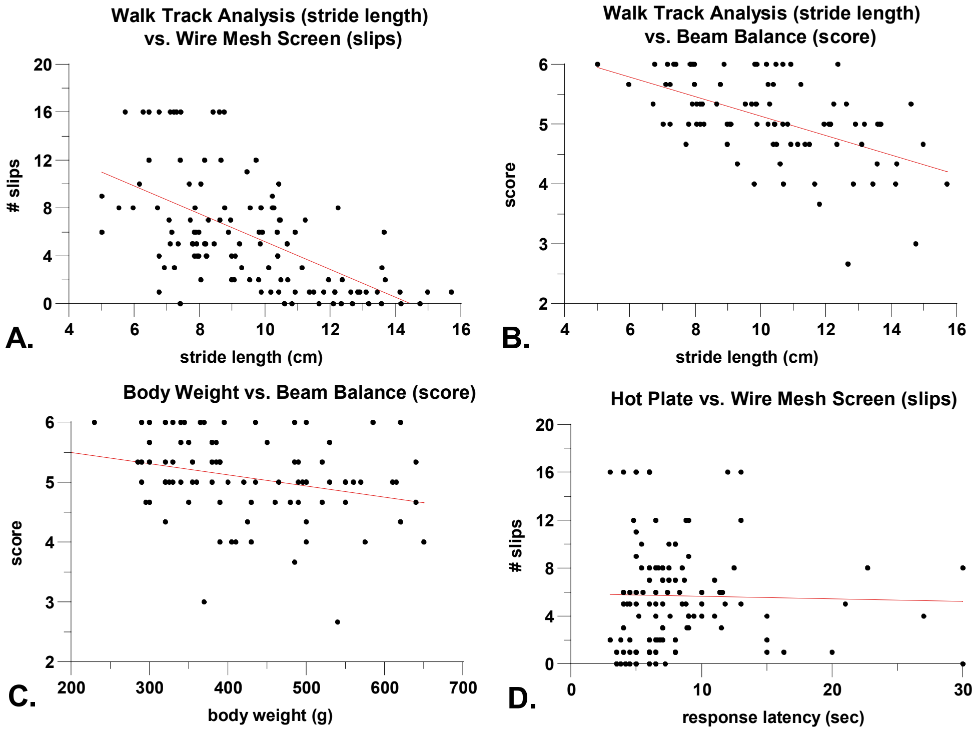

Figure 11. *Analysis of correlation between different behavioral test parameters in individual aged (28-30 months old) female Sprague-Dawley rats. Stride length showed an inverse correlation to the number of slips in the wire mesh screen test (A; r=0.58) and to beam balance score (B; r=0.55). In contrast, only a weak relation was observed between body weight and beam balance score (C; r=0.28), while no correlation was found between hot plate response latencies and numbers of slips in the wire mesh screen test (D; r=0.02)*

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