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Behavioral dysfunction, brain oxidative stress, and impaired mitochondrial electron transfer in aging mice

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Navarro, Ana, María Jesús Sánchez Del Pino, Carmen Gómez, Juan Luis Peralta, and Alberto Boveris. Behavioral dysfunction, brain oxidative stress, and impaired mitochondrial electron transfer in aging mice. *Am J Physiol Regulatory Integrative Comp Physiol* 282: R985–R992, 2002; 10.1152/ajpregu.00537.2001.—Behavioral tests, tightrope success, and exploratory activity in a T maze were conducted with male and female mice for 65 wk. Four groups were defined: the lower performance slow males and slow females and the higher performance fast males and fast females. Fast females showed the longest life span and the highest performance, and slow males showed the lowest performance and the shortest life span. Oxidative stress and mitochondrial electron transfer activities were determined in brain of young (28 wk), adult (52 wk), and old (72 wk) mice in a cross-sectional study. Brain thiobarbituric acid reactive substances (TBARS) were increased by 50% in old mice and were ~15% higher in males than in females and in slow than in fast mice. Brain Cu,Zn-superoxide dismutase (SOD) activity was increased by 52% and Mn-SOD by 108% in old mice. The activities of mitochondrial enzymes NADH-cytochrome *c* reductase, cytochrome oxidase, and citrate synthase were decreased by 14–58% in old animals. The cumulative toxic effects of oxyradicals are considered the molecular mechanism of the behavioral deficits observed on aging.

neuromuscular impairment; NADH-cytochrome *c* reductase

BOTH AGING AND AGE-ASSOCIATED neurodegeneration are related to the development of behavioral impairments; consequently, decreased performances in neuromuscular coordination and exploratory tests are considered markers of neurological aging (15). The life span of rodent strains was found inversely related to the intensity of their behavioral and neuroendocrine responses to stress, this type of evidence suggesting a genetic linkage between the quality of response to stress, the performance in behavioral tests, the rate of age-dependent neurodegeneration, and life span (12, 13, 19).

The likely molecular candidates responsible for the neuromuscular deficits are oxidizing free radicals and the consequent oxidative stress they generate. The free radical theory of aging, understood as the decline of biological function on time, is complemented with the concept that life span is a consequence of oxygen toxicity at 20 kPa O_2 (18, 21). When the free radical theory of aging (21) is focused in mitochondria, it becomes more attractive as the mitochondrial hypothesis of aging (22, 30, 37). This hypothesis considers mitochondria as the pacemaker of tissue aging due to the continuous production of reactive oxygen and nitrogen species ($O_2^{\cdot-}$, H_2O_2, NO, $ONOO^-$, $HO^\bullet$, and $^1O_2$), which are kept in regulated steady-state concentrations (6, 8). The increased steady-state concentrations of reactive oxygen species constitute the chemical basis of the biological situation of oxidative stress and imply an increased rate of intramitochondrial free radical reactions (20). These reactions generate the free radicals $HO^\bullet$, $R^\bullet$, $ROO^\bullet$ that act as intermediates and, among others, the stable products of the oxidation of unsaturated fatty acids, proteins, and DNA. Thiobarbituric acid reactive substances (TBARS), protein carbonyls, and 8-hydroxy-deoxyguanosine (8-OH-dG) are the usual markers of oxidative stress as byproducts of free radical-mediated oxidation of cell components. The first aim of this study, in accordance with the mitochondrial hypothesis of aging, was to assess oxidative stress and mitochondrial electron transfer in aging animals. The second aim was to establish the relationships between oxidative stress markers, mitochondrial function, and behavioral dysfunction on aging.

MATERIALS AND METHODS

Animals. One hundred eighty CD1-Swiss outbred strain mice were received at 10 wk of age and housed in groups of five in a temperature-controlled (25°C) animal room on a 12:12-h light-dark cycle. Mice had free access to water and food (A04 diet, Panlab LS, Barcelona, Spain) in accordance with the recommendations of the American Institute of Nutrition and the regulations of the Department of Experimental Animals of the University of Cadiz. Mice were periodically checked to verify their pathogen-free condition. Animal ex-

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periments were carried out in accordance with European Community regulations (86/609/CEE) and the work fully conforms with the *Guiding Principles for Research Involving Animals and Human Beings* of the American Physiological Society.

Behavioral tests. Mice (40 males and 40 females) were used for a longitudinal study of behavioral indicators. Tests were continuously performed every 2 wk with each individual mouse and started with mice at 13 wk of age and were extended up to the 78-wk-old mouse. Experiments were carried out in similar environmental conditions and in the morning to diminish circadian effects. Individual mice were tested every 2 wk. In the tightrope test for evaluation of neuromuscular coordination (11, 29), mice were placed in the middle of a 60-cm tightrope, they hung from the rope with the anterior legs and moved to a column at the end of the rope. The test was considered successful when mice reached the column in 60 s or less. The spontaneous exploratory activity of mice was assayed in a T-shaped maze with 50-cm arms, a test in which animals are challenged by a new environment, and registered as exploratory activity when they moved toward the T intersection.

Oxidative stress and mitochondrial electron transfer. Mice (48 males and 48 females) were killed (16 males and 16 females) by decapitation at 28, 52, and 72 wk of mouse age in a cross-sectional study to evaluate oxidative stress and mitochondrial activities. Brain and liver were rapidly excised, and tissue samples were immediately processed for mitochondrial isolation or placed into liquid nitrogen and kept at −80°C until further use.

The steady-state level of lipoperoxidation products was assayed by determining TBARS in organ homogenates. Frozen samples of brain and liver were ground in a cold glass mortar and homogenized (1 g of tissue/9 ml) in 100 mM phosphate buffer (pH 7.4) in a Tempest-Virtis homogenizer at 2°C. Homogenate samples (1 ml) were added with 2 ml of 0.1 N HCl, 0.3 ml of 10% phosphotungstic acid, and 1 ml of 0.67% 2-thiobarbituric acid. The mixture was heated 30 min in boiling water, extracted with 5 ml 1-butanol, and, after a brief centrifugation, the absorption of the butanol layer was measured at 535 nm (ϵ = 153 mM/cm) and expressed as nanomoles of TBARS (malondialdehyde equivalents) per gram tissue (16).

Cytosolic (Cu,Zn-SOD) and mitochondrial (Mn-SOD) superoxide dismutases were assayed in brain and liver homogenates prepared as previously described by determining their inhibitory effect on 20 μM cytochrome *c* reduction by xanthine oxidase [50% inhibition = 1 unit (14)] both in the absence (total SOD activity) and in the presence of 1 mM KCN (Mn-SOD activity). Cu,Zn-SOD activity was calculated as total SOD activity minus Mn-SOD activity. Activity was expressed as picomole monomer per gram tissue, considering that pure Cu,Zn-SOD and Mn-SOD have specific activities of 2,820 and 3,760 U/mg and 33 kDa (dimer) and 88 kDa (tetramer) for Cu,Zn-SOD and Mn-SOD, respectively (14).

Brain and liver mitochondria were isolated from tissues that were homogenized in 0.23 M mannitol, 0.07 M sucrose, 15 mM MOPS-KOH (pH 7.4) at a ratio of 1 g of tissue/9 ml of homogenization medium in a Potter homogenizer with a Teflon pestle at 0–2°C. The homogenate was centrifuged at 700 *g* for 10 min, and mitochondria were precipitated by centrifugation at 10,000 *g* for 10 min and washed twice in the same conditions. The mitochondrial suspensions were frozen in liquid N_2 and kept at −80°C until use for the determination of mitochondrial enzyme activities. Samples were frozen and thawed twice and homogenized by passage through a 15/10 hypodermic needle and syringe. Proteins were determined by the Lowry method (27).

Membrane bound mitochondrial activities were assayed spectrophotometrically in 100 mM phosphate buffer (pH 7.4) at 30°C. For the determination of NADH-cytochrome *c* reductase activity, mitochondrial fragments were added with 0.2 mM NADH, 0.1 mM cytochrome *c*, and 1 mM KCN and followed at 550 nm (ϵ = 19.6 mM/cm) (40). Enzyme activity was expressed in nanomoles cytochrome *c* reduced per minute per milligram of protein. Succinate cytochrome *c* reductase activity was similarly determined and expressed, except that NADH was substituted by 20 mM succinate.

Cytochrome oxidase activity was determined with the phosphate buffer added with 0.1 mM reduced cytochrome *c*, which was prepared by reduction with excess $NaBH_4$ and HCl (40). Enzymatic activity was calculated in terms of the pseudo-first-order reaction constant for cytochrome *c* oxidation (k′) per milligram of protein and expressed as the initial rate of cytochrome *c* oxidation in nanomoles cytochrome *c* per minute per milligram protein in the presence of 0.1 mM cytochrome *c*.

Mitochondrial citrate synthase activity, as mitochondrial matrix marker of oxidative capacity, was determined in 50 mM Tris·HCl buffer (pH 8.0) added with 0.1% Triton X-100, 0.5 mM 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), 2 mM acetyl-CoA, and 5 mM oxaloacetate followed at 412 nm (ϵ = 13.6 mM/cm) (4, 9, 26).

Statistics. The K-means clustering method, determining the lowest distance between the values corresponding to individual animals in the Euclidian three-dimensional space defined by the three variables: tightrope successes (%), exploratory activity in the T-shaped maze (%), and body weight (g), classified the individual animals in four groups, two by sex and two by behavior.

The numbers in Figs. 1–6 and Tables 1–4 indicate mean values ± SE, and the significance of differences were analyzed by the Student's *t*-test and the ANOVA test.

RESULTS

The behavioral tests for neuromuscular and synaptic functions. Performance in the tightrope test for neuromuscular function decreased with age in both males and females, success was higher in females than in males from 13 to 72 wk of age (Fig. 1). The spontaneous exploratory activity in the T-shaped maze, a test for neuronal and synaptic functions, also decreased with age in both males and females. Again, female mice performed better and showed a greater exploratory activity than males (Fig. 2). When the animals explored the T maze, exploration time was longer in young and old male mice than in young and old female mice, whereas no difference was observed in adult mice (Fig. 2, *inset*).

Grouping of mice according to the results of the behavioral test. The grouping of animals was established by cluster analysis, a method that allocates a set of individuals into groups that are mutually exclusive, so individuals within a group are similar to one another, whereas individuals in different groups are dissimilar. Figure 3 shows the clustering in a two-dimensional space: tightrope success and T maze exploratory activity. In this case, the variable body weight, which clearly differentiates males from females, was ex-

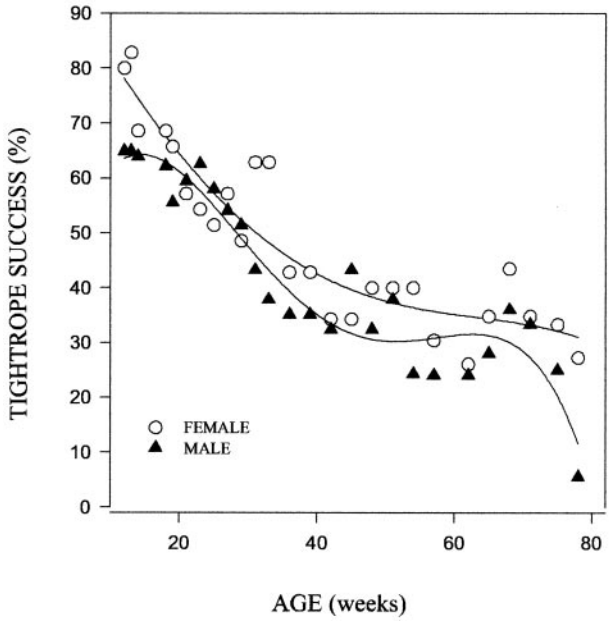

Fig. 1. Mice performance in the tightrope test on aging. Points correspond to mean values of pooled males and females in tests performed every 2 wk. Success was considered reaching, hanging from the rope with the anterior legs, a column 30 cm away in 60 s or less.

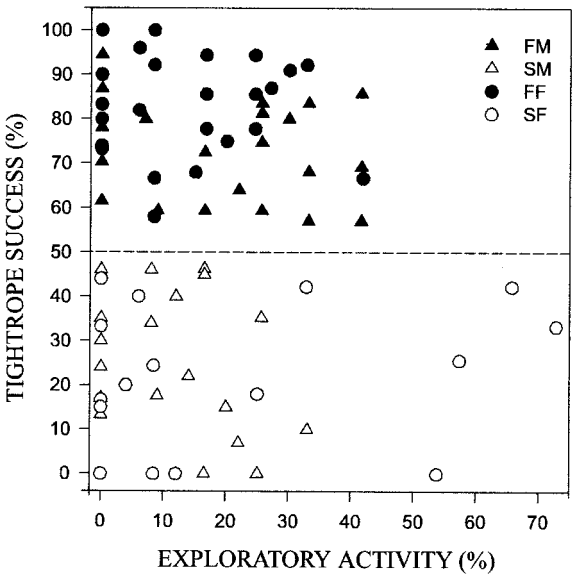

Fig. 3. Cluster analysis in a 2-dimensional space showing tightrope success and T maze exploratory activity of individual mice. The points are mean values of 3 assays performed at 28–30 wk of age. The hyphen line indicates the level of 50% success in the tightrope test that discriminates between slow and fast animals. SM, slow males; FM, fast males; SF, slow females; and FF, fast females.

cluded for simplicity. On the basis of these variables, mice were divided in four groups: the lower performance groups, slow males and slow females (SM and SF), and the higher performance groups, fast males and fast females (FM and FF). The animals remained in the same group throughout their lives.

Body weight and life span. Mice body weight, registered every week, showed that males maintained a higher body weight from 12 to 80 wk of age (Fig. 4). At the same time, females showed a longer life span than males (Fig. 4). It can be seen that the FF group had the longest life span and the SM group was the one with

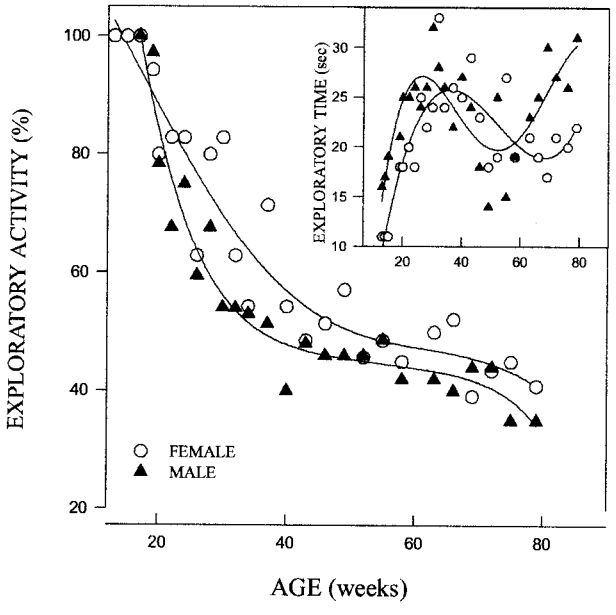

Fig. 2. Mice exploratory activity in a T-shaped maze on aging. Points correspond to mean values of pooled males and females in tests performed every 2 wk in which success was the movements toward the T intersection 50 cm away. *Inset*: time of exploratory activity in the maze. Points correspond to mean values of pooled males and females.

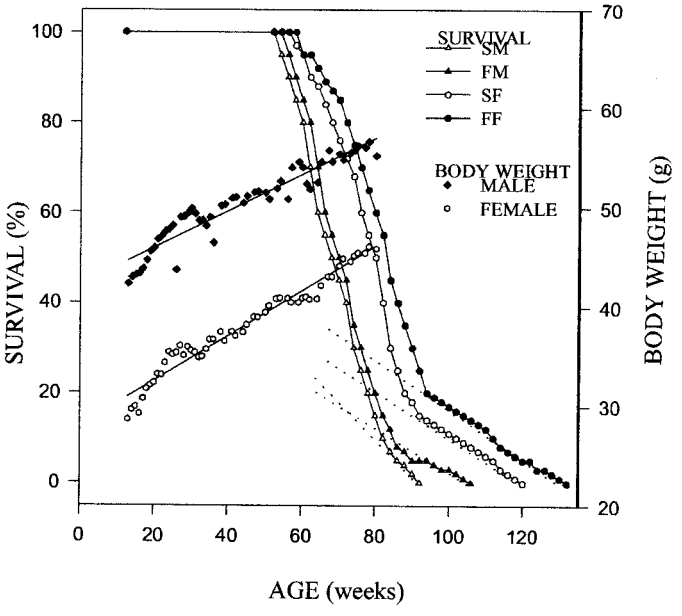

Fig. 4. Mice survival and body weight on aging. 50% survival: SM, 62 wk; FM, 68 wk; SF, 78 wk; and FF, 80 wk. Correlations for the four groups (SM, FM, SF, and FF): life span/brain thiobarbituric acid reactive substances (TBARS) at 72 wk, $r^2 = 0.77$, $P < 0.01$; Life span/brain NADH-cytochrome *c* reductase at 72 wk, $r^2 = 0.75$, $P < 0.01$. The dotted lines identify the high longevity subgroup in each group.

Table 1. *Oxidative stress markers in the brain of aging mice*

Marker/Mice Age	Slow Males	Fast Males	Slow Females	Fast Females
TBARS, nmol/g tissue				
28 wk	123 ± 6	103 ± 4	113 ± 5	108 ± 4
52 wk	168 ± 6†	155 ± 7†	130 ± 6	114 ± 6
72 wk	196 ± 8†	160 ± 7†	174 ± 7†	150 ± 7†
Cu,Zn-superoxide dismutase, pmol/g tissue				
28 wk	22.8 ± 0.7	20.8 ± 0.9	19.4 ± 0.9	21.1 ± 0.6
52 wk	31.5 ± 1.2†	30.5 ± 2.1*	26.8 ± 1.7*	29.1 ± 0.9†
72 wk	37.3 ± 1.7†	33.0 ± 0.9*	27.6 ± 0.9†	30.2 ± 1.2†
Mn-superoxide dismutase, pmol/g tissue				
28 wk	3.1 ± 0.1	3.3 ± 0.3	2.9 ± 0.2	2.7 ± 0.1
52 wk	5.7 ± 0.3†	5.2 ± 0.4*	5.6 ± 0.4†	5.0 ± 0.4†
72 wk	6.8 ± 0.2†	6.1 ± 0.2†	6.4 ± 0.2†	5.6 ± 0.3†

Values are means ± SE and the significance of differences was analyzed by ANOVA and Student's *t*-tests. *$P < 0.01$; †$P < 0.001$, between 28 wk mice and both 52 and 72 wk mice. Significant differences ($P < 0.05$) were observed in thiobarbituric acid reactive substances (TBARS) between males and females and between slow and fast mice.

the shortest life span. It seems that individual and group longevity can be predicted by behavioral differences in young mice. Figure 4 also shows that the four groups had a small subgroup, ~10–20% of mice, that exhibited an increased life span. The fact was marked in the FF group, which showed 20% of mice with a longevity exceeding up to 34% the main group life span.

Oxidative stress in brain and liver. In the description of the results concerning the 12 groups of experimental mice listed in Tables 1-4, the following comparisons will be made for the sake of simplicity: *1*) pooled male and female old mice (72 wk old) compared with adult (52 wk old) and young mice (28 wk old); *2*) male compared with female mice; and *3*) slow mice (male and female) compared with fast mice. Such treatment of the data fully describes the characteristics of all experimental groups.

Brain TBARS levels were increased by ~52% and 20% in old and adult mice compared with young animals, they were 15% higher in males and 14% higher in slow animals. In short, the sex and behavior groups that showed the longer life span presented the lowest level of brain lipoperoxidation (Table 1). In liver, TBARS contents followed the same pattern; they were increased by ~21% and 15% in old and adult animals, but only by ~8% in males and by 2% in slow animals compared with their counterparts (Table 2).

Brain cytosolic Cu,Zn-SOD activity was increased by 52% and 40% in old and adult mice compared with young animals, and was 14% higher in males than in females, but no difference appeared in slow animals compared with the fast ones. In short, the groups with longer life span presented the higher levels of brain Cu,Zn-SOD activity (Table 1). In liver, Cu,Zn-SOD activities followed a similar pattern; they were increased by 54% and 16% in old and adult mice compared with young animals, by ~15% in males, and no differences were found between slow and fast mice (Table 2).

Brain mitochondrial Mn-SOD activities were increased by 108% and 79% in old and adult mice compared with young animals, were 7% higher in males, and 9% higher in slow animals (Table 1). In liver, Mn-SOD was similarly increased by 87% and 16% in old and adult mice compared with young animals, no difference was observed between males and females, and the activity was increased by 11% in slow mice (Table 2).

Impaired mitochondrial electron transfer on aging. An impairment of electron transfer in specific mitochondrial complexes was observed in brain and liver of aging mice. Brain NADH-cytochrome *c* reductase (complexes I and III) was the most affected activity and was decreased by 8% and 48% in adult and old mice (Table

Table 2. *Oxidative stress markers in the liver of aging mice*

Marker/Mice Age	Slow Males	Fast Males	Slow Females	Fast Females
TBARS, nmol/g tissue				
28 wk	75 ± 4	70 ± 5	71 ± 7	69 ± 7
52 wk	85 ± 4	87 ± 4	80 ± 5	76 ± 4
72 wk	92 ± 5*	90 ± 6*	85 ± 6	79 ± 6
Cu,Zn-superoxide dismutase, pmol/g tissue				
28 wk	19.6 ± 1.1	19.3 ± 0.9	21.8 ± 1.2	23.2 ± 1.8
52 wk	26.8 ± 1.2‡	22.6 ± 1.6	23.2 ± 1.7	24.4 ± 1.1
72 wk	33.2 ± 1.1‡	30.1 ± 2.7†	34.6 ± 1.8‡	30.8 ± 1.3†
Mn-superoxide dismutase, pmol/g tissue				
28 wk	3.4 ± 0.1	3.1 ± 0.2	2.6 ± 0.2	3.0 ± 0.2
52 wk	3.7 ± 0.2	3.5 ± 0.3	3.4 ± 0.2	3.3 ± 0.2
72 wk	6.4 ± 0.2‡	5.6 ± 0.3‡	5.5 ± 0.4‡	5.0 ± 0.2‡

Values are means ± SE and the significance of differences was analyzed by ANOVA and Student's *t*-test. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$, between 28 wk mice and both 52 and 72 wk mice.

Table 3. *Mitochondrial enzyme activities in the brain of aging mice*

Activity/Mice Age	Slow Males	Fast Males	Slow Females	Fast Females
NADH-cytochrome *c* reductase, nmol·min^{-1}·mg prot^{-1}				
28 wk	77 ± 3	84 ± 5	86 ± 3	93 ± 3
52 wk	75 ± 3†	76 ± 3†	76 ± 3†	86 ± 5†
72 wk	40 ± 2†	49 ± 2†	51 ± 2†	61 ± 2†
Succinate-cytochrome *c* reductase, nmol·min^{-1}·mg prot^{-1}				
28 wk	52 ± 2	51 ± 2	50 ± 2	51 ± 2
52 wk	48 ± 3	52 ± 3	50 ± 3	54 ± 3
72 wk	54 ± 2	51 ± 3	50 ± 2	54 ± 3
Cytochrome oxidase, nmol·min^{-1}·mg prot^{-1}				
28 wk	103 ± 6	109 ± 6	111 ± 8	118 ± 6
52 wk	99 ± 4	103 ± 4	105 ± 6	113 ± 4
72 wk	90 ± 4	94 ± 4	94 ± 2	105 ± 4
Citrate synthase, nmol·min^{-1}·mg prot^{-1}				
28 wk	70 ± 3	72 ± 3	78 ± 3	80 ± 3
52 wk	58 ± 2*	69 ± 3	70 ± 3	78 ± 3
72 wk	23 ± 2†	25 ± 2†	30 ± 2†	47 ± 2†

Values are means ± SE and the significance of differences was analyzed by ANOVA and Student's *t*-test. *$P < 0.01$; †$P < 0.001$, between 28 wk mice and both 52 and 72 wk mice. A significant difference ($P < 0.01$) was observed in citrate synthase activities between male and female mice.

3). Pooled female NADH-cytochrome *c* reductase activities were 13% higher than the activities of male mice. Interestingly, pooled fast mice (males and females) showed a slightly higher activity (11%) than slow mice. Liver NADH-cytochrome *c* reductase were similarly affected with a 22% and 43% decreased activity in adult and old animals and with a higher activity in females (13%) and in fast mice (6%) (Table 4).

Succinate cytochrome *c* reductase activity (complexes II and III) in both brain (Table 3) and liver (Table 4) was not modified in relation to either age, sex, or behavior, indicating a selective impairment of NADH-dehydrogenase activity (complex I) on aging.

Brain cytochrome oxidase (complex IV) activity was decreased in adult and old animals, 5% and 13%, compared with young mice (Table 3). Brain cytochrome oxidase activity was slightly higher (6%) in females and fast mice. The trend was that the groups with longer life span showed higher values of brain cytochrome oxidase. Liver cytochrome oxidase activity was decreased by 22% and 43% in adult and old mice and females and fast mice showed 22% and 16% higher activities (Table 4).

Brain citrate synthase activity was decreased by 8% and 52% in adult and old animals (Table 3); females also showed here a higher activity (21%), similar to fast mice (13% higher). Liver citrate synthase activity was decreased by 24% and 50% in adult and old animals, and females and fast animals showed slightly higher activities, 7% and 5%, respectively (Table 4).

DISCUSSION

The results of this study show an interesting inverse statistical relationship between mice performance in behavioral tests and cellular indicators of brain oxidative stress. The concept was advanced by Forster et al. (15) that showed a similar inverse correlation between mice maze performance and brain content of protein

Table 4. *Mitochondrial enzyme activities in the liver of aging mice*

Activity/Mice Age	Slow Males	Fast Males	Slow Females	Fast Females
NADH-cytochrome *c* reductase, nmol·min^{-1}·mg prot^{-1}				
28 wk	429 ± 21	442 ± 21	489 ± 25	499 ± 30
52 wk	337 ± 18†	360 ± 18†	376 ± 35*	372 ± 32*
72 wk	223 ± 18‡	258 ± 20‡	267 ± 31‡	317 ± 18‡
Succinate-cytochrome *c* reductase, nmol·min^{-1}·mg prot^{-1}				
28 wk	148 ± 11	150 ± 11	156 ± 11	161 ± 13
52 wk	156 ± 15	159 ± 15	161 ± 16	167 ± 13
72 wk	141 ± 11	143 ± 15	147 ± 13	161 ± 11
Cytochrome oxidase, nmol·min^{-1}·mg prot^{-1}				
28 wk	128 ± 2	136 ± 3	137 ± 3	138 ± 2
52 wk	93 ± 2‡	109 ± 3‡	111 ± 3‡	135 ± 4
72 wk	63 ± 2‡	84 ± 3‡	98 ± 2‡	130 ± 2
Citrate synthase, nmol·min^{-1}·mg prot^{-1}				
28 wk	84 ± 5	86 ± 6	88 ± 4	88 ± 7
52 wk	62 ± 7	66 ± 3	66 ± 5†	70 ± 3
72 wk	40 ± 5‡	40 ± 2‡	41 ± 3‡	51 ± 6†

Values are means ± SE and the significance of differences was analyzed by ANOVA and Student's *t*-tests. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$, between 28 wk mice and both 52 and 72 wk mice. Significant differences ($P < 0.001$) were observed in cytochrome oxidase activity between males and females and between slow and fast mice.

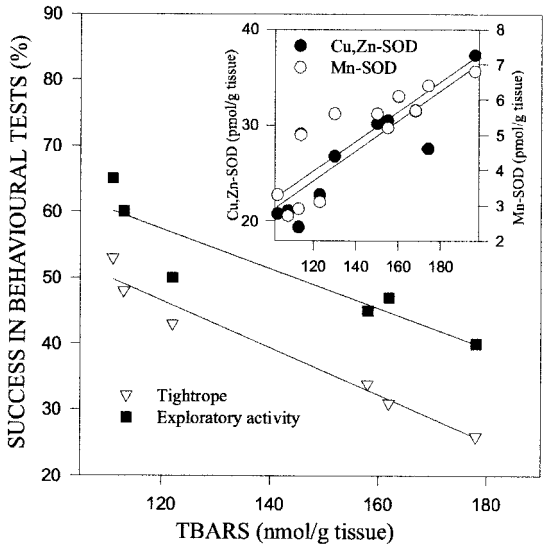

Fig. 5. Correlations between tightrope and T-shaped maze performance and TBARS levels in brain homogenates. Tightrope/TBARS, $r^2 = 0.95$, $P < 0.01$; T-shaped maze/TBARS, $r^2 = 0.83$, $P < 0.05$. *Inset*: correlation between brain Cu,Zn-superoxide dismutase (SOD) and Mn-SOD activities and TBARS in brain homogenates: Cu,Zn-SOD/TBARS, $r^2 = 0.73$, $P < 0.05$; Mn-SOD/TBARS, $r^2 = 0.75$, $P < 0.05$.

carbonyls. In the present study, two behavioral tests were used, tightrope movements and maze exploratory activity, and three indicators of oxidative stress, TBARS, Cu,Zn-SOD, and Mn-SOD, were determined. The dependence of successes in the behavioral tests on aging (Figs. 1 and 2) resembles the form of the plots of survival on time (25) and indicates a continuous and almost linear decline of biological function along time (7, 10). Performance in the behavioral tests significantly correlated with TBARS contents that were taken as the primary indicator of oxidative stress (Fig. 5). Other indicators, such as Cu,Zn-SOD and Mn-SOD, were taken as secondary markers of oxidative stress and significantly correlated with TBARS (Fig. 5, *inset*). Similar increases in TBARS and in Cu,Zn-SOD and Mn-SOD have been reported in the muscle of aging mice (31).

The second interesting observation of this study was the correlation between behavioral tests in young animals and the biochemical markers of oxidative stress and mitochondrial activities in mice populations. Associations of the quality of the response to stressful stimuli, longevity, and decreased neurodegeneration were reported in different mice strains (19). In the present study, a similar relationship was observed in terms of life span, biochemical markers of oxidative stress, and performance in behavioral tests on aging in the four experimental groups: slow males, fast males, slow females, and fast females. The salient observations are that predictions about neurodegeneration and longevity can be done in terms of the early determination of neuromuscular performance and oxidative stress markers and that there is a relationship between brain oxidative stress and behavioral performance. It is apparent that a better behavioral response, both in terms of neuromuscular function and of exploratory activity is linked to a longer life span and to a decreased age-dependent neurodegeneration (19). Mice exhibiting the extended life span, as described in Fig. 4, would be selected individuals that express some longevity genes as recently described in humans (33). As said, Foster et al. (15) advanced the concept that protein oxidative damage is determinant of the age-related decline of specific brain functions related to cognitive and motor capacity, suggesting that the decline of brain performance on aging involves oxidative molecular damage.

It is apparent that aging shifts intracellular redox balance toward a more oxidized state. Increased levels of TBARS, hydroperoxides, protein carbonyls, and 8-OH-dG have been reported in rat brain and liver (15, 17, 28, 36). The oxidation products of intracellular free radical reactions appear to signal, in a homeostatic response, to increase the level of the O_2^- scavenging enzymes, Mn-SOD and Cu,Zn-SOD.

The higher level of TBARS in the brain of old animals indicates increased lipoperoxidation and potential neuronal membrane damage. This fact seems reflected by the decreased activity of mitochondrial electron transfer complexes and by the decreased performance in the behavioral tests that involve neuronal function and firing of action potentials. For both functions, membrane phospholipids have an essential structural role. Brain TBARS also inversely correlated with the activity of two-membrane bound enzymes, NADH-cytochrome *c* reductase and cytochrome oxidase, and with the activity of one matrix enzyme, citrate synthase, that were selectively impaired on aging (Fig. 6).

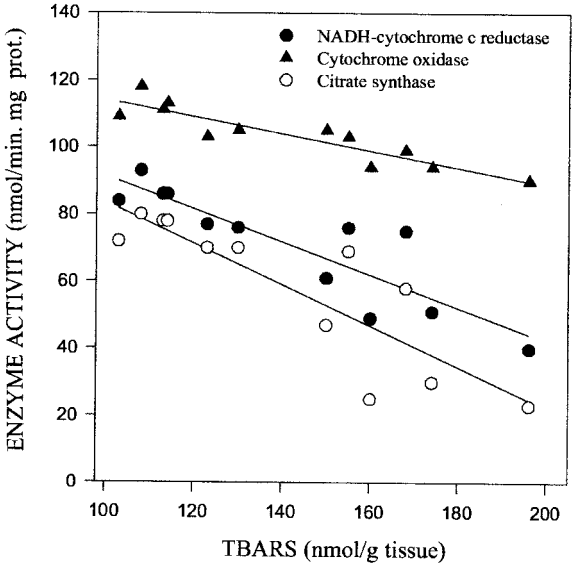

Fig. 6. Correlations between NADH-cytochrome *c* reductase, cytochrome oxidase, and citrate synthase activities in brain mitochondrial membranes in relation to TBARS in brain homogenates. NADH-cytochrome *c* reductase/TBARS, $r^2 = 0.77$, $P < 0.05$; cytochrome oxidase/TBARS, $r^2 = 0.82$, $P < 0.05$; citrate synthase/TBARS, $r^2 = 0.74$, $P < 0.05$.

It seems that the brain and liver mitochondria of old animals are subjected to a complex process of oxidative stress and adaptation. The changes in the activities of mitochondrial enzymes on aging are in accordance with an increased mitochondrial dysfunction on aging. A selective decreased electron transfer activity was observed in brain and liver mitochondria isolated from adult and old mice. The decrease in NADH-cytochrome *c* reductase activity accompanied by an unchanged succinate-cytochrome *c* reductase activity indicates a selective damage of NADH-dehydrogenase. This observation resembles the selective damage to complex I produced by mitochondrial peroxynitrite (34), by dopamine oxidation, and by dopamine-NO mixtures (2, 32). These observations are also in agreement with the reported decreased activities of complex I (NADH-dehydrogenase) and of cytochrome oxidase in the heart and skeletal muscle of aged humans (23, 38) and in the brain of old rat and mice (1) and with the downregulation of genes involved in mitochondrial electron transport reported in aged rhesus monkeys (24, 28). The observed decreased activity of NADH-dehydrogenase in old animals, ~40% (Table 3), is at the threshold of a functional damage in terms of energy production. This is concluded on the basis that the physiological needs for cell ATP demands are provided by the O_2 uptake and ATP production of ~35–40% of mitochondria in heart and liver (5). Then, aged brain and liver cells with their dysfunctional mitochondria may respire normally but they will not be in the condition of responding to a sudden increase in ATP demands.

Citrate synthase activity in brain and liver was related to age, sex, and longevity; the lower values were observed in the groups with lower life span. Decreased citrate synthase was reported in mitochondria from human skeletal muscle (35) and rat heart (39) with increasing age.

The definition of experimental groups based on sex, neuromuscular coordination, and exploratory activity allowed us to recognize significant relationships on aging between molecular markers of oxidative free radical reactions, mitochondrial activities, neuromuscular function, environmental awareness, life span, and the expression of longevity genes.

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