Oxidative stress, neuroendocrine function and behavior in an animal model of extended longevity

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Chapter 4

Deletion of the life span determinant p66^{Shc} prevents age-dependent increases in emotionality and pain sensitivity in mice

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Abstract

Oxidative stress has been implicated in the aging process. Previous studies have determined that mice with a targeted mutation of the p66^{Shc} gene show reduced oxidative stress and extended life span. This study is the first behavioral characterization of mice carrying a deletion of the p66^{Shc} gene. Four-, 11- and 24-months-old homozygous knock-out and wild type mice of the 129Sv/Ev strain underwent a battery of behavioral tests. Locomotion and exploratory activity were tested in the open field test, emotional reactivity was assessed in the elevated plus-maze, while nociception was evaluated by means of the hot plate test (50 °C). In addition, social behavior was assessed in a social interaction test. Our results indicate that pain sensitivity and emotional behavior in wild-type mice increase with age. Deletion of the p66^{Shc} gene results in an increase in pain threshold and reduced emotionality, differences with wild-type subjects becoming more pronounced with age. Thus reduced oxidative stress throughout lifespan is able to prevent some behavioral effects of aging, particularly in response to painful or emotionally arousing stimuli. These data are discussed in relation to recent views indicating new and complex interactions between oxidative stress and emotional stress.

Introduction

Aging is a process associated with a general loss of homeostasis in the organism, leading to an increased risk of neurodegenerative and life-threatening diseases. Indeed, the incidence of cardiovascular disease and cancer rise exponentially with age in the population, Alzheimer's and Parkinson's diseases being commonly referred to as "diseases of old age" (Miller, 1999).

Generation of reactive oxygen species (ROS) appears to be one of the central mechanisms that contribute to aging in a wide range of organisms (Finkel and Holbrook, 2000). Numerous evidence indicates that aged animals accumulate oxidative damage as they grow old (Stadtman, 2001; Floyd and Hensley, 2002). For example, aged rats exhibit enhanced lipid peroxidation (Gupta et al., 1991; Murray and Lynch, 1998; O'Donnell and Lynch, 1998; Devi and Kiran, 2004) and protein oxidation (Cini and Moretti, 1995; Forster et al., 1996; Sohal, 2004) in their brains. Furthermore, it has been demonstrated that aged rats have increased oxidative damage to both nuclear and mitochondrial DNA (Hamilton et al., 2001). Although the damaging actions of ROS are prevented to some extent by a network of cellular defenses, including antioxidant enzymes, these mechanisms are not sufficient to prevent the aging process.

Deletion of the p66^{Shc} gene in mice (p66$^{Shc-/-}$) results in increased resistance to stress and in an approximate 30% extension of lifespan. These mice show an apparently normal phenotype and are characterized by a decreased incidence of aging-associated diseases (Migliaccio et al., 1999; Napoli et al., 2003; Francia et al., 2004). P66^{Shc} is a splice variant of p52^{Shc} /p46^{Shc}, two cytoplasmic adaptor proteins involved in the propagation of intracellular signals from activated tyrosine kinases to Ras (Pelicci et al., 1992). P66^{Shc} has the same modular structure of p52^{Shc} /p46^{Shc} (SH2-CH1-PTB)

and contains a unique N-terminal region (CH2); however, it is not involved in Ras regulation but rather functions in the intracellular pathway(s) that regulates ROS metabolism and apoptosis (Migliaccio et al., 1997; Migliaccio et al., 1999; Trinei et al., 2002). Intracellular ROS levels are decreased in p66$^{Shc-/-}$ cells, as revealed by the reduced oxidation of ROS-sensitive probes and the reduced accumulation of endogenous markers of oxidative stress (8-oxo-guanosine) (Trinei et al., 2002; Francia et al., 2004). Likewise, p66$^{Shc-/-}$mice have diminished levels of both systemic (isoprostane) and intracellular (nytrotyrosines, 8-oxo-guanosine) oxidative stress (Trinei et al., 2002; Napoli et al., 2003; Francia et al., 2004). Indeed, recently a unique role of p66^{Shc} in regulating mitochondrial production of hydrogen peroxide has been described (Giorgio et al., 2005).

The p66^{Shc} gene appears to be one of the converging points linking oxidative stress and the genetics of aging. So far a number of animal models developed to study the aging process have suggested a relationship between changes in metabolism, particularly insulin signalling, and longevity (Kloting and Bluher, 2005). Enhanced resistance to oxidative stress, rather that body size, in p66$^{Shc-/-}$ mice, has been associated with the nature of increased longevity. This mouse model represents thus a unique opportunity to study the role of reduced oxidative damage on the aging process, *in vivo*, and thus the relationship between longevity and health. We hypothesize that reduced exposure to oxidative damage throughout life might attenuate the effects of aging on the nervous system. Aim of this study was thus to investigate whether p66$^{Shc-/-}$ mice might show reduced signs of behavioral aging, compared to same-age wild-type mice. A number of observations indicate an increased level of anxiety with age in mice both in non social and in social situations, in the absence of changes in motor activity (Francia et al., 2006). To this purpose we tested 4-, 11- and 24-months-old homozygous knock out and wild type mice of the 129Sv/Ev strain in a battery of behavioral tests - the open field, the plus-maze, the hot plate and the social interaction test - aimed at assessing age-related changes in the response to painful or arousing stimuli.

Materials and Methods

Animals

Experimental subjects were p66$^{Shc+/+}$ (WT) and p66$^{Shc-/-}$ (KO) mice, of the 129Sv/Ev strain generated as previously described (Migliaccio et al., 1999). Animals were kept under standard conditions. They were housed in an air-conditioned room (temperature 21±1°C, relative humidity 60±10%) with a white-red light cycle (lights on from 08:30 to 20:30). Group housing was chosen to improve animal's welfare. Home cages were Plexiglas boxes (4 in 42 x 27 x 14 cm) with sawdust as bedding. Pellet food (Enriched Standard Diet purchased from Mucedola, Settimo Milanese, I-20019, Italy) and tap water were continuously available.

For hot plate, open field and plus-maze tests experimental subjects were WT and KO mice tested at 4 (Young-Y), 11 (Middle-aged-MA) or 24 (Old-O) months of age (WT-Y and WT-MA, n=10; WT-O, n=7; KO-Y, KO-MA and KO-O, n=10). The so-

cial interaction test was administered to 4- or 11-months-old WT and KO mice (n=10 in each group).

All behavioral tests were conducted under dim light between 9:30 and 16:30, i.e., during the white-light period. Behavioral performances were video recorded using a digital video-camera connected to a professional Sony videocassette recorder V0-5800PS (Model TR 7000E, Sony, Tokyo). The behavioral analysis was carried out from the videotapes, using commercial software ("The Observer 3.0"; Noldus, 1991). All scores were assigned from the same observer who was unaware of the genotype and age of the animals. At the end of each behavioral session apparatuses were thoroughly cleaned with cotton pads wetted with 98% ethanol. All experimental procedures have been carried out in accordance with the EC guidelines (EC Council Directive 86/609 1987) and with the Italian legislation on animal experimentation (Decreto L.vo 116/92).

Open field test

The arena was a cubic black Plexiglas box (35 x 35 x 35 cm) with a white bottom divided into 7 x 7 cm squares by black lines. The test started by placing a mouse in the center of the arena. Subjects were allowed to freely explore the area for 20 min before a stimulus object was placed in the center for 10 min. Thus, overall, the test session lasted 30 min. All scores were assigned from the same observer who was unaware of the age of the animals. The following behavioral categories were scored: frequency of *crossing* (crossing the square limits with both forepaws) and frequency and duration of *rearing* (standing on the hindpaws) and *wall rearing* (standing with the forepaws touching the walls of the arena), *grooming* (licking and rubbing the head or the whole body with both forepaws), *immobility* and *sniffing*. When the stimulus object was present, latency to the first *contact* and frequency of *contacts* with the object were also scored.

Plus-maze test

The apparatus was made of two open (30 x 5 cm) and two enclosed arms (30 x 5 x 15 cm) that extended from a common central platform (5 x 5 cm). The apparatus was made by Plexiglas (dim grey floor, transparent walls) and raised to a height of 60 cm above the floor level. Mice were individually placed on the central platform facing an open arm and allowed to freely explore the maze. The animals underwent a 10 min session. The *time spent* in the open vs the closed arms (both total time and % time in open) was used as a measure of anxiety levels. In addition, frequency and duration of *rearing* (see above), *immobility*, *grooming* (see above) and *head-dipping* (exploratory movement of head/shoulders over the side of maze) were assessed (Fernandes and File, 1996; Rodgers et al., 1999).

Hot plate test

Subjects were placed in the center of the apparatus (Model D837; Basile, Socrel Comerio, Italy), consisting of a hot metal plate set at 50 ±1° C and enclosed by a 19 cm diameter Perspex cylinder. A cut-off time of 60 s was used. Tests were conducted under dim light between 09:30 and 10:30. Behavioral analysis included both nociceptive and exploratory/escape responses. Nociceptive responses were assessed by scoring latency, frequency and duration of the following items: *forepaw* and *hindpaw licking*. In addition, *foot- shaking* was used as a measure of nociception when it was observed that, especially in the case of old subjects, this behavior was frequently performed. Exploratory responses were assessed by scoring both frequency and duration of *rearing*, *wall rearing* and *sniffing* (for a methodological description see Cirulli et al., 2000).

Social interaction test

Subjects were placed in a novel cage with an unfamiliar conspecific and allowed to interact for 20 min. Adult CD-1 male mice were used as social stimulus in order to motivate mice of the 129Sv/Ev strain to interact socially. Behavioral testing took place between 10:00 and 14:00 hr. Social behaviors scored were: *attack, tail rattling*, *offensive upright posture*, *defensive upright posture*. The classical submissive postures, namely *submissive upright posture* and *crouched*, were not observed. Affiliative behaviors scored were *anogenital sniffing* and *body sniffing*. The behavioral categories listed above are mainly based upon the ethological profiles of mouse behavior previously described (for a complete description of the behavioral items please see: Grant and Mackintosh, 1963; Francia et al., 2006).

Statistical analysis

Data were analysed using parametric analysis of variance (ANOVA) (with repeated measures when appropriate) considering Genotype and Age as between-subjects factors and Time blocks as repeated measures, within-subject, factors. Post hoc comparisons have been performed using the Tukey's test.

Results

Open field test

For data analysis purposes, the 30 min session was divided into three 10-minutes time blocks. ANOVA analysis revealed a main effect of Age ($F(2,51)=9.30$; $p<0.0001$) and a significant interaction between Genotype and Age ($F(2,51)=3.21$; $p=0.0485$) for the frequency of *crossing*. Indeed, while in all subjects the amount of locomotion decreased with age, KO subjects did not show a clear age-dependent profile (post hoc comparisons for WT-Y vs WT-MA: $p<0.01$; post hoc comparisons for WT-Y vs WT-O: $p<0.05$) (Fig. 1).

A main effect of Age was found for latency ($F(2,51)=4.94$; $p<0.0109$), frequency ($F(2,51)=3.64$; $p<0.0333$) and duration ($F(2,51)=3.22$; $p<0.0481$) of *wall rearing* (Fig. 1). Overall, this behavior decreased in older subjects. In addition, a main effect of Genotype was found for the latency to perform wall *rearing* ($F(1,51)=4.72$; $p<0.0345$) with KO subjects showing overall lower latencies to perform it compared to WT subjects (Fig. 1). As far as *rearing* behavior is concerned, a significant interaction among Genotype, Age and Time blocks was found for both frequency and duration ($F(4,102)=3.30$; 3.87; $p=0.0138$; $p=0.0057$, respectively for frequency and duration). In particular, at the younger age, WT subjects performed this behavior only over the last time block, KO mice showing it earlier during the session. Latency of *grooming* increased, while frequency decreased, with age (Age main effect: $F(2,51)=10.02$; 5.65; $p<0.0002$; $p<0.0061$, respectively for latency and frequency). A main effect of Age was found also for latency, frequency and duration of *sniffing* ($F(2,51)=14.36$; 41.48; 36.17; $p=0.0001$; $p=0.0001$, $p=0.0001$, respectively for latency, frequency and duration) this behavior decreasing with age, independently from the animal's genotype. ANOVA analysis for the latency to *contact the object* revealed a significant interaction between Genotype and Age ($F(2,51)=3.58$; $p=0.0350$). In particular, WT-Y subjects showed a lower latency to *contact the object* at the younger age (WT-MA vs WT-Y, post hoc comparison: $p<0.05$). By contrast, KO mice decreased their latency to *contact the object* as they become middle-aged.

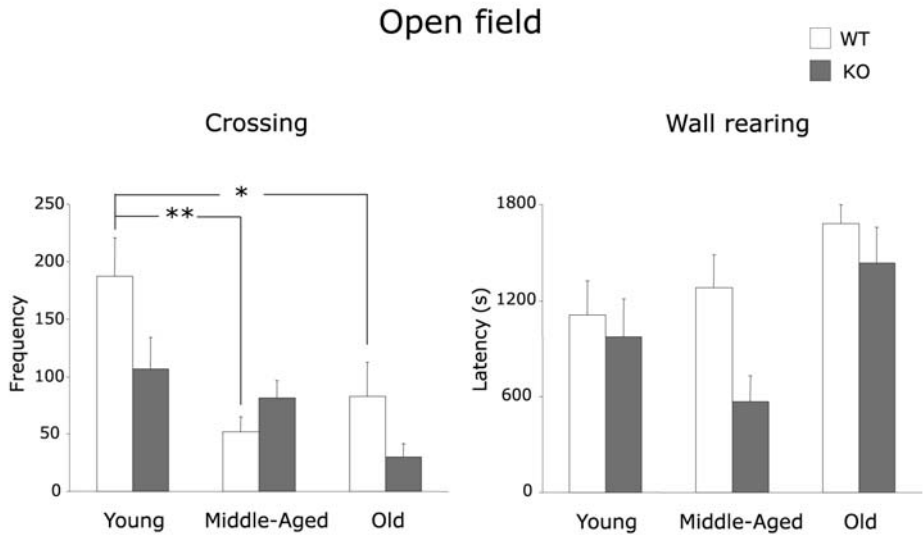

Figure 1 Behavior shown in the open-field test by p66$^{Shc+/+}$ (WT) and p66$^{Shc-/-}$ (KO) mice tested at 4 (Young), 11 (Middle-aged) or 24 (Old) months of age. The session lasted overall 30 minutes. Post-hoc comparisons are shown:* $p<0.05$;** $p<0.01$. Data are mean values ± S.E. (WT-Y and WT-MA, n=10; WT-O, n=7; KO-Y, KO-MA and KO-O, n=10).

Plus-maze test

The behaviors were analyzed separately over two 5-min time blocks. When the frequency of visits to all the compartments of the maze was measured, all subjects showed a reduced exploration with age (Total *entries* frequency, main effect of Age: F(2,50)=13.01; p=0.0001). WT subjects performed a higher number of visits both to the central (*Center entries* frequency, main effect of Genotype: F(1,50)=5.57; p=0.0222) and to the closed parts of the maze (*Closed arm entries* frequency, main effect of Genotype: F(1,50)=7.26; p=0.0096) compared to KO subjects. Thus, as far as locomotor/exploratory activity is concerned, KO mice showed overall less *exploration*, spending more time immobile (Immobility, duration main effect: F(1,50)=5.27; p=0.0260) and performing less *head dipping* during the first part of the session (interaction between Genotype and Time blocks for head dipping frequency, F(1,50)=4.10; p=0.0482). As far as specific measures of anxiety are concerned, ANOVA revealed a main effect of Age for latency and frequency of *entry into the open arms* (F(2,50)=3.35;18.29; p=0.0433; p=0.0001) with older mice showing a higher latency and lower frequency to enter the open arms of the maze (Fig. 2).

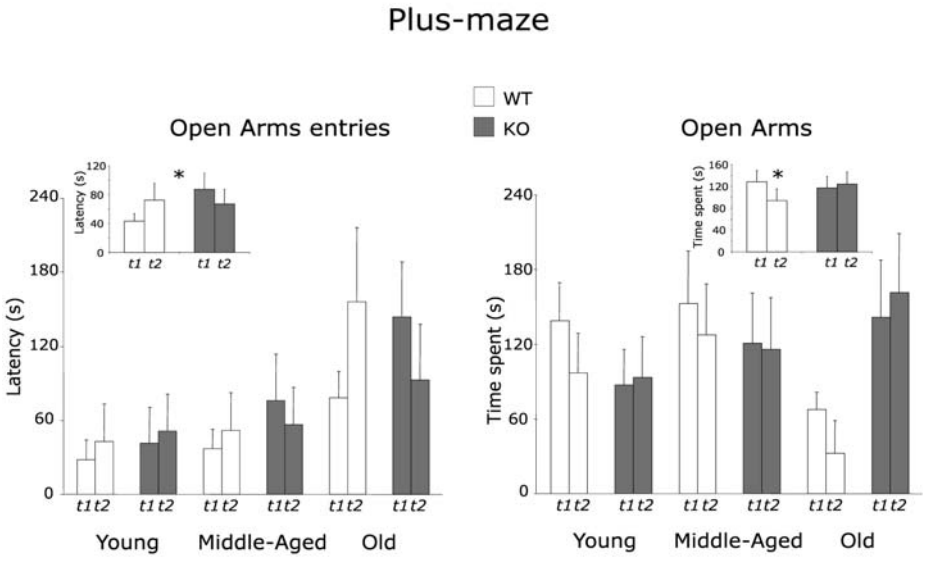

Figure 2 Selected behaviors shown by p66$^{Shc+/+}$ (WT) and p66$^{Shc-/-}$ (KO) mice tested at 4 (Young), 11 (Middle-aged) or 24 (Old) months of age. The session lasted overall 10 minutes. The behaviors were analyzed separately over two 5-min time blocks (t1; t2). Open arm *entries*: in the inset an interaction between Genotype and Time blocks is indicated * p=0.05. *Time* in Open Arms: the main effect of Genotype is shown in the inset. Post-hoc comparison: * p<0.05. Data are mean values ± S.E. (WT-Y and WT-MA, n=10; WT-O, n=7; KO-Y, KO-MA and KO-O, n=10).

This effect was specific for the open arms and did not extend to the closed arms. In addition, a significant interaction between Genotype and Time blocks was found for the latency to visit the open arms of the maze (F(1,50)=4.03; p=0.0502) (Fig. 2). During the first time block, KO subjects showed a longer latency to visit the open arms

when compared to WT mice (post hoc comparisons: $p<0.01$). However, while WT animals showed an increase in the latency to enter the open arms over time (post hoc comparisons $p<0.05$) no difference was found in KO subjects. A significant interaction between Genotype and Time blocks was also found for the time spent in the open arms ($F(1,50)=4.58$; $p=0.0372$) (Fig. 2). In particular, post hoc comparisons confirmed that KO mice spent more time in the open arms of the maze compared to WT during the second part of the test ($p<0.01$), that is when WT subjects usually avoided them ($p<0.01$). This behavior was particularly evident in old subjects. No effect of genotype or age was found for % time spent in the open arms.

Hot plate test

Results from the hot plate test indicate important effects of both Age and Genotype on pain sensitivity. When latency and frequency of *hindpaw licking* were analysed, a significant interaction between Genotype and Age was found ($F(2,51)=4.33$; 4.82; $p=0.0183$; $p=0.0121$ respectively for latency and frequency) with an increase in pain sensitivity in WT-MA subjects, compared to KO mice (post hoc comparisons for hindpaw licking latency and frequency: $p<0.01$). A main effect of Age for latency ($F(2,51)=11.80$; $p=0.0001$) and frequency ($F(2,51)=24.64$; $p=0.0001$) to perform *forepaw licking* was also found. A main effect of Age ($F(2,51)=33.46$; $p=0.0001$) and a main effect of Genotype ($F(1,51)=7.60$; $p=0.0081$) were also found for forepaw licking duration ($F(1,51)=7.60$; $p=0.0081$) (Fig. 3). In addition, a main effect of Age was found for *foot-shaking* frequency and duration (respectively: $F(2,51)=3.65$;3.13; $p=0.0330$; $p=0.0521$). Also, a main effect of Genotype was found for foot-shaking frequency and duration (respectively: $F(1,51)=4.15$; 4.78; $p=0.0468$; $p=0.0335$) (Fig. 3).

A number of behaviors, not directly related to pain can be scored during the test in order to better describe the response of the animal to the noxious stimulus. Thus sniffing behavior was also scored as a measure of exploration. A main effect of Age was found for frequency and duration of *sniffing* behavior, (respectively: $F(2,51)=29.37$; 16.04; $p=0.0001$; $p=0.0001$). Results show that overall KO subjects spent more time exploring the experimental apparatus than the WT animals (Genotype main effect for *sniffing* duration: $F(1,51)=7.36$; $p=0.0091$) (Fig. 3). This might also indicate indirectly that KO subjects were less sensitive to the noxious thermal stimulus. Concerning frequency of *sniffing* episodes, a significant interaction between genotype and age was also found ($F(2,51)=5.90$; $p=0.0049$). In particular, WT subjects showed a decrease in *sniffing* frequency already at middle-age, while in KO subjects this occurred only at the older age (post hoc comparisons: KO-Y=KO-MA vs KO-O $p<0.01$; WT-Y vs WT-MA=WT-O, $p<0.01$).

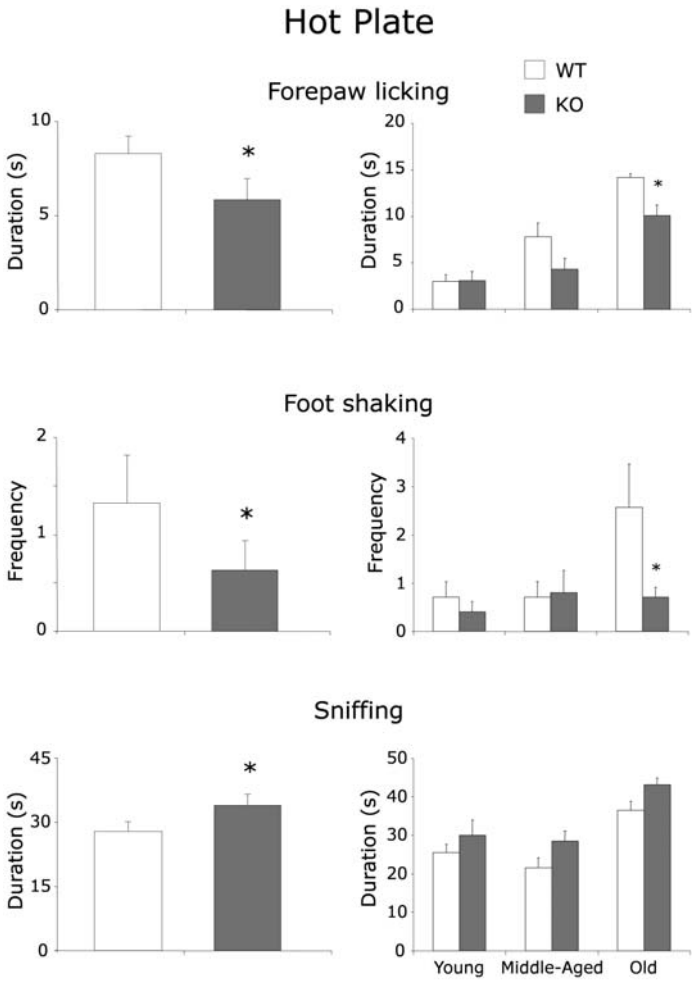

Figure 3 Selected behaviors shown by p66$^{Shc+/+}$ (WT) and p66$^{Shc-/-}$ (KO) mice tested at 4 (Young), 11 (Middle-aged) or 24 (Old) months of age. The hot plate was set at + 50 ±1 °C; cut-off time was set at 60 s. The main effect of Genotype is shown on the left column. Post-hoc comparisons are shown: * $p<0.05$. Data are mean values ± S.E. (WT-Y and WT-MA, n=10; WT-O, n=7; KO-Y, KO-MA and KO-O, n=10).

Social interaction test

Overall, subjects of this strain (129Sv/Ev) showed very little aggression. A nearly significant interaction between Time blocks and Genotype was found for the frequency of *tail rattling* ($F(1,33)=3.86$; $p=0.0579$) with WT subjects performing more of this behavior, compared to KO, especially at the beginning of the session. No differences were found for *attacks*. While all subjects tended to increase the latency to exhibit the *defensive upright posture* with age (main effect of Age ($F(1,33)=10.13$; $p=0.0032$), WT subjects were characterized by a shorter latency to exhibit this behavior (main effect of Genotype ($F(1,33)=4.75$; $p=0.0366$) (Fig. 4). When approached

by an unfamiliar conspecific, KO subjects avoided contact (*flee*) with it less than did WT mice at the younger age (*Flee* frequency: main effect of Age F(1,33)=8.86; p=0.0054, Genotype F(1,33)=5.85; p=0.0212 and Interaction between Genotype and Age F(1,33)=6.38; p=0.0165 see Fig. 4). A significant interaction between Genotype and Age was found for the latency to perform *body sniffing* (F(1,33)=6.08; p=0.0190). In addition, a significant interaction among Genotype, Age and Time blocks was found both for frequency and duration of *body sniffing* behavior (respectively: F(1,33)=5.49; 6.04; p=0.0253; p=0.0194). In particular, KO-Y subjects showed a greater frequency and duration of social investigation compared to WT-Y, while this difference was no longer evident at later ages.

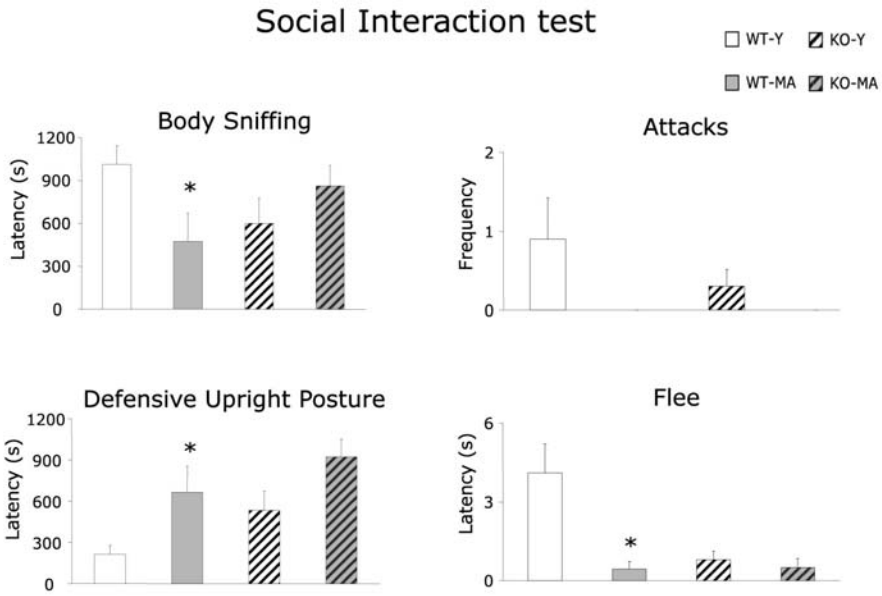

Figure 4 Effect of age on social behavior of Young and Middle-Aged mice. Behaviors were scored during the first, third and fifth daily fighting session. Post-hoc: WT-Y vs WT-MA, *p<0.05. Data are mean values ± S.E. (n=10 in each group).

Discussion

These results represent the first behavioral characterisation of p66Shc-/- mice from adulthood to senescence. Overall they indicate that, in wild-type mice, the aging process results in reduced exploration and increased responses to arousing or painful stimuli. The behavioral phenotype of p66Shc-/- mice is characterized by a reduced response to painful stimuli and reduced emotionality. Differences between KO and WT subjects became more evident with age, suggesting that lack of p66Shc is able to slow the aging process.

The analysis of the open field data confirmed and extended previous observations indicating that, as they age, mice show reduced exploration (Lamberty and Gower, 1992; Boguszewski and Zagrodzka, 2002; Francia et al., 2006). In particular, old sub-

jects showed reduced locomotion, wall rearing and sniffing. Compared to WT mice, p66$^{Shc-/-}$ subjects did not show the same age-dependent profile. This was particularly evident in middle-aged subjects. In fact, at this age, KO mice did not show reduced locomotion (*crossing*) as did WT subjects and were more explorative than same age wild type subjects, showing a greater readiness to perform *wall rearing* and to contact an inanimate object.

Overall, age affected behavior in the plus-maze, independently from the genotype. Older subjects were less explorative and showed a more anxious behavioral profile compared to adults as they moved less in the apparatus and spent less time in the open arms. Independently from age, WT subjects tended to avoid the open arms in the second trial of the session, preferring the protected portion of the maze, while the time spent in both parts of the apparatus did not differ in KO mice. In addition, as they aged, p66$^{Shc-/-}$ mice did not spend less time in the open arms of the maze, like WT mice. It must be emphasized here that reduced emotionality is a double-edge sword. Indeed, some emotional arousal is necessary in order to appraise important environmental cues and to organize efficient responses towards external challenges. We cannot exclude that lack of avoidance of the open arms might depend upon an inability of KO mice to identify an open space as a dangerous zone, where they might be potentially predated upon.

When confronted with a social stimulus in the social interaction test, KO subjects tended to be less aggressive and more affiliative towards the unfamiliar animal. In particular, when approached by an unfamiliar mouse at adulthood, KO subjects did not flee, as did WT subjects. In addition, they were characterized by a longer latency to show defensive behavior. We have previously shown in CD-1 mice that, as they age, social interactions between two unfamiliar males are characterized by increased defensive behavior, lower levels of aggression and greater 'rigidity' (Francia et al., 2006). In particular, greater exploratory activity in the open field (particularly higher levels of rearing and a more direct and rapid approach with a novel object) was correlated with a higher ability to acquire meaningful information from the physical and the social context and resulted in a better performance in a spatial memory task (Francia et al., 2006). The behavioral features characterizing KO mice suggest that these mice might be better equipped to face stressful challenges. Preliminary data suggest that p66$^{Shc-/-}$subjects might also be better in learning and memory performances (data not shown).

Important age-dependent effects were found also in the hot plate test, the aging process being overall characterized by a significant increase in pain sensitivity in all subjects. P66$^{Shc-/-}$mice showed a higher pain threshold compared to WT throughout their life, differences in pain threshold becoming more pronounced in older subjects. Thus, KO mice showed a slower increase in pain sensitivity as they aged. Data concerning pain sensitivity in old mice are scattered and results are often conflicting indicating either a decrease or an increase in pain reactivity (Akunne and Soliman, 1994; Jourdan et al., 2000). These inconsistencies might be due to methodological pitfalls, such as the failure to choose the most appropriate behavioral endpoint, the right

test, or the most appropriate stimulus to assess pain threshold (for a methodological review see Cirulli et al., 2000; Mogil et al., 2001). We have previously shown that, when using the hot plate test, the most appropriate measure to assess pain sensitivity in adult rodents is the latency to lick the hindpaw. However, in this study we found that older mice are often reluctant to lift the hindpaw to lick it, while they readily lick their forepaw. In addition, WT mice of the 129Sv/Ev strain, particularly at the older ages, were found to perform high levels of foot-shaking, a behavior that is able to relieve thermal pain and that is rarely shown at adulthood. Thus, in senescent mice, and differently from adult subjects, both forepaw licking and foot- shaking need to be taken into account as measures of pain sensitivity.

The present findings indicate that reduced oxidative stress characterizing p66$^{Shc-/-}$ mice is associated with a higher pain threshold. Previous observations have indicated that peripheral neuropathies increase with aging, and that ROS contribute to the symptomatology of neuropathic pain disorders since treatment with antioxidants delays the onset of allodynia in a rat model of peripheral mononeuropathy (Crisp et al., 2006). If ROS are generated in excess of the regulatory capacity of the endogenous antioxidants that control them, we might expect the behavioral phenotype of WT and KO mice to become increasingly divergent with age.

Reduced pain sensitivity characterized KO mice already at adulthood. Thus, we cannot exclude the possibility that lack of p66^{Shc} early during development might have affected the ontogeny of sensory neurons or neuronal pathways involved in pain sensitivity. Indeed, it has been previously shown that the mRNAs and functional proteins encoded by the ShcA gene are regulated both spatially and temporally during brain development, being confined to the early embryonic stages, when they can affect the intracellular responses to mitogens during the processes of cellular proliferation and differentiation (Conti et al., 1997). Interestingly, while the two major isoforms of ShcA (p52^{Shc} and p46^{Shc}) are present in the adult brain only in proliferative regions, such as the olfactory epithelium, p66^{Shc} is expressed, though at very low levels, also in total brain lysates (Cattaneo and Pelicci, 1998). The role of p66^{Shc} in differentiated tissues remains to be elucidated.

Overall, data presented in this report indicate that reduced production of ROS throughout lifespan is able to affect the response to painful or emotionally arousing stimuli, producing a phenotype characterized by reduced pain sensitivity and emotionality. These data appear to suggest that common mechanisms might underlie responses to painful or emotionally arousing stimuli and that lack of p66^{Shc} throughout life is able to tap onto these mechanisms to produce the phenotype described. A link between pain perception and emotional behavior has been previously suggested: as an example, targeted deletion of genes encoding for endogenous opioids, as well as for substance P, are able to affect adaptive responses to stressful stimuli (De Felipe et al., 1998; Bilkei-Gorzo et al., 2004). These include both pain sensitivity as well as behavioral responses to threats, such as aggressive behavior, which have an obvious survival value for the animal since they are involved in life-threatening situations, such as the response to predators. This link has been recently strengthened. Indeed,

the use of a convergent functional genomic approach aimed at identifying candidate genes for mood disorders has revealed the existence of a genetic and neurobiological overlap between mood, pain and pleasure pathways (Ogden et al., 2004). The missing link between emotional behavior and oxidative stress might come from a recent paper (Hovatta et al., 2005) showing that the overexpression levels of two enzymes, namely glyoxalase-1 and glutathione reductase-1, in the mouse brain results in increased anxiety-like behavior. Both of these genes are involved in oxidative stress metabolism, linking this pathway with anxiety-related behavior. Although the mechanism by which these enzymes affect anxiety state is not known, they create a novel and unexpected link between oxidative stress and emotional responses.

Conclusions

Life extension is likely to result from a metabolic switch in the use of resources, away from growth and reproduction towards increased maintenance and repair. The accumulation of oxidative damage in late life is unlikely to influence reproductive success and thus there has been no selective pressure to enhance defence systems to prevent it, with the result that the organisms age. The data here reported, in addition to supporting the role of p66^{Shc} in the aging process, indicate that reduced oxidative stress throughout life is able to affect complex physiological regulations, including the orchestration of responses to stressful and painful stimuli. Further studies are needed to identify the mechanisms underlying these effects.

Acknowledgments

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