Can Social Instability, Food Deprivation and Food Inequality Accelerate Neuronal Aging?

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ABSTRACT

Based on both animal and human studies, inequality in food intake and social instability has adverse effects on the health of individuals and the community. However, it is not known whether social instability, food deprivation and food inequality affect neuronal death and premature aging in young animals. To address this question, the effects of these adverse situations, histopathological changes in hippocampal pyramidal cells and aging process were investigated.

Forty eight New Zeeland white male rabbits were divided into six groups and all of them were housed in similar conditions, with 2 animals per cage in a temperature-controlled colony room under light–dark cycle. All experimental animals were fed on standard rabbit commercial pellets and different social situations such as food deprivation, inequality in food intake, and unstable social status were applied to experimental groups during eight weeks. Afterward, lipofuscin accumulation and apoptosis, as main markers of aging, were compared to the control group by Long Ziehl Nelseen staining and the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL reaction) assay to reveal the rate of lipofuscin pigment accumulation and TUNEL-reactive apoptotic bodies in the hippocampal pyramidal cells. Serum cortisol level was also measured. Inequality in social situation raised chronic stress (i.e. food deprivation, social inequality and instability) and caused significant changes in lipofuscin accumulation in hippocampal pyramidal cells in comparison to the control group ($p<0.005$). The results also showed a significant increase in the ratio of apoptotic to normal cells in all of the stressed groups compared to the control group ($p<0.05$). Moreover, application of the social inequality and stresses alone or together modulated levels of cortisol in the experimental group.

These findings suggest that food deprivation, inequality and social instability enhance the susceptibility of hippocampal pyramidal cells to apoptosis and premature aging induced by lipofuscin accumulation.

Key Words:
Social Instability,
Food Deprivation,
Neuronal Aging,
Social Inequality,
Lipofuscin,
Apoptosis.

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1. Introduction

A growing body of research links adverse psychosocial factors such as chronic stressor experience, depressed affect, and lower levels of social integration and social support to be more prevalent in those of lower socioeconomic status (SES), (Williams, 1990). It is well established that individuals from disadvantaged social classes suffer from lower mental and physical health than individuals within higher classes (Lupine et al., 2001). Social position may effect neuroendocrine and physiological functioning and render the body vulnerable to disease (Sonia et al., 1998). Stress is one major factor to explain the association between the social class and health. This suggests that individuals from lower social class may have greater vulnerability to stress and subsequently to disease (Lupine et al., 2001(. Stress has also been correlated with an increased risk for depression (Watt & Panksepp, 2009). The impact of stress is strongly influenced by the type and duration of the stressor. In its acute form, stress is a necessary adaptive mechanism for survival. However, prolonged stress causes over-activation and dysregulation of stress activated systems thus inducing negative effects on the brain morphology and chemistry (Aleisa et al., 2006a & McEwen, 2008(. A recent examination of morphology of hippocampal neurons has found significant shrinkage of apical dendritic arbors of CA3 pyramidal neurons in all animals housed in visible borrow system (Mckittrik, Magarinos, Blanchard, Blanchard & McEwen., 2000) and stress has been shown to accelerate disease processes and cause neuronal degeneration (Gold et al., 1988, Nestler et al., 2002,Tafet & Bernardini, 2003). Individuals of lower social class are exposed to a higher rate of instability in their lives, leading to a higher level of distress (Brodhead et al., 1983 & Dohrenwend et al., 1970). The association between social position and stress may stem from environmental and/or psychological factors (Haan et al., 1989(. Psychological and psychosocial stresses elevate the blood pressure and the plasma levels of glucocorticoids and catecholamine, both by activation of “sympatho-adrenomedullary system” and “hypothalamo-pituitary-adrenal axis” (Gunner et al., 2007). Experimental settings of stressors such as immobilization, forced running or electroshock has been used in many animal studies, however, social interaction is an important source of physiological (and homeostatic) changes (Vermetten et al., 2002). Rodent models have become a widely accepted alternative to study disease mechanisms in chronic stress. In the past decades, numerous animal models of chronic stress in rodents have been developed. Social stress is probably one of the most pervasive stressors in humans and social animals (Bartolomucci et al., 2005). Cellular aging is believed to be associated with progressive accumulation of functionally inert material, including damaged non-degradable organelles and molecular structures often referred to as “biological garbage” (Terman et al., 2006, 2007). An example of such garbage is an indigestible polymeric material called “lipofuscin" or "age pigment" that accumulates in lysosomes as a result of imperfect degradation (Brunk et al., 2002, 1999). Lipofuscin, in turn, is implicated in the formation of free radicals (Terman et al., 2004) which are the major executors of oxidative stress- induced damage and subsequent cell death (Brunk et al., 2001). Apoptosis is a programmed process of cell death that has a tightly regulated initiation and execution. Progressive cell loss mediated by apoptosis is linked to age-related decline in physiologic function or age-related disorders. The loss of neurons is closely associated with functional impairments such as dementia and motor neuron disability in neurodegenerative diseases such as Alzheimer disease, amyotrophic lateral sclerosis, and Parkinson disease (Robert & Friedlander 2003).

Little is known about the effect of food deprivation at the brain level but our recent researches in animals indicated that sense of inequality in food intake rather than food deprivation alone could promote accumulation of aging pigment of lipofuscin in myocardial cells, introducing a premature aging process (Heidary et al., 2008 & Mojarab et al., 2010). However, food restriction in the isolated group of rats caused a significant reduction in their ability for consolidation of memory and recall of information, although this reduction did not appear when the animals were food restricted but considering others unlimited feeding (Vaez Mahdavi et al., 2009).

Stress and its subsequent elevation in serum glucocorticoids level, affect neuronal function and viability in various hippocampal sub regions to a different extent (Lucassen et al., 2001). There are many indications that the hippocampus is strongly affected when animals are socially stressed . Interest in the impact of different social situations has led to the development of several animal models that may be relevant to human social situation-related disorders (Blanchard et al., 2001). In the present study, we conducted animal experiments using different socially affected rabbits as a model similar to that of human psychosocial stress. Several studies indicated that rabbits are suitable representative in studying food deprivation, oxidative stress and aging related changes (Carvalho et

al, 2009 Bharathi et al & 2006. Savory et al). Following our studies about the effects of psychosocial stress on heart and hepatocytes of rabbits, the animal model of psychosocial stress was applied in rabbits to address this hypothesis that "Do psychosocial stresses, food deprivation and food inequality promote neuronal aging?" Lipofuscin accumulation and apoptosis (as two markers of oxidative stress) in hippocampal pyramidal cells, and the serum level of cortisol in rabbits were investigated in exposure to different situations.

2. Methods

2.1. Animal Preparation

This study was approved by the ethical board of the Neuroscience Research Center of Shahid Beheshti Medical University in compliance with European Communities Council Directive (86/609/EEC).

Accordingly, adequate measures were taken to minimize pain and discomfort of animals. The study was conducted on forty eight young New Zealand White male rabbits (9-12 months age) weighting between 2 and 3 kg (obtained from Pasteur Institute of Tehran, Iran). Animals were housed at 21◦C in Plexiglas cages (60cm length, 21.5cm height, 40cm width, Razi Rad company, Tehran, Iran) with 2 animals per cage in a temperature-controlled colony room under light–dark cycle (12 h light: 12 h darkness). All experimental animals were fed on standard rabbit commercial pellets (Pars animal food company, Tehran, Iran) with the following composition: 14.9% crude protein, 2.7% crude fat, 14% crude fiber, and 13% ash. Water was given ad libitum to all groups. The animals were held in the colony room for at least two weeks before being test and then animals were randomly divided into six groups. Each group had eight rabbits. Food intake was weighed and measured daily. Six groups were set in two rooms, five groups in one (general room) and the last group in another (isolated) room. All animals were housed in adjacent areas within visual, auditory and olfactory, but not tactile contact. After two weeks of assimilating period, four different social conditions such as food deprivation to 1/3 of the daily allowance, changing the cage-mate, inequality in food intake and isolation were applied to the groups during eight weeks.

2.2. Experimental Groups

1- Control group (C), free access to diet without any deprivation or alteration of the place or cage-mate (n=8).

2- Food deprivation (FD), 65% food deprivation during 2 months, without any alteration of the place or cage-mate. It is important to mention that this level of food deprivation did not cause any gastrointestinal problems such as stasis, pain or dairies in the present study and similar deprivation level has been used previously in our and other studies (Heidary et al., 2008, Santo et al., 2009 & Carvalho et al., 2009) (n=8).

3-Unstable social status or social instability due to altering the place or cage-mate (Zoladz et al., 2008) and food deprivation (USS+FD). (The residence of the animals in this group was changed every two weeks) (n=8).

4- Similar condition as the third group, but only for four weeks (USS+FD, 4 weeks). Later, the condition became the same as the control group (n=8).

5- This group was similar to the third group, but the animals were separated from other groups and were kept in an isolated room (Motoyama et al., 2009) (USS+FD+IE) (n=8).

6- Unstable social status or social instability (USS), with free access to diet without any deprivation but alteration of the place or cage-mate (n=8).

2.3. Histopathological Evaluation

All animals tolerated the experimental process to the end of the experiment. All animals were deeply anesthetized. Before perfusion the blood sample was collected from the left ventricle for biochemistry assessment at 8-10 AM and the animals were perfused through the ascending aorta with 1000 ml of PBS followed by 1500 ml of 4% phosphate-buffered paraformaldehyde (PH:7.4). After tissue perfusion, the whole brain tissues were removed and subsequently put in tissue processor (DS 2080/H, Tehran, Iran) for processing. Afterward, they were embedded in paraffin for histopathological evaluation. Coronal sections (4-5 µm thickness) of hippocampus formation were prepared using a microtome rotary apparatus (Slee, Germany). At least three sections were assigned to both Long Ziehl Nelseen staining (the specific staining for lipofuscin detection) and TUNEL staining.

2.3.1. Long Ziehl Nelseen Staining

Paraffin embedded tissue sections were soaked in carbol fuscin solution for 3 hours at 60°C. The sections were counterstained with methyl blue and differentiated

in acid alcohol. Then, they were dehydrated in 95% ethanol and absolute alcohol, cleared and mounted (Entellan, MERK).

2.2.3. Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End-Labeling (TUNEL)

TUNEL was performed on 10-µm-thick paraffin-embedded sections using the In Situ Cell Death Detection Kit, POD (Roche Applied Science, Germany). Tissue sections were deparaffinized in xylene, rehydrated, and immersed in 3% hydrogen peroxide to block the endogenous peroxidase activity. After rinsing with PBS, sections were treated with proteinase K solution at 37°C for 30 min to enhance the staining, incubated for 60 min at 37°C with 50 µl of TUNEL reaction mixture, and then incubated for 30 min at 37°C with 50 µl of converter-POD. Sections were rinsed in PBS, then incubated for 10 min at 15–25°C with 50 µl of diaminobenzidine (DAB) substrate solution and rinsed again with PBS. Counter staining was achieved with 0.5% methyl green. For the positive control, the sections were incubated in DNase1 solution at 15–25°C for 10 min, and for the negative control, enzyme solution was omitted. Finally, the sections were dehydrated again and cover-slipped as described above and were analyzed under light microscopy.

2.3.3. Lipofuscin and TUNEL Positive Cell Counting

Lipofuscin analyses were performed in the pyramidal cells of CA1, CA2 and CA3 subfields of the hippocampus. At least five histological sections were processed for each animal to avoid bias in determining the number of lipofuscin pigments in the hippocampal sections. Random numbers were assigned to the X and Y axes of the microscope. The number of lipofuscin pigmentation was counted in cells of each microscopic field. The mean value was calculated for each animal and for each group. Pictures of the histopathological changes were perpetrated by light microscopy.

The number of TUNEL positive pyramidal cells was counted in six adjacent 100X microscopic fields among the pyramidal cells (in the CA1, CA2 and CA3 subfields) of the hippocampus and the ratio of apoptotic cells to normal cells was obtained.

2.4. Cortisol Assay

Cortisol levels were measured by electrochemiluminocense (Elecsys 2010) technique using Cortisol Kit (Cobas, USA).

2.5. Statistical Analyses

Lipofuscin pigmentation, TUNEL positive cell counting and weight measurements were analyzed by one-way analysis of variance (ANOVA) followed by LSD for multiple comparisons, using SPSS 16.0 package program. Data were expressed as mean ± SEM and statistical significance was set at $p<0.05$.

3. Results

3.1. Inequality in Food Intake and Psychosocial Stresses Promoted Accumulation of Lipofuscin pigments in Pyramidal Hippocampal Cells.

Representative photomicrographs of the Long Ziehl Nelseen staining - specific lipofuscin staining - of tissue sections of experimental groups are shown in figure 1a. Lipofuscin pigments are detected in cytoplasm of the cells by their purple appearance.

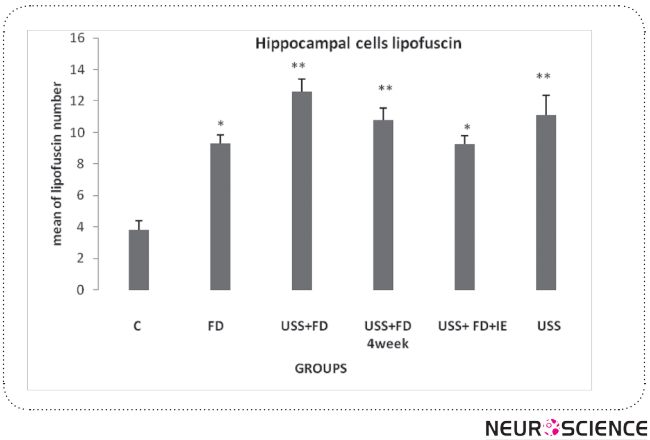

Figure 1(a). Effect of changes in social situations on accumulation of lipofuscin pigments in hippocampal pyramidal cells: The data are Mean+SEM.*$p<0.05$ versus the control group, **$p<0.005$ versus the control group and # $p<0.05$ versus (USS+FD) group .

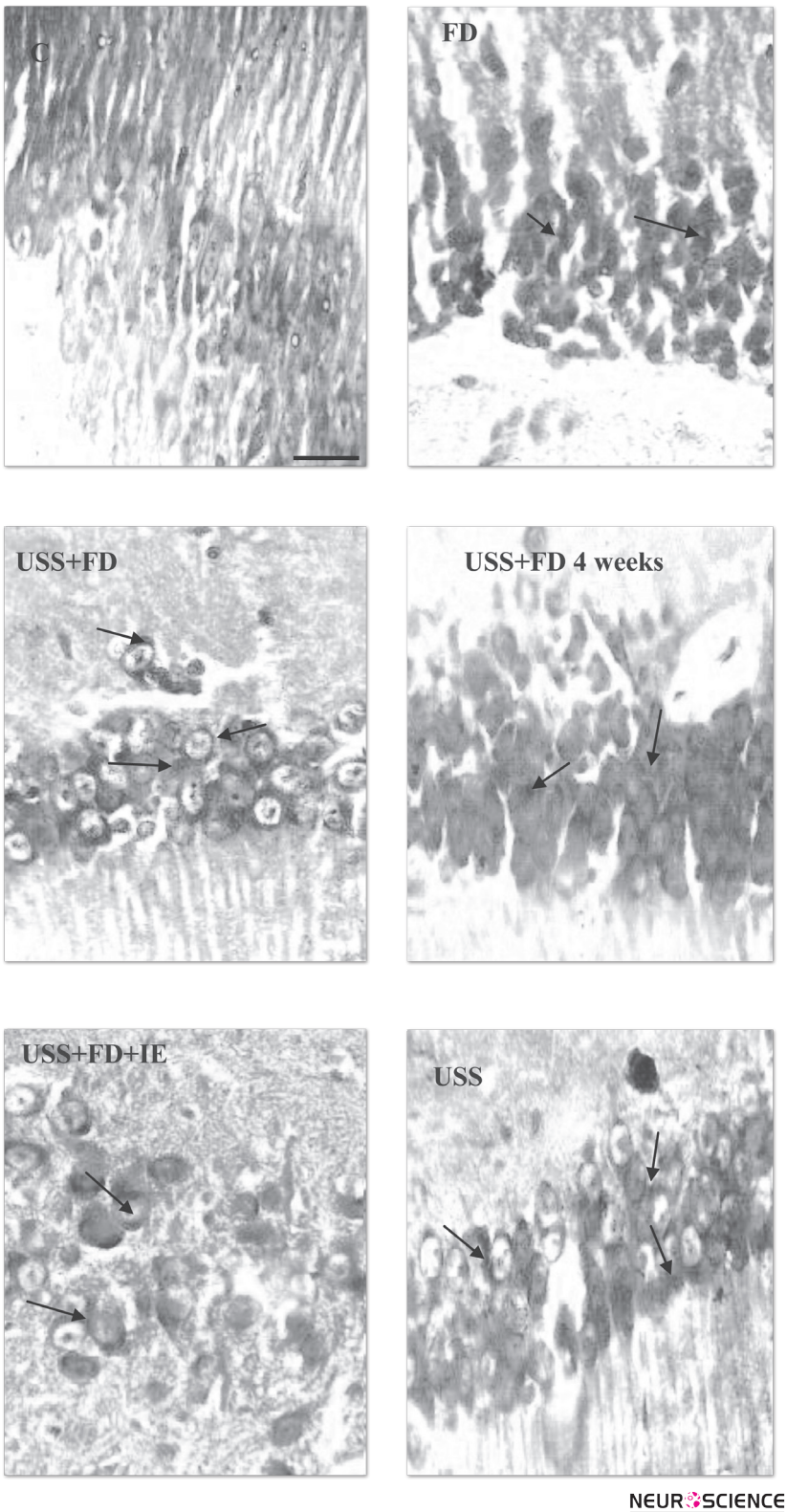

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Figure 1(b). Representative photomicrographs of Long Ziehl Nelseen staining sections respectively from left to right: The control group (C) , The food deprived group (FD), The unstable social status and food deprived group (USS+FD), The group unstable social status and food deprivation for 4 weeks (USS+FD 4 weeks) ,The unstable social status, The food deprived and The isolated group (USS+FD+IE) and the unstable social status group (USS). The arrows show the lipofuscin spots. Lipofuscin has been presented as dark red spots in the cytoplasm. All the pictures have magnification, 40 µm. Scale bar: 40 µm.

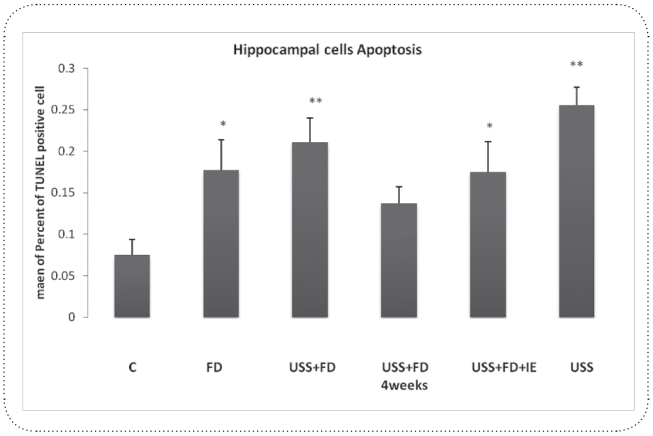

Figure 2(a). Effect of changes in social situations on apoptosis in hippocampal pyramidal cells: *p<0.05 versus the control group,** p<0.005 versus the control group and & p<0.05 versus (USS) group. The data are Mean+SEM.

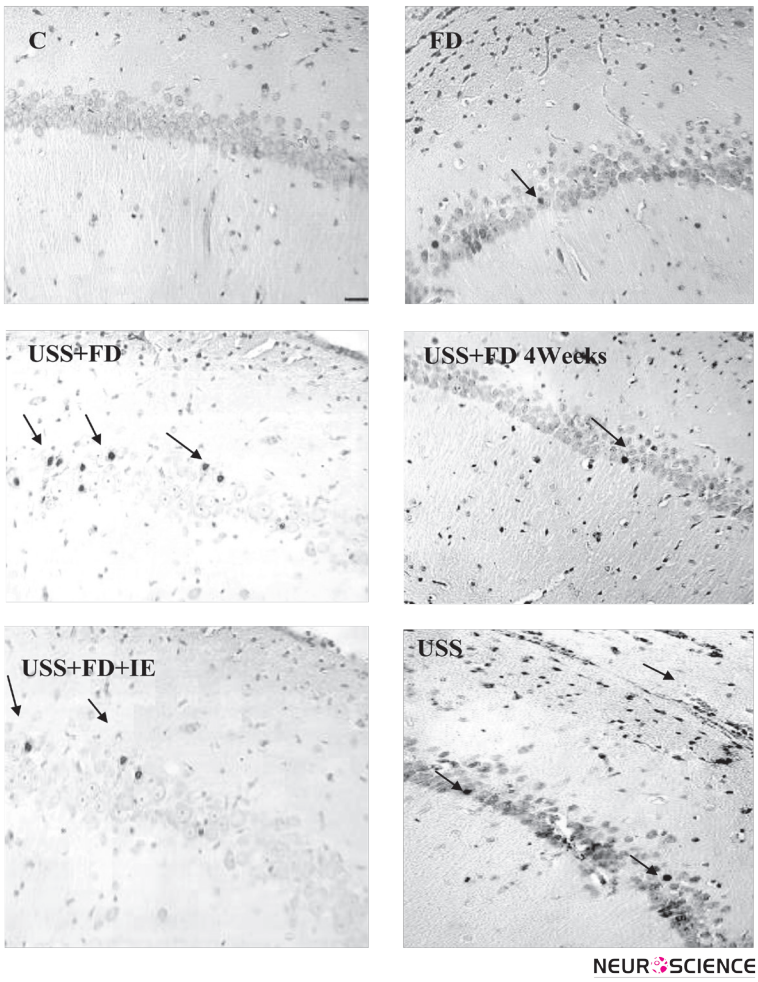

Figure 2 (b). Representative photomicrographs of the TUNEL immunostained sections respectively from left to right: the control group (C), the food deprived group (FD), the unstable social status and food deprived group (USS+FD), the group unstable social status and food deprivation for 4 weeks (USS+FD 4 weeks), the unstable social status, food deprived and isolated group (USS+FD+IE) and the unstable social status group (USS). The arrows show the apoptotic cells. All the pictures have magnification, 40 µm. Scale bar: 40 µm.

Table 3. Effect of changes in social situations on serum cortisol levels. the control group (C) , the food deprived group (FD), the unstable social status and food deprived group (USS+FD), the group unstable social status and food deprivation for 4 weeks (USS+FD 4 weeks) ,the unstable social status, the food deprived and the isolated group (USS+FD+IE) and the unstable social status group (USS).

Groups	C	FD	USS+FD	USS+FD	USS+FD+IE	USS
	4 Weeks					
Mean (µg/dl)	1.71	1.4	2.3	1.7	0.85	2.3

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Application of all types of food deprivation and psychosocial stresses (unstable social status, social inequality and isolation) increased the accumulation of lipofuscin compared to the control group. This accumulation was highest in USS+FD, USS+FD 4 weeks and USS groups. ($p<0.005$, Figure 1b)

3.2. Inequality in Food Intake and Psychosocial Stresses Enhanced Apoptosis in Pyramidal Hippocampal Cells.

Representative photomicrographs of the TUNEL-stained tissue sections of experimental groups are shown in figure 2b. According to the instruction manual of the kit, neurons with double-strand breaks in DNA—a feature suggestive of apoptosis—are detected in TUNEL-staining by their brown nuclei. No evidence of TUNEL reaction was detected in the control animals (Fig. 2a).

Application of the psychosocial stresses and food deprivation caused a significant change in apoptosis of the hippocampal cells. This accumulation was highest in the USS group (in comparison with the control group) ($p<0.005$). (Figure 2(b))

3.3. Serum Cortisol Level

Application of the mentioned stresses, alone or together (i.e. food deprivation, social inequality and changed cage-mate), elevated cortisol levels in stressed groups. However, this elevation was not statistically significant in comparison to the control group. (Table.1)

4. Discussion

Our findings in the present study demonstrated that chronic food deprivation, social inequality and unstable social status are important socio-biologic stressors and drive dramatic accumulation of lipofuscin in hippocampus and apoptosis. Our results showed that unstable social status with or without food deprivation causes significant changes in lipofuscin accumulation and apoptosis in hippocampal cells in comparison with the control group ($p<0.005$). Due to the hippocampus involvement in cognition and its high content of glucocorticoid receptors, exposure to chronic stress may have deleterious effect on the hippocampus structure and function (McEwen, 1999 & Aleisa et al., 2006). Similar to our results some other studies have shown that young animals under stress exhibit increased evidence of aging like neurophysiological changes (Kerr et al., 1991). Chronically elevated physiological stress is considered as a plausible model for how deprivation and poverty could get into the brain and eventually interfere with the achievement (Gary et al., 2009). In addition to contribution to physical morbidity, chronically elevated allostatic load also influences neurobiological processes, particularly in the hippocampus and prefrontal cortex that are capable of disrupting cognitive functioning (McEwen et al., 2005., Sapolsky et al., 2004 &Lupine et al., 2006, 2007). Application of the mentioned stresses alone or together (i.e. food deprivation, social inequality and changed cage-mate), did not cause significant change in the serum levels of cortisol of the stress groups in comparison with the control group. This finding is consistent with extensive work, finding lower baseline cortisol levels in stressed people (Yehuda., 2005) and suggests that the chronic psychosocial stress employed in the present study may have long-term effects on basal HPA axis functioning. Comparable findings have been reported in some animal studies examining the effects of stress–re-stress or single prolonged stress paradigms on animal physiology (Harvey Naciti, Brand & Stein, 2003). Other studies, like our results, showed that food-deprived animals possess a mechanism which suppresses the stress-induced rise of catabolic hormones, saving energy expenditure. the present experiment suggests that food deprivation attenuates a stress-induced increase in catabolic hormones like cortisol (Dantua W et al ., 1993 & Danuta W et al., 1990) (Table.1)

It has been demonstrated that psychosocial stresses induce the production of reactive oxygen species. On the other hand, it has been documented that so-

cial stress causes oxidative stress (Zhang et al., 2006). Findings clearly indicate that social status can be linked with neuroendocrine and metabolic factors. However, the analogy with social gradient in humans can be pursued only cautiously (Steptoe et al., 2002). Lipofuscin accumulation may also diminish autophagocytotic capacity by acting as a sink for newly produced lysosomal enzymes and, therefore, interfere with recycling of cellular components. Lipofuscin, thus, may be much more directly related to cellular degeneration at old age than was hitherto believed (Brunk, 2002). Moreover, it is considered as a predisposing factor for premature cell death. We observed that the percentage of apoptotic cells in the unstable social status group was higher ($p<0.005$) than in the control group (Figure 2(a)).

Inequality in social situation has been shown to cause significant changes in consolidation of learning and memory (Vaez Mahdavi et al., 2009). It might be beneficial to mention that myocardial cell lipofuscin accumulation has been found to be increased under similar stress models (Heidary et al., 2008 & Mojarab et al., 2010). Despite the potential benefits of a moderate food restriction, evidences from literature suggest that a drastic reduction in food intake may have harmful effects in the oxidative status. Domenicali and others (2001) showed that the liver of food-deprived rats presents an increase in protein and lipid oxidation and a decrease in reduced glutathione (GSH) levels. Another study demonstrated that a 36-h fasting protocol increases the levels of free radical generation and lowers the activity of enzymatic defenses like catalase (CAT) and cytosolic superoxide dismutase (CuZn-SOD) (Marczuk-Krynicka et al., 2003). Moreover, it has been suggested that food deprivation enhances ROS production and free radical leak at complex III of the mitochondrial respiratory chain (Sorensen et al., 2006). It has been shown that the effects of caloric restriction depend on different factors, such as the degree of food restriction and its implementation time (Yu 1996). Several studies on mice and rats reported that caloric restriction promotes beneficial effects, including an increase in life span and a delay on the onset of age-associated pathologies (McCarter 1995, Weindruch 1996 & Masoro 2000). However, other studies showed that when rats are submitted to drastic food deprivation, adverse outcomes occur (Santo et al., 2009, Grattagliano et al, 2000 & Dominical et al., 2001). However, the available information concerning the influence of food deprivation in the oxidative status in the brain is scarce. In this line, we evaluated the effects of a 2/3 food deprivation in the oxidative status of Newzeland white rabbit brains. Our results indicate that food deprivation, like social instability, enhances accumulation of pathological markers of oxidative stress in rabbit's brain. Lipofuscin accumulation and apoptosis were significantly increased in (FD) group in comparison with the control group ($p<0.05$), however, this effect was strengthen when both food deprivation and social instability were applied together in (FD+USS) group (Figure 1a and Figure 2a).

Accordingly, Gursoy and others (2001) observed an increase in lipid peroxidation in heart and liver of caloric-restricted rats. It was also reported that under dietary deprivation, fish present high levels of oxidative stress (Pascual et al., 2003 & Morales et al., 2004).

Lipofuscin is well understood as a product of oxidative stress, which contributes to cellular aging (Gray et al., 2005). Therefore, we supposed that severe food deprivation affects brain oxidative status, predisposing brain cells to degeneration and death. While moderate caloric restriction has beneficial effects on animal health status, severe food deprivation may be harmful.

Our research reports, provide an impetus that animals as well as humans sense the inequality and instability and react to them bio-psycho-neuro-socially (Heidary et al., 2008 & Vaez Mahdavi et al., 2009). In this regard, during the present study we finally found that chronically stressed animals displayed a more anxious phenotype immediately after stress induction in the novelty-induced social instability paradigm. For example, after changing the place or cage-mates, animals severely fight together for achieving territory or dominance and at the end of stress period they had sores in their ears, back and genitalia areas. It is important to acknowledge that cellular accumulation of lipofuscin was higher when food deprived animals could observe other animals unlimited food accessibility, as compared with the isolated deprived group (comparison of (USS+FD) with (USS+FD+IE) group $p<0.05$). The latter showed pigment accumulations even lower than the (USS+FD 4 weeks) group, which were under food deprivation associated with observation of others only for half of the period of the experiment. Indeed, sense of inequality in food intake, rather than food deprivation, could promote lipofuscin accumulation and further aging, considering the majority of this effect as instability rather than food deprivation (comparison of FD group who had pure food deprivation with the USS group who had only unstable situation and comparison of FD group with USS+FD group) ($p<0.05$). In (USS+FD 4 weeks) group which were under food deprivation associated

with observation of others only for half of the period of the experiment and (USS+FD+IE) group which were isolated, it seems that shortening of the stress period and isolation without sense of inequality can provide healing role and help cellular recovery. Therefore, in these groups lipofuscin pigments were formed but the ratio of apoptotic cells to normal cells were much lower than others. It is concluded that inequality and instability in social situation promotes biological conditions, in which pre-mature aging can occur. Moreover, our recent findings suggest that this premature aging even provides higher possibility of animal death (data not shown).

There is a poor understanding of animal heterogeneity in patho-physiological mechanisms underlying pathways responsible for oxidative cell injuries due to social stress. Physiological reactivity is associated with psycho-social factors that differentially impact upon the social gradient. Enhanced stress reactivity may be necessary but not sufficient for all psycho-biological pathways. However, all of these pathways in relation to social inequalities and brain cells damage must be evaluated according to the scientific principles that are applied to other pathophysiological processes.

Epidemiological studies clearly show that stress, by itself, is not sufficient to result in the manifestation of a disease. In many of the neuroendocrine and behavioral parameters which were assessed at an old age, we observed a trend which probably did not reach the level of significance due to a high variability in the group. One might speculate that only a sub-population of the previously stressed animals are largely and persistently affected by the stress exposure due to their specific genetic makeup, while other animals "recover" and are not at an enhanced risk for disease. As with the here applied chronic stress model, it is feasible to subject a large number of animals to the stressful situation. Future studies will have to focus on the individual differences between chronic stress in animals in terms of the magnitude of persistent effects and their interaction with genetic vulnerability of the individuals. Furthermore, it will be interesting to study possible pharmacological modification of persistent effects of chronic stress experiences.

The application of chronic social stress during adolescence, exerted robust immediate effects on HPA axis activity, gene expression, and anxiety-related behavior. These data support our previous findings and point to the high face, construct and predictive validity of this animal model for chronic social stress. With respect to the postulated causal relationship between chronic stress and the development of human affective disorders, these data can be the basis of studying the underlying neurobiological mechanisms in detail. Ultimately, social stress can have profound bio-psycho-neurological consequences on the brain of rabbit. This study showed that the chronic stress due to the presence of inequality, social instability and food deprivation affects the nervous system of psychosocially-stressed individuals in a way that increases the rate of premature aging. Further studies is needed to determine which of these changes are adaptive and which ones are associated with continued pathological changes in the brain function and behavior. A better understanding of those pathophysiological mechanisms, in turn, may contribute to the development of novel, innovative, biological and psychological treatments in the future.

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