Oxidative stress (OS) is considered as a main actor in the aging process (Finkel and Holbrook, 2000) because of its ability to cause random damage to macromolecules (Harman, 1998) (oxidative stress theory of aging). A growing body of evidence indicates a redox regulation of several nuclear transcription factors, including the glucocorticoids receptors GR. In particular, it has been suggested that reactive oxygen species (ROS) may affect the HPA axis negative feedback by impairing the GC-mediated translocation of GR from the cytoplasm to the nucleus. This would result in elevated levels of POMC mRNA, an effect that could explain the chronic elevation in GC characterizing inflammation-like states (Asaba et al., 2004).

This interaction between ROS and the HPA axis is particularly intriguing if considered in the context of the p66Shc-/- mice, characterized by a 30% increased longevity and reduced ability to mount oxidative stress responses (compared to their WT counterpart). In the mutants the gene is deleted from GC target tissues, including pituitary and brain. Thus, the objective of the in vivo studies described in this thesis was to elucidate whether the increased longevity of the p66Shc-/- mutants might result from the interaction between OS and the neuroendocrine system. It was hypothesized that the reduced levels of ROS, characteristic of these long-lived subjects, would result in a more efficient regulation of the HPA axis - specifically under stressful conditions - and hence in an overall delay of the aging process. To this aim the effects of the lack p66Shc to behavioral and neuroendocrine regulations were tested from early post-natal life to senescence.

1 Main findings

The lack of the p66Shc gene overall affected the fitness of KO subjects and most notably decreased reproductive success under stressful conditions and over repeated breeding cycles. Maternal behavior of mutant dams was redirected depending upon foraging/metabolic needs possibly affecting the emotional phenotype of the p66Shc-/- mice as well as neuroendocrine responses to stressful stimuli. In addition, in the mutant subjects the onset of puberty was accelerated compared to their same age WT counterpart. This latter feature was likely mediated by maternal behavior. In adult subjects peripheral responses to stressful stimuli did not differ as a function of the genotype, however KOs showed a slight hyperdrive of the HPA axis in response to steroid resistance following a dexamethasone suppression test (DST), possibly underlying a more efficient cross-talk between the neuroendocrine and the immune system. P66Shc-/- mice were also characterized by reduced central responses to stressful/pro-inflammatory stimuli and by a higher neuronal plasticity. These features might in turn be responsible for the better health status overall observed in the KO phenotype. The longevity of p66Shc-/- mice was associated to a delay in the aging process. In particular, the lack of p66Shc resulted in: smoother age-dependent changes in the behavioral profile, lower emotionality, better cognitive plasticity at adulthood and a general better health status at senescence. These features were associated to increased central levels of BDNF under basal conditions suggesting that this neu-
rotrophin might play a key role in shaping the phenotype of the p66Shc-/- mutants representing a converging point linking together many of the features characterizing the mutants.

In the following paragraphs, the main findings of this thesis will be discussed in detail.

1.1 Genetic and nurturing effects of the p66Shc gene: Darwinian fitness and maternal behavior

Chapter 2 describes the effect of the lack of the p66Shc gene on maternal behavior and on the overall fitness.

The lack of the p66Shc gene confers increased longevity and high resistance to oxidative stress to mice carrying a targeted deletion of it. Mutant subjects are also characterized by higher behavioral plasticity at adulthood and better physical performance at senescence (Chapters 4 and 5). In addition p66Shc-/- mice show a higher metabolic rate, with a consequent reduction in body fat, a feature that has been put in relationship with the increased lifespan of the mutants (Berniakovich et al., 2008).

An evolutionary theory of aging states that genes conferring advantages early in life may exert negative pleiotropic effects in successive stages of life (antagonistic pleiotropy or trade-off theory of aging). Given the pleiotropic positive effects of the lack of the p66Shc gene, Chapter 2 investigated the possible reason as to why this gene has not been negatively selected by evolution. Specifically, the hypothesis has been tested that the increased lifespan of the p66Shc-/- mice might have a cost in terms of their overall fitness. To this aim, reproductive success was evaluated by taking into account: number of pregnant females, mean number of pups for each litter, number of litters with dead pups, number of litters in which cannibalistic episodes took place and changes in fertility under stressful environmental conditions and over subsequent breeding cycles. Body weight of virgin females was also taken into account as a possible factor affecting reproductive success. In addition, onset of puberty in both male and female offspring was assessed.

A further interesting feature of the p66Shc-/- phenotype is its overall reduced emotionality (Chapters 4 and 5). Thus Chapter 2 investigated also whether the emotional trait of the p66Shc-/- mutants may be an indirect effect of changes in early environmental factors, such as maternal care. To this purpose, the effects of the p66Shc deletion on maternal behavior were taken into account from postnatal day 1 to 8. In addition, since maternal care affects early neuroendocrine responses to stress of the infant primarily through changes in feeding, possibly through availability of leptin in the milk, leptin levels were also evaluated at PND 8 in dams and pups, both under basal conditions as well as following a 8 hrs maternal separation, a stressful metabolic challenge.

Results indicate that deletion of the lifespan determinant p66Shc leads to a reduced fitness, over repeated breeding cycles and under stressful conditions, overall confirming our initial hypothesis. In addition, it leads to an earlier onset of puberty in both male and female subjects. Thus the p66Shc gene plays an important role in female me-
tabolism, enabling it to carry out the reproduction effort, which involves an extensive energetic demand, more efficiently. Indeed, KO mice showed a higher frequency of cannibalistic behavior, a trait hypothesized to be the result of the metabolic set point characterizing the mutants (higher metabolic rate, reduced body weight and fat mass).

Overall, leptin levels did not differ between the two genotypes (both for mothers and pups). However in KO pups there was no correlation between mothers’ and pups’ leptin while WT subjects showed a direct relationship under basal conditions that was specifically lost as a result of stress (maternal separation). Since during lactation KO dams showed a higher frequency of eating and drinking than their WT counterpart, it was hypothesized that while in the WTs the mother is the primary source of leptin, in the KOs the dam might not provide a sufficient amount of this hormone, triggering an autonomous production by pup tissues to supply to this lack. Further studies are needed to assess the physiological consequences of these observations.

KO lactating dams, in addition to spending more time in foraging also showed a decrease in the overall maternal activity. These two behaviors (foraging and maternal care) were found to be in a strong inverse relationship, especially in the p66Shc-/- dams. In altricial rodents (e.g. mice and rats) alterations in maternal care during early developmental phases can lead to complex and long-term influences on emotionality and neuroendocrine functions of the offspring (Cirulli et al., 2009; Levine, 2000; Pryce et al., 2001). Wild rodents caring for pups are often forced to leave the nest for variable periods of time (hours) to provide their self food (Calhoun, 1963). This pattern of maternal attendance to the nest has been modeled in the laboratory by early handling, this manipulation resulting in important changes in the functionality of the HPA axis (Levine et al., 1957) in a way such that the ability of the adult organism to respond, cope, and adapt to stressful stimuli is increased (Fernandez-Teruel et al., 2002; Meaney et al., 1991). Thus, considering our initial hypothesis, our data suggest that the foraging pattern of KO dams might lead to behavioral and neuroendocrine features characterizing the mutants (Chapters 3, 4 and 5) in a combined nature (the lack of p66Shc)-nurture (maternal cares) effect, this latter driven in turn by the effects of the lack of the gene on dam’s metabolism.

Worth noticing, maternal behavior might also play an important role as a buffer for the reduced fitness observed in mutant subjects leading to the observed acceleration of puberty in the offspring. In fact it has been suggested that a reduced amount of parental care may act as a forecast of negative environmental conditions (e.g. poor food conditions). This environmental context would indeed favor accelerated puberty, higher number of pups and reduced parental investment (r strategy) in a strategy overall resembling the well-known “r strategy” model (McCarthy, 1965), which allows genes to be efficiently inherited maximizing fitness in adverse environmental conditions. This scenario fits well with the above described traits of p66Shc-/- mutants.

Overall data described in Chapter 2 point to a main direct genetic effect of the lack of the p66Shc gene on metabolism which, in turn, in a fine interplay of nurturing effects, also mediated by maternal behavior, is able to affect reproductive pattern.
1.2 Regulation of the HPA axis under conditions of oxidative stress and its relevance to healthy aging in the p66Shc-/- mouse

Chapter 3 was aimed at investigating the neuroendocrine function of KO mice, possibly as a key factor underlying both the emotional phenotype as well as the longevity of mutant subjects.

A wide number of theories exist trying to explain the aging process, each of these focusing on one particular aspect as the main determinant factor. According to one of these theories, damage caused by free radicals may affect the rate of aging of an organism. The neuroendocrine system, which is involved in the response to stressful challenges faced by an organism throughout life, has been hypothesized as a possible target of free radicals.

Chapter 3 specifically focused on the interaction(s) between OS and the neuroendocrine system that might underlie longevity and the delay of the aging process in the p66Shc-/- mutants. A delayed aging in the p66Shc-/- phenotype has been specifically demonstrated in Chapters 4 and 5 through a refined behavioral analysis performed at adulthood, middle-age and senescence. In particular, the question was addressed as to whether the lower susceptibility to OS, characterizing the p66Shc-/- mutants, (resulting in a lower production of ROS in conditions of OS), might favor a more prompt and efficient negative feedback of the GC preventing the deleterious consequences of a prolonged exposure to high levels of GC during lifespan. This would result in a lower accumulation of allostatic load, resulting in a delay of the aging process and in an overall healthier phenotype (as assessed in Chapters 4 and 5) (Sapolsky, 1999).

To answer this question the activation of the HPA axis was assessed as a result of challenges able to induce increased CORT levels and ROS generation, such as acute restraint stress (psychophysical stress) or systemic inflammatory challenge (i.p. LPS injection) (Fontella et al., 2005; Madrigal et al., 2003; Miyake et al., 2005). The GC negative feedback of mutant subjects was specifically assessed under condition of OS by administering LPS and successively a potent GR agonist (dexamethasone - DEX -), while a DEX-suppression-test (DST) was performed to test the ability of the HPA axis to escape from DEX suppression following an immunogenic challenge. In addition, the ability of the central nervous system (CNS) of p66Shc-/- mice to cope with a pro-inflammatory insult (LPS) was also assessed looking at hippocampal levels of isoprostanes - 15-F2t-IsopP - (a marker of lipid peroxidation). Levels of the prostaglandin PGE2 and of the neurotrophin BDNF were also measured in the same brain area as a mean to test neuronal plasticity in response to an inflammatory challenge. As a further index of oxidative status the peripheral total reductive capacity (or anti-oxidant capacity, AOC) was measured, which comprises scavenging enzymes as well as many small anti-oxidant molecules, including vitamins, glutathione and uric acid, and has been recently proved as a useful index of the general health status and prognosis in patients affected by Alzheimer’s disease, likely reflecting the extent to which vulnerable neuronal populations are protected from oxidant processes (Minghetti et al., 2006).

Results indicate that WT and KO mice did not differ in the overall ability of the HPA
axis to cope with stressful stimuli, as shown by the comparable rise in CORT levels following different challenges. Interestingly, when body temperature was measured, KO mice showed a higher basal core temperature suggesting a different set point for basal metabolism. This piece of information, together with data collected in Chapter 2 (lower body weight of mutant virgin females and increased foraging behavior of KO lactating dams), is in agreement with the notion that p66Shc plays a pivotal role on metabolism. In addition, these data are in line with recent findings showing impaired thermo-insulation and increased metabolic rate (Berniakovich et al., 2008), and strengthen the relationship (already established for lower organisms, see Bishop and Guarente, 2007; Kenyon, 2005) between changes in metabolism, energy homeostasis and longevity in higher organisms.

As for the negative feedback, following LPS-DEX administration no difference appeared between WT and KO subjects. By contrast, mutant subjects showed a mild, although significant, escape from suppression when underwent a DST (DEX-LPS administration), as revealed by the increase in CORT levels assessed 4 hrs following DEX administration. This result was quite unexpected given the hypothesized association between the long-lived phenotype of the p66Shc-/- mice and low levels of circulating GC. These findings therefore do not support the hypothesis of a more efficient GC negative feedback in the p66Shc mutants. However, it is important to take into account that a rise in plasma GC levels, which occurs upon inflammation and/or infection, is necessary to prevent the overshooting of the immune functions and that hyporesponsiveness of the HPA axis has been associated to a higher susceptibility to autoimmune diseases (Bakker et al., 2000). Thus, the escape from suppression shown by KO subjects could, at least in part, account for a more efficient cross-talk between the neuroendocrine and the immune system, possibly mediated by a fine GC-dependent tuning of pro-inflammatory responses. This would in turn be responsible for most of the phenotypical traits reported in Chapters 4 and 5 (smoother age-dependent change in the behavioral profile (Berry et al., 2007), better physical performance at senescence and more efficient cognitive strategies at adulthood, increased levels of BDNF and lower levels of OS markers in the hippocampus (Berry et al., 2007; Berry et al., 2008)).

The effects of both the LPS challenge and of the handling procedure (vehicle injection) support this view. In fact, KO mice showed decreased levels of PGE2 and no changes in BDNF and isoprostanes, suggesting that the mutant phenotype might be better equipped to face possible OS-related threats to the CNS. A functional link between PGE2 and BDNF has been suggested in which the expression of this neurotrophin in the rat hippocampus appears to be under the control of COX-2 activity (a main enzyme in the biosynthetic pathway of PGE2) (Shaw et al., 2003). Thus, the lack of response in terms of BDNF synthesis and the decrease of PGE2 levels in KO mice may well reflect a reduced neuronal activation of this genotype in response to a stressful challenge. To further confirm this hypothesis, the specific increase observed in WT adult mice, in the levels of 15-F2αIsopP and in those of BDNF, appears to suggest a lower responsiveness of the p66Shc-/- to stress. In WT subjects, an increase
in BDNF levels could be a countermeasure for increased neuronal activation aimed at neuroprotection. Thus, the specific increase in the levels of this neurotrophin may represent an essential step for WT subjects to buffer the potential deleterious effects of both GC and LPS in the CNS.

Mutant subjects, differently from WTs, were found to be resilient to a change in their anti-oxidant capacity in response to LPS, supporting the hypothesis of a milder exposure of p66Shc-/- mice to OS. In addition, KO subjects showed a trend to increase their AOC, following both LPS and vehicle, suggesting a possible up-regulation of ROS scavengers following exposure to an oxidative stress stimulus (Pani et al., 2009).

Taken together, data in **Chapter 3** suggest that p66Shc-/- mice are characterized by a more efficient homeostatic control and by better abilities to cope with changes in the internal oxidative milieu. This may be achieved by two possible pathways acting synergistically: one involving an OS-resistance mechanism, the other, a fine regulation of the HPA axis, a process independent from the GC negative feedback, as initially hypothesized, that in turn, may be related to a less harsh oxidative milieu.

### 1.3 Behavioral characterization of the p66Shc-/- phenotype from adulthood to senescence: cognitive, emotional and nociceptive traits as landmarks of aging

In **Chapters 4 and 5** the main question addressed was whether “living longer” might also mean “aging better”. Indeed longevity is not necessarily synonymous of health: the increase in lifespan characterizing western societies has been associated to a parallel increase in the incidence of age-related diseases, including neurodegeneration and dementia, that interfere with the quality of life (Barberger-Gateau and Fabrigoule, 1997; Prince et al., 2003).

Work performed in animal models has shown that specific changes in behavior occur with the progression of aging especially in the emotional profile and in pain sensitivity (Francia et al., 2006; Onaivi et al., 1994). Indeed, the mammalian brain is characterized by poor antioxidant defenses, high metabolic rate, and reduced capacity for cellular regeneration resulting particularly susceptible to oxidative stress insults (Floyd and Hensley, 2002). In this scenario, it was hypothesized that in the p66Shc-/- mice a reduced exposure to oxidative damage throughout life might attenuate the overall effects of aging on the nervous system (**Chapter 4**) and might result in improved brain functions and more adaptive behavioral and cognitive performances (Coyle and Puttfarcken, 1993; Siesjo et al., 1989) (**Chapter 5**).

To test this hypothesis, age-related changes in the response to painful or arousing stimuli were tested in 4-, 11- and 24-months-old (respectively adult, middle-aged and old) WT and KO mice in a battery of behavioral tests: the open field (OF), the elevated plus-maze (EPM), the social interaction test (SIT) and the hot plate (HP) (**Chapter 4**). The emotional profile was further characterized at adulthood by means of a refined analysis of the spontaneous behavior in an OF while a tail flick (TF) test allowed to assess peripheral pain sensitivity at the same age. Cognitive abilities were assessed in a Morris water maze (MWM) spatial memory task both at adulthood and
at senescence (4- and 24-months-old subjects). To characterize the neurobiological variables possibly involved in spatial memory performance and emotional reactivity basal levels of the neurotrophin BDNF were evaluated in the brain (hippocampus and cortex) in addition to (basal) levels of isoprostanes 15-F_{2\alpha}-IsoP to monitor the extent of oxidative stress in the same regions (Chapter 5).

During aging mice were found to be characterized by reduced locomotion and exploratory activities (OF) and by a more anxious behavioral profile (lower time spent in the open arms of the EPM). These data confirm and extend the body of evidence on the behavioral profiles characterizing mice at senescence (Boguszewski and Zagrodzka, 2002; Francia et al., 2006; Lamberty and Gower, 1992).

Lack of the p66\textsuperscript{Shc} gene resulted in an overall delay of the aging process, KO mice being characterized by increased pain threshold and reduced emotionality, differences with WT subjects becoming more pronounced with age. KO subjects did not show the decrease in locomotor activity observed in the same age WT counterpart, which is often associated to the old age. In addition, regardles of age, mutant subjects showed a prompt vertical exploration of the apparatus (reduced latency to perform wall rearing) and a greater readiness to contact the object present in the arena, overall suggesting a lower emotional behavioral profile. This piece of information was confirmed both in the EPM, a specific test of anxiety, as well as in a social context such as the SIT. In particular, in the former test KO old subjects spent more time in the anxiogenic part of the maze (open arms), while in the latter they appeared less aggressive and more affiliative towards the unfamiliar partner. At 24 months all subjects showed a significant increase in pain sensitivity, however p66\textsuperscript{Shc-/-} mice were overall characterized by a specific decrease in this parameter compared to WTs, this difference becoming more pronounced at senescence.

In addition, adult p66\textsuperscript{Shc-/-} mice showed greater spatial memory performance in the MWM, possibly associated to increased hippocampal levels of BDNF, while old subjects were characterized by better physical abilities.

Data presented in Chapter 5 were in line with the findings of Chapter 4 and strengthened and extended the knowledge on the behavioral phenotype of mutants at adulthood as well as at senescence.

The OF test confirmed the reduced emotional profile observed in mutant subjects. Pain sensitivity has an important adaptive value and responses to emotionally/arousing stimuli are often accompanied by changes in pain threshold. In Chapter 4 it is shown that the nociceptive threshold was significantly increased in KO subjects only at senescence. However the HP test did not allow distinguishing between peripheral and central component of pain perception. In Chapter 5 the TF test revealed that mutant mice were also characterized by lower peripheral pain sensitivity a feature already present at adulthood, suggesting that lack of the p66\textsuperscript{Shc} gene might have affected the ontogeny of sensory neurons or neuronal pathways involved in pain perception. Alternatively, a reduced exposure to ROS throughout development and at adulthood might account for these findings, since it has been suggested that ROS may contribute to the symptomatology of neuropathic pain disorders (Crisp et al., 2006).
As for cognitive abilities, at adulthood, WT and KO mice were both able to learn the task (acquisition phase in the MWM), while only mutant subjects showed to remember the platform location during the probe trial memory test. A growing body of evidence (both in vivo and in vitro) supports the concept that ROS may be involved in memory impairment (Abidin et al., 2004; Pellmar et al., 1991; Williams and Bliss, 1989). In this scenario, this piece of information enlarges such a body of evidence showing that a genetic manipulation affecting redox balance in favor of an antioxidant milieu may improve cognitive processes already at adulthood.

Although old mice failed to learn the task, KO subjects showed a better physical performance, since a larger number of subjects in this group were able to climb onto the platform at least one time during the acquisition phase. Oxidative stress is primarily involved in the etiology of aging, especially in those tissues with high levels of oxygen metabolism such as skeletal muscles (Carmeli et al., 2005; Fulle et al., 2004; Leeuwenburgh et al., 1998). It is indeed possible to hypothesize that a reduced exposure to oxidative stress at old age might protect p66Shc-/- mice preventing muscles from becoming weak and less powerful. In this context, the decreased pain sensitivity observed in senescent mice might also play a role since the chronic pain often associated to a number of age-related joint disease (Pomonis et al., 2005) might have discouraged WT subjects from further unnecessary movements such as climbing onto the platform (WT old mice overall reached the platform and grasped its edge with their forepaws but did not climb onto it).

Neurotrophins play a main role in learning and memory processes, in particular BDNF has been shown to affect long-term potentiation (LTP) and synaptic plasticity (Thoenen, 2000). At adulthood, basal levels of the neurotrophin BDNF were found to be increased, especially in the hippocampus, while levels of 15-F2t-IsoP were specifically decreased in the same region in KO subjects. Given the role of this neurotrophin in several aspects of neuronal plasticity and memory retention, emotionality and pain sensitivity (Cirulli et al., 2004; Cirulli et al., 2000), it can be hypothesized that the increase in BDNF protein levels observed in the hippocampus of p66Shc-/- mice might underlie their better performance in the MWM test in addition to their reduced pain sensitivity and emotionality.

Old subjects did not differ in their basal levels of BDNF and isoprostanes. This lack of differences might reflect the overall lack of homeostasis, possibly associated to neurodegenerative processes that might occur at senescence (24 months). To further support this hypothesis is the finding that BDNF levels were higher in old subjects, compared to adult mice, a finding which might indicate a compensatory action aimed at neuroprotection. Alternatively, an increase in BDNF protein levels in aged rodents has already been reported and has been related to an impaired retrograde transport mechanism from cortex and hippocampus (area of production) to the basal forebrain (area of need) (Bimonte et al., 2003).

In Chapter 5 a striking result was represented by the specific expression of “backward walking” by p66Shc-/- mice during the OF test. Such a peculiar behavior has been associated to a hyper-activity of both the serotonergic and dopaminergic systems.
and to a higher BDNF tone (Bert et al., 2006; Martin-Iverson et al., 1994). BDNF and serotonin (5-HT) are both known to regulate synaptic plasticity, neurogenesis and neuronal survival in the adult brain (Mattson et al., 2004a). In particular, these two signals co-regulate one another. In addition, impaired 5-HT and BDNF signaling are central to mood disorders since selective serotonin reuptake inhibitors, commonly used to treat anxiety in humans, have been found to increase the expression of BDNF and of its receptor, in the brain (Castren et al., 2007). These data suggest that changes in emotionality and pain sensitivity found in p66Shc-/- mice might be linked to increased serotonergic function, in conjunction with changes in BDNF levels. Recent data (Berry et al., unpublished) support this hypothesis showing that p66Shc-/- adult male mice were characterized by a specific increase in the levels of serotonin, as assessed in the frontal and prefrontal cortex (ng/ml of wet tissue, see Fig 1).

![Figure 1](image)

Figure 1 Under basal conditions adult male mutant mice are characterized by elevated levels of 5-HT both in the pre-frontal (p <0.0001) as well as in the frontal cortex (p<0.05).

In conclusion data reported in Chapters 4 and 5 are in line with the oxidative stress theory of aging and confirm the initial hypothesis showing that deletion of the p66Shc gene, which results in reduced levels of oxidative stress in the brain, is able to prevent some behavioral effects of aging, particularly in response to painful or emotionally arousing stimuli.

Until recently, the main literature describing the role of oxidative stress in the aging process has focused on its contribution to cause random damage to macromolecules or as a worsening factor in (neuro)degenerative processes or as a consequence of chronic or incontrollable inflammatory conditions (Finkel and Holbrook, 2000; Floyd and Hensley, 2002; Harman, 1998). The data presented in this thesis shed a new light on the role of oxidative stress during aging through the study of the lack of the p66Shc gene in a mouse model. We provide evidence that the deficiency of a source of ROS in p66Shc-/- mice leads to a number of effects, emerging already at adulthood, involving specific signaling pathways in the brain, related to energy homeostasis and emotional behavior, which appear as important determinants of health during ageing (see the Perspectives paragraph).
2 Perspectives

It is well established that signaling mechanisms that regulate energy metabolism play important roles in lifespan determination (Bishop and Guarente, 2007; Gems and Partridge, 2001; Guarente and Kenyon, 2000), while a strong positive correlation between stress resistance and longevity has been found such that environmental and genetic factors that increase longevity also increase cellular resistance to stress (Hall et al., 2000; Johnson et al., 2000; Mattson et al., 2001). Thus, signaling pathways involved in both central and peripheral stress responses and regulation of energy homeostasis may share common brain circuitry and biochemical pathways, which may promote successful aging or contribute to the pathogenesis of age-related disease. Intriguingly, it has been suggested that insulin-like growth factors (IGFs), BDNF and serotonin (5-HT) signals - involved in a number of functions ultimately related to survival, such as cellular stress adaptation, growth and repair, neurogenesis, learning and memory and cell survival - may represent a “triumvirate” that, with their cooperative influence on energy metabolism, food intake and stress responses, plays a central role in healthspan during aging (Mattson et al., 2004b).

Data obtained in the present thesis, using a mouse model, indicate that the role of the p66Shc gene in determining lifespan is not merely related to its effects on oxidative stress but to its interaction with metabolic, behavioral and oxidative stress signaling cascades which appear as important determinants of healthspan during aging (see gray box in Fig.2) (Mattson et al., 2004b).

Indeed, genetic evidence suggests that insulin/IGF1 signaling in the adipose tissue plays a critical role in the regulation of lifespan in both invertebrates and vertebrates (Gems and Partridge, 2001), while at the molecular level the most important mecha-
nism of aging involves damage to cellular macromolecules as a result of oxidative stress (Harman, 1998). P66$^{\text{Shc}}$ is an inducible redox enzyme which, when activated by stress, produces ROS (H$_2$O$_2$) to trigger apoptosis and the deletion of it has been proven to increase lifespan in mammals (Giorgio et al., 2007). Intriguingly it has been recently reported that these lifespan pathways (insulin/IGF1 signaling in the fat tissue and oxidative stress) are mechanistically related in a way such that p66$^{\text{Shc}}$ might regulate the effect of insulin on the energetic metabolism in mice, and intracellular oxidative stress might accelerate aging by favoring fat deposition and fat-related disorders (Berniakovitch et al., 2008).

A growing body of evidence suggests that in genetic and environmental models (such as that of dietary restricted animals - DR -) of longevity in which metabolic pathways are disrupted, hormesis may be a common shared and conserved mechanism playing a pivotal role (Arumugam et al., 2006; Gems and Partridge, 2008).

In this context the manipulation of metabolic pathways (for example knocking-out the p66$^{\text{Shc}}$ gene or the dietary restriction regimen etc.) may lead to the activation of stress-related signaling pathways including those of cell survival, resistance to oxidative stress etc., aimed at protecting the organism (Gems and Partridge, 2008). Interestingly, levels of BDNF, a neurotrophin induced by stress and responsible for brain plasticity, were found to be greatly increased in several brain regions of rats and mice maintained on DR (Duan et al., 2001; Lee et al., 2000; Prolla and Mattson, 2001). Thus BDNF appears to act as a transducer of environmental stress signals into changes in energy metabolism and behavior. P66$^{\text{Shc}}$ mutants are characterized by higher basal BDNF levels and also show a decreased emotional profile, higher behavioral plasticity in a cognitive task, and an increased basal metabolism suggesting that changes in the levels of this neurotrophin might be functionally related to the phenotypic characteristics of the mutants. Changes in BDNF levels in p66$^{\text{Shc}}$ mice might also be related to changes in serotonin function (Mattson et al., 2004a) as p66$^{\text{Shc}}$ mice are also characterized by a higher serotonergic tone. This neurotransmitter regulates food intake and stress responses in the CNS (Leibowitz and Alexander, 1998) and recent evidence suggests a role for 5-HT in the modulation of lifespan both in invertebrates (Sze et al., 2000) and in vertebrates (Sibille et al., 2007). Mice lacking the serotonin 1B receptor show age-related motor dysfunction, early onset of brain molecular aging and reduced longevity (Sibille et al., 2007).

In line with the model hypothesized by Mattson (Mattson et al., 2004b) we also propose that metabolic-mitochondrial-oxidative stress signals (p66$^{\text{Shc}}$) might impinge upon signals related to mood balancing (BDNF and 5-HT) to cooperate and determine the quality of life during aging. Notably a growing body of evidence has implicated a role for chronic moderate oxidative stress in the pathogenesis of anxiety in humans (Atmaca et al., 2004). The manifestation of anxiety in a number of psychiatric disorders such as generalized anxiety disorder, depressive disorder, panic disorder, phobia, obsessive-compulsive disorder and posttraumatic stress disorder seriously affects the quality of life, particularly during aging (Gross and Hen, 2004). Recent studies have found a link between genes involved in oxidative stress metabolism and anxiety be-
haviors, supporting the hypothesis of a functional link between these pathways (Fujimoto et al., 2008; Hovatta et al., 2005).

Other prominent features characterizing the p66^Shc/- mutants are reduced pain sensitivity and an overall better physical performance at senescence, suggesting that lack of the p66^Shc gene leads to a plethora of positive effects. Thus the question spontaneously arises as to why has this gene been conserved in the genome. The antagonistic pleiotropy theory of aging predicts that for each benefit there is a cost associated. A large body of evidence reports a negative correlation between longevity and reproductive success in animal models (Kuningas et al., 2008; Partridge et al., 2005) and observational studies in historical human populations have provided similar evidence (Korpelainen, 2000; Westendorp and Kirkwood, 1998).

In our study the deletion of the lifespan determinant p66^Shc in adult mice results in a reduced fitness over repeated breeding cycles and under stressful conditions suggesting that the p66^Shc gene might have been conserved because of its indirect positive role on reproduction. Indeed, given the effects on fat storage the presence of this gene, might ensure that reproduction in general, and lactation in particular, which are energetically-demanding tasks might be “optimally” performed.

Overall, in this scenario our results set the conditions for future studies aimed to better understanding the role of oxidative stress and metabolism signaling pathways in the human brain in the context of mental health. In addition, they point to p66^Shc as a candidate gene in the search for the genetic determinants for the trade-off between fertility and lifespan.
3 Conclusion

I. Lack of the p66Shc gene overall affected fitness of KO subjects, most notably decreasing reproductive success under stressful conditions and over repeated breeding cycles. Maternal behavior was found to be redirected depending upon foraging/metabolic needs and mutant subjects experienced an anticipated puberty compared to their same age WT counterpart;

II. Peripheral responses to stressful stimuli did not differ as a function of the genotype, however mutant subjects showed a slight hyperdrive of the HPA axis and/or CG resistance following a dexamethasone suppression test (DST), possibly underlying a more efficient cross-talk between the neuroendocrine and the immune system. In addition, mutant subjects were characterized by reduced central responses to stressful/pro-inflammatory stimuli and by a higher neuronal plasticity. These features might in turn be responsible for the delay in the aging process and the better health status overall observed in the KO phenotype;

III. The longevity of p66Shc-/- mice was associated to a delay in the aging process. In particular, the lack of p66Shc resulted in: smoother age-dependent changes in the behavioral profile, lower emotionality, better cognitive plasticity at adulthood and a general better health status at senescence;

IV. Central levels of BDNF were increased under basal conditions suggesting that this neurotrophin might play a key role in shaping the phenotype of the p66Shc-/- mutants and represents a converging point linking together many of the features characterizing the mutants.
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General Discussion


