

Behavioral Effects Induced by Mitochondria-Targeted Antioxidant SkQ1 in Wistar and Senescence-Accelerated OXYS Rats

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Abstract. Mitochondrial dysfunction is involved in aging and in neurodegenerative diseases and, therefore, pharmacological agents that alleviate mitochondrial dysfunction are expected to have neuroprotective effects. Promising in this respect is mitochondrial-targeted antioxidant plastoquinonyl-decyl-triphenylphosphonium (SkQ1). We investigated the effects of SkQ1 (250 nmol SkQ1/kg × day with food) on behavior in the elevated plus-maze (EPM) and open field (OF) and on spatial memory in a Morris water maze (MWM) in middle-aged (12 mo) Wistar and senescence-accelerated OXYS rats. Given that changes in the behavior of OXYS rats may be associated with visual impairment, the condition of the retina and the lens was evaluated by ophthalmoscopy. 14-month-old as well as 3-month-old OXYS rats had considerably reduced activities in OF, increased anxiety in EPM, and manifested impaired learning abilities in the MWM in comparison with Wistar rats. SkQ1-treated rats of both strains displayed significantly higher locomotor and exploratory activity in the OF and less anxiety in the EPM compared to age-matched controls. SkQ1 significantly improved visual ability of the rats reducing the severity of the developed signs of retinopathy and cataract but had no impact on OXYS rat's spatial memory in the MWM. SkQ1-treated Wistar rats exhibited slower learning in the MWM task comparison to the control group. Thus, SkQ1 had beneficial effects on locomotor and exploratory functions of the rat brain. Nevertheless, SkQ1 did not alter learning performance in the MWM in OXYS rats and slightly reduced it in the Wistar strain, which may be associated with differences in redox homeostasis.

Keywords: Behavior, brain aging, mitochondrial-targeted antioxidant SkQ1, senescence-accelerated OXYS rats

INTRODUCTION

In humans and in experimental animals, aging is associated with a slow deterioration of cognitive performance, particularly of learning and memory as well as with an increased risk of neurodegenerative disor-

ders [1–3]. Both aging and age-associated neurodegeneration are related to the development of behavioral impairments; consequently, impaired performance on tests of neuromuscular coordination and reduced exploratory activity are considered markers of neurological aging. Brain aging is associated with a growing imbalance between antioxidant defenses and intracellular concentrations of reactive oxygen species (ROS). Overproduction of ROS, which may arise either from mitochondrial electron-transport chain or ex-

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cessive stimulation of NAD(P)H, results in oxidative stress, a deleterious process that can play an important role in the damage to cellular components, including lipids and membranes, proteins, and DNA. At moderate levels, ROS participate in physiological signaling by contributing to the adjustment of brain function to cellular metabolism and metabolic supply. Pathological symptoms as well as modulation of numerous physiological processes may result from both the damaging effects of ROS and from ROS-mediated changes in gene expression [4–6].

Since the formulation of the free radical theory of aging by Harman (1956), antioxidants have been widely recommended as protectors against free-radical damage to be used on a long-term basis at a young and adult age [7]. There are tens of thousands of natural and synthetic compounds that possess antioxidant activity, and a rapidly growing number of these agents have been reported to have beneficial effects in several experimental models of age-related disorders. Animal research as well as observational studies suggests that antioxidant supplementation can slow down aging of the brain and possibly provide some protection against neurodegenerative changes that accompany disorders such as Alzheimer's disease. Nonetheless, there is no evidence that antioxidant supplementation can reduce pre-existing age-related behavioral decline. The correct estimation of the effects of antioxidants on humans is difficult because of many factors, e.g., variation in life span, high costs of clinical trials, ethical issues, as well as individual differences in quality of life, nutrient supply, and age-related deficits in brain function. Studies of animal models of human aging can allow researchers to precisely control these variables and may be used to assess the mechanisms and molecular pathways underlying any positive effects of antioxidant supplementation.

A number of animal models have been successfully used in the studies of aging and age-related disorders. However, there are only a few examples of genetic models with inherited features of accelerated aging. Among these, the strain of senescence-accelerated mice (SAM) represents the only widely used model of accelerated aging [8,9]. Over the last decade, a large amount of experimental data has accumulated which demonstrated that the OXYS strain of rats can be an appropriate model of accelerate senescence. Recently, we showed that senescence-accelerated OXYS rats are a suitable model for studies of aging and age-related cerebral dysfunctions. Our previous studies showed that behavior of young OXYS rats is similar to the be-

havior of old Wistar rats. We found that the specific behavioral alterations in OXYS rats such as increased anxiety and decreased exploratory activity do not appear to be congenital, but rather develop during the period from 4 to 12 weeks of age [10]. As a result, at the age of 3–4 months, OXYS rats exhibit a significantly reduced locomotor and exploratory activity in the open field test and the hole-board task, increased anxiety in the elevated plus-maze test, and abnormal associative learning in passive avoidance task [11–14]. Recently using magnetic resonance imaging, we detected the first signs of neurodegeneration (the diffusion changes) in the brain of OXYS rats at the age of 3 months which progressed, and at the age of 12 months, focal changes were detected mainly in the area of the cortex and the anterior horns of the lateral ventricles [15]. In addition, OXYS rats show an early development of age-associated pathological phenotypes similar to several geriatric disorders observed in humans, including cataract and retinopathy [16,17]. It was hypothesized that the accelerated senescence of OXYS rats is also associated with progressive mitochondrial dysfunction and, indeed, dietary supplementation with antioxidants can prevent the premature deterioration of mitochondrial function typical of OXYS rats [18–20].

Recently, we showed that mitochondria-targeted antioxidant SkQ1 (plastoquinonyl-decyl-triphenylphosphonium, a conjugate of a lipophilic decyltriphenylphosphonium cation with an antioxidant moiety of a plastoquinone) at nanomolar concentrations is capable of preventing some consequences of accelerated senescence in OXYS rats. One of the important advantages of SkQ1 is its rapid reduction by mitochondrial respiratory chain complexes I and II, that is, SkQ1 is a reusable antioxidant [21]. Like other mitoquinone, SkQ1 can behave as an antioxidant or prooxidant in dependency on the concentration and mitochondrial energization. But a risk of enhancing mitochondrial ROS level is extremely low with SkQ1 whose antioxidant effect becomes measurable at about 1000 times lower quinone concentration than prooxidant. In comparison, for MitoQ this value is less than 2 times [22]. According to our data, addition of the above-mentioned SkQ1 amounts to the food completely prevents development of cataract and retinopathy in OXYS rats [23, 24] as well as the age-dependent decline of the immune system [25,26].

The aim of the present study was to investigate the influence of dietary supplementation with SkQ1 on the behavior of middle-aged Wistar and OXYS rats in the open field (OF; locomotor and exploratory behaviors),

elevated plus-maze (EPM; anxiety-like behavior), and Morris water maze (MWM; spatial reference memory) tests and to compare the behavior of middle-aged and young animals. Given that change in behavior of OXYS rats may be associated with visual impairment, we evaluated the condition of the retina and the lens before starting supplementation with SKQ1 and the behavioral tests.

MATERIALS AND METHODS

Animals, diet, and ophthalmoscopic examination

Male senescence-accelerated OXYS and age-matched male Wistar rats were obtained from the Breeding Experimental Animal Laboratory of the Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences (Novosibirsk, Russia). The OXYS rat strain was developed at the Institute of Cytology and Genetics from Wistar stock by selection for susceptibility to cataractogenic effect of a galactose-rich diet and inbreeding of highly susceptible rats [27]. After five cycles of inbreeding, feeding galactose-rich diet and selection, the resulting generations of rats developed cataracts spontaneously without galactose supplementation of the diet. These rats were registered in the Rat Genome Database as the OXYS rat strain (<http://rgd.mcw.edu/>). At this point, we have the 92nd generation of OXYS rats with spontaneously developing cataract and accelerated-senescence syndrome inherited in a linked manner. Cataract still serves as the key parameter for controlling the state of OXYS strain whereas the other features of accelerated senescence observed in these animals, including cognitive and affective alterations, appeared to be concomitant.

At the age of 4 weeks, the pups were taken away from their mothers and housed in groups of five animals per cage (57 × 36 × 20 cm) and kept under standard laboratory conditions (at 22 ± 2°C, 60% relative humidity, and natural light), provided with a standard rodent feed, PK-120-1, Ltd. (Laboratorsnab, Russia), and given water *ad libitum*. To study the influence of SkQ1 supplementation on behavior, 12-month-old males of OXYS and Wistar rats were randomly assigned to one of two groups ($n = 15$): either control diet or control diet supplemented with 250 nmol SkQ1 per kg of body weight per day. The weight gain was measured in the course of the experiment.

Ophthalmoscopic examinations of OXYS and Wistar rats were carried out using a Betta direct ophthal-

moscope (Germany) equipped with a slit lamp, after dilatation with 1% tropicamide. Assessment of stages of cataract and retinopathy were carried out according to Age-Related Eye Disease Study (AREDS) grade protocol (<http://eyephoto.ophth.wisc.edu>).

Behavioral testing began after 2 months of treatment when rats reached the age of 14 months. Simultaneously, the behavior of intact young OXYS ($n = 15$) and Wistar ($n = 15$) rats was tested at age 3 months.

Behavioral testing

Behavioral responses of animals to treatment was assessed in several behavioral tests in the following order: assessment of the degree of anxiety in the elevated plus maze, observation of locomotor exploratory activity in an open field, and spatial memory task in the Morris water maze [28,29]. Each test was performed once for each animal. The test sessions were conducted between 10 a.m. and 2 p.m. to avoid errors attributable to the diurnal variation of motor activity [30].

Elevated plus-maze test. The test was performed as previously described [31]. The plus-maze apparatus was constructed of Plexiglas with two opposite open arms (50 × 10 cm) and two closed arms of the same size but with 40-cm high walls. The four arms were connected by a central square (10 cm²) and thus formed a plus sign. The apparatus was elevated 50 cm above the floor. Each rat was placed in the central square of the plus maze facing one of the closed arms and its behavior was scored for 5 min. The number of entries with all four paws within the arms and the time spent in the arms were scored separately for open and closed arms. A greater amount of time spent in the open arms indicated reduction of anxiety-like behavior.

Open-field test. Forty-eight hours after the completion of the elevated plus-maze test, the animals were subjected to the open-field test normally used to assess emotionality based on the same conflict situation as in the EPM [31]. The open-field area consisted of an enclosed square arena made of Plexiglas (100 × 100 cm) surrounded by walls (40 cm high). The arena was divided by transverse lines into 100 equal squares. A central light source (100 W) on the ceiling gave invariant illumination in an otherwise dark room. The rat to be tested was transported from the home cage to the recording room in a black box. Each rat was gently placed into the same corner of the arena facing the same direction and allowed to freely explore the arena for 5 min. Every time both hind limbs entered a square, a crossing was recorded. Exploratory locomotor activity

was evaluated from measurements of the number of line crossings and the number of rearings (the number of times the animal stood on its rear limbs). The number of grooming episodes, the number of defecations, and the time spent in the center of the field were also scored and interpreted as anxiety-like behavior (for details, see below).

Morris water maze test of spatial memory. Morris water maze test allows for assessing spatial memory by requiring rats to find a submerged platform in a pool of water using external visual cues [32]. Animals were trained in a circular pool (180 cm in diameter) located in a well-lit room with visual cues. An escape platform (13 cm²) was submerged 2.0 cm below the surface of the pool water, which was maintained at $22 \pm 2^\circ\text{C}$, and mixed with milk powder to obscure the platform. The location of the platform remained in the center of northwest quadrant throughout the 5-day training period. Trials (5 consecutive days, 4 trials per day) were conducted with the same hidden platform location, but with varied start locations. Each trial either lasted for 70 s or ended earlier if the rat reached the submerged platform thus escaping from the water maze. If the rat failed to find the platform within 70 s, it was placed on the platform by the investigator. Whether the rat found the platform or was placed on it, the animal was allowed to rest on the platform for 20 s. Latencies to escape from the water maze (finding the submerged escape platform) were collected and recorded with a computerized video system.

Statistical analysis

The data were analyzed using repeated measures ANOVA and nonparametric tests with the statistical package Statistica 6.0. Two-way ANOVA was used to evaluate the differences between OXYS and Wistar rats across ages (age \times genotype) as well as to evaluate effects of treatment (SkQ1 and genotype). To test the effect of the diet on behavior parameters, the genotype and the antioxidant (SkQ1) were chosen as independent variables. A Newman-Keuls *post hoc* test was applied to significant main effects and interactions in order to estimate the differences between particular sets of means. One-way ANOVA was used for individual group comparisons. Data are represented as mean \pm S.E.M. Comparisons between means were analyzed with one-way or repeated measures analysis of variance (ANOVA), as appropriate. Results were considered statistically significant if *p* value was less than 0.05.

RESULTS

Elevated plus-maze

Young OXYS rats had higher scores of anxiety-like behavior in the EPM compared to age-matched Wistar rats, which was manifested as a significantly reduced number of entries into open arms ($p < 0.001$) and more enters into closed arms ($p < 0.04$) (Fig. 1A, C). Nonetheless, there was no difference in time spent by rats in both open and closed arms (Fig. 1B, D). Up to the age of 14 months, anxiety-like behavior increased and rats of both strains manifested significantly lower activity in the EPM compared to young 3-month-old animals. Wistar and OXYS rats spent significantly less time in the open arms ($p < 0.0001$ and $p < 0.02$, respectively) and consequently more time in closed arms ($p < 0.009$ and $p < 0.0001$, respectively). There were no significant differences in the number of closed-arm entries between young and middle-aged OXYS rats, but the number of entries into closed arms made by Wistar rats significantly decreased with age. Nevertheless, this index remained higher in 14-month-old Wistar rats than in OXYS rats of the same age ($F_{1,56} = 6.2$, $p < 0.016$).

The EPM test was performed on the animals during the 10th week of treatment with SkQ1. SkQ1 supplementation had an impact on anxiety-like behavior in both OXYS and Wistar rats. The most impressive effect of this antioxidant was on the number of entries into the open arms ($F_{1,56} = 54.4$, $p < 0.000$) which increased 32.2 and 9.2 times in Wistar and OXYS rats, respectively (Fig. 1A). SkQ1 supplementation significantly increased time spent in open arms ($F_{1,56} = 32.8$, $p < 0.000$): 23 times in Wistar and 5.4 times in OXYS rats (Fig. 1B). At the same time, SkQ1 improved the number of closed arms entries ($F_{1,56} = 8.6$, $p < 0.005$) and increased this parameter to the level of young animals (Fig. 1C). SkQ1 also affected the time spent in closed arms and decreased this parameter ($F_{1,56} = 20.2$, $p < 0.00004$) in both OXYS and Wistar rats. Additionally, when on the antioxidant diet, OXYS rats exhibited the level of anxiety typical of young animals (Fig. 1D).

The number of rearings by a rat in EPM reflects general locomotor and exploratory activity of animals. An age-dependent decrease in this activity was apparent in OXYS rats ($F_{1,29} = 15.6$, $p < 0.0003$), but not in Wistar animals ($F_{1,29} = 0.27$, $p < 0.06$). Consequently, the difference between OXYS and Wistar rats observed at 3 months increased with age ($F_{1,56} = 172.9$, $p < 0.000$; difference for 3 and 14 months $p < 0.0001$

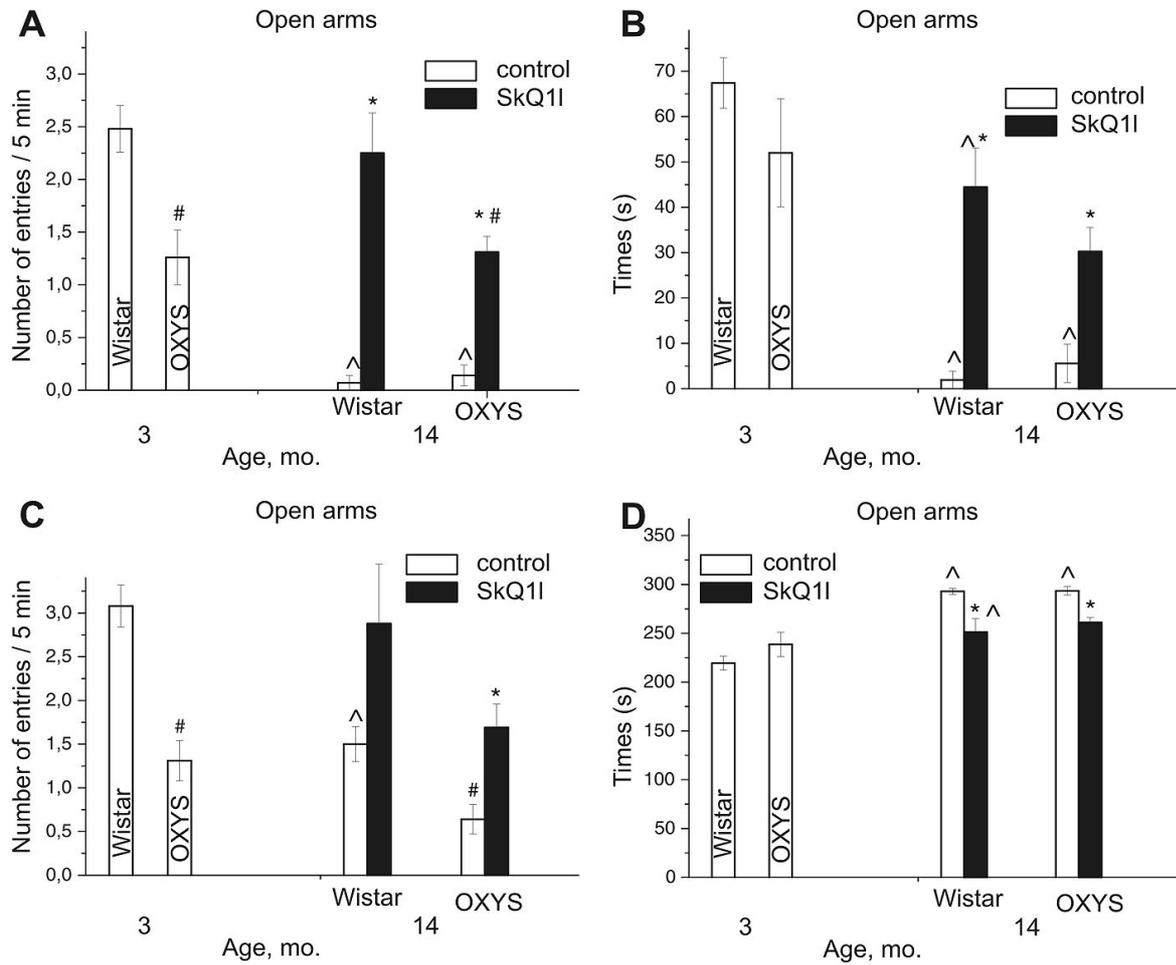


Fig. 1. EPM performance in the control and SkQ1-treated groups comparison with young animals. Young OXYS rats had reduced number of entries to open (A) and into closed arms (C) in comparison with age-matched Wistar rats ($p < 0.05$). Both 14-months-old Wistar and OXYS rats spent less time in the open (B) and more time in closed arms (D) ($p < 0.05$). SkQ1 increased the number of entries to the open arms in both Wistar and OXYS rats, the time spent in open arms, the number of closed arms entries and decreased the time spent in closed arms to the level of young animals ($p < 0.05$). Legend: # – statistically significant differences between the strains of the same age; * – a significant effect of the drug within the strain; ^ – significant age-related differences within the strain.

and $p < 0.0001$, respectively). For locomotor activity, ANOVA showed (Fig. 2A) a significant effect of SkQ1 on rearings in EPM ($F_{1,56} = 25.4, p < 0.000$). The groups of OXYS and Wistar rats treated with SkQ1 had more rearings than corresponding control groups ($p < 0.0002$ and $p < 0.04$, respectively). In addition, there was no significant difference between young and middle-aged OXYS rats treated with SkQ1, while 14-month-old Wistar rats treated with SkQ1 performed more rearings than young animals ($p < 0.009$).

Another important indices of emotional state is a number of head dips that was lower in both young and middle-aged OXYS rats in comparison with age-matched Wistar rats ($p < 0.0001$ and $p < 0.0001$, re-

spectively). However, there were no significant differences detected between young and middle-aged animals within control groups in both OXYS and Wistar rats in the number of head dips made from closed arms of the EPM ($F_{1,29} = 0.1, p < 0.9$ and $F_{1,29} = 2.53, p < 0.12$, respectively). SkQ1 increased this parameter ($F_{1,56} = 9.4, p < 0.003$), but its effect was significant only in OXYS rats and was manifested as a two-fold increase in head dips as compared to control animals (Fig. 2B).

Open field

The open field is a versatile test that permits assess-

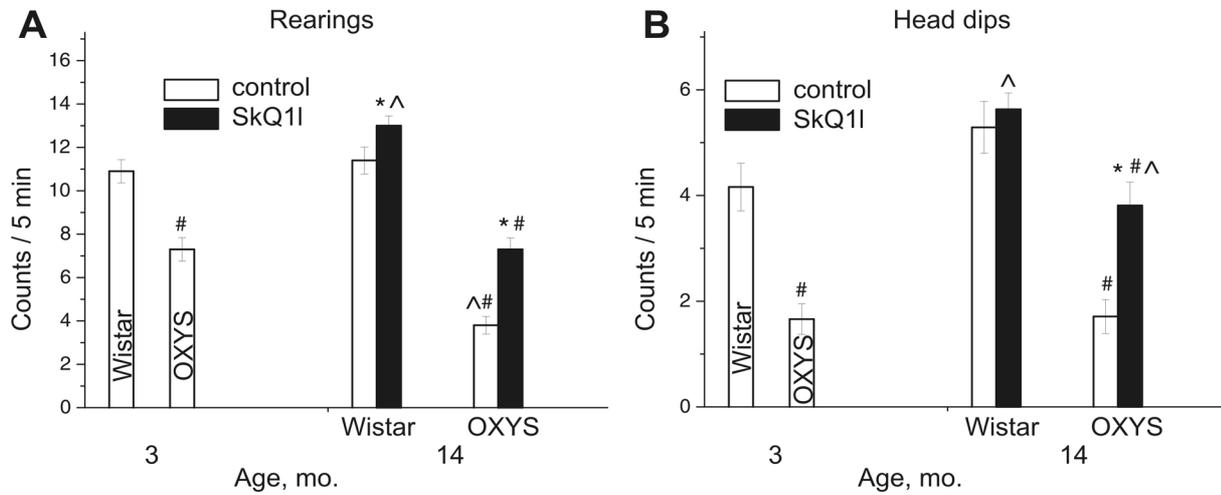


Fig. 2. The number of rearings (A) and head dips (B) in the EPM was lower in OXYS rats at 3 and 14 months of age compared to Wistar rats ($p < 0.05$). SkQ1 increased the number of rearings both in OXYS and Wistar rats ($p < 0.05$) while number of head dips significantly increased only in OXYS rats ($p < 0.05$). Legend: # – statistically significant differences between the strains of the same age; * – a significant effect of the drug within the strain; ^ – significant age-related differences within the strain.

ment of anxiety-like, exploratory, and locomotor behavior [33]. The results of the behavior observations in OF are presented in Fig. 3. ANOVA analysis showed that both age and genotype had an influence on the behavioral parameters. Overall, a clear decline with age in locomotor activity and exploration in OF was observed in both OXYS and Wistar rats. The number of line-crossings, rearings, and grooming episodes decreased with age ($F_{1,26} = 46.8$, $p < 0.000$ for crossings; $F_{1,26} = 55.6$, $p < 0.000$ for rearing; $F_{1,26} = 15.3$, $p < 0.0006$ for grooming episodes). There were no significant differences between young and middle-aged OXYS rats in grooming frequency. In contrast, Wistar rats increased frequency with age ($p < 0.001$); consequently, at the age of 14 months, this parameter was significantly lower in OXYS rats ($p < 0.0007$).

The time of the first entry to the center of the OF arena is associated with less anxious profiles. Analysis of latency to enter the center area of the OF demonstrated effect of age ($F_{1,48} = 6.73$, $p = 0.012$) but not of genotype ($F_{1,48} = 3.05$, $p = 0.087$) and an interaction between these two factors ($F_{1,48} = 8.13$, $p = 0.006$). *Post hoc* analysis revealed a significant difference only between young but not middle-aged Wistar and OXYS rats (mean \pm SEM: young, 179 ± 20.6 and 281 ± 20.8 s, $p = 0.001$; middle-aged 300 ± 22 and 298 ± 18 s, $p = 0.467$). Thus, already at the age of 3 months, OXYS rats had an increased anxiety compared to Wistar animals.

Overall, both young and middle-aged OXYS rats had significantly less activity in OF than age-matched Wis-

tar rats, which was expressed as a significantly reduced number of squares crossed ($F_{1,30} = 8.4$, $p < 0.004$ and $F_{1,56} = 96$, $p < 0.000$, respectively) and frequency of rearing ($F_{1,30} = 51.9$, $p < 0.000$ and $F_{1,56} = 81.3$, $p < 0.000$, respectively).

ANOVA showed that SkQ1 improved locomotor activity in OF ($F_{1,56} = 5.0$, $p < 0.029$), although the significant increase in the number of squares crossed was observed only in OXYS rats ($F_{1,28} = 5.6$, $p < 0.025$), while in Wistar rats, there was only a modest tendency. A higher ambulation caused by SkQ1 in 14-month-old animals was accompanied by increased frequency ($F_{1,56} = 7.0$, $p < 0.011$) and duration of explorative rearings (Fig. 3B). As in the case of horizontal activity, only SkQ1-treated OXYS rats showed a significant increase in rearings ($F_{1,28} = 13.99$, $p < 0.001$). As a result, there were no differences in rearing frequency between young and SkQ1-treated middle-aged Wistar and OXYS rats, but there was an interstrain difference.

Latency to enter the center of OF was affected both by SkQ1 ($F_{1,56} = 6.15$, $p < 0.016$) and by genotype ($F_{1,56} = 14.33$, $p < 0.0004$), and there was also a significant interaction between these two factors ($F_{1,56} = 5.5$, $p < 0.022$). *Post hoc* analysis indicated that SkQ1 reduced latency to enter the center of open field in Wistar rats (from 275 ± 17.9 to 191 ± 16.7 s, $p = 0.0014$) while in OXYS rats it did not affect this parameter ($p = 0.925$). Therefore, there was no difference in the level of anxiety between SkQ1-treated and control OXYS rats.

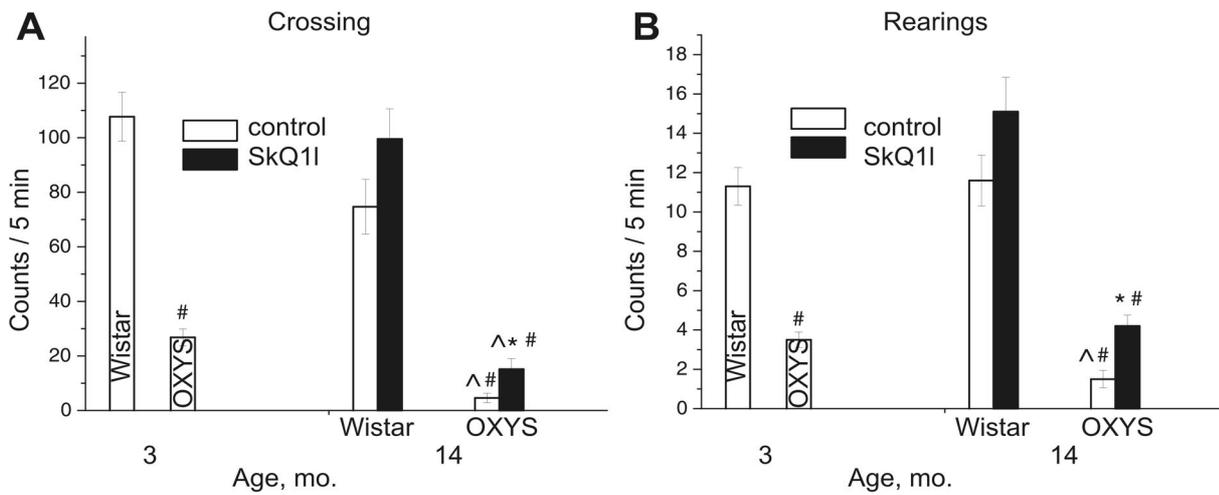


Fig. 3. OF performance in the control and SkQ1-treated groups comparison with young animals. Both young and middle-aged OXYS rats had reduced number of squares crossed (A) and frequency of rearing (B) compared to age-matched Wistar rats ($p < 0.05$). SkQ1 significantly increased the number of squares crossed and frequency of rearing only in OXYS rats ($p < 0.05$). Legend: # – statistically significant differences between the strains of the same age; * – a significant effect of the drug within the strain; ^ – significant age-related differences within the strain.

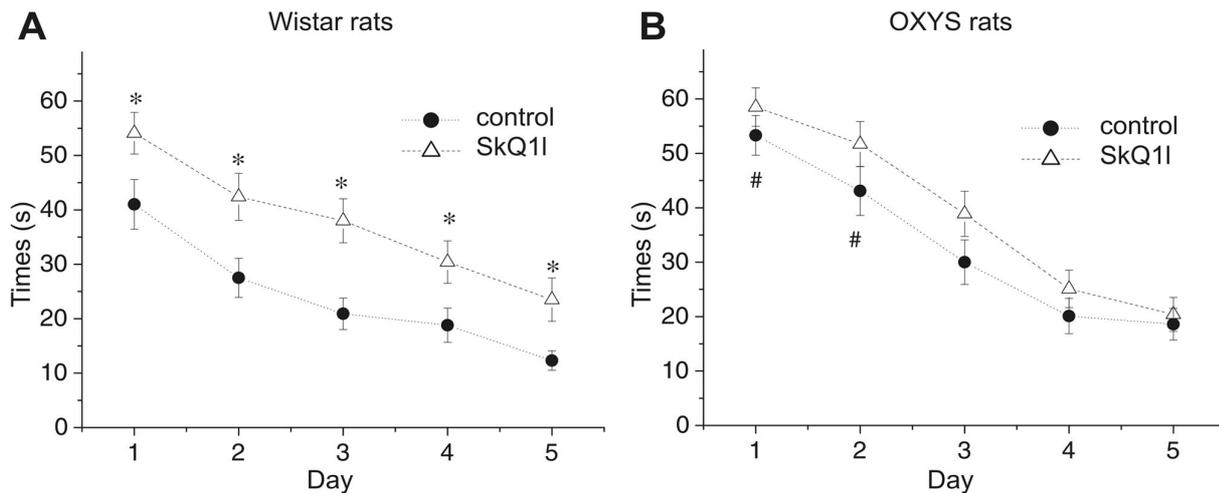


Fig. 4. Effects of SkQ1 on spatial memory in the MWM test of middle-aged Wistar (panel A) and OXYS rats (panel B). SkQ1 significantly reduced spatial learning performances of Wistar rats on each of the five days of testing ($p < 0.05$) (A). Control OXYS rats showed a significantly reduced learning ability on the first two days ($p < 0.05$) in comparison with control Wistar animals (B). Legend: * – a statistically significant effect of the drug within the strain; # – a significant difference with control Wistar rats.

There were no significant differences between control and SkQ1-treated Wistar rats in grooming frequency ($F_{1,28} = 0.33, p = 0.56$); however, the antioxidant significantly increased this parameter in OXYS rats ($F_{1,28} = 15.8, p = 0.0004$).

Morris water maze

In this task, we only used 14-month-old control and SkQ1-treated Wistar and OXYS rats. The effects of

SkQ1 on spatial memory in the MWM performance were evaluated following 3 months of supplementation with SkQ1. Rats are proficient swimmers and are motivated to escape from water based on visual references. Once animals learn where the hidden platform is located, they can remember the location and swim rapidly to it from any starting point.

The results of the behavioral data are presented in Fig. 4A, B. The latency to reach the platform declined progressively throughout the five days of the

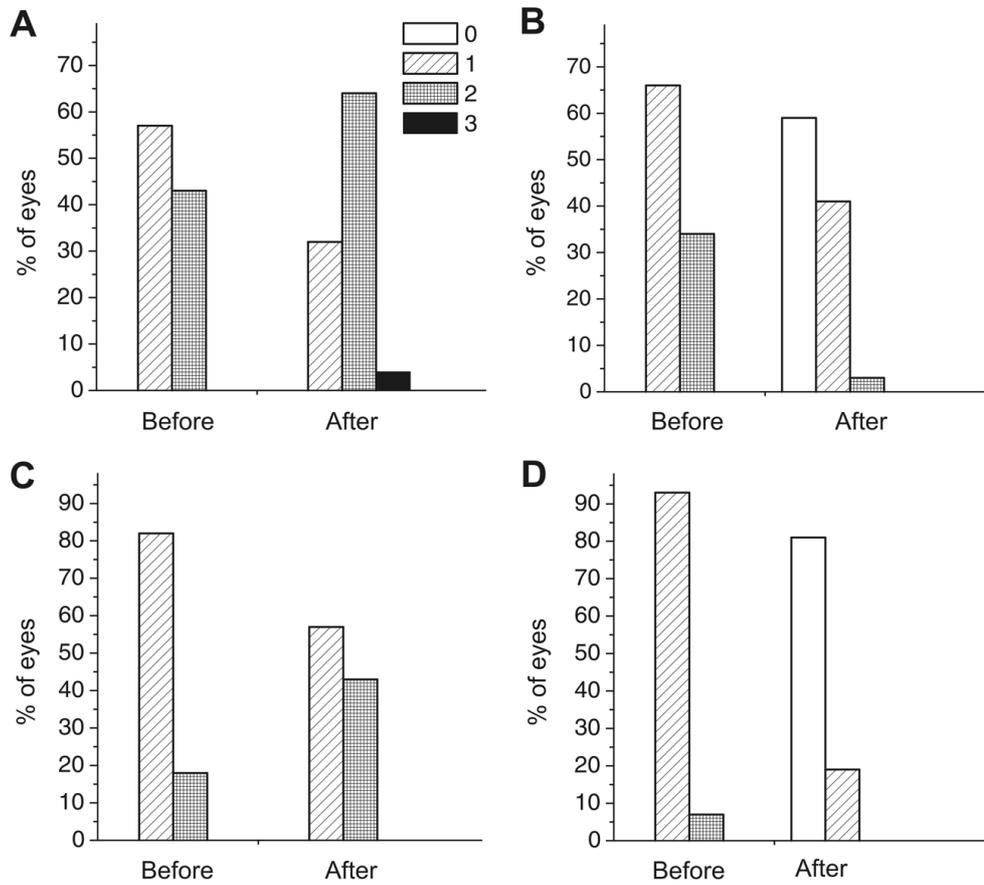


Fig. 5. Effect of SkQ1 on cataract (panels A and B) and retinopathies (panels C and D) in OXYS rats. Data are presented as distribution of eyes of animals within the stages of development of cataract and retinopathy in control (A and C, respectively) and SkQ1-treated OXYS rats (B and D, respectively). 0–3 – corresponding stage of the cataract or retinopathy.

experiment in all animals of control and SkQ1 treatment groups. Repeated measures analysis of performance across all 5 days showed effects of both the genotype and antioxidant: OXYS rats displayed worse spatial learning performance compared to Wistar rats ($F_{1,140} = 5.2, p < 0.007$) and SkQ1 increased the latency time to find the hidden platform ($F_{1,140} = 3.1, p < 0.011$). Nonetheless, Newman-Keuls *post hoc* test revealed that the effect of SkQ1 was significant only in Wistar rats, which was the case on each of the five days (one-way ANOVA, SkQ1 as factor: $F_{1,70} = 5.75, p < 0.02, F_{1,70} = 7.02, p < 0.01, F_{1,70} = 11.8, p < 0.001, F_{1,70} = 5.3, p < 0.025, F_{1,70} = 6.6, p < 0.012$, respectively).

SkQ1 did not have any significant effect on OXYS rats ($F_{1,70} = 1.04, p < 0.03$). In addition, it is noteworthy that the control OXYS rats showed a significantly reduced learning ability only on the first two days ($P < 0.02$ and $P < 0.009$, respectively), which was

manifested as an increased latency to find the hidden platform compared to control Wistar animals.

Ophthalmoscopic examination

Both before supplementation with SkQ1 and before the start of behavioral tests, OXYS rats were examined by an ophthalmologist (two times total for each rat). Data analysis of examinations is shown in Fig. 5. Already during the first inspection, we did not find a single eye that was free of pathological changes. SkQ1 reduced the severity of pathological changes in the lens and eye-ground of OXYS rats, while in the control animals, the cataract and retinopathy had progressed.

By the time of the second inspection, in the control group, the number of eyes with changes in the lens, the corresponding second stage of cataract, had increased from 57% to 64%, and in 4% of eyes, we had found third-stage cataract. In the experimental group before

treatment with SkQ1, in the 34% of eyes, cataract was confirmed as second stage, and in 66% of eyes, as the first stage of cataract. At the time of re-examination, in 59% of eyes, we did not observe pathological changes in the lens, and in 41%, they corresponded to the 1st stage.

By the time of the second inspection, in the control OXYS rats, the number of eyes with problems in eye-ground, the corresponding second stage of retinopathy, had increased from 18% to 43%. In the experimental group before treatment with SkQ1, 93% of eyes were identified with second stage and 7% with the first stage of retinopathy. At the time of re-examination, in 81% of eyes, we did not detect pathological changes in the eye-ground, and in 19%, they corresponded to the 1st stage of retinopathy.

DISCUSSION

In support of our previous reports, the present results indicate that the behavior of young senescence-accelerated OXYS rats is similar to the behavior of middle-aged Wistar animals. Behavioral experiments in the OF showed that locomotor and exploration activity decrease with age in rats of both strains but the degree of age-related decline in exploration was higher in OXYS rats. As we showed previously, this equally applies both to 12-month-old animals [34,35], the age of initiation of treatment with SkQ1, and to 14 month-old OXYS rats as we showed in the present study. Thus, at 14 months, OXYS rats displayed considerably lower exploratory activity in OF test than Wistar rats.

Both Wistar and OXYS rats displayed an age-related increase of anxiety in the EPM. The main emotional indices (open arm entries, time spent in open arms) indicated a higher level of anxiety in middle-aged compared to young rats. According to most researchers [2, 36,37], aging in rodents is associated with a decrease in anxiety and in general locomotor activity. However, Frussa-Filho and colleagues [38] showed that aging appears to be related to an increased sensitivity to anxiogenic effects of the plus-maze and a reduction of the ability to adapt to environmental stress. Moreover, it has been proposed that hyper-reactivity to stressors might be genetically linked to a shorter life span and to accelerated age-dependent neurodegeneration [39]. Taken together, these data and the fact that behavioral alterations in OXYS rats become evident already at 12 weeks of age [10,11] suggest that the behavior of

OXYS rats most likely represents the phenomenon of accelerated senescence.

The MWM has been used extensively to measure cognitive deficits in spatial memory in lesion studies [40,41] and in aging [1,3,42,43]. It is known that old rats show reduced spatial memory on this test compared to young rats, while young and middle-aged animals do not differ from each other [44]. We showed previously that young (age 3 months) and middle-aged Wistar rats (age 12 and 16 months) display the same level of spatial learning, whereas age-matched OXYS rats manifest lower learning abilities in the MWM [35]. In the present work, we assessed spatial learning performance of OXYS and Wistar rats at the age of 14 months. During training in the MWM, all animals were able to learn in the hidden platform spatial task. During acquisition, OXYS rats learned the task more slowly than Wistar rats. Nevertheless, OXYS rats performed the task similarly to Wistar rats starting on the 3rd day of training and latencies remained stable across days 4 and 5.

The main goal of this study was to determine the effects of mitochondria-targeted antioxidant SkQ1 on the behavior of middle-aged Wistar and OXYS rats. The primary findings of the present report are that supplementation of the diet with 250 nmol/kg of SkQ1 was able to reverse age-related motor and exploratory deficits as assessed by OF and partly by EPM tests. SkQ1-treated OXYS and Wistar rats showed considerably more locomotor and exploratory activity in the OF and less anxiety in the EPM compared to their age-matched controls. SkQ1 supplementation improved performance of middle-aged animals in tests of motor function as well as exploratory activity. As a result, the behavior of 14-month-old Wistar rats was similar to that of 3-month-old animals (Figs 1–3).

SkQ1 is a potent antioxidant both *in vitro* and *in vivo* [25]. Previously, we reported the ability of antioxidants (vitamin E and bilberry extract) to influence anxiety in young rats. It is notable that those effects of antioxidants were strain-dependent: vitamin E was weakly effective, while bilberry extract had a strong effect on behavioral characteristics and, in particular, it reduced the elevated anxiety in OXYS rats. On the other hand, both antioxidants, especially vitamin E, increased anxiety-like behavior in Wistar rats, which have a normal level of anxiety [20]. In the present study, we also found that the effects of mitochondria-targeted antioxidant SkQ1 are dependent on the genotype of the animals. Actually, in respect to the reference memory as tested in the MWM, our results show that SkQ1 sig-

nificantly reduced learning ability only in Wistar rats (by increasing the latency to find the hidden platform). It is clear that this effect could not be associated with a decrease in locomotor activity and an increase in anxiety. Therefore, supplementation with SkQ1 had a beneficial effect on locomotor and exploratory functions in the rat brain but did not alter retention performance in the swim maze task.

There is evidence that there is a highly significant association between the age-related deterioration of visual ability and performance levels in MWM [45]. Considering that OXYS rats are characterized by development of early cataract and retinopathy, we examined the impact of impairments of lens and retina on the results of behavioral testing previously [20]. At 3 months of age, OXYS rats develop initial stages of cataract which in relation to humans correspond to subclinical manifestations and can be registered by ophthalmoscope, although generally are not noticed by a person because they do not affect vision. Correlative analysis showed that at 3 months, the behavior of OXYS rats in OF and EPM is linked with manifestations of initial signs of cataract but animals with those cataract signs were unexpectedly more active and less anxious [20]. Up to the age of 14 months, cataract and retinopathy severity in OXYS rats increase considerably and the photoreceptor cell number gradually decreases during the 5–24 months of age in these rats [16,17]. It is possible that the increase of the latency to find the hidden platform in the MWM in OXYS rats at age 14 months is associated with the deterioration visual ability. Be that as it may, we recently showed that SkQ1 supplementation (250 nmol/kg) is able to not only prevent cataract and retinopathy but also to reduce the severity of already developed pathological changes of retina and lens in OXYS rats [24]. Results of ophthalmoscopic examination in the present study confirmed the ability of SkQ1 to reduce signs of developed cataract and retinopathy in OXYS rats (Fig. 5) that implies improvement of animal's vision. Nevertheless, we have not identified a correlation between visual ability and the degree of learning acquisition. Both control and SkQ1-treated OXYS rats were able to successfully find the hidden platform in the MWM and *post hoc* analysis did not reveal group differences in this task. Therefore, it can be concluded that peculiarities of behavior in OXYS rats are not caused solely by the deterioration of visual ability, since the latter had no significant effect on the results of spatial memory testing. A similar phenomenon was observed in the study of behavior in MWM task in Royal College of Surgeons (RCS) dystrophic rats with

hereditary retinopathy that performed very well in the water escape test even though their photoreceptor cell population was decimated [46].

Even more surprising are our results with Wistar rats, which under the influence of SkQ1 trained significantly slower in MWM task compared to controls. As Francia and colleagues [47] showed, in middle-aged mice performance in the MWM task is associated with decreased exploratory behavior. Our results seem to contradict this finding. Schulz et al. [37] surmised that the age-related impairments in the water maze are related to changes in platform behavior, which, in turn, might reflect exploratory activity. Our results confirm the data of Miyagawa and colleagues [29] and suggest that deficits of learning and memory in rats can be dissociated from changes in motor function. Cognitive function might be affected by the subject's emotional responsiveness. Besides, differences in trait anxiety are related to spatial learning abilities [48]. High levels of anxiety might be expected to negatively influence learning and memory. On the other hand, several studies on different mouse strains showed that animals with a lower than normal anxiety level manifest reduced spatial memory in the MWM [49]. It is possible that the effect of SkQ1 on the learning ability of Wistar rats in the MWM is associated with a significant reduction in anxiety.

One question that was not addressed in this study is the potential mechanisms underlying the observed effects of SkQ1. Although aging alone is not sufficient for the development of neurodegenerative disorders, it is likely that a yet unknown combination of environmental and genetic factors is essential for their pathogenesis. Chronic ischemia induced by diffuse insufficiency of blood supply to the brain tissue is a cause of reduced functional potential of the brain and development of cognitive and behavioral deficits in elderly people. Previously, we showed by means of magnetic resonance imaging that early-onset behavioral and synaptic deficits in OXYS rats are associated with structural and functional changes in the cerebral blood flow typical of chronic ischemia. Antioxidant histochrome produced beneficial effects on cerebral vessels in 12-month-old OXYS rats by stimulating collateral blood flow and acting as a vasodilator. Those effects of histochrome were closely related to its capacity to activate locomotor and exploratory activity and to reduce anxiety of OXYS rats in the OF test and hole-board test [37]. In addition, we showed that long-term supplementation with antioxidants (bilberry extract and vitamin E) attenuated cognitive deficits in OXYS rats [20]. Efficient

cy of these antioxidants in the prevention of signs of accelerated senescence in OXYS rats naturally depended on their ability to prevent mitochondrial dysfunction and energy deficiency. As was shown by us and others [24,25], mechanisms of therapeutic action of SkQ1 on retinopathy are associated with renovation of mitochondrial structure and functions, significant improvement of microcirculation in the choroidal vessels and a reversal of functional insufficiency of retinal pigment epithelium in OXYS rats. These mechanisms may also have to do with the reduced level of oxidative damage in tissues and organs. Previously we showed that age-related accumulation of the lipid and protein oxidation levels were abolished by adding to the food of SkQ1 in the same as in the present study, a dose in tissues of Wistar and more significantly of OXYS rats [23].

To summarize, this work and previous studies suggest that the possible mechanisms behind the observed effects of mitochondria-targeted antioxidant SkQ1 on behavior may be associated with a positive effect on cerebral blood flow, improved microcirculation, and energy metabolism. Substantial evidence points to the involvement of mitochondrial dysfunction in the aging and in the pathogenesis of neurodegenerative disorders. Therefore, pharmacological agents that alleviate mitochondrial dysfunction could exert neuroprotective effects. Extremely promising in this respect are mitochondrial-targeted antioxidants including SkQ1, as well as mitochondrial MitoQ [50] which play an important role in modulating ROS-induced mitochondrial permeability transition and cell death, and were found to be protective in several models of ischemia, reperfusion injury, and oxidative stress. Favorable effects of SkQ1 were shown in animal models of some age-related pathologies, namely, heart and kidney infarction, heart arrhythmia, and stroke [51]. It is known that all of these disorders are somehow connected with an increase in the ROS level [52]. Some reports suggest that mitochondrial drug-targeting strategies are effective in several *in vitro* models of neurodegenerative disorders [53–55]. To the best of our knowledge, there are no published studies that use mitochondrially-targeted antioxidants for the treatment of age-related neurodegeneration *in vivo*. Thus, our study appears to be the first to show the ability of a mitochondria-targeted antioxidant to influence age-related changes of behavior. SkQ1 had beneficial effects on locomotor and exploratory functions of the rat brain but did not improve learning in the swim maze task in OXYS rats and slightly decline it in Wistar rats. The effects of SkQ1 on spatial memory depend on the genotype of the ani-

mals. Therefore, the observed interstrain differences in spatial memory experiments could be associated with differences in the redox homeostasis [4] between Wistar and OXYS rats. It would be relevant to mention that the effects of the natural antioxidant carnosine on the physical and behavioral parameters and on the life span were reported to be different (more pronounced) in the senescence-accelerated mice (SAM) compared to the control animals [56,57]. Interesting that decline of different neurochemical processes in OXYS rat's brain may be protected selectively by certain antioxidants [58].

It goes without saying that further investigation is needed to elucidate how exactly SkQ1 affects the brain function. To date, we have confirmed that OXYS rats can serve as a useful animal model of brain aging as well as a suitable platform for studies of the etiopathogenesis of accelerated senescence and for identification of new therapeutic targets in neurodegenerative disorders.

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REFERENCES

- [1] Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, McEwen JJ, Bickford PC (1999) Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. *J Neurosci* **19**, 8114-8121.
- [2] Chen GH, Wang C, Yangcheng HY, Liu RY, Zhou JN (2007) Age-related changes in anxiety are task-specific in the senescence-accelerated prone mouse 8. *Physiol Behav* **91**, 644-651.
- [3] Kiray M, Bagriyanik HA, Pekcetin C, Ergur BU, Uysal N, Ozyurt D, Buldan Z (2006) Deprenyl and the relationship between its effects on spatial memory, oxidant stress and hippocampal neurons in aged male rats. *Physiol Res* **55**, 205-212.
- [4] Dröge W (2002) Free radicals in the physiological control of cell function. *Physiol Rev* **82**, 47-95.
- [5] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telsler J (2007) Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* **39**, 44-84.
- [6] Gerich FJ, Funke F, Hildebrandt B, Fasshauer M, Müller M (2009) H₂O₂-mediated modulation of cytosolic signaling and organelle function in rat hippocampus. *Pflugers Arch* **458**, 937-952.
- [7] Harman D (1956) Aging: a theory based on free radicals and radiation biology. *J Gerontol* **11**, 298-300.

- [8] Takeda T (2009) Senescence-accelerated mouse (SAM) with special references to neurodegeneration models, SAMP8 and SAMP10 mice. *Neurochem Res* **34**, 639-659.
- [9] Brandewiede J, Schachner M, Morellini F (2005) Ethological analysis of the senescence-accelerated P/8 mouse. *Behav Brain Res* **158**, 109-121.
- [10] Sergeeva S, Bagryanskaya E, Korbolina E, Kolosova N (2006) Development of behavioural dysfunctions in accelerated-senescence OXYS rats is associated with early postnatal alterations in brain phosphate metabolism. *Exp Gerontol* **41**, 141-150.
- [11] Loskutova LV, Kolosova NG (2000) Emotional state and one-trial learning in OXYS rats with hereditarily elevated production of oxygen radicals. *Bull Exp Biol Med* **130**, 746-748.
- [12] Loskutova LV, Zelenkina LM (2002) Impairment of latent inhibition in OXYS rats with hereditary syndrome of premature aging. *Zh Vyssh Nerv Deiat Im I P Pavlova* **52**, 366-370.
- [13] Markova EV, Obukhova LA, Kolosova NG (2003) Activity of cell immune response and open field behavior in Wistar and OXYS rats. *Bull Exp Biol Med* **136**, 377-379.
- [14] Markova EV, Kozlov VA, Trofimova NA, Kolosova NG (2005) Stimulation of cell component of the immune response activates exploratory behavior in senescence accelerated OXYS rats. *Bull Exp Biol Med* **140**, 345-347.
- [15] Agafonova IG, Rjtelnikov VN, Mishchenko NP, Kolosova NG (2010) Comparative study of the influence histochrome and mexidol on structural and functional characteristics of premature aging of the brain of rats OXYS by MRI. *Bull Exp Biol Med* **151**, 380-384.
- [16] Zhdankina AA, Fursova AZh, Logvinov SV, Kolosova NG (2008) Clinical and morphological characteristics of chorioretinal degeneration in early aging OXYS rats. *Bull Exp Biol Med* **146**, 455-458.
- [17] Rumyantseva YV, Fursova AZh, Fedoseeva LA, Kolosova NG (2008) Changes in physicochemical parameters and alpha-crystallin expression in the lens during cataract development in OXYS rats. *Biochemistry (Mosc)* **73**, 1176-1182.
- [18] Salganik RI, Shabalina IG, Solovyova NA, Kolosova NG, Solovyov VN, Kolpakov AR (1994) Impairment of respiratory functions in mitochondria of rats with an inherited hyperproduction of free radicals. *Biochem Biophys Res Commun* **205**, 180-185.
- [19] Kolosova NG, Aidagulova SV, Nepomnyashchikh GI, Shabalina IG, Shalbuva NI (2001) Dynamics of structural and functional changes in hepatocyte mitochondria of senescence-accelerated OXYS rats. *Bull Exp Biol Med* **132**, 814-819.
- [20] Kolosova NG, Shcheglova TV, Sergeeva SV, Loskutova LV (2006) Long-term antioxidant supplementation attenuates oxidative stress markers and cognitive deficits in senescence-accelerated OXYS rats. *Neurobiol Aging* **27**, 1289-1297.
- [21] Skulachev VP (2007) A biochemical approach to the problem of aging: "megaproject" on membrane-penetrating ions. The first results and prospects. *Biochemistry (Mosc)* **72**, 1385-1396.
- [22] Antonenko YN, Avetisyan AV, Bakeeva LE, Chernyak BV, Chertkov VA, Domnina LV, Ivanova OY, Izyumov DS, Khailova LS, Klishin SS, Korshunova GA, Lyamzaev KG, Muntyan MS, Nepryakhina OK, Pashkovskaya AA, Pletjushkina OY, Pustovidko AV, Roginsky VA, Rokitskaya TI, Ruuge EK, Saprunova VB, Severina II, Simonyan RA, Skulachev IV, Skulachev MV, Sumbatyan NV, Sviryaeva IV, Tashlitsky VN, Vasiliev JM, Vyssokikh MY, Yaguzhinsky LS, Zamyatin AA Jr, Skulachev VP (2008) Mitochondria-targeted plastoquinone derivatives as tools to interrupt execution of the aging program. 1. Cationic plastoquinone derivatives: synthesis and *in vitro* studies. *Biochemistry (Mosc)* **73**, 1273-1287.
- [23] Kolosova NG, Lebedev PA, Aidagulova SV, Morozkova TS (2003) OXYS rats as a model of senile cataract. *Bull Exp Biol Med* **136**, 415-419.
- [24] Neroev VV, Archipova MM, Bakeeva LE, Fursova AZh, Grigorian EN, Grishanova AY, Iomdina EN, Ivashchenko ZhN, Katargina LA, Khoroshilova-Maslova IP, Kilina OV, Kolosova NG, Kopenkin EP, Korshunov SS, Kovaleva NA, Novikova YP, Philippov PP, Pilipenko DI, Robustova OV, Saprunova VB, Senin II, Skulachev MV, Sotnikova LF, Stefanova NA, Tikhomirova NK, Tsapenko IV, Shchipanova AI, Zinovkin RA, Skulachev VP (2008) Mitochondria-targeted plastoquinone derivatives as tools to interrupt execution of the aging program. 4. Age-related eye disease. SKQ1 returns vision to blind animals. *Biochemistry (Moscow)* **73**, 1317-1328.
- [25] Skulachev VP, Anisimov VN, Antonenko YN, Bakeeva LE, Chernyak BV, Elichev VP, Filenko OF, Kalinina NI, Kapelko VI, Kolosova NG, Kopnin BP, Korshunova GA, Lichinitser MR, Obukhova LA, Pasyukova EG, Pisarenko OI, Roginsky VA, Ruuge EK, Senin II, Severina II, Skulachev MV, Spivak IM, Tashlitsky VN, Tkachuk VA, Vyssokikh MY, Yaguzhinsky LS, Zorov DB (2009) An attempt to prevent senescence: a mitochondrial approach. *Biochim Biophys Acta* **1787**, 437-461.
- [26] Obukhova LA, Skulachev VP, Kolosova NG (2009) Mitochondria-targeted antioxidant SkQ1 inhibits age-dependent involution of the thymus in normal and senescence-prone rats. *AGING* **1**, 389-401.
- [27] Solovyeva NA, Morozkova TS, Salganik RI (1975) Development of a rat subline with symptoms of hereditary galactosemia and study of its biochemical characteristics. *Genetika* **11**, 63-71.
- [28] Handley SL, McBlane JW, Critchley MA, Njung'e K (1993) Multiple serotonin mechanisms in animal models of anxiety: environmental, emotional and cognitive factors. *Behav Brain Res* **58**, 203-210.
- [29] Miyagawa H, Hasegawa M, Fukuta T, Amano M, Yamada K, Nabeshima T (1998) Dissociation of impairment between spatial memory, and motor function and emotional behavior in aged rats. *Behav Brain Res* **91**, 73-81.
- [30] File SE, Day S (1972) Effects of time of day and food deprivation on exploratory activity in the rat. *Anim Behav* **20**, 758-762.
- [31] Redrobe JP, Dumont Y, Herzog H, and Quirion R (2003) Neuropeptide Y (NPY) Y2 receptors mediate behavior in two animal models of anxiety: evidence from Y2 receptor knockout mice. *Behav Brain Res* **141**, 251-255.
- [32] Morris R (1984) Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods* **11**, 47-60.
- [33] Prut L, Belzung C (2003) The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur J Pharmacol* **463**, 3-33.
- [34] Agafonova IG, Kolosova NG, Mishchenko NP, Chaikina EL, Stonik VA (2007) Effect of histochrome on brain vessels and research and exploratory activity of senescence-accelerated OXYS rats. *Bull Exp Biol Med* **143**, 467-471.
- [35] Kolosova NG, Stefanova NA, Sergeeva SV (2009) OXYS rats: a prospective model for evaluation of antioxidant availability in prevention and therapy of accelerated aging and age-related cognitive decline. In: *Handbook of Cognitive Aging: Causes, Processes, and Effects*. Gariépy Q and Ménard R, eds. Nova Science Publishers, NY, pp. 38-51.

- [36] Torras-Garcia M, Costa-Miserachs D, Coll-Andreu M, Portell-Corters I (2005) Decreased anxiety levels related to aging. *Exp Brain Res* **164**, 177-184.
- [37] Schulz D, Kouri C, Huston JP (2007) Behavior on the water maze platform: relationship to learning and open field exploration in aged and adult rats. *Brain Res Bull* **74**, 206-215.
- [38] Frussa-Filho R, Otoboni JR, Giannotti AD, Amaral AC, Conceição IM (1992) Effect of age on antinociceptive effects of elevated plus-maze exposure. *Braz J Med Biol Res* **25**, 827-829.
- [39] Dellu F, Mayo W, Vallée M, Le Moal M, Simon H (1994) Reactivity to novelty during youth as a predictive factor of cognitive impairment in the elderly: a longitudinal study in rats. *Brain Res* **653**, 51-56.
- [40] Brandeis R, Brandys Y, Yehuda S (1989) The use of the Morris Water Maze in the study of memory and learning. *Int J Neurosci* **48**, 29-69.
- [41] Moghaddam M, Bures J (1997) Rotation of water in the Morris water maze interferes with path integration mechanisms of place navigation. *Neurobiol Learn Mem* **68**, 239-251.
- [42] Liu J, Head E, Gharib AM, Yuan W, Ingersoll RT, Hagen TM, Cotman CW, Ames BN (2002) Memory loss in old rats is associated with brain mitochondrial decay and RNA/DNA oxidation: Partial reversal by feeding acetyl-L-carnitine and/or R- α -lipoic acid. *Proc Natl Acad Sci U S A* **99**, 2356-2361.
- [43] Küçük A, Gölgeli A, Saraymen R, Koç N (2008) Effects of age and anxiety on learning and memory. *Behav Brain Res* **195**, 147-152.
- [44] Blalock EM, Chen KC, Sharrow K, Herman JP, Porter NM, Foster TC, Landfield PW (2003) Gene microarrays in hippocampal aging: statistical profiling identifies novel processes correlated with cognitive impairment. *J Neurosci* **23**, 3807-3819.
- [45] Brown RE, Wong AA (2007) The influence of visual ability on learning and memory performance in 13 strains of mice. *Learn Mem* **14**, 134-144.
- [46] DiLoreto DA Jr, del Cerro C, Cox C, del Cerro M (1998) Changes in visually guided behavior of Royal College of Surgeons rats as a function of age: a histologic, morphometric, and functional study. *Invest Ophthalmol Vis Sci* **39**, 1058-1063.
- [47] Francia N, Cirulli F, Chiarotti F, Antonelli A, Aloe L, Alleva E (2006) Spatial memory deficits in middle-aged mice correlate with lower exploratory activity and a subordinate status: role of hippocampal neurotrophins. *Eur J Neurosci* **23**, 711-728.
- [48] Herrero AI, Sandi C, Venero C (2006) Individual differences in trait anxiety are related to spatial learning abilities and hippocampal expression of mineralocorticoid receptors. *Neurobiol Learn Mem* **86**, 150-159.
- [49] Holmes A, Wrenn CC, Harris AP, Thayer KE, Crawley JN (2002) Behavioral profiles of inbred strains on novel olfactory, spatial and emotional tests for reference memory in mice. *Genes Brain Behav* **1**, 55-69.
- [50] Smith RA, Adlam VJ, Blaikie FH, Manas AR, Porteous CM, James AM, Ross MF, Logan A, Cochemé HM, Trnka J, Prime TA, Abakumova I, Jones BA, Filipovska A, Murphy MP (2008) Mitochondria-targeted antioxidants in the treatment of disease. *Ann N Y Acad Sci* **1147**, 105-111.
- [51] Bakeeva LE, Barskov IV, Egorov MV, Isaev NK, Kapelko VI, Kazachenko AV, Kirpatovsky VI, Kozlovsky SV, Lakomkin VL, Levina SB, Pisarenko OI, Plotnikov EY, Saprunova VB, Serebryakova LI, Skulachev MV, Stelmashook EV, Studneva IM, Tskitishvili OV, Vasilyeva AK, Victorov IV, Zorov DB, Skulachev VP (2008) Mitochondria-targeted plastoquinone derivatives as tools to interrupt execution of the aging program. 2. Treatment of some ROS- and age-related diseases (heart arrhythmia, heart infarctions, kidney ischemia, and stroke). *Biochemistry (Mosc)* **73**, 1288-1299.
- [52] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telsler J (2007) Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* **39**, 44-84.
- [53] Reddy PH (2008) Mitochondrial medicine for aging and neurodegenerative diseases. *Neuromolecular Med* **10**, 291-315.
- [54] Reddy PH (2007) Mitochondrial dysfunction in aging and Alzheimer's disease: strategies to protect neurons. *Antioxid Redox Signal* **9**, 1647-1658.
- [55] Chaturvedi RK, Beal MF (2008) Mitochondrial approaches for neuroprotection. *Ann N Y Acad Sci* **1147**, 395-412.
- [56] Boldyrev AA, Gallant SC, Sukhich GT (1999) Carnosine, the protective, anti-aging peptide. *Biosci Rep* **19**, 581-587.
- [57] Gallant S, Semyonova M, Yuneva M (2000) Carnosine as a potential anti-senescence drug. *Biochemistry (Mosc)* **65**, 866-868.
- [58] Salganik R, Dikalova A, Dikalov S, La D, Bulygina E, Stvolinsky S, Boldyrev A (2001) Antioxidants selectively protecting neurochemical functions in rats overproducing reactive oxygen species. *J Anti-Aging Med* **4**, 49-54.